Dhananjaya Pratap Singh
Harikesh Bahadur Singh
Ratna Prabha *Editors*

Plant-Microbe Interactions in Agro-Ecological Perspectives

Volume 2: Microbial Interactions and Agro-Ecological Impacts

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Award by the UP Council of Science and Technology. Dr. Singh has been a fellow of the National Academy of Agricultural Sciences. Currently, he is also serving as an associate/academic/board editor for journals of international repute. Dr. Singh has more than 300 publications to his credit, including several training modules/manuals, 17 edited books, and 20 patents (USA, Canada, PCT).

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Interaction Between Beneficial Bacteria and Sugarcane

1

Guilherme Grodzki Oliveira Figueiredo, Valeria Rosa Lopes, Ricardo Cancio Fendrich, and Vivian Jaskiw Szilagyi-Zecchin

Abstract

Eco-friendly sugarcane production is constantly faced with growing demands for increased productivity. Current biotechnology, based on growth promotion through bacterial inoculants, presents us with the opportunity to increase production without an adverse environmental impact. To this end, plant growth-promoting bacteria (PGPB) with their diverse agricultural characteristics, like nitrogen fixation and production of plant regulators, are a good choice in achieving this goal. Characterization of the abilities of different strains will define their potential use, which for the most part is not limited to a single desirable feature. Therefore, our aim was to contribute to the present understanding of the principal activities of PGPB in sugarcane, to provide some simple and common methods for selecting them, and to draw attention to sugarcane breeding for selection of responsive clones for PGPB inoculation.

Keywords

PGPB • Sugarcane • Inoculation • Biological nitrogen fixation • *Saccharum* sp

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1.1 Introduction

Conventional agriculture has had a considerable negative impact on the environment in recent years, mainly on soil and water sources. Environmental degradation resulting from inappropriate agricultural practices and the indiscriminate use of agrochemicals has changed the way of modern agriculture. New cultural practices that are less aggressive to the environment are necessary and have more sustainable agriculture appeal. Agricultural techniques need to be changed, aiming at "cleaner" practices for the environment.

Many studies have led to the use of "natural products," such as beneficial bacteria for the control of pests and diseases, as well as the promotion of development of plants for greater productivity. One of these new strategies is the use of bacteria to induce plant growth, control plant disease, and produce biodegradation of xenobiotic compounds (Perry et al. 2007). This science is growing rapidly, and in turn, the new biomolecular technologists have contributed significantly to this new agriculture (Moreira and Siqueira 2006).

To date, several microorganisms have been studied and have demonstrated efficiency in controlling diseases, increasing productivity, and improving other desirable traits in various plant species, and sugarcane has been one of the most important crops in this research (Silveira 2008; Moreira and Siqueira 2006).

The interaction of sugarcane with plant growth-promoting bacteria (PGPB) has been extensively studied and various technologies have been developed in the last 50 years. Sugarcane is considered to be one of the best options among the renewable energy sources, with a promising future in a global scenario (Maule et al. 2001). In addition, sugarcane is propagated in a vegetative way, by clones (Matsuoka et al. 2005), which facilitates the selection of bacteria with greater interaction among the cultivars, ensuring greater success in obtaining inoculants.

This chapter thus covers the new knowledge about this mechanism of interaction and its implications for sugarcane agriculture.

1.2 Sugarcane

Sugarcane (*Saccharum* spp.) is one of the most important species cultivated commercially in the tropics and subtropics for renewable energy sources (Bonnett et al. 2004; Manners et al. 2004). It is propagated vegetatively by stems and produces a large amount of biomass, which requires a high application of nutrients, mainly nitrogen. Commercial sugarcane is also propagated by allowing the growth of the stems of the stools that remain in the soil after harvesting the previous crop (ratooning).

The production chain of sugarcane, its products and byproducts, is an important source of distribution of wealth (Matsuoka et al. 2005). In addition to alcohol and sugar, it has other byproducts, such as bagasse, various types of paper, pharmaceutical products, yeast, and various products resulting from the alcohol chemistry such as polyethylene, ether, acetone, and others (Vian 2009).

Brazil is the world's largest producer of sugarcane, followed by India, China, and Thailand (FAO 2016). In fact, this crop occupies an area of 8,654 hectares and has a production of 665,586 thousand tons (data from the last harvest, 2015/2016 (CONAB 2016)). Brazil is also a world leader in sugar production and is responsible for more than half of the world's sugar market (MAPA 2012), exporting to countries such as China, Russia, and Egypt (USDA 2012).

The genus *Saccharum* is characterized by high levels of polyploidy (polyploids have more than two sets of chromosomes) and frequently by unbalanced numbers of chromosomes (aneuploidy) (Blackburn 1984; Jannoo et al. 1999). These characteristics increase the genetic complexity of the cultivars (Jannoo et al. 1999), and confer to this culture a certain adaptability that allows its cultivation in different environments, soil types, and relief (Santos 2008), and adaptability is favorable for interaction with different beneficial bacteria.

1.3 The Activities of Plant Growth-Promoting Bacteria (PGPB) in Sugarcane

The binding between sugarcane culture and PGPB is of great importance for sustainable cultivation once the bacteria can promote the growth of the plant, reducing the use of chemical fertilizer by different mechanisms.

The PGPB are able to promote plant development by means of different mechanisms (Silveira 2008). These bacteria are able to fix nitrogen from the atmosphere, induce plant defense mechanisms responsible for diseases protection, solubilize phosphorus, produce siderophores that sequester and provide ferric ions, oxidize sulfur, and produce hydrocyanic acid (HCN) and other substances (Luz 1996; Rodríguez and Fraga 1999; Arencibia et al. 2006; Tortora et al. 2011).

Beyond those properties, these bacteria have the capability of producing precursor substances of plant growth regulators such as adenine derivatives (precursors in cytokinin biosynthesis) and growth-promoting compounds that have a similar activity to plant regulators (Silveira 2008). The main classes are auxins, cytokinins, gibberilines, ethylene, and abscisic acid (ABA) (Moreira and Siqueira 2006).

The first report of these beneficial mechanisms came from the fact that some commercial cultivars of sugarcane did not present symptoms of nutritional deficiency, mainly nitrogen, after many years of cultivation without fertilization (Boddey et al. 1995).

This and similar reports brought about the discovery of the nitrogen-fixing bacteria in sugarcane that have been studied since then. Recently, Magnani et al. (2010) and Moreira (2013) described the existence of a large bacterial community associated with sugarcane. This association explained the lesser requirement for soil fertility by some cultivars in the last 50 years, like the most planted sugarcane type in Brazil, the RB867515 cultivar. According to Beneduzi et al. (2013), there is a wide spectrum of bacterial populations associated with sugarcane, increasing the cultural potential in restrictive soils. The presence of these bacteria in the cane plantation is

confirmed by its survival capacity in cultivated soils and by propagation of infected stalks (Olivares et al. 1996, 1997).

There are in fact more than 40 bacterial genera that are known to be involved in growth promotion and disease occurrence in sugarcane. Among the beneficial bacteria with biological nitrogen fixation (BNF) or other properties are the genera *Azospirillum*, *Azotobacter*, *Beijerinckia*, *Burkholderia*, *Herbaspirillum,* and *Gluconacetobacter*. The most well-known genera are *Azospirillum* and *Gluconacetobacter*, both of which are endophytic (Oliveira et al. 2004; Moreira and Siqueira 2006; Hungria 2011; Mehnaz 2013). Rodrigues et al. (2016a) showed the wide spectrum of PGPB involved in sugarcane plant development. These authors isolated 136 bacteria, of which 83 bacteria presented with some plant growth mechanism.

G. diazotrophicus is the most common species associated with sugarcane and is found in leaves, stalks, and, especially, intercellular spaces, even sub-stomatal cavities. Usually in the roots this genus is presented between apoplast cells (Dong et al. 1994; James et al. 2001).

The association between PGPB and sugarcane is complex and is closely dependent on an environment-genotype-bacterium interaction. For example, the bacterium-environment interaction was studied by Pereira et al. (2012), where the genus *Burkholderia* spp. associated with sugarcane presented low bacterial growth, low BNF, and auxin production in a high salinity environment. On the other hand, *Burkholderia tropica* described by Reis et al. (2004), was demonstrated to be a good alternative for sugarcane in other environments. Oliveira et al. (2006) affirmed the necessity to combine bacteria and environmental conditions to achieve the maximum potential.

Studying the association between bacterium and genotype, Fuentes-Ramírez and Caballero-Mellado (2006) demonstrated in their review that bacteria also have an "embracing" interaction with many cultivars or specific cultivars. Schmatz et al. (2012) confirmed the response dependent on the different genotypes. For sugarcane, most distinct groups of bacteria are linked more with the rhizospheric region of sugarcane than other areas. The rhizospheric bacteria intensify root development, influencing the whole plant (Costa et al. 2014; Fuentes-Ramírez and Caballero-Mellado 2006; Oliveira et al. 2006).

For Tejera et al. (2005) working with sugarcane cultivars cultivated in Spain, the *Azospirillum* genus is more closely linked to sugarcane colonization and better associated with it than the *Azotobacter* genus, which did not show an affinity to the sugarcane rhizosphere.

Moutia et al. (2010) observed the interaction between cultivar-irrigation-inoculation of *Azospirillum* strains. These authors obtained different results for cultivars when inoculated under drought stress conditions. For cultivar R570 the inoculation did not differ from the non-inoculated treatment in both environmental conditions, while the cultivar M1167/77 presented a positive response to inoculation in drought stress. Drought tolerance in sugarcane was described by Vargas et al. (2014) using *G. diazotrophicus* species inoculated with SP70–1143 cultivar. The plants under watered conditions had less *G. diazotrophicus* concentration than

unwatered plants, 3 days after water deficit. The mechanisms that play an important role in stress tolerance were evaluated by molecular analysis, mainly in the roots. Ethylene and ABA biosynthesis were greater in non-inoculated roots, indicating tolerance to drought stress on inoculated *G. diazotrophicus* treatments.

For these reasons, the beneficial mechanisms may change depending on the cultivar, environment, and bacteria. However, the two principal mechanisms of action are described below—nitrogen fixing and the production of phytohormones.

1.3.1 Nitrogen Fixing

The measure of nitrogenase activity in sugarcane roots provided some of the earliest evidence of bacterial contribution to the rhizosphere, as related by Döbereiner et al. (1972). Some descriptions attribute about 70% of obtained nitrogen by cultivar for BNF to be *G. diazotrophicus,* one of the main bacteria that contribute to this mechanism (Boddey et al. 1991; Moreira and Siqueira 2006). Urquiaga et al. (1992) suggested through a ^{15}N enrichment method that most genotypes assimilated a large amount of nitrogen through BNF. However, Polidoro et al. (2001) observed that factors like soil fertility and plant nutrition influenced the bacterial contribution to available nitrogen to the plants. With regard to sugarcane development, Pedula et al. (2016) demonstrated recently that dry matter and nutrition increase on application of PGPB in sugarcane with or without nitrogen fertilizer.

According to Garcia et al. (2013), an inoculation of a mixture of five diazotrophic bacteria[1] strains provided an increase in chlorophyll content and plant development similar to nitrogen fertilizers. The chlorophyll content may be related to the effect of nitrogen content increasing when the BNF mechanism is activated. BNF provides organic nitrogen, which has a strong influence on the plant's photosynthesis. This may explain the higher chlorophyll content in plants inoculated with *Azospirillum*, relating to a high photosynthesis tax (Zaied et al. 2003; Donato et al. 2004; Bashan et al. 2006; Wolff and Floss 2008). For Marcos et al. (2016), the modifications in sugarcane physiology caused by PGPB inoculation do not change dry matter.

The BNF in sugarcane occurs by association, and to reach similar results to those obtained in leguminous plants may be impossible. Nevertheless, sugarcane has been even more widely studied in this area, due to promising culture associated with PGPB, either through BNF or some other mechanism (Moreira and Siqueira 2006). On the other hand, according Magnani et al. (2010), it is important to consider that not all bacteria associated with sugarcane can be considered nitrogen fixers. The authors found that only 10% of all isolates in stalks and leaves are linked to nitrogenase activity.

[1]The mix of bacteria: BR11335 (*Herbaspirillum seropedicae*), BR11504 (*Herbaspirillum rubrisubalbicans*), BR11281T (*Gluconacetobacter diazotrophicus*), BR11366T (*Burkholderia tropica*) e BR11145 (*Azospirillum amazonense*) (Garcia et al. 2013).

1.3.2 Growth-Promoting Regulators (Phytohormones)

Since the 1990s, the investigation of PGPB has been intensified by researchers, and this has revealed secondary products produced by bacteria, such as growth regulators, that bestow advantages on plant growth development and productivity. One of the growth regulators is from the auxin group, the major group linked to growth development in bacteria-sugarcane associations (Fuentes-Ramirez et al. 1993; Mirza et al. 2001; Reinhold-Hurek and Hurek 2011).

Auxin mainly alters root growth, and this aspect has been recognized as a marker of beneficial bacterial effects. The rapid establishment of roots, either by elongation of primary root or by increments in lateral roots, is "gainful" to the plants, thereby enabling the plants to absorb more nutrients and water due to the increased contact surface (Silveira 2008).

Fuentes-Ramirez et al. (1993) demonstrated a wide spectrum of the presence of *G. diazotrophicus* in sugarcane cultivars inside tissues and producing auxins (IAA), and investigated the metabolic effects on promoting growth. Mirza et al. (2001) observed auxin production by PGPB, which promoted micropropagation in sugarcane.

Beyond auxins, gibberellin production (GA1 e GA3) was found by Bastián et al. (1998) in controlled assays with *G. diazotrophicus* and *Herbaspirillum seropedicae*. Leite et al. (2014) detected PGPB salinity tolerance in soils, producing auxins, fixing nitrogen, and solubilizing phosphate, and investigated sugarcane development in soils restricted by high salinity.

The growth regulators produce secondary effects on plants, affecting sugarcane production positively or negatively. There are many related effects that have been noted in the scientific community, among which are: effect on sprouting, stalk and saccharose accumulation, height of plants, and leaf area index (de La Cruz et al. 2012; Schultz et al. 2012; Beneduzi et al. 2013; Oliver 2014; Gírio et al. 2015).

Because of the discovery of these effects, research has been intensified in sugarcane, as the application of microorganisms may be less costly and easier to handle than chemical fertilizers. Pérez and Casas (2005) isolated *Azospirillum* strains from sugarcane roots and introduced those bacteria in micropropagated sugarcane, noting greater development in the inoculated plants.

In controlled conditions, Ferrel-Caballero and Soriano (2014) applied *Rhizobium* on *Saccharum officinarum* obtaining superior results to those obtained with chemical fertilizers (33% N) applied to roots and aerial parts. Similarly, Toledo (2014) observed that micropropagated plants that had been inoculated with *G. diazotrophicus* show earlier maturity than non-inoculated plants.

The mixed strain inoculation helped the initial development in the RB867515 cultivar and, according to Gírio et al. (2015), increased sprout index and dry matter of all plants. Similarly, Chaves (2014) studied the effect of those bacteria alone and together in different cultivars, and found a positive response for some treatments according to the sprout index and macronutrient content; however, in some cases there was a reduction in biomass accumulation. Therefore, it can be speculated that the cultivar environment and cultivar genotype may influence microbiological activity.

In agreement with other obtained results, Pérez et al. (2015) studying other authors, submitted that inoculation with *G. diazotrophicus* and *Kleibisiella* sp. GR9 contributed to sugarcane biomass in an order of 50%, demonstrating the ability of the bacteria to develop beneficial conditions for plants without having to use synthetic products. These results reinforce the importance of developing standardized methods of using commercial inoculants (biofertilizers) in non-leguminous plants (Vessey 2003; Fuentes-Ramírez and Caballero-Mellado 2006).

1.4 Strain Selection of Agricultural Interest, *in vitro* Methods

Desirable characteristics of agronomic and agricultural interest are always the driving force in the selection of bacterial strains for agricultural use. Their technical features are often associated with the desire to increase yield.

It was hypothesized early on that sugarcane could benefit from nitrogen-fixing bacteria (Döbereiner et al. 1972). Ever since, many selection programs for isolation and testing have been established, as reviewed by Baldani et al. (2002). A remarkable milestone for sugarcane cultivation was the isolation of *G. diazotrophicus* (formerly known as *Acetobacter diazotrophicus*) from sugarcane, a potential plant promoter (Boddey et al. 2003). Furthermore, it has been recognized that some endophytic bacteria substantially affect sugarcane physiology but without changing plant growth (Marcos et al. 2016).

Many factors are randomized in the *in vivo* situation, whether they are beneficial or not. Nevertheless, selection always occurs under conditions that are quite different from those found in the field. These attempts are put into practice because they are a part of a process of choosing the most promising microorganisms. The more advantages it has, the better its adaptability for performance and success in the plant. To date, we have seen that these experiments under controlled conditions, e.g., *in vitro* selection, make approximate admeasurements of strain abilities, making it possible to indicate which are the most appropriate strains for undergoing *in vivo* tests.

However, this is not the only way to proceed. In conjunction with *in vitro* tests, some experiments may also indicate bacterial abilities that will certainly promote plant growth. Many are based on experiments that determine the production of some key compounds. In general, this is a stage performed *a posteriori* of the isolation and the *in vitro* tests. Nonetheless, depending on the goals and availability of resources, nothing prevents the order from being changed.

The use of both PGPB and transgenic plants will be the support basis for sustainable agriculture in the present and the future (Lucy et al. 2004; Glick 2012). The potential of the PGPB isolates can be evaluated, as widely reported, in terms of nitrogen fixation, production of plant growth-regulating substances (phytohormones), phosphorus-solubilizing activity, and siderophore production, amongst many other assays. Some strategies and their respective applied protocols are summarized in Tables 1.1 and 1.2.

Table 1.1 Evaluation according to secretion of plant growth-regulating substances

Protocol	Results of microorganisms and cultures	References
Quantitative estimation of indole-3-acetic acid (IAA) production	Detection of PGPR from roots and rhizosphere of sugarcane (Pakistan)	Ashraf et al. (2011)
(IAA) determined by Salkowski colorimetric method	*Identification of genes involved in IAA biosynthesis of Gluconacetobacter diazotrophicus*	Rodrigues et al. (2016b)
IAA colorimetrically by standard procedure (Gordon and Weber 1951)	All Endophytic bacteria isolates from sugarcane (India) were able to produce IAA (4.8–9 μg ml^{-1})	Chauhan et al. (2013)
Estimation of indolic compounds (Glickmann and Dessaux 1995)	Rhizospheric and root endophytic bacteria isolated from sugarcane (Brazil) showed high indolic compound production (N = 39) 51–100 μg ml^{-1} (N = 16) >100 μg ml^{-1}	Beneduzi et al. (2013)
Effects of exogenous abscisic acid (ABA)	Different hormone ratios influenced growth in diverse sugarcane varieties	Huang et al. (2015)
ACC deaminase (Glick 2005)	Deaminase production was mainly detected in species belonging to *Streptomyces* and *Bacillus* (from endophytes associated with sugarcane)	Kruasuwan and Thamchaipenet (2016)

One of the main targets in bacteria selection programs is to take advantage of the inoculation of sugarcane-associated nitrogen-fixing bacteria due to their capacity to reduce nitrogen fertilization and improve sugarcane production (Lin et al. 2012). Some species of bacteria are able to perform the biological fixation of nitrogen because these microorganisms have the enzyme nitrogenase, which is an enzymatic complex that breaks the triple bond of the atmospheric nitrogen (N2) allowing the formation of ammonia (NH^{4+}). To determine in the microorganism the ability to fix atmospheric nitrogen requires more than one method, since none of them can cover all the variables that this process involves, given the enormous richness of bacteria that have this characteristic.

There are tests that detect the activity of the enzyme nitrogenase by the relative reduction of acetylene (ARA) in ethylene (C_2H_4); this technique has great advantages for high sensitivity (nmoles of C_2H_4 per hour by gas chromatography) and speed. This allows the detection of nitrogenase activity in 2–3 *Azotobacter* cells (Hardy et al. 1968). In general, bacteria that reduce acetylene to ethylene also reduce nitrogen to ammonia, but the reverse is not true because it has been known for a long time that some microorganisms like *Methylosinus* oxidize ammonia into nitrate (de Bont and Mulder 1976). Therefore, it is important to conjugate more than one method to estimate the BNF. In this sense, the amplification of the *nif* genes (encoding proteins of the enzyme nitrogenase-1) has been useful, especially *nif*D, *nif*K, and *nif*H, which function as the structural genes of the nitrogenase enzyme (Dean and Jacobson 1992). In studies with sugarcane bacteria, ARA and *nif*H were used together with success to estimate nitrogen-fixing bacteria (Ashraf et al. 2011; Kruasuwan and Thamchaipenet 2016). Investigators usually find more than one

Table 1.2 Methods and protocols used for study of microorganisms with agricultural interest

Parameter evaluated	Results	References
Biological nitrogen fixation		
Nitrogenase activity by acetylene reduction assay (ARA)	Endophytic nitrogen-fixing bacteria were isolated from the leaves, stems, and roots of industrial variety (cv. U-Thong 3; UT3), wild and chewing sugarcane plants grown for 6 weeks in nitrogen (N)-free sand	Muangthong et al. (2015)
nifH gene amplification by PCR (Rösch et al. 2002)	Detection of nitrogenase producers	Kruasuwan and Thamchaipenet (2016)
Partial amplification *nifH* gene	Detection of nitrogenase producers	Ashraf et al. (2011)
Phosphorus solubilization		
Phosphorus-solubilizing activity on agar (Pikovskaya 1948)	Bacteria isolates from the rhizosphere of crop plants	Chung et al. (2005)
Siderophore production		
Chrome-azurol sulphonate assay (CAS) (Schwyn and Neilands 1987)	Field experiment on sugarcane was conducted with five plant growth-promoting bacterial endophytes Pseudomonas spp. and *Bacillus* spp.	Chauhan et al. (2013)
Chrome-azurol sulphonate including additional control (Schwyn and Neilands 1987; Beneduzi et al. 2010)	Identification of 390 siderophore producers	Beneduzi et al. (2013)

function in the same bacteria, as described by Rodrigues et al. (2016a, b), who identified genes of the beneficial nitrogen-fixing endophyte *Gluconacetobacter diazotrophicus* PAL5 in association with indole acetic acid (IAA) production and its effects on sugarcane and other important crops.

PGPB have often been selected because of their potential to secrete plant growth-regulating substances. Auxins, for instance, have effects on sugarcane dedifferentiation and embryogenic-cell initiation (Nadar et al. 1978); they promote *in vitro* sugarcane regeneration (Franklin et al. 2006) and establish lateral and adventitious root systems in grasses (McSteen 2010). Several developmental effects over sugarcane have been reviewed by Moore and Botha (2013).

There are simple tests that can be performed for qualitative and quantitative estimation of IAA production, an auxin that plays a crucial role in plant growth and development. They are easily accomplished colorimetrically with increased specificity to IAA by means of ferric chlorideperchloric acid procedure (Gordon and Weber 1951), or by adaptation of Salkowski's reagent use (Pilet and Chollet 1970; Glickmann and Dessaux 1995),or by confirming best colorimetric results by gas chromatography tandem mass spectrometry (GCMS) analysis (Ullah et al. 2013), which better indicates how to prepare and prepurify samples before colorimetric measurement. Otherwise, microplate experiments can be similarly performed with some scale adaptations (Sarwar and Kremer 1995).

In an investigation over beneficial sugarcane endophytes, the production of IAA was colorimetrically measured, according to Gordon and Weber (1951), by growing the cultures with or without tryptophan (100 μg ml^{-1}) for 48 h at 30 °C, in triplicates. All the isolates of interest were able to produce IAA ranging from 4.8 to 9 μg ml^{-1} (Chauhan et al. 2013). Several endophytes associated with sugarcane roots have also been evaluated by Kruasuwan and Thamchaipenet (2016) for IAA production, but were rather inoculated into glucose-beef extract broth supplemented with 10 mM L-tryptophan and incubated at 28 °C for 7 days in the dark using Salkowski's reagent colorimetric method (Pilet and Chollet 1970). Isolates from rhizospheric soil, roots, and stems of sugarcane from southern Brazil showed indolic production ranging from 0.16 to 160.4 μg ml^{-1} (Beneduzi et al. 2013). All these protocols used the supernatant of broth after growth and centrifugation of isolates.

In a more laborious study, quantification of IAA was performed by chromatography, comparing the retention time of samples to the IAA standard peak, using specific computer software (Ashraf et al. 2011). For this investigation, an high-performance liquid chromatography (HPLC) system with a UV detector and C-18 column was used, using as the mobile phase methanol:acetic acid:water (30:1:70 v/v/v), pumped at a rate of 0.6 ml min^{-1}. Injected samples have been obtained from the culture of bacteria isolated from the rhizosphere of sugarcane, which were extracted from ethyl acetate and re-suspended in ethanol, according to Tien et al. (1979). A more refined experiment for quantification of IAA makes use of ultra-high performance chromatography with tandem mass spectrometry (UPLC/MS/MS) (Khan et al. 2016), where details of the analysis are appropriately described. Briefly, tandem MS uses the mode of multiple reaction monitoring (MRM) to trace the transition of an IAA precursor ion from 175.65 to 129.8 m/z.

It has been proved that bacteria are able to synthesize many other plant growth regulators such as other auxins (besides IAA), gibberellins, cytokinins, and abscisic acid (Karadeniz et al. 2006). By measuring the enzymatic activity of 1-aminocyclo propane-1-carboxylate (ACC), for example, one can indirectly make an estimate of the potential of soil microorganisms to promote plant growth (Glick 2005). According to this reference, the enzyme promotes plant growth by sequestering and cleaving plant-produced ACC, and thereby lowers the level of ethylene in the plant. This allows the plant to be more resistant to a wide variety of environmental stresses.

Gene promoters are those that allow the binding of transcription factors that modulate the expression of a particular gene. There are several types of promoters, but some are directly involved with ABA. ABA is a cis-element involved in abiotic stress response. It is a phytohormone that induces leaf stomata closure and triggers the activation of many stress-related genes under abiotic stress (Lata and Prasad 2011). ABA is ubiquitous in plants, but it is also produced by some bacteria and fungi (Nambara and Marion-Poll 2005). There are many bacteria that synthesize ABA through the mevalonic acid pathway, in inter-relation with plants (Wasilewska et al. 2008). This hormone plays a pivotal role in a variety of developmental processes and adaptive stress responses to environmental stimuli in plants (Fujita et al. 2011).

Some promoters are particularly useful because they only function after being induced by certain stimuli (microorganisms, temperature, chemical compounds,

wounds) (Canhoto 2010). Thus, their manipulation can be controlled. Any foreign gene transferred to a plant can be expressed only when it has been provided with a suitable promoter sequence, many of which are already included in the commercially available vectors (Heldt and Piechulla 2004).

Sugarcane (*S. officinarum*) is worth mentioning as in it the precise sequences of plant promoters must be determined by plant genomics. However, some performances can in part be paralleled with those of maize, rice, sorghum, or wheat. According to Canhoto (2010), promoters that showed good expectation of use in monocotyledons are Ubiquitin-1 for maize and Actin-1 for rice. These promoters are activated by heat shock proteins (HSPs), which are derived from various stress factors to which the plant is subjected, including thermal stress, a factor that has been reported for its important contributions of HSPs in various abiotic stresses (Scharf et al. 2012). According to Fujita et al. (2011), the ABA-responsive element (ABRE; PyACGTG/TC) is a well-studied cis-element involved in ABA-induced gene expressions. Moreover, phytohormone ABA is involved in dehydration responsive element binding (DREBs) (Lata and Prasad 2011). Thus, ABA should be considered for bacterial selection interaction between beneficious bacteria and sugarcane, since many already culturable areas and future culturable areas of sugarcane suffer from drought. The practical and application value of ABA and DREBs in crop improvement, such as stress tolerance engineering, has been reviewed by Lata and Prasad (2011). Some physiological roles of ABA have been reviewed by Finkelstein (2013), who stated that "Although ABA has historically been thought of as a growth inhibitor, young tissues have high ABA levels."

Another role of bacteria is their phosphate-solubilizing activity. Phosphorus is among the essential nutrients applied to sugarcane (Muwamba et al. 2016). To maintain the sustainability of agriculture, it is imperative that the reliance of crops on inorganic phosphorus (P) fertilizers is reduced (George et al. 2009). This kind of bacteria, which most commonly belongs to the genera *Pseudomonas, Bacillus*, and *Rhizobium,* used as inoculants can increase P uptake by the plant and crop yield at the same time. The mechanism for mineral phosphate solubilization, in general, is the production of organic acids and acid phosphatases for the mineralization of organic phosphorous in soil (Rodríguez and Fraga 1999). In a selection program of strains for this characteristic, is very common to use the assay of solubilized insoluble phosphorus on Pikovaskaya's agar (PVK) (Pikovskaya 1948), a fast and simple method that is adequate for large samples with many isolates. Another medium, NBRIP (National Botanical Research Institute's phosphate), was used with good results for bacteria isolation; it is more efficient than PVK in a broth assay (Nautiyal 1999). It is very common to find IAA production and phosphate solubilization capability in the same bacteria, and these have been used as parameters of potential plant growth promotion (Ullah et al. 2013).

Siderophore production by the isolates is commonly qualitatively estimated by the Chrome-azurol S assay in solid medium (Schwyn and Neilands 1987). It is useful to determine its bacterial production because siderophores are a class of organic compounds with low molecular masses and with iron-chelating properties. Taken from Greek, it means "iron carrier," and this is the actual way that it increases iron

bioavailability to plants. It is produced by aerobic and facultative anaerobic bacteria (frequently by PGPB) and some fungi. Although iron is common in soils, it has a low solubility for plants and microorganism utilization.

1.5 Inoculants for Sugarcane

The PGPB inoculants available are of great importance to sustainable agriculture, as they aim to reduce the environmental impact through the use of fewer chemical fertilizers and also through a reduction in production costs. The bacteria may replace fertilizers, maintaining productivity and improving conditions for soil microbiota. In this way, the input costs can be reduced, because there is a certain fragility and dependence of the political external market on fertilizer prices (Hungria 2011).

According to Vessey (2003), inoculants can be called by biofertilizers, because their composition consists of live microorganisms that are beneficial to plant development like the chemical fertilizers but in a different way. The studies in this area are mainly from India and South America (Vessey 2003; Stamford et al. 2006; Okon et al. 2015). Biofertilizer use may reduce chemical fertilizer application, as demonstrated by Kumar and Yadav (2015) in Indian soils. The biofertilizer from that study contained BNF bacteria, phosphate-solubilizing bacteria, and bio-control agents. In South America, the commercial use of biofertilizers in sugarcane is common, therefore most of them are applied to other *Poaceae* cultures, mainly focusing on rhizospheric bacteria. Mostly of those biofertilizers are *Azospirillum* genus-based (Okon et al. 2015).

The inoculants are the vehicle in which beneficial bacteria survive, and when applied to the roots or leaves the bacteria act in symbiosis or association with plant. The inoculant must have the capacity to keep live bacteria at low metabolic activity, otherwise they will still multiply and probably compromise the stability of the inoculant when applied to plants. Thus, most bacteria provide phytohormones from bacterial "mechanisms" to plants, and on the other hand bacteria survive on plant exudates (Vessey 2003; Fuentes-Ramírez and Caballero-Mellado 2006; Moreira and Siqueira 2006).

Plants with the greatest potential to produce photoassimilates, allied carbon sources on the rhizosphere or bacteria action site, probably gain more success in the plant-bacteria association (Fuentes-Ramírez and Caballero-Mellado 2006). Accordingly Reis (2007) defined an inoculant as: "*...utilization of alive microorganisms, capable of promoting the vegetal growth in a direct or indirect way, through different mechanisms, and worldwide being named as biofertilizers...*" A good inoculant maintains the appropriate quality and quantity of bacteria to be available in the appropriate place for symbiosis or association with the plants; however, some factors may influence the viability for maintenance of the inoculant, including temperature. Inoculants based on *Azospirillum* genus present a slight decrease in the live cell quantity over time, and consequently maintain viability when inoculated in the plant. The maintenance of live cells occurs through poly-β-hydroxybutyrate production, which is produced by bacteria generally in a high C/N relation condition to maintain cell reserve. This polysaccharide also provides protection from the

deleterious effects of oxygen over nitrogenase in *Beijerinckia* genus (Barbosa and Alterthum 1992; Reis 2007).

Although viability is of great importance, the inoculant should go through many trials before being released, which involves laboratory and field tests. For laboratory tests, including *in vitro* tests and greenhouse trials, the identification of bacterial strains, microorganism benefit to the plants, and how to multiply the microorganisms are important issues. After that, agronomic efficiency must be tested, accompanied by field tests. Thus, it could result in the recommendation of an inoculant for a determined plant, place, or region (Polidoro et al. 2001; Reis Junior et al. 2000; Silva et al. 2009; Torriente 2010; Xavier 2006).

In soybean cultivation, the first Brazilian experience with inoculation with beneficial bacteria, many studies were carried out in the 1930s and 1950s. This cultivation is marked by the relationship with *Bradyrhizobium,* which participates routinely in soybean breeding. In the 1950s the commercial soybean inoculant utilization began on a large scale, initially developed as a peat inoculant and later in response to the market demand as a liquid way, oil, or polymer (Freire and Vernetti 1999).

With the inoculant success in soybean and other leguminous plants, other bacteria were discovered in association with the *Poaceae* family, *e.g.*, sugarcane, maize, and wheat. The most studied genus was *Azospirillum*, with the ability to develop inoculant with a very good response in cultures like maize and wheat, increasing the radicular system of these cultures. However, the application of the technology may modify plant response, as well as vehicle concentration (Araujo 2008; Reis 2007).

Commercially, inoculants started to make their presence known on the global market in recent years, and are aimed at *Azospirillum* utilization, and usually in association wtih wheat, maize, and rice cultures. In the Brazilian market, one of the largest, public and private partners have arisen to release and produce access to the benefits of the inoculants. Income in the order of one to two billion dollars more a year for maize and wheat may be involved (Okon et al. 2015; Hungria 2011; Parnell et al. 2016). Fuentes-Ramírez and Caballero-Mellado (2006) demonstrated that *Azospirillum* may result in profit in the order of 4–60% from increased productivity in cereals.

In recent years, research into *Azospirillum* has produced new perspectives and discoveries of species that primarily colonize the plant interior (endophytic), resulting in increases in productivity experimental trials. *G. diazotrophicus*, *Burkholderia* spp., *H. seropedicae,* and *H. rubrisubalbicans* have been well researched in sugarcane cultivation. The ecological advantage of these bacteria over naturally occurring conditions is that the inside tissues of sugarcane are protected from high concentrations of oxygen, which inhibit nitrogenase activity and reduce the ability to fix nitrogen (Perin et al. 2007). Other avenues are to mix different species and strains, reflecting more expressive effects when applied to plants (Reis 2007).

The commercial product of PGPB is commonly used for *Poaceae* cultures like wheat and maize, while for sugarcane no commercial product has been registered. Many inoculants have been developed, mainly from University research. Sugarcane presents a strong genotype-PGBG interaction, interfering directly for a better strain-plant relationship. Therefore, many field results are controversial, where in some cases PGPB works with a sugarcane genotype and in other cases it does not work.

Because of these results, it may be assumed that the environmental and strain affinity could be influenced by the results, besides the quality of inoculants, viability of microorganism maintenance, applied technology, and culture management. Researchers have been working for the past few years to test the consistency of different inoculants, better vehicles, and type of application that will benefit and result in better effects for sugarcane (Oliveira et al. 2006; Silva et al. 2009; Bhattacharyya and Jha 2012).

Sugarcane is usually given credit as being a low exigency nutritional product producing good stalk quantity. For many cultivars nutrient quantity, as well as nitrogen, application is important for the plant. Therefore, many researchers have asked in the past how sugarcane grows without exogenous nutrient application. Thus, the nutrient exigency fluctuates among cultivars but productivity remains high (Urquiaga et al. 1992; Boddey et al. 1995; Fuentes-Ramírez et al. 1999; Rossetto 2008; Vitti et al. 2008).

These characteristics were assigned by the PGPB-sugarcane association, with a large contribution from BNF. Therefore, recent studies show an increase in bacteria application aiming at other effects, like biocontrol and biofertilization (*e.g.*, phytohormone production) (Arencibia et al. 2006; Ashraf et al. 2011; Pereira et al. 2013; Souza 2011).

Ashraf et al. (2011) have found endophytic and facultative bacteria-fixing nitrogen in sugarcane, producing amino acids, IAA, and ethylene, and inhibiting pathogenic fungi. In this way, Pandya et al. (2011) obtained high gibberellin (GA_3) concentrations produced by rhizospheric bacteria associated with sugarcane roots. Oliveira et al. (2015) studied a biofertilizer with diazotrophic bacteria and found great success in stalk production.

The establishment of bacteria in tissues or the rhizosphere is very important, so that the relationship with the plant is established. If not, this relationship may be unreliable, and depends on the application form of the bacterial inoculation, as a solid or a liquid, or as a furrow or sprinkling, etc. Toledo (2014) observed that *G. diazotrophicus* bacteria inoculated *in vitro* took 15 days to establish inside sugarcane and present as a growth-plant contributor.

Oliveira et al. (2006) experimented with the five diazotrophic strains (Table 1.3) consolidated as inoculant, along the lines of previous studies like those of Boddey et al. (1991) studying *G. diazotrophicus* and Reis Junior et al. (2000) studying *Herbaspirillum spp.* and *Azospirillum spp*. These bacteria were isolated from cultivars and presented positive behavior for sugarcane inoculation. The inoculant that was developed by Embrapa[2], which was scientifically based on those studies, consists of a formulation comprising the five diazotrophic strains separated into a peat vehicle (Embrapa 2007). Table 1.3 presents the tissues that were isolated for the five bacteria in extracted by Oliveira et al. (2002).

Earlier results with these five bacteria (Table 1.1) were presented by Silva et al. (2009), who developed a polymeric inoculant vehicle that was gel- and liquid-based,

[2] Embrapa – Empresa Brasileira de Pesquisa Agropecuária (Brazilian Agricultural Research Corporation).

Table 1.3 Diazotrophic endophytic bacteria isolated from sugarcane

Scientific name	Strain	Tissue (isolation spot)	Sugarcane cultivar
G. diazotrophicus	BR11281	Roots	Saccharum sp.
H. seropedicae	BR11335	Roots	SP 70-1143
H. rubrisubalbicans	BR11504	Stalks	SP 70-1284
A. amazonense	BR11115	Roots	SP 77-5181
Burkholderia sp.	BR11366	Germinated buds	SP 71-1406

Adapted from Oliveira et al. (2002)

and conducted trials testing inoculant viability, in which they showed the efficiency of *rhizobia* inoculants (Silva et al. 2012). Chaves (2014) carried out tests in polymeric and peat vehicles, obtaining success with both inoculants. The first trial was in a field condition and the second one in greenhouse conditions. These studies considered a concentration of 10^6 a 10^9 cells ml^{-1} satisfactory for successful inoculation (Reis et al. 1999; Oliveira et al. 2002; Schultz et al. 2012; Silva et al. 2009; Suman et al. 2005, Table 1.3).

With regard to the application of technology, major studies have applied peat vehicle with a recommendation of bud immersion for 30 min (Chaves 2014) or 1 h (Garcia et al. 2013; Gírio et al. 2015; Schmatz et al. 2012). De la Cruz et al. (2012) considered a dipping method (fast immersion) for 2 min adequate for *Gluconacetobacter diazotrophicus*. Reis et al. (1999) and Schultz et al. (2012) inoculated micropropagated plants with a 0.1 ml solution.

Prado Junior (2008) prepared a liquid inoculant in a pulverized method, which positively influenced increased productivity in sugarcane. Oliver (2014) applied by means of pulverization in furrows an injected inoculant implementing a nematicide (adapted) and foliar spray after 60 days with a manual backpack-type sprayer. This was found to have similar results to nitrogen fertilization.

On *Poaceae* like maize and wheat, the utilization of *A. brasilense* species has been intensified in the last few years. These species have the ability to develop liquid inoculant, which demonstrated easier applicability through foliar pulverization or in a furrow (Hungria 2011). This will probably be used in future in the inoculation of sugarcane, as Lopes et al. (2012) showed.

1.6 Sugarcane Breeding

An increase in productivity may be determined by adequate management; however, many plant genotype characteristics influence productivity directly. For detecting productivity in plant genotypes, breeding knowledge is of paramount importance. In sugarcane, breeding has been a better strategy to "fix" the best genotype to meet the international market demand (Cheavegatti-Gianotto et al. 2011; Daros et al. 2015; Segalla 1964; Stevenson 1965).

The most well-known sugarcane variety is the *Saccharum officinarum* species, which has a high sugar and low fiber content, but it is susceptible to diseases like mosaic. The sugarcane breeding programs were started in 1858 after the first flowering

report, consolidated years later with crossings in Barbados and Java. Interspecific crossings were important, obtaining cultivars resistant to diseases, and maintaining quality in sugar production (Blackburn 1984c; Scarpari and Beauclair 2008).

Seeds originating from interspecific crossings started the modern cultivars, which have more resistance to adversities. These cultivars are known as *Saccharum* spp., hybrids with approximately 75% of *S. officinarum* and about 25% of *S. spontaneum*. The first one is important to sugar production and the second one is used to confer biotic and abiotic stress resistance (Blackburn 1984; CIB 2009; Scarpari and Beauclair 2008).

The occurrence of interspecific crossings is based on cultivar characteristics; sugarcane is cross-pollinated by over 95% and is vegetatively propagated for commercial purposes. Beyond the interspecific crossings, it is believed that sugarcane originated from closely crossed groups, called the "*Saccharum* complex," which included different genera, like *Saccharum*, *Erianthus* sect. *Ripidium*, *Sclerostachya*, *Narenga*, and even *Miscanthus* (Matsuoka et al. 2005). These hypotheses have been tested with the aid of molecular markers and DNA sequencing (Hodkinson et al. 2002).

Recent studies have indicated that commercial sugarcane is the result of limited crossings and backcross series from domesticated *Saccharum officinarum* species combined with wild *Saccharum spontaneum*, a process known as "nobilization" (Scarpari and Beauclair 2008). The current classification of sugarcane is as a member of the *Poaceae* family (late *Gramineae*), and belongs to the *Saccharum* genus, containing the species *Saccharum officinarum*, *S. barberi*, *S. robustum*, *S. spontaneum*, *S. sinensis*, and *S. edule* (Cesnik and Miocque 2004; Scarpari and Beauclair 2008).

Currently, the selection of genotypes is a long and expensive process that consists of a set number of steps (Calija et al. 2001; Rattey et al. 2004). At each step, evaluations are carried out to identify promising clones, which will pass to the next phase, but only a small percentage of these clones reach the final stages (Landell et al. 1999; Calija et al. 2001; Kimbeng and Cox 2003). These steps in sugarcane breeding entail long periods of research, as it is a semi-perennial plant with a high complexity in cultivar (Cesnik and Miocque 2004; Daros et al. 2015). The phases involved in genotype selection (cultivar) may vary in different breeding programs. For example, in the *Rede Interuniversitária para o Desenvolvimento do Setor Sucroalcooleiro* (RIDESA),[3] the breeding phases of sugarcane may take 15 years (Daros et al. 2015).

1.6.1 Selection of Responsive Sugarcane Clones to PGPB Inoculation

Genotypes adapted to environmental conditions are a priority in sugarcane research, as they obtain higher productivity. Adaptability is necessary for environmental diversity, where genotypes are susceptible to general soil conditions, temperature,

[3] Interuniversity Network to the Development of Sucroenergetic Sector—Brazilian breeding program focused on the obtaining of sugarcane genotypes. One of the most important global programs.

humidity, and light intensity as well as the influence of soil microorganisms (Cesnik and Miocque 2004; Cheavegatti-Gianotto et al. 2011; Magnani et al. 2010).

For many years the influence of microbiota in cultivated sugarcane soils has been studied, particularly with regard to beneficial bacteria and fungus. However, research has also been conducted that seeks to select more effective strains of PGPB and apply them to different genotypes (Baldani et al. 2002; Moreira and Siqueira 2006; Xavier 2006).

Currently, in a sugarcane-breeding program that seeks to select genotypes with better responses to PGPB inoculation, the inoculation of seedlings during the first phase of selection is recommended. Moreover, to optimize the interaction between PGPB and plants, the breeder must carefully choose the bacterial strain or strains that will be used in inoculation of the seedlings (Lopes et al. 2012; Figueiredo et al. 2013). This was a consideration in the soybean evolution, where the selection of cultivars was made according to agronomic interest, in combination with the nitrogen-fixing bacteria *Bradyrhizobium* (Okazaki et al. 2004; Tang et al. 2012).

The association of sugarcane with PGPB depends on how the interaction occurs between plants and bacteria, *e.g*. whether the bacteria are found inside the vegetal tissues (entophytic), around tissues (association), or close to or in the rhizospheric region (facultative or free life) (Gnanamanickam and Immanuel 2007; Hungria 2011; Gaiero et al. 2013).

The specificity between strains of PGPB and sugarcane families or genotypes was reported by Baldani et al. (1997), Reis Junior et al. (2000), Muñoz-Rojas and Caballero-Mellado (2003), and Lopes et al. (2012). These studies indicate the possibility of finding commercial cultivars that are responsive to inoculation in the first stages of sugarcane breeding, without agreement on which approach is the best.

The authors Lopes et al. (2012) and Figueiredo et al. (2013) showed even in initial phases of sugarcane development that it is possible to identify families that show response to PGPB. Xavier (2006) working with commercial sugarcane cultivars found a difference within genotypes and the response of each one to BNF. The author also found more potential in some genotypes for BNF than others, exemplifying the bacteria-genotype interaction.

1.6.2 The Plant Genotype and Microbiology of Soil

There is an interaction between plant genotypes, the environment, and soil microbiota, modifying vegetal reaction as well productivity and quality production. The environmental variation that occurs in different places involves climatic and soil differences, and this variation may even occur within the same field, and microorganisms may also be different within the same field. Salinity is one of the environmental influences on sugarcane development that directly affects plant growth. Lamizadeh et al. (2016) reported the large effect on microbiota caused by salinity and suggested a large number of bacteria with PGPB properties linked to salinity in soils, which could help sugarcane under those conditions.

The interactions between *Rhizobia* bacteria and leguminous plants are more common due their high specificity, where nodule formation often only occurs with certain bacterial species and/or strains and leguminous species (Glick 2012; Perry et al. 2007).

Can sugarcane behave in the same way as leguminous plants?

Muñoz-Rojas and Caballero-Mellado (2003) related one of the first cases of specificity to bacterium species and cultivar. Another example of this delicate interaction in sugarcane is with the bacterial species *Herbaspirillum rubrisubalbicans*, which is responsible for the disease called mottled streak only in susceptible cultivars, namely B4362. In other cultivars, although there is infection of the tissues by the bacterium, there are no characteristic symptoms of the disease (Baldani et al. 1997).

The interaction within sugarcane genotypes and beneficial microorganisms improves the developmental conditions of the plant, making possible the promotion of growth and even increasing resistance of plants to pathogen infection (Glick 2012; Perry et al. 2007; Reinhold-Hurek and Hurek 2011).

However, the great challenge for the plant-bacteria interaction is that the plant needs to recognize the beneficial microorganisms and not resist the interaction. Microorganisms both in a pathogenic and a beneficial way may have similar mechanisms, as well as infection of plants. Such mechanisms induce plants to protect themselves from infections, explaining the failure of many inoculations, which are eliminated by plants' defense mechanisms. On the other hand, both types of microorganisms (beneficial and harmful) may "resist" plant defenses to obtain infection success (Perry et al. 2007).

As discussed before, Reinhold-Hurek and Hurek (2011) in their revision raised the question: "How can endophytes reach relatively high numbers in plant tissue without eliciting strong defense responses or plant damage?"

Some bacteria have an adaptive ability to the environment in which they are found, and usually the genotype of plant interacts positively with these bacteria. However, bacteria develop special mechanisms to infect and interact with plant genotypes. The endophytic bacteria mechanisms comprise specific enzyme production, which degrade the cell wall, thus infecting plants. With regard to the plant defense systems, when encountering beneficial bacteria, many of them express a lower number of defense genes, facilitating endophytic action (Reinhold-Hurek and Hurek 2011).

The PGPB-plant interaction is facilitated by many different molecules, including phytohormones, flavonoids, siderophores, exopolysaccharide, lectins, antibiotics, etc. (Moreira and Siqueira 2006; Bashan and Bashan 2011). The communication between hostage roots and bacteria in soils usually occurs by chemotaxis, explaining the microbial attraction for root exudates, like lectins, proteins linked to carbohydrates that attract beneficial microorganisms. The beneficial bacteria can interact with the plant in three main ways: bacteria are present in the rhizosphere, or they are present on the root surface, or they colonize root and shoot tissues. According to the kind of plant and genotype, the microorganisms interacts "more or less" with plant exudates. One example is the bacterium *A. brasilense*, which has more affinity with

cereals root exudates than leguminous plants, revealing a special interaction (Glick 2012; Perry et al. 2007).

Bacteria act according to plant genotype, therefore they still may be effective when environmental conditions are favorable. This dimension of the complex soil-plant system involves microorganisms. According to Marcos (2012), in hydric normal conditions, sugarcane genotypes could not benefit from PGPB interactions; however, when they faced hydric restrictions the bacterial isolates acted to maintain sugarcane plant growth, ensuring better efficiency of nitrogen use.

Exemplifying the environmental effect, despite controlling the conditions, some Brazilian cultivars like RB867515 show a distinct reaction to biometric characteristics in the first 120 days after planting in different sites (Gonzaga 2012). According to Morais et al. (2011) and Pereira et al. (2013), RB867515 is a promising cultivar for PGPB inoculation for biomass and BNF, while according to Schultz et al. (2014), the cultivar does not interact with PGPB. Therefore, the environmental conditions may determine inoculation performance, despite effectiveness of the genotype and bacteria.

1.7 Conclusions

The need for and use of new technologies with less environmental impact and greater agronomic efficiency increases each year, and consequently the use of PGPB in the promotion of plant growth. Many advances have been observed in this area, both in a plant-bacterial interaction and in the identification, isolation, and use of PGPB in sugarcane. The first results can already be seen through the development of inoculants and in the detection of sugarcane cultivars that are more responsive to inoculation.

However, the studies point to the need to increase knowledge of the main mechanisms involved in the plant-bacterial interaction, and the discovery of more efficient strains and sugarcane cultivars more responsive to inoculation. These are the major challenges that the research still needs to clarify so that the interaction between sugarcane and PGPB becomes as efficient as the symbiosis between *Rizhobium* and leguminous plants.

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Potential for Developing Low-Input Sustainable Agriculture in the Tropical Andes by Making Use of Native Microbial Resources

2

Luis Andrés Yarzábal and Eduardo J. Chica

Abstract

The Tropical Andes, a vast region spanning over 1,540,000 km^2 from Western Venezuela to Northern Chile and Argentina, faces huge challenges. Among these are a rapid demographic change and an increasing demand of agricultural goods to satisfy the needs of both rural and urban population. Unfortunately, crop production in this mountainous region is severely constrained by adverse natural factors, among which low soil fertility and cold climates occupy the top positions in the ranking. Considering that agriculture intensification, following the traditional approaches that made possible the Green Revolution, may cause further disruption and degradation of Andean agroecosystems, new strategies are being explored by researchers and farmers to deal with that dilemma. It has been proposed that partial replacement of agrochemicals (fertilizers and pesticides) with *bioinoculants* – products formulated with living microorganisms with plant-promoting abilities – is one of the measures that might allow to intensify even more agriculture in the Andes, without seriously affecting the environment or threatening human health. In order to maximize profits following this approach, it is imperative to study in depth the microbial diversity present in the Andean ecosystems, to select microbes exhibiting the best plant growth-promoting traits, and optimal performances in the rhizosphere of crops. Here we review some of the recent advances concerning the description of the microbes colonizing the rhizosphere of some important Andean crops; we further highlight important local and regional experiences showing that the development of efficient

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bioinoculants may certainly contribute to intensify agriculture in the Tropical Andes and, subsequently, to create better life conditions for the Andean small farmers and their families.

Keywords
Agriculture • Plant growth-promoting microorganisms • Tropical Andes • Sustainable intensification • Biofertilization • Biocontrol

2.1 Introduction

Over a period spanning several decades of the last century, fantastic increases in agricultural yields saved over a billion people from starvation. Thanks to the success of the Green Revolution (Pingali 2012), it seemed likely that hunger could be eradicated from the globe; however, although the number of undernourished people has decreased steadily over the last decades, about 795 million people remain undernourished worldwide, most of whom live in developing countries (FAO 2015). Even acknowledging the benefits of the Green Revolution in helping balancing population growth and food production, it soon became evident that intensification of agriculture through adoption of agro-technologies by farmers had led to degradation of fragile agroecosystems, due to loss of soil fertility, erosion, ecological imbalances, and pollution. These problems were the consequence of the injudicious and disproportionate use of the technologies developed within the Green Revolution, exacerbated by short-sighted developmental policies (Pingali 2012; Rahman 2015). Unfortunately, almost 50 years after its beginning, the dire legacy of the Green Revolution in many places is the degradation of agronomic and natural ecosystems and an important fraction of the population that has not reaped the promised hunger-ending benefits of these technologies.

According to the Food and Agriculture Organization of the United Nations (FAO), the global agricultural production in 2050 should be 60% higher than that of 2005/2007 (Alexandratos and Bruinsma 2012). This means an 11% increase in total crop production from 2006 to 2050 as compared to the production reached from 1962 to 2006 (Searchinger et al. 2013). To achieve this goal, agriculture will require to be intensified even more, if one considers that the amount of arable land is finite and that the expansion of the agricultural frontier will worse even more the current global environmental crisis. There is, therefore, an obvious and urgent need for a second Green Revolution or an Evergreen Revolution as proposed by Swaminathan (1996, 2006). This Evergreen Revolution must rely on sustainable agricultural practices, in order to not harm delicate ecological balances and to prevent further disruption and degradation of agroecosystems. Considering that the prevailing model of agricultural intensification has not been effective at eliminating hunger and has seriously harmed the environment and biodiversity (Tittonell 2014), scientists have explored new models of agricultural intensification, aimed at maintaining and enhancing ecosystem functions, that is, by adding an ecological dimension to crop productivity improvement (Swaminathan 2006).

Today, new paradigms have arisen and one in particular, *sustainable intensification*, has gained wide acceptance. According to Pretty and co-workers (2011), sustainable intensification refers to "…producing more output from the same area of land while reducing the negative environmental impacts and at the same time increasing contributions to natural capital and the flow of environmental services." This concept was adopted by international policy and research organizations like the Consultative Group on International Agricultural Research (CGIAR), the Food and Agriculture Organization of the United Nations (FAO), the World Economic Forum, the Montpellier Panel, or the Sustainable Development Solutions Network (SDSN) among others (Tittonell 2014).One possible way to achieve agricultural intensification without seriously compromising other ecosystem functions is to make proper and adequate use of an often neglected resource: the soil microbiome. In fact, microbe-based technologies have been repeatedly shown to increase agricultural productivity and sustainability without harming the environment (Fuentes-Ramírez and Caballero-Mellado 2006; Singh et al. 2011a; Velivelli et al. 2014a). For that reasons microbial inoculants are becoming increasingly popular, and represent today a real alternative to synthetic agrochemicals.

In the following pages, we will review some successful examples emphasizing the role of native soil microbes as key drivers of this paradigm switch from conventional to sustainable intensification. We will also highlight their importance to restore soil functioning and ensure long-term sustainability, particularly in Tropical Andean agroecosystems. Furthermore, we will highlight the need of mining on Andean soils microbial diversity in order to gain more information on the potential uses of this biologic and genetic resource.

2.2 The Role of Microbes in Sustainable Agriculture

Among the reasons explaining the decrease in fertility of soils under the current model of agricultural production, it is widely accepted that besides other deleterious effects, misuse and abuse of agrochemicals negatively impact the communities of soil microbes by damaging their habitats, disrupting their functions, altering their populations, and modifying their structure and diversity (Kibblewhite et al. 2008; Geiseller and Scow 2014). This, in turn, affects the soil by modifying its structure and decreasing its productivity (Aeron et al. 2011). Indeed, microbes are the *architects* of soils (Rajendhran and Gunasekaran 2008). They catalyze the weathering of mineral surfaces and promote the formation of fertile soils from inorganic bedrock through a series of complex interactions between physical, chemical, and biological processes. Soil microorganisms play significant roles in the major global biogeochemical cycles and, consequently, their activity is of paramount importance for the development of agriculture. Among many important functions fulfilled by microorganisms, we can mention their fundamental roles in regulating the dynamics of organic matter decomposition as well as in mobilizing and transforming plant nutrients such as N, P, and S. Consequently, soil microorganisms promote plant growth and indirectly increase their productivity (De La Peña and Loyola-Vargas 2014).

Soil microbes of all kinds, bacteria, fungi, nematodes, protists, and viruses, thrive in the area surrounding plant roots and influenced by root depositions: the "rhizosphere." In fact, organic molecules excreted by plant roots including sugars, amino acids, flavonoids, etc. act as attracting signals for soil microorganisms (Zhang et al. 2014). While some of these rhizospheric microorganisms are pathogens, a significant proportion of them promote plant growth and development, the *good* ones (Mendes et al. 2013), and are collectively known as "plant growth-promoting microorganisms" (PGPM). PGPM can be grouped into several categories: those able to inhibit or antagonize plant pathogens are called "biocontrollers" or "bioprotectants"; others release phytohormone-like metabolites and promote plant growth directly ("biostimulants"); many microorganisms may mobilize mineral nutrients such as P, which otherwise are chemically unavailable to plants or fix atmospheric nitrogen ("biofertilizers"); another group may activate plant defense systems ("bioelicitors"); finally, many microorganisms are able to chemically modify toxic xenobiotics functioning as "bioremediators" (Glick 2012). Intriguingly, some of these microorganisms can display insect pathogenicity, thus acting as "bioinsecticides" able to protect plants from root-feeding insects (Kupferschmied et al. 2013). As it has been repeatedly confirmed, the same microorganism may exhibit several of these traits simultaneously (Ahemad and Kibret 2014).

Some current practices of sustainable agriculture rely on the use of PGPM. Perhaps the best known example of this are nitrogen-fixing bacteria (NFB). As mentioned before, NFB fix N_2 in root nodules of legumes, thus contributing to enhance their growth and yield (Herridge et al. 2008). By 2012, biofertilizers based on NFB were the most consumed around the globe, accounting for almost 80% of the global biofertilizer's demand (Transparency Market Research Report 2012).

Phosphate-solubilizing bacteria (PSB) are another group of PGPM which is receiving increasing attention (Sharma et al. 2013). PSB can readily and effectively solubilize inorganic P from sparingly soluble minerals like hydroxyapatite or phosphatic rock, through the release of organic acids, namely, gluconic acid (bacteria) and citric acid (fungi) (Jones 1998; Rodríguez and Fraga 1999; Sharma et al. 2013). These organic acids may also mobilize P from Fe or Al oxides, particularly abundant in acidic soils, by efficiently chelating the metal ions (Johnson and Loeppert 2006). Much less frequent is the use of organic P mineralizing microorganisms, able to hydrolyze organic forms of P (phosphate esters, phosphonates, and anhydrides) by means of specific enzymes (mainly phosphatases) to release PO_4^{2-} (Turner et al. 2006; Richardson and Simpson 2011). This is striking, considering that organic forms of P may constitute up to 90% of the total soil P (Khan et al. 2009) and that world resources of rock phosphate, suitable for fertilizer production, are decreasing and will be depleted in the midterm (Cordell et al. 2009; Edixhoven et al. 2014; Scholz and Wellmer 2016). Some microorganisms may even solubilize inorganic P and mineralize organic P simultaneously (Oliveira et al. 2008).

Besides bacteria, some fungi are also very efficient at promoting plant growth. Arbuscular mycorrhizal fungi (AMF) are among the best known and studied. These fungi colonize the cortical cells roots of vascular plants where they develop their characteristic arbuscular structures that allow nutrient exchange between partners. By means of their hyphal growth, AMF extend the volume of soil that can be reached

by plant roots to uptake and transfer water and nutrients, especially phosphorus, but also sulfur, nitrogen, and some micronutrients (Parniske 2008; Baum et al. 2015). Among other important functions played by AMF, we can mention their ability to alleviate plant stress caused by abiotic as well as biotic factors (Gianinazzi et al. 2010; Singh et al. 2011b) and their role as biocontrollers of a wide range of plant pathogens, mostly fungi, but also bacteria and nematodes (Harrier and Watson 2004; Whipps 2004; Jung et al. 2012; Schouteden et al. 2015).

In addition to their roles in fertilization and pathogen control, both bacteria and AMF promote soil aggregation and improve soil structure both of which improve nutrient availability by means of an abundant production and secretion of polysaccharides and the formation of an intricate network of AMF hyphae (Wright and Upadhyaya 1998).

Since the early twentieth century, PGPM of different kinds have been used to develop bioinoculants, and, nowadays, they are considered as fundamental biological resources which sustain a well-established biotechnology (Parnell et al. 2016). Through their rational use, it has been possible to significantly reduce the use of agrochemicals, both fertilizers and pesticides, by concomitantly lowering the production costs and minimizing the environmental hazards derived from agricultural activities (Adesemoye et al. 2009). With an expected increase in the consumption of leguminous and non-leguminous plants, concerns on the environmental impacts of agrochemicals and promotion of this kind of bioproducts by emerging economies such as China and India, it is expected that the demand for bioinoculants will increase substantially in the following years.

2.3 Bioinoculants and Agriculture Intensification in Mountainous Regions

The potential role of PGPM to develop agriculture in mountainous regions has also been intensively studied in different countries. This subject has been particularly explored by Indian researchers during the last two decades, owing to the fact that much of this country's agricultural production originates in the Indian Himalayan Region (IHR), a vast territory spanning ten Indian states. As it is characteristic in many mountainous regions of the world, agriculture is severely limited in the IHR due to climate constraints (low temperatures, intermittent frosting conditions), soil characteristics (severe erosion, low nutrient availability, low organic matter content, lack of irrigation), and social and/or financial matters (inadequate infrastructure, geographical isolation, lack of technical assistance). Consequently, mountainous agriculture in the IHR and elsewhere is a subsistence though sustainable system, characterized by a low productivity (Trivedi et al. 2012).

The Tropical Andes is also a vast region that follows the path of the Andes Mountains and runs through seven countries from the Southwestern Venezuela to Northern Chile and Argentina, including mountainous regions of Colombia, Ecuador, Peru, and Bolivia (Fig. 2.1). Over 100 million people live in the Tropical Andes or in regions that depend directly on their natural resources (United Nations 2006). Like in other mountainous regions, the population inhabiting the Tropical

Fig. 2.1 The Tropical Andes region. Reproduced with permission of GRID-Arendal and CartografareilPresente/Riccardo Pravettoni (http://www.grida.no/graphicslib/detail/the-tropical-andes-region_12ab)

Andes highlands (>2,500 masl) depends largely on agriculture and related activities for their livelihoods (see next section). Unfortunately, owing to the harsh climatic conditions prevailing in these mountains, agriculture is negatively affected (Stadel 1991).

Among the reasons explaining such low productivity, the infertility and fragility of Andean soils stand as two of the most important (Poulenard et al. 2003; Dahlgren et al. 2004). In fact Andosols, the most extended soil type above the forest line (Poulenard and Podwojewski 2006), are in essence volcanic ash soils that seriously limit agriculture development owing to their strongly capacity to fix phosphate ions, their low pH and their high levels of Al (Saigusa and Matsuyama 1998; IUSS Working Group WRB 2014) (Fig. 2.2).

Moreover, small Andean farmers have a limited access not only to a number of basic agricultural inputs such as improved crop varieties, pesticides, and fertilizers but also to machinery and irrigation systems, all of which contributes to the low agricultural productivity in this region.

Agriculture in the Tropical Andes

Agriculture in the Tropical Andes is nowadays the result of a centuries-old process of developing and adapting technologies and practices of different origins. The earliest evidence of agriculture in this region dates back to ~9,000 years before present (Dillehay et al. 2007; Piperno 2011). Since then, many indigenous peoples inhabiting the region continued to develop agricultural technologies that were highly adapted to the environments in which they lived. Different processes of trade and conquest among these peoples led to an exchange of knowledge and technologies that characterized Andean agricultural systems before the arrival of the European settlers. When that happened, indigenous agricultural practices were further hybridized with newly introduced technologies and crops. These new hybrid technologies were quickly assimilated and practiced throughout the region. Since then, Andean agriculture has evolved greatly influenced by western agriculture; however, it retains to these days many traditional indigenous practices. This long process of technological mingling, alongside the preservation of indigenous identity across the Andes, has resulted in the very diverse agricultural landscape of the region.

Agriculture in the Tropical Andes is primarily developed by smallholder farmers (Figs. 2.3 and 2.4). These farmers satisfy most of the domestic demand for fresh fruits and vegetables in their countries and, thus, have a significant socioeconomic impact (Devenish and Gianella 2012). Furthermore, many smallholders, particularly those living in the communities farther away from large cities, use a greater share of their agricultural production for their own consumption and manage their own seed stocks of native varieties. This increases even more the social impact of their production systems, by contributing to strengthen their food security and sovereignty and by promoting the conservation of agricultural biodiversity (Mulligan et al. 2009). Nonetheless,

(continued)

low productivity, inappropriate and hazardous technology adoption, and the lack of smallholder-oriented new technologies threaten the sustainability of these agricultural production systems (Bojacá et al. 2010; Cole et al. 2011).

Following global trends, as Tropical Andean countries continue to grow both economically and demographically, their per capita demand of crops and produce is also expected to increase (Tilman et al. 2011). To meet this future production demand sustainably, agriculture in the Tropical Andes would need to increase crop yields per area without clearing new land for agriculture, reducing their reliance on greenhouse gas-emitting technologies and becoming more resilient to changing climatic patterns. Furthermore, it would need to make a more efficient use of human labor as the rural population ages, migrates to urban centers and younger generations feel less attracted to the physically demanding work typical of traditional smallholder agriculture. Thus, the technologies and innovations to be developed to satisfy those needs will be oriented toward optimizing the functioning of the agroecosystems by taking advantage of natural processes of nutrient recycling and biological control of pests, in order to maximize economic returns that will help maintaining farmers in business and attracting new generations to this activity.

In recent years, the use of bioinoculants started also to emerge as one of several important strategies aimed at intensifying agricultural practices in mountainous regions, without the negative outputs highlighted in the previous sections (Trivedi et al. 2012; Fonte et al. 2012). Unfortunately, currently available commercial bioinoculants are not useful under the environmental conditions that prevail in mountainous ecosystems (Pandey et al. 1998). Indeed, in a certain way, Andean soils are to be considered "extreme," since they support colonization by organisms exhibiting some specific and common adaptations, due to particular physical, chemical, climatic, and/or geological conditions (Dion 2008). Among the various soilborne challenges faced by exogenous microbes – used to formulate commercial bioinoculants – when applied to local soils, two are of utmost importance: *(i)* cool temperatures, which imposes serious constraints to the metabolic activities of microorganisms by negatively affecting the catalytic efficiency of enzymes (Feller and Gerday 2003), and *(ii)* low survival/colonization rates, frequently observed when these microorganisms are introduced in local soil environments and must escape predators and/or compete with autochthonous microorganisms to colonize the rhizosphere of plant crops (Bashan 1998).

Still, bioinoculants remain a very attractive alternative to chemical fertilizers and pesticides. Therefore, some strategies have been proposed to develop more efficient products based on the use of microorganisms with enhanced PGP abilities, well adapted to low temperatures (cold-tolerant or cold-loving) and to the particular conditions of the local soils they will encounter. Consequently, bioprospection of mountainous ecosystems to search for such kind of indigenous PGPM strains is receiving increasing attention worldwide, and the Andes are no exception.

Fig. 2.2 Andosol soil profile from the Ecuadorian Andean highlands (páramos), showing a dark-colored surface horizon derived from volcanic ash or dust. Copyright: Luis Andrés Yarzábal (Reproduced with permission)

2.4 Bioprospection of Andean Soils/Crops and PGPM

In the past 15 years, Andean environments have been prospected to isolate, characterize, and identify indigenous PGPM in order to develop efficient bioinoculants. Very often, this search has been oriented toward microorganisms that naturally colonize the rhizosphere of several important crops which are at the base of Andean agroecosystems. In the following pages, we will review the most important results on this matter, concerning Andean tubers, grains, vegetables, and fruits.

Fig. 2.3 Small farms in the Andean region of Ecuador (highlands or páramos). Copyright: Luis Andrés Yarzábal (Reproduced with permission)

2.4.1 Potato

Potato (*Solanum tuberosum*) is, by far, one of the most important food crops native to the Central Andes. After maize, wheat, and rice, it is the fourth most important food crop worldwide with an annual production that surpasses 385 Mt. (FAO Stat 2014). According to international agencies, potato is considered a food security crop, particularly important for human kind in a scenario of increasing population and hunger (http://cipotato.org/; Birch et al. 2012). Potato has been cultivated traditionally in the Andes by small farmers for centuries (Spooner et al. 2005) and is

Fig. 2.4 Small farmers in the Ecuadorian Andes. Copyright: Juan Vasquez (Reproduced with permission)

nowadays considered the main staple crop in these mountains. However, potato average production yields are very low, mainly owing to unfavorable conditions prevailing in the high mountains (high soil acidity, low nutrient content, frequent freezing conditions) (Aubron et al. 2009).

Potato production requires not only addition of large inputs of expensive chemical fertilizers (mainly N, P, and K) – unaffordable to many smallholder farmers – but the plants are also severely affected by fungal pathogens (*Phytophthora infestans*, *Rhizoctonia solani*, and *Fusarium solani*) which reduces crop yield and tuber quality (Finckh et al. 2006).

In order to explore sustainable ways to intensify potato production, many research groups around the world focused on potato rhizosphere microbes (Wu et al. 2013; Hopkins et al. 2014).To reach a similar goal, the microbial diversity in the Andean potato fields soils, has also been the subject of several studies. For example, in 2005 the International Potato Center implemented a strategy to improve potato production in a sustainable way through the development of biofertilizers – to be delivered to small Andean farmers – by means of native PGP rhizobacteria. The studies were initially conducted with two commercially available bioproducts: Azotolum (based on a Peruvian strain, *Azotobacter* sp.) and FZB24 (a commercial *Bacillus subtilis* strain from Germany). Field trials conducted at 3,200 and 4,000 masl showed that some native potato varieties increased their tuber yield and/or tuber number when biofertilized, sometimes up to 25% and 35%, respectively, as compared with control assays supplemented with manure only (Oswald et al. 2007).

Following these initial studies, rhizospheric bacteria were isolated from potatoes sampled in different provinces in Peru (Calvo-Vélez et al. 2008; Calvo et al. 2009).

Some PGP *Bacillus* isolates were subsequently tested in potatoes grown in aeroponic systems and resulted in significant increases in tuber numbers per plant, ranging from 40 to 100%, depending on the potato cultivar (Oswald and Calvo 2009). Later, Oswald et al. (2010) showed that around 80% of total PGP bacterial isolates naturally colonizing the rhizosphere of potatoes cultivated in Huancavelica and Puno – between 3,900 and 4,200 masl in the central Andean highlands of Peru – increased total plant and tuber weights between 50% and by more than 200%, when tested on a pot experiment under greenhouse conditions with seven commercial potato varieties. Some of these isolates – belonging to the genera *Bacillus*, *Azotobacter*, and *Actinomycetes* – were later on selected for a field trial conducted in Puno (3,820 masl), under rain-fed low-input conditions. Inoculated potato plants exhibited higher tuber yields than the uninoculated controls; however, the results were highly variable and needed further verification. The same year, Calvo and Zuñiga (2010) and Calvo et al. (2010) described the PGP abilities of some *Bacillus* spp. strains, isolated in the highland Peruvian regions of Huancavelica and Puno – Titicaca Lake – at 3,900–4,200 masl by Calvo-Vélez et al. (2008). Besides exhibiting several PGP in vitro, the isolates inhibited *R. solani* and *F. solani* growth and were therefore proposed as good candidates for developing bioinoculants. Some field trials have been also conducted in different regions of Bolivia. For example, Franco and co-workers (2011) investigated on the effects of some indigenous PGPM on potato productivity at altitudes ranging from 2,650 to 3,453 masl. Their results showed that, when applied to potato cultivar Waycha (*S. tuberosum* subsp. *andigena*) in combination with organic amendments, a native *Bacillus subtilis* isolate resulted in superior and uniform plant emergence, higher plant vigor and development, improved plant health, and higher yield of tubers. On the other side, infection of tubers with *R. solani* was suppressed by means of the same treatment. More recently, Ghyselinck et al. (2013) isolated 585 bacterial strains from potato fields in the central Andean highlands of Peru and Bolivia, differing in altitude, soil composition, and agrochemicals use. From these, 58 strains inhibited growth of *R. solani* and *P. infestans*, whereas 12 strains significantly increased plant growth and development in vitro. Additionally, 14 isolates (belonging to the *Pseudomonas koreensis* subgroup, the *P. fluorescens* subgroup, and the *Bacillus* genus) protected potato plantlets challenged with *R. solani*.

Five of these strains were later shown to increase potato tuber number and yield when applied to soils amended with organic manure, during field trials conducted in Bolivia, Peru and Ecuador (Velivelli et al. 2014b). Additionally, a suppressive effect of one *Pseudomonas* strain against *R. solani* was also observed. In a similar study performed in Bolivia, two *Pseudomonas* sp. isolates and one *Bacillus* sp. isolate, from an initial group of 17 bacterial strains showing PGP abilities in vitro, increased tuber weight per plant when tested in potato fields(Franco et al. 2015).

Arbuscular mycorrhizal fungi (AMF) have also been tested with encouraging results as potential biofertilizers/biocontrollers for potato production in different regions of the world (Hijri 2016). For example, positive responses of *S. tuberosum* were recorded by McArthur and Knowles (1993) after inoculation with AMF, in experiments conducted in growth chambers under P deficiency. At almost the same

time, some pioneering studies – performed in Perú and Colombia – confirmed the positive effects of AMF inoculation on potatoes (Moreno Díaz 1988; Sieverding et al. 1991). However, at least in one of these studies, non-native AMF isolates were used. In 2005, Davies and co-workers showed that a mixed inoculum – containing native strains of *Acaulospora* spp., *Glomus* spp., *Scutellospora* spp., *Gigaspora* spp., and *Sclerocystis* spp. – resulted in enhanced growth and yield of Yungay potatoes, an important Peruvian cultivar, cultivated at low P supply (Davies et al. 2005a). The positive effect recorded was attributed to an enhanced nutrient uptake (P, Fe, and Mg). Incidentally, as an unexpected output of the same work, the authors demonstrated the possibility of using a radically different approach –*biostimulation* – to reach more cost-effective levels of AMF inoculums, rather than solely relying on indigenous AMF present in the soil of the crop production site. Indeed, direct application of a flavonoid produced by a number of plants (Formononetin), previously shown to enhance AMF sporulation and effectiveness of mycorrhizal plants, induced greater extraradical hyphae formation and a better development of plants and tubers. Further work on the stimulating effect of Formononetin was performed under field conditions at 3,900 masl in San Jose de Aymara (Department of Huancavelica), in the central highlands of Peru (Davies et al. 2005b). Six Andean potato cultivars were treated directly with Formononetin, applied when shoots from tubers began to emerge. The biostimulation treatment increased tuber dry mass and/or grade quality in three out of six cultivars and also induced greater soil sporulation levels of naturally occurring AMF (predominantly *Gigaspora*, *Glomus*, and *Scutellospora* spp.). By combining the PGP abilities of bacteria and the biocontrol capacities of some particular fungi, it is also possible to develop mixed bioinoculants with enhanced effects. This was shown by Franco and co-workers (2011) in a field assay with potato cultivars Waycha (*S. tuberosum* subsp. *andigena*) and Desiree (*S. tuberosum* subsp. *tuberosa*). In this assay, co-inoculation with *B. subtilis* and the AMF *Glomus fasciculatum* allowed a significant increase in tuber yield and a suppressive/inhibitory effect on soil borne diseases. The effectiveness of mixed bioinoculants was also demonstrated by Pérez et al. (2015), by monitoring the effect of bioinputs on tolerance to drought stress of 15 native potato cultivars at 3,309 masl in Cochabamba (Bolivia). Indeed, the combined use of earthworm humus and Mibac (a commercial product that combines native strains of *B. subtilis* and *G. fasciculatum*) allowed to record satisfactory results. However, as shown by others, some of the responses were cultivar dependent. One among several positive outputs of these series of assays was the development of a commercial biofertilizer – BioFert, formulated with a native isolate of *B. amyloliquefaciens*, by PROINPA, a Bolivian national foundation, and used by farmers dedicated to organic production – both as a single treatment or mixed with *G. fasciculatum* and vermicompost (PROINPA Catalog).

2.4.2 Quinoa

Quinoa (*Chenopodium quinoa* Willd.) is an exceptionally rich seed crop whose contents often surpass recommended values for many nutrients (Hirose et al. 2010).

This crop, a pseudo-cereal cultivated for millennia in the Central Andes, has experienced a spectacular increase in demand over the last few years, a rise that can be largely attributed to its high protein and fiber content, the absence of gluten and the health trend among consumers, particularly in the Western Hemisphere (Vega-Gálvez et al. 2010). In fact, the European Union imported in 2014 more than twice as much quinoa than in 2012, approximately 15 thousand tons, of which around 95% came from Peru and Bolivia (CBI Product Fact Sheet 2015). Quinoa is remarkably well adapted to harsh environmental conditions, including frost, salinity, and drought (Ruiz et al. 2014). For such reasons, it is also considered a food security crop potentially important for the human kind in the face of the predicted future world scenario of increasing salinization and aridity.

Quinoa has been traditionally grown by small farmers in the Andes using traditional methods. This is more evident in the Bolivian Altiplano (highlands), where most of the quinoa production is said to be organic and cultivated using ancestral techniques passed on through generations from parents to children. However, in recent years, increasing worldwide demand for quinoa has boosted the rapid development of large farms in Peru, where traditional techniques have been replaced by more conventional methods of intensification that resulted in higher yields. The subsequent industrial development associated to quinoa production is, however, putting in danger the cultural legacy associated with the crop and its diversity (Jacobsen 2011).

The demand for quinoa in many developed countries is driven by the "organic" movement, and this trend is expected to persist for many years. Therefore, preservation of small-scale farming in order to continue cultivating quinoa in a sustainable (and organic) way is necessary for many reasons.

Bolivia is leading research efforts toward the sustainable intensification of quinoa farming by making use of microbial resources. Ortuño et al. (2013, 2014) have isolated PGP microbes from different organs of organically grown quinoa plants to screen potential bioinoculants that could reduce production costs of organic quinoa. They verified the PGP and/or antagonistic capacities of their isolates against quinoa pathogens and identified two microbial strains of interest, a *Bacillus* sp. bacteria and a *Trichoderma* sp. fungus. These two microbes were later produced at large scale using simple and inexpensive media and distributed among local farmers. Application of these bioinoculants increased quinoa yield compared to the untreated controls when tested in the field (Ortuño et al. 2014). In addition to the yield advantage, inoculated plants exhibited a healthier and more vigorous appearance than untreated plants.

2.4.3 Vegetable Crops

A great variety of vegetable crops are cultivated by smallholder farmers in the Tropical Andes. This production is of great relevance for the food security and sovereignty of Andean countries and to maintain rural livelihoods. Although most of the vegetables produced in the Tropical Andes are introduced species or varieties of native species developed outside the region, its cultivation over decades has resulted in their

assimilation as traditional crops. Although vegetable varieties cultivated in the Tropical Andes do not differ significantly from those found in other parts of the world, yields and crop development are markedly influenced by climatic conditions such as the lack of seasonal variations in climate, constantly cool temperatures, microclimatic variations, and erosion-prone nutrient-locked soils (Borsdorf and Stadel 2015).

The general effects of PGP and pathogen antagonist microbes on vegetable production have recently been reviewed elsewhere (Baum et al. 2015; Zaidi et al. 2015), and many commercial inoculants are currently marketed to Andean farmers. However, as noted earlier in the chapter, because of the highly specific environmental conditions found in the Tropical Andes and the fact of being composed of living organisms, the effects of inoculants observed in other regions cannot be directly extrapolated to this region. A recent survey of Colombian research on bioinoculants shows that ~30% of the publications reviewed are related to the discovery and application of these inoculants to vegetable crops (Zambrano-Moreno et al. 2016).

Local research on inoculants has focused mainly on prospecting and evaluating the biocontrol capacity of native and introduced microorganisms. The introduced (or not locally generated) commercial inoculants most commonly evaluated include *Bacillus thuringiensis*, *Beauveria bassiana*, *Pseudomonas* spp., and some NFB strains. These organisms are introduced through the application of commercial inoculant formulations and will not be discussed further.

Among the native organisms that have been the subject of more interest by researchers are different species of *Trichoderma*. These fungal species have been studied mainly for their biocontrol properties against several pathogens such as *Sclerotinia sclerotiorum*, *Sclerotium cepivorum*, and *Fusarium oxysporum* (Jaimes et al. 2009; Rojas et al. 2010; Smith et al. 2013). Although members of the *Trichoderma* genus are considered cosmopolitan species adapted to a wide variety of ecosystems, the introduction of exotic *Trichoderma* species or strains in commercial formulations remains a matter of concern because of potential undesirable effects on nontarget species (Zambrano-Moreno et al. 2016).

Other organisms prospected as inoculants for vegetable crops are dark septate endophytes such as *Leptodontidium orchidicola*, DSE48, and DSE49 that have reduced the negative effects of *Verticillium dahliae* in tomato plants (Andrade-Linares et al. 2011); *Pseudomonas* spp. that stimulated growth in lettuce by solubilizing P contained in phosphate rock (Sánchez López et al. 2014); N-fixing *Azospirillum* spp. and *Azotobacter* spp. that increased yields in hydroponic strawberry (Jiménez et al. 2011; Rueda et al. 2016); enthomopathogenic nematodes that controlled *Delia platura* larvae (Jaramillo et al. 2013); and the yeast *Candida guilliermondii* that controlled postharvest *Rhizopus stolonifer* infections in tomato (Celis Zambrano et al. 2014).

2.4.4 Tamarillo

Tamarillo (*Solanum betaceum*, also known as tree tomato) is a native Andean, emerging exotic fruit (for the rest of the world), cultivated in South America but also in other tropical and subtropical countries like New Zealand, Australia, and India

(Bohs 1989; Carrillo-Perdomo et al. 2015). The fruits can be eaten fresh, blended with milk or water (the most popular and common use), cooked in stews and sauces, and incorporated into desserts and salads. Owing to its high content in ascorbic acid, provitamin A, carotenoids, and vitamin B6, and also to its excellent antioxidant activity (Vasco et al. 2009; Acosta-Quezada et al. 2015), the demand for tamarillo has been increasing. For instance, by 2012 China's imports reached almost 800,000 tons, equivalent to 31.6% of the world demand (Cámara de Comercio de Bogotá 2015). In South and Central America, tamarillo is frequently produced in small orchards, between 1,800 and 2,600 masl, using traditional management systems (Prohens and Nuez 2000); however, due to the increasing demand, more intensive and larger plantations have been developed, particularly in Colombia. Even though precise statistical data are hard to reach, by 2013 almost 8,400 Ha were dedicated to tamarillo in Colombia (Cámara de Comercio de Bogotá 2015); the same year, Ecuador dedicated 5,900 Ha to this fruit (ProEcuador Bulletin 2013) with another 41,900 ha suitable for the expansion of this crop (Carrillo-Perdomo et al. 2015). Despite this potential, research on tree tomato diversity, conservation, and breeding has been limited. Interestingly, contrary to countries where it has been introduced such as New Zealand, the cultivation of tamarillo in its native countries remains underdeveloped.

Tamarillo is highly susceptible to several diseases, including anthracnose and powdery mildew (Tamayo 2001). Among the most important pests affecting tamarillo, three nematode species, namely, *Meloidogyne incognita*, *M. java*, and *M. hapla*, can cause serious diseases to young trees (Prohens and Nuez, 2000). In a pioneering work published in 1987, Cooper and Grandison demonstrated the protective effect of several AMF on infection of tamarillo plants by *M. incognita*. Indeed, mycorrhizal infection of tamarillo plantlets at transplanting with a mixed inoculum containing seven AMF strains did improve not only plant growth but suppressed nematode reproduction and development in roots. The protective effect was more pronounced when plantlets were inoculated with AMF before infection with nematodes. Nevertheless, the protective effect varied according to the AMF tested, but a combination of three of them (*Glomus fasciculatum*, *G. mosseae*, and *G. macrocarpum*) protected tamarillo plants to the same extent than the seven-strain inoculum.

Despite these rather encouraging results, only a few papers have been published on this matter since then. For example, in 2013 Orrico et al. reported protective effects exerted by a mixed inoculum of native Ecuadorian AMF and *P. fluorescens* strains isolated from organically grown tamarillo trees against *M. incognita*, *M. java*, and *M. hapla*. Co-inoculation of these microbes counteracted efficiently the deleterious effects of nematode infection and stimulated root and shoot development in infected plants, grown under controlled conditions in a mixture of sterile soil (50% w/v), sand (25% w/v), and peat (25% w/v). The results also suggested a synergistic effect between *P. fluorescens* and AMF in reducing knot formation and nematode proliferation while simultaneously stimulating spore formation, mycorrhizal proliferation, and bacterial colonization. The same year, Ramírez et al. (2013) obtained almost identical results (also in Ecuador) but this time by combining native strains of AMF and *P. putida*.

Besides protecting tamarillo plantlets against *M. incognita* infection, AMF also stimulate plantlet development and acclimatization. Espín et al. (2010) showed that mycorrhization of tamarillo plantlets with an unidentified native AMF isolate from Ecuador naturally colonizing tamarillo trees, stimulated plantlet growth, biomass accumulation, root biomass, leaf area, and P content. In 2013, Echeverría et al. compared the effect of a consortium of native AMF and three *Pseudomonas fluorescens* isolates in mixed or single infections on the development of tamarillo plantlets. Their results were very similar to those described before and confirmed that mixed infections induced higher growth and increased P and N contents in plantlets after a 4-month period.

2.5 Is It Really Possible to Intensify Agriculture in the Tropical Andes through Adoption of Microbial-Based Technologies?

As we have seen in the previous pages, the prospection of natural and/or agroecosystems permitted the isolation, identification, and use of native microorganisms for the development of bioinoculants that performed adequately, even when tested under field conditions in the Andean mountains. Unfortunately, the adoption of new technologies by farmers, irrespective of their scale or educational level, has been always being a matter of debate and controversy. In the particular case of microbe-based technologies, an additional barrier to this adoption is imposed by the very nature of these bioproducts. In fact, microbes have been traditionally associated with diseases and pests and not only in the less developed countries. Therefore, alongside with technical optimization and in order to take fully advantage of the potential benefits these bioinputs offer to farmers, important efforts must be devoted to modify this flawed perception.

A good starting point to overcome this cultural barrier and to increase confidence of users is to communicate knowledge concerning the use of these technological assets, both objectively and transparently (Wolt and Peterson 2000). As we will see below, another fundamental strategy is to include concerned parties in the research programs aimed at developing new bioinoculants, particularly during the final steps (field trials).

On the other hand, it is also of utmost importance to facilitate access to newly developed technologies and at low cost in order not to create new dependencies. In fact, it is well known that large corporations control the market of agricultural biotechnologies and that the evolution of bioinoculants – far from being powered by the desire of fighting against hunger and poverty in poor countries – is driven largely by the interest of increasing corporate profitability and competitiveness (Glover 2003). Unfortunately, this holds true even in countries like Brazil and Argentina that devote substantial academic and scientific efforts to develop new agriculture biotechnologies (Eakin and Lemos 2006). Therefore, even accepting that bioinoculants can be very helpful for the sustainable intensification of agriculture in the less developed countries, care must be taken in order to not fall in the *input substitution* trap already warned by Rosset and Altieri (1997).

One effective way of avoiding this is by effectively transferring technology to local stakeholders and/or small farmer's cooperatives. By doing this, local production of bioinputs becomes an option for the creation of micro- or small enterprises in underdeveloped countries. With the cooperation of academia and governments, strengthening of this sector will certainly favor sustainable farming, concomitantly benefiting scientific and technological development in these countries, as already demonstrated in Cuba (Altieri and Funes-Monzote 2012).

In a recent work, Barragan-Ocaña and del Valle Herrera (2016) investigated on some of these issues by addressing the impact of endogenously generated biotechnologies on the lives of the peasant producers from underdeveloped countries. The investigation, conducted in the State of Morelos (Mexico), confirmed that a group of peasants who currently use two biofertilizers produced by a local company – with strong academic connections – perceived favorably the effect of those bioinputs both on the environment and on their lives. Nevertheless, it was also established that producers with more education and greater technical and financial resources were the most benefited through the use of such a biotechnology, confirming the necessity of implementing the above mentioned measures.

Another important conclusion of the aforementioned study highlighted the validity of using biofertilizers as an option for peasant producers in the developing world. This is in frame with some experiences conducted in the Andean context. For example, the Andean Foundation for Research and Promotion of Andean Products (PROINPA) – a Bolivian institution dedicated to promote the sustainable use of natural resources through research and innovation – together with the International Potato Center (IPC), already developed a series of bioinoculants with the aim of increasing the productivity of some Andean crops, like potato and quinoa, by making profit of native microorganisms (Ortuño et al. 2010; Oswald et al. 2007; Ortuño et al. 2013). In their path, researchers and developers took constant care in generating and facilitating technology transfer mechanisms and, at the same time, educated and trained local farmers on the correct use of these biotechnologies. More recently, the results of a collaborative project financed by the EU and including partners from five European and three Latin American countries – VALORAM project (http://valoram.ucc.ie/) – seem to confirm that when properly addressed, newly developed biotechnologies may be socially accepted and adopted by farmers. The project – aimed at developing alternative, efficient technologies to intensify potato-based farming systems in a sustainable way by making use of the Andean microbial diversity – included several participatory field trials in Bolivia, Ecuador, and Peru. During these trials, the participants (including local farming communities and NGOs) evaluated actively the potential of Andean microorganisms to increase the productivity and plant health of high- and low-input potato-based cropping systems. Furthermore, they also monitored the success or failure of the trials and selected the most accepted technologies in each community, thus strengthened the decision-making capacity of the potential users.

Altogether, the results obtained through the VALORAM initiative seem to confirm that it is possible not only to intensify potato-based farming systems in the Andean region in a sustainable way, but to do it cost-effectively (VALORAM Final

Report). Furthermore, through the combination of suitable plant genotypes and useful microbes, together with appropriate land management, a valuable opportunity result may be well perceived and properly adopted by local populations.

2.6 Concluding Remarks

In their paramount work on alternative pathways to intensify soil fertility management by smallholder farmers in the Andean highlands, Fonte et al. (2012) highlighted the necessity of developing proper strategies to take full advantage of the functioning of soil microbial communities, either by adequately managing the soil or by inoculating beneficial microbes. As we have seen in the previous pages, the development and use of such kind of microbial inoculants may certainly contribute to agriculture intensification in the Tropical Andes. The potential of native microbes in this regard is enormous and deserves to be explored in depth. However, before this potential can be translated into useful products and better life conditions for the Andean small farmers and their families, a long journey awaits.

One of the problems that remain to be solved deals with the proper selection of microbial strains, a task which is far from simple. Microorganisms must not only exhibit excellent PGP and/or biocontrol abilities during experiments in the laboratory but should also be able to efficiently colonize the rhizosphere of plant crops under more realistic conditions. To do so, they will have to compete favorably with other microorganisms already present in the soil and escape predation. Furthermore, they should also endure the challenges imposed by the harsh climatic conditions prevailing in the high mountains, among which low temperatures is one of the most important (Yarzábal 2014).

On the other hand, the substitution of agrochemicals with microbial inoculants is only one of several aspects that should be taking into consideration to achieve agriculture intensification in a sustainable way. It would be naïve to think that only through input substitution the problems associated with monocultures and dependence on off-farm inputs will be solved. As proposed by Baulcombe et al. (2009), the sustainable intensification of agriculture would demand an effective integration of social, economic, environmental, scientific, and technological factors recognizing the complexities and particularities of the problems associated with agriculture in different places.

The Tropical Andes is considered a “hot spot” of biodiversity, containing about one sixth of all plant life in the world, as well as the largest variety of amphibian, bird, and mammal species (Myers et al. 2000). However, only a few studies have focused on the diversity of microbial communities colonizing different ecosystems in this region. In the particular case of microbes colonizing Andean crops, their prospection has relied until now – almost exclusively – on cultured-based techniques. Consequently, the great majority of microbes – the unculturable ones – remain to be identified and their functions revealed. Luckily, with the progressive inclusion of *omic* technologies (metagenomic, metatranscriptomic, metabolomic, and so on), the real complexity of these communities is starting to emerge (Senés

Guerrero and Schüssler 2016), helping us to understand in depth their multiple functions and ecosystem services. By doing this, chances are that more efficient bioinoculants will be developed in the near future, allowing thus intensifying agriculture in this vast region.

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Arbuscular Mycorrhizal Fungi Improve Tolerance of Agricultural Plants to Cope Abiotic Stress Conditions

3

Pablo Cornejo, Alex Seguel, Paula Aguilera, Sebastián Meier, John Larsen, and Fernando Borie

Abstract

Abiotic stresses have strong impact on agriculture, decreasing the stability of agroecosystems worldwide, due mainly to water and nutrient limitations and the presence of toxic elements. Several studies have demonstrated that soil microorganisms can improve plant growth, even more when plants are under stressful conditions, being probably the most important are the arbuscular mycorrhizal fungi (AMF). This kind of fungi forms symbiosis with approximately 80% of plant species, including the majority of agricultural plants, and is present in all terrestrial ecosystems. Via its extraradical mycelium, the AMF can improve the absorption of water and nutrients of their host plants under stress conditions, as well as contribute to cope with the presence of toxic elements such as phytotoxic aluminum and other toxic metal(loid)s, increasing plant growth and crop production. Moreover, several studies have determined that AMF strains isolated from agroecosystems affected by different abiotic limiting conditions enhance the

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growth of plants than those isolated from soils without such limiting condition. In this chapter we describe the main ways by which AMF contribute to the plant tolerance to cope the abovementioned abiotic stresses. Moreover, the physiological, biochemical, and molecular bases that explain the responses mediated by AMF in host plants are covered. Finally, biotechnological prospects of AMF present under stress conditions and their potential use as bio-inoculants are presented.

Keywords
Acid soils • Fungal diversity • Nutrient limitations • Osmotic stress • Potentially toxic elements

3.1 Introduction

Plants are subjected to stress when the environmental conditions are significantly different from those optimal for their establishment, growth, and development (Sade et al. 2013). In general, plants are commonly exposed to different kinds of abiotic stresses, highlighting the water excess or limitation, extreme temperatures, low nutrient availability, and presence of toxic elements, which reduce plant growth and productivity (Atkinson and Urwin 2012; Sánchez-Rodríguez et al. 2012). Moreover, due to the global climatic changes generated by the continuous increase of greenhouse gas emissions that produce changes in environmental temperatures, many of these stresses are becoming common (Walter et al. 2010; IPCC 2012; Fischer and Knutti 2015; Teskey et al. 2015).

A series of studies have shown that beneficial soil microorganisms improve plant tolerance to abiotic, principally through the generation of a root-soil interface that enhances the absorption of water and nutrients, determining a better plant condition. These microorganisms include some endophytes as the most important ones, with special emphasis on nitrogen-fixing bacteria and arbuscular mycorrhizal fungi (AMF) (Barea et al. 2005; Hidri et al. 2016). In this sense, AMF generate a strict symbiosis called arbuscular mycorrhiza (AM), which protects plants against the vast majority of abiotic stresses through different mechanisms. Some of the principal mechanisms are the improvement of nutrient absorption (Evelin et al. 2012; Estrada et al. 2013; Krishnamoorthy et al. 2016), exacerbation of photosynthetic activity and increase of water use efficiency (Asghari et al. 2005; Porcel et al. 2015), higher accumulation of osmoprotectant solutes, control of reactive oxygen species (ROS), increased activity of antioxidant enzymes (Evelin et al. 2013; Manchanda and Garg 2011; Barzana et al. 2015; Calvo-Polanco et al. 2016), and modification of the rhizosphere environment (Asghari et al. 2005; Yin et al. 2016), including the accumulation of glomalin-related soil protein (GRSP). Also, the increase in the production of hyphae and spores able to sequester and accumulate significant amount of toxic elements (Cornejo et al. 2008a; Aguilera et al. 2011; Meier et al. 2012b),

which can reduce the toxicity in the soil environment, is an interesting mechanism recently describe in this kind of fungi.

Given the importance that the AM symbiosis represents for the establishment and growth of plants under the conditions of diverse abiotic stress, it is necessary to broaden the knowledge for a more efficient use of this symbiosis that increases plant tolerance to the limiting conditions. In this sense, this chapter incorporates state-of-the art information on the role of the AM symbiosis in environmental stress conditions, with special emphasis on water and nutrient limitations, and the presence of toxic elements in the soil, mainly aluminum (Al) and toxic metal(loid)s. Additionally, proposals for the biotechnological utilization of these microorganisms are given in order to obtain agronomic advantages on lands characterized by the limitations here addressed. Particular emphasis has been done on the potential use of AMF as useful bio-inoculants.

3.2 Principal Abiotic Stress Conditions

Although it is well known, there are several environmental conditions of abiotic nature that can have a strong and negative repercussion on plant growth and development; the cases in which AM symbiosis has an important role in increasing plant tolerance are related basically to improved nutrient and water acquisition, as well as the increasingly studied role in reducing the toxicity of some elements. This section will address the basis for stress in plants due to limitation of water and nutrients, as well as the excess of potentially toxic elements (PTE).

3.2.1 Nutrient Limitations

Thirteen of the mineral nutrients required for plant growth come from the soil, dissolved in the soil solution and adsorbed through plant roots. However, soils not always have sufficient nutrients to grow healthy plants, which is why farmers continuously need to apply fertilizers. Fertilizer application is expensive, and the bulk of worldwide soils present deficits of some of these nutrients, mainly P and N. While N can be better managed with the use of legumes, which fix N from the atmosphere through the symbiosis with *Rhizobium* or other free-living microorganisms (*Azotobacter*, *Azospirillum*, among others), it is not the case for P where its availability in the most of soils is scarce due to its low solubility and high anion-fixation capacity to soils, especially acid ones. This involves low diffusion rate in the soil and consequently low plant root efficiency. To cope with this problem, the most commonly adopted measure is the continuous application of high amounts of P fertilizers, but only a minor part of the total P applied is effectively acquired by plants, with the remaining P being immobilized in the soil (Borie and Rubio 2003). Therefore, efforts to support the productive use of high P-fixing soils must be focused on developing strategies to use the "P bank" accumulated in the soil due to the continuous fertilization performed annually, especially under a scenario in

which various abiotic limitations of importance (drought, low P availability, among others) are expected to coexist permanently. On the other hand, it is globally recognized that natural P sources used in agriculture are decreasing, with their total depletion anticipated within a few decades, which has also caused an explosive increase in the costs of P fertilizers (Cordell et al. 2009; Cordell and White 2011; Elser and Bennett 2011).

One of the most high P-fixing soils is the order andisols presenting serious fertility problems due to its acidity and Al phytotoxicity. In such soils, the climate change has generated drought stress, which coexists with the low P availability, becoming the main factor limiting plant growth. In contrast, andisols have high amounts of total P (more than 1000 ppm), but a significant fraction is associated with sources that are unavailable to plants, mainly as organic complexes in inositol penta- and hexa-phosphates (Borie and Zunino 1983; Escudey et al. 2001; Pinochet et al. 2001; Borie and Rubio 2003), tightly fixed to the mineral fraction of the soil and/or precipitated with Al, which is an abundant chemical species present particularly when acidic conditions increase the presence of this phytotoxic element (Mora et al. 2005; Seguel et al. 2013; Aguilera et al. 2014).

In addition, healthy plant growth requires of some micronutrients such as Cu, Mn, and Zn. Unfortunately the threshold of these elements for optimal plant growth is very close to toxic levels, and plants growing at high levels of metal concentrations present serious phytotoxicity symptoms, which must be adequately overcome.

3.2.2 Aluminum Phytotoxicity

Aluminum phytotoxicity is the second most important limiting factor for crops growing in acid soils being more severe when pH is below 5.0 (Kochian et al. 2004; Imadi et al. 2016). Aluminum interferes with a range of physical and cellular processes in plants (Kochian et al. 2005), and the major symptom observed is the inhibition of root growth (Delhaize and Ryan 1995; Imadi et al. 2016) with a concomitant limited water and nutrient acquisition. Such damage is due to the increase of phytotoxic Al forms including Al^{3+}, which is reported as the most toxic chemical form. Therefore, from a practical point of view and similar to that with phosphorus (P) deficiency, farmers apply liming materials (lime and gypsum) and phosphate fertilizers for avoiding such Al phytotoxicity, to finally decrease Al activity, applied alone or together with Al-tolerant genotypes of agricultural plants (Seguel et al. 2015, 2016b). This last strategy has received increasing interest in recent years breeding for genotypes with Al tolerance, which is crucial for overcoming acid soil limitations.

There is consensus that it is possible to distinguish two different kinds of Al tolerance mechanisms: (*i*) those that exclude Al from plant tissue (Al excluders) and (*ii*) those that allow the plant to tolerate high internal Al concentrations (Al accumulators) (Miyasaka et al. 1991; Kochian 1995, Ma et al. 1997; Watanabe and Osaki 2002; Kochian et al. 2004; Seguel et al. 2013). There is considerable evidence

supporting that the major proposed mechanisms for Al exclusion is the formation of stable complexes with chelating substances such as organic acid (OA) anions exuded by roots (Li et al. 2000; Ma 2000) including citrate (Ma et al. 1997; Zhao et al. 2003), malate (Ryan et al. 1995), and oxalate (Ma et al. 1997), among others. In addition, it has been suggested that P efflux observed in roots of *Triticum aestivum* can be the result of a potential Al tolerance mechanism (Pellet et al. 1996), evidencing the important role of plant P status in the Al tolerance by agricultural plants. This theory was supported by Zheng et al. (2005) who found that genotypic differences in Al resistance in buckwheat were unrelated to oxalic acid secretion, whereas most likely related to a higher accumulation of Al and P in the roots. Dong et al. (2004) also reported Al and P interactions influencing plant growth and OA exudation in soybean, but whereas oxalate and malate release were influenced by P deficiency, the citrate exudation was activated by Al toxicity. Similar behaviors have been reported by Ligaba et al. (2004) in lupine, where citrate exudation was enhanced by P deficiency but not by Al toxicity.

Genotypic differences in Al tolerance shown by some crops occur in relation to Al-P interactions. Therefore, P produced an enhancement of Al resistance in the Al-tolerant *Lespedeza bicolor* in comparison with Al-sensitive *L. cuneata*, which may be associated with a more efficient P accumulation and translocation to shoots and a greater Al exclusion (through complexation or precipitation mechanisms) from root tips, but not with an increased root OA exudation (Sun et al. 2008). In the same way, Nakagawa et al. (2003) reported that in rice plants precultured with phosphate, addition of Al did not inhibit shoot growth, whereas Al retarded shoot growth in plants precultured without phosphate. However, Al inhibited plant root elongation independent of the presence of P in the precultured solution.

When P addition alleviates Al toxicity in plants, such effects are commonly based on two potential mechanisms: (*i*) direct interactions of Al-P precipitation in the soil solution as well as at plant level (root surface, root cell wall) (Zheng et al. 2005) and (*ii*) indirect effects such as improving root morphology, increasing nutrient uptake, or secreting root exudates (Liao et al. 2006). The latter aspect is crucial to take into account demonstrating high interdependence of two of the main agricultural limitations coexisting (P deficiency and Al toxicity) in a vast surface of acid and acidic soil globally (Seguel et al. 2013; Aguilera et al. 2015).

3.2.3 Presence of Potentially Toxic Elements

Toxicity of an element is denoted by the term "potentially toxic elements" (PTE) (Gadd 1993). Some PTE do not have biological functions in plants, such as Cd, Hg, and Pb (Adriano 2001), which are also toxic when present even in low concentrations. Some metals are micronutrients for plants such as Co, Cu, Mn, and Zn but behave toxic when present at high concentrations. Therefore, the accumulation and the migration of these contaminants through dust or leachates into non-contaminated areas are examples of events that contribute toward the contamination of our ecosystem even in agricultural soils (Meier et al. 2012b).

Potentially toxic elements enter into ecosystems through two major pathways. The first one is natural (lithogenic), in which metals are derived from primary minerals through geological weathering, which contributes only to a small fraction of the total input (Wuana and Okieimen 2011). The second one is anthropogenic, which is the major contributor of metal(loid)s in the ecosystems. The anthropogenic activities related are industrial processes, mining, and agriculture practices. The first two of these produce metal-enriched solid and effluent wastes. Some agricultural practices like fertilization with untreated biosolids may increase PTEs in soils (Wuana and Okieimen 2011), and the use of phosphate rock in high amounts is closely related with the increment of Cd in soils. The discharges of the metal-enriched wastes into the ecosystems pose risks and hazards. Firstly, the hazards may be caused through direct contact with contaminated soils, drinking of contaminated groundwater and consuming toxic food (Wuana and Okieimen 2011). In such cases, the PTE can be accumulated in the tissues of living organisms (bioaccumulation). Their concentrations increase as they pass from lower trophic levels to higher trophic levels (biomagnification). Secondly, irreversible soil degradation may be caused by the presence of high concentrations of PTE. In such case, its physical, chemical, and biological properties are degraded, thus limiting vegetation and agriculture use and affecting the ecosystem functions and sustainability (Bolan et al. 2014).

The PTE in the soils undergo both chemical and biological transformations including retention, redox, and methylation reactions. They are retained in the soil by sorption, precipitation, and complexation or removed by plant uptake and/or leaching (Adriano et al. 2004). When the concentration of PTE in the soil solution is low and sorption surface is large, processes of sorption/desorption will govern the metal concentration (Bolan et al. 1999). The fate of metals in the soil depends on both soil properties and environmental factors.

3.2.4 Hydric Stress

Globally, drought, soil salinity, and extreme temperatures represent the environmental abiotic stresses that most limit the growth of agricultural plants (Kramer and Boyer 1995; Cattivelli et al. 2008; Lambers et al. 2008; Trenberth et al. 2014), with greater effects being registered in arid and semiarid areas (Knapp et al. 2001; Seki et al. 2003; Fischlin et al. 2007). These stresses are produced by a water imbalance in ecosystems caused mainly by decreased rainfall volume, together in many cases with rising average temperatures that induce a higher rate of evapotranspiration, and reduced capacity of some soils to store water (Wery et al. 1994; Rapti-Caputo 2010). Drought, high temperatures, and increase of soluble salts in soils reduce water absorption by the roots causing tissue dehydration, which finally determines the occurrence of osmotic stress (Zhu et al. 1997; Seki et al. 2003; Aroca et al. 2012).

The osmotic stress caused by drought interferes with normal growth and development of plants by altering physiological and biochemical processes, thereby affecting their survival and productivity (Kramer and Boyer 1995; Bray 2004; Silva

et al. 2009). The osmotic stress caused by drought negatively affects water relations, gas exchange, photosynthesis, nutrient absorption, metabolism of carbohydrates, proteins, amino acids, and other organic compounds (Sircelj et al. 2005; Ashraf and Foolad 2007; Anjum et al. 2011). Similar effects are produced by salt stress, adding in this case the toxic effect of Na^+ and Cl^- ions, which produce homeostatic imbalances and denaturation of proteins and other organic molecules (Adiku et al. 2001; Evelin et al. 2009).

Additionally, high environmental temperatures increase the water demand of plants causing stress, manifested as denaturation and aggregation of proteins, hyperfluidity of membrane lipids losing their permeability, and direct chemical decomposition by the accumulation of toxic elements and the ion efflux (Levitt 1980; Gong et al. 1998).

3.3 Biological Basis of the Arbuscular Mycorrhizal Symbiosis

The role that certain soil microorganisms have in improving the use of diverse resources that plants need for growth and development is remarkable. In general term, and as a principal microbial component in plant production emerges the mycorrhizal symbiosis, strict relationships occur between plant root with fungi in several different forms (from the Greek *mycos*, meaning fungus, and *rhiza*, meaning root). These are mutualistic symbionts, in which the fungus obtains sugars from the plant, while the plant benefits principally from the increased uptake of mineral nutrients and water through the fungal hyphae (Smith and Read 2008).

The AM symbiosis is developed by more than 80% of vascular plant (Jeffries et al. 2003), including about 90% of plants of agricultural interest (Finlay 2008; Smith and Read 2008). According to fossil and molecular evidence, the AMF belonging to phylum *Glomeromycota* and terrestrial plants generated this symbiotic association around 460 million years ago in the Devonian period. This association allowed aquatic plants to colonize the land, producing adaptations that favored the passage from an aquatic environment to a terrestrial one, especially by the incorporation of P as a nutrient (Simon et al. 1993; Redecker et al. 2000). Specifically, AMF stand out in terms of their contribution to the mineral nutrition of host plants (Barea et al. 2005; 2011; Smith and Read 2008; Borie et al. 2010). These fungi are strict biotrophs, depending on the host plant to complete their life cycle producing asexually resting spores.

The AM symbiosis occurs when the fungal spores in the soil germinate forming the germ tube, a stage that depends on physical factors such as temperature and humidity (Giovannetti et al. 1993). Recognition between the fungus and the plant is run by molecular signals, which interact to generate the establishment of the symbiosis (Requena et al. 2007; Hause and Schaarschmidt 2009). At this point, strigolactones exudated by the roots of the host plant have been considered a significant component in the initiation and establishment of this association, stimulating branching and presymbiotic hyphae metabolism by increasing ATP production and

mitochondrial division (López-Ráez et al. 2011; Kohlen et al. 2012; Bonfante and Genre 2015). Subsequently, the germ tube (or active mycelia in the soil) contacts the root, forming an appressorium to penetrate it (Genre et al. 2005).

Genetic studies using the AM fungus *Rhizophagus irregularis* as a model have shown that these fungi lack exoenzymes degrading cell wall, necessary for root penetration, suggesting that the plant causes distension of the intercellular spaces so that the hyphae can penetrate, which is a mechanism that can be regulated by gibberellin (GA) hormones (Takeda et al. 2015, 2016). Meanwhile, other studies have determined that jasmonic acid (JA) (Nagata et al. 2016), abscisic acid (Martin-Rodriguez et al. 2016), cytokinin (Cosme et al. 2016), auxin (Etemadi et al. 2014), and flavonoids (Abdel-Lateif et al. 2012) promote root colonization, length of extraradical and intraradical mycelium, and production of spores and arbuscules. The mycelium penetrates the root intercellularly, producing intraradical colonization without invading the conducting vessels. Subsequently it divides dichotomically to form the so-called arbuscules, which are responsible for the exchange of water and nutrients to the plant and simple carbohydrates to the fungus. In parallel, the extraradical mycelium is produced, which provides a larger contact surface between plant and soil, acting as a complementary root system that increases the absorption of nutrients and water beyond the root depletion zone (Peterson et al. 2004; Mikkelsen et al. 2008).

In addition to the well-known nutritional role of AM symbiosis, the fungal mycelia form networks in the soil that help in the formation and maintenance of stable aggregates, increasing the cohesion between particles and preventing surface erosion (Barea et al. 2005; Curaqueo et al. 2010; Mardhiah et al. 2016). This fungal network can range from a few centimeters to several meters and can account for up to half of the microbial biomass in grassland soils (Soka and Ritchie 2014). Moreover, it produces recalcitrant forms of C such as chitin and glomalin (Zhu and Miller 2003). The latter is a hydrophobic natural glycoprotein that participates in the formation and stabilization of soil aggregates, being affected by different tillage systems, soil management, and even other abiotic environmental stresses (Miller and Jastrow 2000; Cornejo et al. 2008a; Aguilera et al. 2011; Curaqueo et al. 2011; Seguel et al. 2015).

Likewise, the AM colonization causes changes in root exudates, selectively affecting the communities of other microorganisms in the rhizosphere (Marschner and Timonen 2005). As an example of this, inoculation with *Glomus versiforme* resulted in a greater abundance of ammonificant bacteria, nitrobacters, denitrifying bacteria, and phosphobacteria, which improves urease and alkaline phosphatase activity in soils cultivated with *Lolium multiflorum* (Ye et al. 2015). An increase in plant growth-promoting rhizobacteria (PGPR) by the presence of AMF interacts positively with the absorption of P by the host plants (Fernández-Bidondo et al. 2012). Similarly, the PGPR produce auxins able to promote the development of mycorrhizal mycelium (Fernández-Bidondo et al. 2011). Another effect of the AMF in the soil is the extraradical mycelium capacity to produce enzymes that contribute to the solubilization of nutrients. As an example, recently Sato et al. (2015) detected a 187 kDa acid phosphatase in monoxenic culture of *Rhizophagus clarus*, which

supports a new line of research about the direct role of AMF in the solubilization of low-labile P forms in the soil.

3.4 Contribution of AM Symbiosis to Cope with Abiotic Stresses

The AM symbiosis is highly effective in providing access to nutrients and water from areas that plant roots cannot access, so the volume of the (myco)rhizosphere is highly increased (Smith and Read 2008). However, because of the biotrophic nature of AMF, the study of the biological basis of these fungi cannot be performed in the same way as for other free-living microorganisms that are able to grow in axenic media. Therefore, the development of AMF-based bio-inoculants has been hampered, particularly due to the support systems that must be considered, based on the growth substrate and the use of fragments of AMF-colonized roots. Based on this condition of strict biotrophy, AM colonization is known to trigger a series of physiological responses, including an increase in photosynthetic efficiency due to the consumption of C needed by the fungus for its functioning and growth (Ferreira et al. 2015). This highly efficient symbiosis, involving bidirectional nutrient exchange, is based on a high level of physiological adaptation between both symbionts, which in some cases reaches an extreme level of adaptation by some plants that cannot survive if the AM symbiosis is absent, particularly in environments subject to abiotic restrictions (Barea et al. 2011). As an example, many of the plant ecosystems of arid and semiarid environments are a priori associated with some indigenous AMF ecotypes that may provide significant benefits to their hosts. This is of great interest because strict specificity between both symbionts does not exist in AM symbioses, making it theoretically possible to establish AM colonization in any root of a plant able to be colonized by virtually any AMF strain (Smith and Read 2008). Therefore, the possibility of studying diverse combinations of plant species × AMF ecotypes that result in greater functional compatibility emerges as a noticeable alternative to undertake in research for the development of biotechnological tools to cope with the environmental limitations here addressed.

3.4.1 AM and Nutrient Limitations

The omnipresence of AM symbiosis, even in greatly disturbed ecosystems as arid and low-fertility soils, suggests that AMF is playing a crucial role on plant establishment and growth through helping plants to acquire nutrients from such adverse environmental conditions. In this sense, it is well known that AM hyphae absorb N, P, K, Ca, S, Cu, and Zn translocating them from soil to inside associated roots (Gildon and Tinker 1983). However, the most recognized improvements have been reported for the immobile nutrients, particularly P, Cu, and Zn (Liu et al. 2000; Cornejo et al. 2008b), which are largely determined by the rate of diffusion. When plants are not adequately supplied with nutrients, the absorption by the roots largely

exceeds the rate at which such nutrients are diffusing toward the root resulting in a zone of depletion, which has been evidenced by using labeled isotopes. AM hyphae extend its absorptive action beyond this depletion zone exploring a greater soil volume compared to non-mycorrhizal roots, which present a lower explorative capacity.

Briefly, plants have evolved adaptive mechanisms for growth in stressed environments like P deficiency or scarcely P availability such as (a) increased root length or a better architecture for exploring capacity, (b) organic acids and phosphatase root exudation into the rhizosphere to solubilize or mineralize inorganic or organic P with low availability, (c) the association with bacteria or free-living fungi producers of quelant compounds or phosphatase, and (d) the establishment of mutualistic associations between the roots of vascular plants and AMF, being this last the most powerful mechanisms in P acquisition by plants. Therefore, the role played by AMF in P acquisition has been reported for a long time in almost all soils worldwide (Smith and Read 2008; Barea 2015) especially in soils where available P is scarce.

The implementation of isotopic dilution methodology by using labeled P isotope dilution approach has allowed to verify and quantify the AM contribution to plant P needs (Azcón-Aguilar and Barea 2015) showing that the bulk of such nutrient is acquired via the fungal performance. As mycorrhizal network mycelia can be seen as root prolongation, it has been suggested that the AMF take up the soluble P from the same pool that roots do. The positive effect of AMF on plant P acquisition is well known and has been attributed to the following:

(a) A greater exploration of a large soil volume by the extraradical mycelium in which P is scavenged more efficiently due to the lower threshold compared with plant roots
(b) The small hyphal diameter that allows the fungus to penetrate into small aggregates in searching occluded P enhancing absorption efficiency per root surface unit
(c) The capacity of AM structures to store P as phospholipids
(d) The production and secretion of acid phosphatases and organic acids facilitating the phosphate release from insoluble P forms or mineralizing P organic complexes
(e) The AM structures can “protect” and “store” high quantities of P as polyphosphates, which is the chemical form in which P is transported through the hyphae from the soil, transferring P far from the P depletion zone of the non-colonized root directly to the colonized root and hydrolyzing it to inorganic P prior to being transferred to the plant cell (Hijikata et al. 2010)
(f) The AM symbiosis can modify the molecular mechanisms related to P uptake and phosphatase production by plants (Cornejo et al. 2007; Guo et al. 2014), with the high affinity of P transporters (Karandashov et al. 2004; Oono et al. 2013), being one of the most interesting topics to be addressed, particularly for AM-colonized plants growing in acid soils.

Taking into consideration that most agricultural plants are associated with AMF by multiple ways contributing to crop P nutrition, the AM symbiosis should be part of the integrated nutrient management strategy to improve the P acquisition efficiency (PAE). In this context, there has been relatively little discussion of AMF in relation to their role to improve the PAE because it is not considered an economic substitute for P fertilizer (Elbon and Whalen 2015). However, there are studies where the AMF have demonstrated their efficiency to increase the PAE in *Allium porrum* (Kahiluoto and Vestberg 1998), *Zea mays* (Gill et al. 2013), *Lotus japonicus* (Zhang et al. 2015), and *Hordeum vulgare* (Seguel et al. 2015) and where non-AM treatments need more P fertilization in *Capsicum annuum* (Olsen et al. 1999). In this sense, P-efficient wheat cultivars reach the same marketable yield without P fertilization but with high response to AMF (Seguel et al. data no published), and greater yields are expected for mycorrhizal than non-mycorrhizal crops in soils with low plant-available P concentration in *Allium cepa* and *Capsicum annuum* (Krikun et al. 1990), *Glycine max* (Plenchette and Morel 1996), *Allium fistulosum* (Tawaraya et al. 2012), *Hordeum vulgare* (Seguel et al. 2015), and *Tritucum aestivum* (Smith et al. 2015). It is noticeable that AM produced similar or greater yields as non-AM crops receiving P fertilizer demonstrating the potential benefits for farmers or crop producers with the help of AMF (Tawaraya et al. 2012).

The positive effects of the symbiosis in P nutrition are beyond of any doubt; however, the contribution of AMF to the N nutrition is still controversial (Smith and Smith 2011). Whereas NO_3^- is the form that is available in most agricultural soils, NH_4^+ is predominant in unaltered or very acid soils, but AMF can take up both N forms although NH_4^+ is preferred (Bûcking and Kafle 2015). Recent studies have reported that AMF have much higher affinity for NH_4^+ compared with plant roots, which would suggest that AMF facilitate plants to acquire N under that form under very low N supply conditions (Pérez-Tienda et al. 2012). On the other hand, it has been reported negative, neutral, and positive effects of AM symbiosis on N nutrition, and even some of them affirm that the improved N acquisition by AM plants is a consequence of the improvement of P acquisition. On the other hand, in relation to the N uptake from organic N sources, especially in organic soils, some studies using ^{15}N have demonstrated that AMF hyphae can transfer N to the host plant from organic patches (Leigh et al. 2009).

In addition to P and N, AMF are able to improve the acquisition of other nutrients as have been reported by Liu et al. (2000), who have found that when plants are growing in soils deficient in low mobility micronutrients, such as Zn, Cu, or Fe, their uptake by plants is often increased by the formation of AM symbioses which is attributed to the capacity of the external mycelium to exploit larger volumes of soil beyond the zone of depletion. Similar to the N acquisition by AM, fungal mycelia is dependent on P nutrition; these authors have shown that the mycorrhizal contribution to Zn, Cu, Mn, and Fe uptake is significantly influenced by the plant P status.

3.4.2 AM and Al Phytotoxicity

In studies where multiple AMF species have been screened for attenuation of acidic soil stresses, there is a wide range of variation in effectiveness across AM fungal species investigated. In maize, three *Glomus* species, *G. diaphanum*, *G. etunicatum*, and *G. intraradices*, were equally effective at promoting plant growth in acid soils (Clark and Zeto 1996). Cavallazzy et al. (2007) noted that *G. etunicatum* and *S. pellucida* were the most effective strains conferring Al resistance in *Malus prunifolia*. *Glomus clarum* was superior in conferring Al resistance to *Liriodendron tulipifera* (Klugh and Cumming 2007), and *G. clarum* and *S. heterogama* were most effective in the case of *Andropogon virginicus* (Klugh-Stewart and Cumming 2009). Recently, in a study related to Al-tolerant wheat cultivars in an andosol with phytotoxic Al levels, the AMF diversity was described, and the authors have suggested the presence of a degree of coadaptation among wheat cultivars and indigenous AM fungal communities that could have greater specialization (Aguilera et al. 2014). In particular, AM fungal species belonging to *Scutellospora* and *Acaulospora* genera were found in this study (Aguilera et al. 2014).

In addition to all the known benefits of this symbiosis in nutrient acquisition (some of them with amelioration capacity for overcoming Al damage), it may play an important role in conferring Al resistance to host plants as it has been probed in *Panicum virgatum* (Koslowsky and Boerner 1989), *Andropogon virginicus* (Cumming and Ning 2003; Klug-Stewart and Cumming 2009), *Vigna unguiculata* (Rohyadi et al. 2004), *Ipomoea batatas* (Yano and Takaki 2005), *Hordeum vulgare* L. (Seguel et al. 2015), and *Tritucum aestivum* (Seguel et al. 2016a, b), among others. In all these plant species, AM-colonized plants have had higher Al tolerance than non-mycorrhizal ones and absorbed more water and nutrients. Moreover, reactive Al concentration in roots differed significantly in plants growing in symbiosis (Lux and Cumming 2001; Cumming and Ning 2003). Such effect was also reported in Al-tolerant cultivars of *Hordeum vulgare* (Mendoza and Borie 1998; Borie and Rubio 1999); *Clusia multiflora*, a tropical woody species (Cuenca et al. 2001); and *Eucalyptus globulus* (Arriagada et al. 2007). Among others, Klug and Cumming (2007; 2009) concluded that some strains of AMF gave higher Al tolerance to plants through an increased OA exudation, which decreased the concentration of free Al on their root zones. Some authors have evidenced that the benefit is variable among AMF species in terms of nutrient acquisition or in plant effects (Bever et al. 1996; Clark et al. 1999a, 1999b; Borie and Rubio 1999). This is a consequence of a substantial genetic variation among and within AMF species (Bever et al. 1996), which may provide different benefits depending on the edaphic environments (Kelly et al. 2005). Such studies have provided evidence that AM fungal diversity may play an important role in decreasing the adverse conditions for plant growth in acid soils. In the case of soils with or without high Al contents, there is a variation between tolerant AM fungal ecotypes showing increased adaptation to these conditions by a difference in spore germination, hyphal growth, and colonization percentage (Klugh and Cumming 2007), and an early colonization can be an important factor in Al tolerance against Al toxicity (Seguel et al. 2012, 2016a).

A more recent study reported a total of 24 AMF species in an andisol with strong acidity and an extremely high Al saturation (pH 4.5, 70% Al sat.). These fungal species were associated with Al-tolerant wheat cultivars. The AMF identified here belonged to all classes and orders of *Glomeromycota*, including 9 families and 12 genera, with *Acaulospora* and *Scutellospora* being the most dominant (Aguilera et al. 2014). However, the main conclusion of this study was that there is a high influence even at the cultivar level (genotype) on the composition and structure of AMF communities. This fact allows us to hypothesize that other characteristics displayed by different cultivars in other agricultural plant species may be due to the associated AMF ecotypes and/or the presence of the most efficient AMF species, which can confer a particular phenotypic response to cope abiotic stress.

3.4.3 AM and Presence of Potentially Toxic Elements

In recent years there has been great interest in using plants and their associated microorganisms to rehabilitate and/or restore degraded soils, processes generically known as phytoremediation (Schwitzguébel 2001; Meier et al. 2012b). The phytoremediation refers to technologies that use plants (as well as their associated microorganisms) to remove, transfer, stabilize, decrease, and/or decompose pollutants present in the environment (Meier et al. 2012b). Nevertheless, the phytoremediation techniques have some disadvantages because it is a slow process, which could take several years or decades to reduce metal concentrations in soil to acceptable levels (McGrath and Zhao 2003). This slowness could be due to the limited growth rate or biomass production of plants in metal-polluted soils (Cornejo et al. 2008a; Meier et al. 2012b). In addition, the lack of knowledge about the interactions between soils, plants, and microbial communities contributes to this issue (Barea et al. 2005; Leung et al. 2007). Recently, a number of soil microbe-assisted strategies were suggested for enhancing the efficiency of phytoremediation processes (Meier et al. 2012b, 2015, 2016). Nevertheless, the most prominent symbiotic microorganisms for potential use in phytoremediation are the AMF due to its ubiquity in soil environments (even in metal-contaminated soils) and also because AMF can develop several strategies that allow the plant to tolerate high concentrations of toxic elements in the soil (Cornejo et al. 2008a; Meier et al. 2011, 2015; Cornejo et al. 2013).

The ability of AMF to confer resistance to plants against toxic metals has been studied in several conditions (Hildebrandt et al. 2007; Meier et al. 2011; Cornejo et al. 2013; Meier et al. 2015). Therefore, the manipulation and use of AMF as a tool for contaminated soils must be one of the most important aspects to be considered when phytoremediation programs are designed. However, in order to analyze the specific role of AM in the host's exposure to metal stress and in the progression of the host's stress response depends on a variety of factors, including the plant species, the diversity of species, and the metal (bio)availability (Meier et al. 2012b).

The presence of AMF in metal-contaminated soils must be considered in terms of its ecological diversity (i.e., qualitative aspect) and functional compatibility with

the endemic metallophytes and hyperaccumulator plants in the agroecosystem. The presence of AMF in metal-contaminated soils and their ability to develop an effective AM symbiosis have been extensively investigated (del Val et al. 1999; Lehmann et al. 2003; Meier et al. 2012c). As example, del Val et al. (1999) found that the diversity of AMF ecotypes in metal-polluted soils was low, because high metal concentrations reduce both density and diversity of fungal populations. The AMF genera reported in the abovementioned study were principally *Glomus* and *Gigaspora* species (da Silva et al. 2006). However, AMF taxonomic diversity in metal-contaminated soils also includes other genera such as *Acaulospora* (González-Chávez et al. 2002), *Entrophospora*, *Paraglomus*, and *Scutellospora* (da Silva et al. 2006).

With respect to the AM-induced tolerance to cope with PTE, most of the mechanisms that plants and AMF have developed to alleviate metal stress are quite similar, due to the strict biotrophy of AMF product of a coevolution of both organisms (Meier et al. 2012b). The mechanisms used by AMF include immobilization of PTE by chelating substances secreted to soil (González-Chávez et al. 2002); PTE binding to biopolymers in the cell wall, such as chitin and glomalin (González-Chávez et al. 2004); immobilization of PTE in the plasmatic membrane once it crosses the cell wall (González-Chávez et al. 2004); membrane transporter that mobilizes PTE from the soil to the cytosol; intracellular chelation through metallothioneins (Meier et al. 2012b); exudation of OA (Meier et al. 2012a); transference of metals from the cytosol by membrane transporters; and confinement of metals into the vacuoles.

A mechanism against PTE, present exclusively in AMF, involves transporting PTE by means of the fungal hyphae (González-Chávez et al. 2002). Additionally, membrane transporters in AMF arbuscules may carry metals to the interfacial matrix (the contact zone between the plasma membrane of the fungus and the plant cell) followed by their subsequent incorporation inside the plant (Meier et al. 2012b). This may explain why some plants can accumulate metals in their shoots (Kraemer 2003) and also the accumulation of metals in AMF resistance structures (spores) (Ferrol et al. 2009). Moreover, Cornejo et al. (2013) demonstrated the accumulation of Cu in mycorrhizal spores in soils contaminated with Cu. Thus, AMF can play a role in protecting plants against toxicity in metal-polluted ecosystems, which should be considered as a biotechnological tool for phytoremediation programs.

3.4.4 AM and Hydric Stress

It is well known that under drought conditions, some metabolic and biochemical processes must be adapted, such as an increase in the activity of antioxidant enzymes and their substrates in response to the production of reactive oxygen species (ROS) (Bhargava et al. 2013). In the previous studies, it has been observed an important influence of AM colonization in reducing oxidative stress in plants growing under toxic levels of Cu, which produces a similar unspecific oxidative response in plants (Meier et al. 2011; Ferreira et al. 2015). In the case of wheat catalase, superoxide dismutase and peroxidase are the enzymes with the most important activity under

drought conditions, due to the accumulation of significant concentrations of ROS (Babeanu et al. 2010), especially H_2O_2, which is produced in the early stages in drought-tolerant cultivars but later in drought-sensitive cultivars (Huseynova et al. 2015). This response must also be considered due to the intrinsic characteristic of development and growth in contrasting cultivars.

Additionally, diverse studies reflect strong changes in photosynthetic mechanisms and respiration under drought conditions (Soja and Soja 2005; Guan et al. 2015), which generally tend to reduce leaf gas exchange, PSII maximal photochemical efficiency, and the concentration of pigments to avoid damage by photoinhibition (Guan et al. 2015). As observed, this type of approach has been successfully used to indicate drought stress in wheat; however, very few studies include AM symbiosis as a principal participant modifying the physiological responses in agricultural plants.

On the other hand, from a metabolic point of view, phenolic compounds (PCs) have an important role in some biological and biochemical processes related to plant growth, development, reproduction, and stress defense (Ma et al. 2014). Such PCs can be classified as flavonoids and non-flavonoids, with flavonoids being the most common group of polyphenolic secondary metabolites in plants (Schijlen et al. 2004). As is well known, PC function is related to defense against biotic and abiotic stresses, including drought stress, although the effects of drought on the profiles and concentrations of PCs have been scarcely studied. As an example, in wheat, phenolic acids from the hydroxycinnamic and hydroxybenzoic families have been detected as soluble-free form (9%), soluble conjugated (1%), and insoluble bound (90%) linked to cell wall components such as cellulose, lignin, and proteins (Luthria et al. 2015). Regarding the composition of the individual compounds, the presence of p-hydroxybenzoic, vanillic, syringic, p-Coumaric, ferulic, and sinapic acids has been described (Martini et al. 2015), while information about the profiles of flavonoids or anthocyanins are not currently available. In wheat leaves, an increase in PC levels, total flavonoids, and total anthocyanins during drought stress has also been reported (Ma et al. 2014). Other important metabolites that play a role in the control of drought stress are osmolytes (osmoregulatory compounds), highlighting the proline that accumulates in plants to cope with physiological drought, especially when the plants are colonized by AMF (Pinior et al. 2005; Barzana et al. 2015). Considering the above, the study of PC profiles and other compounds in agricultural plants is relevant because their composition may be responsible for antioxidant activity, may be highly correlated with the abovementioned enzymatic activities, and may be related to the protection of plants against (unspecific) stresses.

As an example, Al-Karaki et al. (2004) found responses in various agronomic parameters and yield in plants of winter wheat growing under field conditions inoculated with two AMF species, *Funneliformis mosseae* and *Claroideoglomus etunicatum*. Inoculated plants showed a better tolerance to decreases in soil moisture, but this behavior was better in plots where *Cl. etunicatum* was used. Therefore, in conclusion and supporting the previous comments, it is necessary to evaluate the existing fungal diversity to identify strains that can promote better performance in plants in response to increasing drought.

Additionally, considering the use of powerful modern tools to elucidate molecular mechanisms, there are limited studies that include transcriptome or gene expression for the plant × AMF interaction. However, some related studies have shown the AM regulation of important mechanisms leading to increased water use efficiency in the plant. In this regard, the most important and studied mechanisms are related to aquaporins (AQP), which mediate the bidirectional flux of water across cell membranes (Hove et al. 2015). In the case of AM-colonized plants, this is most likely locally regulated in the roots, which has been evaluated as plasma membrane intrinsic protein (PIP)-aquaporin gene expression in the roots of soybean, lettuce (Porcel et al. 2006), and maize (Barzana et al. 2015). This effect could be systemically mediated in other plant compartments, as observed in barley leaves (MIPs-aquaporins), which has even been proposed as tool for the selection of drought-tolerant genotypes (Hove et al. 2015). Nevertheless, enhanced AQP activity can be related to better performance in other important parameters, such as leaf photosynthetic rate, transpiration rate, and leaf water use efficiency, resulting in an increased tolerance to drought stress (Zhao et al. 2010; Zhou et al. 2012). Therefore, as it is possible to observe, the elucidation of AMF-mediated mechanisms in fungus x plant interactions deserves to be deeply studied in the case of agricultural plants subjected to water limitations, especially in a context of climate change.

3.5 Conclusions and Future Directions

It is expected that given the non-specific condition of the AM interaction, the study of compatibility between AMF strains and genotypes of agricultural plants will be in the near future highly increased. Moreover, given the several ecosystem services provided for the AM interaction in natural and agricultural conditions, to take advantage of the symbiosis would be a first-order point to consider in the planning of cropping systems. Here it is important to consider the possible impact agronomic practices (fertilization, tillage, rotations, among others) on the viability and functionality of AM propagules, especially in extensive crops where the use of directed mycorrhizal inoculation would be impracticable. However, in other production systems, especially those that include nursery stages, the use of AM inoculants presumably will be one of the most important practices to be normally incorporated.

Currently, some bio-products based on AMF are available on the market, but their effectiveness has not been proven at all, especially because the active organisms have been isolated from environments very different from the conditions of target soils. Therefore, they presumably do not show adaptations to the particularities of the agroecosystems, especially the specific conditions of abiotic stress. Thus, the opportunity to generate deep knowledge for further technological-productive innovation in this field is pivotal. Moreover, despite the numerous studies linking productive parameters of agricultural plant species with environmental variables, studies involving the crucial role of the AM symbiosis in the physiological changes in plants under field conditions are extremely scarce but are very important considering the significant effects of these changes a posteriori on various yield

components. Contrasting the results from various biochemical, physiological, and ecological methodologies with the information that we can obtain from the powerful modern molecular tools at the genomic and transcriptomic levels will serve as a basis for robustly defining the best indicators (including specific genes) to be used to select desirable crop genotypes having drought, Al and PTE tolerance, as well as efficiency in P acquisition.

Finally, it must be considered that future research and biotechnological use of AMF require as a starting point the controlled maintenance of trap pots (micro-, mesocosms) to analyze the AMF communities and check the functional compatibility with different agricultural plant species. Undoubtedly such systems will make available fungal material to be used as bio-inoculants for further studies or other uses considering abiotic stress conditions, highlighting among others the following:

1. The production of conditioned plants of endangered plant species
2. The identification of new AMF species into communities established in soils with abiotic limiting conditions
3. The study of AMF ecotypes to promote agricultural production, soil stabilization, and remediation
4. The transference of the research results to other plant production models, such as cereals, pastures, rotations, forestry, phytoremediation, and others

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The Actions of PGPR on Micronutrient Availability in Soil and Plant Under Calcareous Soil Conditions: An Evaluation over Fe Nutrition

4

Muzaffer İpek and Ahmet Eşitken

Abstract

The autotrophic plants need minerals for life cycle. An adequate supply of mineral nutrients is necessary for optimum plant growth. However, when adequate amounts of essential nutrients are present in soil, plants may still show deficiencies due to the non-availability of these mineral nutrients. Availability of plant nutrients such as Fe, Mn, Cu, B, and Zn are generally low in calcareous soils. Fe deficiency-induced chlorosis is the main limiting factor restricting plants growing worldwide. Microorganisms play an important role in enhancing nutrient availability to plant roots. Some PGPR increase the Fe availability in soil by decreasing pH by releasing organic acids or synthesizing low-molecular-weight iron-chelating agents (siderophores). In addition, some PGPR may increase Fe translocation and availability in plants via enhancing organic acid contents and FC-R activity in the root and leaves.

Keywords

PGPR • Micronutrients • Calcareous soils • Iron nutrient • Siderophores

4.1 Introduction

The autotrophic plants need minerals for life cycle. They absolutely require 16 elements: carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), molybdenum (Mo), boron (B), and chlorine (Cl). The plants derive C, H, and O from air and water. The rest of the 13 elements are totally

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obtained from the soil-forming minerals. They are very important for plant growth and metabolism. Each element is specific and/or cannot be replaced by another element (Arnon and Stout 1939).

The mineral nutrients divide into two or more categories according to their chemical nature, form taken up by plants, biochemical functions, and quantitative differences in functional requirements. The difference between macro- and micro-elements is based on the requirement of plants, and they are widely called as macro- and micro-nutrients (Loomis and Shull 1937). If the elements are required at a concentration exceeding 1 mgl^{-1} in solution cultures, they are classified as macronutrients (N, P, K, Ca, Mg, or S). The elements which are required in < 1 mgl^{-1} quantity are classified as micronutrients (Cu, Zn, Mn, B, Mo, Cl). Though, Iron (Fe) which quantitatively behaves as a macronutrient is considered as micronutrient (Arnon 1950).

An adequate supply of mineral nutrients is necessary for optimum plant growth. However, when adequate amounts of essential nutrients are present in soil, plants may still show deficiencies due to the non-availability of these mineral nutrients. Plants depend for their micronutrient needs on their availability in the rooting zone. This is a function of their total content, derived from the soil-forming minerals in the parent material and the several soil chemical and physical properties. The latter determines the dynamics of the equilibrium between the total content of a micronutrient in the soil and its labile pool, from which they are acquired by plant roots. The acquisition of micronutrients from the labile pool is also affected by biological activities in the soil, physical factors of the environment (temperature, pH, light intensity, etc.), and cultural practices. Genetic attributes and stress disposition of plants also influence the acquisition of micronutrients.

Factors influencing the availability of micronutrients to plants have been discussed in general terms in several publications on soils, soil fertility, and plant nutrition (Barber 1995; Black 1993; Brady and Weil 1999; Marschner 2011; Mengel and Kirkby 2001) and in a more specific manner in publications dealing with one or more micronutrients (Abadía 1993; Graham et al. 1988; Gupta 1993, 1997; Katyal and Randhawa 1983; Loneragan 1981; Mortvedt et al. 1991; Rengel 1999; Robson 1993). Soil fertility is a complicated quality which is associated with plant nutrient management. It is the ingredient of soil productivity which is interested on its available nutrient status and ability to provide nutrients out of its own reserves. It combines the biological, chemical, and physical properties of soils, all of which affect directly or indirectly nutrient dynamics and availability. Soil fertility is controllable, and its control is the most important for optimization of crop nutrition on both short-term and long-term bases to achieve sustainable crop production. Approximately 25% of cultivable soils in the world have acute chemical problems. Moreover, these conditions cannot easily be ameliorated because of their extent, the cost of improving the soils, or both (Vose 1983). More than one third of the earth's land surface is affected by calcareous soil. Availability of plant nutrients such as Fe, Mn, Cu, B, and Zn are generally low in these soils (Marschner 2011).

Microorganisms play an important role in enhancing nutrient availability to plant roots. Solubilization of mineral nutrients such as Fe, Mn, Cu, B, and Zn by plant growth-promoting rhizobacteria (PGPR) makes them more readily available for

plant uptake, and this should be considered as a mechanism for enhanced plant growth (Glick 1995).

Nowadays, PGPR is produced commercially and is marketed to plant growers whose numbers increase day by day (Lucy et al. 2004). PGPR inoculation with beneficial effect on plant growth is an attractive and alternative agricultural practice (Antoun and Prévost 2005). Although they colonized on all plant parts, rhizosphere is the main source of PGPR. PGPR have the ability to colonize the roots and promote plant growth either via biological control of plant diseases or through direct action (Kloepper and Schroth 1978). They are related to many plant species and are prevalently present in different environments. Reinhold-Hurek and Hurek (2000) reported that PGPR strains take part in many bacteria genera such as *Azoarcus*, *Azospirillum*, *Azotobacter*, *Arthrobacter*, *Bacillus*, *Clostridium*, *Enterobacter*, *Gluconacetobacter*, *Pseudomonas*, and *Serratia*.

4.2 Micronutrients

All micronutrients form stable complexes with organic ligands. When complexed to proteins, they function as biological catalysts (metalloenzymes). Except for zinc, they all exist in more than one oxidation states, which enable them to participate in redox reactions and in electron transport. Micronutrients are important for plants because they take part in organic structures. They also play important roles as components or activators of enzymes. Moreover, they work as electron carriers or osmoregulators. Micronutrients function in regulation of metabolism, reproduction, and protection against abiotic and biotic stresses.

Sustained growth and yield of crop plants is a function of the interactive influences of the soil, plant, and environmental factors. Any one or more of these factors may adversely affect the availability of micronutrients and reduce it to deficiency concentrations. Some common factors influencing the availability of the micronutrients are listed in Table 4.1.

Comprehensive information about the geographic distribution of micronutrient deficiencies all over the world is provided in the articles by Welch et al. (1991) and White and Zasoski (1999) and in several publications on particular micronutrients referred above.

4.2.1 Manganese

Manganese is a constituent or an activator of enzymes, and it plays a catalytic role in cellular metabolism including CO_2 fixation in C4 and CAM plants and detoxification of oxygen-free radicals. Plants take up manganese as Mn^{2+} via diffusion. Long-distance transport of manganese through xylem takes place either in the ionic form or as chelate (Tiffin 1972). White et al. (1981) reported that manganese is transported from roots to shoots in the form of complexes of citric and malic acids.

Table 4.1 Factors affecting the availability of micronutrients to plants

(a) *Soil factors*	Minerals, total content
	Soil reaction (pH), redox
	Cation exchange capacity
	Organic matter
	Nutrient balance
	Moisture
	Aeration
	Soil microorganisms
(b) *Plant factors*	Nutrient-uptake efficiency
	Root-shoot transport
	Nutrient accumulation, compartmentalization
	Nutrient utilization, transformation into biogenic molecules
	Metabolic activity, rate and stage of growth
(c) *Environmental factors*	Light intensity
	Temperature
	Drought
	Flooding, hypoxia
(d) *Cultural practices*	Cultivation practices
	Fertilizer use

4.2.2 Copper

Copper is a redox-active transition metal. Copper is similar to iron in terms of providing sites for reaction with molecular oxygen. It has great affinity to combine with organic ligands. Many copper proteins function as enzymes and electron carriers catalyzing oxidation-reduction reactions in cellular metabolism. Copper is also involved in the detoxification of superoxide radicals, lignification of plant cell walls, and in pollen fertility. Plants take up copper as Cu^{+}, to which state it is reduced by plasmalemma-bound cupric reductases (Hassett and Kosman 1995; Georgatsou et al. 1997). This possibly involves the same plasma membrane reductase system, which carries out the reduction of Fe^{3+} (Welch et al. 1993). Long-distance transport of Cu^{2+} takes place in the form of copper complex with some amino acids (Loneragan 1981). Both xylem and phloem mobility of copper is low (Hocking 1980) and influenced by the copper status of plants. In the xylem sap of tomato and chicory, about 100% of Cu transportation is in a bound form. When copper concentration increases, some amino acid (nicotianamine, histidine, asparagine, glutamic acid) synthesis is induced. Nicotianamine and histidine has the highest association with Cu. These amino acids can be used in account for carrying Cu bound in xylem sap (Liao et al. 2000).

4.2.3 Zinc

Zinc is an important element for all organisms. It acts a functional and structural role in plant metabolism and structure. Zinc is taken up by plants in the ionic form, as free Zn^{2+} (Blindauer 2015). Zn uptake was inhibited by low temperatures indicating dependence on active metabolism. Phytosiderophores help to mobilize not only iron but also zinc in calcareous soils (Treeby et al. 1989).

4.2.4 Molybdenum

Molybdenum is essential for nitrogen fixation by free living as well as symbiotic bacteria. It is taken up as the molybdate anion (MoO_4^{2-}). Molybdenum is a cofactor for 30 or more bacterial and plant enzymes. There is limited information about molybdenum transportation from roots to shoots.

4.2.5 Boron

Boron plays a structural and functional role in plant cell walls and plasma membrane. Although, it is widely accepted that boron is taken up by a passive mechanism (Power and Woods 1997; Hu and Brown 1997; Abadía 1993; Dordas and Brown 2000), studies showed that boron uptake also involves an active metabolic process (Shu et al. 1991; Hu and Brown 1997). Boron is quite mobile in phloem (Oertli 1993; Oertli and Richardson 1970). Boron is transported long-distance on xylem and its transportation relates to rate of transpiration (Raven 1980).

4.2.6 Chlorine

Chlorine is a structural component of the manganese cluster of photosystems and involved in the regulation of enzyme activities and stomatal functioning (Xu et al. 1999). Chlorine is taken up by plant roots either as free anion (Cl^-) or in association with a monovalent cation. Rainwater and environmental pollutants also contribute substantial amounts of $C1^-$ in plants. $C1^-$ is transported through the xylem from roots to shoots. The transpiration rate and the rate of shoot growth influence the transportation of $C1^-$ from roots to shoots (Greenway 1965; Pitman 1982; Storey 1995; Moya et al. 1999). The nitrogen also influences xylem transportation of $C1^-$.

4.2.7 Iron

Iron is a prominent micronutrient, which acts as a cofactor for about 140 enzymes (Brittenham 1994). This element affects many physiological functions of plants

including participation in electron transfer reactions (Zhang et al. 2009), chlorophyll biosynthesis (Miller et al. 1995), and protection of chloroplasts from the reactive oxygen species (ROS) (Murgia et al. 2004). However, calcareous soil and lime-induced iron deficiency (chlorosis) may cause reduction in fruit yield and quality loss in fruit species (Tagliavini and Rombolà 2001; Molassiotis et al. 2005). Furthermore, iron deficiency leads to the impairment of chlorophyll synthesis (Morales et al. 2000) that decreases photosynthesis.

4.3 Fe Nutrition of Plants in Calcareous Soil Conditions

Iron is generally present in inorganic form in soil, and the solubility of Fe oxides depends on the soil's pH. Many soil-based properties such as lime and high pH reduce Fe availability and/or uptake, and that leads to the development of Fe chlorosis. Fe deficiency-induced chlorosis is the main limiting factor restricting plants growing worldwide. Calcareous soils possess high carbonate contents, high pH, and high HCO_3 concentrations. This lime-induced chlorosis was described precisely for the grape vine at the beginning of the twentieth century by Molz (1907), quoted by Mengel (1994), and was a severe problem even at that time. Chlorosis related to disturbed Fe metabolism on high Ca-containing soil is generally referred to as lime-induced iron chlorosis (Faust 1989). The result of bicarbonate presence in calcareous soil is the decrease in Fe uptake and translocation in plants (Wallace and Abou Zamzam 1986). Under high soil moisture conditions, particularly in association with a poor soil structure, HCO_3^- may accumulate to concentrations as high as 6 to 8 mol/m^3 HCO_3^-. Fe chlorosis is likely to be present under rainy weather conditions (Gärtel 1974), when soil moisture is high and soil aeration is poor. In the root and leaf apoplast, Fe^{3+} reduction may decrease or even be prevented in calcareous soil conditions (Toulon et al. 1992; Kosegarten et al. 1998). Therefore, Fe availability is limited under calcareous conditions (Tagliavini and Rombolà 2001). Fe^{+3} must be reduced to Fe^{+2} to be taken up by plants. Plants mobilize iron from the rhizosphere and transport it across the plasmalemma (Brown and Jolley 1989; Römheld and Marschner 1986; Römheld and Marschner 1981; Marschner et al. 1986). A lot of experimental evidence was reported, and it is now generally accepted that the transport of Fe across the plasma membrane is closely linked to Fe^{3+} reduction (Crowley et al. 1991). It is the Fe^{2+} which is taken up (Fox et al. 1996) and which passes through a specific channel of the plasma membrane (Fox and Guerinot 1998). Iron can be taken up by plants via some other ways also despite calcareous conditions. Iron deficiency induces two distinct and mutually exclusive mechanisms or strategies for iron acquisition (Bienfait 1988; Marschner and Römheld 1994): through reduction (strategy I) which is used by the dicotyledons and monocotyledons except the *Graminaceae* and chelation (strategy II) which is used by the members of the *Graminaceae*. In strategy I plants, an enzyme called ferric-chelate reductase (FC-R, EC 1.6.99.13) can ensure Fe availability by reducing Fe^{3+} into Fe^{2+} (Curie and Briat 2003; Schmidt 2003). Many researchers stated that elevation in FC-R activity hampers Fe deficiency in calcareous soil (Gogorcena et al. 2000; Molassiotis et al.

2005). Beside FC-R activity, organic acids influence Fe uptake and availability. Many researches demonstrated that organic acid excretion makes iron available to plants under Fe-absence conditions (Jones et al. 1996; Abadía et al. 2002). There are also reports that Fe-efficient plants have higher concentrations of organic acids than Fe-inefficient ones (Fournier et al. 1992). Moreover, excretion and accumulation of organic acids such as citrate and malate facilitate Fe transport and Fe availability in the rhizosphere (Tyler and Ström 1995; Jones 1998; Abadía et al. 2002). Strategy II plants lack this mechanism or express it at a very low level (Zaharieva and Römheld 2000). They mobilize iron from the rhizosphere by producing and releasing ferric (Fe^{3+})-solubilizing compounds termed as phytosiderophores (Takagi 1976; Römheld and Marschner 1986). The phytosiderophores, exemplified by mugineic acids, are produced by *graminaceous* species against lack of iron and Zn. They are non-proteinogenic amino acids. Strategy II plants take up iron in the form of Fe^{3+} chelates of mugineic acids. Fe^{3+} siderophores produced in the soil are transported to the roots by diffusion or mass flow and enter the root-free space through which they move to the plasmalemma-bound Fe^{3+} reductase.

4.4 Possible Action of PGPR on Fe Nutrition Under Calcareous Soil Conditions

Some PGPR increase the Fe availability by decreasing pH by releasing organic acids or synthesizing low-molecular-weight iron-chelating agents (producing siderophores).

4.4.1 Production of Phytosiderophore

Siderophores (Greek *sideros* meaning iron and phores meaning bearer) are the metal-chelating agents that primarily function to capture the insoluble ferric iron from different habitats (Nagoba and Vedpathak 2011). Siderophores are low-molecular-weight compounds that complex with Fe^{+3} (Leong 1986). Existing literature showed that both gram-negative and gram-positive bacteria synthesized siderophore under iron-deprived condition (Tian et al. 2009; Saharan 2011). Generally, most of the aerobic and facultative anaerobic bacteria were found to produce siderophore in lack of Fe (Neilands 1995).

Siderophores may be classified in three major groups which include the hydroxamates, catecholates (phenolates), and carboxylates. These groups are created by the ligands used to chelate the ferric iron (Hider and Kong 2010).

Iron plays a limiting role on plant growth because of the insoluble iron form (Fe^{3+}). This scenario is seen in soil having rich iron. The availability of iron in soil solutions is 10^{-18} M, a concentration which even cannot sustain the microbial growth. Several soil microorganisms produce siderophores and low-molecular-weight iron-chelating compounds that bind Fe^{3+} with very high affinity and help in iron uptake. It is possible for the rhizosphere microorganisms to use siderophores

provided they contain the appropriate uptake protein (Raaijmakers et al. 1995). It was reported in many studies that PGPR may increase the mobility and availability of micronutrients by the formation of high-affinity siderophores. *Pseudomonas fluorescent* produce a siderophore having yellow-green fluorescent pigment, and Kloepper et al. (1980) named it as "pseudobactin." *Pseudomonas* spp. which are among the gram-positive PGPR are the effective siderophore producers. They produce pseudobactin, pyochelin, pyoverdine, quinolobactin, and salicylic acid, and the structure of the outer membrane receptor proteins complementary to some of these siderophores has been determined (Cobessi et al. 2005). The role of pseudobactin in promoting the growth of potato was demonstrated when 10 mg of pseudobactin increased growth to the same size as when the fluorescent pseudomonad was treated to a potato. The widespread production of siderophores by microbes at low iron levels is reviewed by Neilands (1986). Organisms as diverse as *Bacillus*, *Rhizobium*, *Pseudomonas*, *Agrobacterium*, *Escherichia coli*, and many fungi produce a wide range of iron-chelating compounds (Esitken 2011).

4.4.2 Release of Organic Acids

Organic acids, such as citrate and malate, are involved in many metabolisms including plant mineral uptake and mitigation of stress in roots (Jones 1998). Many of them are complex of Fe in soil and dissolver of unavailable iron (Gerke 1992). Jones et al. (1996) found that citrate takes a prominent role in supplying Fe to dicotyledonous plants. Transportation form of Fe in xylem is mainly Fe^{3+} citrate. In that case, a wealth of citrate in xylem increases Fe transport from roots to above parts of a plant. One of the most known effects of PGPR is secreting organic acids and decreasing soil pH. In calcareous and high-pH soils, especially, decrease in pH has important roles in conversion of insoluble Fe to soluble form for plants. Furthermore, many researchers suggested that bacterial treatments led a decrease in soil pH and an increase in nutrition availability in soil (Sharma et al. 2003; Orhan et al. 2006; Karlidag et al. 2007; Zhang et al. 2009). Studies by Esitken et al. (2016) in peaches, Ipek et al. (2016) in pears, and Aras et al. (2016) in apples determined that there was a considerable increase in Fe nutrition, FC-R activity in roots and leaves, and leaf organic acid contents such as citrate and malate as a result of rhizobial root inoculation.

In strategy I plants, high lime in soil affects Fe nutrition detrimentally in various aspects. At first, availability of Fe in soil is decreased under lime and high-pH conditions. Fe is trapped in bicarbonate soils and it turns into a non-uptake form. Fe acquisition is deteriorated due to high pH as a result of high bicarbonate (Nikolic and Römheld 1999). Afterward, Fe entered into the root apoplast must be carried into the xylem. However, some parts of Fe^{3+} remains in the root apoplast under lime-contained soil conditions and cannot be carried into the plant shoot as a result of high pH in the root apoplast (Kosegarten and Koyro 2001; Molassiotis et al. 2005). Even if this transportation occurred to the Casparian strip via apoplastic way, Fe must pass into the cell inside and pass the Casparian strip via symplastic way. In this

step, entering of Fe present in the apoplast to the cell inside could not be sufficient due to lime and high pH in soil. Thus, part of the uptaken Fe remains in the root apoplast and could not be carried into the shoots. Mengel (1994) reported that root Fe concentration of chlorotic plants and chlorosis could be related to the mobilization of root Fe and its translocation to upper plant parts. Furthermore, reduction of Fe^{3+} to Fe^{2+} occurs in order to enter the cell by crossing the plasma membrane (PM). In that case, the activity of PM-bound FC-R is a requisite (Schmidt 2003; Vert et al. 2003). Fe uptake into the cell inside is maintained by PM-bound FC-R enzyme that is dependent on pH. Moreover, FC-R activity increases when apoplastic pH is at 6.5 and declines while pH elevates (Mengel 1994). It has been supposed that some parts of Fe taken up from soil is present in the root apoplast (Bienfait et al. 1983; Longnecker and Welch 1990). In a previous experiment, it was found that chlorosis and the root Fe content of chlorotic plants could be related to removing of root Fe into plant shoots (Mengel 1994). Iron (Fe^{3+} citrate)-loaded xylem must be distributed into the leaf from veins after removing the leaf. There must be re-reduction of Fe^{3+} citrate into Fe^{2+} for distribution in leaves (Brüggemann et al. 1993; Mengel 1994; Toselli et al. 2000; Bohórquez et al. 2001). The chlorosis is not solely caused by absolute Fe deficiency. In contrast to most other plant nutrients, Fe concentration in the chlorotic leaves can be higher than in the green leaves (Carter 1980; Rashid et al. 1990; Bertoni et al. 1992). But, in the plant leaves with Fe chlorosis, active Fe content is lower than non-chlorosis plants although the total Fe content is same in both plant leaves (Mengel 1994; Toselli et al. 2000). In this step, malignant effect of soil lime and high pH appears. Iron present in the leaf apoplast must enter the cell inside in order to maintain distribution of Fe in the leaf vein to the leaf. In that case, Fe could not enter the cell inside due to high pH in the leaf apoplast, and Fe remains in the leaf veins. Reduction of Fe^{3+} to Fe^{2+} in the leaf apoplast occurs with FC-R activity. Therefore, the leaf FC-R enzyme possesses a remarkable importance for elevating Fe availability in the leaves. Leaf apoplast FC-R activity also depends on apoplastic pH (Mengel 1994; Marschner 2011; Kosegarten et al. 1999). In lime-contained soils, HCO_3^- causes an increase in leaf apoplastic pH that leads to decrease in FC-R activity, and in that case the Fe removed from the leaf remains in the veins. Thus, under calcareous soil conditions chlorosis occurs in the interveinal space because of no distribution of Fe from the veins to leaf.

In these steps, it is possible to see some mitigations via Fe nutrition by PGPR (Fig. 4.1). Rhizobacterial inoculation to root increases Fe availability in the way of decreasing soil pH via released organic acids (Fig. 4.1a). The rhizosphere's microbial community takes an important role in Fe acquisition (Glick 1995; Sharma et al. 2003). That could be related with the elevation of available Fe content in the rhizosphere and becoming appropriate soil pH in Fe acquisition. Iron located in soil complexes with many organic acids such as citrate and malate increases availability of insoluble ferric oxyhydroxides (Gerke 1992; Jones et al. 1996). Thus, the increase of active iron (Fe^{2+}) in soil may have led to Fe uptake by plant from soil. As a matter of fact, rhizobacteria remarkably increase active Fe in soil by releasing organic acids in peaches (Esitken et al. 2016), pears (Ipek et al. 2016), and apples (Aras et al. 2016). Besides that, Fe mobilization in the rhizosphere may be in consequence

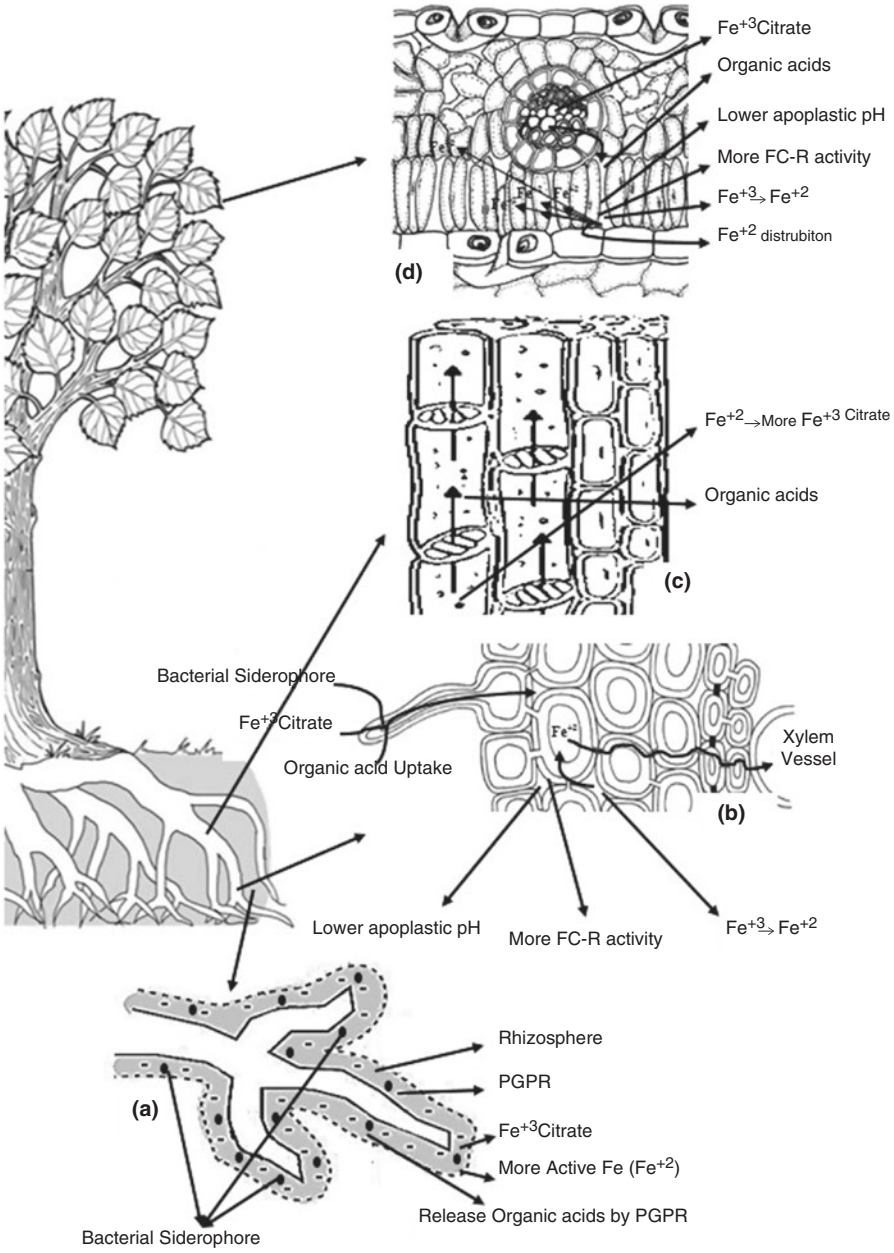

Fig. 4.1 Effects of organic acid-releasing and siderophore-producing rhizobacteria on Fe nutrition and FC-R activity under calcareous soil conditions. (**a**) Organic acid releasing by rhizobacteria decrease soil pH and increase active Fe (Fe+2) and Fe+3 citrate, and siderephore producing by rhizobacteria increase Fe uptake from soil. (**b**) Organic acids take up by plant from rhizosphere decrease root apoplastic pH and increase root FC-R activity. (**c**) More bacterial citrate in xylem sap enhance formation and translocation of Fe+3 citrate to leaves. (**d**) Bacterial organic acids in the leaves decrease leaf apoplastic pH, increase leaf FC-R activity, and enhance Fe+2 distribution in the leaves

of the combination of the acidification power of the H^+-ATPase together with the complexing characteristic of citrate under calcareous soil conditions. Fe^{3+} citrate complex in the soil is also carried into the roots by the way of diffusion or mass flow and steps into the root-free space (Mengel and Kirkby 2001). PGPR decrease rhizospheric pH by releasing organic acids in the rhizosphere zone. This case may also decrease root apoplastic pH. The root FC-R activity is so prominent in this step. However, exposure to ammonium-containing solution or to the water with pH 3.5 may lead to decreased root apoplastic pH (Mengel and Geurtzen 1988). PM-bound FC-R enzyme provides Fe entering the cell inside in the way of reducing Fe^{3+} to Fe^{2+}. FC-R activity depends on apoplastic pH and works in pH 6 as the highest level. Organic acids released by bacteria may have entered the root apoplast and lead to decrease in apoplastic pH. In that case, bacteria may have increased FC-R activity by the way of decreasing root apoplastic pH (Fig. 4.1b). Thus, Fe^{2+} entered to root apoplast may have been loaded to xylem with symplastic way and afterward elevated Fe transportation to shoots in the way of promoting Fe loading to xylem. The Fe^{2+} transported to xylem is re-oxidized to Fe^{3+} and then complexed by citrate as Fe^{3+} citrate. The transportation form of iron in xylem is mainly Fe^{3+} citrate. In this regard, citrate in xylem promotes Fe transport from the roots to shoots. Therefore, transport of citrate released by PGPR from soil to root and xylem is useful for Fe translocation (Fig. 4.1c). Fe^{3+} citrate transported to leaves with xylem must be re-reduced to Fe^{2+} in the veins in order to be distributed in the leaves (Mengel 1994; Nikolic and Römheld 1999; Toselli et al. 2000; Bohórquez et al. 2001). Fe^{3+} present in the leaf apoplast must be reduced to Fe^{2+} by PM-bound FC-R enzyme in order to enter the cell inside (González-Vallejo et al. 2000). In lime-induced Fe chlorosis, chlorosis occurs in interveinal areas due to nondistribution of Fe from the veins to leaf. Thus, the treatments decreased leaf apoplastic pH which provides distribution of Fe to leaves and re-greening. In fact, sprayed diluted acid (Plänker 1991) or citric acid (Tagliavini et al. 1995) to leaves or ammonium fertilizer application to soil (Mengel and Geurtzen 1988) decreases leaf apoplastic pH; thus iron in the veins can be distributed in the leaves. Rhizobacteria located in the rhizosphere zone may have led to lower leaf apoplastic pH by the way of carrying of released organic acids in the rhizosphere to the leaf (Fig. 4.1d). In this regard, intake and transportation of organic acids released by bacteria in the rhizosphere may be effective in decreasing leaf apoplastic pH. PGPR increased the organic acid content in leaves that may lead to a decrease in leaf apoplastic pH and followed by an increased leaf FC-R activity. Just as it was established that there was a significant increase in leaf FC-R activity by rhizobacteria treatments and led to an increase in active Fe content of peach (Esitken et al. 2016), pear (Ipek et al. 2016), and apple (Aras et al. 2016) leaves. This rhizobacterial effect may be related with the leading changes in leaf apoplastic pH. Therefore, PGPR may have decreased the apoplastic pH on both root and leaf by synthesizing of organic acids in the rhizosphere and increasing root and leaf FC-R activity.

4.5 Effects of PGPR on Microelement Nutrition

Microorganisms are the main agents in natural nutrient element cycle. Mineral nutrient solubility may be increased due to PGPR which release organic and sugar acids to the rhizosphere and create acidic condition by CO_2 (respiration). In general, acid-producing bacteria readily accumulate in the rhizosphere because of favorable habitat for its (Mohite 2013). There are numerous studies on a lot of PGPR strains and species. These studies include their ability to take up some minerals especially Fe, Zn, Cu, Mn, and B. PGPR take up these minerals via the production of organic acids such as citric, glutamic, succinic, lactic, oxalic, malic, fumaric, and tartaric acid (Orhan et al. 2006; Sabir et al. 2012; Ipek et al. 2014; Shaheen et al. 2014; Pratiwi et al. 2016). Numerous rhizobacteria were determined as producer of siderophore. PGPR may also increase the mobility and availability of micronutrients by producing siderophores (Ghavami et al. 2016; Radzki et al. 2013; Sadeghi et al. 2012; Sharma et al. 2015).

In plants applied with PGPR strains, the nutrient element amount of plant may provide important information about the effect of bacterial inoculation in nutrient element uptake. Rhizobacteria treatments affect not only availability of Fe but also other micronutrients. Generally, the enhancements in micronutrient contents such as Fe, Zn, Mn, Cu, and B were more pronounced in organic acid-releasing and phytosiderophore-producing bacterial inoculations, just as many researchers have announced beneficial effects of PGPR inoculations on many different plant species (Tables 4.2 and 4.3).

4.6 Conclusions and Future Prospects

Micronutrient unavailability is a widespread and damaging disorder in several important agricultural crops under high calcareous content soils. Most of agricultural crops such as fruits, vegetables, grapes, and other dicot species are generally considerable sensitive to high calcareous content of soil. There are some ways to cope with high calcareous content of soils such as using tolerant rootstocks in fruit crops and grapes and chelated fertilizers. Using tolerant rootstocks is not sufficient, and synthetic chelates are able to overcome micronutrient deficiency, but these fertilizer costs are high and pose environmental concerns. In this regard, sustainable agricultural techniques and biofertilization could be a solution, just as PGPR can appear to decrease in sensitivity to these conditions. PGPR can affect micronutrient availability like Fe by releasing organic acids and producing siderophore. It is shown in current studies that PGPR root inoculation significantly affected plants not only by decreasing pH and increasing Fe availability in soil but also increasing organic acid content of leaves and raising root and leaf FC-R activity.

For future studies, performance of PGPR under calcareous soil conditions in particular Zn, Mn, Cu, and B availability and translocation should be investigated. In addition, the mechanism of actions of PGPR in micronutrient availability should be studied in the future.

Table 4.2 Response of different agricultural crops to organic acid-releasing rhizobacteria for micronutrients availability

Species	Bacteria strain	Results	References
Raspberry	M3, OSU-142	Fe, Zn, Mn	Orhan et al. (2006)
Barley	M-13, OSU-142, RC01, RC02, RC04, RC05, RC06	Fe, Zn, Cu, Mn	Cakmakci et al. (2007)
Strawberry	FS-3, FS-9	Fe, Zn, Cu, Mn	Güneş et al. (2009)
Apple	A-18, OSU-142	Fe, Zn	Karakurt and Aslantas (2010)
Hazelnuts	2/3, 5/8, 13/4, 21/1, 29/6, 42/1, 47/6, 55/1, 59/8	Fe, Zn, Mn, B, Al	Erturk et al. (2011)
Grape rootstocks	BA-7, OSU-142, Sp 245	Fe, Zn, Mn	Sabir et al. (2012)
Hungarian vetch	PPB310, BCB51, PAB58, PFC82, PF84, PP315, PAA362, BA361, BMA424, BMA479, AM235, BS521	Fe, Zn, Cu, Mn, B	Yolcu et al. (2012)
Strawberry	EY 2, EY 6, EY 30, EY 37, EY 43	Fe, Zn, Cu, Mn	Karlidag et al. (2013)
Cauliflower	TV-3D, TV-91C, RK- 92, TV-17C, TV-87A, KBA-10	Fe, Zn, Cu, Mn	Ekinci et al. (2014)
Strawberry	637Ca, FF1, MFDCa1, MFDCa2, M3, A18	Fe, Cu, Mn, B	Ipek et al. (2014)
Tomato	N 52/1, N17/3, F 21/3, Fe 43, 637Ca, MFDCa1, 52/1 Zeatin	Fe, Zn, Cu, Mn, B	Seymen et al. (2014)
Olive	*Bacillus polymyxa*	Fe, Zn, Mn	Shaheen et al. (2014)
Cabbage	TV-91C, RK- 92, TV-17C,	Fe, Mn	Turan et al. (2014)
Pepper	N 52/1, N17/3, Fe 43, F 21/3, 637Ca	Fe, Zn, Cu, Mn	Seymen et al. (2015)
Cabbage	RC 14	Fe, Zn, Cu, Mn	Yildirim et al. (2015)
Strawberry	RC05, RC06, RC35, RC77, RC86, 29/2	Fe, Zn, Cu, Mn	Erdogan et al. (2016)
Sour cherry	OSU-142, T8	Fe, Zn, Cu, Mn	Arikan and Pirlak (2016)
Groundnut	*Pseudomonas fluorescens*	Fe	Pratiwi et al. (2016)

Table 4.3 Response of different agricultural crops to siderophore-producing rhizobacteria for micronutrients availability

Species	Bacteria strain	Microelements	References
Pigeon pea	*Pseudomonas pseudoalcaligenes*	Fe, Mn, Zn, Cu, Co, Ni, Al	Gamit and tank (2011)
Wheat	*Streptomyces* (isolate C)	Fe, Mn	Sadeghi et al. (2012)
Tomato	*Chryseobacterium* spp. C138	Fe, Zn	Radzki et al. (2013)
	Pseudomonas fluorescens N21.4		
Maize	*Arthrobacter globiformis*	Fe	Sharma et al. (2015)
Maize and canola	*Stenotrophomonas pavanii*	Zn	Ghavami et al. (2016)
	Isolate B2 (*Enterobacteriaceae*)		
	Stenotrophomonas chelatiphaga		
	Micrococcus yunnanensis		

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Microbe-Mediated Induced Abiotic Stress Tolerance Responses in Plants

5

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Abstract

Abiotic stresses, including salinity, drought, high temperature, chilling injury, and heavy metal toxicity, have become major limiting factors for the global agricultural production. Moreover, these environmental conditions have increased over time due to change in global climate pattern and human interference. Using a diverse array of microorganisms harbored by plants to improve plant growth and host stress tolerance may benefit in sustaining the increases in food production in many regions of the world. Microbes help in rendering plants tolerant to these unfavorable environmental stresses. This cross-stress protection provided by microbial inoculants plays an important role in maintaining ecological balance and holds promise for generating more tolerant crops. Microorganisms not only provide "non-nutritional" effects in stabilizing soil aggregates, prevent erosion, detoxify pesticides, and suppress plant diseases and soilborne pathogens, but they can also fix atmospheric nitrogen, solubilize mineral phosphate, decompose organic wastes and residues, improve nutrient cycling, produce bioactive compounds, produce phytohormone and siderophore, as well as enhance osmolyte production, plant–water relation, photosynthetic capacity, protein assimilation, plant hormonal status, ionic balance, antioxidant production, and other physiological parameters inside the plant. In addition, using compatible multiple microbial consortia consisting of bacterial symbionts and fungal symbionts acting synergistically, providing various beneficial effects, is also a potential technical tool. Furthermore, intensive selection of stress-tolerant bioinoculants could improve plant abiotic stress tolerance and thus enhance crop productivity under

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stressful conditions. This chapter documents the potential of microorganisms and highlights insights into the mechanisms underlying improved stress tolerance in plants by microbial colonization.

Keywords
Abiotic stress • Arbuscular mycorrhizal fungi • Microbes • Plant growth-promoting bacteria • Plant microbe interaction • Plant stress tolerance

5.1 Introduction

Plants face several unfavorable abiotic stresses including salinity, drought, heat, chilling injury, and heavy metal toxicity that severely impact plant growth and development and finally decrease their overall yield to about 70% (Saxena et al. 2013). Practical strategies were developed worldwide for mitigating stress toxic effects through the development of tolerant varieties, shifting crop calendars, using chemicals that may be toxic for the environment, resource management practices, etc. (Grover et al. 2011). While most of these practices are time-consuming and costly, microbial inoculation as a safe, low-cost, effective, environment-friendly approach can increase plant stress tolerance and can also maintain ecosystem health (Naveed et al. 2014; Talaat and Shawky 2014a, 2015; Meena et al. 2015; Talaat et al. 2015a). Indeed, microbe's ability to confer plant stress resistance may open a new avenue for alleviating the adverse effect of global climate change on agricultural production (Grover et al. 2011).

Plant roots are colonized by soilborne bacteria and fungi that may have beneficial effects on the agriculture crops by inducing plant adaptation to abiotic stresses (Grover et al. 2011). Arbuscular mycorrhizal fungi (AMF) are microscopic filamentous fungi that colonize cortical tissues and extend hyphae into the rhizosphere (Shokri and Maadi 2009). It has a positive effect on the poorly mobile nutrients such as phosphorus and other nutrients such as N, K, Ca, Mg, Fe, Zn, and Cu of plants subjected to unfavorable environmental stresses (de Andrade et al. 2008; Bagheri et al. 2012; Talaat and Shawky 2011, 2013). AM symbiosis helps plants to cope up with abiotic stresses by defending roots against soilborne pathogens, improving rhizosphere and soil conditions, modifying microbial communities, maintaining membrane integrity, stimulating plant growth regulator production, enhancing/selective nutrient uptake and preventing nutritional disorder, inducing osmoregulator accumulation, controlling reactive oxygen species (ROS) accumulation by enhancing antioxidant enzyme activity and antioxidant molecule content, improving photosynthesis process, enhancing protein synthesis, and changing transcript levels of genes involved in signaling pathway or stress response, as well as structural adaptations (Zhang et al. 2010b; Maya and Matsubara 2013; Talaat and Shawky 2014a, b, 2015; Shabani et al. 2016).

Nitrogen-fixing bacteria which are also called diazotrophic bacteria can fix atmospheric nitrogen for the plant. They can act as free-living bacteria or form a

symbiosis with legumes and establish root nodules where biological nitrogen fixation occurs (Gomez-Sagasti and Marino 2015). Rhizobia, a group of associative diazotrophic bacteria, are often used as co-inoculants with other microbes, either bacteria or fungi, to enhance plant growth and productivity under different stressful conditions (Figueiredo et al. 2008; Ahmad et al. 2013; Gomez-Sagasti and Marino 2015).

Other types of beneficial soilborne microbes that are defined as plant growth-promoting bacteria (PGPBs) can colonize the rhizosphere/endorhizosphere of plants, stimulate plant growth, and confer enhanced resistance to biotic and abiotic stresses. Rhizospheric bacteria capable of promoting plant growth under different conditions are known as plant growth-promoting rhizobacteria (PGPRs). They colonize the rhizosphere of many plant species and impart benefit to the plants indirectly by reducing plant pathogens or directly by releasing phytohormones (auxins, cytokinins, gibberellins, and abscisic acid); producing essential enzymes, 1-aminocyclopropane -1-carboxylate (ACC) deaminase, to reduce ethylene level in the root of developing plants; fixing nitrogen; solubilizing and mineralizing nutrients, particularly mineral phosphate; mobilizing nutrients in the rhizosphere; producing siderophores to facilitate root uptake of metal nutrients; emitting volatile organic compounds (VOCs); producing antioxidants; producing exopolysaccharides (EPS); and biofilm formation (Grover et al. 2011). The term induced systemic tolerance (IST) is referred as PGPR-induced physical and chemical changes in plants to respond to changing environmental conditions and mitigate the impacts of stress (Belimov and Wenzel 2009; Cohen et al. 2015; Singh and Jha 2016). Bacteria belonging to different genera including *Achromobacter*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Microbacterium*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Serratia*, *Streptomyces*, etc. can improve the host plant growth under abiotic stress conditions (Grover et al. 2011; Nadeem et al. 2016).

Today, sustainable agricultural practices should be productive, profitable, energy-conserving, eco-friendly, conserving of natural resources, and ensuring food safety and quality. Effective microorganism (EM) application can minimize the investment of money and labor, minimize the environmental impact, and support food safety and food security. It consists of naturally occurring beneficial microorganisms: photosynthetic bacteria (*Rhodopseudomonas* spp.), lactic acid bacteria (*Lactobacillus* spp.), yeast (*Saccharomyces* spp.), actinomycetes, and fermenting fungi (*Aspergillus* and *Penicillium*) (Higa 2004). It can enhance crop production and protection by promoting seed germination; enhancing plant growth, root development, flowering, fruiting, and ripening; increasing the efficacy of organic matter as fertilizers; increasing nutrient availability in the rhizosphere of plants; developing resistance of plants to pests and diseases; suppressing soilborne pathogens and pests; and increasing the production of antioxidants that suppress the negative impact of free radicals in plant metabolism (Higa 2004; Talaat 2015a). It can also decrease the damage to plants caused by soil salinization by improving various physiological and biochemical processes inside the plant cell (Talaat 2014, 2015b; Talaat et al. 2015a).

Interestingly, the use of multi-strain microbial inocula is a potential biotechnological approach to ameliorate the deleterious effects of the stressful conditions on

plant and enhance its fitness. Co-inoculation with *Paenibacillus polymyxa* and *Rhizobium tropici* ameliorated the harmful impact of water deficit on *Phaseolus vulgaris* L. (Figueiredo et al. 2008). The combined interaction of *Pseudomonas* and *Rhizobium* protected mung bean from saline soil (Ahmad et al. 2013). Co-inoculation of PGPBs and *Rhizobia* with legume plants also supported the growth of plant during metal phytostabilization and phytoextraction strategies (Gomez-Sagasti and Marino 2015). Furthermore, co-inoculation of PGPR *Dietzia natronolimnaea* strain STR1 and AMF *Glomus intraradices* alleviated the negative impact of salinity on the growth of *Ocimum basilicum* (Bharti et al. 2016a).

The present chapter appraises the crucial role of useful soil microbes in plant tolerance to major abiotic factors, such as salinity, drought, heat, chilling injury, and heavy metal toxicity. Special emphasis is given to the physiological impacts and how the compatible multiple microbial consortia mitigate the abiotic stress symptoms in the plants. Moreover, it explores the beneficial effects of using stress-tolerant bioinoculants to develop plant stress tolerance. Finally, major aspects for future work in the current direction have also been highlighted.

5.2 Salt Stress

Soil salinization is a devastating ecological and agronomical problem that limits agricultural production and land development in many areas on the earth. Approximately 7% of the global land surface is salt-affected. More than 800 million hectares of global land are affected by salinity (Munns and Tester 2008). Plant cells can sense salt stress through membrane disorganization; enzyme damage; metabolic toxicity; inhibition of photosynthesis, respiration, and protein synthesis; disturbance in nutrient accumulation; toxicity of excessive Na^+ and Cl^-; disturbance in water and osmotic potential; reduction in assimilate translocation to sinks; and increasing ROS production in chloroplasts (Talaat and Shawky 2013, 2014a, b; Pedranzani et al. 2016). Indeed, increasing salinization of soils, increasing costs of fertilizers, and increasing need for food to feed the global population are the main factors that stressed the need for full exploitation of soil microbes. Exploitation of soil microbes for utilizing salt-stressed land is an effective tool and may provide a quick-fix solution to this problem. The benefit to mankind and the economic potential make it a worthwhile task.

5.2.1 Plant Growth-Promoting Bacteria in Salt Stress Mitigation

PGPRs can act as elicitors of tolerance to salt stress. They facilitate growth in the saline environment via inducing different physical and biochemical changes in stressed plants, to confer IST. Certain PGPR strains confer salt tolerance by protecting the plants from the negative impact of high Na^+ concentrations in the soil. For example, soil bacterium *Bacillus subtilis* strain GB03 conferred salt tolerance in *A. thaliana* by tissue-specific regulation of *HKT1* (high-affinity K^+ transporter 1).

GB03 under saline condition concurrently downregulates *HKT1* expression in roots and upregulates its expression in shoots, which lowered the accumulation of Na^+ throughout the plant compared with controls (Zhang et al. 2008). Similarly, soil bacterium inoculation with GB03 promoted the growth of white clover under salt stress by decreasing shoot and root Na^+ accumulation, thereby improving K^+/Na^+ ratio. GB03 also regulated chlorophyll content, leaf osmotic potential, cell membrane integrity, and ion accumulation in salt-affected plants (Han et al. 2014). Furthermore, inoculation with *Bacillus* sp. strain L81 and *Arthrobacter oxidans* strain BB1 significantly reduced *A. thaliana* Col 0 mortality under salt stress, which might be due to the induction in the expression of *PR1* that is a gene associated to the SA-dependent pathway (Barriuso et al. 2008). Co-inoculation of *B. subtilis* and *Arthrobacter* sp. alleviated the deleterious effects of salt stress on wheat growth by increasing plant dry biomass and by inducing the accumulation of sugars and proline (Upadhyay et al. 2011). Recently, the multi-strain consortium of three bacterial species *P. fluorescens*, *B. megaterium*, and *V. paradoxus* enhanced the leaf chlorophyll content in cucumber plants subjected to saline conditions (Nadeem et al. 2016). Hence, the multi-strain bacterial inoculum can play a crucial role in protecting the plant from saline soil.

PGPR improves salt stress tolerance in different plant species by producing ACC deaminase, phytohormones, siderophores, exopolysaccharides (EPS), antioxidants, and volatile organic compounds (VOCs) (Grover et al. 2011). PGPB with ACC deaminase activity colonizes the rhizosphere and keeps ethylene levels low that is beneficial for root growth and plant survival under saline conditions. High K^+/Na^+ ratio, relative water content, chlorophyll level, and low proline content were detected in salt-stressed maize inoculated with *Pseudomonas syringae*, *Enterobacter aerogenes*, and *P. fluorescens* containing ACC deaminase activity (Nadeem et al. 2007). Similarly, improved root length, shoot height, as well as fresh and dry weight was observed in salt-stressed wheat plants inoculated with ACC deaminase-producing *Klebsiella* spp. SBP-8 (Singh et al. 2015). Inoculation with *Enterobacter* sp. UPMR18 containing ACC deaminase activity induced antioxidant enzyme activities (SOD, APX, and CAT) and upregulated ROS pathway genes (CAT, APX, GR, and DHAR) of salt-affected okra plants (Habib et al. 2016). PGPB also promotes plant growth and development under soil salinization by enhancing the synthesis of plant hormones. Production of indoleacetic acid, gibberellins, and some unknown determinants by PGPR improved wheat salt tolerance as a result of increasing root length, root surface area, and number of root tips, which increased nutrient uptake (Egamberdieva and Kucharova 2009). Salt-stressed *Medicago* plants infected with IAA-overproducing PGPR *Sinorhizobium meliloti* strain showed high antioxidant enzyme activity (Bianco and Defez 2009). Inoculation with *Streptomyces* producing the PGP activity of an auxin and siderophore significantly improved the germination rate, shoot length, dry weight, and N, P, Fe, and Mn concentrations in salt-stressed wheat plants (Sadeghi et al. 2012). Furthermore, exopolysaccharide-producing bacteria inoculation could restrict Na^+ influx into roots. EPS production by PGP strains helps in binding cations, including Na^+, and thus decreases the content of Na^+ available for uptake by plants, which is especially beneficial for alleviating salt

stress in plants. Wheat seedling inoculation with bacteria that produce EPS restricted sodium uptake and stimulated plant growth under salt stress (Grover et al. 2011). PGPR can also enhance salt tolerance via enhancing ROS-scavenging system. Inoculation of paddy with two root-associated bacteria *Pseudomonas pseudoalcaligenes* and *Bacillus pumilus* enhanced salt tolerance via regulating lipid peroxidation, SOD activity, caspase-like protease activity, and programmed cell death (Jha and Subramanian 2014). In addition, plant perception of bacterial VOC causes a tissue-specific regulation of HKT1 that controls Na^+ homeostasis under saline conditions. VOCs from PGPB downregulated high-affinity K^+ transporter 1 (HKT1) expression in roots, but upregulated it in shoot tissues and thus reduced the Na^+ levels in the whole plant (Yang et al. 2009). Soybean plants exposed to volatile emissions from *Pseudomonas simiae* strain AU not only decreased root Na^+ levels but also enhanced the proline accumulation, which protect the plant cells from osmotic stress. Consistent with induced systemic tolerance under salt stress, inoculation with AU VOCs resulted in increase in the vegetative storage protein and several other proteins that can help the stressed plants to sustain their growth and development (Vaishnav et al. 2015). Recently, *Herbaspirillum* sp. strain GW103, which is capable of producing plant beneficial factors, such as auxin, siderophore, and ACC deaminase, alleviated salinity stress in Chinese cabbage by increasing the root K^+/Na^+ ratio that generated balance in the ion homeostasis and thus contributed to biomass increase (Lee et al. 2016). Inoculation with *Enterobacter* sp. SBP-6 containing ACC deaminase activity and showing other PGP traits like phosphate solubilization, phytohormone production, and nitrogen fixation to wheat plants ameliorated the negative impact of saline conditions. It significantly improved plant biomass, chlorophyll content, K^+ uptake, and K^+/Na^+ ratio, while it diminished Na^+, proline, and malondialdehyde contents (Singh and Jha 2016).

Co-inoculation of PGPR with symbiotic bacteria under saline conditions is a potential biotechnological approach that improves both the plant productivity and the soil health. Co-inoculation of *Serratia proteamaculans* ATCC 35475 and *Rhizobium leguminosarum* bv. *viciae* 128C56G alleviated the inhibitory effect of salinity on plant growth, antioxidant enzyme activity, photosynthesis process, and mineral content of *Lactuca sativa* (Han and Lee 2005). Inoculation by *Pseudomonas* sp. 54RB and *Rhizobium* sp. Thal-8 decreased the electrolyte leakage, while it increased the proline production, the relative water content, and the K^+ ion uptake of maize plants subjected to salinity stress (Bano and Fatima 2009). Likewise, co-inoculation of *Pseudomonas* and *Rhizobium* enhanced growth, nodulation, and ionic acquisition of mung bean grown under saline conditions (Ahmad et al. 2013).

Halotolerant bacteria isolated from saline environments have potential to improve plant establishment under saline conditions through direct or indirect mechanisms and would be most appropriate as bioinoculants under such conditions. Inoculation with 14 halotolerant bacterial strains ameliorated salt stress in canola plants through the reduction of ethylene production *via* ACC deaminase activity (Siddikee et al. 2010). Inoculation with the salt-tolerant rhizosphere bacteria (*Bacillus pumilus*, *Pseudomonas mendocina*, *Arthrobacter* sp., *Halomonas* sp., and *Nitrinicola lacisaponensis*) enhanced chlorophyll, carotenoids, and protein contents, as well as

the accumulation of individual phenolics (gallic, caffeic, syringic, vanillic, ferulic, and cinnamic acids), flavonoid quercetin, and IAA, which induced salt stress tolerance in wheat (Tiwari et al. 2011). Wheat plants inoculated with saline-adapted *Azospirillum* strains had higher shoot dry weight, grain yield, and N concentrations than the uninoculated ones under saline conditions (Nia et al. 2012). Inoculation by halotolerant bacteria *Halobacillus* sp. and *B. halodenitrificans* ameliorated salinity stress in wheat by improving root elongation and root dry weight (Ramadoss et al. 2013). *Bacillus* sp. and *Arthrobacter pascens* sp. isolated from rhizospheric soil of halophyte regions promoted the growth of salt-stressed maize plants by enhancing sugar and proline accumulation as well as increasing SOD, POX, CAT, and APX activities (Ullah and Bano 2015). Another PGPB, *Pseudomonas koreensis* strain AK-1, mitigated salt stress and promoted soybean growth by reducing Na^+ level while increasing K^+ level, stress enzyme activity, and proline content in leaves and roots (Kasotia et al. 2015). Inoculation with ACC deaminase-producing PGPB *Enterobacter cloacae* strain KBPD isolated from salt-affected soil alleviated salt toxicity in *Vigna radiata* L. by increasing shoot length, root length, fresh and dry weights, as well as total chlorophyll content. Salt-affected plants had higher proline content, while inoculation with *E. cloacae* KBPD reduced its content (Bhise et al. 2016). Carotenoid producing halotolerant PGPR *Dietzia natronolimnaea* STR1 promoted growth and protected wheat plants from damage to salt stress via modulating the expression of stress-responsive genes. It modulated ABA-signaling of cascade genes (*TaABARE* and *TaOPR1*), expression of SOS pathway-related genes (*SOS1* and *SOS4*) and ion transporters (*TaNHX1*, *TaHAK*, and *TaHKT1*), expression of *TaST* (a salt stress-induced gene), and gene expression of various antioxidant enzymes (*APX*, *MnSOD*, *CAT*, *POD*, *GPX*, and *GR*), which contributed to increased salt stress tolerance in these plants. Inoculated plants subjected to salinity stress recorded also higher proline and lower MDA levels in comparison to the uninoculated ones (Bharti et al. 2016b).

5.2.2 Arbuscular Mycorrhizal Fungi in Salt Stress Mitigation

Mycorrhizal infection has gained a considerable importance as a shotgun approach to overcome the salt-induced reduction in crop production by altering the physiological and biochemical properties of the host plant. It has been considered a bioameliorator of saline soils. Several mechanisms have been reported behind mycorrhizal elicited plant stress tolerance. It alleviated salinity-induced injuries by regulating water uptake, increasing nutrient acquisition, inducing organic solute accumulation, maintaining photosynthetic capacity, improving protein synthesis, altering plant hormonal status, and enhancing ROS-scavenging activity resulting in higher crop yield (Talaat and Shawky 2015).

The improved salt tolerance of AM plants could be due to a more efficient uptake of nutrients. Symbiosis between AMF and most plant species provides nutrients such as phosphorus and others including N, K, Ca, Mg, Fe, Zn, and Cu (Shokri and Maadi 2009). Arbuscular mycorrhizal colonization and arbuscule formation

effectively alleviated salinity-induced injuries by increasing N, P, K^+, Ca^{2+}, and Mg^{2+} uptake and decreasing Na^+ concentration in wheat (Talaat and Shawky 2011). Indeed, AMF may act as a first barrier for ion selection during the fungal uptake of nutrients from the soil or during their transfer to the host plant. For example, *Rhizophagus intraradices* can take up K^+, Mg^{2+}, and Ca^{2+} while avoiding Na^+ uptake, which suggest that AMF can induce a buffering effect on the Na^+ uptake (Hammer et al. 2011). AMF colonization can also improve the water absorption capacity of maize plants grown under saline conditions, by increasing root hydraulic conductivity and by adjusting the osmotic balance (Sheng et al. 2008). Moreover, mycorrhizae can increase the root growth and thus could enhance the plant adaptation to adverse soil conditions (Talaat and Shawky 2012; Pedranzani et al. 2016). Indeed, root growth promotion can lead to a larger root surface and consequently increase the water acquisition and nutrient uptake. Change in polyamine balance is a frequent response of plant metabolism to the mycorrhizal colonization influencing many physiological aspects including stress resistance and resulting in better performance of plants under stressful conditions (Smith and Read 2008). Mycorrhization improved wheat salt stress tolerance by altering polyamine balance; it changed Put, Spd, and Spm content as well as reduced the activities of diamine oxidase and polyamine oxidase. It also improved host plant nutrient status via increasing N, P, K^+, Fe, Zn, and Cu acquisition and diminishing Na^+ uptake and thus increased the fitness of wheat plant to salt stress (Talaat and Shawky 2013).

Mycorrhizal symbiosis can also play as an ecosystem service provider to guarantee plant productivity in saline soils by improving carbon and nitrogen metabolisms. It alleviated the deleterious effect of salinity through improving relative water content and membrane stability index; inducing a better osmotic adjustment via compatible solute accumulation such as soluble sugars, free amino acids, proline, and glycinebetaine; altering ionic balance via increasing N, K^+, and Mg^{2+} acquisition and decreasing Na^+ uptake; enhancing photosynthetic efficiency via improving photochemical reactions of photosynthesis, gas exchange capacity, chlorophyll content, Chl a/b ratio, carbonic anhydrase activity, and carbohydrate content; promoting protein synthesis via enhancing nitrate content and nitrate reductase activity; as well as preventing oxidative stress via alleviating membrane lipid peroxidation and decreasing H_2O_2 content (Talaat and Shawky 2011, 2014a). Inoculation via *Rhizophagus irregularis* to salt-affected *Populus cathayana* plants enhanced plant–water status (relative water content and water-use efficiency), which could increase the capacity of photosynthesis and thus biomass production (Wu et al. 2015). Furthermore, AMF can improve plant adaptation to saline soils by eliminating the ROS. Mycorrhizal symbiosis altered the plant physiology of salt-stressed wheat plants by reducing membrane lipid peroxidation, membrane permeability, and H_2O_2 content as well as by enhancing ROS-scavenging system activity via increasing the antioxidative enzyme (SOD, POX, CAT, and GR) activity and the antioxidant molecules (glutathione, ascorbate, and glycinebetaine) concentration (Talaat and Shawky 2014b). AM *Digitaria eriantha* plants grown under saline conditions had higher CAT, APX, and SOD activity, higher stomatal conductance value, higher endogenous jasmonate level, and lower hydrogen peroxide level than the non-AM ones (Pedranzani et al. 2016).

Influence of AMF on organic acids in maize leaves under salt stress was studied by Sheng et al. (2011), who found that AM symbiosis increased the accumulation of organic acids such as oxalic acid, fumaric acid, acetic acid, malic acid, and citric acid, whereas the concentrations of formic acid and succinic acid decreased, and no significant effect was found on lactic acid concentrations. Mycorrhizal infection can also alter plant hormonal status in salt-stressed plants. Lower ABA levels were detected in *Glomus intraradices*-colonized lettuce plants indicating that AM plants were less strained than non-AM plants by salinity stress imposed; hence, they accumulated less ABA (Jahromi et al. 2008). An increase in strigolactone, a new class of plant hormone, in mycorrhizal-treated plants was demonstrated to overcome salinity effects in lettuce plants (Aroca et al. 2013). Higher cytokinin concentration and higher translocation of photosynthetase were detected in AMF-inoculated plants subjected to salt stress (Hameed et al. 2014).

Potential molecular mechanisms underlying AMF-mediated plant salt stress tolerance were reported. AMF-induced plant salinity tolerance may be influenced by genes encoding Δ1-pyrroline-5-carboxylate synthetase (*LsP5CS*), late embryogenesis-abundant protein (*LsLea*), and ABA (*Lsnced*) (Kapoor et al. 2013). Cyclic nucleotide-gated ion channels (CNGCs) assisted the AM-inoculated plants to survive under saline conditions by supplying the sodium reallocation within the plant tissues (Ruiz-Lozano et al. 2012). Although the Na^+/H^+ antiporters – *LeNHX1* and *LeNHX2* – catalyze the transfer of Na^+ out of the cytoplasm into either vacuole or apoplasm, the AM symbiosis under salt stress did not alter the expression of *LeNHX1* and *LeNHX2* genes (Ouziad et al. 2006). Inoculation of three native AMF from a Mediterranean saline area to salt-stressed maize plants showed significant increase in K^+ and reduction in Na^+ accumulation as compared to salt-stressed non-mycorrhizal ones, concomitantly with higher K^+/Na^+ ratios. This effect correlated with the regulation of *ZmAKT2*, *ZmSOS1*, and *ZmSKOR* genes in their roots (Estrada et al. 2013). Mycorrhizal infection enhanced the rice salt tolerance by decreasing Na^+ root-to-shoot distribution and increasing Na^+ accumulation in rice roots. In aerial plant tissues, the AM symbiosis may favor Na^+ extrusion from cytoplasm, its sequestration into the vacuole, the unloading of Na^+ from the xylem, and its recirculation from photosynthetic organs to roots through regulation of the expression of *OsNHX3*, *OsSOS1*, *OsHKT2;1* and *OsHKT1;5* genes encoding plant transporters involved in ion homeostasis (Porcel et al. 2016).

Mycorrhizal-colonized plants can also interact with several soil microorganisms including PGPR to increase the plant salt tolerance. Inoculation with PGPR *P. mendocina* alone and in combination with an AMF *Glomus intraradices* or *G. mosseae* improved the biomass of salt-stressed *Lactuca sativa* cv. *Tafalla* plants, along with antioxidant enzymes and proline content in foliage (Kohler et al. 2009). The combined application of AMF and PGPB attenuated the negative salinity effects on the plants by producing phytohormone and increasing nutrient uptake (Dodd and Perez-Alfocea 2012). Co-inoculation with a mixture of AMF from the genera *Glomus*, *Gigaspora*, and *Acaulospora* and the rhizobia *Sinorhizobium terangae* resulted in a positive osmotic adjustment that improved salinity tolerance in *Acacia saligna* (Soliman et al. 2012). Co-inoculation of AMF (*Glomus etunicatum*) and PGPB

(*Methylobacterium oryzae* CBMB20) alleviated salt stress and significantly increased dry biomass and nutrient accumulation, while it significantly reduced proline content and Na^+ uptake in maize plants (Lee et al. 2015). Co-inoculation of PGPR *Dietzia natronolimnaea* strain STR1 and AMF *Glomus intraradices* positively influenced the growth of *Ocimum basilicum* plants grown in salt-affected soils (Bharti et al. 2016a).

5.2.3 Effective Microorganisms in Salt Stress Mitigation

Using EM application as a biological strategy to enhance plant salt stress tolerance can increase the saline soil utilization and become an emerging challenge as a promising environmentally friendly method. It provides an inexpensive and viable method for alleviating the effect of soil salinization on crop production (Talaat 2014, 2015b; Talaat et al. 2015a). It protected the plant cell against the oxidative damage and enhanced the plant survival under soil salinization by countering the lipid peroxidation via enhancing the enzymatic activities of antioxidative enzymes involved in the ascorbate–glutathione cycle and a higher redox status of the antioxidants ascorbate and glutathione. Indeed, EM treatment enhanced the H_2O_2-scavenging capacity of the ascorbate–glutathione cycle to attenuate the activation of plant defenses (Talaat 2014). Preventing oxidative stress and eliminating ROS are the most effective mechanisms used by EM-treated plants to cope with salinity stress (Talaat 2015a). EM application can also increase plant salinity tolerance by enhancing the nutrient acquisition and improving the osmotic adjustment via compatible solute accumulation. EM treatment reduced Na^+ uptake, increased N, P, K^+, Ca^{2+}, Mg^{2+}, Fe, Zn, and Cu absorption, and enhanced important osmolytes such as soluble sugar, free amino acid, proline, and glycinebetaine accumulation in *Phaseolus vulgaris* plants subjected to saline conditions (Talaat et al. 2015a). Moreover, the application of EM could improve salt stress tolerance by the regulation of protein synthesis and the modulation of polyamine pool. It activated the nitrate uptake and enhanced the NR activity, which could be a reason for the observed increase in the protein content. It also regulated the ionic homeostasis, modified the biosynthesis of polyamines, decreased the activity of the polyamine catabolizing enzymes, prevented the oxidative stress via decreasing the MDA and H_2O_2 contents, and enhanced the membrane stability index in *Phaseolus vulgaris* plants grown in salty soils (Talaat 2015b).

5.3 Water Stress

Drought is one of the major environmental factors that limit crop production worldwide by altering a series of physiological, biochemical, and molecular responses. It disrupts photosynthesis and protein synthesis, increases photorespiration, affects plant hormone balance, alters the normal homeostasis of cells, and induces high levels of ROS in plant cells (Cohen et al. 2015; Talaat et al. 2015b; Talaat and

Shawky 2016). Therefore, there is a serious need to find new ways to cope with the threat of global water deficiency on agricultural production. Microbial inoculants are a powerful strategic tool in inducing plant drought tolerance.

5.3.1 Plant Growth-Promoting Bacteria in Water Stress Mitigation

Microbial inoculants can alleviate the negative impact of water deficit by inducing different physical and biochemical changes in plants. Probably specific mechanisms are responsible for plant performance under this condition. *Azospirillum*-inoculated wheat seedling showed better phospholipid composition in its root than that in non-inoculated ones when exposed to water-deficit condition, which suggested that bacterial inoculation led to changes in the root cell membrane elasticity and thus improved tolerance to water deficiency (Pereyra et al. 2006). Root colonization with rhizobacteria *Pseudomonas chlororaphis* 06 enhanced water stress tolerance in *Arabidopsis* by reducing water loss and increasing stomatal closure. The volatile metabolite 2,3-butanediol produced by *P. chlororaphis* 06 increased salicylic acid (SA) content and thus induced the stomatal closure and subsequently drought resistance. Hence, induction of drought tolerance in *Arabidopsis* by *P. chlororaphis* 06 is through a SA-dependent mechanism (Cho et al. 2008). Indeed, studies with *Arabidopsis* mutant lines revealed that induced water stress tolerance requires SA, ethylene, and jasmonic acid signaling pathways. Additionally, many bacteria can ameliorate the adverse effect of water deficiency by increasing osmoprotectant accumulation. Both Gram-negative (*Azospirillum* and *Pseudomonas*) and Gram-positive (*Bacillus*) strains promoted water stress resistance of basil plants by inducing the production of proline and soluble carbohydrates in root and leaf tissues. Inoculated plants had also higher chlorophyll content, confirming the positive effect of bacteria under drought conditions (Heidari et al. 2011). Wheat seedlings inoculated by *Azospirillum* under osmotic stress had better water status, which could be attributed to the morphological changes in xylem vessels of the coleoptiles, upregulation of its own indole-3-pyruvate decarboxylase gene, and enhanced bacterial IAA synthesis (Pereyra et al. 2012). As a drought tolerance mechanism, plants overexpress zeatin to delay the leaf senescence. Inoculation by engineered strains of *Sinorhizobium meliloti* with *ipt* gene enhanced the concentration of zeatin and cytokinin and improved the activity of antioxidant enzymes in the leaves of alfalfa plants grown under severe drought conditions (Xu et al. 2012). Furthermore, drought stress amelioration was detected in wheat inoculated with *Burkholderia phytofirmans* strain PsJN by modulation of metabolism and improving the ionic balance (Naveed et al. 2014). *Azospirillum brasilense* Sp 245 strain ameliorated the deleterious effect of drought on *Arabidopsis thaliana* via altering root architecture, stimulating photosynthetic and photoprotective pigments, and retarding water loss in correlation with enhancement of ABA levels (Cohen et al. 2015). Overall, modulating water stress tolerance and minimizing the stress damage could be induced in the plant tissues by the microbial activities irrespective of the microbial origin.

PGPR ameliorates the negative effect of water deficiency on plant cells via a so-called process induced systemic tolerance (IST), which includes:

(a) Production of cytokinins that causes abscisic acid accumulation in leaves and results in the stomatal closure
(b) Production of indoleacetic acid which improves root growth and nutrient uptake
(c) Degradation of the ethylene precursor ACC by bacterial ACC deaminase
(d) Production of antioxidants that causes ROS degradation and reduces damage to cells and biomolecules
(e) Production of volatile organic compounds (VOCs)
(f) Production of exopolysaccharides which tends to improve soil structure by facilitating the formation of macroaggregates
(g) Production of siderophores to facilitate root nutrient uptake (Grover et al. 2011)

PGPR containing ACC deaminase significantly lowered the ACC level in water-stressed plants and thus decreased the ethylene synthesis and the plant damage. Inoculation with rhizobacteria containing ACC deaminase, *Pseudomonas fluorescens* biotype G (ACC-5), increased fresh weight, dry weight, root length, shoot length, number of leaves per plant, and water-use efficiency of water-stressed peas. Longer roots might increase the water uptake from deep soil, thus increasing water-use efficiency (Zahir et al. 2008). PGPB *Pseudomonas* sp., *P. putida*, and *B. megaterium* with IAA-producing abilities alleviated water stress in *Trifolium repens* (Marulanda et al. 2009). Certain PGPBs alleviated plant drought stress via VOC production. Osmotic-stressed *Arabidopsis* plants inoculated by the soil microbe *Bacillus subtilis* GB03 VOCs accumulated higher levels of choline and glycinebetaine, which are important osmoprotectants that confer dehydration tolerance in plants than plants without VOC treatment (Zhang et al. 2010a). PGPR-induced water stress tolerance can also be achieved via enhancing the antioxidant enzyme activity and the antioxidant molecule concentration. *Pseudomonas fluorescens* Pf1 ameliorated drought by increasing catalase and peroxidase activities, as well as proline accumulation in green gram plants (Saravanakumar et al. 2011). Water stress amelioration and plant growth promotion were detected in wheat plants inoculated with *Bacillus safensis* strain W10 and *Ochrobactrum pseudogregnonense* strain IP8. These PGPBs enhanced antioxidant responses via elevating activities of antioxidant enzymes catalase, peroxidase, ascorbate peroxidase, superoxide dismutase, and glutathione reductase as well as increasing accumulation of antioxidants carotenoids and ascorbate (Chakraborty et al. 2013). PGPR-treated potato plants had higher gene expression of ROS-scavenging enzymes as well as higher photosynthetic performance, which displayed increased tolerance to various abiotic stresses (Gururani et al. 2013). Certain PGPR may also indirectly ameliorate drought by enhancing the production of exopolysaccharide. Rhizobacteria *Pseudomonas putida* strain P45 with exopolysaccharide-producing abilities improved sunflower drought resistance. Exopolysaccharides possess unique water-holding and water-cementing properties, thus helping in the formation and stabilization of soil aggregates and regulation of nutrients and water flow across plant roots through biofilm formation (Sandhya

et al. 2009). *Pseudomonas aeruginosa* strain Pa2 produce exopolysaccharides that enhanced bacterial ability to maintain soil moisture content and increased maize drought tolerance (Naseem and Bano 2014).

Rhizosphere bacteria also induce plant drought tolerance when applied in combination with rhizobial strains. Co-inoculation with *Paenibacillus polymyxa* and *Rhizobium tropici* alleviated drought stress in common bean (*Phaseolus vulgaris* L.) by increasing the plant growth, nitrogen content, and nodulation as well as by altering the phytohormone content (Figueiredo et al. 2008).

Moreover, adapted drought-tolerant microorganisms may compensate for the stress effect and can be active in promoting plant establishment. The use of adapted autochthonous microorganisms to regenerate arid soils is an attractive possibility. Inoculation with five drought-tolerant PGP *Pseudomonas* spp. strains, namely, *P. entomophila*, *P. stutzeri*, *P. putida*, *P. syringae*, and *P. montelli*, ameliorated maize drought stress by modifying compatible solute accumulation and antioxidant status (Sandhya et al. 2010). The stress protecting agent *Stenotrophomonas rhizophila* DSM14405T produced spermidine, which is a general, highly efficient stress protectant (Alavi et al. 2013). Autochthonous bacteria strains of *Bacillus megaterium*, *Enterobacter* sp., *Bacillus thuringiensis*, and *Bacillus* sp. alleviated water stress in *Lavandula* and *Salvia* by increasing K content, depressing stomatal conductance, and controlling shoot proline accumulation (Armada et al. 2014). The autochthonous bacteria *Bacillus thuringiensis* and the allochthonous bacteria *Pseudomonas putida* alleviated drought stress impact on wheat plants through improving the content of nutrients and relative water as well as decreasing the stomatal conductance, electrolyte leakage, proline content, and APX activity (Ortiz et al. 2015).

5.3.2 Arbuscular Mycorrhizal Fungi in Water Stress Mitigation

Mycorrhizal soil community is a vital component in the plant–soil system. AM symbiosis facilitates the nutrient transfer from soil to host plants. It also improves drought tolerance through enhancing water uptake by extraradical hyphae, improving gas exchange and water-use efficiency, improving osmotic adjustment as a result of the enhancement in compatible solute accumulation, altering plant hormonal status, regulating antioxidant system, and improving soil structure by glomalin (Gong et al. 2013; Yooyongwech et al. 2013; Ortiz et al. 2015; Pedranzani et al. 2016). AMF enhancing the host plant drought tolerance can also be achieved by proteins with chaperone-like activity, such as that of luminal binding protein (BiP), which prevent intramolecular and intermolecular interactions in endoplasmic reticulum that can result in permanent misfolding or aggregation and thus loss of their function (Porcel et al. 2007).

AMF-induced drought tolerance can be mediated through lowered oxidative burst via increasing antioxidant enzyme activity and antioxidant molecule content. AM associations improved the *C. equisetifolia* drought tolerance via lowering the plasma membrane permeability and malondialdehyde content as well as by enhancing peroxidase activity and P, soluble sugar, and soluble protein concentrations

(Zhang et al. 2010b). Mycorrhization enhanced photosynthetic efficiency, induced glutathione accumulation, as well as reduced hydrogen peroxide accumulation and oxidative damage to lipids in rice plants grown under drought stress conditions (Ruiz-Sanchez et al. 2010). AMF inoculation mitigated the oxidative stress generated in *Poncirus trifoliata* under water deficiency by increasing the mRNA levels of four stress-responsive genes *CSD1* (copper/zinc SOD), *MIOX1* (myo-inositol oxygenase), *GLX1* (glyoxalase), and TTC5 (transparent testa 5), which encode enzymes responsible for elimination of ROS, alleviating oxidative stress and detoxification of cytotoxic compounds (Fan and Liu 2011). Higher shoot and root biomass as well as higher flavonoids as one of the ROS scavengers were observed in *Glomus etunicatum*-colonized pistachio plants as compared to non-colonized ones under drought conditions (Abbaspour et al. 2012). Colonization of pomegranate plants by *Rhizophagus intraradices* resulted in considerably higher shoot superoxide dismutase and catalase activity under water-deficit conditions (Bompadre et al. 2014). AM symbiosis improved plant drought tolerance by elevating the production of isoprenoids, nonvolatile compounds, and antioxidants (Rapparini and Penuelas 2014). In addition to antioxidant concentration and antioxidant enzyme activity, mycorrhizal plants possess a H_2O_2 efflux pathway to prevent oxidative burst being induced under water stress conditions. *Funneliformis mosseae*-colonized trifoliate orange seedlings had lower oxidative burst under drought conditions, which resulted from a combination of higher antioxidant enzyme activity (superoxide dismutase and catalase), lower O_2^- accumulation, higher H_2O_2 effluxes, and higher Ca^{2+} influxes (Zou et al. 2015). Increases in shoot/root biomass ratio; shoot dry matter content; stomatal conductance value; CAT, APX, and SOD activity; and endogenous jasmonate accumulation and decreases in H_2O_2 level were recorded in AM *Digitaria eriantha* plants grown under drought conditions (Pedranzani et al. 2016).

The symbiotic association formed by AMF with higher plants under water-deficit conditions could increase the water uptake from the soil and improve the regulation of stomatal aperture to get higher water-use efficiency. AMF inoculation improved maize drought tolerance by affecting plant–water relation through inducing changes in cytokinin and auxin concentrations, enhancing stele tissue size, increasing soil aggregate stability and soil available water, as well as enhancing stomatal conductance (Boomsma and Vyn 2008). AMF can alter water regulation in water-stressed *Poncirus trifoliata* plants through modulation in hormonal signaling or by stimulating the accumulation of osmolytes (Fan and Liu 2011). Abscisic acid plays an important role as one of the non-nutritional mechanisms by which AM symbiosis influences stomatal conductance in drought-exposed plants (Ludwig-Muller 2010). In addition, improving plant–water status in AM-inoculated *Zea mays* plants played an indirect role in enhancing osmotic adjustment, gas exchange capacity, effectiveness of photochemistry of PSII, and nutrient uptake under water-deficit conditions (Zhu et al. 2012). Greater leaf water potential, gas exchange, stomatal conductance, and photosynthetic rates were detected in mycorrhizal sunflowers grown under drought stress (Gholamhoseini et al. 2013). Furthermore, AM symbiosis can enhance drought tolerance through improving soil structure by glomalin. AM symbiosis increased plant growth under drought stress indirectly via affecting soil

moisture retention via glomalin's effect on soil water-stable aggregates (Wu et al. 2008). Extensive hyphal network formation and glomalin secretion by AMF can improve soil structure and enhance water and nutrient uptake, thus improving plant growth under drought conditions (Gong et al. 2013). Mycorrhization has also been known to ameliorate the drought-induced deficiency in nutrients such as P, K, Ca, Mg, Fe, and Zn. Pistachio plants inoculated with two AMF species (*G. mosseae* and *G. intraradices*) under soil water deficit showed significant increases in P, K, Zn, and Mn concentrations (Bagheri et al. 2012). *G. mosseae* inoculation improved P concentration in sunflowers and thus improved plant drought tolerance and seed oil yield (Gholamhoseini et al. 2013). Mycorrhizal inoculation significantly minimizes drought stress-imposed effects on wheat plants by enhancing plant growth, nutrient uptake, and the relative water content (Ortiz et al. 2015).

AMF could also mitigate the adverse effects of drought stress by regulating plant endogenous ABA concentration. Changes in plant ABA concentration caused by AMF have been postulated to induce the expression of many stress-related genes including aquaporin genes encoding membrane intrinsic proteins that facilitate transport of certain small molecules in addition to water across biological membranes. Expression of two aquaporin genes (*GintAQPF1* and *GintAQPF2*) was improved in both root cortical cells holding arbuscules and extraradical mycelia of *Zea mays* plants colonized by *Glomus intraradices* under drought stress. Thus, the fungal AQPs could mediate the AM plant's ability to enhance water uptake under water deficiency (Li et al. 2013). Moreover, in *Rhizophagus intraradices*, 14-3-3 protein and aquaporins (GintAQPF1 and GintAQPF2) could be activated by the simultaneous increase in the expression of plant genes encoding D-*myo*-inositol-3-phosphate synthase (IPS) and 14-3-3-like protein GF14 (14-3GF) that are responsible for ABA signal transduction. These findings suggested that co-expression of *IPS* and *14-3GF* is responsible for the crosstalk between maize and *R. intraradices* under drought stress and potentially induces the synergistic actions of the symbiotic partners in enhancing plant drought tolerance. Hence, mycorrhizal colonization decreased root ABA concentration mainly by downregulating *AO* expression under drought stress. Consequently, *Rhizophagus intraradices* improved plant–water status by modulating ABA-mediated abiotic signaling pathway involving IPS and 14-3-3 proteins (Li et al. 2016).

Compatible solute accumulation is another important mechanism underlying AMF-mediated protection of drought-exposed plants. Inoculated citrus plants by *Glomus versiforme* enhanced the plant osmotic adjustment under drought conditions via improved nonstructural carbohydrates and K^+, Ca^{2+}, and Mg^{2+} levels (Wu and Xia 2006). Sugars and proline accumulation as osmolytes decreased the osmotic potential in *Macadamia tetraphylla* water-stressed mycorrhizal plants, which lead to subsequent plant drought tolerance (Yooyongwech et al. 2013). In contrast, AM-mediated decrease in soluble sugar concentration in *Erythrina variegata* drought-exposed plants (Manoharan et al. 2010) was related to the lower levels of drought injury in these plants. Inoculation of drought-exposed *Poncirus trifoliata* plants with *Funneliformis mosseae* decreased proline accumulation and improved its growth performance and biomass production (Zou et al. 2013). Furthermore,

AMF colonization improved free polyamines and soluble nitrogenous compound levels in water-stressed plants (Rapparini and Penuelas 2014).

Higher upregulation of the message levels of phospholipase D delta, calcineurin B-like proteins (CBL 1), and histone acetyltransferase (HAT) was detected in *Arabidopsis thaliana* seedlings colonized by *Piriformospora indica*, an endophytic fungus, under drought conditions. These increases could result from the priming of the expression of a quite diverse set of stress-related genes in the leaves (Sheramati et al. 2008).

Interaction between AMF and PGPR could have beneficial effect on the development of revegetation in water limitation soils. Inoculation with the PGPR *P. mendocina* alone or in combination with an AMF, *G. intraradices* or *G. mosseae*, conferred drought resistance to lettuce plants by stimulating nitrate reductase, phosphatase, and catalase activities in plant roots and proline accumulation in leaves significantly, which support the potential use of dual application of PGPR and AMF as an inoculant to ameliorate the adverse effect of water stress on the plant antioxidant system (Kohler et al. 2008).

5.4 Heat Stress

With the recent advent of global warming, heat stress has become a major area of concern to crop production. Plants react to temperature changes at cellular, tissue, and organ levels. Generation of several toxic ROS in cells, protein denaturation and aggregation, fluidity of membrane lipids, inactivation of enzymes in chloroplast and mitochondria, inhibition of protein synthesis, and loss of membrane integrity are the major types of cellular damage that result from higher temperatures (Ali et al. 2011; Meena et al. 2015). Rhizosphere microbes have positive impact on counteracting the adverse effects of heat stress on plants.

5.4.1 Plant Growth-Promoting Bacteria in Heat Stress Mitigation

PGPB may enhance plant thermotolerance through several mechanisms including biological nitrogen fixation, enhancing the bioavailability of phosphorous, iron, and other mineral nutrients; production of phytohormones including indoleacetic acid, abscisic acid, gibberellic acid, brassinosteroids, jasmonates, and salicylic acid; production of ACC deaminase; as well as generation of antioxidants (Grover et al. 2011). In this respect, three PGPR isolates *P. alcaligenes* PsA15, *Bacillus polymyxa* BcP26, and *Mycobacterium phlei* MbP18 conferred heat and salt stress tolerance by enhancing growth and nutrient acquisition of maize plants and consequently improve their survival under these stressful conditions (Egamberdiyeva 2007). More interestingly, heat tolerance induced by bacteria can be due to reducing the ROS generation and thus less cell damage as well as to inducing changes in the activation of certain heat shock transcription factors (Abd El-Daim et al. 2014).

Pseudomonas aeruginosa strain 2CpS1 showing ACC deaminase activity ameliorated the deleterious impacts of temperature stress on wheat by increasing plant height, root length, leaf area, dry matter, chlorophyll content, relative water content, and decreasing cell membrane injury (Meena et al. 2015).

Furthermore, adapted thermotolerant microorganisms can promote plant establishment and alleviate heat stress effects. Thermotolerant strain of *Pseudomonas* sp. AKM-P6 possessing PGPR activities helped sorghum seedlings to withstand heat stress by inducing heat shock proteins (HSPs) in leaves, reducing membrane injury, and increasing proline, chlorophyll, sugar, amino acid, and protein content (Ali et al. 2009). Inoculation with a PGP-thermotolerant *Pseudomonas putida* strain AKMP7 improved survival and growth of heat-stressed wheat plants via increasing root and shoot length, dry biomass, tiller, spikelet, and grain formation; improving proline, chlorophyll, sugar, starch, amino acid, and protein level; and reducing membrane injury and the activity of several antioxidant enzymes such as SOD, APX, and CAT (Ali et al. 2011).

5.4.2 Arbuscular Mycorrhizal Fungi in Heat Stress Mitigation

Mycorrhizal infection evokes various physiological and biochemical processes to help plants to sustain their development under heat stress. Antioxidant compounds such as polyphenol and ascorbic acid were enhanced in the leaves of mycorrhizal strawberry compared to that in non-mycorrhizal ones under heat stress conditions (Matsubara 2010). Anti-oxidative activity of superoxide dismutase and ascorbate peroxidase, content of ascorbic acid and polyphenol, and scavenging activity of 2,2-diphenyl-1-picrylhydrazyl radical were increased in mycorrhizal cyclamen under heat stress, which suggested that the AM symbiosis can alleviate ROS damage, protect plants against oxidation, and improve heat stress tolerance during plant production (Maya and Matsubara 2013).

Colonization of *Arabidopsis thaliana* plants by *Paraphaeosphaeria quadriseptata* as a rhizosphere fungus improved plant heat stress tolerance by induction of HSP101 and HSP70 proteins, the conserved components of the stress response (McLellan et al. 2007). Endophytic fungus *Paecilomyces formosus* LWL1 mitigated heat damage in japonica rice by improving plant growth attributes (plant height, fresh weight, and dry weight), downregulating the stress-related signaling molecules (abscisic acid and jasmonic acid), as well as increasing the contents of total protein and chlorophyll (Waqas et al. 2015).

5.5 Chilling Injury

Low temperature stress (cold or chilling) is a serious problem that reduces agricultural output potential by influencing cellular metabolism, macromolecule activity, antioxidant–ROS balance, decreasing osmotic potential in the cellular milieu, solidification or rigidification of plasma membrane, and destabilization of protein

complexes (Barka et al. 2006; Chen et al. 2013; Pedranzani et al. 2016). Microbial inoculants are potential candidates that can improve plant cold stress tolerance.

5.5.1 Plant Growth-Promoting Bacteria in Chilling Injury Mitigation

PGPR root colonization can improve the plant ability to withstand cold stress. Epiphytic bacterial species with ice-nucleating activity (ice$^+$ bacteria), such as *Pseudomonas syringae*, contribute to the frost injuries of many cold-sensitive plants via reducing the plants' ability to supercool, a process that prevents the formation of membrane-damaging ice crystals (Lindow and Leveau 2002). Inoculation of grapevine (*Vitis vinifera*) with a PGPR, *Burkholderia phytofirmans* strain PsJN, lowered the rate of biomass reduction and electrolyte leakage – an indicator of cell membrane injury – during cold treatment (4°C) and promoted post-chilling recovery. Levels of starch, proline, and phenols and rates of photosynthesis and starch deposition were also enhanced (Barka et al. 2006). *Burkholderia phytofirmans* PsJN acclimated grapevine to cold by improving plant photosynthesis and regulating carbohydrate metabolism (Fernandez et al. 2012). Moreover, *Burkholderia phytofirmans* PsJN primed *Vitis vinifera* L. and conferred cold stress tolerance by modulating stress-related gene expression, carbohydrate metabolism, and metabolite accumulation (Theocharis et al. 2012).

Cold-tolerant PGPB *Pantoea dispersa* strain 1A isolated from a subalpine soil in the North Western Indian Himalayas and cold-tolerant *Serratia marcescens* strain SRM (MTCC 8708) isolated from flowers of summer squash exhibited PGP characteristics like IAA production, P-solubilization, HCN, and siderophore production. Higher biomass and nutrient acquisition were observed in cold-stressed wheat seedlings when their seeds were bacterization with these strains (Selvakumar et al. 2007a, 2007b). *Pseudomonas lurida* M2RH3 (MTCC 9245), a psychrotolerant bacterium, solubilized phosphate; produced siderophores, IAA, and HCN; and promoted the growth of cold-stressed wheat seedling (Selvakumar et al. 2011). Cold-tolerant *Pseudomonas* spp. and *Rhizobium leguminosarum*-PR1 acclimated lentil to cold and improved its iron acquisition, nutrient uptake, and growth (Mishra et al. 2011).

5.5.2 Arbuscular Mycorrhizal Fungi in Chilling Injury Mitigation

The underlying potential mechanisms improved plant cold stress tolerance as a result of AMF inoculation. AM symbiosis enhanced photosynthetic characteristics, chlorophyll synthesis, plant–water status, water-use efficiency, and SOD, CAT, POD, and APX activities in plants grown under cold stress (Zhu et al. 2010; Abdel Latef and Chaoxing 2011). Furthermore, osmotic adjustment is one of the most important mechanisms in plants to achieve low temperature tolerance. Mycorrhization enhanced the accumulation of osmoprotectants such as soluble sugar, soluble protein, and proline in tomato plants under low temperature stress (Abdel Latef and Chaoxing 2011).

AM mediated also increase in the accumulation of phenolics, flavonoids, and lignin accompanied with significant decrease in the H_2O_2 accumulation in cucumber subjected to low temperature stress (Chen et al. 2013). AM symbiosis enhanced shoot dry matter content, photosynthetic efficiency, and CAT, APX, and SOD activities, while it decreased H_2O_2 and MDA contents in *Digitaria eriantha* plants under cold stress condition, which could help plants to cope with stressful conditions (Pedranzani et al. 2016). At the molecular level, Aroca *et al.* (2007) found that AM symbiosis enhanced *Phaseolus vulgaris* tolerance to cold, drought, and salt stress by regulation of root hydraulic properties, which were closely correlated with the regulation of PIP2 protein levels and phosphorylation state.

5.6 Heavy Metal Toxicity

Heavy metal toxicity is an increasingly serious problem worldwide that reduces agricultural output potential and damages the health of ecosystem. Some of these metals are essential plant micronutrients such as Cu, Fe, Mn, Ni, and Zn and are required for beneficial plant growth and development, while others have no known biological function such as Cd, Pb, and Hg. High contents of heavy metals in soils are generally considered a matter of concern as they can accelerate rate of mortality, reduce potential survival, and induce toxicity symptoms. They severely damage plant metabolic activities by altering the structure and function of enzymes, the permeability and function of plasma membrane, the uptake and distribution of macro- and micronutrients, the hormonal balance and water movement, the photosynthetic process, the nitrogen assimilation, as well as the production of ROS (Garg and Singla 2012; Islam et al. 2016). Therefore, interaction between the rhizospheric microorganism and the plant activity related to soil metal toxicity is inevitable. Restoration and remediation of metal-polluted soils through biological remediation is safe, low-cost, effective, eco-friendly, and socially accepted strategy. Microbial inoculants such as PGPRs or mycorrhizae could protect plants from the harmful effects of heavy metal-contaminated areas.

5.6.1 Plant Growth-Promoting Bacteria in Heavy Metal Toxicity Mitigation

PGPB as bioinoculants can improve plant heavy metal stress tolerance via inducing different biochemical changes in stressed plants. PGPR *Pseudomonas aeruginosa* OSG41 enhanced chickpea growth under chromium stress with considerable decrease in proline content (Oves et al. 2013). PGPR inoculation increased SOD, CAT, DHAR, GR, and APX activities in potato under salt, water, and heavy metal stresses, which enhanced photosynthetic efficiency and ultimately plant growth (Gururani et al. 2013).

Furthermore, PGPB was used as a tool for rhizoremediation in contaminated soils. They can reduce the injury effect of heavy metals on plant and enhance its fitness by employing various mechanisms, such as:

(a) Influencing the pH and the redox potential in the rhizosphere through releasing organic acids
(b) Reducing the heavy metal mobilization in contaminated soils
(c) Improving the bacterial migration from the rhizoplane to the rhizosphere, which can reduce Cd plant uptake
(d) Forming the iron–siderophore complexes, which can be taken up by the host plant
(e) Forming the bacterial exopolysaccharides, which can develop the soil sheaths around the plant root, thus reducing the sodium flow into the stele
(f) Production of indoleacetic acid
(g) Production of ACC deaminase (Gadd 2004; Dimkpa et al. 2009)

Inoculation with PGPR strain, *Klebsiella mobilis* CIAM 880, resulted in 120% higher grain yield and twofold decreased in grain Cd content of barley plants grown on Cd-contaminated soil. Here, free Cd ions can be bound by bacteria into complex forms that cannot be taken up by the plant (Pishchik et al. 2002). *Methylobacterium oryzae* and *Burkholderia* sp. reduced nickel and cadmium stress in tomato via reducing their uptake and translocation (Madhaiyan et al. 2007). Moreover, some bacteria can protect plants against nickel, lead, iron, or zinc toxicity by siderophore production. Microbial siderophores were able to alleviate metal-induced oxidative stress in plants. By chelating and reducing toxic metal concentrations in the root zone, siderophores exerted a bioprotective effect by lowering the formation of cell-damaging free radicals, thereby enabling a microbial IAA-mediated plant biomass increase (Dimkpa et al. 2009). Rhizobacteria containing ACC deaminase activity improved plant growth and development as well as enhanced plant heavy metal stress tolerance (Belimov and Wenzel 2009). Several PGPR modulated plant–soil chemistry by mediating the methylation of Pb, Hg, Se, As, Tn, and Sn. These bacteria can transfer a methyl group to the metals, resulting in volatile methylated metal compounds that can easily excavate the soil zone (Bolan et al. 2014).

An extensive range of PGPR has been identified as most efficient candidates in phytoremediation. Significant enhancement of heavy metal phytoremediation by *Alnus firma* with an endophytic strain of *Bacillus thuringiensis* GDB-1 was reported by Babu *et al.* (2013). *Enterobacter* sp. 192 and *Klebsiella* sp. strains inoculated in *Brassica napus* L. improved its growth and resulted in bioaccumulation of Cd, Pb, and Zn (Jing et al. 2014). The recombinant strain KT2440-spPCS, which developed through the cloning of phytochelatin synthase (PCS) genes from *Schizosaccharomyces pombe* expressed in *Pseudomonas putida* KT2440, enhanced resistance to Hg, Cd, and Ag and a three- to fivefold increase in Cd accumulation (Yong et al. 2014). Introducing glutathione synthase gene *gcsgs* into endophytic *Enterobacter* sp. CBSB1 improved phytoremediation efficiency of host plant (Qiu et al. 2014). PGPR increased the phytoextraction ability of plants via enhancing the mobility of heavy

metal and improving their bioavailability by releasing chelating agents, acidification, phosphate solubilization, and redox changes. Some others produce organic acids, such as gluconic, oxalic, and citric acids that can mobilize and solubilize the heavy metals (Ullah et al. 2015).

More interestingly, the use of multi-strain inocula could be one of the better strategies to improve plant growth under contaminated soil. Co-inoculation of PGPBs with *Rhizobia* allowed a longer exudation of nod-gene-inducing flavonoids, which improve the performance of symbiotic nitrogen fixation. This co-inoculation with legume plants also supported the seedling vitality and their survival during metal phytostabilization and phytoextraction strategies (Gomez-Sagasti and Marino 2015).

In addition, for effective microbe-assisted bioremediation, metal-resistant PGPB can facilitate the growth and development of plants by restricting their uptake of excess metal and thus prevent its bio-amplification in the ecosystem. Clover-inoculated with a Cd-adapted autochthonous PGPR, *Brevibacillus* and grown in soil contaminated with Cd, resulted in growth-promoting effects and a reduction in Cd transfer from soil to plants. Cd accumulated by PGPR in their cells and, thus, reduced the bioavailable Cd concentrations, thereby reducing its uptake by plants and rhizobia (Vivas et al. 2005). Lupine inoculated with a consortium of metal-resistant PGPR (including *Bradyrhizobium*, *Pseudomonas* sp., and *Ochrobactrum cytisi*), for reclamation of multi-metal-contaminated soil, showed increment in plant growth. This mixture also succeeded to reduce plant toxicity symptoms and metal accumulation in both shoots and roots. This ameliorating impact might also be due to more intimate bacteria–plant relationships such as those ensured by endophytic PGPRs (Dary et al. 2010). Inoculation with copper-resistant bacteria had a positive effect on the upregulation of antioxidative defense mechanism (improved SOD, CAT and APX, and GPX activities) that eliminated the ROS and reduced the MDA content in wheat. They also found that bacterial inoculation prevented the negative impact of copper stress on protein synthesis/production by lowering the metal toxicity, which might be due to the fact that bacterial inoculation activates the gene expression profile of metal detoxifying enzymes to cope with the metal stress (Wang et al. 2013). Cd-tolerant PGP *Bradyrhizobium* sp. exhibit several PGP traits (synthesis of IAA, ACC deaminase, siderophores) increased shoot dry weight and Cd accumulation in roots of *Lolium multiflorum* grown in Cd-contaminated soil. They also detected that *Bradyrhizobium* sp. improved the extractable Cd concentrations in the rhizosphere, as well as it diminished the accumulation of Cd in root and shoot of *Glycine max* by increasing Fe availability (Guo and Chi 2014). Copper-resistant bacteria *Providencia vermicola* with different PGP traits (synthesis of the plant required hormone (IAA), P solubilization, siderophore production, and efficient ACC deaminase activity) protected lentil plants grown in copper-contaminated soil from copper toxicity. It increased root and shoot length, plant dry weight, leaf area, pod number, seed weight, gas exchange characteristics, N and P accumulation, leaf chlorophyll content, and root nodulation. Anti-oxidative defense mechanism also improved by inducing the expression of ROS-scavenging enzymes, such as ascorbate peroxidase, superoxide dismutase, catalase, and guaiacol peroxidase with

alternate decrease in malondialdehyde, H_2O_2, proline, and total phenolic contents and electrolyte leakage (Islam et al. 2016). Thus, using multifarious growth-promoting bacteria with metal resistance properties holds a great potential to be used as biofertilizer in metal-contaminated soils.

5.6.2 Arbuscular Mycorrhizal Fungi in Heavy Metal Toxicity Mitigation

AMF play one of the most important ecological roles in phytostabilization of toxic trace elements in soil by sequestration and thus can increase the survival of mycorrhizal plants in polluted soils. AM plant in heavy metal-polluted soil shows higher biomass and more tolerance because of:

1. Metal adsorption to chitin in the cell wall
2. Chelation of metals inside the fungus
3. Metals bound to metallothioneins or PCs inside the fungal or plant cells
4. Sequestration of heavy metals by siderophores, which deposit the heavy metals in root apoplasm or in soil
5. Restriction of metals by compounds secreted by the fungus
6. Immobilizes heavy metal on its hyphae and sequesters it inside the cell, thereby lessening its transfer to shoot
7. Changes in rhizophere pH and microflora, thereby decreasing heavy metal availability
8. Precipitation in polyphosphate granules in the soil
9. Plasma membrane acts as a living and selective barrier of toxic metals
10. Promotes plant growth and thus dilutes metal concentrations in plant tissues
11. Regulation of gene expression (Hossain et al. 2012; Shirmohammadi et al. 2014)

AMF enhanced plant Zn tolerance by absorbing and crystallizing it in AMF hyphae and cortical cells of mycorrhizal root, and thus Zn transfer to shoot was decreased (Khan et al. 2000). AMF enhanced Fe and Mn uptake in plants, at high concentrations, while it decreased Mn translocation in shoots and retain Fe in roots (Leyval et al. 2002). AMF produced glycoprotein (Glomalin), which has a metal chelating function and thus reduces the metal availability and decreases toxicity risk. One gram of glomalin could extract up to 4.3 mg of Cu, 0.08 mg of Cd, and 1.12 mg of Pb from the polluted sites (Gonzalez-Chavez et al. 2004). Furthermore, AMF colonization also enhances nutrient and water uptake, thereby maintaining better nutrient status. Mycorrhizal sunflower plants showed higher P/Cd, N/Cd, and S/Cd ratios in both shoots and roots than non-mycorrhizal ones. Higher N and S uptake in mycorrhizal plants leads to higher production of thiol-rich proteins, while higher P status leads to phosphate complexation with metal ions inside the cells that could have an important role in heavy metal detoxification (de Andrade et al. 2008). AMF in the roots of *Pteris vittata* modulated the activity of two major enzymes, namely, glutamine synthetase (which control the use of nitrogen inside the cells) and S-adenosyl methionine (SAM) synthase (which catalyze the SAM formation

from methionine and ATP) under arsenic exposure (Bona et al. 2010). AMF induced a clear protective effect against the high concentration of Mn in the soil which was related to "dilution" of the metal in plant tissues, because of increased growth and, to a varying degree, of mechanisms of exclusion, immobilization, or retention by which uptake and root-to-shoot transport of heavy metals are restricted (Bati et al. 2015).

Four AM fungal genes (*GrosMT1*, *GinZnT1*, *GmarMT1*, *and GintABC1*) play a vital role in maintenance of the cellular homeostasis against metals. Zn transporter *GinZnT1* helps in vacuolar Zn compartmentalization. *GmarMT1* codes for metallothioneins (MTs), the major metal chelators, regulates the fungal redox potential, and protects it against oxidative stress. *GintABC1* codes for a polypeptide of 434 amino acids and participates actively in Cu and Zn detoxification. *GintMT1* contributes to the pool of cytosolic thiols and regulates redox status of the extraradical mycelia of *G. intreradices* through its metal chelation activity or its –SH group (Gonzalez-Guerrero et al. 2010; Azcon et al. 2013). AMF colonization improved plant heavy metal tolerance/detoxification by inducing the expression of several plant genes coding for proteins. Inoculation with AMF *F. mosseae* or *G. intraradices* restored normal growth in a white poplar clone grown on Cu- and Zn-polluted soil, and this was associated with upregulation of foliar metallothionein and polyamine biosynthetic gene expression (Cicatelli et al. 2010). Moreover, *Glomus intraradices* colonization increased transcriptional of a GSH-dependent GST gene in *Medicago truncatula* Zn-stressed plants (Hossain et al. 2012). Inoculation with AMF *Funneliformis mosseae* significantly lowered nickel (Ni) translocation from roots to the aboveground parts of tall fescue plants, which may be due to the activation of mechanisms in mycorrhizal plants roots, viz., chelation of Ni and/or compartmentation within vacuoles. The ATP-binding cassette (ABC) transporter and metallothionein (MT) transcripts accumulated to considerably higher levels in the roots of mycorrhizal plants than in the corresponding non-mycorrhizal ones, which probably made metal levels insufficient for the expression of these genes in the shoots (Shabani et al. 2016).

Furthermore, AMF symbiosis can ameliorate the injury of heavy metal by stimulating or modifying specific physiological mechanisms related to the adaptation to stressful environments. AMF colonization alleviated cadmium stress in *Medicago truncatula* via promoting photosynthesis process through increasing the plant's ability to use light energy, maximizing the area available for CO_2 assimilation, facilitating the electron transport, preventing inhibition of aminolevulinic acid synthesis and protochlorophyllide photoreduction, increasing the density of photosynthetic units, increasing the photosynthesis-related proteins, as well as reducing the gluconeogenesis/glycolysis and the antioxidant processes (Aloui et al. 2011). *Glomus mosseae* colonization promoted the relative water and chlorophyll contents, cellular sucrose and glycinebetaine accumulation, as well as enzymatic components of antioxidant defense system in pea plants subjected to arsenic contaminated soil, which signifying the role of AM colonization in the higher turgor maintenance and lower leaf chlorosis (Garg and Singla 2012). AMF were confirmed to be strong growth stimulants in olive Mn-stressed plants by optimizing P absorption and ensuring a greater supply of macronutrients and micronutrients (Bati et al. 2015). *Funneliformis mosseae* not only improved nutrition and water absorption, but there was also a

significant increase in the content of leaf pigments (chlorophyll, carotenoid) under Ni stress. Mycorrhization increased the carotenoids amount in tall fescue nickel-stressed plants. Carotenoids as antioxidants can quench singlet oxygen and can scavenge free radicals (Shabani et al. 2016).

5.7 Conclusion and Future Perspectives

The beginning of the twenty-first century is marked by global scarcity of water resources, global warming, environmental pollution, and increased salinization of soil and water, which cause major reductions in crop productivity and quality. A significant increase in agricultural productivity is required to fulfill the food supply requirements to feed the world's growing population, and that should be based on sustainable practices that minimize the environmental impact but also support food safety and food security. Plant-associated microorganisms are a powerful strategy in this regard. Plants in their natural environment are colonized by both endocellular and intracellular microorganisms. Rhizosphere microorganisms, particularly beneficial bacteria and fungi, can control abiotic stresses and are considered as eco-friendly strategies to improve crop yield. Utilizing microbial inoculation subordinates the plant stresses and is an alternative to traditional remediation methods that involve the addition of synthetic chemicals, which are time-consuming and increase the cost of the final crop. Some PGPR can enhance plant growth and productivity via providing plants with fixed nitrogen, soluble phosphate, iron, and phytohormones. Others can do this indirectly by protecting the plant against soilborne diseases. PGPR can also adapt plants to different abiotic stress factors through the presence of the ACC deaminase enzyme, the production of exopolysaccharides, the enhancement of defense-related enzymes, the production of phenolic compounds, and the eliciting of jasmonic and ethylene pathways in plants.

To help plants to combat abiotic stresses, selection of the appropriate microbial inoculants (mycorrhizae or PGPRs) is one of the most important technical traits. In addition, using compatible multiple microbial consortia consisting of bacterial symbionts and fungal symbionts acting synergistically, providing various beneficial effects, is also a powerful strategic tool. Hence, future research has to be focused on the application of multi-microbial inoculation, which could be an effective approach to reduce harmful impact of stress on plant development, but prerequisites for effective combinations need to be established. Furthermore, the challenge in the twenty-first century lies on developing stable multiple stress tolerance traits, thus improving yields particularly in areas with adverse environmental conditions and contributing to global food security.

Genetic techniques may point out to new insight in the alleviating role of microbial inoculants under abiotic stresses. Therefore, using microorganism application as an elicitor to increase plant abiotic stress tolerance and to incorporate microbial genes into stressed plants is now being addressed and getting the interest of scientists in such studies. Furthermore, special attention should be drawn on isolating

bacteria from stressful conditions. Indeed, their use as bioinoculants could help to emerge a new dimension into the microbial inoculant application to plants under abiotic stress conditions. The application of isolated, characterized, and tested stress-tolerant microbial strains can enhance plant stress tolerance and could be used as a feasible strategy for improving crop production under the stressful conditions.

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Plant Growth-Promoting Fungi (PGPF): Phytostimulation and Induced Systemic Resistance

6

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Abstract

The associations between plants and multipurpose plant growth-promoting fungi (PGPF) have been proven extremely to be beneficial to plants. This review describes new knowledge about the interactions between plants and their associated PGPF in determining improved plant growth and induced systemic resistance (ISR) to invading pathogens. It has been shown that fungi of heterogeneous classes and habitats function as PGPF. The well-known fungal genera *Aspergillus*, *Fusarium*, *Penicillium*, *Piriformospora*, *Phoma*, and *Trichoderma* are the most frequently reported PGPF. On comparing the results of different studies, it appears that plant-PGPF interactions can have positive effects on belowground and aboveground plant organs. The most commonly reported effects are significant improvement in germination, seedling vigor, biomass production, root hair development, photosynthetic efficiency, flowering, and yield. Some strains have the abilities to improve plant biochemical composition. It has now known that PGPF can also control numerous foliar and root pathogens by triggering ISR in the host plants. These capabilities are driven by their abilities to enhance nutrient uptake and phytohormone production as well as to reprogram plant gene expression, through differential activation of plant signaling pathways. The PGPF-triggered plant growth and ISR responses to pathogen attack may work through genetype-dependent manner in plants.

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Plant growth promoting fungi • Induced systemic resistance • Defense response • Priming • Plant signaling pathway • Genetic variability

6.1 Introduction

Fertilizers and pesticides are the integral parts of the modern crop production inputs. Adequate access to pesticides and fertilizers is a prerequisite for smooth agricultural production and growth. The benefits of synthetic fertilizer and pesticide use in the crop field have been immense. They reduce crop losses due to nutrient deficiencies, weeds, diseases, and insect pests. The crop losses due to pests and diseases for eight of the world's major crops are estimated at US$244 billion per annum, accounting for 43% of world production (Oerke 2006), and postharvest losses contribute a further 10% (Edwards and Poppy 2009). Thus, the collective effects of increased fertilizer and pesticide use coupled with improved varieties and irrigation have significantly contributed to the improvement of grain yields since the late 1960s (Otsuka and Larson 2013). Consequently, the grain production per capita and the food-population balance have substantially been improved in many low-income countries and lagging regions, especially in Asia. Despite this success, the Green Revolution has yielded a range of unintended negative consequences on environment. Excessive use of fertilizer and pesticides has been associated with potentially highly detrimental effects on nontarget species and soil and water quality. Moreover, continuous use of pesticides over a long period results in developing resistance of the pest (Aktar et al. 2009). Overcoming these widespread hazards is a major challenge in contemporary agriculture, and the problem must be seriously addressed before their impacts on environment become irremediable.

It is well known that farm practices define the level of food production and, largely, the state of the global environment. The resource intensive current firm practices have been proven costly, as the environmental and health costs associated with fertilizer and pesticide use are higher (Soares and de Souza Porto 2012). Such big costs have already raised questions about the sustainability of the current production system. Sustainability is important as it ensures social, environmental, and economic acceptability of the farm practices. A sustainable production system relies on firm practices that seek to protect the environment by making a significant reduction in environmentally detrimental amounts of chemical inputs to the crop fields, while ensuring higher firm returns. Needless to say, efforts must be given in favor of green strategies, which are characterized by the development and diffusion of nontoxic and/or least-toxic alternatives for plant disease and nutrient management. Environmentally friendly preparations of multipurpose beneficial microbes seem to be one of the major substitutes of chemical inputs in agriculture. Currently huge research inventiveness is underway for the identification and utilization of beneficial microbes for plant growth and disease control.

Rhizosphere, the narrow zone of soil surrounding and influenced by plant roots, is a natural habitat for numerous beneficial microorganisms and represents a biologically complex ecosystem on Earth (Mendes et al. 2013). This biologically active zone is critical for plant-microbe interactions and, as a consequence, for nutrient cycling, plant growth, and resistance of plants to diseases. During positive plant-microbe interaction, rhizosphere colonization by soil microorganisms is beneficial for both plant and the microorganisms. Both partners derive benefits from the intimate association and vitalize each other. The large amount of rhizodeposits released by the plant roots is a key determinant of microbial activity and community structure in the rhizosphere (Gahan and Schmalenberger 2014). The rhizosphere microbes utilize the rhizodeposit carbon as a major energy source for their growth and development (Denef et al. 2007). Consequently, plant roots can manipulate the rhizosphere microbiome to its own benefit by selectively stimulating microorganisms with traits that are beneficial to plant growth and health (Mendes et al. 2013). Mutual interdependence and interplay between the rhizosphere microbiome and the plant result in the overall quality of plant productivity (Lakshmanan et al. 2014).

The rhizospheric microbial forms vary in diversity, which includes bacteria, fungi, nematodes, viruses, arthropods, oomycetes, protozoa, algae, and archaea. Beneficial effect of number of rhizosphere fungi with respect to plant growth promotion has long been known (Hyakumachi 1994). These plant growth-promoting fungi (PGPF) include species of the genera *Aspergillus*, *Fusarium*, *Trichoderma*, *Penicillium*, *Piriformospora*, *Phoma*, and *Rhizoctonia*, which have the natural ability to stimulate various growth-related traits of plants (Hossain et al. 2007, 2014; Shoresh et al. 2010). Many studies in dicots and monocots have shown that PGPF mimic the well-studied plant growth-promoting rhizobacteria (PGPR) in their interaction with host plant. As examples, treating seeds with PGPF inoculum can improve germination and seedling vigor of different plants. They can also induce longer and larger shoots. Some may exert effect on root development and performance. There are PGPF that may stimulate early and vigorous flowering of plants. Photosynthetic ability of the plant can also be enhanced by PGPF inoculation. Some PGPF have the ability to increase crop yield. They have also the ability to stimulate production of host secondary metabolites. These abilities are important to agriculture.

It is now established that plant growth-promoting activities by PGPF are only a fragment of their abilities. They also have the abilities to protect plant against the deleterious microorganisms. Suppression of plant diseases by PGPF can be achieved in many ways. Some PGPF produce antibiotics, some are parasite, while others compete with pathogens for food and space. Along with these direct antagonistic effects against pathogens, PGPF also protect plants by inducing systemic resistance. Induced systemic resistance (ISR) can be defined as the phenomenon by which plant exhibits increased level of resistance to broad spectrum of pathogens in a plant portion distant from the area where PGPF is active, caused by the triggering of active plant defenses (Pieterse et al. 2014). PGPF reduce the impacts of various fungi (Fontenelle et al. 2011; Murali et al. 2013; Tohid and Taheri 2015; Nassimi

and Taheri 2017), bacteria (Hossain et al. 2008a; Yoshioka et al. 2012; Hossain and Sultana 2015), viruses (Elsharkawy et al. 2012), and nematodes (Gottlieb et al. 2003; Vu et al. 2006) by eliciting ISR. These plant growth-promoting and disease control abilities are frequently considered to be the basis for how PGPF expedite the beneficial effects on plant (Fig. 6.1).

Over recent decades, interdisciplinary researches have made significant advances in understanding how these microorganisms interact with the host plants. It has been revealed that various signaling cascades modulate interaction of plants with PGPF. Furthermore, transcript-profiling analysis shows that plant response to PGPF depends on the complete reprogramming of a high number of genes or proteins in plants. Current knowledge also suggests that genetic variability in plant genotypes determines the outcome of phytostimulation and ISR interactions with PGPF. These illuminate the intensity of the interaction between plant and PGPF and favor the plasticity of the plant response to fine-tune the precise mechanisms. This chapter describes recent knowledge regarding PGPF's abilities and the underlying mechanisms for induction of plant responses.

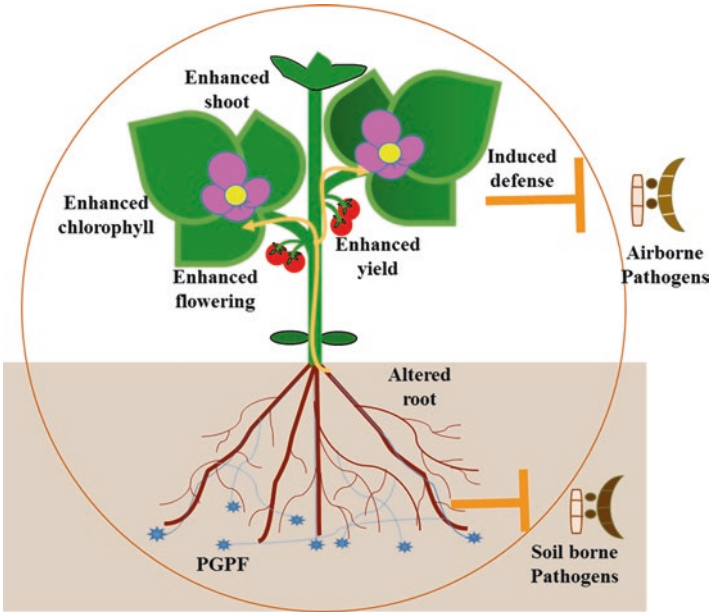

Fig. 6.1 Impact of plant growth-promoting fungi (PGPF) on plant growth promotion and disease suppression. PGPF stimulate shoot growth, root growth, photosynthetic efficiency, flowering, and yield. PGPF play a role in protection of plants against deleterious microorganisms by inducing systemic resistance

6.2 Nature and Diversity of Plant Growth-Promoting Fungi (PGPF)

Plant growth-promoting fungi (PGPF) are heterogeneous group of nonpathogenic fungi that are associated with plant and mediate improvements in plant growth and health. The classification of different fungi as PGPF does not represent any real biological similarity between fungi. Results from different studies indicate that the fungi under PGPF may differ distinctly from one another in taxonomy, in habitats, in physiology, and in their interaction with plants. Despite the name, PGPF do not always increase plant growth (Bent 2006). In reality, a fungus that promotes the growth of a given plant may not have same effect upon the growth of another plant, or the effect may vary under different set of environmental conditions. Similarly, not all fungi that promote plant growth are considered PGPF. For example, symbiotic mycorrhizal fungi are known to improve growth of the plants, but they are not considered as PGPF. Mycorrhizal fungi behave as obligate biotrophs and establish an intimate association with the roots of most host plants (Mehrotra 2005; Corradi and Bonfante 2012). On the other hand, PGPF are nonsymbiotic saprotrophic fungi that live freely in the root surface or the interior of the root itself or the rhizosphere. Therefore, the term PGPF is not any absolute term, rather it is an operational term (Bent 2006).

Microorganisms identified as PGPF have diverse taxonomy. According to the reported literatures, majority of true fungi characterized as PGPF primarily belongs to the phylum *Ascomycota* (*Aspergillus*, *Aureobasidium*, *Chaetomium*, *Cladosporium*, *Colletotrichum*, *Exophiala*, *Penicillium*, *Trichoderma*, *Fusarium*, *Gliocladium*, *Phoma*, *Phomopsis*, *Purpureocillium*, and *Talaromyces*), and a few of them belongs to *Basidiomycota* (*Limonomyces*, *Rhodotorula*, *Rhizoctonia*, and sterile fungi) and *Zygomycota* (*Mucor* and *Rhizopus*) (Table 6.1). A small number, like *Fusarium oxysporum*, *Colletotrichum*, and binucleate *Rhizoctonia*, is phylogenetically much related to plant pathogens but lack functional virulence determinants for many of the plant hosts from which they can be recovered. PGPF in mycelial fungi that do not produce any spores are known as sterile fungi. Most members in the *Oomycota* are usually virulent plant pathogens, while a few are nonpathogenic (Thines and Kamoun 2010). The nonpathogenic oomycetes *Pythium oligandrum* and *Phytophthora cryptogea* colonized the root ecosystems and acted as PGPF (Attitalla et al. 2001; Benhamou et al. 2012).

Species of PGPF are ubiquitous saprobes. Most PGPF have origin either in the soil or in the roots of large host range. On average 44% of the rhizosphere fungal isolates were PGPF (Hyakumachi 1994). This suggests that large portions of rhizospheric microorganisms are PGPF. However, the frequency of PGPF occurrence in the rhizosphere varies with crop plants. Some of the fungi that live inside root tissues or endophytes have also diverse positive effects on plant growth and are PGPF (Waqas et al. 2015). The most dominant endophyte appears to be *Fusarium* (25%), followed by *Penicillium* (12.5%) and *Alternaria* (7.5%) (Khalmuratova et al. 2015). Subsequent studies have also demonstrated the potential of phyllosphere fungi as PGPF (Limtong and Koowadjanakul 2012; Voříšková and Baldrian 2013), although the vast majority of studies have focused on phyllosphere bacteria and, to a lesser

Table 6.1 Different fungi reported as plant growth-promoting fungi (PGPF) with their original source of isolation

Systematics	PGPF strain(s)	Original source of isolation	References
Ascomycota	*Alternaria* sp.	Flower roots (*Rosa rugosa*, *Camellia japonica*, *Delonix regia*, *Dianthus caryophyllus*, and *Rosa hybrid*)	Zhou et al. (2014)
	Aspergillus sp.	Chili (*Capsicum annuum*)	Islam et al. (2014a)
	As. fumigatus	Soybean roots (*Glycine max*)	Hamayun et al. (2009a), and Khan et al. (2011a)
	As. niger	Tropical and subtropical soil, chickpea (*Cicer arietinum*) rhizosphere soil	Chuang et al. (2007), and Yadav et al. (2011)
	As. terreus	*Elymus mollis* roots	Waqas et al. (2015)
	As. ustus	Potato (*Solanum tuberosum*)	Marina et al. (2011)
	Aureobasidium pullulans	Dark Chestnut soil	Ignatova et al. (2015)
	A. pullulans	Golder shower tree leaves	Limtong and Koowadjanakul (2012)
	Candida maltosa	Unknown plant leaves	
	Chaetomium globosum	Pepper (*Capsicum annuum*) roots	Khan et al. (2012)
	Cladosporium sp.	Cucumber (*Cucumis sativus*) roots, flower roots (*Rosa rugosa*, *Camellia japonica*, *Delonix regia*, *Dianthus caryophyllus* and *Rosa hybrid*)	Hamayun et al. (2010), and Zhou et al. (2014)
	Colletotrichum sp.	Flower roots (*Rosa rugosa*, *Camellia japonica*, *Delonix regia*, *Dianthus caryophyllus* and *Rosa hybrid*)	Zhou et al. (2014)
	Exophiala sp.	Cucumber (*C. sativus*) roots	Khan et al. (2011b)
	Fusarium sp.	Bermuda grass (*Cynodon dactylon*)	Islam et al. (2014b)
	F. equiseti	Zoysiagrass (*Zoysia tenuifolia*) rhizosphere, *Lygeum spartum* roots	Kojima et al. (2013), and Maciá-Vicente et al. (2009)
	F. oxysporum	Cooking banana (*Musa* sp.) roots, diverse environment	Waweru et al. (2014), Bitas et al. (2015)
	Penicillium sp.	Halophyte roots	You et al. (2012)

	Penicillium koreense sp. *nov*	Rhizosphere soils of various Korean regions	You et al. (2014)
	Pe. chrysogenum	Rhizosphere soil and vegetables, sunflower, legumes, and cereal roots	Jogaiah et al. (2013)
	Pe. citrinum	Chickpea (*C. arietinum*) rhizospheric soil	Yadav et al. (2011)
	Pe. citrinum	*Ixeris repenes* (L.) roots, *Elymus mollis* roots	Khan et al. (2008), Waqas et al. (2015)
	Pe. janthinellum	*Zoysiagrass (Z. tenuifolia)* rhizosphere	Hossain et al. (2008a)
	Pe. kloeckeri	Soil	Yamagiwa et al. (2013)
	Pe. menonorum	Crop field soil	Babu et al. (2015)
	Pe. resedanum	*Capsicum annuum* roots	Khan et al. (2013)
	Pe. simplicissimum	Zoysiagrass (*Z. tenuifolia*)	Hossain and Sultana (2015)
	Pe. viridicatum	Zoysiagrass (*Z. tenuifolia*)	Hossain et al. (2014)
	Phoma sp.	Zoysiagrass *(Z. tenuifolia)* rhizosphere, flower (*Rosa rugosa*, *Camellia japonica*, *Delonix regia*, *Dianthus caryophyllus*, *Rosa hybrid*) roots	Sultana et al. (2008), and Zhou et al. (2014)
	Phoma herbarum	Soybean (*G. max*) roots	Hamayun et al. (2009b)
	Phoma multirostrata	Rhizosphere soil and vegetables, sunflower, legumes, and cereal roots	Jogaiah et al. (2013)
	Phomopsis sp.	Flower (*Rosa rugosa*, *Camellia japonica*, *Delonix regia*, *Dianthus caryophyllus*, *Rosa hybrid*) roots	Zhou et al. (2014)
	Phomopsis liquidambari	Inner bark of *Bischofia polycarpa*	Siddikee et al. (2016)
	Purpureocillium lilacinum	Soil	Cavello et al. (2015)
	Talaromyces wortmannii	Soil	Yamagiwa et al. (2011)
	Trichoderma asperellum	Soil	Yedidia et al. (2001)

(continued)

Table 6.1 (continued)

Systematics	PGPF strain(s)	Original source of isolation	References
	T. atroviride	Soil	Contreras-Cornejo et al. (2011)
	T. hamatum strain	Soil	Shaw et al. (2016)
	T. harzianum	Soil, rhizosphere soil, Zoysiagrass (*Z. tenuifolia*) rhizosphere, vegetables, sunflower, legumes, and cereal roots	Hyakumachi (1994), Brotman et al. (2013), Jogaiah et al. (2013), and Akhter et al. (2015)
	T. longibrachiatum	Soil	Zhang et al. (2016)
	T. pseudokoningii	Decorticated wood	Lee et al. (2016)
	T. viride (BBA 70239)	Water-damaged building	
	T. virens Gv. 29-8	Soil	Contreras-Cornejo et al. (2009)
Basidiomycota	Non-sporulating sterile fungi *Limonomyces roseipellis* SRF1	Wheat (*Triticum aestivum*) root	Andjic et al. (2005)
	Non-sporulating sterile fungi	Zoysiagrass *(Z. tenuifolia)* rhizosphere, wheat (*T. aestivum*) roots	Sultana et al. (2008), Andjic et al. (2005)
	Rhizoctonia solani	Tomato (*Lycopersicon esculentum*) roots	Muslim et al. (2003)
	Piriformospora indica	Thar desert, India	Bhuyan et al. (2015)
	Rhodotorula mucilaginosa	Dark chestnut soil	Ignatova et al. (2015)
Zygomycota	*Mucor* spp.	Zoysiagrass (*Z. tenuifolia*) rhizosphere	Hyakumachi (1994)
	Rhizopus	Arsenic-contaminated soil	Srivastava et al. (2012)
Oomycota	*Phytophthora cryptogea*	Tomato (*L. esculentum*)	Attitalla et al. (2001)
	Pythium oligandrum	Soil	Benhamou et al. (2012)

extent, phyllosphere fungi (Vorholt 2012). However, there are fewer number of PGPF in the phyllosphere as opposed to the rhizosphere. This is because the phyllosphere is a short-lived habitat for microorganisms and, more importantly, the rhizosphere microbes have better nitrogen capacity than those at the phyllosphere (Mwajita et al. 2013).

6.3 Impact of the PGPF on Plant Growth and Development

Plant growth-promoting fungi are generally believed to be beneficial for all plant species they associate with, because of their conserved beneficial abilities. PGPF directly and indirectly influence the growth and productivity of a wide range of host plants. The reported benefits derivable from plant-PGPF interactions include the improvements in seed germination rate, seedling vigor, root development and morphogenesis, shoot growth, yield, photosynthetic efficiency, flowering, and plant composition (Table 6.2). Recent studies have reported that certain PGPF strains promote plant growth through the production of plant growth-promoting compounds such as phytohormones and volatiles (Harman et al. 2004; Naznin et al. 2013). Plant growth promotion by PGPF may also variously arise from enhanced nutrient availability, amelioration of abiotic stresses, and antagonism to phytopathogens (Wakelin et al. 2007; Hossain et al. 2014). PGPF, most likely, stimulate plant growth through one or more of these remarkably diverse arrays of mechanisms.

6.3.1 Seed Germination and Seeding Vigor

The beneficial effects of PGPF are observed from the very early stage of plant development influencing germination and seedling growth. Various species of PGPF differ greatly in their effect on seed germination and seedling growth. Cucumber seeds sown in soil amended with *T. harzianum* propagules showed a ~ 30% increase in seedling emergence, 8 days after sowing (Yedidia et al. 2001). A significant increase in early seedling emergence and vigor was observed in tomato after seed priming with *T. harzianum* TriH_JSB27, *Phoma multirostrata* PhoM_JSB17, *T. harzianum* TriH_JSB36, and *Pe. chrysogenum* PenC_JSB41, *T. harzianum* Bi application (Jogaiah et al. 2013). Similarly, it was shown that treatment with *Trichoderma* spp. SL2 enhanced rice seed germination and vigor (Doni et al. 2014a). As per the findings of Mushtaq et al. (2012), presoaking of seeds in the culture filtrates of the nine *Penicillium* isolates was highly effective in significantly increasing seed germination in tomato when compared with the control seeds. Similar improvement in seed germination and seedling vigor in different plants was also found with treatment by other PGPF (Vujanovic and Goh 2012; Islam et al. 2014a, b) (Table 6.2).

PGPF colonization at the seed state has been proved to be beneficial for plant survival and timely seedling establishment (Baskin and Baskin 2004). Fungal isolates belonging to *Clonostachys rosea* controlled pre- and postemergence death caused by *A. dauci* and *A. radicina*, resulting in a higher number of healthy seedling

Table 6.2 Effect of different plant growth-promoting fungi on seed germination, plant growth, and yield in various plants

Growth traits	PGPF strain	Test crop	Specific effects	References
Germination and seedling vigor	*Talaromyces wortmannii* FS2	*Brassica campestris* L. var. *perviridis*	Enhanced seedling growth	Yamagiwa et al. (2011)
	Clonostachys rosea IK726	Carrot (*D. carota*)	Improved emergence and emergence time	Bennett et al. (2009)
		Onion (*Allium cepa*)		
	Cl. rosea	Carrot (*D. carota*)	Higher healthy seedling stand due to reduction in damping off caused by *A. dauci* and *A. radicina*	Jensen et al. (2004), and Szopińska et al. (2010)
	T. harzianum	Cucumber (*C. sativus*)	~ 30% increase in seedling emergence	Yedidia et al. (2001)
	Aspergillus spp. PPA1		Increased seed germination and seedling vigor	Islam et al. (2014a)
	Fusarium spp. PPF1	Indian spinach (*Basella alba*)	Higher germination percentage and increased vigor index	Islam et al. (2014b)
	T. harzianum	Maize (*Zea mays*)	Reduced *F. verticillioides* and fumonisin incidence and increased field emergence	Nayaka et al. (2010)
	T. harzianum Bi	Muskmelon (*C. melo*)	Augmented seed germination	Kaveh et al. (2011)
	Pe. chrysogenum, *Phoma* sp., and *T. koningii*	*Opuntia streptacantha*	Broke seed dormancy	Delgado-Sanchez et al. (2011)
	T. harzianum	Rice (*Oryzae sativa*)	Improvement in plant stand establishment	Rahman et al. (2015)
	Trichoderma spp. SL2		Increased seed germination and seedling vigor	Doni et al. (2014a, b)
	T. harzianum	Sunflower (*Helianthus annuus*)	Increased seed germination and seedling vigor	Nagaraju et al. (2012)
	Rhizopus sp.	*Thelocactus hexaedrophorus*	Broke seed dormancy	Arredondo et al. (2007)
	Penicillium spp.	Tomato (*Lycopersicon lycopersicum*)	Increased seed germination	Mushtaq et al. (2012)
	T. harzianum T-22		Under stress, treated seed germinated consistently faster and more uniformly	Mastouri et al. (2010)
	T. harzianum TriH_JSB27, TriH_JSB36		Enhanced early seedling emergence and seedling vigor	Jogaiah et al. (2013)
	Phoma multirostrata PhoM_JSB17			
	Pe. chrysogenum PenC_JSB41			

	Sphaerodes mycoparasitica	Wheat (*T. aestivum*)	Improved seed germination and seedling growth	Vujanovic and Goh (2012)
	Pi. Indica	Wheat (*T. aestivum*), chickpea (*Cicer arietinum*), bean (*Phaseolus vulgaris*)	Broke seed dormancy	Varma et al. (2012)
Shoot growth	*Pe. janthinellum* GP16-2	*Arabidopsis thaliana*	Increased shoot biomass and number of rosette leaves per plant.	Hossain et al. (2008a)
	Pe. simplicissimum GP17-2		~72% and 55% increase in shoot fresh and dry biomass, respectively, and one more rosette leaf per plant	Hossain et al. (2007)
	T. viride (BBA 70239)	*A. thaliana*, tomato (*L. lycopersicum*)	Increased fresh shoot weight	Lee et al. (2016)
	Fusarium oxysporum NRRL 38499, NRRL 26379, and NRRL 38335	*A. thaliana*, tobacco (*Nicotiana tabacum*)	~85% increase in shoot fresh and dry biomass	Bitas et al. (2015)
	T. virens Gv. 29-8	*A. thaliana*	Increased shoot biomass production	Contreras-Cornejo et al. (2009)
	Pe. citrinum IR-3-3	*Atriplex gemelinii*, *Waito-c* rice *(S. japonica)*	Enhanced shoot length	Khan et al. (2008)
	Preussia sp. BSL10	*Boswellia sacra*	Increased shoot length, number of internodes and leaf number	Khan et al. (2016)
	A. niger 1B and 6A	*Brassica chinensis*	Enhanced shoot biomass	Chuang et al. (2007)
	Pe. resedanum LK6	*Capsicum annuum* L.	Improved shoot growth	Khan et al. (2015)
	A. niger BHUAS01	Chickpea (*C. arietinum*)	Enhanced shoot length and shoot biomass	Yadav et al. (2011)
	Pe. citrinum BHUPC01			
	T. harzianum			

(continued)

Table 6.2 (continued)

Growth traits	PGPF strain	Test crop	Specific effects	References
	Pe. Menonorum	Cucumber (*C. sativus*)	~52% increase in shoot dry biomass	Babu et al. (2015)
	Pe. viridicatum GP15-1		Enhanced shoot length and shoot biomass	Hossain et al. (2014)
	Pe. simplicissimum GP17-2		Enhanced the plant shoot dry weight	Chandanie et al. (2009)
	T. harzianum GT3-2			
	F. equiseti GF19-1		Enhanced the shoot dry weight of cucumber plants	Saldajeno and Hyakumachi (2011)
	Aspergillus spp. PPA1		Augmented the shoot length as well as fresh and dry biomass	Islam et al. (2014a)
	Exophiala sp. LHL08		Enhanced shoot length, fresh weight, and dry weight	Khan et al. (2011b)
	Phoma sp. and sterile fungus		Increased shoot length, dry biomass, and number of leaves	Shivanna et al. (2005)
	Phoma sp.		Increased the shoot dry weight	Chandanie et al. (2005)
	T. harzianum T-22	GiSeLa6® (*Prunus cerasus* × *P. canescens*)	Improved shoot growth	Sofo et al. (2012)
	Fusarium spp. PPF1	Indian spinach (*Basella alba*)	Increased shoot length, shoot fresh and dry biomass	Islam et al. (2014b)
	F. oxysporum MSA 35	Lettuce (*Lactuca sativa*)	Increased shoot length (75.0%) and fresh weight (85.8%)	Minerdi et al. (2011)
	T. harzianum T-22	Maize (*Z. mays*)	Produced larger shoots	Harman et al. (2004)
	T. harzianum	Melon (*C. melo*)	Increased shoot fresh weight	Martínez-Medina et al. (2014)
	T. ghanense			
	T. hamatum			
	T. atroviride	*Miscanthus* × *giganteus*	Enhanced plant height	Chirino-Valle et al. (2016)
	Penicillium spp. NICS01 and DFC01	Sesame (*Sesamum indicum*)	Enhanced shoot length and biomass	Radhakrishnan et al. (2014)
	Aspergillus ustus	*Solanum tuberosum*, *A. thaliana*	Increased shoot fresh weight	Salas-Marina et al. (2011)

	Phoma herbarum TK- 2-4	Soybean (*Glycine max*)	Enhanced plant length and biomass	Hamayun et al. (2009b)
	A. fumigatus HK-5-2		Enhanced shoot length, shoot fresh and dry weight	Hamayun et al. (2009a)
	F. equiseti GF183	Spinach (*Spinacia oleracea*)	Improved shoot growth	Horinouchi et al. (2010)
	Alternaria sp. A7, A38	Tobacco (*N. tabacum*)	Increased shoot dry biomass and leaf area	Zhou et al. (2014)
	Phomopsis sp. H25			
	Cladosporium sp. B50			
	Cladosporium sp. MH-6	*Waito-c* rice *(Suaeda japonica)*	Improved shoot length, shoot fresh and dry biomass	Hamayun et al. (2010)
	Penicillium sp. Sj-2-2		Enhanced shoot length	You et al. (2012)
	A. niger NCIM	Wheat (*T. aestivum*)	~200% increase in shoot: Total length ratio	Gujar et al. (2013)
Root growth	*T. virens* Gv. 29-8	*Arabidopsis thaliana*	Induced production of lateral root (LR)	Contreras-Cornejo et al. (2009)
	P. indica		Increased number of RH	Peskan-Berghofer et al. (2004)
	As. ustus	*A. thaliana*, potato (*S. tuberosum*)	Increased root fresh weight and number of LR and RH	Salas-Marina et al. (2011)
	Ta. wortmannii FS2	*Br. campestris* L. var. *perviridis*	Increased root length (22.37%) and fresh biomass	Yamagiwa et al. (2011)
	T. harzianum T-22	Cherry rootstocks (*Prunus cerasus* x *P. canescens*)	Produced larger root	Sofo et al. (2011)
	Pi. indica	Chinese cabbage (*Brassica rapa*)	A twofold longer elongation zone, a 1.5-fold thicker epidermal and cortex layer, and a 1.4-fold higher biomass of the LR	Dong et al. (2013)
	Pi. indica	Chinese cabbage (*B. rapa*), *A. thaliana*	Promoted RH development	Lee et al. (2011)
	Pe. viridicatum GP15-1	Cucumber (*C. sativus*)	Enhanced fresh and dry weight and root length	Hossain et al. (2014)
	Epichloë festucae	*Festuca rubra*	Greater root biomass	Vázquez-de-Aldana et al. (2013)

(continued)

Table 6.2 (continued)

Growth traits	PGPF strain	Test crop	Specific effects	References
	T. harzianum T-22	GiSeLa6® (*Prunus cerasus* × *P. canescens*)	~76% increase in mean root length	Sofo et al. (2012)
	Fusarium spp. PPF1	Indian spinach (*Basella alba*)	Increased root length, root fresh and dry biomass	Islam et al. (2014b)
	F. oxysporum MSA 35	Lettuce (*Lactuca sativus*)	Increased root length and fresh weight	Minerdi et al. (2011)
	T. harzianum T-22	Maize (*Zea mays*)	Promoted deeper and robust roots with greater surface area	Harman et al. (2004)
	T. harzianum	Melon (*C. melo*)	Increased root fresh weight	Martínez-Medina et al. (2014)
	T. ghanense			
	T. hamatum			
	Dark spot endophytic fungus EF-37	*Saussurea involucrate*	Increased number of RH	Wu et al. (2010)
	Penicillium spp. NICS01 and DFC01	*Sesame (Sesamum indicum)*	Enhanced root length and biomass	Radhakrishnan et al. (2014)
	Mycocentrospora spp. (EF-37)	Snow lotus (*S. involucrate*)	Promoted root growth and number of RH	Wu et al. (2010)
	A. fumigatus HK-5-2	Soybean (*G. max*)	Enhanced root length, root fresh and dry weight	Hamayun et al. (2009a)
	F. equiseti	Spinach (*Spinacia oleracea*)	Improved root growth.	Horinouchi et al. (2010)
	M. robertsii	Switch grass (*Panicum virgatum*)	Increased root lengths, root hair (RH) density, and LR emergence	Sasan and Bidochka (2012)
		Haricot beans (*Phaseolus vulgaris*)		
	T. viride	Tomato (*L. lycopersicum*)	Enhanced LR development	Lee et al. (2016)
Photosynthetic efficiency	*T. viride* (BBA 70239)	*A. thaliana*, tomato (*L. lycopersicum*)	Increased chlorophyll content	Lee et al. (2016)
	Epichloë endophyte	*Achnatherum Inebrians*	Increased the chlorophyll content and net photosynthetic rate under *Blumeria graminis* infection	Xia et al. (2016)
	Pi. indica	*Aloe vera*	Increased Chl a, Chl b, and total Chl under high salinity	Sharma et al. (2016)

	Preussia sp. BSL10	*Boswellia sacra*	Enhanced photosynthetic pigments	Khan et al. (2016)
	Pe. menonorum	Cucumber (*C. sativus*)	Increased leaf chlorophyll contents	Babu et al. (2015)
	F. oxysporum MSA35	Lettuce (*L. sativa*)	~68% increase in leaf chlorophyll content	Minerdi et al. (2011)
	T. hamatum DIS 219b	Maize (*Z. mays*)	Increased chlorophyll contents in the drought-tolerant plant	Bae et al. (2009)
	T. virens		Increase photosynthetic rate	Vargas et al. (2009)
	T. atroviride TaID20G		Improved the chlorophyll under drought stress	Guler et al. (2016)
	Epichloë typhina	Orchard grass (*Dactylis glomerata*)	Improved chlorophyll b contents, abundance of LHCI and LHCII proteins and photosynthesis efficiency	Rozpądek et al. (2015)
	Metarhizium anisopliae LHL07	Soybean (*G. max*)	Higher chlorophyll contents and photosynthetic rate under salt stress	Khan et al. (2012)
	Pe. funiculosum LHL06		Chlorophyll contents in soybean plant under Cu stress	Khan and Lee (2013)
	As. fumigatus sp. LH02		Increased leaf area, chlorophyll contents, and photosynthetic rate	Khan et al. (2011a)
	Alternaria sp. A7, A38	Tobacco (*N. tabacum*)	Increased leaf chlorophyll content	Zhou et al. (2014)
	Phomopsis sp. H25			
	Cladosporium sp. B50			
Flowering	*Pe. chrysogenum*, *Saccharomyces cerevisiae*, *Pe. Aurantiogriseum*	*A. thaliana*	Promoted flowering	Sánchez-López et al. (2016)
	Pochonia chlamydosporia		Led to accelerated flowering	Zavala-Gonzalez et al. (2017)
	T. viride	*A. thaliana*, *C. forskohlii*	Showed robust and early flowering phenotype	Hung et al. (2014)
	Pi. indica	*Coleus forskohlii*	Induced early and vigorous flowering	Das et al. (2012)
	Sebacina vermifera	*Nicotiana attenuate*	Flowered earlier, produced more flowers, and matured more seed capsules	Barazani et al. (2005)

(continued)

Table 6.2 (continued)

Growth traits	PGPF strain	Test crop	Specific effects	References
	T. harzianum	Periwinkle (*Catharanthus roseus*), alyssum (*Lobularia maritima*), and marigold (*Tagetes erecta*)	Enhanced numbers of flower buds in chrysanthemum and petunia	Chang et al. (1986)
			Early flowering occurred in periwinkle, alyssum, and marigold	
	Pochonia chlamydosporia	Tomato (*L. lycopersicum*)	Led to accelerated flowering	Zavala-Gonzalez et al. (2015)
	T. viride (BBA 70239)		Acceleration of flower and fruit development	Lee et al. (2016)
	T. harzianum TriH_JSB27, and *Pe. Chrysogenum* PenC_JSB41		Induced early flowering	Jogaiah et al. (2013)
	Trichoderma spp.	*Verbena*, *Petunia*	Enhanced numbers and weight of flowers in verbena and numbers of flowers and buds in petunia	Ousley et al. (1994)
Crop yields	*Pochonia chlamydosporia*	*A. thaliana*	Increased seed production per plant	Zavala-Gonzalez et al. (2017)
	F. oxysporum V5 W2, Eny 7.11o, and Emb 2.4o	Banana (*Musa* sp.)	Up to ~20 to ~36% yield increase	Waweru et al. (2014)
	Pi. indica	Barley (*Hordeum vulgare*)	Increased grain yield	Waller et al. (2005)
	T. harzianum T-75	*Chickpea* (*Ci. arietinum*)	Enhanced grain yield in the field	Hossain et al. (2013)
	Phoma sp. and sterile fungus GU21-2	Cucumber (*C. sativus*)	Enhanced number and fresh biomass of marketable cucumbers	Shivanna et al. (2005)
	Pe. menonorum		Enhanced yield	Babu et al. (2015)
	T. harzianum	Mustard (*B. nigra*), Tomato (*L. lycopersicum*)	Enhanced yield	Haque et al. (2012)
	T. harzianum T-3	*Pea (Pisum sativum)*	Enhanced grain yield in the field	Akhter et al. (2015)
	T. viride	Sugarcane (*Saccharum officinarum*)	Increased cane yield	Yadav et al. (2009)
	T. harzianum		Increased millable canes, yield, and commercial cane sugar (CCS t/ha)	Srivastava et al. (2006)
	T. viride			
	Pi. indica	Thyme (*Thymus vulgaris*), *Foeniculum vulgare*	Increased essential oil yield	Dolatabadi et al. (2011)
	Sebacina vermifera			

	Po. chlamydosporia	Tomato (*L. lycopersicum*)	Increased number of marketable fruits, and fruits per plant, total fruit weight, and mature fruit weight per plant	Zavala-Gonzalez et al. (2015)
	T. viride (BBA 70239)		Enhanced tomato fruit yield	Lee et al. (2016)
	R. solani		Higher marketable and total yield	Muslim et al. (2003)
Photosynthetic and bioactive compounds	*Pi. indica*	*Aloe vera*	Higher phenol, flavonoid, flavonol, aloin contents, and radical scavenging activity at different salinity concentrations	Sharma et al. (2016)
	Pi. indica	*Artemisia annua* L.	Enhanced artemisinin content	Sharma and Agrawal (2013)
	Gilmaniella sp. AL12	*Atractylodes lancea*	Higher Sesquiterpenoid content	Wang et al. (2012)
	T. viride	*Coleus forskohlii*	Enhanced forskolin yield in roots	Boby and Bagyaraj (2003)
	Pi. indica	*Centella asiatica*	Enhanced asiaticoside content	Satheesan et al. (2012)
	Pi. indica	*Chlorophytum* sp.	Enhanced saponin content	Gosal et al. (2010)
	Pi. indica	*Coleus forskohlii*	Enhanced *p*-cymene in aerial parts	Das et al. (2012)
	Pe. menonorum	Cucumber (*C. sativus*)	Enhanced starch and protein content in leaves	Babu et al. (2015)
	Pi. indica	Fennel (*Foeniculum vulgare*)	Increased anethole level in fruit	Dolatabadi et al. (2011)
	Sebacina vermifera			
	Pi. indica	*Linum album*	Enhanced production of podophyllotoxins in *L. album* cells	Baldi et al. (2010)
	Neotyphodium lolii	*Lolium perenne* cv SR4000	Higher accumulation of soluble sugars in leaves under mild drought stress and starch under severe drought stress	Ren et al. (2006)
	Westerdykella aurantiaca FNBR-3	Rice (*O. sativa* L. var. IR-36)	Enhanced carotenoid and protein content	Srivastava et al. (2012)
	T. longibrachiatum FNBR-6	Pea (*P. sativum* L. var. PG-3)		
	T. atroviride D16	*Salvia miltiorrhiza*	Higher tanshinone I (T-I) and tanshinone IIA (T-IIA) content	Ming et al. (2013)
	Pi. indica	*Spilanthes calva*	Increase in spilanthol content	Rai et al. (2004)

(continued)

Table 6.2 (continued)

Growth traits	PGPF strain	Test crop	Specific effects	References
	T. harzianum	Sunflower (*H. annuus*)	Starch, total soluble sugars, reducing sugar, phenol, lipid and linoleic acid content	Lamba et al. (2008)
	Pi. indica	Thyme (*Thymus vulgaris*)	Enhanced level of thymol in fruit	Dolatabadi et al. (2011)
	Se. vermifera			
	As. niger	Tomato (*L. lycopersicum*)	Increased accumulation of salicylic acid, total phenolic and chlorophyll contents of plant, as well as lycopene, ascorbic acid (Vitamin C), and Brix index of fruit	Anwer and Khan (2013)
	T. longibrachiatum T6	Wheat (*T. aestivum*)	Higher soluble sugar and protein content	Zhang et al. (2016)

stand in carrot (Jensen et al. 2004; Szopińska et al. 2010). Priming seed with the same fungus also improved rate and time of seedling emergence in carrot and onion (Bennett et al. 2009). Maize seed treated with *T. harzianum* reduced the *F. verticillioides* and fumonisin incidence and increased the field emergence (Nayaka et al. 2010). Rahman et al. (2015) reported that *T. harzianum* seed treatment significantly contributed to the improvement of plant stand establishment in rice. These demonstrate that PGPF facilitate seed germination by nullifying adverse effects of dangerous seed-borne pathogens (Szopińska et al. 2010). Some PGPF may also function to overcome seed dormancy. Seed treatment with *P. indica* culture filtrate was effective in breaking the seed dormancy of *Triticum aestivum*, *Cicer arietinum*, and *Phaseolus vulgaris* (Varma et al. 2012). Arredondo et al. (2007) found that *Rhizopus* sp. was moderately effective in breaking dormancy of *Thelocactus hexaedrophorus* seeds. Olvera-Carrillo et al. (2009) observed that 7-month-old exhumed seeds of *Opuntia tomentosa* were colonized by fungal hyphae that penetrated the funicular envelope through the openings and favored germination of the weak embryo. Delgado-Sánchez et al. (2011) reported that inoculation of *O. streptacantha* seed with *P. chrysogenum*, *Phoma* sp., and *T. koningii* helped to break seed dormancy. Scanning electron microscopy revealed that these fungi had been able to erode the funiculus, thus reducing its resistance to germination. It may be possible that enzyme production by the fungal hyphae assists in seed stratification or replacement of scarification process. Fungi may also grow on the testa and erode or crack the hard stony endocarp. Consequently, they can potentially reduce mechanical resistance to germination (Morpeth and Hall 2000). The other possibilities are production of germination-inducing volatiles and degradation of water-soluble germination inhibitors associated with the outer surface of the seed (de Boer et al. 2005).

Orchid seeds also need a fungus for germination in nature. Orchid seeds lack endosperm and no significant food reserves. Exogenous supply of carbohydrates is required for orchid seed germination. After the formation of the protocorm, additional development does not occur until sugar molecules are supplied. Symbiotic fungi are the main source of sugars. When hyphae are broken, sugars are released into the orchid cells. The most common genus of fungi that stimulates germination of orchids and promotes growth of protocorms and seedlings is *Rhizoctonia* (Chou and Chang 2004). In addition, *Penicillium*, *Chaetomium*, *Choanephora*, and some other fungi are also known to stimulate germination in orchid seeds (Baskin and Banskin 2014). This improvement in germination and seedling vigor is attributed to the provision of compounds essential to germinating seeds and young plants by PGPF. Production of hormones such as gibberellins (GAs) and cytokinin (CK) by the fungi may also have a role in stimulating seed germination (Gupta and Chakrabarty 2013).

6.3.2 Shoot Growth

Although PGPF is restricted to roots, there are numerous changes in the phenotypic responses of shoots, indicating that the effects of these fungi are systemic. There are numerous field and growth chamber experiments, which have reported the shoot growth enhancement by PGPF. Members of the genus *Aspergillus*, *Fusarium*, *Trichoderma*, *Penicillium*, *Rhizoctonia*, *Exophiala*, *Phoma*, *Alternaria*, *Phomopsis*, *Cladosporium*, and *Colletotrichum* were often the most effective in eliciting their effects on shoot growth (Table 6.2). Shoot growth enhancement has been observed across a broad range of species, including Arabidopsis, tomato, tobacco, *brassica chinensis*, chilli, chickpea, cucumber, Indian spinach, lettuce, maize, melon, sesame, potato, soybean, spinach, wheat, etc. Reported studies have revealed that inoculation of these plants with PGPF promotes significantly greater shoot length and/or shoot biomasses in these plants. Application of root endophytic *Trichoderma* isolates significantly enhanced plant height of a second-generation energy crop *Miscanthus* × *giganteus* (Chirino-Valle et al. 2016). Similarly, inoculation with a *Pe. menonorum* isolate significantly increased the dry biomass of cucumber shoots (~52%) (Babu et al. 2015). Some species have been shown to produce large-leaved plants. Cucumber plants inoculated with a PGPF *Pe. simplicissimum* GP17-2 grew larger and produce ~1.5–2.0 times larger leaf than normal plants (Fig. 6.2). The results are in agreement with numerous growth chamber and field experiments, which have shown that PGPF inoculants can modulate plant shoot growth (Table 6.2).

Proteomes or genes triggered by PGPF in treated plants exhibit the mechanisms associated with the enhanced stem and leaf growth. Shoresh and Harman (2008)

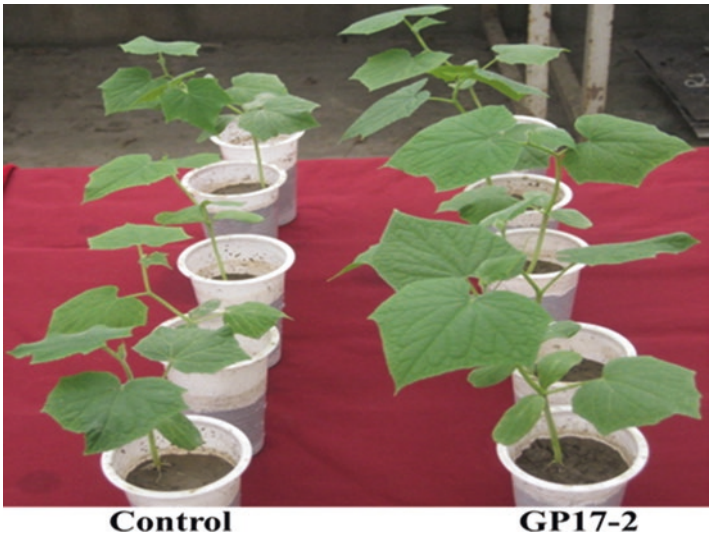

Fig. 6.2 Seedlings of cucumber cv. Baromashi (21 days old) grown in soil treated with (GP17-2) or without (control) a PGPF *Penicillium simplicissimum* GP17-2

revealed that proteins involved in carbohydrate metabolism were strongly affected in the shoots due to *Trichoderma* colonization of maize roots. The important identified proteins included fructokinase (FRK), Fru-bisphosphate aldolase (FBA), glyceraldehyde-3-P dehydrogenase (GAPDH), malate dehydrogenase (MDH), β-glucosidases, 3-phosphoglycerate kinase, and oxalate oxidases. FRK2 from tomato was shown to be expressed abundantly in leaves and essential for stem growth and vascular development (Odanaka et al. 2002; Damari-Weissler et al. 2009). Suppression or reduced expression of this gene resulted in smaller cell size in the xylem and phloem and much shorter plants (Odanaka et al. 2002; Damari-Weissler et al. 2009). Strong expression of FRK2 in stems confirms a similar role. Cotton plants transformed with a tomato fructokinase gene (*LeFRK1*) had larger leaf areas and stem diameters (Mukherjee et al. 2015). Increased FBA in plastids enhances growth of tobacco plants (Uematsu et al. 2012). As a member of the tricarboxylic acid cycle, MDH is involved in providing reducing power and is involved in photosynthetic fixation of CO_2 (Nunes-Nesi et al. 2005). Single- and double-knockout mutants of the mitochondrial MDH isoforms in Arabidopsis showed no detectable MDH activity, and the resulted plants were small and slow growing. These confirm that activation of carbohydrate metabolism in plants by PGPF contributes to the enhanced shoot growth.

Plant growth-promoting effect of PGPF is not necessarily limited to direct interaction of plants with fungi in the rhizosphere. Fungal elicitors such as culture filtrate produced by PGPF have also demonstrated a strong positive influence on the shoot growth of plants. Addition of *T. harzianum* culture filtrate in the growth medium of *Centella asiatica* resulted in significantly higher shoot dry weight (Prasad et al. 2012). Culture filtrate of *F. oxysporum* and *T. viridi* also significantly enhanced shoot growth of Arabidopsis and tomato, respectively (Bitas et al. 2015; Lee et al. 2016). The presence of gibberellic acids (GA4, GA9, and GA34), indole-3-acetic acid (IAA), and high concentrations of phosphate in the fungal culture filtrate is responsible for promoting host shoot growth (Khan et al. 2008; Kang et al. 2015). PGPF species are also abundant producers of small volatile metabolites. Co-cultivating plants with volatile-producing fungi or exposure of plants directly to volatile organic compounds (VOCs) induces shoot growth. Fungal VOCs emitted by different species and strains of *Trichoderma* augmented plant biomass and size of Arabidopsis (Lee et al. 2016). Similarly, tobacco plant growth was enhanced significantly, when they were grown in the presence of VOCs produced by *Phoma* sp. (Naznin et al. 2013). The PGPF VOCs have diverse chemical structures and are produced as mixture of hydrocarbons, ketones, amines, thiols, terpenes, alcohols, aldehydes, acids, ethers, esters, and their derivatives (Korpi et al. 2009; Lemfack et al. 2014; Lee et al. 2016). Their effects on plant growth depend on fungal species, culture conditions, plant developmental stage, and duration of the exposure (Hung et al. 2013; Lee et al. 2015). It is thought that promotion of plant growth by microbial VOCs is mainly due to CO_2 enrichment during co-cultivation (Kai and Piechulla 2009). However, Bitas et al. (2015) found no significant difference in CO_2 production among volatile-producing and neutral strains of *F. oxysporum*. Therefore, increased CO_2 production solely may not drive plant growth enhancement by PGPF VOCs.

6.3.3 Root Growth and Performance

The main functions of plant roots are to explore soil and acquire nutrients to support growth and development of the plant. The plant root system is in closest contact with soil microbial populations; therefore, the root system functions under the direct influence of microbial interaction. Many of the reported PGPF have long been known to significantly enhance the root growth. Plants inoculated with some PGPF had greater root biomass of the root system than the control plants (Zhang et al. 2012; Vázquez-de-Aldana et al. 2013; Hossain et al. 2014; Islam et al. 2014b). Other effects associated with PGPF colonization on roots were faster-growing roots and roots that grew for prolonged periods, causing the development of longer and larger root systems (Björkman et al. 1998; Hossain et al. 2014). Maize roots inoculated with *Trichoderma* were deeper, more robust, and had greater surface area (Harman et al. 2004). Similarly, the treatment of potting medium with barley grain inoculum of *Pe. simplicissimum* GP17-2 significantly increased root growth of cucumber plants, producing a longer and larger root system 3 weeks after planting (Fig. 6.3). There are also PGPF strains that can cause alterations in the root system architecture (RSA) of host plants. RSA is a complex notion that captures aspects of root structure and root shape (Pages 1992). The importance of RSA lies in the fact that it is a key determinant of nutrient- and water-use efficiency in plants. Moreover, RSA determines largely the extent of contact and interaction between the plant and the rhizosphere (Orman-Ligeza et al. 2013). The RSA is evolved from three main processes: (1) indeterminate growth of the main root, a process originated by the root meristem; (2) lateral root (LR) formation; and (3) root hair (RH) formation (Scheres et al. 2002). Each of the apparatuses that constitute the RSA has distinct roles. However, LR and RH constitute the most important traits of the root architecture that facilitate plant anchorage and increase the root's exploratory capacity for water and minerals. PGPF are well noted for their effects on LR and RH morphology.

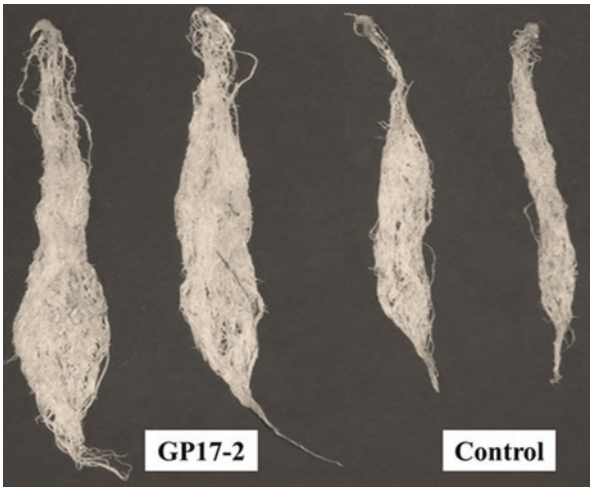

Fig. 6.3 Roots of cucumber cv. Baromashi (21 days old) grown in soil treated with (GP17-2) or without (control) a PGPF *Penicillium simplicissimum* GP17-2

Increased root branching via LR formation has been observed as a response to colonization by some PGPF species (Harrison 2005). *Trichoderma* spp. were highly efficient in inducing LR production in *A. thaliana* (Contreras-Cornejo et al. 2009). Inoculation of *As. ustus* on *A. thaliana* and *S. tuberosum* roots induced an increase in root growth and LR and RH numbers (Salas-Marina et al. 2011). The dark spot endophytic fungus EF-37 increased the RH number in *Saussurea involucrata* (Wu et al. 2010). RH development was strongly promoted in Chinese cabbage and *A. thaliana* by *Pi. indica* (Lee et al. 2011). On average, *Pi. indica* colonization resulted in a ~ 2-fold longer elongation zone, a ~ 1.5-fold thicker epidermal and cortex layer, and a ~ 1.4-fold higher biomass of the lateral roots, compared with the uncolonized control (Dong et al. 2013). This basidiomycete alters root growth in a number of other plant species (Varma et al. 1999; Peskan-Berghofer et al. 2004). Other endophytic fungi also cause similar changes in LR and RH (Malinowsky et al. 1999; Sasan and Bidochka 2012). There are also fungi that stimulate lateral root formation and increase root hair length through release of VOCs (Felten et al. 2009).

The mechanisms by which PGPF alter root systems have recently been started to be dissected at the genetic and molecular levels (Contreras-Cornejo et al. 2009). Stimulation of LR development seems to be an early phase of interaction in nonphytopathogenic, root-colonizing fungi (Felten et al. 2009). Microbial-induced increase in the number and/or length of LR and RH is thought to be caused by reduction in growth rate of the primary root (Contesto et al. 2008; Combes-Meynet et al. 2011; Chamam et al. 2013). Signals originating from the fungi target primarily the meristematic elongation zone in roots and activate the growth-stimulating programs (Dong et al. 2013). Auxin has a critical role during this developmental process from founder cell specification to LR emergence (Dubrovsky et al. 2008). However, high fungal IAA (auxin) production does not always lead to the highest rooting frequency (Niemi et al. 2002). Similarly, exogenous application of auxin did not stimulate the morphological changes in Chinese cabbage roots, which were observed after *Pi. indica* colonization (Lee et al. 2011). These observations are in line with a study by Hilbert et al. (2012) which have also demonstrated that production of indole derivatives by the fungus is not required for growth promotion of barley root. Therefore, the root-stimulating effects are suggested to be mediated by auxin of plants and not fungal auxin (Lee et al. 2011).

A decrease in CK content was induced by the isolates of *Trichoderma* that promoted the root growth of melon plants (Martínez-Medina et al. 2014). Sofo et al. (2011) also observed a significant decrease in trans-zeatin and in dihydrozeatin, two of the most active CKs in plants shoots and roots, following the inoculation with *T. harzianum* T-22. This indicates that CK has an opposing role in root development, although major sites of CK synthesis are considered to be root tips (Aloni et al. 2005). Exogenous application of CK at physiological concentrations suppresses root growth and reverses the IAA effects (Lloret and Casero 2002). A low CK level in CK-deficient transgenic plants overexpressing the CK oxidase/dehydrogenase (*CKX*) genes is seen to cause an enlarged root meristem, formation of LR closer to the root apical meristem, increased root branching, and promotion of adventitious root formation (Lohar et al. 2004). Similarly, abscisic acid (ABA) and ethylene (ET)

cascades share some common features in terms of mediation of root growth. The concentration of ABA and the ET precursor 1–aminocyclopropane-1–carboxylate (ACC) was decreased by isolates of *T. harzianum* (T-4, T-7, and T-22) (Martínez-Medina et al. 2014). A low concentration of both promotes root growth, and high concentrations inhibit root growth (Joshi-Saha et al. 2011; Arc et al. 2013). Previous studies have demonstrated root that growth inhibition by high concentrations of ABA requires ET signaling components but not ET production (Beaudoin et al. 2000; Ghassemian et al. 2000). This discussion implies that, as for other physiological processes, root growth is usually not regulated by hormonal levels per se but rather the complex balances between various hormones (Müller and Leyser 2011).

6.3.4 Photosynthetic Efficiency

The main source of carbon for green plants is photosynthesis. Higher photosynthetic potential may result in increased carbon assimilation in plants, which is the basis for faster development and higher biomass production. It has been reported that many of the studied PGPF clearly influence photosynthesis-related mechanisms in plant allowing to meet elevated energy demands. The changes in leaf architecture, leaf numbers, leaf chlorophyll levels, and photosynthetic rate are often the effects associated with plant's response to PGPF colonization. According to earlier reports, Arabidopsis plants treated with *Pe. simplicissimum* GP17-2 and *Pe. janthinellum* GP16-2 increased number of rosette leaves per plant (Hossain et al. 2007, 2008a), while soybean plants inoculated with *As. fumigatus* sp. LH02 significantly increased leaf area, chlorophyll contents, and photosynthetic rate as compared to non-inoculated plants (Khan et al. 2011b). Similar increases in the content of photosynthetically active pigments as well as the photosynthesis efficiency were reported in plants upon different PGPF colonization (Babu et al. 2015; Rozpądek et al. 2015; Khan et al. 2016; Per et al. 2016). Additionally, the abundance of light-harvesting chlorophyll a−/b-binding proteins LHCI and LHCII was significantly higher in *Epichloë typhina*-treated orchard grass (Rozpądek et al. 2015).

Many of these studies also show that PGPF is utilized to enhance photosynthesis under suboptimal conditions. Bae et al. (2009) observed increased chlorophyll contents in the drought-tolerant *T. hamatum* DIS 219b–colonized seedlings. *Metarhizium anisopliae* LHL07-inoculated soybean plants showed significantly higher chlorophyll contents, transpiration rate, photosynthetic rate, and leaf area, under salt stress as compared to non-inoculated control plants (Khan et al. 2012). Similarly, *Pe. funiculosum* LHL06 symbiosis increased chlorophyll contents in soybean plan under Cu stress (Khan and Lee 2013). Root colonization with *T. atroviride* TaID20G improved the chlorophyll and carotenoid synthesis in maize seedlings, contributing to the alleviation of the drought stress (Guler et al. 2016). PGPF also increase the chlorophyll content and photosynthetic rate in host plant under pathogen stress (Vargas et al. 2009; Xia et al. 2016). Loss of chlorophyll and carotenoid contents under biotic and abiotic stress regimes are frequently the primary causes of inactivation of photosynthesis (Xia et al. 2016). Hence, the positive effects of PGPF on

photosynthesis in plants can be ascribed, at least partially, to very efficient use of light as a consequence of enhanced accumulation of photosynthetic pigments and improved net photosynthetic rate (Sánchez-López et al. 2016).

Until recently, little is known about the molecular mechanisms of PGPF-mediated photosynthesis improvement in plants. PGPF may have the ability to switch the cellular mechanisms in the shoot, in consequence increasing photosynthetic efficiency. In order to elucidate the key changes in photosynthesis-related protein levels in plant shoots, Shoresh and Harman (2008) have examined the expression of proteins in maize shoot after root colonization by *T. asperellum* T-22. Upregulation of four spots associated with photosynthesis, including two forms of Rubisco large subunit, Rubisco, and PSII oxygen-evolving complex protein 2, were observed in shoots of *T. harzianum* T-22-treated plants. Similarly, Vargas et al. (2009) detected the transcriptional upregulation of two photosynthetic genes, rubisco small subunit (*rbc*S) and the oxygen-evolving enhancer 3–1 (*oee3–1*), in leaves of maize plants inoculated with *T. virens.* Upregulation of *rbc*S was also identified in the leaves of *Trichoderma*-challenged common bean plants (Pereira et al. 2014). The increased expression of these photosynthesis genes is suggestive of a higher photosynthetic rate in PGPF treated than control plants. Moreover, photosynthesis is generally subject to feedback inhibition by elevated sugar levels in plants (Rolland et al. 2006). Degradation of sucrose inside fungal cells might have a positive effect on the photosynthesis, as it reduces sugar levels. Vargas et al. (2009) demonstrated that the upregulation of the photosynthetic genes and photosynthetic rate in leaves were dependent on sucrose degradation in *T. virens* cells during mutualistic association. Consequently, when *Trichoderma* colonizes roots, the increased demand of photoassimilates alters the carbon partitioning toward the organs, causing a stimulation of the photosynthetic process in leaves (Vargas et al. 2013). On the contrary, *Alternaria alternata* VOC-promoted enhancement of photosynthesis was accompanied by accumulation of high levels of soluble sugars in the leaves (Sánchez-López et al. 2016). The lack of photosynthetic inhibition by high sugar content in leaves of VOC-exposed plants might be due to enhanced CK production, as CKs and sugars work antagonistically in gene-regulated responses (Kushwah and Laxmi 2014).

6.3.5 Flowering

The application of some PGPF strains seems to influence phenotypic plasticity of flowering, an important ecological trait for plants and their communities (Forrest and Miller-Rushing 2010). Although flowering phenology is known to be under strong genetic control, it also responds to different stimuli including temperature (Aikawa et al. 2011), water availability (Crimmins et al. 2013), herbivory (Brys et al. 2011), and pathogen infection (Korves and Bergelson 2003). Similarly, PGPF have also been found as a possible driver of flowering phenology in plants. It has shown that root inoculation with PGPF may stimulate flowering time, flower numbers, and/or size in the host plant (Table 6.2). Early reports of the effects of the *Trichoderma* spp. on floricultural crops indicated that when the fungus was applied

to soil as a peat-bran formulation, the numbers of flower buds were enhanced in chrysanthemum and petunia, while early flowering occurred in periwinkle, alyssum, and marigold (Chang et al. 1986). Similarly, adding *Trichoderma* as dried fermenter to the growing medium of flower plants enhanced the numbers and weight of flowers in verbena and the numbers of flowers and buds in petunia (Ousley et al. 1994). Early and vigorous flowering was also observed in *C. forskohlii* after inoculation of its root with *Pi. indica* (Das et al. 2012). Under greenhouse conditions, two PGPF *T. harzianum* TriH_JSB27 and *Pe. Chrysogenum* PenC_JSB41 induced early flowering in tomato (Jogaiah et al. 2013). The root-colonizing nematophagous fungus *Pochonia chlamydosporia* hastened flowering in tomato and Arabidopsis (Zavala-Gonzalez et al. 2015). Plants grown in the presence of VOCs emitted by different fungal species have also been reported to show robust and early flowering phenotype. Arabidopsis plant exposed to VOCs emitted by phylogenetically diverse fungi such as *T. viride*, *Pe. chrysogenum*, *Saccharomyces cerevisiae*, and *Pe. aurantiogriseum* had increased number of flowers in Arabidopsis (Hung et al. 2014; Sánchez-López et al. 2016).

Plants often exploit various interconnecting mechanisms, including photoperiod, vernalization, hormone biosynthesis, nutrient uptake, and aging pathways to shorten the vegetative growth period and hasten flowering (Song et al. 2013). Enhancement of flower production in PGPF-treated plant may be due to an increase in plant nutrient (especially K+) uptake in combination with one or more of the abovementioned mechanisms (Perner et al. 2007). Hormones, such as GAs, are involved in the regulation of bud production and early flowering in plants (Zhang et al. 2014). Higher levels of K+ in the plant are responsible for faster transport of GAs (Das et al. 2012). Some studies have emphasized the importance of phosphorus on the impact on bud formation and development and the number of flowers (Poulton et al. 2002). Furthermore, CKs also play important roles in flowering by stimulating floret primordia differentiation and ovule development (Riefler et al. 2006; D'Aloia et al. 2011; Zhang et al. 2014). In contrast, nitric oxide (NO) is known to participate in plant flowering repression (Shi et al. 2012). Fungal VOC-promoted early flowering involves suppression of NO action through the scavenging of NO molecules by CKs (Sánchez-López et al. 2016). It is likely that PGPF may utilize one or more of these flowering mechanisms.

6.3.6 Crop Yields

Global yields of many crops have been somewhat static during the last two decades (Gopalakrishnan et al. 2015). Many studies have proposed to use PGPF as an eco-friendly and sustainable tool to enhance the yield of different crop plants (Table 6.2). Commercial trials on several *T. harzianum* T-22-treated hybrids and inbred lines have revealed the yield increases in most genotypes (Harman et al. 2004). Application of *T. harzianum* and *T. viride* was significantly effective in improving millable canes (~5–30%), yield (~6–38%), and CCS (commercial cane sugar) t/ha (~30–34%) over the control in plant cane (Srivastava et al. 2006). Similarly, application of 50% N fertilizer

along with 50% *Trichoderma*-enriched biofertilizers has resulted in ~ 108% and ~203% yield increase in mustard and tomato, respectively, over the control (Haque et al. 2012). In strawberry, lettuce, chickpea, and pea, crop yields were also increased significantly following the application of *Trichoderma* spp. (Elad et al. 2006; Bal and Altintas 2006; Hossain et al. 2013; Akhtar et al. 2015). Treatment with *Pe. menonorum* was useful in increasing the yields of cucumber plants (Babu et al. 2015). Inoculation of banana plants with *F. oxysporum* strains resulted in up to ~20 to ~36% yield increase (Waweru et al. 2014). Root colonization by *Pi. indica* results in an overall increase in grain yields in barley (Waller et al. 2005) and oil yields in *Thymus vulgaris* and *Foeniculum vulgare* as compared with non-colonized plants (Dolatabadi et al. 2011). Application of HBNR isolates to tomato plants in greenhouses resulted in consistent and higher marketable and total yields, which were ~70–73% higher than untreated plants (Muslim et al. 2003). These examples are a few of many that demonstrate the yield benefit from plant-PGPF interactions (Table 6.2).

The exact reason for increased yields seems to be unclear yet, but in most cases, it is probably due to greater supply of nutrients by PGPF to plants. Yedidia et al. (2001) suggested that presence of PGPF in the rhizosphere increases root surface area allowing the roots to explore larger volumes of soil; thus, more nutrients become available to the plants especially under nutrient-stressed soil environments. In vitro studies have shown that micronutrients and insoluble phosphates become soluble and available by PGPF treatments, therefore useful to the roots interacting with PGPF in the root zone (Waklin et al. 2007). PGPF also have the ability to increase nitrogen-use efficiency in crops (Alberton et al. 2013) and to ameliorate biotic and abiotic stresses (Shoresh et al. 2010). Some PGPF strains show abilities to improve photosynthetic efficiency (Babu et al. 2015). All of these capabilities singly or in combination contribute to improve crop yield.

6.3.7 Photosynthetic and Bioactive Compounds

Positive effects of PGPF are not always limited to the growth and yields; rather many species of PGPF are associated with the biochemical changes in the colonized plants. It is believed that some PGPF are quality enhancers and treatment with them alters the photosynthetic product content in plants (Table 6.2). The application of *T. harzianum* and *Ps. fluorescens* led to increases in starch, total soluble and reducing sugar, and phenol contents in leaves of sunflower (*Helianthus annuus*). There was also a significant increase in seed lipid content and the proportion of linoleic acid (Lamba et al. 2008). In a greenhouse study, plants inoculated with inocula of *Westerdykella aurantiaca* FNBR-3 and *T. longibrachiatum* FNBR-6 significantly improved total carotenoid and protein contents of the plant leaves in rice and pea (Srivastava et al. 2012). Application of isolates of *As. niger* significantly caused higher accumulation of total phenolic, salicylic acid, and chlorophyll contents of plant, as well as lycopene, ascorbic acid (vitamin C), and Brix index of tomato fruit compared to untreated control (Anwer and Khan 2013). PGPF inoculation also improve the levels of different photosynthetic compounds under stress and help the

plants ameliorate oxidative stress resulting from high stress. Under mild drought stress, endophyte fungus *Neotyphodium lolii* enhanced the accumulation of soluble sugars in *Lolium perenne* cv SR4000 plants to improve their osmotic ability (Ren et al. 2006). When stress have been intensified, the improvement by endophyte no longer sustained, but other photosynthetic products such as starch were accumulated in the endophyte-infected plants to survive through the undesirable conditions. Similarly, application of *T. harzianum* T6 increased the soluble sugar and protein contents in the wheat seedlings grown under salt stress, compared to the control (Zhang et al. 2016). Sharma et al. (2016) investigated the effect of *Pi. indica* inoculation on salinity stress tolerance of *Aloe vera* plant and observed significantly higher phenol, flavonoid, flavonol, and aloin contents as well as improved radical scavenging activity in the inoculated plantlets as compared to non-inoculated controls at all salinity concentrations. The increased accumulation of these compounds in plants usually indicates a highly protective mechanism against oxidative damage caused by high stress in the plant environment (Bartels and Sunkar 2005). Accordingly, PGPF-inoculated plants are likely to recover from undesirable conditions more rapidly than non-inoculated plants.

Many of the PGPF have developed the ability to enhance the production of bioactive substances originated from the host plants. In addition to their role in conferring fitness benefits to host plants, many of these secondary metabolites have interesting applications in industry. For example, *Coleus forskohlii* is a perennial medicinal shrub of the mint family (Lamiaceae) and has been used in traditional medicine for treating a broad range of human health disorders (Lukhoba et al. 2006). The main active compound of *C. forskohlii* is forskolin, which is known for its broader pharmacological activities (Li and Wang 2006; Wagh et al. 2012). The forskolin concentration in roots of *C. forskohlii* was enhanced by dual inoculation with *Glomus mosseae* and *T. viride* (Boby and Bagyaraj 2003). Others report that the effect of bioinoculation on the production of secondary metabolites was negative. For example, Das et al. (2012) found the reduced contents of forskolin in *Pi. indica*-colonized plants as compared with the non-colonized plants. Singh et al. (2012) reported that it is not the forskolin content of the root, rather the forskolin yield which is increased significantly by treatment with bioinoculants. Another essential oil, p-cymene, is frequently utilized in pharmaceuticals or in fine chemical industries for syntheses of fragrances, p-cresol, flavorings, herbicides, non-nitrated musks, etc. (Martín-Luengo et al. 2008). The level of p-cymene increased in the aerial parts of the *Pi. indica*-colonized *C. forskohlii* plants as compared with the non-colonized plants (Das et al. 2012). Likewise, inoculation of *Sebacina vermifera* and *Pi. indica* significantly increased the level of thymol in thyme, anethole in fennel, and podophyllotoxin and 6-methoxypodophyllotoxin in *Linum album* as compared to non-inoculated control plants (Baldi et al. 2010; Dolatabadi et al. 2011). Similar cases of enrichment of bioactive compounds such as artemisinin in *Artemisia annua* L. shoots (Sharma and Agrawal 2013), spilanthol in *S. calva* (Rai et al. 2004), saponin from *Chlorophytum* sp. (Gosal et al. 2010), and asiaticoside from *Centella asiatica* (Satheesan et al. 2012) were also reported in earlier studies with *P. indica* treatment. As biotic elicitors, PGPF or constituents of their cells can equally be used to stimulate the

secondary metabolite production in plant cells. As reported by Ming et al. (2013), both the mycelial extract and the polysaccharide fraction produced by *T. atroviride* D16 could stimulate the biosynthesis of tanshinones in hairy roots of *Salvia miltiorrhiza*. The data presented here show that PGPF can increase industrial advantages of the host plants by producing scarce and valuable bioactive compounds for human use. Moreover, understanding the effects of PGPF on plant secondary metabolite production may help produce targeted drugs through bioengineering.

6.3.8 Plant Signaling Pathways Leading to Enhanced Growth

The interaction between host plant and PGPF involves the exchange of signal molecules by the two partners. This initial exchange leads to recognition of the appropriate partner and thus plays an integral role in establishing successful association. Plant responses to microbial association are translated into massive changes in biochemical reactions, metabolic adjustments, and physiological state. With current advances in molecular biology, many components of the signal transduction pathways in beneficial plant-microbe interaction have now been characterized. It has now become obvious that plant signaling pathways leading to enhanced growth by PGPF rely on endogenous regulators, such as auxin, ET, and CKs. Other plant hormones such as GAs and ABA represent additional classes of signaling molecules that influence beneficial plant-PGPF interactions.

As noted earlier, plant-PGPF interactions can employ direct or indirect influences on belowground and aboveground plant structures. The frequently reported effects are enhanced biomass production, flowering, root hair development, and increased yield (Björkman et al. 1998; Harman et al. 2004; Contreras-Cornejo et al. 2009). Several interesting studies have pointed to the role of auxin as plant signaling hormones in plant responses to PGPF and especially describing their participation in controlling shoot and root development. Wild-type Arabidopsis seedlings inoculated with *T. virens* showed augmented biomass production and lateral root development (Contreras-Cornejo et al. 2009). The inoculated plants exhibited the expression of auxin-regulated genes. As it was expected, mutations in genes involved in auxin transport or signaling, *AUX1*, *BIG*, *EIR1*, and *AXR1*, reduced the plant growth-promoting and root developmental effects of *T. virens* inoculation in Arabidopsis. These results indicate that plant growth promotion by *T. virens* operates through the classical auxin response pathway (Contreras-Cornejo et al. 2009). Similarly, *Pi. indica*-induced expression of auxin-regulated genes was reported in barley (Schäfer et al. 2009) and in Chinese cabbage (Lee et al. 2011), and their induction was instrumental for the strong growth-promoting effect by the fungus. It is assumed that microbial auxin may have a role in altering auxin biosynthesis or signaling in the host (Sukumer et al. 2013). Previously, Sirrenberg et al. (2007) have noted that the phenotype obtained from interactions of Arabidopsis with *Pi. indica* is mimicked by an external application of IAA, at a concentration lower than produced by the fungus, suggesting a role for exogenous auxin. Similarly, Contreras-Cornejo et al. (2009) showed that treatment with IAA and indole-3-acetaldehyde was found to

rescue the root hair-defective phenotype of *rhd6* mutant. This result may imply that the microbial auxin may take part in suppressing the root hair formation defects of *rhd6.* Therefore, auxin can act as a reciprocal signaling molecule in plant-microbe interaction.

Ethylene, the gaseous phytohormone, is important for plant growth and development as well as plant response to environmental signals (Vandenbussche et al. 2012). The growth-promoting endophytic fungus *Sebacina vermifera* significantly increases the growth of *Nicotiana attenuata*. When the *N. attenuata* plant was transformed to silence ET production, growth promotion effect by the fungus was not observed (Barazani et al. 2005). DNA microarray-based gene expression analysis revealed a differential induction of genes related to ET synthesis and signaling in barley roots colonized by endophytic fungus *Pi. indica* (Schäfer et al. 2009). Mutants *etr1*, *ein2*, and *ein3/eil1* impaired in ET signaling showed compromised or inhibited growth and seed production responses by this fungus compared with the wild type. These results are the indication of involvement of ET signaling in the beneficial interaction between the two symbionts (Camehl et al. 2010). Impaired ET signaling resulted in reduced root colonization by the fungus, while Arabidopsis mutants exhibiting constitutive ET signaling and synthesis or ET-related defense were hypersusceptible to *Pi. indica* (Khatabi et al. 2012). This suggests that ET signaling influences plant growth by affecting fungal colonization on the roots.

Although several VOCs from PGPF are known to affect plant growth, the signaling pathways mediating VOC sensing are not fully understood. The major natural antifungal VOC isolated from *Trichoderma* was 6-pentyl-2H-pyran-2-one (6-PP) (Lee et al. 2016) which induces *A. thaliana* root morphogenesis via auxin transport and signaling and the ET-response modulator EIN2 (Garnica-Vergara et al. 2016). Ryu et al. (2003) reported that CK signaling plays a role in growth promotion with exposure to *Bacillus subtilis* GB03 VOCs. CKs are also essential for *Pi. indica*-induced growth promotion in Arabidopsis (Vadassery et al. 2008). Moreover, in response to *Pi. indica* colonization, the ABA pathway was proposed to enhance plant growth via cellular [Ca^{2+}] elevations, phosphoinositide, and particular protein kinases (Vadassery et al. 2009; Camehl et al. 2011). Additional phytohormones synthesized or manipulated by the growth-promoting fungi include GAs and brassinosteroids (Schäfer et al. 2009). In summary, almost the whole phytohormone signaling networks appear to be involved in generating compatible interactions between the fungus and host, which lead to growth promotion and finally to greater biomass.

6.3.9 Plant Genetic Variability Affecting Induced Plant Growth

The expected beneficial effects of microbial application are frequently influenced by treated plant genotype. While plant growth promotion by PGPF has been well documented, this trait rarely occurs across all plant-PGPF combinations. It is assumed that a preferential interaction exists between strains of PGPF and a

particular host. Similarly, plant-dependent differences in response to PGPF inoculation may also occur at the cultivar level. Both fungi and plant cultivars have their own sets of characteristics that ultimately define the intimate interaction between them and the beneficial outcomes resulting from the developed interaction. There are cultivar genotypes for which the use of particular PGPF strain may be either endorsed or contraindicated. The use of a responsive cultivar may help maximize the efficacy of PGPF, and new inducer strains should be explored for the less responsive cultivars. Despite the obvious significance for agriculture, there are still a few studies on how the plant response to PGPF is influenced by plant genotypes in terms of growth promotion.

Earlier, Shivanna et al. (1994) tested seven zoysiagrass sterile fungal isolates and a wheat rhizosphere isolate (K-17) on two wheat varieties in field conditions. The growth of one variety was enhanced by most of the isolates, except K-17, while only a few isolates increased the growth of the other variety. There are at least four PGPF isolates which increased yields of both varieties. The authors concluded that the effectiveness of PGPF isolates in terms of plant growth promotion depends on the crop variety besides their inherent growth promotion abilities. In another study, Shivanna et al. (2005) examined the ability of a few of *Phoma* sp. isolates and one non-sporulating fungal isolate to promote plant growth of four cucumber cultivars: Aodai kyuri, Jibai, Ochiai fushinari, and Shogoin fushinari. All isolates enhanced plant length in cucumber cv. Shogoin fushinari, while nine isolates except the sterile fungal isolate GU21-1 improved the plant length in cv. Aodai kyuri. On the contrary, stimulated plant length was not observed in cucumber cv. Jibai and Ochiai fushinari, when the plants were treated with one (GS6-4) and five fungal isolates (GS6-1, GS7-4, GS8-6, GS10-2, and GU21-2), respectively. These results also suggest that the tested PGPF isolates caused cultivar-specific plant length promotion in cucumber. Harman (2006) reported that maize inbreeds treated with T-22 strain of *T. harzianum* showed three different types of growth responses such as strongly positive, little effect, and negative. Thus, there clearly are strong genetic components to the response of maize to T-22. Further, analysis of T-22-induced growth responses of hybrids derived from parent with dissimilar growth responses suggests that the T-22 responses in maize are largely conditioned by dominant genes (Harman 2006). In a growth chamber study, Tucci et al. (2011) demonstrated that substantial differences in the growth response to the symbiotic interaction with two selected strains of *Trichoderma* spp. occurred when different tomato varieties were tested. Consequently, the plant response to *T. harzianum* T-22 or *T. atroviride* P1 is affected by plant genetic variability and thus is under genetic control in tomato. Since plant response to PGPF is a heritable trait (Harman 2006), its extrapolation to crop plants by breeding would be significant for plant improvement. The possible mechanisms that underlie plant genetic control of the interaction may include the genotype ability to support and sustain root colonization by the PGPF, different sensitivities to the effectors produced by the fungus, variability in the perception and signal transduction of any of the hormones whose concentrations are controlled by it, and so on (Tucci et al. 2011).

6.4 Induction of Systemic Resistance by PGPF

Additional interests in the biological control of soil-borne diseases of plants led to the useful discovery of a specialized type of induced resistance as resulting effects of the colonization of plant roots by certain PGPR, referred to as induced systemic resistance or ISR (van Loon et al. 1998). ISR is known to reduce the incidence and/or severity of various fungal, bacterial, viral, nematode, and oomycete diseases on a diversity of plants (Walters et al. 2013). In contrast to constitutive defense, ISR is considered cost saving. ISR reduces physiological costs of the plants by better matching between resource investment into defense and potential threats (Gómez et al. 2007). Therefore, ISR could offer the most efficient means of defense against invading pathogens.

Research in the last decade in plant-fungal biocontrol agent interactions has made it clear that elicitation of ISR is a widespread phenomenon. It is not limited only for PGPR but also for a variety of other microorganisms including PGPF. PGPF of different taxa have been found as potential inducers of systemic resistance against pathogens. Among them, members of *Trichoderma* (Shoresh et al. 2005), *Penicillium* (Hossain et al. 2007; Hossain et al. 2008a), nonpathogenic *Fusarium* (Kojima et al. 2013), *Piriformospora* (Stein et al. 2008), *Pythium* (Hase et al. 2008), *Sebacinales* (Waller et al. 2008), *Phoma* (Sultana et al. 2009), and sterile fungi (Sultana et al. 2008) are well studied for their roles as elicitors of ISR (Table 6.3). The classical biocontrol agents *Trichoderma* spp. have frequently been shown to suppress the severity of diseases, particularly those caused by soil-borne plant pathogens through mycoparasitism and antibiosis (John et al. 2010; Akhter et al. 2015). However, *T. virens* mutants deficient in mycoparasitic ability and/or inability to produce antibiotics had no effect on the biological activity of these strains. Instead, there seemed to have a very strong correlation between the abilities of these strains to trigger terpenoid phytoalexin defense in cotton seedlings and control of *R. solani* (Howell et al. 2000). These examples clearly demonstrate the importance of ISR by PGPF. The ability of *Trichoderma* spp. to trigger ISR has been shown in agriculturally important crops such as rice, wheat, bean, maize, cucumber, lettuce, cotton, tobacco, and tomato and *Rhododendron* against fungi to oomycetes to bacteria and even virus (Ahmed et al. 2000; Koike et al. 2001; Yedidia et al. 2001; Howell 2003; Harman et al. 2004; Shoresh et al. 2005; Hoitink et al. 2006; Saksirirat et al. 2009; Elsharkawy et al. 2014; Vitti et al. 2016). Several *Penicillium* spp. have also been extensively tested for their ability to elicit ISR in plants and were very much effective against fungi (Hossain et al. 2014), bacteria (Hossain and Sultana 2015), and viruses (Elsharkawya et al. 2012). *Phoma* sp. and sterile fungi have similar capabilities (Hossain et al. 2008b; Sultana et al. 2008; Sultana et al. 2009). ISR has been reported to be a mechanism of action for some nonpathogenic strains of *F. oxysporum*. ISR by *Fusarium* isolates have been reported against root-knot nematodes (Dababat and Sikora 2007) and *Radopholus similis* in banana (Athman et al. 2006); *Pythium ultimum* infection in cucumber (Benhamou et al. 2002); *Verticillium* wilt in eggplant (Ishimoto et al. 2004); *Fusarium* wilt in watermelon (Larkin and Fravel 1999), sweet potato (Ogawa and Komada 1986), and tomato (Patil et al.

Table 6.3 Induction of systemic resistance by different plant growth-promoting fungi against diverse pathogens in various plants

Host Plant	PGPF	Pathogen	Effect	References
Arabidopsis (A. thaliana)	*Ampelomyces* sp., *Cladosporium* sp.	*Pseudomonas syringae* pv. *tomato* DC3000	Reduction in disease severity and pathogen proliferation	Naznin et al. (2014)
	Aspergillus ustus	*Botrytis cinerea*	Reduction in disease incidence	Salas-Marina et al. (2011)
		Ps. s. pv. *tomato* DC3000		
	Fusarium equiseti GF19-1	*Ps. s.* pv. *tomato* DC3000	Reduction in disease severity and pathogen proliferation	Kojima et al. (2013)
	P. simplicissimum GP17-2	*Ps. s.* pv. *tomato* DC3000	Reduction in disease severity and pathogen proliferation	Hossain and Sultana (2015)
	Pe. simplicissimum GP17-2	*Ps. s.* pv. *tomato* DC3000	Reduction in disease severity and pathogen proliferation	Hossain et al. (2007) and Hossain and Sultana (2015)
	Penicillium sp. GP16-2	*Ps. s.* pv. *tomato* DC3000	Reduction in disease severity and pathogen proliferation	Hossain et al. (2008a)
	Phoma sp. (GS6-2 and GS7-3) and sterile fungus (GU23-3)	*Ps. s.* pv. *tomato* DC3000	Reduction in disease severity and pathogen proliferation	Sultana et al. (2008)
	Phoma sp. GS8-1	*Ps. s.* pv. *tomato* DC3000	Reduction in disease severity and pathogen proliferation	Hossain et al. (2008b)
	Phoma sp. GS8-3	*Ps. s.* pv. *tomato* DC3000	Reduction in disease severity and pathogen proliferation	Sultana et al. (2009)
	Pi. indica	*Golovinomyces orontii*	Reduction in conidia per leaf fresh weight	Stein et al. 2008
	Rhizoctonia spp.	*Rhizoctonia* spp. isolate RS13	Increase plant survival	Sharon et al. (2011)
	T. asperellum SKT-1	*Cucumber mosaic virus* (*CMV-Y*)	Reduction in CMV symptoms	Elsharkawy et al. (2013)
	T. hamatum T382	*B. cinerea* B05-10	Reduction in average lesion diameter	Mathys et al. (2012)
	T. harzianum Tr6	*B. cinerea*	Reduction in disease incidence index	Alizadeh et al. (2013)
	T. harzianum Rifai T39	*B. cinerea* Strain B4	Reduction in percentage of mean lesion area	Korolev et al. (2008)

(continued)

Table 6.3 (continued)

Host Plant	PGPF	Pathogen	Effect	References
Cucumber (*C. sativus*)	*Alternaria cucumerina* *Cladosporium fulvum*	*Sphaerotheca fuliginea*	Reduction in number of powdery mildew colonies on each leaf	Reuveni and Reuveni (2000)
	Nonpathogenic *F.oxysporum* Fo47	*Py. ultimum*	Reduction in the number of root lesions	Benhamou et al. (2002)
	P. simplicissimum GP17-2	*C. orbiculare*	Reduction in the number and size of the lesions	Shimizu et al. (2013)
	Pe. chrysogenum	*Meloidogyne javanica*	Reduced root galling	Gotlieb et al. (2003)
	Penicillium sp. isolate GP15-1	*C. orbiculare*	Reduction in lesion number per leaf and total lesion area	Hossain et al. (2014)
		R. solani AG-4 RO2	Reduction in disease severity	
	Phoma sp. GS8-1	*Colletotrichum orbiculare*	Reduction in lesion area and lesion number	Elsharkawy et al.(2015)
	Sterile fungi GU21-2			
	T. asperellum T203	*Ps. s.* pv.*lachrymans*	Inhibited multiplication of bacterium	Shoresh et al. (2005)
	T. harzianum Tr6	*F. o.* f. sp. *radicis-cucumerinum*	Reduction in disease incidence index	Alizadeh et al. (2013)
	Trichoderma sp. GT3-2, *Fusarium* sp.GF18-3, *Penicillium* sp. GP17-2, *Phoma* sp. GS8-2, sterile fungus GU23-3	*C. orbiculare*	Reduction in total lesion area, lesion diameter, disease index and severity	Koike et al. (2001)
		F. oxysporum f. sp. *cucumerinum*		
		Ps. s. pv. *lachrymans*		
Rice (*O. sativa*)	*Meyerozyma guilliermondii* TA-2	*Magnaporthe oryzae*	Reduction in disease severity	Elsharkawy et al. (2015)
	Phoma spp.	*F. semitectum*	Reduction in disease severity	Elsharkawy et al. (2014)
	T. harzianum GT3-2	*Rhizopus* sp.		
		F. moniliforme		
	Pi. indica	*Magnaporthe oryzae*	Reduction in disease symptoms	Mousavi et al. (2014)
	Pi. indica	*F. proliferatum*	Reduction in *F. proliferatum* DNA in roots	Hajipoor et al. (2015)
	T. virens Gv29-8	*C. graminicola*	Reduction in lesion area	Djonović et al. (2006)

Maize (*Z. mays*)	*Pi. indica*	*R. solani* AG1-IA	Delayed the infection process of *R. solani* and decreased sheath blight severity	Nassimi and Taheri (2017)
	T. virens Gv29-8	*C. graminicola*	Reduction in lesion area	Djonović et al. (2007)
Wheat (*T. aestivum*)	*T. virens*	*Cochliobolus heterostrophus*	Reduction in lesion size	Gaderer et al. (2015)
	T. atroviride			
	Aureobasidium pullulans	*F. culmorum*	Reduction in *Fusarium* head blight severity	Wachowska and Głowacka (2014)
Barley (*H. vulgare*)	*Pi. indica*	*F. culmorum*	Reduction in *Fusarium*-induced shoot and root fresh weight loss	Waller et al. (2005)
		Blumeria graminis f. sp. *hordei*	Reduction in disease index	
Cabbage (*B. oleracea*)	*Meyerozyma guilliermondii* TA-2	*Alternaria brassicicola*	Reduction in disease severity and pathogen proliferation	Elsharkawy et al. (2015)
Banana (*M. acuminata*)	*F. oxysporum*	*Radopholus similis*	Reduction in root penetration rates by nematode	Vu et al. (2006)
	F. cf. *diversisporum*			
	F. oxysporum	*Radopholus similis*	Reduction in total number and percentage of *Ra. similis* attracted to banana root segments	Athman et al. (2006)
			Reduction in percentage of *Ra. similis* that migrated toward banana plants	
Tomato (*L. esculentum*)	*Fusarium* spp. UPM31P1	*F. o.* f. sp. *cubense* race 4	Reduction in disease incidence	Ting et al. (2010)
	Meyerozyma guilliermondii TA-2	*Ralstonia solanacearum*	Reduced symptom development, disease severity, and pathogen proliferation	Elsharkawy et al. (2015)
	Pe. chrysogenum	*Phytophthora infestans*	Reduction in development of necrotic leaf spot and leaf area	Unger et al. (2006)
	T. harzianum T-22	*Cucumber mosaic virus* (CMV)	Reduction in CMV infection severity and accumulation	Vitti et al. (2016)

(continued)

Table 6.3 (continued)

Host Plant	PGPF	Pathogen	Effect	References
	Pe. chrysogenum	*Me. javanica*	Reduced root galling	Gotlieb et al. (2003)
	Phy. cryptogea	*F. o.* f. sp. *lycopersici*	Reduction in disease incidence	Attitalla et al. (2001)
	Phy. cryptogea	*F. o.* f. sp. *lycopersici*	Reduction in disease incidence	Attitalla et al. (2001)
	R. solani AG 4	*R. solani*	Reduction in pre and post-emergence seedling mortality caused by *R. solani.*	Cardinale et al. (2006)
		B. cinerea	Reduction in *B. cinerea* lesion size	
	T. hamatum 382	*X. euvesicatoria* 110c	Reduction in disease severity	Alfano et al. (2007)
	T. harzianum	*Xanthomonas campestris* pv. *vesicatoria*	Reduction in spot numbers, disease severity and pathogen proliferation	Saksirirat et al. (2009)
	T. harzianum T-78	*B. cinerea*	Reduction in lesion size	Martínez-Medina et al. (2013)
Mustard (*B. campestris*)	*Talaromyces wortmannii* FS2	*C. higginsianum*	Reduction in infection frequency	Yamagiwa et al. (2011)
Cotton (*Gossypium barbadense*)	*Penicillium janczewskii*	*R. solani*	Reduction in the incidence of damping-off	Madi and Katan (1998)
	T. virens strain Gv29-8	*Colletotrichum* sp.	Reduction in lesion area	Djonović et al. (2006)
Melon (*C. melo*)	*Pe. janczewskii*	*R. solani*	Reduction in the incidence of damping-off	Madi and Katan (1998)
Pepper (*C. annuum*)	*T. harzianum* isolate 2413	*Phytophthora capsici*	Reduction in length of necrosis in the stem	Ahmed et al. (2000)
Kidney bean (*P. vulgaris*)	*R. solani* AG-Bb	*R. solani*	Reduction in disease severity	Tohid and Taheri (2015)

2014); *Phytophthora infestans* in potato (Quintanilla 2002); pea root rot pathogen (Peters and Grau 2002); and *Ps. syringae* in Arabidopsis (Kojima et al. 2013). The hypovirulent *Rhizoctonia* isolates protect bean and tomato (Cardinale et al. 2006), Arabidopsis (Sharon et al. 2011), and kidney bean (Tohid and Taheri 2015) against important pathogens through mechanisms associated with ISR. Evidence also suggests that *Pi. indica* induces systemic resistance in rice against bakanae disease caused by *F. proliferatum* (Hajipoor et al. 2015), leaf blast caused by *Magnaporthe oryzae* (Mousavi et al. 2014), and sheath blight caused by *R. solani* (Nassimi and Taheri 2017). The fungus reduces fusarium head blight severity in wheat (Rabiey and Shaw 2016) and powdery mildew disease caused by *Blumeria graminis* f. sp. *hordei* in barley (Waller et al. 2005; Harrach et al. 2013). These results show that PGPF strains can effectively enhance disease resistance of plants.

Colonization of plant roots by PGPF seems an essential step for eliciting ISR. However, studies revealed that culture filtrates of certain *Penicillium*, *Fusarium*, *Phoma*, and sterile fungi afforded better protection than living inocula, suggesting that not only the effect of root colonization but also the triggering of host defense mechanisms by certain chemical factors produced by fungi is responsible for the induction of resistance in plants against pathogens (Hossain et al. 2008a; Sultana et al. 2008; Kojima et al. 2013). Various microbial metabolic molecules such as protein with enzymatic activity, cell wall lipid, chitin oligomers, and glycopeptides have been described with elicitor activity. Hyakumachi (1997) revealed that the lipid fraction of mycelial cell walls of non-colonizing PGPF and the cell wall lipid fractions as well as polysaccharides of root colonizing PGPF were effective in eliciting a resistance response. Koike et al. (2001) reported that both the MW 12,000 D fraction and the lipid fraction of culture filtrate of *Pe. simplicissimum* GP17-2 induce resistance, lignification at the site of pathogen infection, and generation of reactive oxygen species. The peptaibols (peptide antibiotics) and the small protein Sm1 produced by *T. virens* have been shown to be responsible for the systemic activation of the defense responses against *Colletotrichum graminicola* and *Cochliobolus heterostrophus* in maize leaves (Djonović et al. 2007; Viterbo et al. 2007; Gaderer et al. 2015). Similarly, its homologue Epl1 from *T. atroviride* induces plant resistance responses to a lesser extent against *Cochliobolus heterostrophus* (Gaderer et al. 2015). Recent studies have also revealed that VOCs emitted by some PGPF strains can effectively enhance disease resistance. A terpenoid-like volatile β-caryophyllene emitted by *Talaromyces* wortmannii FS2 significantly enhanced the resistance to *Colletotrichum higginsianum* (Yamagiwa et al. 2011). Two VOC blends extracted from *Ampelomyces* sp. and *Cladosporium* sp. containing m-cresol and methyl benzoate (MeBA) as major active volatile compounds, respectively, were found to elicit ISR in Arabidopsis plants against *Ps. s.* pv. *tomato* DC3000 (Naznin et al. 2014). These observations imply the use of VOCs emitted from beneficial fungi as a novel strategy for biocontrol. However, they are difficult to apply in the field because of their high evaporative nature, and additionally, their efficacy seems to be low compared with other chemical elicitors (Naznin et al. 2014).

6.4.1 Defense Responses During PGPF-Mediated ISR

Plants defend themselves against phytopathogenic attacks by activating a wide spectrum of defense-related genes or compounds that enhance both cellular protection and disease resistance. Often, the induced effects of PGPF on the plant defenses are not limited to the root, but they are also exhibited in aboveground plant tissues (Martínez-Medina et al. 2010), providing the whole plant more resistance to a wide range of plant pathogens. Various transcriptomic studies have provided evidences that ISR may result in the direct activation of cellular defense responses in systemic tissue after local stimuli and/or of the priming, which involves activation of systemic responses, but only when the pathogen reaches these sites (Aranega-Bou et al. 2014). Some of PGPF-mediated ISR result from direct activation of defense genes than priming, while others are most frequently associated with priming for boosted defense rather than direct activation. There are also PGPF-mediated ISR which are partly associated with the direct activation of defense-related genes and partly associated with priming (Hossain et al. 2008a). These differential mechanisms of ISR by different PGPF could possibly be due to strain-specific differences in elicitor substances.

6.4.1.1 Direct Activation of Defense Responses

Direct activation of various defense responses and a significant reduction in pathogen growth are observed in different PGPF-mediated ISR. In a growth chamber study, examination of local and systemic gene expression revealed that *Pe. simplicissimum* GP17-1-mediated ISR is accompanied by direct activation of *PR-2* and *PR-5* genes in leaves and roots of Arabidopsis plants, while increased expression of *PDF1.2* was seen in the leaves of treated plants (Hossain et al. 2007). In another study, *Pe. chrysogenum* PenC_JSB4 and *T. harzianum* TriH_JSB27 treatments directly activated phenylalanine ammonia lyase (PAL) activity in tomato plant (Jogaiah et al. 2013). Similar results have been reported with increase in PAL activity in sunflower plants treated with *T. harzianum* (Lamba et al. 2008). Mathys et al. (2012) reported that addition of *T. hamatum* T382 to the roots of the plant triggers a clear and pronounced induction of *PR-1*, *PR-2*, and *PR-5* on the first 3 days of post-T382 inoculation, while the expression of the *PDF1.2a* was not affected in the leaves on the second day after the treatment. Moreover, comparing plants treated with *T. hamatum* T382 with mock-treated controls, they identified 2075 genes that are differentially expressed during T382-mediated ISR. Several other studies also suggested the direct activation of defense-related genes during *Trichoderma*-induced systemic resistance (Alfano et al. 2007; Salas-Marina et al. 2011; Morán-Diez et al. 2012). Root treatment with nonpathogenic *F. oxysporum* modulates the expression of systemic acquired resistance (SAR) marker genes in tomato (Duijf et al. 1998). Similarly, the onset of resistance induced by *F. equiseti* GF19-1 in the leaves of Arabidopsis plant was associated with a significant induction of *PR-1*, *PR-2*, and *PR-5* genes (Kojima et al. 2013). Not only the root colonization by PGPF but also the culture filtrates produced by them modulate the direct activation of defense genes, leading to enhanced resistance to invading pathogens (Hossain et al. 2008a; Sultana et al. 2009; Kojima et al. 2013; Shimizu et al. 2013). Enhanced expression

of *PR-1*, *PR-2*, *PR-5*, *ChitB*, and *Hel* genes was observed in Arabidopsis plants treated with culture filtrate of *Phoma* sp. GS8-1 (Hossain et al. 2008b). Two VOC blends extracted from *Ampelomyces* sp. and *Cladosporium* sp. containing m-cresol and MeBA induced *PR-1* and *PDF 1.2* genes in leaves of *A. thaliana* (Naznin et al. 2014). The correlation between ISR and presence of constitutive induction of defense genes postulates the assumption that constitutively activated defense responses are essential mechanisms in the PGPF-mediated ISR response of plants.

6.4.1.2 Priming (Sensitization) of Defense Responses During PGPF-Mediated ISR

There are PGPF, which are believed not to significantly alter gene expression upon treatment or show minimal induction of defense genes. Rather, they acquire a second line of defense, in which they prime or sensitize plants to express resistance response more rapidly and/or more robustly upon pathogen attack. Upon pathogen infection, there is an activation of cellular defense responses in attacked cells of both ISR-expressing and non-expressing plants. However, in case of ISR-expressing, cellular defense responses are induced more rapidly and stronger than in a non-induced plant. The primed state develops from the enhanced perception and/or amplification of defense signals (Aranega-Bou et al. 2014). Thus, ISR orchestrates an enhanced ability of the plant for the fast and effective activation of defense responses that are triggered not until challenged pathogen attack (Conrath 2009). This process of priming has been demonstrated in various plant species protected by ISR triggered by PGPF. Hossain et al. (2008a) analyzed the expression of a set of defense-related genes, locally, in roots as well as, systemically, in the leaves of *Penicillium* spp. GP16-1-colonized plants. The leaves and roots of the GP16-2-treated plants did not show enhanced expression of any of the genes studied over untreated plants. However, upon infection with *P. syringae* pv. *syringae*, activation of the *ChitB* gene was greatly enhanced in GP16-2-treated plants. Despite no induction of the *Vsp* gene was observed in *Pe. simplicissium* GP17-2-treated plants before pathogen inoculation, transcript levels accumulated to greater levels in these plants at 4 and 6 days post-infection by *P. s.* pv. *syringae* (Hossain et al. 2007). Likewise, although systemic induction of three defense genes (*PI II*, *PS*, and *MC* coding for the proteinase inhibitor II, prosystemin, and multicystatin) was relatively weak in plant colonized by *T. harzianum*, the expression of these genes has been boosted in the induced plants, upon *Botrytis cinerea* infection (Martínez-Medina et al. 2013). Similar activation of a priming state in plants by *Trichoderma* has been observed previously in Arabidopsis, tomato, and grapevine plants (Segarra et al. 2009; Tucci et al. 2011; Perazzolli et al. 2012; Alizadeh et al. 2013). These solid evidences substantiate that priming is a major defense mechanism in PGPF-mediated ISR. PGPR and SAR activators have also been demonstrated to enhance the plant's defense capacity by priming for potentiated expression of defense genes (Verhagen et al. 2004; Tjamos et al. 2005; Conrath et al. 2006). Ryu et al. (2004) demonstrated that some PGPR can even induce priming by the release of volatiles. This indicates that priming is, indeed, a very common mechanism underlying plant's various induced responses (Bruce et al. 2007). From an economic context,

priming appears to offer an overall advantage to plant over the direct induction of the plant defense responses. Direct induction of defense mechanisms is known to seriously affect the growth and seed set, while priming had only marginal effects (van Hulten et al. 2006). Priming conditions plants to trigger appropriate set of defenses without misuse of resources in every situation and reduces trade-offs between defenses against various pathogens. Biochemical and histological changes characteristic of ISR-expressing plants become apparent only in plant organs where an effective resistance is essential.

6.4.2 Plant Signaling Pathways Leading to ISR

SAR and ISR are two classes of inducible resistance where plant defense systems are sensitized by prior infection or treatment with a stimulus that triggers putative resistance against succeeding challenge inoculation by a pathogen (Choudhary et al. 2007). These different forms of resistance are usually associated with the generation of defense-eliciting signals that stimulate a series of downstream events. The key downstream elements of defense signal transduction that warrants particular importance are SA, jasmonic acid (JA), and ET. SA signaling through NPR1 is necessary to trigger SAR (Withers and Dong 2016). Different from SAR, ISR elicited from *Ps. fluorescens* colonization is independent of SA accumulation but requires responsiveness to JA and ET. Besides SAR, NPR1 is also needed for ISR triggered by rhizobacteria (Pieterse et al. 1996, 2009). Some studies have indicated that similar signaling pathways of PGPR-mediated ISR are likely to have required in PGPF as well. ISR triggered by *Trichoderma* spp. involves responsiveness to JA and ET pathways (Shoresh et al. 2005; Segarra et al. 2009; Perazzolli et al. 2011; Tucci et al. 2011). Similarly, ET- and JA-signaling pathways with mediation of NPR1 are key players in the regulation of ISR elicited by *Penicillium* sp. GP16-2 (Hossain et al. 2008a). However, others have disputed this generalization (Hossain et al. 2007; Korolev et al. 2008; Niu et al. 2011), an indication that is established by the results of many studies. As examples, ISR mediated by *Pe. simplicissimum* GP17-2 against *P. syringae* pv. *tomato* only partially requires the SA pathway, while it shows complete independency on the JA and ET pathways (Hossain et al. 2007). The same PGPF elicits resistance to *cucumber mosaic virus* (CMV) in Arabidopsis independent of SA, JA, and ET pathways (Elsharkawya et al. 2012). Although ISR elicited by *Penicillium* spp. GP16-2 against *P. syringae* pv. *tomato* follows JA- and ET-dependent pathways, its cell-free filtrate mediates resistance independent of SA, JA, and ET pathways (Hossain et al. 2008a). Similarly, differences from the reported pathways were noted with mycelial extract of *Pe. chrysogenum* and culture filtrate of *Phoma* sp. (Thuerig et al. 2006; Hossain et al. 2008b; Sultana et al. 2008).

It has been proven that other forms of induced resistance exist. A study by Korolev et al. (2008) using multiple mutant lines of Arabidopsis has shown that the induction of resistance by *T. harzianum Rifai* T39 against *B. cinerea* requires responsiveness to JA, ET, and ABA signalings. Stein et al. (2008) showed that induction of systemic resistance in Arabidopsis by *Pi. indica* to powdery mildew

(*Golovinomyces orontii*) requires JA signaling and function of NPR1. Mathys et al. (2012) reported a role of the SA pathway in *T. hamatum* T-382-induced ISR against *B. cinerea* in Arabidopsis. Similarly, the phenotypic analysis of disease development in the JA (*def1*)- and SA (*Nah*G)-impaired mutants demonstrated that *T. harzianum*-induced systemic resistance against *B. cinerea* requires not only the JA but also the SA signaling pathways (Martínez-Medina et al. 2013). Investigation of ISR in various signaling mutants and transgenic plants showed that the induced protective effect conferred by *F. equiseti* GF19-1 against *P. s.* pv. *tomato* requires responsiveness to an SA-dependent pathway (Kojima et al. 2013). The examination of plant hormones revealed that treating tomato plants with *T. harzianum* T-22 before or simultaneously to CMV infection leads to a systemic resistance that requires JA/ET and SA signaling pathways. Conversely, systemic resistance occurs in an ABA-dependent manner when T-22 treatment was administered after the CMV infection (Vitti et al. 2016). Therefore, the role of plant signaling pathways in the regulation of ISR is complex. The nature and composition of signaling pathways and the regulated defenses during PGPF-mediated ISR distinctively depend on the tripartite combination plant-PGPF-pathogen, and the overlap between SAR and ISR is very common.

6.4.3 Plant Genetic Variability Affecting Induced Systemic Resistance

In nature, plants within a population generally vary in different traits, which include yield potential, large seed, disease resistance, etc. Natural variation in plants is prerequisite for biological effects of genetic diversity and for the adaptive potential of a species to environments that vary in space and time (Shindo et al. 2007; Hossain and Sultana 2015). From the very beginning of modern agriculture, breeders make use of the trait diversities in plant population to develop new and improved cultivars with desirable characteristics. These improved cultivars have been crucial in producing surplus food for growing populations. ISR has been emerging as an important mechanism, which allows conditioning of plant defense system by rhizosphere microorganisms to promote desirable traits in plant. Exploitation of this mechanism is extremely valuable in reducing yield losses to diseases in susceptible crops in a cost-efficient way. So far, various application methods have been attempted to integrate ISR into conventional agriculture and in a few cases with improved efficacy (Hossain and Sultana 2015). Existing data support the heritability in the ISR and a link between basal and induced resistance (Ton et al. 2001a). Therefore, breeding efforts to add ISR to commercial cultivars could be a feasible option that, overall, would have much significant impact on resistance breeding.

The variation in morphological and physiological traits among plant genotypes is known to affect relative benefits and efficacy of induced resistance (Tucci et al. 2011). Walters et al. (2011) have examined the effect of host genotype on the expression of chemical elicitor-induced resistance in barley to foliar pathogens and noticed that manifestation of induced resistance differed widely across a range of spring

barley varieties. This implies that genetically different genotypes vary in the extent to which induced resistance is expressed. Until now, only a few studies have examined the genotypic effects of plants on PGPF-mediated ISR. In tomato, genetic variability among cultivated and wild lines influenced the consequence of the interaction with strains of *T. harzianum* and *T. atroviride*, with ISR to *B. cinerea* being observed in some, but not all, tomato lines examined (Tucci et al. 2011). In table and wine grapes, treatment with *T. harzianum* T39 reduced downy mildew symptoms, but the degree of efficiency varied greatly among grapevine cultivars (Banani et al. 2013). In Arabidopsis, Hossain and Sultana (2015) investigated the variation in basal as well as *Pe. simplicissimum* GP17-2-mediated resistance to *P. s.* pv. *tomato* among a worldwide collection of 75 Arabidopsis accessions. A wide variation was observed in basal as well as induced resistance among the accessions infected with the bacterium. Only 49 accessions manifested GP17-2-mediated ISR to the pathogens, while 26 accessions were non-responsive to GP17-2 treatment. This indicates that the observed GP17-2-mediated ISR is ecotype specific in Arabidopsis. Interestingly, accessions non-inducible to GP17-2 treatment appeared to be marked with higher basal resistance to infection by *P. syringae* pv. *tomato* (Hossain and Sultana 2015). Hence, GP17-2-ISR in Arabidopsis does not require components of the basal resistance pathway. Future study with these parental lines could be undertaken to map and introgress major trait loci responsible for PGPF-mediated ISR in plant.

6.5 Conclusion and Future Perspectives

Understanding the induction of plant responses by PGPF is essential for developing new strategies for managing plant growth and diseases. The enormous benefits of their exploitation are related to their use as innovative microbial sources for plant growth promotion and induced resistance to a diverse range of pathogens. Some of these fungi are already being used successfully in a number of countries, and this practice is expected to grow. However, practical use of PGPF is often hindered by inconsistency and relatively poor plant growth and disease control compared with their chemical alternatives, and as such, their effects are greatly influenced by genotype, environment, and other factors. Eventually, for PGPF to gain widespread use in farmer fields, a number of issues should be addressed. It is crucial to develop effective and practical techniques for mass culture, storage, shipping, formulation, and application of these fungi. More importantly, effort is needed to convince the growers that PGPF can provide a useful addition to their existing crop management programs.

Recent advances in molecular tools continue to give more insight into the cellular process and signaling mechanisms, related to growth and defense, resulting from plant-PGPF interactions. The current demand for high-performing PGPF could be achieved by applying innovative biotechnology to generate genetically modified strains with improved characteristics. Likewise, PGPF genes can be expressed functionally in plants to confer beneficial properties. Concern exists about the nontarget activities of the genetically modified plant or microbes, which needs to be carefully

and thoroughly assessed in non-field studies. Moreover, market failure of the developed products illustrates one aspect of the problem of externalities. Active and justified participation of private industry in product research and development may help overcome the problem.

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Plant-Microbe Interactions in the Rhizosphere: Mechanisms and Their Ecological Benefits

7

Kulandaivelu Velmourougane, Garima Saxena, and Radha Prasanna

Abstract

Plant and microbes interact with each other and with other fauna and flora in the environment, and these interactions are modulated by abiotic factors. The rhizosphere is one of the active zones for such activities, which facilitate nutrient transformations and cross talk among beneficial and pathogenic flora and fauna. The net results are manifested in improved plant growth, yields and soil fertility. Several processes operate in this niche – quorum sensing, volatiles, defence and pathogenicity-related enzymes, nitrogen fixation, mobilisation and immobilisation of macro- and micronutrients, etc. Abiotic factors, including salinity, drought, high/low temperature and humidity, play significant roles in fine-tuning these interactions. Rhizosphere engineering or making targeted attempts to increase or decrease the populations of microorganisms or their metabolites or introduction of new organisms can bring about modifications in the plant and soil microbiome. These are influenced through changes in diversity and abundance of microbial communities and in terms of ecological balance in the rhizosphere. Strategies for improving plant-microbe interactions require more efforts to gain better understanding of these interactions using molecular, bioinformatics and modelling tools.

Keywords

Cross talk • Nutrients • Rhizosphere • Quorum sensing • Volatiles

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7.1 Introduction

Plant-microbe interactions facilitate a number of transformations in the rhizosphere, such as nutrient cycling, particularly, carbon and nitrogen sequestration, which influence various facets of ecosystem functioning. Rhizospheric microbes include *Eubacteria*, *Actinobacteria*, archaea, viruses and fungi; they can be beneficial or pathogenic, exhibiting free living or mutualistic/commensal associations (Philippot et al. 2013). Plant-microbe interactions may be beneficial, harmful or neutral. The population density and community structure in the rhizosphere influence the plants, particularly in terms of nutrient availability. The soil and plant microbiome are increasingly being viewed as valuable assets for improving crop yields, developing better varieties and sustaining soil fertility (Gopal and Gupta 2016).

Plant root exudates are among the primary factors involved in the attraction or inhibition of proliferation of microbes in the rhizosphere. These exudates are composed of smaller molecular weight compounds – including amino acids, polysaccharides, acids, mucilage and other cell components. Plants release a diverse range of secondary metabolites, which include terpenes, flavonoids, glucosinolates and phenylpropanoids through their roots (Moore et al. 2014). Even minor modulation in the amounts or types of secondary metabolites can bring about distinct influence on the microbial communities, in terms of diversity or abundance (Bressan et al. 2009; Nguyen 2003; Jones et al. 2004). Thus, root exudates exhibit specificity in selecting microorganisms for root colonisation from the bulk soil population and the rhizosphere microbiome, which in turn are fine-tuned by other biotic and abiotic factors (Grayston et al. 1998).

Beneficial and positive interactions among microorganisms/microfauna and nutrients lead to favourable crop and soil environment, forming the basis of good agricultural practices (Fig. 7.1). A healthy soil is the base for plant productivity. The soil is a dynamic system, which is influenced by several biotic and abiotic parameters, and in turn affects plant productivity. Soil microorganisms are among the most dominant biological components of the soil ecosystem. Plants have close interactions with soil; hence, plant-microbe interactions are not only essential for the plant in terms of their nutrition, growth promotion, biocontrol, stress alleviation, etc., but their interactions also influence soil physical, chemical and biological properties through biogeochemical cycles facilitating soil nutrient balance. Hence, understanding of plant-microbe interactions in soil is essential for developing future sustainable crop production and protection strategies. In the present review, we discuss briefly about the strategies for enhancing plant-microbe interactions with special reference to rhizosphere and their ecological benefits.

7.2 Types of Plant-Microbe Interactions

Plant-microbe interactions can be classified either as beneficial or harmful based on the interaction effects on the host (plants) by the colonising microorganisms or vice versa.

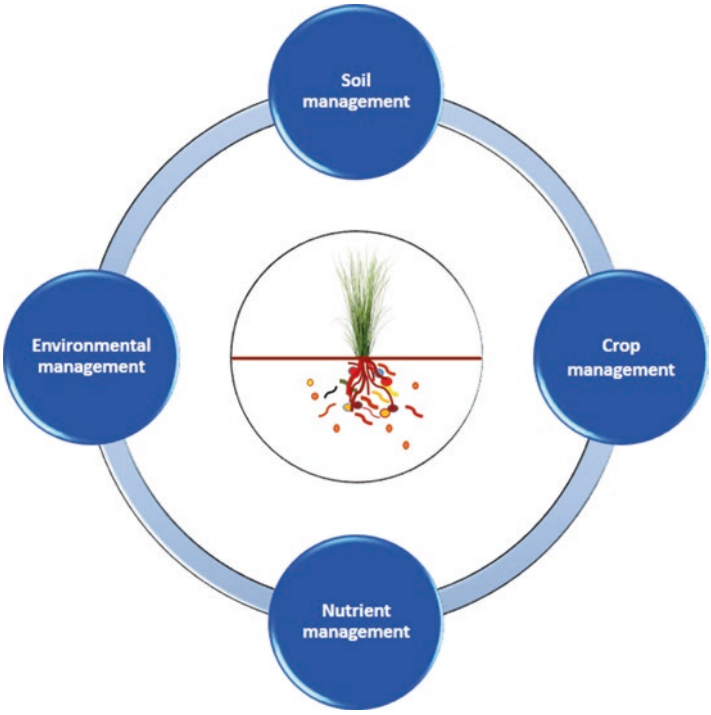

Fig. 7.1 Overview of sustainable agricultural practices, through plant-microbe interactions

7.2.1 Beneficial Plant-Microbe Interactions

Beneficial interactions are those which benefit the host or the inhabiting microorganisms in one or many ways. In general, beneficial plant-microbe interactions help the partners to acquire unavailable soil nutrients through solubilisation and mobilisation, aid in abiotic stress tolerance, protect against pests and pathogens, facilitate plant growth promotion, etc.

7.2.1.1 Nitrogen-Fixing Microorganisms

Nitrogen-fixing microbes are grouped as asymbiotic/free living, symbiotic, associative symbiotic and endophytes based on their relationships with their host plants (Morgan et al. 2005). Symbiotic fixation of nitrogen by microbes is the primary contribution to plant-available nitrogen in the soil. The symbiotic nitrogen-fixing group includes bacterial genera – *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Azorhizobium* and *Photorhizobium* which form either root nodules or stem nodules and represent rod-shaped *Proteobacteria* (Deaker et al. 2004). Recent investigations have shown that not only rhizobia form nodules in roots of legumes, but several other α-*Proteobacteria* including *Phyllobacterium*, *Methylobacterium*, *Ochrobactrum* and β-*Proteobacteria* like *Burkholderia*, *Cupriavidus* and *Devosia* also form nitrogen-fixing nodules in legumes (Moreira

2008). Actinobacterial symbiosis (*Frankia*) is also another important contributor to nitrogen in selected crop plants (Daniel et al. 2007). Asymbiotic and free-living N_2-fixer genera include *Azotobacter*, *Azoarcus*, *Beijerinckia*, *Klebsiella*, *Pantoea*, *Bacillus*, *Burkholderia*, etc. Apart from bacteria, few Cyanobacteria (*Nostoc*, *Anabaena*, *Calothrix*, etc.) are also contributors to asymbiotic N_2 fixation (Prasanna et al. 2009). *Azospirillum* is the dominant genus involved in associative symbiotic N_2 fixation (Bashan and de-Bashan 2010). *Herbaspirillum* and *Gluconacetobacter* are the common endophytes involved in N_2 fixation. Obligate and facultative anaerobes fix N_2 only in the absence of oxygen (e.g. *Clostridium*, *Desulfovibrio*, *Klebsiella*, *Enterobacter* and *Bacillus*) (Unkovich and Baldock 2008).

7.2.1.2 Mycorrhizae

Mycorrhizal symbiosis is the mutualistic association between fungi and higher plants, and this association has been reported in several terrestrial plants (Morgan et al. 2005). Based on their interactions and localisation in plant roots, mycorrhiza is classified as ecto- and endomycorrhiza. While ectomycorrhiza has been studied in mostly trees, endo or arbuscular mycorrhizal fungal (AMF) associations have been reported from several agricultural crops. The commonly occurring genera of AM fungi are *Glomus*, *Gigaspora*, *Acaulospora*, *Scutellospora* and *Entrophospora*. AM fungi are reported to have synergistic interactions with soil microorganisms like nitrogen fixers, phosphate solubilisers and other plant growth-promoting rhizobacteria (PGPRs) (Bagyaraj 2011). Mycorrhizal association in crop plants is reported to provide innumerable benefits to crops such as improved nutrient mobilisation (phosphorus) and uptake of macro- and micronutrients (Bago et al. 2003), besides tolerance towards drought, heavy metals and increased biocontrol potential and disease suppressiveness (Buee et al. 2000).

7.2.1.3 Plant Growth-Promoting Rhizobacteria (PGPRs)

In the last decade, microorganisms in rhizosphere of crop plants have been studied for their role in improvement of plant growth and productivity and broadly termed as plant growth-promoting rhizobacteria (PGPRs) (Bloemberg and Lugtenberg 2001). PGPR can be broadly classified as extracellular PGPR and intracellular PGPR (Viveros et al. 2010). The extracellular PGPRs mainly reside in the rhizosphere or on the rhizoplane, while intracellular PGPR localises inside the specialized nodular structures of root cells, sometimes referred to as endophytes. Common extracellular PGPR belong to the bacterial genera such as *Bacillus*, *Burkholderia*, *Agrobacterium*, *Erwinia*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Caulobacter*, *Chromobacterium*, *Flavobacterium*, *Micrococcus*, *Pseudomonas* and *Serratia* (Ahemad and Kibret 2014). The genera *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium, Rhizobium*, *Frankia* and certain endophytes are generally classified as intracellular PGPR (Bhattacharyya and Jha 2012). These rhizospheric microbes improve plant growth either by increasing nutrient uptake (major, secondary, and micronutrients – nitrogen fixation, phosphorus and potassium solubilisation, iron sequestration, etc.) (Cooke et al. 2002; Singh et al. 2007) or through the secretion of growth-promoting substances (IAA, GA, cytokinins, ethylene, etc.) or through

inhibition of specific plant pathogens through its biocontrol activity (Kloepper 1993). Several cyanobacteria are involved in plant growth promotion, enhanced nutrient uptake and soil fertility enhancement in crops when inoculated as single or with bacterial or fungal partners (Bidyarani et al. 2015; Karthikeyan et al. 2007; Kumar et al. 2013; Manjunath et al. 2011, 2014; Prasanna et al. 2011, 2014, 2015b; Rana et al. 2012; Swarnalakshmi et al. 2013).

7.2.1.4 Endophytes

Endophytes are microbes which may be a bacterium or fungus that lives within the tissues or inside the plant cells without adversely affecting them. Endophytes commonly depend upon the host plant for their nutrition and protection and benefit the plants in several ways. Many endophytes are reported to accelerate seedling emergence, promote plant growth, enhance yield, help in bioremediation, improve nutrient cycling and reduce the proliferation of pathogens. Endophytes produce phytohormones and indirectly increase tolerance against abiotic stresses (Arnold 2007). Endophytes can colonise nearly all the plant tissues like roots, stem, bark, leaves, floral parts and seeds. The plant microbiome harbours diverse endophytes which show a high level of abundance (Ganley et al. 2004), and play beneficial roles in plant nutrition and eliciting defence mechanism, through modulation of gene expression. Some commonly observed bacterial endophytes belong to the genera *Azospirillum, Gluconacetobacter*, *Herbaspirillum*, *Azoarcus*, *Achromobacter*, *Acinetobacter*, *Pseudomonas*, etc. and actinomycetes like *Streptomyces*, *Microbispora*, etc. (Coombs and Franco 2003). Among fungi, *Basidiomycetes* group were found to be the most dominant endophytes. Other fungal endophytes include unknown taxa *Exophyla*, *Cladophialophora*, *Harpophora*, *Periconia macrospinosa* and *Ceratobasidium/Rhizoctonia* complex which were found to be involved in the enhancement of plant growth and nutrient uptake (Jumpponen 2001).

7.2.2 Harmful Plant-Microbe Interactions

Harmful plant-microbe interactions are detrimental to the host (plant) as the infecting microbes may be saprophytic in nature or cause necrotrophy in the colonising plants. On the other hand, several plants produce allelochemicals, which inhibit the growth of microorganisms in their vicinity.

7.2.2.1 Root Exudates and Allelopathy

Allelochemicals are chemical compounds belonging to various chemical families and include plant growth regulators, including salicylic acid, gibberellic acid and ethylene, which give such plants an edge in terms of competing for limited resources (Gioria and Osborne 2014). Ferulic acid, p-hydroxybenzoic acid and hydroxamic acid act on soil microflora, inhibiting soil nitrification, reducing emission of N_2O and improving the utilisation efficiency of nitrogen fertilizers, thereby reducing pollution in wheat crop (Ma 2005). Allelopathic compounds produced as root exudates generally enter the rhizosphere soon after their release (Inderjit 2001). These

compounds may be released in large amounts but are subjected to sorption (physical), metal oxidation (chemical) and microbial degradation (biological) within the rhizosphere (Huang et al. 1999). Significant correlations were observed between crop growth and the activity of soil microbes, particularly due to the application of allelochemicals or when grown in the presence of allelopathic plants (Mishra et al. 2013). The indirect effects of allelopathy as a mediator of plant-plant interactions were observed to be more important than the direct effects of an inhibitor (Zeng 2014). Microorganisms possess the ability to modify the nature or breakdown allelochemicals released signifying their key role in plant-plant interactions (Fernandez et al. 2013). Additionally, bacteria, growing as biofilms in the rhizosphere, prevent the direct interactions of phytotoxic allelochemicals with the colonisation sites, thereby reducing the toxicity of these chemicals (Mishra et al. 2012).

7.2.2.2 Plant Pathogens

Higher colonisation of plant pathogens vis-a-vis beneficial microbes in the rhizosphere of susceptible host plants will not only affect the survival of plants but also disturb the diversity of native rhizosphere microflora. Community structure and population density changes in native microorganisms in rhizosphere may bring harmful effects on host plant as well as the associated microbes in terms of nutrient availability or plant- and microbial-related metabolic activities. Economically important fungal plant pathogens include pathovars belonging to genera *Phytophthora* (potato late blight), *Puccinia* (wheat rust), *Magnaporthe* (rice blast), *Ustilago* (maize), *Fusarium* (root rots), *Alternaria* (leaf spots), *Pythium* (stem rot), *Rhizoctonia* (rots), etc. (Stukenbrock and McDonald 2008). Common bacterial pathogens of agricultural crops include species of *Agrobacterium*, *Xanthomonas*, *Pseudomonas*, *Erwinia*, *Ralstonia*, etc. (Lacombe et al. 2010).

7.3 Plant-Microbe Interactions in the Rhizosphere

The region surrounding the roots or the rhizosphere is microbially active (Hiltner 1904). While rhizosphere zone varies according to the type of plant and their root volume/biomass, rhizoplane is the exact root surface, which harbours microbes that are closely adapted to the particular crop plants (Baudoin et al. 2002). Rhizosphere is the most important niche for plant-microbe interactions (White 2003) in terms of nutrient uptake, exudation and nutrient transformations (biogeochemical cycles).

Rhizosphere is highly dynamic, and the rhizospheric associated microbes are often transient in nature according to the quality and quantity of root exudates (Yang and Crowley 2000). The spatial variation in microbes associated with the rhizosphere highly depends on the biological and chemical parameters of the roots (Morgan et al. 2005). In general, the rhizospheric microbial population exceeds the population of nearby bulk soil (Pinton et al. 2001) due to the differences in physical, chemical and biological parameters of the microenvironment (Benfey and Scheres 2000). The rhizosphere may exhibit either beneficial or harmful interactions, depending upon the interactions between plant and microorganisms, which include bacteria or fungi.

Diverse groups of microorganisms live around the plant roots based on their nutritional requirements. Several heterotrophic bacteria utilise organic compounds excreted by the root exudates (Sorensen et al. 2001). The congregation of microbes around the zone of root exudation is termed as "rhizosphere effect" (Whipps 1990). At present, the rhizosphere is further subdivided into the endo-rhizosphere (root cortex, epiderma and root hairs) and the ecto-rhizosphere (root associated soil compartments up to a distance of 5 mm). The diversity of rhizosphere and associated rhizospheric microbes in terms of their structure and function varies according to the plant species, age, root volume, quality and quantity of root exudation and soil physical and chemical attributes (Narula et al. 2007). Plant roots exudate ions, free oxygen and water, enzymes, mucilage, metabolites and other nutrients including carbon/peptide compounds, which support the growth of an array of microbes (Nardi et al. 2000; Walker et al. 2003) that may help in promotion of plant growth, nutrient uptake (Barea et al. 2005), alleviation of stress and disease suppression (Abbott and Murphy 2003). Root exudates can be divided into two groups, based on the molecular weight of the exuded compounds: (a) low molecular weight compounds (amino acids, organic acids, sugars, phenols and other secondary metabolites) (Rougier 1981) and (b) high molecular weight compounds – mucilage (polysaccharides) and proteins (Abbott and Murphy 2003; Walker et al. 2003). Because of their high nutrient content, root exudates also recruit plant pathogenic fungi such as *Rhizoctonia*, *Fusarium*, *Sclerotium*, *Aphanomyces*, *Pythium*, *Verticillium Phytophthora*, etc. in the rhizosphere of susceptible host plants. Some plants exudate toxic substances such as glycosides and hydrocyanic acid, which may inhibit proliferation of the pathogens in the rhizosphere (Rangaswami 1988).

Root exudates are known to serve as signalling molecules between plant roots and microbial partner; interestingly, the same chemical molecule can act as an inhibitory signal for undesirable organisms. Secretion of isoflavones by soybean roots attracts a mutualist (*Bradyrhizobium japonicum*) and a pathogen (*Phytopthora sojae*) alike (Bais et al. 2006). Flavonoids present in root exudates of legumes activate the *Rhizobium meliloti* genes, coding for the nodulation process (Becard et al. 1995). The root cells are protected by defence proteins like phytoalexins and other unknown chemicals from pathogenic bacteria (Flores et al. 1999). Recently, rosmarinic acid was found to be elicited by fungal cell wall extracts from *Phytophthora cinnamomi* in the root exudate of hairy root cultures of sweet basil (Bais et al. 2006). In some cases, the plant and microbially produced compounds are further degraded to yield allelopathic or other toxic compounds, which are inhibitory to pathogenic microbes (Yang et al. 2001).

7.4 Mechanisms of Plant-Microbe Interactions in Rhizosphere

Plant-microbe interactions, either beneficial or harmful, occur in the rhizosphere through diverse mechanisms. Some of the widely investigated mechanisms in plant-microbe interactions include quorum sensing, volatile production and plant or microbial signalling.

7.4.1 Quorum Sensing

Quorum sensing (QS) is mediated by small diffusible signal molecules (autoinducers), which can regulate the gene expression of the population in response to their density in an environment (Miller and Bassler 2001; Hooshangi and Bentley 2008). The *N*-acyl homoserine lactones (AHL) serve as signalling molecules in Gram-negative bacteria and regulate the population density (von Bodman et al. 1998) (Table 7.1), while the altered oligopeptides, which generate signals from membrane-bound sensor histidine kinases, act as receptors. Several plant-secreted constituents imitate bacterial AHLs in effecting quorum-sensing signals in plant-associated bacteria (Teplitski et al. 2000). Most of the work on QS in the rhizosphere has focused on plant pathogens (von Bodman et al. 2003). Reports on microbes possessing the ability to 'quench' the QS systems via quorum interference are available (QI) (Crepin et al. 2012; Uroz et al. 2009). QS plays an important role in legume symbiosis (Gurich and Gonzalez 2009; Rinaudi and Gonzalez 2009).

7.4.2 Volatiles

Volatile metabolites are chemicals, which facilitate communication across all kingdoms of life (Dweck et al. 2015). They play a major role in diverse microbial interactions and are capable of manipulating physiological processes in other bacteria, as well as in fungi and plants (Audrain et al. 2015; Schmidt et al. 2015). Involvement of bacterial volatiles in cross-species interactions (bacterium-bacterium and bacterium-host) has also been studied (Blom et al. 2011). Qualitative and quantitative differences in the volatiles produced regulate several bacterial processes, including biofilm formation and motility (Lowery et al. 2008). Root associated/secreted volatiles are also involved in the initiation of biofilm formation in plants (Rudrappa

Table 7.1 Signalling mechanisms of plant-microbe interactions in rhizosphere

Signalling mechanisms	Compounds involved	References
Quorum sensing	*N*-Acyl homoserine lactones, altered oligopeptides	von Bodman et al. (1998), Teplitski et al. (2000), Hooshangi and Bentley (2008), Gurich and Gonzalez (2009), Rinaudi and Gonzalez (2009), Uroz et al. (2009), and Crepin et al. (2012)
Volatiles	2-Amino acetophenone, acetoin, 2,3-butanediol, 2-pentylfuran, 13-tetradecadien-1-ol, 2-butanone, and 2-methyl-n-1-tridecene	Ryu et al. (2003), Dickschat et al. (2005), Lowery et al. (2008); Rudrappa et al. (2008, 2010), Zou et al. (2010), Blom et al. (2011), Kesarwani et al. (2011), Groenhagen et al. (2013), Audrain et al. (2015), Park et al. (2015), Schmidt et al. (2015), and Kai et al. (2016)
Plant-mediated signalling	Jasmonic acid	Walling (2000), Glazebrook (2005), Doornbos et al. (2011), Lakshmanan et al. (2012), Landgraf et al. (2012), Carvalhais et al. 2013; Soler et al. (2013), Stam et al. (2014), Lebeis et al. (2015), and Rosier et al. (2016)
	Ethylene	
	Salicylic acid	
Plant hormones	Abscisic acid, cytokinin, gibberellin, auxins	Pieterse et al. (2012), and Giron et al. (2013)

et al. 2008). The production of 2-amino acetophenone, a QS-regulated volatile by bacteria, has been reported in *Pseudomonas aeruginosa*, *Streptomyces* spp. and *Burkholderia ambifaria* (Dickschat et al. 2005; Kesarwani et al. 2011; Groenhagen et al. 2013) (Table 7.1). Since volatiles are QS controlled, the pattern of volatile emission of bacteria is influenced by plant-microbe interactions (Kai et al. 2016).

7.4.3 Plant-Mediated Signalling

Plant signals influence the microbial assemblages in the rhizosphere (Rosier et al. 2016). Specifically, in response to the biotic stress (pests, pathogens, etc.), plants activate signalling molecules involved in the defence system, popularly called induced systemic resistance (ISR) (Walling 2000). Predominantly, the signalling pathways in plants are based on the jasmonic acid (JA)/ethylene-dependent (ET) ISR (Walling 2000) and salicylic acid (SA)-dependent systemic acquired resistance (SAR) systems (Glazebrook 2005) (Table 7.1). Apart from these signals, other hormones, viz. abscisic acid, cytokinin, gibberellin and auxins, also play a role as modulators of the signalling network (Pieterse et al. 2012; Giron et al. 2013). The activation of jasmonic acid/salicylic acid-dependent signalling largely depends on the type of stress (Soler et al. 2013; Stam et al. 2014). These plant signalling and defence mechanisms influence distinctly the rhizospheric microbial community (Doornbos et al. 2011). Lee et al. (2012) observed that feeding by aphids stimulated the plant to attract beneficial *Bacillus subtilis* GB03 populations in the rhizosphere of sweet pepper. Repeated wounding and foliar application of JA in *Medicago truncatula* influences upregulation of JA-signalling and leads to enhanced colonisation of beneficial mycorrhiza (Landgraf et al. 2012). Activation of JA signalling through the application of methyl jasmonate (MeJA) leads to significant changes in the composition of the rhizosphere community, as compared to bulk soil microbial communities (Carvalhais et al. 2013). Salicylic acid modulates the colonisation of the root by selected bacterial groups (Lebeis et al. 2015). The root exudates released in response to changes in JA signalling may influence the relative abundances of bacteria and archaea in the rhizosphere (Carvalhais et al. 2015). Application of plant hormones such as SA, MeJA, ABA and ET to *Arabidopsis thaliana* results in changes to the belowground bacterial richness and evenness (Carvalhais et al. 2013, 2014). Foliar infection by necrotrophic bacteria influences root exudate composition, possibly mediating the colonisation of roots by beneficial rhizobacteria (Rudrappa et al. 2008; Lakshmanan et al. 2012, 2014).

7.5 Factors Affecting Plant-Microbe Interactions

Several factors, such as host genome, the developmental stage of the plants and its root architecture, are known to modulate the community structure and diversity of rhizospheric microbiome members. Other factors (Fig. 7.2) including soil pH,

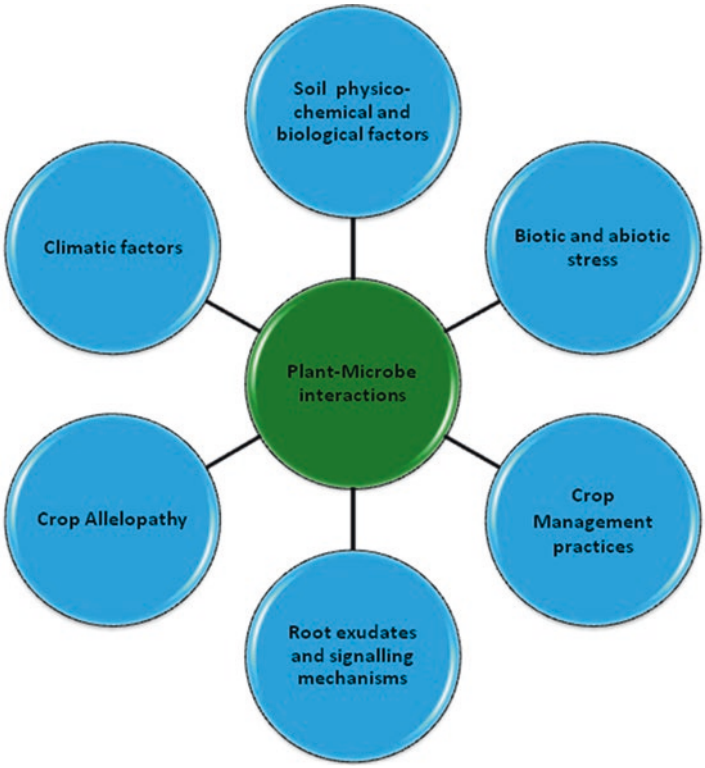

Fig. 7.2 Factors influencing effective plant-microbe interactions

temperature, moisture, pesticide application, grazers and other fauna also play important roles (Philippot et al. 2013; Spence and Bais 2013; Turner et al. 2013; Lakshmanan et al. 2014).

Lately, soil erosion, compaction, acidification, organic matter losses, nutrient losses, desertification, etc. have deteriorated soil health, which in turn reduces the agricultural productivity of soils. The inaccessibility of organic manures and composts and questions regarding their quality and public health issues have made agricultural activity depend more on synthetic fertilizers and other plant-promoting/protecting chemicals. Continuous and non-judicious use of synthetic chemicals has led to for soil contamination and degradation. Owing to these problems, soil faces functionality losses such as nutrient imbalance, nutrient deficiencies and biodiversity losses in many parts of the world. Microorganisms play a key role in ecologically important biogeochemical processes (Kennedy 1999). Additionally, microbiological properties represent among the most sensitive and rapid indicators of land use changes (Kuramae et al. 2012). Soil microbial diversity can bring about direct effects on plant productivity and diversity, by their effects on plant growth and development, competition and nutrient and water uptake (Schloter et al. 2003). Differences in soil physical and chemical properties can also contribute to variations in microbial populations and their diversity (Singh et al. 2006; Velmourougane et al. 2014).

Researchers have studied the connection between microbial biomass and soil properties like moisture (Herron et al. 2009), temperature, soil organic matter content (Bardgett and Shine 1999), texture (Grandy et al. 2009) and depth (Velmourougane et al. 2013). Optimum microbial activity is often limited by low organic matter and poor moisture availability of soils (Rao and Venkateswarlu 1983). Soil pH affects microbial population and community composition (Jesus et al. 2009). Rietz and Haynes (2003) concluded that agriculture-induced salinity and sodicity not only bring about distinct changes in the chemical and physical characteristics of soils but also induce major changes in soil microbial and biochemical properties. Populations of bacteria, total fungi and biomass are significantly larger in uncompacted soil than in compacted soil (Smeltzer et al. 1986).

Microbial community structure in the rhizosphere varies according to the crop types and varieties (Grayston et al. 1998; Velmourougane et al. 2014). Although multi-cropping systems increase microbial activity and diversity, monocropping systems reduce microbial diversity in the rhizosphere (Moore et al. 2000). Application of leguminous green manure crops, as organic manure, encourages beneficial soil microflora, as compared to farming systems, which receive application of chemical fertilizers (Bolton et al. 1985). Various soil management and cultural practices influence soil microbial populations and their activities (Wu et al. 2008). Type of cultivation and management practice show more influence on soil biota than soil types (Fromm et al. 1993). Differences in tillage intensity have an impact on microbial community composition (Jackson et al. 2003). Soil contamination by chemicals and pollutants also affects the soil biological functions, which is reflected in poor crop establishment and productivity. Soil factors along with management factors affect the crop performance by way of modifying the rhizosphere environment, which modifies the root exudate's characteristics and affects the microbial function and diversity along with alterations in nutrient transformations and uptake. Typically, healthy soils should possess high structural stability, which reduces soil degradation and produces good aggregate stability, which allows better porosity, aeration, water holding capacity and root penetration. Better physical properties aid in organic matter accumulation, nutrient adsorption and release, buffering capacity, etc., which in turn enhance higher soil biological activities and diversity.

Among different management factors, tillage influences soil structure and aggregate stability. Tillage disrupts soil aggregates, leading to compaction of soil, which affects plant and animal communities (Plante and McGill 2002). No-till management systems have more stable aggregates and soil organic carbon (SOC) (Filho et al. 2002). Reduced tillage enhances higher macropores and bio-channels that influence water movement and availability (Warkentin 2001). The addition of mulches to soil surface provides protection against soil erosion, evaporation and raindrop impact, thereby increasing aggregate stability (Layton et al. 1993). Mulches also increase the soil organic carbon pool (Duiker and Lal 1999) and modify temperature and moisture regimes and soil fauna. The application of plant residues to soil, particularly amount and quality of the residues, brings about significant improvement in soil structure (Martens 2000). Manuring improves soil structure and increases macroaggregation and resistance to slaking; however, it can decrease the stability of soil

aggregates (Hao and Chang 2002). It also increases the SOC, which results in higher soil biological activity, and results in increased porosity, aggregate stability (Martens and Frankenberger 1992) and decreased bulk density (Kay 1998).

The quantum and quality of fertilizers affect soil properties. Fertilizer application generally improves soil aggregation; however, under some conditions, fertilizers may also decrease SOC content, reduce aggregation and reduce microbial communities compared to manured soils (Haynes and Naidu 1998). Optimum fertilizer application will act as a good nutrient source and facilitate normal microbial functions, but inappropriate quantities may cause adverse effects on soil microbial population and diversity. Generally, crop cultivation decreases nutrient availability in soil by plant nutrient uptake if it is not properly replenished. Total microbial SOC pool and soil fauna decreases by continuous crop cultivation but increases metabolic CO_2 (Saggar et al. 2001). Several management interventions have been promoted to increase SOC and aggregation; these include fertilisation, grazing management, allowing native vegetation to grow instead of cultivation, growing cover crops, legumes and grasses, earthworm inoculation and irrigation (Bronick and Lal 2005). Retention of cover crop residues enhances microbial biomass, soil respiration and N mineralization and brings about changes in microbial communities (Schutter and Dick 2002). The inclusion of leguminous trees in agricultural systems also reduces soil erosion and improves soil productivity (Buresh and Tian 1997).

7.6 Ecological Benefits of Plant-Microbe Interactions

Plants are constantly in interaction with soil, which is rich in microbial diversity, and the use of beneficial microbes is an age-old practice in agriculture (Berg 2009). Plant root exudation enables the formation of beneficial associations with diverse groups of microbes. Many studies revealed plants' benefits through microbial associations in the rhizosphere, in most cases (Bouffaud et al. 2014; Edwards et al. 2015; Prasanna et al. 2016a, b; Ranjan et al. 2016). Several studies reported that rhizospheric microbiome can protect plants against both biotic and abiotic stresses (Zolla et al. 2013; Sugio et al. 2015; Harris et al. 2015).

7.6.1 Plant Growth Promotion and Nutrient Availability

Microbial inoculation to crop plants and subsequent enhancement in plant growth and yield are due to enhanced nutrient uptake and healthy nutrient status of plants. Mechanisms including asymbiotic nitrogen fixation, nutrient solubilisation/mobilisation, iron sequestration through siderophore production, etc. are some of the potential mechanisms for improved plant growth promotion (Boddey and Dobereiner 1995; Döbereiner 1997). Some of the important genera of bacteria involved in the supply of nitrogen to plants include asymbiotic/free living [*Azoarcus* spp. (Hurek et al. 1994), *Beijerinckia* spp. (Baldani et al. 1997), *Klebsiella* spp. (Riggs et al.

2001), *Pantoea* spp. (Riggs et al. 2001), *Azotobacter* spp. (Mrkovacki and Milic 2001), *Bacillus* spp. (Omar et al. 1996), *Burkholderia* spp. (Baldani et al. 2000)], symbiotic (*Rhizobium* spp., *Azorhizobium* spp., *Frankia* spp., etc.), associative symbiotic [*Azospirillum* (Bashan and de-Bashan 2010)] or endophytic [(*Herbaspirillum* spp. (Pimentel et al. 1991), and *Gluconacetobacter diazotrophicus* (Boddey et al. 2001)].

Bacterial genera including *Pseudomonas* spp. (Park et al. 2009), *Bacillus* spp. (Zaidi et al. 2006), *Burkholderia* spp. (Tao et al. 2008), *Streptomyces* spp. (Chang and Yang 2009), *Achromobacter* spp. (Ma et al. 2009), *Micrococcus* spp. (Dastager et al. 2010), *Flavobacterium* spp. (Kannapiran and Ramkumar 2011), *Erwinia* spp. (Rodríguez et al. 2001) and *Azospirillum* spp. (Rodríguez et al. 2004) have been identified as efficient phosphorus solubilisers which help in phosphorus supply to crop plant. The primary mechanism of phosphorus solubilisation is through production of organic acids (Kpomblekou and Tabatabai 1994) and phosphatases (acid or alkaline) enzymes (Rodríguez et al. 2006). Arbuscular mycorrhizal fungi play a major role in mobilisation of phosphorus and serve as a primary contributor of phosphorus nutrition in several crops (Smith et al. 2011). In recent years, use of potassium-solubilising microbes in agriculture is gaining importance. Solubilisation of potassium-bearing minerals, viz. micas, illite and orthoclases by bacterial species through production of organic acids, was reported earlier (Parmar and Sindhu 2013). Especially *Bacillus* spp. were widely explored in solubilisation of potassium and its subsequent availability to crops (Han and Lee 2005).

Apart from macronutrients (N, P and K), microbes are also reported to help plants to enhance their uptake of other secondary and micronutrients. *Pseudomonas* spp., *Acinetobacter* spp., *Azospirillum* spp., *Bacillus* spp. and arbuscular mycorrhizal fungi were reported to increase uptake of zinc (Kohler et al. 2008; Yazdani and Pirdashti 2011), copper, manganese (Liu et al. 2000), calcium, magnesium (Giri and Mukerji 2004; Khan 2005), sulphur (Banerjee et al. 2006) and iron (Rungin et al. 2012; Sharma et al. 2013). Co-inoculation of cyanobacteria along with bacteria in rice crop was found to enhance C-N sequestration in soil (Prasanna et al. 2012). Volatile organic compounds including alcohols, aldehydes, ketones, hydrocarbons, etc. produced by microbes, plants or interaction of plant-microbes enhance plant growth and biocontrol activities in the rhizosphere (Vespermann et al. 2007; Zhuang et al. 2007). Several microbial species and their interactions with crop plants can modify the quantity and quality of plant growth regulators/hormones (auxins, cytokinins, gibberellins and ethylene) (Dodd et al. 2010; Bhattacharyya and Jha 2012), which have profound effect on plant physiological functions.

7.6.2 Alleviation of Biotic and Abiotic Stress

Under natural conditions, crop plants face different kinds of stresses (biotic and abiotic) due to changes in climatic and soil conditions. While biotic stresses mainly involve pests and diseases, abiotic stress includes climatic variation, drought, salinity, sodicity, etc. Among abiotic stress, drought and salinity are important (Kramer and Boyer 1997), as they cause considerable yield loss and soil degradation in

agriculture. Recent studies indicate that the application of selective microbes as bioinoculants to crop plants can alleviate abiotic stress and sustain plant productivity, e.g. *Rhizobium* spp. and *Azospirillum* spp. enhanced the tolerance to salinity (Cordovilla et al. 1999; Hamaoui et al. 2001). *Azospirillum lipoferum* application to wheat decreased the ill effects of salinity (Bacilio et al. 2004). Inoculation of maize seedlings with *A. brasilense* reduced the negative impact of drought stress (Casanovas et al. 2002), which may due to increased production of osmoprotectant proline. *Azospirillum*-inoculated wheat showed increased Mg, K and Ca content, compared to non-inoculated plants under drought conditions (Creus et al. 2004). Under drought conditions, *Pseudomonas* spp. and *Bacillus* spp. stimulate plant growth (Marulanda et al. 2009). Microbial inoculation to crops under dry conditions improved root development and nutrient uptake (Padilla and Pugnaire 2007). Bioinoculant inoculation to crops under drought can also alleviate stress by production of IAA (Marulanda et al. 2009) and ABA (Cohen et al. 2008). Deactivation of stress hormone ethylene by ACC-deaminase led to enhanced drought tolerance in inoculated plants (Arshad et al. 2008).

Arbuscular mycorrhizal fungi were able to enhance crop establishment and abiotic stress tolerance (Porcel et al. 2012), e.g. corn, soybean, wheat, onion and lettuce showed enhanced drought tolerance through mycorrhizal symbioses (Augé 2001; Brundrett 1991). Inoculation of maize plants with *Glomus mosseae* (AMF) resulted in salt tolerance, which was attributed to soluble sugars accumulating in the roots (Feng et al. 2002). Fungal-based inoculants induced drought tolerance in plants (Singh et al. 2011). Microbial inoculation apart from inducing drought tolerance also modified the plants to adapt to various types of biotic stresses (Kim et al. 2012). Strawberry inoculation with *B. subtilis*, *Bacillus atrophaeus*, *Bacillus sphaericus* and *Staphylococcus kloosii* showed enhancement in the content of chlorophyll, nutrients and yield (Karlidag et al. 2013). Inoculation of canola with *Azospirillum lipoferum* increased shoot and root weights and antioxidant levels in the plants (Baniaghil et al. 2013). Microbial inoculants induced changes in the phytohormones of the inoculated plants and increased their salinity tolerance (Dodd and Pérez-Alfocea 2012).

Many indigenous rhizosphere microorganisms, including several bacterial genera – *Streptomyces*, *Agrobacterium*, *Enterobacter*, *Erwinia*, *Bacillus*, *Serratia*, *Azotobacter* and fluorescent *Pseudomonas* – suppress or control plant diseases (Kloepper 1993). Previous work showed that plants grown under biotic stress conditions tend to select and associate more with beneficial microbes (Rudrappa et al. 2008; Lakshmanan et al. 2012; Kumar and Bais 2012). Beneficial rhizobacteria such as *Azotobacter* and *Gluconacetobacter* exhibited antagonism against a variety of plant parasitic nematodes, including *Meloidogyne incognita* (Bansal et al. 2005). Ammonium ions produced by rhizosphere bacteria repel *M. incognita* juveniles, and acetic acid produced by rhizobacteria inhibited egg hatching and movement of infective juveniles (Bansal and Bajaj 2003). Studies have also reported biocontrol activities of mycorrhizal symbiosis in controlling root disease-causing pathogens in several crop plants (Chhabra et al. 1996). The mechanism of biocontrol activity of rhizospheric microbes is through direct killing or suppression of the pathogenic

organisms or by indirect competition for nutrients or inhibiting the establishment of pathogens due to the greater competence and ecological fitness of the biocontrol agent.

Primary metabolites (root exudates) modify the physical and chemical attributes of the soil (Dakora and Phillips 2002; Badri et al. 2009) and make available nutrients for microbial growth in the rhizosphere (Jones et al. 2004). Synthesis of phytochemicals by plant roots and their influence on structure and function of the soil microbiome is well studied (Bakker et al. 2012; Hartmann et al. 2009). Regulation of soilborne fungal endophytes and pathogens by the production of phytochemicals in chickpea was reported (Bazghaleh et al. 2016). Cyanobacterial inoculation in crops as single or in combination with bacterial or fungal partners showed potential biocontrol activity in several crops, besides their well-established role as nitrogen supplements in rice (Prasanna et al. 2008, 2015a). Apart from protection from fungal pathogens, biofilm biofertilisers, involving cyanobacteria and or fungal associations, showed enhanced plant growth and crop productivity (Babu et al. 2015; Prasanna et al. 2013; Triveni et al. 2013, 2015).

7.6.3 Environmental Remediation (Phyto- and Rhizoremediation)

Soils are often contaminated with heavy metals due to application of chemical fertilizers (rock phosphate, e.g. contains residues of uranium) pesticide-containing metals or sewage sludge and industrial wastewaters. Bioremediation is an emerging technology, involving the use of living organisms to manage or remediate polluted environments, particularly soils. It can be defined as the biologically mediated processes by which elimination, attenuation or transformation of polluting or contaminating substances into their less toxic forms takes place (Vidali 2001). Soil is a reservoir of thousands of contaminants that vary in quality and quantity. These pollutants enter the soil or water system through several ways including deliberate application, inadequate residue disposal, accidental wastes and inappropriate use (Knaebel et al. 1994). The soil is polluted by inorganic compounds, explosives (Kitts et al. 1994), monoaromatic hydrocarbons (Rooney-Varga et al. 1999), polycyclic aromatic hydrocarbons (Wang et al. 1990), herbicides (Fantroussi et al. 1999) and heavy metals (Glick 2003). Plants can also be employed in environmental remediation as an emerging alternative to chemical restoration of contaminated sites (Siciliano and Germida 1998). Plant-based remediation (phytoremediation) came way as eco-friendly solution for the pollution problem. Plant-assisted bioremediation (phytoremediation) involves the deployment of green or higher terrestrial plants for ameliorating chemically or radioactively polluted soils. Utilisation of crop plants combined with selective microorganisms to increase the efficiency of contaminants extraction is termed as rhizoremediation (Jing et al. 2007).

In rhizoremediation, the selective microorganisms make the unavailable form of pollutant to plant available form, thereby help in higher uptake (extraction) by the plants (Chaudhry et al. 2005). In rhizoremediation, plant and microbe work in a

synergistic way. Microbes derive their essential nutrients from plants for their growth, multiplication and pollutant degradation, while plants receive the altered form of pollutant, which is less toxic; this helps to clean up the pollutant site (Siciliano and Germida 1998). However, screening for heavy metal-resistant soil microbes was viewed as a challenge (Haferburg and Kothe 2007), but currently, many PGPRs find use in rhizoremediation (Narasimhan et al. 2003). Bacteria have been isolated which are capable of degrading organic pollutants such as polychlorinated biphenyls (PCBs), and the pathways and encoding the genes responsible have been elucidated (Brazil et al. 1995). The use of PGPR as adjuncts in metal phytoremediation helps the host plant to survive under the high concentrations of metals (Zhuang et al. 2007; Glick 2010). Use of endophytes along with *Thlaspi goesingense*, a hyperaccumulator plant of nickel, has been reported (Idris et al. 2004).

Apart from bacteria, mycorrhizae were widely used for remediation of contaminated sites. (Leyval and Binet 1998; Khan 2006). Mycorrhizae play a protective role in plant-microbe associations, through enhanced degradation of organic pollutants in the mycorrhizosphere (Meharg and Cairney 2000), thus lowering the bioavailable concentration of the pollutant in soil. The intermediary role of mycorrhizal fungi in the protection of roots from heavy metal toxicity has been investigated (Leyval et al. 1997). The use of *Streptomyces* spp. in the bioaccumulation of copper, cadmium and nickel has been investigated (Albarracin et al. 2008). The use of fungi and ectomycorrhizal fungi for bioaccumulation of heavy metals has been tested (Merten et al. 2004; Terpitz and Kothe 2006). To overcome the limitation of organic pollutant degradation by the microbes, in recent times, with the advent of genetic engineering, pollutant-degrading genes are introduced in microbes for application in bioremediation (Glick 2010). Sriprang et al. (2003) introduced the gene for phytochelatin synthase from *Arabidopsis thaliana* into *Mesorhizobium huakuii* subsp. *rengei* strain B3, which was then used to establish symbiosis between *M. huakuii* subsp. *rengei* strain B3 and *Astragalus sinicus*. The gene was expressed to produce phytochelatins and accumulate Cd^{2+}, under the control of bacteroid-specific promoter, the *nif* H gene (Perret et al. 1999).

7.7 Summary and Conclusions

Today, the world faces several challenges to supply food, fibre and feed for satisfying the needs of the ever-growing populations globally. Soil health deterioration and limited availability of productive land for agricultural use, coupled with climate change, are major challenges for crop production. Scientific advances in various spheres have paved the way for increased yield and quality in crops and improved health awareness among consumers regarding benefits of the product, besides a reduction in the costs involved. Their impact on soil and climate, as evaluated though the application of molecular tools, has illustrated the long-term impact of these technologies for crop production. Plant-microbe interactions, particularly harnessing the soil and plant microbiome and their ecological benefits to crop production, have gained greater interest in recent years. Application of modern techniques such as metagenomics, metatranscriptomics, proteomics, metabolomics, etc., in combination with plant genomics and transcriptomics, aids in better understanding

of the plant-microbe interactions in the soil. Analyses of beneficial and detrimental interactions between plants and microbes can offer unprecedented opportunities to increase crop productivity and sustain soil fertility.

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Strategies for Biological Control and Antagonisms

8

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Abstract

Microorganisms play an important niche in the control of soil populations producing a variety of bioactive compounds as an ecological strategy for competition for space and nutrients. Thus, the bioprospecting of microorganisms as potential antagonists for pathogen biocontrol, or obtaining their bioactive metabolites, is one of the alternatives currently studied for the control of diseases, especially in species of agronomic importance. In this chapter, we reviewed several microorganisms and how, in general, the products of their metabolism are obtained to be used in the control of pathogens.

Keywords

Bioactive compounds • Secondary metabolism • Bioprospecting • Purification

8.1 Introduction

Agricultural products are constantly under the pressure of phytopathogenic microorganisms, both during cultivation and after harvesting, causing significant economic losses. Fungi of several species, including *Penicillium* and *Botrytis cinerea*,

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besides bacteria such as *Erwinia carotovora* and *Xanthomonas vesicatoria*, have been mentioned as the main cause of diseases in plants (Trias et al. 2008). Currently, the control of these phytopathogens is carried out, almost exclusively, with bactericides, fungicides, and chemical insecticides, which can often cause serious environmental problems, besides selecting resistant strains due to unrestrained use. These problems, coupled with the society's growing demand for new products that present lower risks to the environment, encourage the search for alternatives that are less damaging to the environment.

One of the most interesting proposed alternatives is biological control. It can be defined as the use of a living, nonpathogenic, preexisting organism, antagonistic to pathogenic microorganisms, which are used to eliminate or control the outcome of disease. *Pantoea*, *Bacillus*, and *Pseudomonas* are among the bacteria most used and described as biocontrol agents (Trias et al. 2008).

According to Borrero et al. (2006 and 2009), some mechanisms can be used in the biocontrol activity against pathogens: (A) competition for nutrients in the rhizosphere and deprived sites for colonization, (B) production of iron siderophores chelated by microorganisms (e.g., *Pseudomonas* spp.), (C) antimicrobial production, and (D) production of cell wall degrading enzymes (CWDEs).

As for antimicrobials, it is known that many substances produced by biocontrol agents have broad-spectrum activity against many pathogens. Many examples can be found in literature, such as the antifungal activity of pyrrolnitrin isolated from *Pseudomonas* and *Burkholderia* species and its antibacterial activity against Gram-positive bacteria (El-Banna and Winkelmann 1998, Ligon et al. 2000) and the antibiotic activity of phenols, produced and isolated from *Pseudomonas aeruginosa*, against *Xanthomonas oryzae* pv. *oryzae* (Shanmugaiah et al. 2010). Therefore, the selection, production, and use of antagonistic microorganisms, or antimicrobial molecules produced by them, are potential alternatives for the biological control of pathogens and pests, without damaging the development of plants and with reduced harm to the environment (Harman 2000).

In this chapter, we will discuss how interactions between biocontrol agents and the effect of natural compounds produced by the secondary metabolism of some bacteria occur against phytopathogenic microorganisms, as well as some commercial products currently used and future perspectives in the development of new antimicrobials.

8.2 Microorganisms and Biological Control

Phytopathogenic microorganisms are able to colonize the crop tissues through the natural openings of the plant or through wounds, causing serious diseases during handling and processing of agricultural products, leading to economic losses.

The integrated control used to minimize the damages related to these infections include the application of antibiotic compounds, copper-based compounds, cultural treatments, production of pathogen-free seeds or seedlings, and development of resistant varieties. These alternatives do not seem sufficient to minimize the impacts

caused by pathogens, mainly because of the development of resistance by these microorganisms. The environmental damage caused by the application of chemical compounds is an even greater problem, once they have high toxicity.

There is a growing demand for an eco-friendly crop production, being necessary to find alternative control systems to replace the traditional chemical treatments in agriculture. Biological control, also called biocontrol, is defined "as the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be" (Eilenberg et al. 2001).

The use of microorganisms as biological control agents has been the focus of many researches in the last two decades, aiming to create more sustainable agriculture methods to control diseases caused by plant pathogens. These microorganisms are able to compete with the pathogens by different modes of action, producing antagonistic effects or inducing a plant defense mechanism. These microorganisms have a well-marked potential (Axel et al. 2012).

Many researches demonstrated the efficacy of biological control using microorganisms. On the control of phytopathogenic fungi, Banani et al. (2015) reported that the yeast *Metschnikowia fructicola* has been opportunely used in the control of a number of pathogens on fruits and vegetables, such as *Penicillium expansum* on apple and *B. cinerea* on grapes and on strawberries. Cordero-Ramírez et al. (2013) showed that strains of *Bacillus subtilis* and *Bacillus cereus* could control *Fusarium oxysporum f.* sp. *radicis-lycopersici*, a fungus that infects tomatoes and has the potential to reduce crop yield by 50%. Elkahouia et al. (2014) concluded that *Bacillus* sp. strain BCLRB2 produced various lipopeptides, with specific and broad spectrum of antifungal activities, presenting high antagonistic effect against *Rhizoctonia solani*, *Sclerotinia sclerotiorum, Fusarium graminearum*, *F. oxysporum*, and *F. oxysporum cicero* and a moderate antagonistic activity against *F. culmorum*. Ge et al. (2016) isolated a strain of *Bacillus methylotrophicus* able to inhibit mycelial growth and conidial germination of several plant pathogenic fungi in vitro and the growth of *B. cinerea* (the cause of gray tomato mold) by 60% in greenhouse conditions. Haidar et al. (2016) demonstrated the capability of two bacterial strains, *B. pumilus* and *Paenibacillus* sp., isolated from grapevine wood, to suppress *Phaeomoniella chlamydospora* via direct and/or indirect mechanisms.

The control of phytopathogenic bacteria is reported by Großkinsky et al. (2016). They identified the ability of *Pseudomonas fluorescens* G20-18 to efficiently control *P. syringae* infection in *Arabidopsis* sp., by producing microbial cytokinin and maintaining tissue integrity and, ultimately, biomass yield. Wu et al. (2016) showed the antagonistic effect of *Bacillus amyloliquefaciens* in the control of *Ralstonia solanacearum*, a well-known soilborne pathogen and causative agent of tobacco bacterial wilt.

Another strategy that has been studied is the use of bacterial strains that interrupt the quorum-sensing signaling by the pathogen. This mechanism of control is known as quorum quenching (QQ) and has been highlighted as a promising approach in biological control. Quorum-quenching bacteria (QBs) belong to various prokaryotic taxa and live in niches rich in nutrients, like rhizospheres and phyllospheres, and

have been used to reduce the virulence factors of plant pathogenic bacteria (Alymanesh et al. 2016). *Rhodococcus erythropolis* and *Bacillus* sp. significantly reduced the pathogenicity of *Pectobacterium carotovorum* subsp. *carotovorum* and *Erwinia amylovora*, respectively. *Pseudomonas* was the most abundant and strongest QQ-based biocontrol agent in rhizospheres, and QBs with extracellular enzymatic QQ activities are among the best biocontrol agents (Alymanesh et al. 2016). *P. aeruginosa*, which produces enzymes with QQ capabilities, may be used to suppress the quorum-sensing apparatus of pathogens (Bokhove et al. 2010). *Pseudomonas fluorescens* strongly reduced symptom development of a soft-rot casual pathogen, *E. carotovora*, which is dependent on the quorum-sensing-mediated production of CWDEs by the QQ mechanism (Molina et al. 2003). Information found in literature support the hypothesis that QQ is a promising mechanism to control bacterial pathogens in agriculture (Zhang and Dong 2004).

These are only a few examples of the potential of microorganisms in the control of plant disease pathogens. Several other studies in the past years showed the use of bacteria, fungi, and actinomycetes as biocontrol agents. However, it is noticeable that the development of effective alternative products able to resist in inhospitable ecosystems is very difficult. Many experiments using biological control agents were conducted so far, but most of them were tested in in vitro and ex situ, with assays generally being carried under very simplified conditions (Axel et al. 2012). The application of microorganisms directly on plants or on the soil, as biological control agents, can fail because of their sensitivity to the new ecosystem. The isolation of their metabolites and its use in pathogen control can be a suitable alternative to overcome this problem.

8.3 Biocontrol by Secondary Metabolites

The search for new natural products increases daily, especially the ones looking for bioactive compounds from the secondary metabolism of microorganisms and plants (Bérdy 2005). Metabolism is a set of biochemical reactions occurring within the cells. In case of microorganisms, it can be divided into primary and secondary metabolism. In primary metabolism, components that are essential for cell survival during an exponential growth phase are produced. Secondary metabolites of microorganisms, produced for the final phase of exponential growth or near the beginning of the stationary phase, are complex organic molecules and require specific enzymatic reactions for their synthesis (Madigan et al. 2010).

Secondary metabolites are not essential to the growth and reproduction of the microorganism. Their synthesis depends almost exclusively on the conditions of cultivation, especially in relation to the composition of the environment and the environment (temperature, luminosity, agitation, among others). The vast majority of secondary metabolites are complex organic molecules and requires a large number of enzymatic reactions for their synthesis. These compounds are very important for survival. Even in small concentrations, they inhibit the growth of other microbial

species, diminishing competition for nutrients and thus collaborating for species survival and selection (Madigan et al. 2010).

Among the groups of microorganisms studied in relation to the production of bioproducts, the group of filamentous actinomycetes has more than 10 thousand bioactive compounds, being considered the largest group of microbial metabolites ever studied (45%). In the group of bacteria, the genera that stand out are *Pseudomonas* and *Bacillus*, with about 3800 compounds studied, representing around 17% of all microbial metabolites with antibiotic activity (Bérdy 2012; Oliveira et al. 2016).

The search for bioactive compounds began with the discovery of lysozyme and penicillin by Alexander Fleming in the 1920s. Since then, new classes of compounds have been discovered, with antitumor, antiviral, and antiparasitic activities. Since the 1990s, there has been an exponential increase in the numbers of new metabolites discovered (mainly non-antibiotic compounds), but the occurrence of new chemical groups has decreased (de Oliveira et al. 2016).

Due to the growing problems with the arising new strains of multiresistant microorganisms and new pathologies, the need for new natural therapeutic agents is an emergency. New technologies promote rapid progress in phytopathological disease therapies, potentially renewing classical treatment methods and supplying the great demand for new products (Bérdy 2005).

Trichoderma used as controller agent of diseases in plant has great potential because of many factors: competitive activity for energy source, antibiotic metabolites that inhibited pathogen activity, and mycoparasitism (Chet 1987). These fungi also grow fast, with few nutritional requirements; produce CWDEs, factors that stimulate plant growth; make spores and chlamydospores; and acquire resistance to fungicides, and it is somewhat easy to obtain stable mutants (Melo 1991). During plant-fungus interaction, numerous elicitors released may induce signals transmitted in the plant, i.e., salicylic acid (SA), jasmonic acid (JA), and reactive oxygen species (ROS), triggering expression of defense proteins. Because of gene activation, the plant produces enzymes involved in direct suppression of pathogens and enhances the biochemical and structural barriers in plant organism. Depending on the *Trichoderma* strain, the defensive reactions activated by fungi may oscillate between the two types of systemic resistance, induced systemic resistance (ISR), and systemic acquired resistance (SAR) (Nawrocka and Małolepsza 2013).

Actinomycetes are gram-positive bacteria with typical filamentous growth. They are present in several environments, most frequently within soils, where they act as decomposers of organic matter. They are widely studied due to the bioactive metabolites they produce, like antimicrobials and enzymes, with biotechnological application.

Within this group, the genus *Streptomyces* sp. is one of the most studied producers of bioactive compounds, with a broad range of compounds from antimicrobial to antitumor activity. One of the antifungal compounds produced by this genus is the enzyme chitinase. It is a large and diverse group of glycosyl hydrolase enzymes ranging in size from 20 kDa to 90 kDa, and it is present in a broad spectrum of organisms (Kasprzewska 2003). Chitinase has the ability to degrade chitin in low

molecular weight chitooligomers, and it is used as a source of energy for bacteria (Hamid et al. 2013). Chitin is present in the cell wall of fungi, algae, insect exoskeleton, and other invertebrates. As a result, the chitinase of some microorganisms has become an important tool in the biocontrol of pests in agriculture. In addition to the chitinases, *Streptomyces* genus also produces β-1,3-glucanase, which also acts on the degradation of fungal cell wall components (Singh et al. 1999).

Other compounds with antimicrobial activity, produced by *Streptomyces* sp., were the focus of studies of bioactive compounds, such as 3-phenulpropionic acid and 8-hydroxyquinoloine against *Aspergillus flavus*, *Aspergillus niger*, *F. oxysporum*, and *Penicillium citrinum* (Narayana et al. 2008), and chloroxaloterpin A and B (diterpenoids) against *B. cinerea* (Bi and Yu 2016) among others.

The genus *Bacillus* contains strict aerobic or facultative anaerobic Gram-positive bacteria. When under stress, they form an endospore, with the ability to survive and remain metabolically active under extreme conditions. *Bacillus* sp. present antagonistic properties and many species produce extracellular hydrophilic enzymes that break down polysaccharides, nucleic acids, and lipids, allowing the use of these products as carbon sources and electron donors. They also produce lipopeptides that act as biosurfactants and phosphate solubilizers. *Bacillus* spp. are good secretors of proteins and secondary metabolites with antimicrobial activity (bacitracin, polymyxin, thyrocytin, gramicidin, and circulin). Additionally, they are easy to grow and maintain and highly efficient for the biocontrol of pathogenesis (Han et al. 2015).

One of *Bacillus* mechanisms of action as an antagonist for fungi and bacteria is antibiosis. Isolated bacterial and fungal inhibitory compounds are very similar throughout the genus *Bacillus*, i.e., the three broad families of cyclic lipopeptides (CLPs), including zwittermicin, kanosamine bacillomycins, iturin, fengycins, and surfactins. The purified compounds suppressed the disease and inhibited development of *oomycetes* by stunting and deforming germ tubes of germinating cysts. They controlled damping-off disease of tomato seedlings, caused by *R. solani,* and presented an even higher inhibition activity against *Plasmodiophora brassicae* and *Fusarium solani* (Schneider et al. 1999, Suk et al. 1999, Yu et al. 2002).

The genus *Pseudomonas* is extensively studied in relation to the bioactive compounds that it produces in its secondary metabolism, for evidences of their application in biocontrol, plant growth promotion, bioremediation, and induction of resistance. Much alike the genus *Streptomyces*, they produce lytic enzymes, such as chitinases, β-1,3-glucanase, and proteases, which affect pathogenic fungi and bacteria (Gupta et al. 2006). In addition to these compounds, they produce pseudomonic acids, phenazines, indoles, pyrrolnitrins, and some peptides with diverse bioactivities.

Phenazines are a broad group of aromatic heterocyclic substances produced almost exclusively by bacteria, which can be easily extracted from the microbial culture, analyzed, and quantified by chromatographic methods. Their antifungal property has been well known and studied for a long time, but the mechanism of action is poorly understood. It is known that phenazine diffuses through or enters the membrane of the microorganism, acting as a reducing agent, resulting in the

decoupling of oxidative phosphorylation and generating intracellular superoxide radicals and hydrogen peroxide that are fatal to the cell (Chin-a-Woeng et al. 2003). Phenazine compounds also have antibacterial action against Gram-positive bacteria, potentiated by the action of silver nanoparticles (Cardozo et al. 2013). Additionally, tumor cells are susceptible to respiratory interference and the generation of ROS by phenazine compounds (Pierson III and Pierson 2010). In plants, phenazines have been shown to induce systemic resistance against numerous pathogens and may influence growth. The main phenazines studied are phenazine-1-carboxylic acid (PCA), pyocyanine (PYA), and phenazine-1-carboxyamide (PCN).

Both PCA and PCN produced by *Pseudomonas* sp., have proved antifungal activity against several pathogenic fungi, such as *B. cinerea* (Zhang et al. 2015), *R. solani* (Olorunleke et al. 2015; Niu et al. 2016), *Benjaminiella poitrasii* (Tupe et al. 2014), *Fusarium graminearum* (Hu et al. 2014), among others.

The *P. aeruginosa* LV strain produced an unidentified organometallic compound with strong activity against various phytopathogens, such as *Xanthomonas axonopodis* (Lopes et al. 2012), *Xanthomonas citri* pv. *citri* (de Oliveira et al. 2011 and Oliveira et al. 2016), *Xanthomonas arborícola* pv. *pruni* (Vasconcellos et al. 2014), and *S. sclerotiorum* (Emiliano 2016). In scanning electron microscopy (SEM), it is possible to observe the population decline of *X. citri* pv. *citri*, morphological changes, and the reduction of extracellular polysaccharides when treated with the fraction called F3d (containing organometallic and phenazine compounds) (Fig. 8.1) (De Oliveira et al. 2016).

8.4 Obtaining Secondary Metabolites of Microorganisms for the Biological Control of Phytopathogens

In competitive terms, microorganisms that produce antimicrobial components are favored over nonproducers. These compounds have the advantage of species selection; they are very important for survival, because they can inhibit the growth of other microbial species even in small concentrations, reducing competition for nutrients.

Secondary metabolites are often produced after cell-associated growth processes, usually in the stationary phase. Secondary metabolism can be recognized as a general maintenance phenomenon for some species, and it is usually associated with plants and microorganisms. However, there is a variety of examples in the animal kingdom, such as the antibodies (Jung et al. 2008).

The following subsections will describe the main methodologies for the search, production, identification, and evaluation of natural compounds with antimicrobial properties.

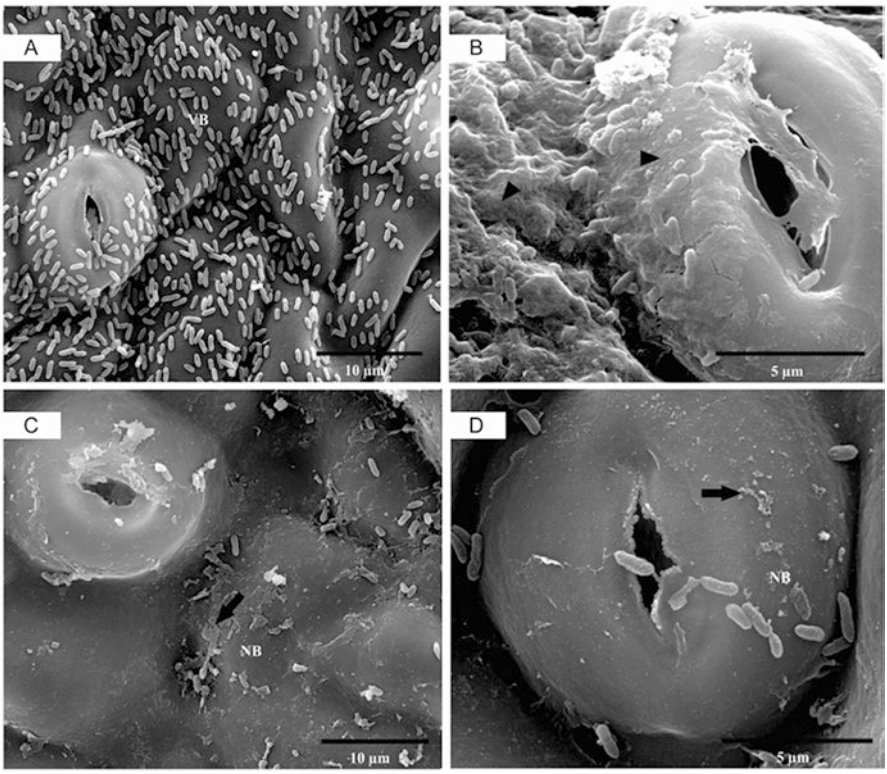

Fig. 8.1 Scanning electron micrographs of orange leaf (*Citrus sinensis* cv. *Valence*) inoculated with *X. citri* pv *citri* (Xcc). (**a**) Control 24 h after inoculation; (**b**) Higher magnification of control, with extracellular polysaccharides (EPS) on the leaf surface; (**c**) Curative treatment 24h after F3d application; (**d**) Higher magnification of curative treatment, with morphological changes in bacterial shape and EPS absence. (VB, viable cell; NB, nonviable cell) (de Oliveira et al. 2016)

8.4.1 Bioprospecting

The microbial metabolism has led to many studies with bioactive substances that can be used both in the control of diseases in agriculture and in therapeutic medicine, performing antimicrobial, antifungal, and antiviral functions among others (Lemire et al. 2013). The methodology for locating, evaluating, and systematically exploring microbial diversity in a given location, the main purpose of which is to search for genetic and biochemical resources for commercial purposes, is known as bioprospecting.

Bioprospecting mainly involves strategies for exploring the biodiversity of cultivable and noncultivable microorganisms, as well as genomic sequences already available in the database. All this has the purpose of identifying microorganisms, genes, enzymes, and/or metabolic pathways for subsequent strategic biotechnological applications in the industry or in the research itself.

Bioprospecting cultivable microorganisms require the cultivation and selection of microorganisms from existing microbial biodiversity in a given habitat or stored in collections for the application of a specific purpose. It is of great interest in the research to select microorganisms that grow specifically in a particular nutrient source. Less traditional and more efficient selection techniques, including mimicking of industrial conditions and automated process conditions, allowed the sterile cultivation of hundreds of microorganisms for desirable, even complex, characteristics in a single day. For example, miniaturized tests and online detection systems can be used to prospect large numbers of microorganisms in little time, producing bioactive compounds and identifying molecules of interest faster than before (Embrapa 2015). In addition, the cultivation of previously noncultivable microorganisms has been improved by increasing the knowledge on physiology, biochemistry, and microbial ecology, using large-scale phenotyping techniques, which permits the analysis of several characteristics simultaneously and facilitates the optimization of culture media.

Nonarable microorganisms account for approximately 99% of all species in outdoor environments and are an unexplored source of new antibiotics. Bioprospecting noncultivable microorganisms (microorganisms that cannot be cultivated with current techniques of microbiology) happened mainly due to the use of metagenomics. In this technique, the genetic material of microorganisms present in a given environment is collected, isolated, and amplified in DNA libraries. Metagenomic libraries allow not only the identification of the main microbial groups present in the sampled environment but also the genetic characterization (DNA sequence of the microbial pool) and its functional prospection. Microsystems have been constructed, and information on the microbiota of complex systems has been effectively obtained and used for biotechnological applications (Embrapa 2015). Several methods have also been developed to grow non-cultured in situ culture organisms such as diffusion chambers or the use of specific growth factors such as iron chelating siderophores (Losee et al. 2015).

Once colonies are produced and able to grow, a large number of substances can be obtained in the laboratory by in vitro culture.

8.4.2 Antimicrobial Natural Production

A bioreactor is basically a container that must be able to guarantee production under the desired conditions, to meet local containment regulations, and to monitor and control parameters such as pH, temperature, pressure, oxygen, and foam, among others. The three main fermentation techniques are batch, continuous, or fed-batch and continuous.

In industry, batch and fed fermentations have been used for the production of alcohol and fermented foods for 3000 years BC. At the beginning of the twentieth century, other applications have been popular, especially in World War II, with the production of antibiotics by culture submerging strains of bacteria and filamentous fungi. In human history, most fermentation processes were by batch. Feeding

fermentation became common in antimicrobial production (Tempest and Wouters 1981). Products not associated with microbial growth, such as antibiotics, are not well produced by continuous fermentation.

Most antibiotics are products of secondary metabolism. The fermentation must be stopped at the stationary phase, just before the cells begin to die. The use of bioaccumulating microbial metabolites was investigated in order to evaluate the effect of microorganisms on the microbial activity of the actinomycete. Among fungi, ascomycetes, species of filamentous fungi and endophytes, are the most significant producers of bioactive compounds. Basidiomycetes are also frequently reported as good producers, while yeasts seldom produce these metabolites. The total number of bioactive fungal products is approximately 8.600, representing 38% of all microbial products. Of the approximately 22.500 antibiotics and bioactive microbial compounds, less than one percent, only about 150 compounds, is in direct use in human medicine, veterinary medicine, and/or agriculture (de Oliveira et al. 2016).

8.4.3 Characterization of Antimicrobials

One of the researcher's tasks is to extract, isolate, and identify one or more pure substances from a crude extract. There are many processes and extraction systems described in the literature that can be used and adjusted if necessary. However, a trial and error approach is often necessary. The isolation of bioactive compounds is usually filled with difficulties and at every step requires judgment, improvisation, and new discoveries. The techniques most commonly used and described for extraction and/or pre-concentration of natural antimicrobial compounds or any bioactive compound are liquid phase extraction, solid phase extraction, supercritical fluid extraction, and solid membrane extraction (Gade et al. 2010).

The isolation of one or more substances from fractions of an extract can be a long and expensive process. Obtaining a pure compound many times requires several purification steps involving different techniques. This is particularly the case when it comes to bioactive metabolites wherein the target compound (e.g., natural antimicrobials) may be present only in trace amounts in a complex matrix of hundreds of other constituents or even have no standard for comparison. Chromatography is a physicochemical method of separating components from a mixture, performed by distributing these components between two phases, which are in close contact. One phase remains stationary while the other moves through it. During the passage of the mobile phase through the stationary phase, the components of the mixture are separated between the two phases, so that each component is selectively retained by the stationary phase, resulting in differential migration patterns of these components (Gade et al. 2010).

After the entire process of production, extraction, and purification of the natural antibiotic, it is possible to carry out the molecular identification. With the molecular structure and the functional groups that it possesses, it is possible to determine its physical properties and reactivity and to infer other biological activities and characteristics (Solomons and Fryhle 2000). One of the classic techniques for molecular

determination is spectroscopy. When we apply an energy to matter, it can be absorbed and emitted and/or cause a chemical modification and be transmitted. Spectroscopy is the study of the interaction between energy and matter, and its results can provide detailed information on the molecular structure of the compound (Silvertein et al. 2005).

8.4.4 From Laboratory to Field

After the initial studies, the isolation of biological control from the laboratory phase to obtaining a commercial product is a difficult task. Information is needed regarding the efficacy, mode of action of the agent, survival, colonization, and toxicity potential for nontarget species. In addition, studies on formulation, stability, and shelf life are also needed (Mathre et al., 1999, Harman 2000).

The fact is that most agents selected for biological control, despite being antagonistic at in vitro stages, are not successful in in vivo or field conditions (Aysan et al. 2003). Therefore, in vitro antagonism should not be used as the sole criterion for the selection of potential biocontrol agents (Tani et al. 1990). A possible justification for the difference between in vitro and in vivo is that the success of the agents depends on controlled environmental conditions, such as greenhouses or seedlings, favoring their efficiency (Paulitz and Bélanger 2001).

Another problem is that the solid culture medium does not reflect the actual physical and chemical conditions of the environment (Rampazo 2004). One of the fundamental differences is that the leaf has two dimensions, with little vertical diffusion of solute occurring, in contrast to the high degree of nutrient diffusion that occurs in a solid medium (McCormack et al. 1994). In addition, the solid medium favors the action and development of the antagonist agent.

8.5 Commercial Products

Although the first report of the antagonistic interaction between microorganisms was carried out in 1874, when William Roberts demonstrated that the fungus *Penicillium glaucum* inhibited the growth of bacteria, the use of these agents as biocontrol in crop protection against diseases is relatively new and not yet consolidated, even more when compared to the use of chemical compounds. In 1979, just over 100 years after William's discovery, the first commercial product containing an active bacterium, *Agrobacterium radiobacter* strain k 84, was registered in the United States, which was intended to control crown gall. As early as 1989, the use of *Trichoderma harzianum* ATCC 20476 was registered at the United States Environmental Protection Agency (EPA) for plant diseases control, originating the first commercial fungal product. According to the latest CPL survey in 2013, the biopesticide (products containing microorganism) market accounted for a total of $ 3 billion, representing only 5% of the total plant protection market. Also in the same year, approximately 2300 commercial products intended for plant protection

contained microorganisms in their formulation, with a total registered species of 77 bacteria, 68 fungi and yeasts, 36 viruses, and 2 protozoa (Ravensberg 2015). The increase in the number of registered products that use microorganisms in their formulation is mainly due to the lower cost of developing when compared to the chemicals. While the cost of developing a chemical molecule since the discovery to commercialization is around $ 256 million and takes an average of 9 years, the same process for marketing a biological product ranges from $ 20 to $ 50 million and takes only 5 years (Olson 2015).

Among the species of bacteria commercially used in the biocontrol of plant diseases are those of the genus *Bacillus*, especially *B. amyloliquefaciens*, *B. pumilus*, and *B. subtilis*, widely used for the control of soilborne pathogens such as *Fusarium*, *Rhizoctonia*, and *Alternaria*. As previously mentioned, the genus *Agrobacterium,* mainly represented by *A. radiobacter,* is also used against soilborne pathogens, especially *Agrobacterium tumefaciens*, the causative agent of crown gall. Some products containing *Pseudomonas* strains are effective in controlling foliar diseases, especially those caused by bacteria, besides guaranteeing fruit sanity after harvest (Fravel 2005; Junaid et al. 2013).

The genus *Trichoderma* is the most registered genus of fungi for commercial use. Its use is based on the control of soil diseases as mentioned above; however, the genus *Trichoderma* is known as a generalist biocontrol, due to its action against a broad spectrum of pathogens such as *B. cinerea*, *S. sclerotiorum*, *Sclerotium* spp., *Pythium ultimum*, *Phytophthora* spp., *Armillaria* spp., *Verticillium* spp., *Gaeumannomyces graminis*, *R. solani*, and *F. oxysporum*. Approximately 250 products containing *Trichoderma* are registered worldwide (Woo et al. 2014). Other fungi are widely marketed, mainly to control soil diseases and even nematodes. Among them are *A. flavus*, *Clonostachys rosea*, *Gliocladium* spp., *Paecilomyces* spp., *Pochonia*, *Ampelomyces quisqualis*, and others (Bettiol et al. 2012). There are also products that use yeasts such as *Aureobasidium pullulans* and *Candida oleophila*, used to control some foliar diseases after harvest.

Despite being relatively recent, the market for microorganism-based products for plant protection presents a promising future with an annual growth rate of 15.6% (Marrone 2014) and some optimistic projections that, in the future, the biological products market will take the place of the chemical market (Olson 2015). Such projection does not appear to be so distant from reality when considering the advantages provided by the use of bioproducts in agriculture when compared to the use of chemicals. Bhattacharjee and Dey (2014) summarize these advantages: (1) less environmental pollution; (2) less impact on beneficial organisms; (3) lower production cost and less probability of resurgence; (4) several applications; (5) biopesticides are highly efficient in controlling soil pathogens, where chemical control is not as effective; and (6) they can induce systemic resistance in plants.

Table 8.1 presents part of the worldwide market for biocontrol agents of plant diseases available. The products are assembled according to the group of microorganisms to which they belong. For each product, the following are presented: commercial name, diseases and pathogens target, mode of action and specific characteristics of the strain, and the culture where it is commonly applied.

Table 8.1 Commercial products based on biocontrol agent

Antagonist	Product	Phytopathogen	Activity	Crop
Ampelomyces quisqualis	AQ10 – Biofungicide®	*Erysiphe, Oidium, Podospora, Sphaerotheca*	*A. quisqualis* is a hyperparasite of causal agents of powdery mildew. *A. quisqualis* M-10 colonizes hyphae and conidiophores of pathogens of the orders *Erysiphales*, *Mucorales* and *Perisporiales*, forming picnids	Apple, ornamental plants, strawberry, tomato, grape
Arthrobotrys spp.	Nemout 0.65 WP®	*Helicotylenchus, Meloidogyne, Pratylenchus, Radopholus*	Nematode control	
Aspergillus flavus	AF36®, Afla-guard®, Aflasafe™	*A. flavus,* aflatoxin producer	*A. flavus* non-aflatoxin producing strain. Competition for space and nutrients with isolates producing aflatoxin, preventing the colonization of toxicogenic isolates. Aflatoxin is produced by *A. flavus* and can cause various damages to human and animal health, as it presents carcinogenic and abortive properties	Cotton, peanut, corn

(continued)

Table 8.1 (continued)

Antagonist	Product	Phytopathogen	Activity	Crop
Aureobasidium pullulans	Blossom ProtectTM, Boni Protect®	*E. amylovora* (fire blight), *Botrytis, Penicillium, Monilia*	*A. pullulans* competes for space on the surface of plant tissues and stimulates the vitality of plants	Pomegranates, ornamental
Clonostachys rosea	Clonosnat®, Clonotri®, EndoFine®, Kamoi	*Botrytis, Rhizoctonia, Sclerotinia, Sclerotium, Cylindrocladium, Fusarium, Phytophora*	*C. rosea.* It is a saprophytic fungus that acts preventively inhibiting the colonization of *B. cinerea* in the plant and sporulation in the cultural remains	Diverse
Cryptococcus albidus	Yield Plus®	*Botrytis, Penicillium*	*C. albidus* control post-harvest pathogens	Apple, citrus
Gliocladium spp.	Gliomix®, Prestop®	*Botrytis, Rhizoctonia, Sclerotinia, Sclerotium, Cylindrocladium, Fusarium, Phytophora, Alternaria, Helminthosporium, Penicillium*	*Gliocladium* sp. Produces enzymes that act on the pathogen. Also, the antagonist stimulates seedling emergence and root growth, ensuring uniformity and vitality to plants, even in the absence of pathogens	Diverse

Antagonist	Product	Phytopathogen	Activity	Crop
Bacillus spp.	Avogreen®, Ballad®, Cease®, Companion®/Kodiak®, EcoGuard®, FZB24®, HiStick N/T®/Subtilex®/ Pro-Mix®, Nacillus®, Rhapsody®, Rhizo Plus®, RhizoVital®, Serenade®, Sonata®, Sublic®, Yield Shield®	*Alternaria, Botrytis, Curvularia, Clavibacter, P. carotovorum, Fusarium, Gaeumannomyces, Gerlachia, Phomopsis, Phytophthora, Pyrenochaeta*	*B. subtilis* and *Bacillus* sp. Competition, antibiosis, and induction of resistance: stimulates plant growth, increases yield, and induces resistance against stress and infection by plant pathogens. Also acts by producing antibiotics and hydrolytic enzymes	Diverse
Burkholderia cepacia	Botrycid®	*Rhizoctonia, Thielaviopsis, Verticillium, Fusarium, Pythium, Botrytis, Mycosphaerella, Erwinia, Xanthomonas, Agrobacterium, R. solanacearum*	*B. cepacia* acts by competition, production of siderophores, and production of hydrogen cyanide, phenazines, and pyrrolnitrin	Diverse
Candida oleophila	Aspire®	*Penicillium digitatum, Botrytis*	*C. oleophila* I-182 competes with the pathogen for nutrients in the injuries and prevents infection	Stone fruits
Coniothyrium minitans	Contans WG®, Koni®	*S. sclerotiorum, Scletorinia minor*	*C. minitans* acts by hyperparasitism on sclerotia of the pathogen	Sunflower, canola, carrot, cabbage, bean, tomato, lettuce, celery, tomato, chili, pumpkin, and flowers

(continued)

Table 8.1 (continued)

Antagonist	Product	Phytopathogen	Activity	Crop
M. fructicola	Shemer®	*Penicillium digitatum, Penicillium italicum, P. expansum, Botrytis cinérea, Rhizopus stolonifer, A. niger.*	*M. fructicola* compete for space and nutrients	Citrus, fruits of heart, grape, strawberry, potato, carrot
Myrothecium verrucaria	Ditera®	*Meloidogyne, Pratylenchus, Trichodorus, Belonolaimus, Radopholus, Heterodera, Globodera, Tylenchulus semipenetrans, Trichodorus, Longidorus, Paratylenchus, Rotylenchulus, Xiphinema, Belonolaimus, Criconemoides, Criconemella, Tylenchorhynchus, Hoplolaimus, Rotylenchus, Helicotylenchus*	*M. verrucaria* acts on phyto-parasites and does not impair free-living nematodes	Diverse

Antagonist	Product	Phytopathogen	Activity	Crop
Paecilomyces spp.	BioAct®WG, Biomyces®, Biostat®, MeloCon WG®, Nemakontrol®, Nemata®, Paecil®, Safelomyces® WP	*Meloidogyne, Radopholus similis, Heterodera, Globodera, Pratylenchus, Rotylenchulus reniformis, Tylenchulus semipenetrans*	*Paecilomyces lilacinus* is parasitic at all stages of phytohematoid development, especially eggs. Antagonist spores are also attached to the cuticle of vermiform stages of the nematodes when they move in the soil. These spores germinate; the fungus penetrates the cuticle and colonizes the nematode, feeding on the contents of your body. The fungal hyphae also penetrate through openings in the body, such as the anus and the vulva	Vegetables, strawberry, pineapple, ornamental plants, tobacco, citrus, walnut, peach, grape, grass, banana, flowers, tomato, sugarcane, wheat
Pantoea agglomerans	Bloomtime Biological®, Blossom Bless™	*E. amylovora*	*P. agglomerans* E325 and P10c act by competition of space and nutrients	Apple, pear
Phlebiopsis	Rotstop®	*Heterobasidion annosum*	*Phlebiopsis gigantea* is a natural competitor of *Heterobasidion annosum*	Conifers

(continued)

Table 8.1 (continued)

Antagonist	Product	Phytopathogen	Activity	Crop
Pochonia	Pochar®	*Meloidogyne, Heterodera, Globodera*	*Pochonia* sp. and *Arthrobotrys* sp. protect against diseases caused by nematodes endo- and ectoparasites	Diverse
Pseudomonas spp.	Biomonas, Biosave® 100/110, BlightBan A506®, Cedomon®, Cerall®, Spot-Less Biofungicide®,	*Sclerotinia, Rhizoctonia, Pythium, Alternaria, Ascochyta, Cercospora, Macrophomina, Myrothecium, Ramularia, Xanthomonas, Erwinia, Fusarium, Verticillium*	The mode of action of the bacteria is not fully elucidated. Probably includes competition for nutrients and space, stimulus to plant growth, induced resistance, and antibiosis	Diverse
Pseudozyma	Sporodex L®	*Sphaerotheca pannosa, Sphaerotheca fuliginea*	*Pseudozyma flocculosa* PF-A22 UL control of powdery mildew, acting by antibiosis, producing a fatty acid toxic to the pathogens	

Antagonist	Product	Phytopathogen	Activity	Crop
Pythium oligandrum	Polyversum®	*Alternaria, Sclerotinia, Botrytis, Tilletia, Peronospora*	With its hyphae, *P. oligandrum* penetrates inside the cells of the pathogen, where it feeds. It competes with pathogens for nutrients and space to prevent attack and penetration of the pathogen. There is production of enzymes and parasitism that eventually exhaust the pathogen. Pythium oligandrum also induces the plant defense reaction	Sunflower, grape, wheat, cabbage, hops
Rhizobium radiobacter (Agrobacterium radiobacter)	Dygall®, Galltrol-A®, Nogall™	*A. tumefaciens*	*R. radiobacter* (antiga *Agrobacterium radiobacter*) cepa K84 acts by antibiosis mediated by the production of bacteriocin and agrocin. The bacterium biocontrol has a conjugative plasmid that codifies for the synthesis of agrocin 84, as well as for resistance to the agrocin	Apricot, cherry, nectarine, peach, grape, plum, pear, blackberry, raspberry, chestnut, walnut, ornamental plants
Serratia plymuthica	Rhizo Star®, Mycostop®	*Verticillium dahliae*	*S. plymuthica* HRO – C48	Strawberry

(continued)

Table 8.1 (continued)

Antagonist	Product	Phytopathogen	Activity	Crop
Streptomyces	Actinovate® SP, Mycostop®	*Fusarium, Alternaria brassicicola, Phomopsis, Botrytis, Pythium, Phytophthora, Rhizoctonia*	*Streptomyces* sp. acts through different modes such as competition for space and nutrients, antibiosis, hyperparasitism, and growth promotion	Agricultural and ornamental
Ulocladium oudemansii	Botry-Zen®	*Botrytis cinérea, S. sclerotiorum*	*U. oudemansii* U3 occupies the same space and competes for nutrients against pathogens	Apricot, cherry, nectarine, peach, grape, plum, pear, blackberry, raspberry, chestnut, walnut, ornamental plants

8.6 Future Perspectives

There are many challenges in biological control: the diversity of agents, the interaction with the host plant, the spectrum action of the metabolite produced by these agents, and the persistence of these metabolites in the environment, among other questions.

The use of live microorganisms as biocontrol agents is not a simple task. There are many environmental factors that make difficult the survival of these microorganisms in the environment, like climatic conditions and the interactions with the host and other microorganisms.

It is necessary to enlarge the range of biocontrol agents suitable for commercial use, either in the search for microorganisms that persist in the environment or in the isolation of secondary metabolites that can be applied to the crops.

Researches that focus on investigating the potential effects of these agents on the environment and human and animal health have to be improved. To combine biocontrol methods with other sustainable management techniques and to guide producers on the correct use of these agents is another challenge.

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9 Toward an Integrated Resource Management: Harnessing *Trichoderma* for Sustainable Intensification in Agriculture

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Abstract

Trichoderma has proved its diverse role in agriculture as an efficient microorganism to overcome numerous challenges associated with it. Being ubiquitous in nature, studies conducted on it are totally safe and involve low-cost implementation. Initially the research works highlighted this microbe as a suitable biocontrol agent against most phytopathogens. Many strains of *Trichoderma* have been successfully screened out for its beneficial effects on soil fertility and plant health aspects, but we need an environment which is free of pollution, and therefore focusing on multiple functions of *Trichoderma* to fight against various biotic and abiotic stresses and the hazardous pollutants which can affect our food chain is important to maintain sustainability. This mini review attempts to include the potentials of *Trichoderma* in present and upcoming condition of resource management.

Keywords

Abiotic and biotic stress • Tolerance • Plant growth promotion • *Trichoderma*

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9.1 Introduction

Trichoderma is a genus of filamentous ascomycete fungi that are among the most commonly isolated soil microorganisms; a gram of tropical soils contains 10^1–10^3 culturable vegetative structures (Harman et al. 2004; Druzhinina et al. 2011). The accomplishment of *Trichoderma* in the rhizosphere is owed to their towering reproductive capacity and capability to endure under extreme stress, efficiency in the exploitation of nutrients, ability to amend the rhizosphere, and sturdy antagonism against plant pathogenic fungi (Benitez et al. 2004; Harman 2006). These species can colonize woody as well as herbaceous plants, in which the sexual teleomorph (genus *Hypocrea*) has been observed. There are many *Trichoderma* strains together with the majority biocontrol strains with no sexual stages. *Trichoderma* are strong opportunistic invaders; fast-growing, prolific producers of spores; and also powerful antibiotic producers even under highly competitive environment for space, nutrients, and light (Schuster and Schmoll 2010; Herrera-Estrella and Chet 2004; Montero-Barrientus et al. 2011). *Trichoderma* are ubiquitous colonizers on cellulosic materials and can thus often be found wherever decaying plant material is available (Kubicek et al. 2009; Jacklitsch 2009). They are also present in the rhizosphere of plants from where they can induce systematic resistance against pathogens (Harman 2000). Although *Trichoderma* spp. have an intrinsic ability to attack plants, they usually are avirulent; they invade the superficial layers of the root, but do not penetrate further, and elicit plant defense reactions. *Trichoderma* spp. are characterized in culture media by a large number of small green or white conidia from phialids present on the profusely or nearly branched conidiophores. *Trichoderma* are found in diversified environments as decaying plant material, cockroaches (Yoder et al. 2008), marine mussels and shellfish (Sallenave et al. 1999), termite guts (Sreerama and Veerabhadrappa 1993), and inside dark and sterile biotechnological fermentor or shake flask. Thus, *Trichoderma* spp. adjust its lifestyle in both light and dark by regulation of growth, condition, and enzyme production. There is surprising link between light response and metabolic processes which is revealed with studies on carbon source utilization (Friedl et al. 2008) and cellulase-related gene expression (Schmoll et al. 2005).

9.2 *Trichoderma* and Stress Tolerance

Trichoderma spp. have been well known to assist in inducing biotic as well as abiotic tolerance to plants, so many species of *Trichoderma* are extensively analyzed for their diverse abilities of biocontrol, plant growth promotion, and bioremediation properties (Fig. 9.1).

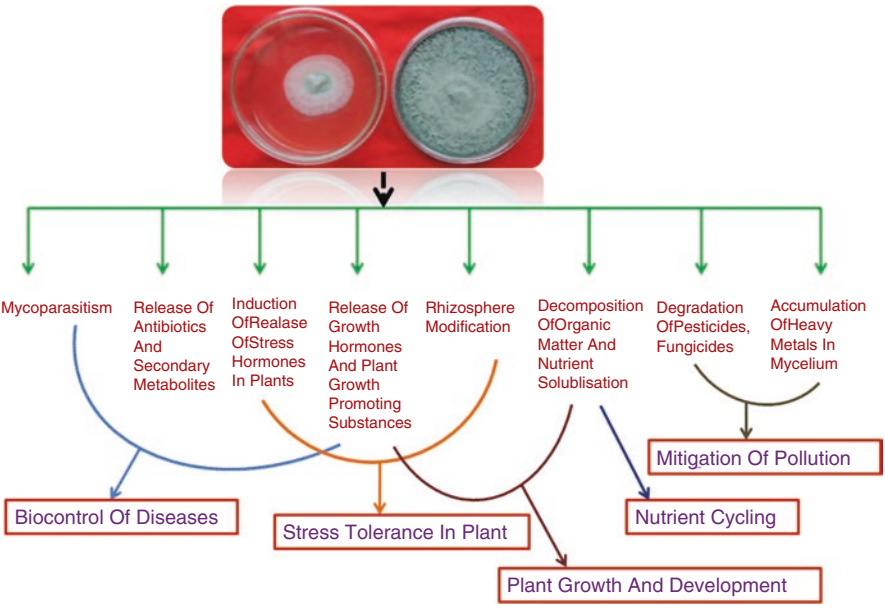

Fig. 9.1 The multifaceted role of the *Trichoderma* in soil-plant system

9.2.1 Biotic Stress

Competition among microbes and death due to starvation decides the dominating microbe in the soil (Benitez et al. 2004). Nonpathogenic *Trichoderma* becomes more competitive and efficient compared to other detrimental soil fungi by being better mobilizers for limiting nutrients (Chet and Inbar 1994). *Trichoderma* strains can compete for the key metabolites exudated from seeds that stimulate the germination of propagules of phytopathogenic fungi in soil (Howell 2002). Mobilizing soil nutrients could be related to the synthesis of organic acids, such as gluconic, citric, and fumaric acids which decrease the soil pH and help in solubilizing phosphates; mineral cations like iron, manganese, and magnesium; and other micronutrients (Vinale et al. 2008). *T. atroviride*, *T. virens*, and *T. reesei* have been found to secrete a compound ferricrocin, a siderophore in the rhizosphere which is the key metabolite that helps in the competition for iron uptake (Kubicek et al. 2011). The release of siderophores by microbes can be beneficial to plants in two ways – siderophore production suppresses the growth of plant pathogens by creating iron-starving conditions and siderophores solubilize unavailable iron. *Trichoderma harzianum* CECT 2413 contains a gene that encodes for expression of a high-affinity glucose transporter (GH 1). This gene is only expressed during very low glucose concentrations (Delgado-Jarana et al. 2003; Benitez et al. 2004). Plant-derived sucrose is an important resource to *Trichoderma*, and *T. virens* intracellular invertase (TvInv) has been identified responsible for sucrose hydrolysis in nutrient-poor soils (Vargas et al. 2009). Some proteins playing a vital role in root colonization by *Trichoderma*

are found to be crucial in competition with other root colonizers (Saloheimo et al. 2002; Viterbo et al. 2004; Brotman et al. 2008). Thus, the most common reason for the death of many microorganisms growing in the vicinity of *Trichoderma* strains is the starvation and scarcity of limiting nutrients (Table 9.1).

9.2.2 Antibiosis

Associated with competition for nutrients in the rhizosphere, antibiotics and/or hydrolytic enzymes are produced by *Trichoderma* which help in antagonism (Yedidia et al. 1999; Harman et al. 2004a). These metabolites include harzianic acid, alamethicins, tricholin, peptaibols, antibiotics, 6-pentyl-α-pyrone, massoia lactone, viridian, gliovirin, glisoprenins, and heptelidic acid (Vey et al. 2001; Raaijmakers et al. 2009).

It has been seen that in tobacco plants, exogenous application of peptaibols activated defense-responsive genes and showed reduced susceptibility to tobacco mosaic virus (Wiest et al. 2002). Antibiotic production varies according to different species such as *Trichoderma brevicompactum, T. viride, T. harzianum, Trichoderma atroviride, T. longibrachiatum, Trichoderma erinaceum, Trichoderma citrinoviride* although the main antibiotics from *T. virens* are peptabiols (Velazquez-Robledo et al. 2011; Mukherjee et al. 2013).

9.2.3 Mycoparasitism

Mycoparasitism is the antagonistic property with which *Trichoderma* attack and lyse plant pathogenic fungi. Seventy-five *Trichoderma/Hypocrea* species have been reported to attack fungi such as *Alternaria alternata*, *Botrytis cinerea*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Pythium* spp., and *Fusarium* spp. (Harman et al. 2004; Druzhinina et al. 2011). The process of mycoparasitism apparently includes chemotropic growth of *Trichoderma*, recognition of the host by the mycoparasites, secretion of extracellular enzymes, penetrations of the hyphae, and lysis of the host (Zeilinger et al. 1999). *Trichoderma* possess remote-sensing activity, i.e., it can grow toward fungal hosts by recognizing them. This characteristic is because of the sequential production of pathogenesis-related proteins mostly glucanase proteases and chitinase (Harman et al. 2004). This is a complex process that involves trophic growth of the biocontrol agent toward the targeted fungi, lectin-mediated coiling of *Trichoderma* hyphae to the pathogen, and finally the attack (Harman 2000). The mode of action involves release of some chemical compounds which inhibits the metabolic process in the mitochondria of cell (Miyadera et al. 2003). Constitutive secretion of exochitinases at low level, which degrade fungal cell walls releasing oligomers, plays a central role in growth inhibition of pathogenic fungi strains (Gajera et al. 2013). At least 30 proteins and other metabolites are involved in the process of attacking the target pathogenic fungus. The cell wall degradation of the target fungus results in the parasitism.

Table 9.1 Mechanisms imparted by *Trichoderma* for moderation of stresses in different crop species

Crop	Species involved	Stress factor	Mechanism	References
Biotic stress				
Green bean	*T. harzianum*	*Fusarium solani* and *Rhizoctonia solani*	Induced systemic resistance	El-Mohamedy et al. (2015)
Tomato	*T. harzianum*	*Rhizoctonia solani*	Root colonization and chemical communication	Singh et al. (2014)
Chickpea	*T. harzianum and T. viride*	Root lesion nematode (*Pratylenchus* spp.) and *Fusarium* spp. the causal agent of wilt/root-rot disease complex	Competition, antibiosis, parasitism, and systemic-induced resistance	Mudawi and Idris (2015)
Bean	*T. album*, *T. hamatum*, *T. harzianum*, and *T. viride*	*F. solani* and *R. solani*	Colonization, plant growth stimulation, biocontrol of diverse plant pathogens, decomposition of organic matter, symbiosis, and nutrient exchange	Abd-El-Khair et al. (2010)
Chili	*Trichoderma* spp.	*Colletotrichum capsici*	Elevated defense response	Saxena et al. (2016)
Tomato	*T. harzianum*	*Pythium ultimum*	Activation of the induced systemic resistance pathway	Mastouri et al. (2010)
Cucumber	*T. asperellum*	*Pseudomonas syringae* pv. *lachrymans*	Secretion of phenylalanine ammonia lyase (PAL) and hydroperoxide lyase (HPL) enzymes	Yedidia et al. (2003)
Squash, tomato, and brinjal	*T. harzianum*	*Sclerotinia sclerotiorum*	Mycoparasitism, secretion of lytic enzymes, inhibition of the myceliogenic germination of *Sclerotia*	Abdullah et al. (2008)
Abiotic stress				
Rice	*T. harzianum*	Soil salinity	Root colonization resulting increased level of plant enzymes like peroxidases (PODs), chitinases (CHIs), α-1,3-glucanase (Glc), lipoxygenases (LOXs), hydroperoxide lyase (HPLs)	Rawat et al. (2012)
Tomato	*T. harzianum*	Osmotic and salinity stress	Control of damage caused by the reactive oxygen species	Mastouri et al. (2010)

(continued)

Table 9.1 (continued)

Crop	Species involved	Stress factor	Mechanism	References
Cocoa	*T. hamatum*	Water deficit	Elongation of root	Bae et al. (2009)
Eucalyptus	*T. harzianum*	Arsenic (As)-contaminated soil	Adsorption of as on mycelia	Arriagada et al. (2009)
Mustard	*T. atroviride*	Cadmium (Cd)-contaminated soil	Increased plant biomass coupled with phytoremediation property	Cao et al. (2008)
Tomato	*T. harzianum*	Salinity and drought	Reduce stress-induced ROS generation	Azad and Kaminskyj (2016)
Maize	*T. atroviride*	Drought	Induction of antioxidant enzyme activities and decrement of H_2O_2 level	Guler et al. (2016)
Faba bean	*T. harzianum*	Salinity	Changes in protein pattern/ Activation of gene producing ACC deaminase	Abd El-Baki and Mostafa (2014)

9.2.4 Induced Resistance

In the genus *Trichoderma*, some species colonize and penetrate plant root tissues and initiate a series of morphological and biochemical changes in the plant physiology, related to plant defense response, giving rise to induced systematic resistance (Bailey and Lumsden 1998). *Trichoderma* can activate local and systematic resistance in plants. In *Trichoderma harzianum* strain T-39, bean plants showed resistance to diseases caused by fungal pathogen *Botrytis cinerea* when the strain was applied to roots (Bigirimana et al. 1997). Several metabolites produced by *Trichoderma* are involved in the induction of plant resistance which involve (1) proteins with enzymatic activity, i.e., "xylanase," (2) avirulence-like gene products to induce defense reactions in plants, and (3) low molecular weight compounds released from fungal cell walls through enzymatic activities (Harman et al. 2004). Roots inoculated with *T. harzianum* strain 203 exhibited higher activities of chitinase, β-1,3-glucanase, cellulose, and peroxidase when compared to untreated control, 72 h post inoculation (Yedidia et al. 1999).

9.3 Abiotic Stress

9.3.1 Inorganic Pollutants

Trichoderma has the property of phytoremediation of heavy metals and other inorganic pollutants. Strains of fungus *Trichoderma* proved to be very effective due to

high biodegradation potential (Harman et al. 2004; Lorito et al. 2010). Multiple heavy metal tolerance against Ni, As, and Zn has been proved in *Trichoderma* isolates (Kredics et al. 2001; Errasquin and Vazquez 2003), so it helps in high accumulation of multiple metals which is advantageous in phytoremediation because soils are often contaminated with various metals. Bioaccumulation of heavy metals such as Cu, Zn, Cd, and As is proved in in vitro conditions by some species (Harman et al. 2004; Zeng et al. 2010). Biovolatilization process with *T. asperellum* and *T. viride* is reported in arsenic-contaminated liquid media (Urik et al. 2007; Zeng et al. 2010). *T. harzianum* strains can detoxify potassium cyanide which in turn helps in mitigating cyanide toxicity in soil and promotes root growth of As hyperaccumulating fern *Pteris vittata* increasing the efficiency of accumulation (Lynch and Moffat 2005). Glutathione transferase (GST) enzymes secreted from *Trichoderma virens* are important for combating oxidative stresses induced by various heavy metal toxicity. Cloning of glutathione transferase gene from the fungus, and introducing it into tobacco by *Agrobacterium*-mediated gene transfer helped the plant to cope up with cadmium (Cd) toxicity (Dixit et al. 2011).

9.3.2 Organic Pollutants

Trichoderma can also be used effectively in the remediation methods for organic pollutants as some of the isolates of the fungus are found to be tolerant to crude oil (coil), naphthalene (NAPH), phenanthrene (PHE), and benzo[a]pyrene (B[a]P) in in vitro systems (Argumedo-Delira et al. 2012). *T. reesei* can promote plant growth in soil polluted with diesel (Mishra and Nautiyal 2009).

9.3.3 Agrochemicals

Extracellular enzyme system of *Trichoderma* catalyzes reactions that can degrade aromatic toxic compounds. They degrade various chemicals, including pesticide residues like chlordane, lindane, and DDT (Ezzi and Lynch 2005; Zhou et al. 2007). *T. viride* has been reported to efficiently degrade chlorpyrifos and photodieldrin (Mukherjee and Gopal 1996).

9.3.4 Water Stress

When plants are subjected to abiotic stress, reactive oxygen species (ROS) increase to toxic concentrations. *Trichoderma* augments protection against ROS by increasing ROS scavenging antioxidative enzymes. Proteomics of plants inoculated with *Trichoderma* show an increase in levels of antioxidative enzymes mainly superoxide dismutase (SOD) in roots as well as of peroxidase, glutathione reductase, and glutathione S-transferase (GST) in leaves (Shoresh and Harman 2008). These enzymes helped plant through different mechanisms as SOD converts toxic

superoxide (O_2^-) to hydrogen peroxide and oxygen and protects against tissue damage due to oxidative stress; CAT and POD, antioxidative enzymes, are able to convert toxic H_2O_2 to water and oxygen. Water use efficiency is studied on plants with and without *Trichoderma* which show that plants in symbiotic association use significantly less fluid while achieve increased biomass levels (Rodriguez et al. 2008).

9.3.5 *Trichoderma* and Nutrient Use Efficiency

Trichoderma enhances root growth and development, which increases the absorptive surface of roots and volume of soils explored by the roots. This feature directly contributes to uptake potential of plants for soil nutrients. Moreover, they have role in humification of compost which in turn helps in building the reserve of organic matter. Humification results in soil buffering neutralizing both acidic and alkaline soils and brings pH to the optimum range. When the soil pH is within optimum range, nutrients are easily available to crops. *Trichoderma* produces plant cell wall degrading enzymes, and as a result, both nutrient uptake efficiency and nutrient utilization efficiency are enhanced. Seed treatment with *Trichoderma* reduced requirement of nitrogen application to the extent of 30–50%, i.e., increase in nitrogen use efficiency by plant (Harman and Mastouri 2010; Shoresh et al. 2010). They also help in solubilization of tricalcium phosphate and results enhanced phosphorus availability to plants (Azarmi et al. 2011; Saravanakumar et al. 2013). *T. harzianum* in combination with other biofertilizers showed significant increase in N, P_2O_5, K_2O, Fe, and Mg content in leaves and grains of chickpea (Mohammadi et al. 2010). *Trichoderma* can thus serve the purpose of being one of the most prominent contributors of promoting increased nutrient use efficiency of crops.

9.4 Conclusions

Trichoderma, initially identified as an efficient biocontrol agent, is now known to have other potentials also which can be harnessed to increase agriculture production in a sustainable manner. Induction of biotic stress tolerance in plants by *Trichoderma* application reduces the use of plant protection chemicals and fertilizers. Tolerance to abiotic stress as by application of *Trichoderma* to plants prepared them to adapt under the climate change situation and sustain production on degraded lands also. It can also be used for remediation of environmental degradations caused by anthropogenic process as *Trichoderma* have capacity to degrade organic chemical residue and bioaccumulation of heavy metals. The multiple role of *Trichoderma* makes it an eligible candidate to incorporate it as one of the component in integrated resource management as it reduces complexity created by using number components for every function. Still researches on *Trichoderma* are required to develop it as a package for harnessing the potential of it in sustainable intensification of agriculture.

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Cyanobacteria: Role in Agriculture, Environmental Sustainability, Biotechnological Potential and Agroecological Impact

10

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Abstract

Cyanobacteria, a group of photosynthetic prokaryotes, have drawn the attention of agricultural scientists due to their notable key features such as the presence of oxygenic photosynthesis along with nitrogen fixation, ease in genetic manipulation and excellent adaptability to various environmental vagaries. Moreover, they have been recognized as an opulent source of various bioactive compounds possessing antibacterial, antiviral, antifungal and anticancer activities. They are also contributing positively in bioremediation and sustainable development of ecosystem. Furthermore, the presence of novel genes opens new ways for generation of transgenic crops with improved productivity and nutritional values. In view of the above, the present chapter is an attempt to cast light on cyanobacterial assistance and their potential role in sustainable development of agriculture and ecosystem.

Keywords

Cyanobacteria • Biofertilizer • Transgenics • Biotechnological potential • CO_2 sequestration

10.1 Introduction

Increasing population has raised a serious concern in front of global agriculture sector, as ~33% increase in population is expected by the end of year 2050. In order to meet the global food demand, current agricultural practices are injudiciously utilizing synthetic fertilizers and pesticides, which obviously helped to achieve the

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arduous target of agriculture sector, however upraised serious health and ecological complications. In view of the above, eco-friendly, sustainable and low-cost farming techniques have drawn the attention of agriculture scientists in recent years. One of the eco-friendly agricultural practices involves use of beneficial microorganisms that improve soil fertility and crop yield thus contribute in sustainable management.

Among beneficial microorganisms, cyanobacteria hold a prominent position due to their (1) unique and intrinsic capability of both N_2-fixation and photosynthesis; (2) excellent adaptive strategies towards various stress such as salinity, drought, heavy metal, UV-B radiation and temperature extremes; and (3) being a renewable source of biomass that produce organic substances (20 metabolites) useful for crops (Zulpa et al. 2003).

Cyanobacteria are a group of photosynthetic prokaryotes, where many of them can fix biological N_2 and not only fulfil their own requirement but also contribute significantly to global nitrogen economy (Pandey et al. 2012; Song et al. 2005). Moreover they are known to provide a wide range of bioactive compounds possessing antibacterial, antifungal, antialgal and antiviral activities (Dahms et al. 2006; Teuscher et al. 1992) thus serving as a biocontrol agent. These bioactive compounds are either toxins or phenols and polysaccharide (Mohamed et al. 2011; Tiwari and Kaur 2014; Flores and Wolk 1986). Few reports also suggest their effectiveness against plant pathogens (Yuen et al. 1994). Recently few studies witnessed the role of cyanobacteria in wastewater management, degradation of toxic compounds and pesticides (Cohen 2006; Yan et al. 1998; Radwan and Al-Hasan 2000; Raghukumar et al. 2001; Agrawal et al. 2015). Apart from this, cyanobacteria have gained attention due to their potential application in biotechnology in the past few years. Exclusive properties that make them potential candidate for biotechnology applications are (a) low-cost growth requirement, (b) short generation time and (c) ease of genetic manipulation. There are a number of reports that witnessed the potential of cyanobacterial gene pool in offering stress tolerance (Narayan et al. 2016; Mishra et al. 2009; Chaurasia et al. 2008; Shrivastava et al. 2012, 2014; Pandey et al. 2013a,b; Agrawal et al. 2015; Banerjee et al. 2015; Chakravarty et al. 2016). Furthermore, few cyanobacterial genes involved in photosynthesis, carbon metabolism, fatty acid biosynthesis and carotenoid biosynthesis have been also used to improve crop plant quality and yield. Overexpression of photosynthesis-related genes from cyanobacteria helped in improvement of crop yield (Häusler et al. 2002). In view of the above, the present chapter is an attempt to review key contribution of cyanobacteria in sustainable agriculture and crop improvement along with recent developments.

10.2 Cyanobacteria: Role in Agriculture

10.2.1 Biofertilizer

The ever-increasing demand of food supply has led to the use of chemical fertilizers. Due to large-scale application of chemical fertilizers, crops are becoming more susceptible to diseases leading to a decline in the soil fertility (Aktar et al. 2009;

Dong et al. 2012). Moreover, prolonged use of chemical fertilizers has been found to change the pH of the soil and destroy the beneficial microbes present in the soil. These chemicals also decreased water holding capacity, increased soil salinity and inequality in soil nutrients (Savci 2012). Therefore, considering all the adverse effects of these fertilizers, organic fertilizers have been initiated which act as natural stimulators for plant growth and development (Jardin 2015). In agricultural sector, utilization of microbes as biofertilizers is an alternative of chemical fertilizers because of their capability of enhancing crop production and food safety. They improved soil fertility by fixing the atmospheric nitrogen and solubilizing insoluble phosphates in the soil (Sahu et al. 2012). Cyanobacteria or blue-green algae (BGA) are a group of microorganism that can fix the atmospheric nitrogen. Cyanobacteria, for example, *Nostoc*, *Tolypothrix*, *Anabaena* and *Aulosira*, fix atmospheric nitrogen under nitrogen-deficient conditions and enrich the soil fertility. Nitrogen is one of the major nutrients and required in large amount (Wagner 2012; Santi et al. 2013). Some of the cyanobacterial members have specialized cells for nitrogen fixation, named as heterocyst. The nitrogen-fixing ability has been showed by both heterocystous cyanobacteria or by several non-heterocystous unicellular and filamentous cyanobacteria (Singh et al. 2016). *Anabaena* form symbiotic association with ferns *Azolla* that can be used as biofertilizer. After the decomposition of *Azolla* in soil, its nitrogen is accessible in the form of ammonia, polypeptides or free amino acids to the rice plants. Additionally, it also provides other nutrients like phosphorus, potassium, zinc, iron, molybdenum and other micronutrients to the plants (Al Abboud et al. 2013). Cyanobacteria contributed approximately 20–30 kg $N^{ha\text{-}1}$ organic matter to the soil, which is substantial for farmers unable to spend for costly chemical nitrogen fertilizer (Issa et al. 2014). It has been seen that application of cyanobacteria (in vitro) in rice as well as wheat fields enhanced the plant shoot/root length, dry weight and yield (Karthikeyan et al. 2009; Singh et al. 2016).

Phosphorus is the most important key element in the nutrition of plants, next to nitrogen. Cyanobacteria along with bacteria, fungi and actinomycetes and mycorrhiza showed phosphorus solubilization activity. Cyanobacteria can improve the accessibility of phosphorus to the plants by solubilizing and mobilizing the insoluble organic soil phosphates with the help of phosphatase enzymes (Wolf et al. 1985; Cameron and Julian 1988). It has been hypothesized that cyanobacteria synthesized a chelator for Ca^{2+} and breakdown $Ca_{10}(OH)_2(PO_4)_6$ into $10Ca^{2+} + 2OH^- + 6PO_4^{3-}$ (Cameron and Julian 1988; Roychoudhury and Kaushik 1989). Also, cyanobacteria solubilized phosphorus by releasing organic acid (Bose et al. 1971). Furthermore, many studies reported that soluble phosphate (PO_4^{3-}) scavenged by the cyanobacteria for their own food and after their death, PO_4^{3-} released in the soil and accessible for plants (Arora 1969; Saha and Mandal 1979; Mandal et al. 1992, 1999). Beneficial effects of applying cyanobacterial inoculation are also reported on various other crops such as barley, oats, tomato, radish, cotton, sugarcane, maize, chilli and lettuce (Thajuddin and Subramanian 2005).

10.2.2 Plant Growth Promoter

In addition to contributing as biofertilizer, cyanobacteria also produce several growth-promoting substances such as hormones, amino acids, vitamins and antibiotics that stimulate plant development. Plant hormones play an important role in plant growth. Under the stressful environmental condition, plants adjusted their endogenous hormonal level to combat the stresses (Peleg and Blumwald 2011). Cyanobacteria are known to release phytohormones such as auxin, gibberellin, cytokinin and abscisic acid (Singh et al. 2016). Auxin (IAA/indole-3-acetic acid) is known to enhance plant root system, hence increasing the possibility of improved nutrient acquisition by the roots (Spaepen et al. 2007). Gibberellin has very important role in controlling and promoting seed germination, as it is required to break seed dormancy. It has been well established that gibberellin-deficit mutants failed to germinate in the absence of exogenous gibberellin (Gupta and Chakrabarty 2013). Cytokinin works as a positive regulator in shoot development and a negative regulator in root development. Cytokinin is majorly contributed as a promoter of cell division, as well as in the identity of the shoot meristem. In roots, cytokinins control the rate of meristematic cell differentiation (Werner et al. 2010). ABA (abscisic acid) regulates various aspects of plant development such as embryo maturation, seed dormancy, germination, cell division and elongation, floral induction and responses to diverse environmental stresses (Finkelstein 2013). Marine cyanobacteria serve as valuable sources of vitamins and being used for the large-scale production of vitamins. For instance, *Spirulina* is a rich source of vitamin B12, beta-carotene, thiamine and riboflavin (Lau et al. 2015). Three cyanobacteria species *Anabaena oryzae*, *Nostoc calcicola* and *Spirulina* decreased the number of galls and egg masses in infected cowpea which is caused by the nematode *Meloidogyne incognita* and improved the plant growth criteria (Youssef and Ali 1998). Quite a few reports emphasized that using cyanobacterial inoculation in paddy crop field could enhance plant seed germination and root and shoot growth (Misra and Kaushik 1989a,b; Kausik 2012; Singh et al. 2016). Co-inoculation of cyanobacteria with wheat boosted root dry weight and chlorophyll as a consequence of extracellular substances released by cyanobacteria that colonized wheat plant roots (Obreht et al. 1993; Gantar et al. 1995a,b). Due to the fast growth and simple nutritional requirement of cyanobacteria, it is suggested that they can be commercially used as plant growth promoter.

10.2.3 Biocontrol Agents

The concept of agricultural sustainability is not promising without looking for an approach to control the infestation caused by the pests. Synthetic chemicals are used primarily to suppress pests and maintain high crop yields, but emphasis should be given to the role of microorganisms in achieving the cost-effective, long-term durable food security without any side effects on environment. Although execution of cyanobacteria as biofertilizers is well-known fact, recently we are focusing our attention towards the role of secondary metabolites produced by them in the management

of phytopathogen (Kulik 1995) and their further implementation in crop protection (Singh et al. 2014). These organisms can be exploited as biocontrol agents for plant pathogens particularly associated with soilborne diseases. Cyanobacteria produce a variety of secondary metabolites belonging to various chemical groups, i.e. polyketides, amides, alkaloids, fatty acids, indoles and lipopeptides (Abarzua et al. 1999; Burja et al. 2001) which are effectively potent against bacteria, fungi, algae and virus (Teuscher et al. 1992; Dahms et al. 2006). Allelopathic efficiency of cyanobacteria (i.e. growth inhibition of one species by the biologically active metabolites produced by other sympatric species) can be employed as biocontrol agents against pathogenic fungi and bacteria as these organisms can grow with minimum nutrients, cost-effective and eco-friendly manner. Table 10.1 represents the antagonistic effects of cyanobacteria in response to different plant diseases. Growth of pathogen is inhibited by disturbing their metabolic and physiological activities using array of secreted metabolites (Dahms et al. 2006). Algicidal properties were observed in selected number of nitrogen-fixing heterocystous genera *Fischerella*, *Nostoc* and *Calothrix.* Most of these algicides are functionally similar to natural herbicides as their prime target is PS II of photosynthetic apparatus. Similarly, Biondi et al. (2004) reported production of cryptophycin (natural pesticide) by *Nostoc* sp. that is effective against fungi, insects and nematodes. *Fischerella* is effective towards various plant pathogenic fungi, e.g. *Uromyces appendiculatus, Erysiphe graminis, Phytophthora infestans* and *Pyricularia oryzae*. *Anabaena* spp., *Scytonema* spp. and *Nostoc* spp. were used in control of damping off fungi such as *Fusarium* sp., *Pythium* sp. and *Rhizoctonia solani.* Kim and Kim (2008) reported inhibition of *Fusarium oxysporum* f. sp. *lycopersici* by extracts of *Nostoc commune* FA-103. It was observed that the growth of *Candida albicans* and *Sclerotinia sclerotiorum* was significantly restrained by the culture filtrates of *Nostoc muscorum* and the effect of the extract is species specific. Tiwari and Kaur (2014) observed that the growth of *Aspergillus niger* is severely influenced by cyanobacterial extract as compared to *Alternaria solani.* These extracts not only act as antifungal agent but also help in augmentation of the biocidal capability of fungal, bacterial and yeast. The prevalence of *Botrytis cinerea* on strawberries and *Erysiphe polygoni* causing powdery mildew on turnips and damping off disease in tomato seedlings is reduced by the cyanobacterial extracts. In addition, these extracts help in inhibiting the growth of saprophytes – *Chaetomium*

Table 10.1 Cyanobacterial species exhibiting antagonistic effects against different plant pathogens

Cyanobacteria	Plant diseases and pathogens	References
Calothrix elenkenii	Damping off (*Rhizoctonia solani*)	Manjunath et al. (2009)
Fischerella muscicola	Brown rust (*Uromyces appendiculatus*), Powdery mildew (*Erysiphe graminis*)	Hagmann and Juttner (1996)
Nostoc muscorum	Rice blast (*Pyricularia oryzae*),	De Caire et al. (1990)
	Cottony rot of vegetables and flowers (*Sclerotinia sclerotiorum*),	Kulik (1995)
	Damping off (*Rhizoctonia solani*)	Tassara et al. (2008)

globosum, Cunninghamella blakesleana and *Aspergillus oryzae* – and plant pathogens such as *Rhizoctonia solani* and *Sclerotinia sclerotiorum* (Kulik 1995). Mohamed et al. (2011) suggested that phenol and polysaccharide content is responsible for the allelopathic activity of cyanobacteria. Tiwari and Kaur (2014) observed that due to higher phenol and polysaccharide concentration, *Spirulina platensis* showed more allelopathic activity as compared to cyanobacterial strains like *Anabaena variabilis* and *Synechococcus elongatus*.

Certain toxic cyanobacteria excrete linear cyanotoxins that may possibly be made for defence towards other microbial pathogens including fungi, bacteria, virus and algae. These cyanotoxins inhibit physiological activities of other algae also (Flores and Wolk 1986; Shunmugam et al. 2014) and higher plants (MacKintosh et al. 1990; Lehtimaki et al. 2011; Shunmugam et al. 2014). Apart from their role as biocontrol agents cyanobacteria can also be applied for integrated disease management. Haggog et al. (2015) observed that cyanobacterium – *Oscillatoria agardhii* – was effective in integrated disease management of barley against foliar pathogens including net blotch, spot disease, powdery mildew and rust. According to Gol'din (2012), the antagonistic activity of cyanobacteria is similar in plants and other microbes with respect to their mode of action and wide spectrum. Yanni and Abdallah (1990) observed significant reduction in natural infestation of Indian rice with the stem borer *Chilo agamemnon* and leaf miner *Hydrellia prosternalis* when *Anabaena oryzae, Nostoc muscorum* and *Tolypothrix tenuis* were used in combination. *Synechococcus leopoliensis* and *Anabaena variabilis* have also shown biocidal activity against the Colorado potato beetle and fall webworm. The cultural extract of *Nostoc* is lethal to *Helicoverpa armigera* larvae at specific concentration of 2.20 mg cm^{-2}. Certain cyanobacterial metabolites, e.g. nostocyclyne A, nostocin A, ambigol A and B, hapalindoles, tjipanazoles and scytophycins, reveal fungicidal activity towards important plant pathogens. The biocidal activity has been observed in recombinant cyanobacteria with *Bacillus thuringiensis* subsp. *Israelensis* (Bti) toxin genes, which is effective against the fourth-instar *Aedes aegypti* larvae (Kiviranta and Abdel-Hameed 1994). Similarly, *Oscillatoria agardhii* strain 27 is toxic to *Aedes albopictus* larvae (closely related to *Aedes aegypti*) due to secretion of biochemical substance with mixture of unsaturated fatty acids (Harada et al. 2000). Zaritsky et al. (2010) reported the role of *cry* genes found in genetically engineered cyanobacterium *Anabaena* PCC 7120 and *Anabaena siamensis* in biocontrol of the larvae of *A. aegypti*. It was observed that Cry genes in association with Cyt1Aa are sevenfold more toxic as compared to Bti gene itself (Manasherob et al. 2003), and mutually the two genes prevent its degradation/toxicity from the sunlight in the field conditions.

10.2.4 Reclamation of Salt-Affected Soils

Salinity adversely affects 19.5% of the irrigated agricultural lands, and around 2.1% of dry land is facing the different ranges of salt conditions, limiting the productivity of crops including staple diet all over the world (Boyer 1982; Nelson et al. 1998). Salinity and alkalinity are the major problems associated with the arid and semiarid climatic regions of the world. Saline soils are the soils that have developed due to

prevalence of sodium salt (mainly NaCl or Na_2SO_4) whereas alkaline soils have developed mainly due to prevalence of Na_2CO_3 and $NaHCO_3$ (Szabolcs 1993). Salt-affected soils (commonly known as Usar soil in India) occupy 7.0–26.0 Mha of area as measured by different agencies. Water logging and insufficient drainage are the root cause of saline soil. Saline soil is infertile because of excess salt accumulation on its surface, which leads to less permeability, poor hydraulic conductivity, less aeration, high pH, high exchangeable Na and other soluble salts. The physiochemical properties of such soil can be conventionally changed by the addition of gypsum ($CaSO_4$, $2H_2O$) or pyrite ($FeSO_4$) followed by drainage of excess salts by flooding or extensive irrigation. However, such chemical methods are eco-unfriendly and not so cost-effective. In view of the above, reclamation of saline soil by biological means through invasion of cyanobacteria is a better option. Singh (1961) suggested that due to their archaic origin, cyanobacteria can survive under extremities of climatic conditions like high temperature, high pH, desiccation and high salinity, where most plants are incapable to survive (Stal 2007) and hence could be used to reclaim alkaline soils. Cyanobacteria helps in reclamation of alkaline soil as they form a thick layer on the surface of soil which store organic carbon, nitrogen, phosphorous, retain moisture and also converts Na^+ to Ca^{2+}. It will lead to enhancement in soil aggregation by lowering the pH and electrical conductivity and by increasing hydraulic conductivity (Kaushik and Subhashini 1985). Enhanced hydraulic conductivity results in better root penetration and increased nutrient uptake from nutrient-limiting sodic and saline soils (Fernandez et al. 2000). Invasion of cyanobacterial filaments in such soil promotes soil genesis, adds humus, dissolves certain minerals, increases moisture content (10–15%) and polysaccharide content, reduces soil loss and improves texture by increasing soil-binding properties. Cyanobacteria are also proficient in converting insoluble salts into soluble form by secreting certain exopolysaccharides (Flaibani et al. 1989) and metabolites like oxalic acid. The mucilaginous sheath of *Aphanothece* sp. forms a grey substratum which is capable of absorbing and retaining water and increases the soil aggregation, reducing erosion particularly in light and sandy soils (Singh 1961). Cyanobacterial filaments aggregate soil-binding properties as well as enhance the N and C content of the soil. Changes in physiochemical properties of soil were more rapid after the incorporation of pyrite (FeS_2). Venkataraman (1981) and Kaushik (1994) observed reclamation of saline soil by a filamentous heterocystous cyanobacterium *Nostoc calcicola* and its bicarbonate-resistant (HCO_3-R) mutant. Besides bringing physiochemical changes in soil, cyanobacteria improve the yield of crops especially rice and subsequently enhance cultivation of cereal and horticultural crops (Singh 1950, 1961; Aziz and Hashem 2003). They enhance the nutrient content by adding N and C in the soil as well as adopt a variety of mechanism to survive under salt stress conditions, which includes exchange of ions, accumulation of certain osmolytes and production of proteins in response to stress. *Anabaena*, *Aulosira*, *Calothrix*, *Nostoc*, *Plectonema* and *Westiellopsis* are the predominant genera that are ubiquitous in tropical soils, while *Hapalosiphon*, *Scytonema* and *Cylindrospermum* sp. have been locally distributed (Gopalaswamy et al. 2007). Blue-green algae are proficient in reclamation of saline and sodic soils, and their efficiency can further be enhanced if

gypsum at 50% of its required dose is applied along with blue-green algae. A combination of halotolerant cyanobacterium (*N. calcicola*) and gypsum is a considerable option for saline-alkaline/Usar soil reclamation, and it should also be a matter of great concern in future experiments related to soil reclamation (Jaiswal et al. 2010; Singh and Singh 2015).

10.3 Biotechnological Potentials for Crop Improvement

Keeping in view, the rising challenges of agriculture and environment, improvement of crop productivity through genome manipulation has become a prime target of plant biotechnology. The presence of robust type of genes in cyanobacteria and high similarity with plant genetic system offers special opportunity for crop improvement. Key contributions of cyanobacterial gene pool in crop improvement have been witnessed through various evidences. Cyanobacterial genes used for genetic manipulation in plants are listed in Table 10.2. Major groups of plants belong to C_3 group therefore lacking CO_2 concentrating machinery. Therefore in an attempt to enable C_3 plants to raise CO_2 concentration around Rubisco, cyanobacterial *ictB* gene involved in HCO_3^-accumulation from *Anabaena* PCC7120 and *Synechococcus* PCC7942 was overexpressed in *Arabidopsis* and tobacco (Lieman-Hurwitz et al. 2003). Transgenic *Arabidopsis* and tobacco displayed significant upsurge in photosynthetic rates under CO_2-limiting conditions. Similarly, Miyagawa et al. (2001) introduced FBP/SBPase and FBPase-II from *Synechococcus* PCC7942 into tobacco chloroplast and found significant increase in growth rate of transgenics probably due to enhanced C assimilation and various other metabolite contents. FBPase and SBPase are key enzymes of Calvin cycle and involved in hydrolysis of fructose-1,6-bisphosphate and sedoheptulose-1,7-bisphosphate.

Moreover, their survival in extremely hostile environments makes them an attractive candidate for fishing out genes offering stress tolerance. In view of the above, various attempts were made. Chamovitz et al. (1991) constitutively expressed phytoene desaturase (PDS) in tobacco from the herbicide-resistant mutant *Synechococcus* PCC7942. Transgenic tobacco plants demonstrated increased tolerance to photooxidative damage (Wagner et al. 2002). Flavodoxin, an electron carrier flavoprotein present in cyanobacteria and not found in plants (Park et al. 1998) from *Anabaena* PCC7120 was overexpressed in tobacco. Transgenics exhibited increased tolerance following various oxidative stresses such as herbicide, high irradiation, UV-B, heat, cold and water deficiency (Tognetti et al. 2007).

In another attempt, overexpression of acyl-lipid desaturase of mesophilic cyanobacterium *Anacystis nidulans* in tobacco chloroplast resulted in tolerance to cold stress in transgenic plants (Ishizaki-Nishizawa et al. 1996). In the same way, overexpression of delta 9- or 12-desaturase from *Synechococcus vulcanus* and *Synechocystis* sp. PCC6803 in tobacco lead to enhanced oleic acid and linolenic acid content, thus protecting from cold stress (Orlova et al. 2003; Reza et al. 2007) (Table 10.2).

Apart from this aspect, introgression of few cyanobacterial genes also resulted in improved nutritional value. For instance, ketolases crtR and crtO from *Synechocystis* PCC6803 were introduced in potatoes, and transgenic potatoes displayed

Table 10.2 Cyanobacterial genes used to improve plant productivity

S. No.	Genes (proteins)	Sources	Recipient plant	Major impacts	References
1.	*fld (isiB)* (flavodoxin)	*Anabaena* sp.	Tobacco	Enhanced tolerance to various oxidative stress	Tognetti et al. (2006), and (2007)
2.	*Pds* (phytoene desaturase)	*Synechococcus* sp. PCC 7942	Tobacco	Herbicide and oxidative stress resistance	Wagner et al. (2002)
3.	*Pepc* (phosphoenolpyruvate carboxylase)	*Synechococcus vulcanus*	*Arabidopsis*	Altered amino acid metabolism	Chen et al. (2004)
4.	*FBP/SBPase* or *FBP-I* (fructose-1,6–/ sedoheptulose-1,7-bisphosphatase)	*Synechococcus* sp. PCC 7942	Tobacco	Enhanced photosynthesis	Miyagawa et al. (2001)
5.	*FBPase-II* (fructose-1,6-bisphosphatase	*Synechococcus* sp. PCC 7942	Tobacco	Increased photosynthesis	Tamoi et al. (2005)
6.	*sps* (sucrose-phosphate synthase)	*Synechocystis* sp. PCC 6803	Tobacco, rice and tomato	Resistant to insect	Lunn et al. (2003)
7.	*crtO* (*b*-carotene ketolase)	*Synechocystis* sp. PCC 6803	Potato tuber	Commercial production of astaxanthin and others	Gerjets and Sandmann (2006)
8.	*desA* (acyl-lipid D12-desaturase)	*Synechocystis* sp. PCC 6803	Potato	Increased lipid content unsaturation for chilling stress resistant	Reza et al. (2007)
9.	*desC* (D9-desaturase)	*Anacystis nidulans*	Tobacco	Increased production of poly unsaturated fatty acid and chilling resistance	Ishizaki-Nishizawa et al. (1996)
10.	*desC* (D9-desaturase)	*Synechococcus vulcanus*	Tobacco	Increased production of poly unsaturated fatty acid and chilling resistance	Orlova et al. (2003)
11.	*desD* (D6-desaturase)	*Synechocystis* sp. PCC 6803	Tobacco	Increased production of poly unsaturated fatty acid	Reddy and Thomas (1996)

accumulation of astaxanthin, a carotenoid able to stimulate immune function (Lagarde et al. 2000). Above-mentioned illustrations cast light on potentials of cyanobacterial gene pool for crop improvement through genetic manipulation.

10.4 Cyanobacteria and Environment Sustainability

Environmental sustainability is the capability to retain things or traits that are essential for the physical environment like land, waters and atmosphere (Sutton 2004). Sustainability of the environment issues arises whenever the existing value of environmental system, object, process not being maintained and its quality could be at risk. Thus the sustainable environment needs to balance among the natural resources availability, quality and their uses. Population explosion, industrial production, irregular distribution of resources and agriculture land degradation are the main cause of degradation of the sustainability of the environment. In the last few decades, cyanobacterial research was focused for the suitability of the environment owing to their efficient application in environment management.

10.4.1 A Tool for Bioremediation

Cyanobacteria used as bioremediation tools due to their photoautotrophic nature and ability to fix atmospheric N_2, which makes them independent for growth and maintenance and adaptability to survive in extreme adverse condition (Sokhoh et al. 1992; Singh et al. 2016). Cyanobacteria are promising tool for the treatment of different types of hazardous environmental contaminates such as pesticides (Megharaj et al. 1994), crude oil (Sokhoh et al. 1992; Al-Hasan et al. 1998, 2001), naphthalene (Cerniglia et al. 1980a,b), phenanthrene (Narro et al. 1992), phenol and catechol (Shashirekha et al. 1997), heavy metals (Singh et al. 2011b; Rai et al. 1998), radioactive compounds (Acharya et al. 2012) and xenobiotics (Megharaj et al. 1987) either through their accumulation or degradation. Cyanobacteria metabolized environmental toxic pollutants to nontoxic form (Quintana et al. 2011) by the process of biosorption and active uptake cumulative known as "bioaccumulation" (Malik 2004; Sharma 2012). Due to high potential of metal sorption capacity and high multiplication rate, they play an important role in the detoxification of various industrial effluents such as from oil refinery, brewery and distilleries, paper mill, sugar mill, dye and pharmaceuticals industries. Cyanobacteria help in mitigation of eutrophication and metal toxicity problems in aquatic ecosystems so they can be for wastewater and agro-industrial effluents treatments (Vílchez et al. 1997; Singh et al. 2016). Cyanobacterial members display biosorption of different heavy metals and tolerance towards them, like *Nostoc calcicola* for Cu (Verma and Singh 1990); *Spirulina platensis* for Cu, Pb, Zn, Ni and Cd (Greene et al. 1987); *Oscillatoria angustissima* for Cu and Zn (Ahuja et al. 1999); *Microcystis* for Ni and Cd (Rai et al. 1998; Pradhan and Rai 2000); and *Synechococcus* sp. for Cu, Pb, Ni and Cd (Yee et al. 2004). Moreover, cyanobacterial mats possess excellent metal sorption

abilities which is attributed to their exopolysaccharide-enriched matrix as well as to entrapped cyanobacterial filaments and bacterial cells which provide numerous sites for the binding of metal ions (Mehta and Gaur 2005; De Philippis et al. 2011). *Phormidium bigranulatum*-dominated mats have potential for removal of Pb(II), Cu(II) and Cd(II) from aqueous solution (Kumar et al. 2012a, c; Kumar and Gaur, 2014). Kumar and Gaur (2014) demonstrated 80–94% removal of Cu^{2+} from the growth medium containing 10–100 μM Cu^{2+} by *Phormidium bigranulatum* dominated mat. Cyanobacteria exhibit high range of pesticide tolerance either via accumulation of huge amount of pesticide or through degradation (Ahmad and Venkatraman 1973; Kaushik and Venkatraman 1983; Pabbi and Vaishya 1992). Several cyanobacterial genera *Oscillatoria*, *Synechococcus, Nodularia*, *Nostoc*, *Microcystis*, *Cyanothece and Anabaena have* the potent capacity to remove or degrade the lindane residues (g-hexachlorocyclohexane) (Kuritz and Wolk 1995, El-Bestawy et al. 2007). Kumar et al. (2010) demonstrated an *Oscillatoria* sp.-dominated cyanobacterial mat is proficient sorbent of two pesticides, paraquat (PQ) and 2,4-dichlorophenoxyacetic acid (2,4-D). Microflora of cyanobacteria also increased alteration and degradation of some organic compounds, polycyclic aromatic hydrocarbon and organophosphorus compound. *Nostoc* sp. and *Aulosira fertilissima* are capable to degrade organic compounds, and *Synechococcus elongatus*, *Anabaena* sp., *Lyngbya* sp., *Microcystis* sp. and *Nostoc* sp. degrade the wide range organophosphorous and organochlorine (Semple et al. 1999; Vijayakumar 2012, Forlani et al. 2008). *Synechocystis sp*. plays important role in mineralization of aniliofos herbicide and used the product as phosphate source. The removal of synthetic dyes has become an area of immense concern because of their carcinogen, mutagenesis and toxic nature, besides their low biodegradability that causes environmental pollution. Certain species of cyanobacteria were reported for the removal of the synthetic dyes such as *L. lagerteimii*, *N. linckia* and *Oscillatoria rubescens.* Different species of *Phormidium* degraded the wide range of dyes like indigo, acid red 97 and 119, Ff sky blue, e.g. *P. valderianum*, and *P. autumnale* UTTEX1580 removes 90% of textile and indigo dye, respectively (Dellamatrice et al. 2016). It is also reported that many species of cyanobacteria like *Oscillatoria salina*, *Plectonema terebrans*, *Aphanocapsa* sp. and *Synechococcus* sp. degrade crude oil and other surfactant by forming mats in aquatic environments which were effectively used in the bioremediation of oil spills (Radwan and Al-Hasan 2000; Raghukumar et al. 2001; Cohen 2002). It is also reported that *Oscillatoria* sp. and *Agmenellum* sp. oxidize naphthalene to 1-naphthol and n alkanes (Cerniglia et al. 1979, 1980a). Furthermore, role of cyanobacterial mats in dye removal was also studied. Kumar et al. (2012b) have investigated that an *Oscillatoria* sp. dominated cyanobacterial mat has efficient potential for sorbing methylene blue (MB), through the batch contact method.

Several investigation showed that many cyanobacterial species like *Nostoc carneum*, *Nostoc insulare*, *Oscillatoria geminata* and *Spirulina laxissima* remove radioactive pollutants like Cs, Sr, Ra and Am (Pohl and Schimmack 2006) and

Anabaena torulosa eliminates uranium (Acharya et al. 2012) from waste and maintained the sustainability in environment.

10.4.2 Cyanobacteria and CO_2 Sequestration

Carbon sequestration is a natural phenomenon of removing CO_2 from environment. Cyanobacteria are photoautotrophic microorganism, which use CO_2 for photosynthesis. Therefore they are gaining attention for carbon sequestration due to alleviation of CO_2 concentration in the environment. Moreover, their cosmopolitan and tolerant habit in extreme stress condition makes them significant for it (Sundquist et al. 2008). Cyanobacteria decline the greenhouse gases by CO_2 sequestration and diminish the global warming (Singh et al. 2016). This is due to the reason that cyanobacteria are capable to fix CO_2 10–50 times faster than the terrestrial plants. Global CO_2 emission is primarily due to combustion of fossil fuels such as coal, oil, gas, etc. which produces flue gas (mixture of N_2, CO_2, O_2 and water vapours) with high temperature of around 1200C. Jacob-Lopes et al. (2008) reported increased CO_2 uptake in various cyanobacterial spp., such as *Aphanothece microscopica*, in areas exposed to higher CO_2 levels from flue gas. Some thermophilic members such as *Synechococcus aquatilis*, *Chlorogloeopsis* sp. etc. can be also used for CO_2 sequestration from flue gas. *Anabaena*, *Spirulina* and *Scenedesmus* species have the ability for high biomass production (Kanahiya and Das 2013). Furthermore, few genetically modified cyanobacteria are also developed for mitigating atmospheric CO_2 concentration.

Chen et al. (2012) raised transgenic *Synechococcus elongatus* PCC7942 with secreted carbonic anhydrases CynT and Can thus increasing cell growth by catalysing the hydration of CO_2 to produce HCO^{3-}. Another approach towards CO_2 sequestration employs direct conversion of CO_2 into useful bioproducts with aid of transgenic cyanobacteria. In this context, Miyasaka et al. (2013) have developed transgenic *Synechococcus* sp. PCC7002 that can convert CO_2 into polyhydroxyalkanoate (PHA) onsite. This approach can be a promising option for biological conversion of CO_2 into useful products.

10.4.3 Cyanobacteria and Nutrient Management

Cyanobacteria play an important role in the management of the nutrient in the soil as they fix the free atmospheric nitrogen and also produce the organic substances and maintain the soil structure.

It accumulates organic matter in the soil, which contains nutrient like phosphorus, nitrogen and also enhance organic carbon in the soil. An application of cyanobacteria in agriculture ecosystems, mainly the rice fields, enhanced availability of N to plants (Stewart et al. 1968; Peters et al. 1977; Singh and Singh 1987; Kaushik 1998). As cyanobacteria have the ability for nutrient management, bioremediation and biodegradation, they are used as biofertilizer, which improves the productivity of plants

with diminishing harmful effects of chemical fertilizer and maintains the sustainability of environment. There are several cyanobacterial species such as *Anabaena variabilis*, *Nostoc muscorum*, *Aulosira fertissima* and *Tolypothrix tenuis* found to be effective biofertilizers, discussed in detail in Sect. 10.2.1 (Singh et al. 2016).

10.5 Conclusion

In summary, cyanobacteria not only serve as excellent source of biofertilizer but also improve soil organic carbon and phosphorus bioavailability to the plants. Moreover, they are outstanding accumulators of heavy metals and degraders of various environmental pollutants such as pesticides and other toxicants. They are also the source of variety of bioactive compounds with evident antagonistic properties. Furthermore, the presence of agriculturally significant gene pool offers striking opportunity for exploitation in crop improvement through genetic manipulation. At present, due to growing number of cyanobacterial genome sequencing projects, post-genomics analysis is accelerating, resulting in a great number of useful cyanobacterial genes. Therefore, there is vast scope of cyanobacteria in sustainable agriculture that may eventually result in declines in the agricultural costs. Figure 10.1 summarizes key roles and potential of cyanobacteria for agroecosystem.

However, very limited information exists regarding the use of cyanobacteria or their product as biocontrol agent, their biotechnological prospective and their use in establishment of sustainable agroecosystem. Profound investigation is needed to address certain key issues of exploiting cyanobacteria in a healthier manner.

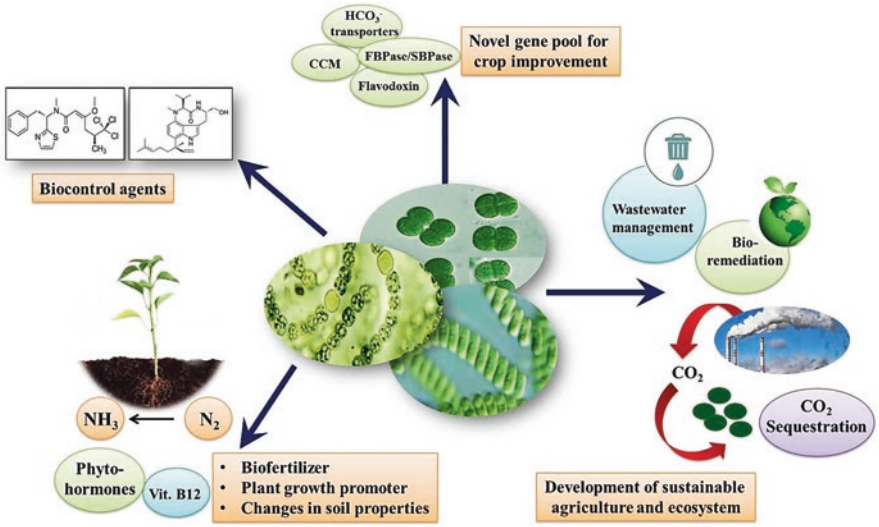

Fig. 10.1 Summary of key roles and potential of cyanobacteria in agriculture and ecosystem

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Green Input in Agriculture: An Overview

11

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Abstract

Agriculture, the mainstay of every country's economy, contributes to the overall economic growth, and change in its structure has a subsequent impact on the present socioeconomic life of the population. World population is expected to grow over a third or 2.3 billion people between 2009 and 2050 and nearly this entire forecast to take place in the developing countries. In this stage natural disaster like floods, droughts, climate change, and volatility has played a major role in raising the risk of production deficits. Moreover the increased rate of population growth demands more production of food. Therefore to achieve the increasing demand of agricultural production, a sizable quantity of mineral fertilizers will be needed to accept the challenge. Agricultural fertilizers are indispensable to enhance proper growth and crop yield. To raise the productivity, farmers have been using chemical fertilizers and pesticides. The high input of chemical fertilizers and pesticides makes threats for disproportionate supplement of nutrients to crops and deterioration of soil health and endangers ecosystems, plants, human, and animal lives. Therefore, there is an urgent need for proportionate application of green inputs, viz., microbe-based biofertilizers to stop the adverse effect of chemical fertilizers which would unravel these problems and make the ecosystem healthier and improve the physicochemical properties of the soil. The demand for biofertilizers goes on increasingly due to its eco-friendly nature, and therefore intensive research is needed to improve the quality and activity to achieve food security for the growing population and restore soil

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health. This book chapter exhibited the necessary information on PGPRs and their immense potentiality on crop development and their future outlook for the economic development.

Keywords
PGPR • Biofertilizer • Biopesticides • Nematode

11.1 Introduction

Entering the new millennium, it was apparently increasing the demand for food by the growing population throughout the world. In the last 45 years, the population has increased twice around the globe, and that number is expected to grow to nine billion by the year 2050 (Planning Commission 2002). But the agricultural crop production rate and yield have comparatively slowed with the world human population. This leads to uncertainties that the world may not be able to grow enough food and other merchandise to ensure that future populations are sufficiently fed. Besides, developing countries will turn out to be more reliant on agricultural production, but the food security is not yet improved in many poor areas. Agriculture, as a global practice, has been exploiting resources faster than they could be renewed.

Agriculture is crucial for a wide range of development as it can allow for improved livelihood. Among the greatest challenges for agriculture is to develop the agriculture production by boosting crop yield growth rates on existing agricultural land. Therefore to meet up the increasing global demand, agriculture has to follow different advances in production techniques. On the other hand, rapid population growth and increased human activities have resulted in the overexploitation of the environment. Moreover, indiscriminate use of different synthetic chemicals, pesticides, insecticides, and colors are spilled out from the agricultural lands, which ultimately has effect on the health and environment. The increased use of chemical fertilizers in agriculture helped in achieving the food production rate, but it has impact on environmental pollution and also human health. To obviate these tribulations and higher plant yields, green technology is now being greatly used to turn agriculture along a sustainable path along with the advancement of economic and efficient production of harmless and high-quality food. It has the potential of massive yield gains, economically viable and environmentally sustainable for large-scale farming (Simmons 2011).

Current trends in agriculture are focused on the organic farming methods, which are supposed to be more environmentally effective than chemical pesticides and inorganic fertilizers for improvement of soil fertility and crop production. The chemical fertilizers and pesticides have caused dreadful effect on soil fertility, soil quality, water pollution, genetic variation in plants, and also human health. On the other hand, biofertilizers and PGPR have shown great potential as a renewable and environment-friendly source of plant nutrient and also improve the soil health. The

use of biopesticides can also play an important role to achieve the challenges of agricultural production growth.

In the present article, we have reviewed the different types of biofertilizer, biopesticides, and PGPR and their different application and future possibility on agricultural productivity.

11.2 Agriculture Input Market Trend and Potential

The agricultural marketing is essential for product marketing, consumption, and acceleration of economic development. An efficient agricultural marketing system leads to optimize the use of raw material, which leads to increase in marketed surplus by decreasing the losses coming out of inefficient processing, storage, and transportation. For example, farmers in the past used raw material, which was available to them; input amount was negligible, and therefore credit in the production of farm products has increased. The new agricultural technology is input responsive and thus gained higher level of income by reducing the middle marketing services. An efficient system undertakes the farmers to invest their surpluses on modern input purchase so that the productivity and production may increase which ultimately increases in the marketed surplus and income of the farmers. Nowadays agro-based industries are very much conventional for improved and efficient agricultural marketing. Moreover, the marketing system provides employment to millions of persons engaged in various activities, such as packaging, transportation, storage, processing, and supplying.

Agriculture and associated sector contribute 24% of the total GDP and provide 67% of Indian employment (Pawan 2001). The chemically produced fertilizers and pesticides have increased the agricultural productivity, and thereby India is becoming independent of producing food grain. Yield of food-grain in India increased from 644 kg per hectare in 1966–1967 to 1636 kg per hectare in 2000–2001. This has mainly brought about more than 12-fold increase in the consumption of chemical fertilizers during the same period (Garibay and Jyoti 2003). Since the use of chemical fertilizer is not eco-friendly, demand for green agricultural products like biofertilizers, biopesticides, vermicompost, green manure, and FYM is increasing. Different agencies like FIBL and ORG-MARG have anticipated differently the area under organic agriculture for illustration (Mayak et al. 1999). According to SOEL-Survey, the estimated number of total organic farms is 5661, but ORG-MARG reviewed it as 1426. The Garibay S V and Jyoti K. (Mayak et al. 1999) reported that according to FIBL and ORG-MARG survey, total commodity-wise demand has been estimated in some selected export markets (Germany, Holland, the UK, Switzerland, the USA, and Japan) which shows for banana it is 6410 tons, for wheat and soybean 1000 tons, for pineapple around 900 tons, and for mango around 650 tons. Moreover the attractiveness of organic market gets enhanced due to premium price, which varies (30–50% trader level) in different countries depending upon the distribution channels and market conditions. India has established potentiality of exporting agricultural merchandise like rice, wheat, tea, coffee, spices, oil meals, sugar, fruits, vegetables, etc.

to countries like the USA, the UK, Germany, Japan, France, Saudi Arabia, South Africa, CIS countries, Poland, the Netherlands, Italy, etc. It also shows that there is an increasing demand for organically produced supplies in most of the countries, which attract price premiums ranging from 10 to 100%.

11.3 Magic Bullets PGPR

Plant growth-promoting rhizobacteria (PGPRs) are the free-living soil bacteria residing around/on the root surface of plants and are responsible for the plant growth promotion and development (Glick et al. 1995; Ahemad and Kibret 2014) through the use of a variety of regulatory chemicals produced. The PGPR replaces chemical fertilizer, pesticides, and supplements, which have their adverse effect, and most of the PGPR isolates are responsible for significant increase in plant height, root length, and dry matter production of shoot and root of plants. Joe Kloepper of Auburn University coined the name PGPR in the 1980s. It can help in plant growth directly by helping in the attainment of different minerals or modulating plant hormone levels or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development. PGPR can improve the biomass production in plants at their early stage of development by inducing the growth of root and shoot when inoculating with them.

The PGPR can be classified into two categories: extracellular plant growth-promoting rhizobacteria (ePGPR) and intracellular plant growth-promoting rhizobacteria (iPGPR). ePGPR (like *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, and *Serratia*) which may be present in the rhizosphere or in the intracellular spaces of root cortex and iPGPR (like *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Rhizobium*, endophytes, and *Frankia* species) reside inside the root nodules (Graham 1988).

A number of direct mechanisms like nitrogen fixation, growth promotion through hormonal involvement, and secretion of different metabolites like siderophore, ACC deaminase, etc. are significantly influenced by PGPR strains for plant growth enhancement. Application of consortium, i.e., involvement of two or more PGPR strains, offers diverse advance in promoting plant growth and improving yield worldwide, PGPRs can help the nitrogen-fixing bacteria for fixation of nitrogen in leguminous plants; enhancement of the supply of iron, sulfur, copper, and phosphorus; production of plant hormones; enhancement of beneficial bacteria or fungi; and control of microbial diseases and also insect pest control.

11.3.1 Plant Growth Promotion: Mechanism of Action by PGPR

Growth promotion in plants by plant growth-promoting rhizobacteria occurs through a broad variety of mechanisms, which leads to improvement of plant growth and progress in the varied environmental conditions. Their mode of action can be

grouped into the following categories: (1) synthesis of substances (nitrogen, phosphorus, potassium, and essential minerals) that can be easily assimilated by plants, (2) nutrient mobilization, (3) prevention of plant diseases through decreasing the inhibitory effect of plant pathogens, and (4) introduction of plant stress resistance.

Moreover the mechanisms of plant growth promotion can be varied among different types of PGPR strains.

11.3.1.1 Direct Mechanisms

The direct mechanisms of plant growth-promoting rhizobacteria to promote plant growth include providing nutrients/resources to plants. Farmers have become increasingly dependent on chemical fertilizer sources, as many agricultural soils are unable to provide sufficient nutrients to the plants. As a result, the use of nonrenewable chemical fertilizer laid awful ecological impact on the environment. It would obviously be advantageous if efficient biological means of providing nitrogen and phosphorus to plants could be used to substitute for at least a portion of the chemical nitrogen and phosphorus that are currently used.

Nitrogen Fixation

All life forms are dependent on nitrogen since it is the most essential nutrient for growth and productivity, but plants cannot avail nitrogen from nature directly even though a large portion is present in the atmosphere. Therefore different nitrogen-fixing microorganisms are involved in the conversion of atmospheric nitrogen into utilizable nitrogen so that plants can get directly for its growth. Plant growth-promoting rhizobacteria have varying capabilities to fix atmospheric nitrogen, occur regularly in diverse soils. Two different types of mechanisms (i.e., symbiotic and nonsymbiotic) are associated to fix atmospheric nitrogen in the soil.

Graham PH (Kundan et al. 2015) reported that about 180×10^6 metric tons/year of nitrogen is produced globally with the help of biological nitrogen fixation, out of which symbiotic association produces 80% and the rest comes from free-living or associative systems (Muhammad et al. 2013). In the first type, microbes and plants follow mutualistic relationship where microbes first enter the roots of the plants and form nodules in which nitrogen is fixed to ammonia and make it easily accessible to the plants. Different bacterial species like *Arthrobacter*, *Azospirillum*, *Acinetobacter*, *Bacillus*, *Burkholderia*, *Flavobacterium*, *Enterobacter*, *Pseudomonas*, *Rhizobium*, and *Serratia* are capable of fixing nitrogen for the plant growth (Ahemad and Kibret 2014). On the other hand, in nonsymbiotic nitrogen fixation, free-living diazotrophs are basically involved in the fixation and stimulate nonleguminous plants. *Enterobacter*, *Azotobacter*, *Azospirillum*, *Acetobacter*, *Burkholderia*, *Gluconacetobacter diazotrophicus*, *Pseudomonas*, and *Cyanobacteria (Anabaena, Nostoc*) are some of the examples of nonsymbiotic nitrogen-fixing bacteria (Zahran 2001; Gupta et al. 2000). It was reported that nitrogen fixation rate is about 30–40 kg/ha/year by the associative bacteria (Patten and Glick 1996). Nitrogenase (nif) genes are involved in the nitrogen fixation, which are present in both symbiotic and free-living systems (Muhammad et al. 2013). Inoculation of these biological nitrogen-fixing plant growth-promoting rhizobacteria on crop provides different beneficial

effect, i.e., growth promotion activity, maintains nitrogen level in the soil, and increases nutrient availability to the plants and also disease management. For example, *Azotobacter* has been reported to increase seed germination and growth of seedlings. Different species of *Bacillus* are known to release a number of metabolites, which increase the availability of nutrients to the plants (Sharma et al. 2013). Moreover *Pseudomonas* is one of the good PGPR and can increase plant productivity when used in combination with biofertilizers.

Phosphate Solubilization

Phosphorus is the most essential nutrient for plants, next to nitrogen. It plays a crucial role in plant growth mechanism. Phosphates are mostly present in inorganic forms, and therefore plants could not absorb directly. PGPRs play an important role to make use of unavailable forms of phosphorus in the soil through phytase action or the production of organic acids (Pii et al. 2015) and in turn also help in making phosphorus available for plants which increases growth and yield directly. The exact mechanism of phosphorus uptake by PGPR is not truly understood (Bhattacharyya and Jha 2012).

The mechanism of phosphate solubilization by PGPR includes (1) release of organic acids and affecting the mobility of phosphorus by means of ionic interactions, (2) liberation of extracellular enzymes, and (3) release of phosphatases which help to unbind the phosphate groups from organic matter. The most efficient phosphate solubilizing bacteria include the genera *Bacillus*, *Rhizobium*, *Arthrobacter*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Microbacterium*, *Pseudomonas*, *Rhodococcus*, and *Serratia* (Rivas et al. 2007) *Mesorhizobium ciceri* and *Mesorhizobium mediterraneum* are the examples of potential rhizobium species for phosphate solubilization (Vikram and Hamzehzarghani 2008). Gupta et al. (2015) also reported the effect of the inoculation with phosphate solubilizing bacteria used alone or in combination with other rhizospheric microbes.

Potassium Solubilization

The third major macronutrient is the potassium, which has major impact on plant's growth, yield, and rate of development. It is also present in the soil in insoluble form. Potassium solubilizing PGPR species such as *Acidithiobacillus ferrooxidans*, *Bacillus edaphicus*, *Bacillus mucilaginosus*, *Burkholderia*, *Paenibacillus* sp., and *Pseudomonas* has been reported to release potassium in accessible form through the solubilization of insoluble potassium sources through the production and liberation of organic acids (oxalate, succinate, and citrate) in the rhizosphere (Pii et al. 2015). The use of potassium solubilizing PGPR improves the soil fertility and availability of soluble potassium to the plants, which acts as eco-friendly biofertilizer and can reduce the use of agrochemicals (Rajkumar et al. 2010).

Siderophore Production

Among the bulk minerals present on earth, iron (Fe) is one of the most vital micronutrient for all life forms in the biosphere. But the fact is that iron is commonly present in the soil in the form of ferric ion Fe^{3+}, which is highly insoluble, thus making it generally inaccessible to both plants and microbes (Wandersman and Delepelaire 2004). Microorganisms have the ability to assimilate iron by producing low molecular weight (400–1500 Dalton) iron-chelating compounds known as siderophores, which transport this element into their cells (Crowley and Kraemer 2007). Siderophores are divided into three main families depending on the presence of functional groups, i.e., hydroxamates, catecholates, and carboxylates. Siderophore-producing rhizobacteria is reported to improve plant health by improving iron nutrition, inhibit growth of other microorganisms with release of their antibiotic molecule, and hamper the pathogens' growth by limiting the available iron for the pathogen. The bacterial genera *Burkholderia*, *Enterobacter*, and *Grimontella* presented *s*trains that produce high siderophore, while the genera *Klebsiella*, *Stenotrophomonas*, *Rhizobium*, *Herbaspirillum*, and *Citrobacter* presented strains that produce less siderophore. Crowley and Kraemer (2007) inferred that under iron-limited conditions, siderophores produced by rhizosphere microorganisms transport iron to oat plants by using Fe-siderophore complexes. Sharma et al. (2003) reported the role of siderophore on the nutrition of *Vigna radiata* by *Pseudomonas* strain GRP3. They found that the plants inoculated with GRP3 showed decline of iron content and chlorotic symptoms and increase in chlorophyll a and b content, compared to control. Similarly in rice roots, the rhizospheric isolates *Enterobacter* and *Burkholderia* species produced the highest levels of siderophores (Souza et al. 2014; Szilagyi-Zecchin et al. 2014). Szilagyi-Zecchin et al. (2014) reported that endophytic *Bacillus* strains can produce siderophore in maize which was the most efficient against the growth of *Fusarium verticillioides*, *Colletotrichum graminicola*, *Bipolaris maydis*, and *Cercospora zeae-maydis* fungi.

Phytohormone Production

Plant hormones or phytohormones are natural organic compounds, which can influence plant's ability to react with the environmental conditions at very low concentration. They are synthesized inside the plants and transported to different locations for the processes like growth, differentiation, and development, and other processes, such as stomatal movement, could also be affected (Patten and Glick 1996). Wide ranges of PGPR are capable of producing different types of phytohormones, i.e., auxins, gibberellins, cytokinins, and ethylene (Bhattacharyya and Jha 2012).

Indoleacetic Acid (IAA)

Among phytohormones, indoleacetic acid (IAA) plays a vital role on the effect of root growth of plants as natural auxin. It is reported that up to 80% of rhizobacteria are capable of producing IAA as secondary metabolites (Kaminek et al. 1997) the ability to synthesize and release as secondary metabolites. IAA has been applied in almost each aspect of plant growth and development such as cell division, extension, and differentiation; stimulates seed and tuber germination; develops xylem

and root, controlling vegetative growth processes; arbitrates light responses, gravity, and florescence; and affects pigment formation, photosynthesis, biosynthesis of various metabolites, and resistance toward stress conditions (Graham 1988) as well as defense responses. Moreover, in several microorganisms, IAA is responsible for gene expression which acts as reciprocal signaling molecule expression. It was reported that bacteria such as *Bradyrhizobium*, *Pseudomonas*, *Agrobacterium*, *Enterobacter*, and *Klebsiella* have the capability of synthesizing IAA (Graham 1988). Nevertheless, phytohormones produced by microbes are more effective due to their slow release, and the threshold level is low between inhibitory and stimulatory levels of chemically produced hormones.

Cytokinins and Gibberellins

Cytokinins are purine derivative phytohormones which can support and maintain the cell division in roots and shoots of plants and also involve in various differentiation processes. Cytokinins are involved in delaying the senescence or aging of tissues and thereby effecting the leaf growth and helping the plant. Other growth regulators, e.g., auxins, can also influence the balance of cytokinin (Nieto and Frankenberger 1989). This phytohormone can be produced in soil and pure culture by PGPR, which is an alternative to enhance plant growth and to improve yield and quality of crops. Etesami et al. (2009) reported that many soil bacteria and PGPB are capable of producing cytokinins or gibberellins alone or both. Cytokinins have been identified in the cell-free medium of certain strains of *Pantoea agglomerans*, *Rhodospirillum rubrum*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Paenibacillus polymyxa*, *Azotobacter* spp., and *Rhizobium* spp. However, it was found that PGPB produce lower level of cytokinin compared to phytopathogens so that the effect of the PGPB on plant growth is stimulatory while the effect of the cytokinins from pathogens is inhibitory.

Gibberellins are one of the important phytohormones produced naturally by plants and are involved in several aspects of germination by stimulating alpha amylase and help in hydrolysis of starch present in many seeds into glucose to be used in cellular respiration. Gibberellins are also involved in the processes like stem elongation, dormancy, flowering, sex expression, and leaf and fruiting senescence. Several PGPR strains like *Pseudomonas fluorescens*, *Bacillus subtilis*, *Pantoea agglomerans*, *Rhodospirillum rubrum*, *Azotobacter* sp., *Rhizobium* sp., and *Paenibacillus polymyxa* can produce both cytokinins and gibberellins which can influence the plant growth promotion (Lugtenberg and Kamilova 2009).

11.3.1.2 Indirect Mechanisms

In major indirect mechanism, PGPR is a promising candidate acting as eco-friendly biocontrol agents (Glick 2012; Tariq et al. 2014) instead of chemical pesticides to obtain sustainable fertility of the soil and plant growth promotion. This approach led to reducing the need for agrochemicals (fertilizers and pesticides) for improving soil fertility by a variety of mechanisms like fabrication of siderophores, antibiotics, HCN, hydrolytic enzymes, etc. (Lugtenberg and Kamilova 2009; Shilev 2013).

Antibiosis

The production of low molecular weight antibiotic compounds is considered to be one of the most powerful and biocontrol mechanism of PGPR against phytopathogens (Loper and Gross 2007) and thus retards the growth. Different types of antibiotics such as amphisin, oomycin A, 2,4-diacetylphloroglucinol (DAPG), phenazine, pyoluteorin, pyrrolnitrin, tensin, tropolone, and cyclic lipopeptides produced by pseudomonads (Compant et al. 2005) and oligomycin A, kanosamine, zwittermicin A, and xanthobaccin produced by *Bacillus*, *Streptomyces*, and *Stenotrophomonas* sp., respectively, have been identified to prevent plant pathogens (Weller 2007). To prevent from resistance against the single antibiotic dose, researchers have utilized biocontrol strains that synthesize one or more antibiotics (Tariq et al. 2014). Weller (2007) reported that when wheat seeds inoculated with *P. fluorescens* strains, they produced antibiotic phenazine-1-carboxylic acid (PCA) which resulted in significant suppression of disease (60%) in field trials.

Lytic Enzymes

Plant growth-promoting rhizobacteria play a significant role in growth enhancement of the plants by producing some enzymes such as chitinases, dehydrogenase, β-glucanase, lipases, phosphatases, proteases, etc., which exhibit hyperparasitic activity by attacking pathogen with cell wall hydrolases and thus protecting the plants from biotic and abiotic stresses. It was reported that *Pseudomonas fluorescens* CHA0 and *P. putida* suppress black root rot of tobacco caused by the fungi *Thielaviopsis basicola* (Maheshwari et al. 2012) and *Macrophomina phaseolina* in chickpea, respectively, and *Azotobacter chroococcum* can suppress *Fusarium oxysporum* in *Sesamum indicum* in field condition (Avis et al. 2008).

Induced Systemic Resistance (ISR)

Plant growth-promoting rhizobacteria can generate a physiological defensive capacity known as induced systemic resistance (ISR), which can occur inside the plants only when plants can activate their defensive mechanisms against subsequent biotic challenges (Doornbos et al. 2012). The ISR-acquiring plants are referred as primed plants which have systemic resistance mechanism against a broad spectrum of plant pathogens. ISR is not specific against particular pathogen but effective for controlling the diseases caused by different pathogens when pathogenic attack occurs. Additionally, ISR can help in the signaling of hormones like jasmonate and ethylene inside the plants, which can stimulate the host plant's defense responses to a range of plant pathogens (Tariq et al. 2014). Besides ethylene and jasmonate, many individual bacterial components such as lipopolysaccharides (LPS), flagella, siderophores, cyclic lipopeptides, 2,4-diacetylphloroglucinol, homoserine lactones, and volatiles like acetoin and 2,3-butanediol have also been reported to induce ISR (Glick et al. 1999).

Exopolysaccharides Production or Biofilm Formation

Exopolysaccharides (EPS) are secreted by a wide variety of PGPR and remain associated with the cell wall as extracellular slime (Vimala and Lalithakumari 2003).

Pseudomonas aeruginosa, *Bacillus subtilis*, and *Streptococcus mutans* are reported for the production of EPS (Parada et al. 2006). EPS have crucial role in different processes like biofilm formation, protection of bacterial cell from desiccation, maintaining cellular function, antibacterial activity against pathogens, and circulation of essential nutrients to the plants for proper growth and development. PGPRs producing EPS are very much important in plant growth promotion as they work as an active signal molecule during beneficial interactions and provide defense response during infection process (Tewari and Arora 2014). EPS have the impact on cell aggregation and also helps in nitrogen fixation by preventing high oxygen tension (Vimala and Lalithakumari 2003).

11.4 Biofertilizers

Biofertilizers are biologically active products or microbial inoculants, which when applied to soil enhance the growth and yield of crops, improve fertility of soil, and reduce the risk of pollution. It is one of the most widely approved methods for organic farming. Since long past, chemical fertilizers have become a backbone of highly productive agricultural system. However, extreme use of chemical fertilizers leads to numerous environmental disorders. Therefore, current agricultural practices emphasize on environmental sustainability by limiting the use of chemical fertilizers owing to the risk of pollution, severe changes in ecological equilibrium, and poisoning (Rai 2006). As such several sustainable alternative methods are being introduced to fulfill the needs of chemical fertilizers. These biofertilizers make up nutrients that are naturally rich in the soil and atmosphere, relatively cheap, and more convenient to use (Gosh 2004). Although India has shown positive response for green agriculture, there are some limitations that hinder its growth. Lack of proper infrastructure for production, distribution, and conservation of bio-products and lack of awareness among the farmers regarding organic farming also somehow hinder the growth of organic agriculture in India.

However during the last 10 years, subsequent changes have taken place in Indian agriculture system. According to the data provided by the National Centre of Organic Farming (NCOF), production of biofertilizers in India has increased from 25065.04 to 40324.21 metric tons from the year 2008–2012, with Maharashtra being the top in state-wise production (8743.69) followed by Uttar Pradesh (8695.08) and Karnataka (5760.32). The result shows the awareness among the people regarding harmful effects caused by chemical fertilizers and the subsequent shifting of Indian agriculture system toward green agriculture.

Microbial inoculants have attained significant importance in Indian agriculture system. *Rhizobium* inoculants are commonly used in leguminous crops, and *Azotobacter* are used in different crop plants like wheat, maize, mustard, potato, cotton, and other vegetables. *Azospirillum* inoculants are mostly recommended for cereal plants like sorghum, millet, maize, sugarcane, wheat, etc. In addition to this, there are also some free-living and symbiotic nitrogen-fixing cyanobacteria and fungi that directly or indirectly act as biofertilizers, thereby increasing the fertility

of the soil. Accordingly they are grouped as nitrogen-fixing bacteria, phosphorous solubilizing bacteria, plant growth-promoting rhizobacteria (PGPRs), *Cyanobacteria*, and *Mycorrhiza*:

1. **Nitrogen-fixing bacteria as biofertilizer**: Nitrogen-fixing microbes directly or indirectly fix atmospheric nitrogen, thus making it available for the plants. More than 90% of the N_2 fixation is affected by these organisms. These include free-living bacteria—*Cyanobacteria* (*Nostoc*, *Anabaena*, *Tolypothrix*), *Azotobacter*, *Beijerinckia*, and *Clostridium*— and symbiotic bacteria such as *Rhizobium* associated with leguminous crops, *Frankia* with dicotyledonous plants (Rai 2006), *Azospirillum* with cereal plants, *Anabaena azollae* in the roots of *Azolla* plant, etc. Cyanobacterial biofertilizers are most commonly applied in paddy field. Mazid and Khan (2014) examined an increase in crop yield up to 10–14% after application of cyanobacterial biofertilizer. Nowadays new systems are being introduced in biofertilizer technology to increase the biological nitrogen fixation (BNF) with cereals and other nonlegumes by incorporating nitrogen-fixing bacteria within the roots (Cocking 2000; Baset Mia and Shamsuddin 2010).
2. **Phosphate solubilizing bacteria as biofertilizer**: These bacteria solubilize insoluble phosphates and make it available for the plants. PSB have attracted attention of agriculturist to improve plant growth and yield as soil inoculums (Zaidi 1999; Gull et al. 2004) and have greater capacity to compensate inorganic sources of P fertilizers (64). PSB also has the potentiality toward enhancing the phosphate-induced metal immobilization for remediation of contaminated soil, e.g., *Pseudomonas* and *Bacillus*. Studies have found that increase in yield of vegetable crops was due to inoculation of plants with peat-based cultures of *Bacillus* and *Pseudomonas* (Khan et al. 2009; Qureshi et al. 2012).
3. **Plant growth-promoting rhizobacteria (PGPRs) as biofertilizer**: Rhizobacteria are important microorganisms which help in promoting plant growth by fixing nitrogen, producing phytohormones, solubilizing phosphates, decomposing organic matter, degrading organic pollutants, and stimulating root growth. They colonize the roots or the rhizosphere or may even be endophytic. Studies have shown that for better plant growth, endophytic N_2-fixing bacteria are more beneficial than rhizobacteria as they are more competitive, and also they remain in close contact with plant tissues (Dobereiner 1992; Assmus et al. 1995; Ramizez and Mellado 2005). In recent years, much awareness has been given regarding the replacement of chemical fertilizers and pesticides with PGPRs and to develop new techniques to produce genetically modified PGPRs with increase in antibiotic, phytohormone, and siderophore production (Gupta et al. 2015).
4. **Mycorrhiza as biofertilizer**: Mycorrhiza biofertilizer also known as VAM (vesicular arbuscular mycorrhiza) plays a great role in inducing plant growth. It helps in retaining moisture around the root region and increasing resistivity toward different root- and soilborne pathogens and nematodes. They also help in the uptake of different nutrients like Cu, K, Al, Mn, etc. from the soil and remove toxic chemicals which otherwise hinder nutrient availability (Sullia 1991).

Mycorrhiza biofertilizer is very useful in organic farming as well as in normal commercial farming. Arbuscular mycorrhiza fungi (AMF), a group of obligate biotrophs, are considered as natural biofertilizer because they provide the host with nutrients and water and protection from pathogen (Aguilar and Barea 1997; Sadhana 2014; Gentili and Jumpponen 2006).

5. **Compost as biofertilizer**: Compost includes remains of organic matter that are aerobically decomposed. It serves as a naturally grown medium for plants that holds moisture and soluble minerals, thereby providing support and nutrients. Vermicompost is another type of pure organic fertilizer produced by using various worms. It provides N, P, K, organic carbon, etc. which helps in increasing the quality and quantity of yield.

Liquid Biofertilizers

Liquid biofertilizers are suspensions provided with useful microorganisms and substances that improve stickiness, stabilization, and dispersal abilities. Liquid biofertilizers have gained much attention in developed countries mainly for legume inoculation. Liquid inoculants having concentration of 2×10^9 cells/ml are most commonly preferred (Schulz and Thelen 2008). A major advantage of liquid biofertilizer over solid is that they allow production workers to add sufficient amount of nutrients, cell protectants, and inducers responsible for formation of cell, spore, and cysts.

Nowadays some additives are used in liquid inoculants to improve the performance of microbes and have longer shelf life, e.g., Taurian et al. (2010) used sucrose to improve performance of PSB for peanuts and glycerol in cell culture medium to preserve the viability of *Pseudomonas fluorescens* cells. Similarly Singleton et al. (2002) used different additives in *Rhizobium* formulation medium to improve their growth performances. Encapsulated formulations are newly developed and are currently in field trials (Bashan et al. 2014). This technique involves encapsulation of living microorganisms (mainly PGPR) into a polymeric matrix to maintain their viability. The entrapped bacteria are liberated from the matrix when the native soil microbes degrade the polymer thereby releasing the microbes to the soil. At present there is no any commercial bacterial product using this technology but is believed to come in the near future.

11.5 Biopesticides

Biopesticides are biological products based on pathogenic microorganisms effective against different plant pathogens. It is one of the most reliable and eco-friendly alternative approaches to chemical pesticides and a major component of integrated pest management (Gupta and Dikshit 2010). Pest control by biological means has emerged as an effective tool in recent years. Due to increase in demand for healthy crop production and products, the demand for biopesticides is believed to boost in the near future. The global market value of biopesticides was $1.3 billion in 2011,

and by 2017 it is expected to become $3.2 billion (Raja 2013). Biopesticides originating from *Bacillus thuringiensis* (Bt) was the first to be used on commercial scale in the world.

India has huge potential for biopesticide production, but its use by farmers has to be accelerated to gain maximum yield. In India biopesticide contributes only about 2.89% (as of 2005) of the overall pesticide market and is expected to increase in the coming years. Till now only 12 types of biopesticides have been registered under the Insecticide Act 1968. Among them neem-based biopesticides, *Bacillus thuringiensis* (Bt), nuclear polyhedrosis virus (NPV), and *Trichoderma* are the major biopesticides produced in India (Kandpal 2014). Some of the biopesticides are being used on commercial scale, and the microorganisms involved are virus, bacteria, protozoa, fungi, and mites. Based on the type of active ingredients present, biopesticides are of three types—microbial biopesticides, biochemical biopesticides, and plant-incorporated protectants:

1. **Microbial biopesticides**: Include either spores or the organism itself as the active ingredients. They suppress pests by producing toxic or by other means of action (Clemson 2007; O'Brien et al. 2009). Microbial biopesticides are categorized into different types based on main organism present as bacterial, fungal, and viral biopesticides.
2. **Bacterial biopesticides**: Bacterial biopesticides are the most commonly used biopesticides to control unwanted bacteria, viruses, and fungi. Biopesticides produced from *Bacillus thuringiensis* (Bt) are efficient against harmful pests like caterpillars, insects, and moths (Vora et al. 2008; Dutta 2012; Kumar 2012) and *Bacillus subtilis* against certain mosquito (Revathi et al. 2013). *Pseudomonas fluorescens* act on several fungal (Lee and Kim 2001; Haas and Defago 2005) and bacterial diseases (Commare et al. 2002).
3. **Fungal biopesticides**: Biopesticides consisting of entmopathogenic fungal spores produces compounds that dissolve plant cell walls, commonly used to control insects, other fungi, bacteria, nematodes, etc. For example, *Trichoderma* are effective against soilborne fungi (Harman et al. 2004; Vinale et al. 2006; Verma et al. 2007; O'Brien et al. 2009) and *Muscodor albus* against bacterial and soilborne pests (O'Brien et al. 2009).
4. **Viral biopesticides**: These act on insects and arthropods only when they are ingested by the host. Baculovirus is effective against different varieties of moths and arthropods (Gramkow et al. 2010; Senthil-nathan 2015; Nawaz et al. 2016).
5. **Biochemical biopesticides**. Biopesticides include naturally occurring biochemical substances that control pests (biochemical pesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants. Biochemical pesticides can hinder with the growth that prevent pest multiplication (Gupta and Dikshit 2010).
6. **Insect pheromones**: These are chemicals through which insects interact with other members. Insect pheromones themselves do not kill the target pests; they only act as trap containing lethal pesticides. The main advantage of insect pheromones is that they are highly specific and have low toxicity (Senthil-nathan

2015). Plant extracts or oils are much diverse in their mode of action and target pest. Neem oil extract, recognized as a good insect repeller, is the most commonly used biopesticide in India (Mondal et al. 2007). Similarly citronella oil obtained from *Cymbopogon* species, *Pyrethrum* obtained from *Chrysanthemum* sp., is mostly used as insect repellent in organic farming (Senthil-nathan 2015).

7. **Plant growth regulators**: Plant growth regulators alter the growth of the plants or bring about certain biological changes. They do not target any insect pest; instead they enhance the crop viability or shelf life (Lovatt 2008; Senthil-nathan 2015).
8. **Insect growth regulators** (IGR): These are like plant growth regulators that control population of harmful pest like cockroach and fleas (Elahi 2008; Senthil-nathan 2015). Different insect growth regulators that are registered as biopesticides are juvenile hormone-based insecticides. The advantage of IGR is that they are effective when used at very small amount.
9. Plant-incorporated protectants include substances produced by the plant in which the genetic material has been incorporated to give the desired product. Application of such material has led to significant decrease in insecticide use (Kennedy 2008). According to EPA (Environmental Protection Agency), such genetically modified plants when given desired product that acts as pesticides can be regarded as biopesticides.

11.6 Role of PGPR in Control of Biotic and Abiotic Stress

Plant growth-promoting rhizobacteria (PGPRs) are mainly associated with plant roots and augment plant productivity and immunity, but however, PGPR can efficiently lessen the damages and help in the management of the stresses caused by biotic and abiotic stresses on crop plants.

Biotic stresses in plants are mainly corresponded to pests and diseases, which include many thousands of different types of phytopathogens (fungi, bacteria, and viruses), insects, nematodes, weeds, and other organisms. Though recent farming practices are good applicant for controlling the pests and diseases, they are not environment-friendly and have harmful effects on nontarget organisms. Naturally occurring PGPR plays a significant role as biological control agents to deal with the biotic stresses on plants.

11.6.1 PGPR in Control of Phytopathogens

PGPRs have significant role in the damage control in plants caused by the phytopathogens through different indirect mechanisms such as induction of antibiotics, antifungal metabolites, defense enzymes, and siderophores, exhibiting rhizospheric competition with phytopathogens and inducing plant systemic resistance (Glick 1995; Glick et al. 1999).

11.6.1.1 Antibiotics Producing PGPR

One of the most effective mechanisms through which a PGPR can protect plants from phytopathogen explosion is the synthesis of antibiotics and acts as antagonistic agents (Glick et al. 2007). Over the past two decades, the activity of antibiotics based on the secretion of molecules, which can kill or reduce the growth of target pathogen, was better understood (Dowling and O'Gara 1994; Whipps 2001; Lugtenberg and Kamilova 2009). Haas and Défago (2005) reported the impact of antibiotic compounds such as phenazines, phloroglucinols, pyoluteorin, pyrrolnitrin, cyclic lipopeptides, and hydrogen cyanide on the biocontrol of root diseases in plants. For example, antibiotic pyrrolnitrin produced by *P. fluorescens* is able to prevent the cotton plants from the damage of *Rhizoctonia solani* (Hill et al. 1994). Maurhofer et al. (1992) and Schnider et al. (1994) stated that genetically manipulated *Pseudomonas fluorescens* can defend the cucumber plants from the disease caused by *Pythium ultimum than the wild-type strain* by overproducing the antibiotics pyoluteorin and 2,4-diacetylphloroglucinol. The 2,4-diacetylphloroglucinol (DAPG) produced by pseudomonads is a very much effective antibiotic, which causes membrane damage to *Pythium* spp. and predominantly inhibits the zoospores of this oomycete (de Souza et al. 2003). Maksimov et al. (2011) reported that majority of *Bacillus* sp. producing antibiotics such as polymyxin, circulin, and colistin are active against both Gram-positive and Gram-negative bacteria and also to various pathogenic fungal species.

11.6.1.2 PGPR Producing Antifungal Metabolites

PGPR can produce a broad range of low molecular weight metabolites with antifungal activity capable of reducing or suppressing infection by pathogenic fungi in several crops (Ongena et al. 1999). Voisard et al. (1989) reported that pseudomonad strains could synthesize hydrogen cyanide that inhibits the pathogenic fungi *Thielaviopsis basicola* causing black root rot of tobacco. Different researchers have reported that *Pseudomonas cepacia*, *P. solanacearum*, and *Cladosporium werneckii can hydrolyze the* fusaric acid which is responsible for *Fusarium* infection to the plants, and by hydrolyzing this chemical, the bacterial strains can prevent damage (Toyoda and Utsumi 1991). Moreover fluorescent *pseudomonads* have been effectively controling the major fungal diseases of plants by introducing antifungal metabolites. Reddy et al. (2009) reported that antifungal metabolites produced by *Pseudomonas fluorescens* can prevent the plants from the damage of rice blast and sheath blight caused by *Magnaporthe grisea* and *Rhizoctonia solani*, respectively. Meena et al. (2016) isolated four PGPR strains which show 89% antifungal activity against *Fusarium oxysporum* and can significantly enhance the overall growth of wheat.

11.6.1.3 PGPR Producing Defense Enzymes

Several bacteria have the ability to produce enzymes, which are able to hydrolyze chitin, proteins, cellulose, and hemicellulose, thus controlling plant pathogens. Similarly, a number of PGPR strains have been found to produce enzymes such as chitinase, β-1,3-glucanase, protease, and lipase that can lyse fungal cells (Chet and Inbar 1994). Lim et al. (1991) reported that *Pseudomonas stutzeri* could be able to digest and lyse *Fusarium solani* mycelia by secreting extracellular enzyme

chitinase and laminarinase thereby preventing from root rot disease on the plants. Correspondingly a β-1,3-glucanase-producing strain of *Pseudomonas cepacia* significantly damages the fungal mycelia of *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Pythium ultimum* and thus decreased the disease incidence (Fridlender et al. 1993). Sneh et al. (1984) and Frankowski Lorito et al. (2001) reported that some bacteria producing lytic enzymes are able to destroy oospores of fungal pathogens and affect the spore germination and germ tube elongation of phytopathogenic fungi. Root and crown rot of cucumber caused by *Pythium aphanidermatum* can be suppressed by treating them with *Pseudomonas corrugata* 13 or *Pseudomonas aureofaciens* 63–28 (Chen et al. 2000). Phenylalanine ammonia lyase (PAL), peroxidase (PO), and polyphenol oxidase (PPO) enzymes were secreted by the bacterial isolate, activated, and suppressed the pathogen. Ramamoorthy et al. (2002) reported *Pseudomonas fluorescens* Pf1 can prevent tomato plants from wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici* by introducing defense proteins and chemicals secreted by the bacterial strain. Moreover *Bacillus licheniformis* and *B. pumilus* SG2 also show antifungal activity and controlled their spore germination (Xiao et al. 2009; Shali et al. 2010). A novel strain *Brevibacillus laterosporus* is also reported to possess antifungal and pesticidal activity by producing two thermostable chitinase (Prasanna et al. 2013).

11.6.1.4 PGPR Producing Siderophores

One way of preventing the proliferation of phytopathogens is by means of siderophore production by bacteria. Siderophores bind most of the Fe^{3+} ions available in the plant rhizosphere region and significantly reduce the amount of Fe^{3+} ions available to certain rhizosphere microflora and thereby inhibit the growth of the fungal pathogens (Kloepper et al. 1980). Siderophores act as effective disease-suppressive agents in agriculture for plant disease management and improve plant growth (Patel et al. 2010). A mutant strain of *Pseudomonas putida* producing siderophores was more effective than the wild-type bacterium in controlling *Fusarium oxysporum* causing disease on tomatoes (Vandenbergh and Gonzalez 1984). Sharma and Johri (2003) demonstrated that when maize seeds were bacterized with siderophore-producing fluorescent *Pseudomonas* spp. GRP3A and PRS_9 in iron-stressed conditions, maize seeds showed enhancement of germination and growth. Patil et al. (2014) reported that siderophore-producing *Bacillus subtilis* CTS-G24 can significantly control the disease caused by fungal pathogens *Fusarium oxysporum* f. sp. *ciceris* (FOC) and *Macrophomina phaseolina* in chickpea. Moreover some other rhizobacterial species such as *Acinetobacter baumannii*, *Aeromonas hydrophila*, *Acinetobacter* sp., *Pseudomonas alcaliphila*, *Klebsiella pneumonia*, and *Pseudomonas brassicacearum* have also significant antagonistic effect against phytopathogen *Fusarium oxysporum* in addition to plant growth promotion (Singh and Varma 2015). Nowadays, research is going on cloning of the genes for iron-siderophore receptors from one PGPR and introduced them into other strains for better efficiency.

11.6.1.5 PGPR Inducing Plant Systemic Resistance (ISR)

For the reduction of disease incidence on different variety of hosts, practicing of induced systemic resistance (ISR) by plant-associated bacteria was demonstrated earlier using *Pseudomonas* spp. and other Gram-negative bacteria for the reduction of disease incidence on different variety of hosts. PGPR-elicited ISR was first noticed on carnation (*Dianthus caryophyllus*), which effectively prevent the plants from wilt disease and foliar disease caused by *Fusarium* sp. and *Colletotrichum orbiculare*, respectively (Van Peer et al. 1991; Wei et al. 1991). Choudhary and Johri (2009) tested several *Bacillus* spp. eliciting ISR showing considerable amount of reduction in the occurrence of various diseases on different tropical crops caused by a diversity of phytopathogens. Actually PGPR-triggered ISR strengthens the plant cell wall and changes the host physiology and metabolic responses, thereby enhancing synthesis of plant defense chemicals upon challenge by pathogens. Presently for different types of plant-pathogen systems, a variety of numerous PGPR-mediated induction of ISR has been reported (Maurhofer et al. 1994; Zhang et al. 2002; Iavicoli et al. 2003; Ryu et al. 2004; Meziane et al. 2005).

11.6.2 PGPR in Management of Insects

The management of insect pest has been exclusively based on the application of chemical-based pesticides, but not considered as a long-term solution due to pesticide residual risks, health and environmental hazards, persistence of residue, pest renaissance, expenses, and elimination of natural enemies. Therefore, the need for alternative methods of controlling the pests has become essential. The use of PGPR in the management of plant pests is restricted to very few crops. Application of *Pseudomonas maltophilia* in corn has been reported 60% reduction in adult emergence of the corn earworm *Helicoverpa zea* (Bong and Sikorowski 1991). Similarly, the rate of growth, digestibility, and consumption of *Helicoverpa armigera* larvae has been affected when fed with cotton plants treated with *Pseudomonas gladioli* due to the increase in their polyphenol and terpenoid content (Qingwen et al. 1998). The talc-based bioformulation containing two *Pseudomonas fluorescens* strains (PF1 and FP7) and their mixture with or without chitin can significantly reduce the leaf folder incidence both under greenhouse and field conditions (Commare et al. 2002). It was found that the two strains in combination showed better performance and can increase the larval mortality by decreasing the larval and pupal weight. Fluorescent pseudomonas strains Pf1, TDK1, and PY15 can effectively reduce the development of leaf folder, *Cnaphalocrocis medinalis* Guen., in rice by inducing defense molecules in the rice plants and enhancing the resistance of leaf folder attack (Saravanakumar et al. 2007). Karthiba et al. (2010) also reported that the bioformulations containing the consortium of bacterial strains *Pseudomonas fluorescens Migula* strains Pf1 and AH1 and *Beauveria bassiana* (Balsamo) *Vuill.* isolate B2 were efficient against leaf folder pest on rice plants and have significant increase in rice grain yield. Moreover, it was also reported that transgenic *P. cepacia*

strain 526 has insecticidal activity against tobacco hornworm (Stock et al. 1990). Thus, PGPR can be effective for insect pest management on crop plants and has a great potential for future use.

11.6.3 PGPR in Management of Nematodes

Due to non-eco-friendly nature of the conventional nematicides, there is a need for the emergence of a new method for managing plant-parasitic nematodes. PGPR showing nematicidal activity by inducing systemic resistance against nematode pests is a well established environment friendly method (Oostendorp and Sikora 1990; Sikora 1992; Sikora and Holmann-Hergarten 1992). For example, in roots of sugar beet, *P. fluorescens* inhibited the early penetration of cyst nematode, *Heterodera schachtii*, by in*ducing systemic resistance* (Oostendorp and Sikora 1990). Similarly, *B. subtilis* has induced protection against *Meloidogyne incognita* and *M. arenaria* in cotton, and also some other PGPR strains have been reported in the management of sugar beet and potato cyst nematode (Sikora 1992). *P. fluorescens* strain Pf1 and *P. chitinolytica* have been reported to reduce galls and egg masses of root-knot nematode *M. incognita* on tomato crop (Santhi and Sivakumar 1995; Spiegel et al. 1991). Burkett-Cadena et al. (2008) stated that PGPR can induce significant reductions in nematode eggs and juvenile nematodes on plants along with growth promotion. It was found that *Pseudomonas fluorescens*, *Pichia guilliermondii*, *Paecilomyces lilacinus*, and *Calothrix parietina*, singly or in combination, show nematicidal effect against the root-knot nematode *Meloidogyne incognita* and found 30–45% of juvenile mortality after 48 h of exposures (Hashem and Abo-Elyousr 2011).

11.7 Role of PGPR in Control of Abiotic Stress

Abiotic stresses such as prolonged droughts, intense rains and flooding, salinity, metal toxicity, heat waves, and frost damages conferred great impact on the productivity of principal crops all over the world. Therefore, a wide range of improvement and adaptations are required to cope with such problems. Different strategies were being implemented but due to being long drawn and cost intensive, there is a need to develop simple, low-cost, and short-term biological methods for the management of abiotic stress. Application of PGPR plays an imperative role for better plant growth under stress conditions. The expression of an ACC deaminase gene in PGPR can be increased under anaerobic conditions and facilitate to lower the level of endogenous ACC and, hence, the concentration of ethylene in a plant (Grichko and Glick 2001). Grichko and Glick (2001) reported that tomato plants grown from the seeds treated with bacteria showing ACC deaminase activity such as *Pseudomonas putida* ATCC17399/pRKACC, *P. putida* ATCC17399/pRK415, *Enterobacter cloacae* UW4, and *E. cloacae* CAL2 showed a substantial tolerance to flooding stress. Mayak et al. (2004) also reported that *Achromobacter piechaudii* with ACC

deaminase activity can significantly enhance the growth of tomato seedlings grown in the presence of salt stress. *Pseudomonas fluorescens* strain TDK1 containing ACC deaminase activity can enhance the resistance to salinity and also increase the yield of groundnut plants as compared with the ACC deaminase activity-deficient *Pseudomonas* strains (Saravanakumar and Samiyappan 2007). Cheng et al. (2007) have also confirmed that ACC deaminase can induce the salt tolerance on plants by decreasing the synthesis of ethylene production responsible for inducing salt and increase the growth rate. Kohler et al. (2009) investigated that the *Lactuca sativa* L. cv. Tafalla plant shows better growth behavior when inoculated with PGPR strain *Pseudomonas mendocina* Palleroni, alone or in combination with an arbuscular mycorrhizal (AM) fungus, *Glomus* sp., under salt stress. In case of moisture stress condition, it was noticed that pea plants (*Pisum sativum* L.) can tolerate and were more consistent under such condition when inoculated with ACC deaminase bacteria *Variovorax paradoxus* 5C-2 (Dodd et al. 2004). In nature, plants are very much sensitive to changes in temperature and respond to the seasonal variants, which lead to hormonal imbalance. The global warming can also significantly affect the growth of plants. Bensalim et al. (1998) reported that potato plants can maintain normal growth under stress condition when inoculated with PGPR strain *Burkholderia phytofirmans* PsJN. Recently, Barka et al. (2006) also experimented that the same PGPR strain *Burkholderia phytofirmans* PsJN can also enhance plant growth and physiological activity of grapevine at both ambient (26 °C) and low (4 °C) temperatures. These studies clearly demonstrated the potential of ACC deaminase in normalizing plant growth when exposed to temperature extremes (Wang and Irving 2011).

11.8 Thrust in Research and Development

A lot of new technologies and advancement have been made in the field of agricultural biotechnology that offer opportunities for identification of novel sources of biopesticides and biofertilizers, for example, exploration of nanosilica particles as a potential agent of biopesticide (Galal and Samahy 2012), improving the lethal activity of bacteria and viruses by insertion of desired gene of interest that acts as next-generation biopesticides (Fitches et al. 2004; Gramkow et al. 2010; Kumar and Singh 2015; Nawaz et al. 2016), etc., and, similarly in the field of biofertilizers, incorporation of N_2-fixing bacteria in the roots (Cocking 2000, Baset Mia and Shamsuddin 2010), development of genetically modified PGPRs with varied functions (Gupta et al. 2015), and encapsulated formulations containing desired microorganisms to be delivered at targeted site (Bashan et al. 2014), etc.

Although the development, production, and distribution of biofertilizers and biopesticides are still at its early stages and have not achieved much importance, these products have an important role to play in crop improvement program and sustainable agricultural management in the years to come. Furthermore, future research in PGPR will be needed for the advancement of molecular and biotechnological techniques for enhancing the knowledge of PGPR technology and to achieve

an integrated management of soil microbial populations. New alternatives should be investigated for the implementation of bioinoculants for other high value crops. Moreover, the application of a variety of proper PGPR bacterial consortium over a single bacterium could be an effective way of reducing the harmful impact of stress on plant growth.

11.9 Conclusions and Future Outlook

The development of a more efficient and sustainable agriculture, sufficient food supply for growing world population and minimizing damage to the environment, is one of the greatest challenges for the present civilization. In this context, the use of PGPR is one of the major pathways to maintain or increase crop yield as well as reduce the environmental footprint. The development of the biofertilizer and biopesticide has made significant improvement, and the promotion of bacterial inoculations in the field is an environment-friendly way to meet the worldwide need to raise crops yields. Nevertheless, their use is still far from that of chemical fertilizers. Numerous recent studies have implemented the modern way of combining beneficial bacterial strains that interact synergistically for proper development. There is an urgent need for research to identify useful and necessary bacterial traits for different environmental conditions and plants so that optimal bacterial strains can be selected. Further research has to be implemented on finding more competent rhizobacterial strains which may work under diverse agroclimatic conditions.

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Harnessing the Plant Microbiome: A Key Towards Sustainable Agriculture

12

Sanjana Kaul, Malvi Choudhary, Tanwi Sharma, and Manoj K. Dhar

Abstract

Plants are no more considered as organisms but as complex communities harbouring diverse microbes both on its outer as well as inner surfaces and environment. Plant microbiome represents the complex microbial communities associated directly or indirectly with a plant. It can be broadly categorized into endophytic, epiphytic and rhizospheric microbiome. Therefore, complex interactions between different said zones lead to a plant microbiome. Interestingly, plant microbiome involves pathogenic as well as non-pathogenic microbes. Non-pathogenic members include neutral as well as symbiotic members. Applications of plant-associated microbes hold a plethora of promises in diverse fields, viz. biotransformation, biodegradation, phytoremediation, seed production, seed predation, plant growth promotion, stress tolerance, biocatalysis, biofuel production, biocontrol, agricultural importance, source of novel natural products, biosynthesis and many more. There is an urgent need to explore and understand the hyperdiversity as well as functional potential of these microbial communities not just for the sake of sustaining ecosystem services but to maintain the beneficial use of biodiversity to mankind. For sustainable development of the human world, sustainable agriculture is the need of the hour. Plant microbiome communities are reported to play important roles in soil improvement, plant growth promotion and stress resistance. They are bestowed with the distinguished features of atmospheric nitrogen fixation, bioactive metabolite and phytohormone production, plant disease suppression, nutrient cycling enhancement and many more. Microbial mutualism offers a novel approach to develop microbial inoculants for use in agricultural biotechnology. The microbial inoculants offer several advantages as they are more safe, have reduced environmental cost, have lesser

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negative impacts on human health, are active in small quantities and have many more positive applications. These products can plausibly be used as biofertilizers and/or biocontrol agents, plant strengtheners, phytostimulators and biopesticides. It is a well-established fact that plants cannot survive in the absence of microbial associations. Therefore, deep understanding of the plant microbiome as a whole is essential in order to explore the same for better and sustainable agriculture.

Keywords
Microbiome • Sustainable agriculture • Plants • Microbes

12.1 Introduction

Almost all higher organisms including plants and humans harbour complex microbial communities constituting their respective microbiomes. A wide range of studies are available which reveal the diverse and complex microbial communities associated with the plant parts, viz. leaf, stem roots, etc., and rhizosphere as well. Microbes are not only restricted on the outer surface of plants as epiphytes but are also present inside the tissues as endophytes. Plant-associated non-pathogenic microbes, commonly regarded as plant growth promoters, are found to be involved in a number of activities, thus benefitting their host plant one way or the other. These microbes endow the plants with resistance towards biotic and abiotic stress as well as improved nutrient acquisition and plant growth promotion features. Such novel characteristics of the selected associated microbes can be exploited to mitigate the repercussions of world climate change on agriculture. Microbial communities associated with different organs of the plant can be similar or different. They may perform the same function or the different one. Actually function of each microbe is the result of their continuous and complex interactions with the host plant and other members of the microbiome. Therefore, unravelling and understanding the plant microbiome as a whole are essential in order to exploit such positive interactions for successful sustainable agriculture in general and microbiome-driven cropping system in particular.

12.2 Importance of Studying Plant Microbiomes

The interplay among plant microbiome components tends to influence plant health and crop productivity. The most influential are the plant-associated microorganisms which are known to be involved in improved nutrient acquisition and plant growth. The beneficial interactions between plants and the surrounding microbial population is a dynamic process and helps in fulfilling important ecosystem functions for plants and soil (Chaparro et al. 2012). The current agricultural practices lead to improper usage of chemical pesticides and fertilizers as a means of achieving pest

and disease resistance which concomitantly create a long list of environmental and many health problems (Bever 2015; Gunnell et al. 2007; Leach and Mumford 2008). Microbial mutualism offers a novel approach to develop microbial inoculants for use in agricultural biotechnology. These microbial inoculants offer several advantages as they are more safe, reduce environmental cost, have lesser negative impact on human health, are active in small quantities and have many more positive applications. These products can plausibly be used as biofertilizers and/or biocontrol agents, plant strengtheners, phytostimulators and biopesticides (Berg 2009). Some of the representative genera which are employed as microbial inoculants for plant growth promotion include *Azospirillum*, *Rhizobium*, *Bacillus*, *Pseudomonas*, *Serratia*, *Stenotrophomonas* and *Streptomyces* besides the fungal genera *Ampelomyces*, *Gliocladium*, *Piriformospora*, *Coniothyrium* and *Trichoderma* (Qiang et al. 2012). The use of these inoculants can help to enhance crop production by improving resistance and/or resilience to biotic and abiotic stresses from both bottom-up and top-down vistas (Hamilton et al. 2016).

Positive or mutualistic symbioses are ubiquitous and are known to be directly or indirectly involved in belowground and aboveground plant growth promotion. Well-known examples of the members of the rhizosphere microbiome influencing the nutrient status of plants are the nitrogen-fixing rhizobia and mycorrhizal fungi. The importance of these symbionts is well documented in translocation of macro- and micronutrients from soil to plant (Gianinazzi et al. 2010; Adeleke et al. 2012; Johnson and Graham 2013), improvement of soil quality, generation of stable soil aggregates (Duchicela et al. 2012), decreased soil erosion (Bever et al. 2012; Govers et al. 2013), phosphorus and iron uptake (Miransari 2011) and suppression of soil-borne pathogens (Pozo and Azcon-Aguilar 2007). The plant-associated microorganisms are also known to produce diffusible antibiotics, volatile organic compounds, toxins, siderophores, extracellular cell wall degrading enzymes, etc. which reduce the activity of pathogenic microorganisms, thereby promoting plant health. Accumulating facts illustrate that plant nutrition acquisition, metabolism, manipulation of plant growth hormones, stress tolerance and disease resistance may be strengthened or modulated via microbial symbionts. Diverse mechanisms are involved in the mutualistic interaction between microbiota and plants. They are based on the exchange of chemical signals such as metabolites, root exudates, substrates, etc. between the two partners. These types of interactions have been proposed as a predictor of aboveground plant diversity and productivity under different environmental conditions (Wagg et al. 2011). Altogether, administration of microbial communities and the exploitation of beneficial plant–microbe interaction should be highlighted and should be considered in future agricultural applications.

12.3 Plant Microbiome and Its Components

Plants being a complex community harbour a number of microbes on its inner as well as outer surface. Microbes residing inside the tissue of healthy plants are termed as endophytes, whereas those inhabiting the outer surface of plants are

termed as epiphytes. These microbes have different types of relationships with their host plant, viz. symbiotic, pathogenic, commensal, etc. A plant along with its associated microbial communities whether residing in endosphere, episphere or rhizosphere constitutes a complete plant microbiome. Most of the microbes associated with a plant are neutral or symbiotic; only a few possess the pathogenic association (Andreote et al. 2014). According to Lederberg and McCray (2001), the ecological community of commensal microorganisms, symbionts or pathogens that literally occupy some space in a body is termed as microbiome. Boon et al. (2014) have proposed microbiome as a set of genes encountered in association with the host or a defined environment, thus diminishing the importance of the link between taxonomy and functionality of the microbial community members. Plant microbiome can be broadly classified into three compartments: episphere, endosphere and rhizosphere (Hirsch and Mauchline 2012). The soil area that is influenced by the plant roots and its metabolites is termed as rhizosphere (Philippot et al. 2013). Due to the presence of root exudates, the microbial community associated with the rhizosphere is different from that of bulk soil. It has been postulated that availability of nutrients (root exudates) in the rhizosphere attracts the specific microbes that are beneficial for the plant (Prashar et al. 2014; Andreote et al. 2014). Moreover, the environment in the rhizosphere is found to be optimum regarding the proliferation of microbes with plant growth promotion potential (Mendes et al. 2013). Endosphere is considered as a habitat for intimate friends. Microbes inhabiting endosphere of a plant are termed as endophytes. Endophytes are found to be involved in bestowing a number of benefits to their host plants, viz. plant growth promotion, biocontrol, nutrient acquisition, phytoremediation, phytohormone production, phytochemical production, etc. Episphere (phyllosphere) is the outer plant surface directly in contact with the air. Microbes residing in the plant episphere are termed as epiphytes. Microbes associated with the phyllosphere are also found to be involved in plant growth promotion activities. Some of the important plant growth promoting properties possessed by the plant associated microbial communities are discussed in the chapter.

12.4 Applications of the Plant Microbiome in Agriculture

12.4.1 Nutrient Acquisition

Today's need of agriculturists and plant biologists is to create clean and efficient means to improve the quality of soil by nourishing and maintaining the useful microbial populations covering plant growth-promoting rhizobacteria, N2-fixing cyanobacteria, mycorrhiza and plant disease-suppressive beneficial bacteria. Most promising performances have been shown by plant growth-promoting rhizobacteria (PGPR) in enhancing nutrient bioavailability. Plant growth-promoting rhizobacteria (PGPR) are soil bacteria that are able to colonize rhizosphere, and several pieces of evidence highlight the beneficial effects of PGPR on plant at both physiological and molecular levels like organic matter mineralization, biological control against soilborne pathogens, biological nitrogen fixation, induction of rhizosphere

acidification, up- and downregulation of genes involved in ion uptake and translocation and root growth promotion (Pii et al. 2015). These microbes exert beneficial effects on plant development by one of the following mechanisms: (1) promote the plant growth either by using their own metabolism (solubilizing phosphates, producing hormones or fixing nitrogen) or directly affecting the plant metabolism (increasing the uptake of water and minerals), enhancing root development, increasing the enzymatic activity of the plant or "helping" other beneficial microorganisms to enhance their action on the plants or may (2) promote the plant growth by suppressing plant pathogens. These abilities are of paramount significance in agriculture in terms of improving soil fertility and crop yield (Perez-Montano et al. 2014). Two plant growth-promoting rhizobacteria (PGPR), i.e. *Pseudomonas fluorescens* and *Azospirillum brasilense*, were evaluated to study their effect on growth and essential oil (EO) composition and phenolic content in marigold (*Tagetes minuta*). Following inoculation, there was substantial increase not only in the growth parameters but also in the biosynthesis of the major essential oil components and total phenolic content which finds application in the food and cosmetic industries (del-Rosario Cappellari et al. 2013). Similarly, Yadav and Verma (2014) studied the effect of indigenous symbiotic N_2-fixing bacteria, *Rhizobium leguminosarum* BHURC04 and *Pseudomonas aeruginosa* on growth and yield of chickpea (*Cicer arietinum* L. var. C-235). It was found that this synergistic relationship resulted in increased N_2 fixation, improved acquisition of P and Fe besides stimulating growth by production of phytohormone (IAA) and suppressed plant diseases like wilt and root rot caused by *Fusarium oxysporum* f. sp., *Cicer* and *Rhizoctonia solani*, respectively. The arbuscular mycorrhiza (AM) symbiosis is the most common type of mycorrhizal association and plays a vital role in micronutrient acquisition (Hodge and Storer 2015). The fungus enhances plant uptake of phosphorus, nitrogen and other nutrients from the soil which are transferred to the plant host in exchange for photosynthetically fixed carbon (C). Qiao et al. (2015) reported the enhancement of faba bean competitive ability by arbuscular mycorrhizal fungi which substantially resulted in enhanced biomass production and N and P acquisition. Similarly, Nath et al. (2015) studied the potential of fungal endophytes in plant growth promotion isolated from tea (*Camellia sinensis*) and may have the potential to develop a biofertilizer consortium. Additionally, methylotrophic bacteria both as epiphyte and endophyte in host plant play a significant role in fortifying plants by increasing phosphorus acquisition, nitrogen fixation, phytohormone production, iron chelation and plant growth promotion and can be used as bioinoculant for sustainable agricultural practices (Kumar et al. 2016). Thus, use of mutualists may represent a promising method to improve plant use efficiency of nutrients, already present in soil or supplied by fertilizers.

12.4.2 Plant Growth Promotion

Microbial communities that promote plant growth are termed as biofertilizers. Such microbes have a role in improving the nutrient status of host plants via nitrogen fixation, potassium solubilization or mineralization, release of plant growth-regulating

substances, production of antibiotics and biodegradation of organic matter in the soil. The potential application of biofertilizers is reflected in increased nutrient profiles, plant growth and productivity, bioavailability of micro- and macronutrients in soil environment and improved tolerance to environmental stress. The growth-promoting factors in biofertilizers are important for regulating cell proliferation and modulating plant growth and development. These growth-promoting factors are phytohormones. They are crucial factors in driving plant growth through the various plant cell cycle checkpoints (Wong et al. 2015). Arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria have paramount significance in production of nutrient-rich high-quality food in sustainable comportment to ensure biosafety and nutrient management and are best employed as organic fertilizers exclusive of alternatives to agrochemicals (Bhardwaj et al. 2014; Paul and Lade 2014). Similarly, Fu et al. (2016) reported that the continuous application of biofertilizer (BIO) (*Bacillus amyloliquefaciens* NJN-6) resulted in significantly reduced *Fusarium* wilt disease of banana but increased crop yields. By artificially inoculating specific endophytes in host/non-host plants, the property of microbes to improve host plant growth potential can be transferred to new and economically important crops. It has been observed that seed bacterization of bhendi plant with some selected endophytic isolates has promoted the plant growth (Vetrivelkalai et al. 2010). Khan et al. (2012a, b) reported an endophyte, *Paraconiothyrium* sp., as potential producer of phytotoxin. So, it can be exploited for weed control strategies.

12.4.3 Siderophore Production

Micronutrients are gaining attention for their essential role in plant growth and productivity. Microbial symbionts are significant in releasing the nutritive cations such as Fe, Zn, etc. from soil minerals required not only for their own nutrition but also for plant nutrition. Iron is among the third most limiting nutrient for plant growth; it exists primarily in the insoluble ferric oxide form in aerobic environments (Samaranayake et al. 2012). In order to regulate intracellular iron concentrations, microbial communities are involved in secretion of siderophores which increase the solubility of inorganic iron in the rhizosphere and are transported into the root tissue via specific uptake system (Mendes et al. 2013). To study the effect of plant growth-promoting bacteria (PGPB) and cyanobacteria on rice and wheat micronutrient enrichment, Rana et al. (2015) inoculated the plants with *Anabaena oscillarioides* CR3, *Brevundimonas diminuta* PR7 and *Ochrobactrum anthropi* PR10 (T6) and found significant increase in nitrogen, phosphorus, and potassium (NPK) content. *Methylobacterium* spp. associated with citrus plant are also reported for its siderophore-producing potential (Vendan et al. 2010).

12.4.4 Rhizoremediation

The importance of increasing crop production while reducing resource inputs and increased utilization of agrochemicals could result in an increase in N emissions and leaching as well as carbon emissions directly from the soil. The negative effect of these pollutants on the environment and human health is devastating and cannot be overstated. Keeping in view these limitations of pollutants, there is a need to look out for alternative methods for excavation and incineration to clean polluted sites resulting in the application of bioremediation techniques. Bioremediation is defined as the action of microbes or other biological systems to degrade environmental pollutants (Sharma 2012) and focuses on the combination of two approaches, phytoremediation and bioaugmentation, resulting in rhizoremediation. In the process of rhizoremediation, exudates derived from the plant can help to stimulate the survival and degradation activities of microbes, which subsequently results in a more efficient mineralization of pollutants (Toussaint et al. 2012). Therefore, the use of pollutant-degrading microbes can serve as an important alternative to restore polluted sites in a less expensive, less labour-intensive, safe and environmentally friendly way. Rylott et al. (2011) reported the biotransformation of 2,4,6-trinitrotoluene (TNT) by *Enterobacter cloacae* PB2 and *Pseudomonas fluorescens* I-C, the major environmental pollutant. A wide variety of microorganisms are available for the elimination of different types of contaminants from aromatic to linear hydrocarbons (Kanaly and Harayama 2010). *Ceratobasidium stevensii,* an endophyte from the plant belonging to family Euphorbiaceae, is found to metabolize 89.51% of phenanthrene which is one of the major environmental pollutants (Dai et al. 2010). *Pseudomonas putida* KT2440, an efficient root-colonizing microorganism, has been explored for bioremediation of naphthalene-polluted soils (Fernandez et al. 2012). Uhlik et al. (2013) investigated the plant secondary metabolite-induced shifts in bacterial community composition and its degradative activity in long-term polychlorinated biphenyl (PCB)-contaminated soil. Isolation of several naphthalene- and anthracene-degrading bacteria (*Kurthia* sp., *Micrococcus varians*, *Deinococcus radiodurans* and *Bacillus circulans*) from rhizosphere of *Populus deltoides* has been reported by Bisht et al. (2010). *Acinetobacter calcoaceticus* P23, a bacterium in the rhizosphere of duckweeds *Lemna aoukikusa*, is studied for the sustainable biodegradation of phenol (Yamaga et al. 2010). Yang et al. (2016) studied the potential application of inoculation of arbuscular mycorrhizal fungi (AMF) in legume tree, *Robinia pseudoacacia*, for the phytoremediation of lead-polluted soil. Apart from decreasing the levels of pollutants in the ecosystem, rhizoremediation facilitates the instauration of natural flora and fauna and is considered ecologically sustainable.

12.4.5 Phytohormone Production

The phytohormones regulate a whole repertoire of plant developmental processes and various signalling networks known to be involved in plant responses to a wide range of pathogenic and mutualistic interactions. Central to these regulations, plant

hormones play a pivotal role in refining plant–microbe interactions, thereby modulating the beneficial or detrimental outcomes of plant–microorganism interactions, a key to improve defence responses without decreasing beneficial (e.g. symbiotic) associations (Boivin et al. 2016). Beneficial associations between plants and symbionts can lead to a profound reconfiguration of the plant primary and secondary metabolic pathways resulting in production of secondary metabolites which serve as key molecules in increasing plant immunity and/or defence against biotic invaders and abiotic stress tolerance besides increase crop productivity by improving nutrient uptake (de zelicourt et al. 2013). The mycorrhizal and rhizobial associations with plant root lead to reciprocal exchanges between fungi or bacterial microorganisms and host plants (Berendsen et al. 2012). Some of the representative genera of these symbiotic associations include ectomycorrhizal (ECM) fungi from the *Basidiomycota* and *Ascomycota* phyla and many forest trees (Diagne et al. 2013; Raudaskoski and Kothe 2015), arbuscular endomycorrhizal (AM) fungi from the *Glomeromycota* phylum which form association with most of land plants (Foo et al. 2013; Gutjahr and Parniske 2013) and nitrogen-fixing bacteria such as *Rhizobium* sp. and *Frankia* sp. (Santi et al. 2013). Endophytic isolates like *Paecilomyces Formosus*, *Micrococcus luteus*, *Penicillium citrinum* and many more are reported to be involved in phytohormone synthesis for growth promotion of the host plant (Khan et al. 2012a, b; Vendan et al. 2010; Khan et al. 2008). Rather than just being a phytohormone, they serve several functions in microbial communities and also in plant–microbe interactions which include (1) enhanced mineral uptake from the soil and root exudation, (2) increased nodulation and N_2 fixation, (3) affect gene expression in some microorganisms, (4) increased tolerance to biotic and abiotic stresses, (5) act as reciprocal signalling molecule in plant–microbe interactions, (6) contribute plant growth and defence system and (7) as an effector molecule in phytostimulation and pathogenesis (Spaepen et al. 2009). All plant growth-promoting rhizobacteria (PGPR) facilitate or promote plant growth under different environmental conditions. This phytohormone production by rhizobia and its subsequent role in plant growth promotion contribute to a process known as phytostimulation. Phytostimulation can be induced either directly or indirectly. Indirect plant growth promotion is induced by biosynthesis of stress-related phytohormones like jasmonic acid (JA) or ethylene which modulate various biochemical and physiological processes of plant growth. Direct growth promotion mechanisms induced by PGPR include production of plant growth regulators such as auxins, gibberellins (GAs), cytokinins (CK) and nitric oxide (NO) with the capacity to control plant development in both beneficial and deleterious ways (Duca et al. 2014; Giron et al. 2013). One of the most representative PGPR belonging to the genus *Azospirillum* is well studied for its phytohormone production and its agronomical impact (Cassán et al. 2014; Castillo et al. 2015). Likewise, Thakur et al. (2015) studied the effects of plant growth-promoting rhizobacteria (*Bacillus licheniformis* CKA 1, *Bacillus subtilis* CB 8 A, *Bacillus* sp. RG1, *Bacillus* sp. S1 and *Bacillus* sp. S2) and GA3 (25, 50 and 75 ppm) on growth, yield and fruit quality of strawberry cultivar Chandler. Similarly, Belimov et al. (2015) studied the role of rhizobacteria in auxin production and various 1-amino-cyclopropane-1-carboxylic acid deaminase containing rhizobacteria

(*Achromobacter xylosoxidans* Cm4, *Pseudomonas oryzihabitans* Ep4 and *Variovorax paradoxus* 5C-2) in improving growth and yield of potato (*Solanum tuberosum*) under abiotic stress. To study the role of fungal endophytes in phytohormone production, Khan et al. (2015) investigate the potential of fungal endophyte *Penicillium janthinellum* LK5 (PjLK5) in synthesizing the defence-related endogenous phytohormone salicylic acid to counteract the adverse effects of aluminium stress in *Solanum lycopersicum*.

12.5 Biotic Stress Tolerance

Plant microbiomes are found to be involved in bestowing disease resistance to host plants. Plant-associated commensals are known not only as plant growth promoters but also as biological agents against plant diseases. Diverse mechanisms are employed by microbial communities for the biocontrol of phytopathogens, which include (1) competition for an ecological niche or substrate, (2) production of inhibitory allelochemicals (3) and the induction of systemic resistance in host plants, against a broad spectrum of pathogens. Induction of systemic resistance against soilborne pathogens includes resistance through salicylic acid-dependent pathway or jasmonic acid pathway and ethylene perception from the plant (Beneduzi et al. 2012). Saraf et al. (2014) studied the role of allelochemicals produced by plant growth-promoting rhizobacteria as a nonhazardous biological control strategy to control phytopathogens. Phyllosphere-harbouring microbes were found to be involved in resistance of the plant *Arabidopsis thaliana* against plant pathogen *Botrytis cinerea* (Ritpitakphong et al. 2016). Singh (2016) also observed the role of several non-pathogenic strains of plant-associated fungi such as *Trichoderma* sp., *Penicillium* sp., *Fusarium* sp., *Phoma* sp., etc. in triggering defence responses via multiple signalling pathways, thereby highlighting its potential in inducing systemic resistance in host plant against phytopathogens.

12.6 Abiotic Stress Tolerance

A number of abiotic factors, viz. drought, flood high soil salinity, heat, cold, oxidative stress, heavy metal toxicity, nutrient deficiency, etc., result in plant stress and frequently limit growth and productivity of major crop species. These unfavourable environmental conditions pose a great hindrance to photosynthetic performance of plants and world agriculture. Agricultural crops in particular are deeply affected by these environmental stresses, which when occurring simultaneously can have severe consequences. The altered climatic conditions, combined with an increasing pressure on global food productivity, are likely to induce changes in plant physiology and result in a demand for stress-tolerant crop varieties (Takeda and Matsuoka 2008; Newton et al. 2011). Understanding the mechanism of cellular and molecular plant responses to multiple simultaneous stresses is therefore fundamental in providing opportunities for developing broad-spectrum stress-tolerant crops (Atkinson

and Urwin 2012). Plant–microbial communities with plant growth-promoting potential can be exploited to overcome the limitations of crop productivity caused by different abiotic stress factors, and it may prove to be a promising alternative strategy. Endophytes are found to produce important stress-relieving phytohormones (Khan et al. 2012a, b). A number of endophytes like *Fusarium culmorum*, *Piriformospora indica* and *Trichoderma hamatum* are reported for endowing their host plants with drought resistance potentials (Redman et al. 2011; Sun et al. 2010; Bae et al. 2009). *Piriformospora indica,* an endophyte associated with *Hordeum vulgare*, is found to be involved in the salinity stress tolerance of the host plant (Waller et al. 2005). Similarly, *Paecilomyces formosus* LHL10, a phytohormone-producing fungus isolated from the roots of cucumber plants, has been evaluated for modulating salinity stress in rice cultivar. A type of multifunctional molecule, popularly known as strigolactones, has been well studied, and its role in combating water-related stress (drought and salinity) is well established besides favouring beneficial symbiosis between the plant and arbuscular mycorrhizal fungi/rhizobacteria (Andreo-Jimenez et al. 2015). Azad and Kaminskyj (2016) indicated the potential of systemic fungal endophyte in alleviating salt and drought stress and proposed the role of these endophytes in agricultural and horticultural important crop plants grown in arid environment. Glutathione S-transferase (GST) gene, from endophytic fungus, *Exophiala pisciphila*, was also identified and functionally characterized. Functional characterization of GST gene in *Exophiala pisciphila* confers tolerance to many biotic and abiotic stresses in plants (Shen et al. 2015; Zhao et al. 2015).

12.7 Techniques for Studying the Plant Microbiome

Apart from the conventional methods which have not been described here, modern 'omics' tools and techniques involving genomics, transcriptomics and proteomics along with their metaomic partners can prove to be helpful in unravelling and better understanding of plant microbiomes. These techniques, along with the analysis of the fungal behaviour on its host, are a valuable tool to understand lifestyle complexity and result in the identification of the symbiosis determinants and their evolution. Metagenomic and comparative metagenomic approach can be successfully used to study microbiomes of the same or different plants under similar or different environmental conditions. Dinsdale et al. (2008) used the comparative metagenomic approach to describe the variation in functional potential of nine different microbiomes. The putative functional characteristics of the root endophytes of rice have been successfully analysed based on metagenome analysis (Sessitsch et al. 2012). Transcriptomic and metatranscriptomic approaches can give us the idea about change in response of plant-associated microbial communities with changing environmental conditions. The metatranscriptomic analysis of soybean plant samples has revealed the presence of various pathogenic, symbiotic and free-living microbes, thus comprising the microbiome of the soybean plant (Molina et al.

2012). In another significant report, the transcriptional profiling of wheat plant has revealed the upregulation of nutrient acquisition and cell cycle genes in the wheat roots specifically colonized by *Azospirillum brasilense* (Camilios-Neto et al. 2014). Interestingly, transcriptomic analysis of endophyte-free and endophyte-infected plants has revealed the differential expression of hundreds of host plant genes (Ambrose and Belanger 2012). Proteomic and metaproteomic techniques can prove to be useful in the assessment and identification of the proteins involved in establishing the relation between host plant and different microbiome entities. Protein fingerprints can be used to assess the role of different microbes, constituting host plant microbiome, under different stress conditions (Bhuyan et al. 2015). Recently, insights on the 'omics' approaches for understanding the plant–microbe interaction have been reviewed by Kaul et al. (2016).

12.8 Conclusions

Microbe-based growth promotion in plants could provide effective ways of developing sustainable agriculture in order to ensure human and animal food production with a minimal disturbance of the environment. Keeping an eye on the plethora of potentials promised by plant-associated microbes, it becomes indispensable to include plant microbiomes in future breeding programmes. For the successful introduction of microbiome components in breeding programmes, identification of the genetic elements responsible for plant–microbe interactions is the need of the hour (Schlaeppi and Bulgarelli 2015). It is also important to understand the factors responsible for the distinct composition of the plant microbiomes as well as the factors governing the selection of specific microbial communities as a member of a plant microbiome. System biology techniques (genomics, transcriptomics, proteomics and metabolomics) along with their metaomic partners can be successfully used for such investigations (Kaul et al. 2016). Moreover more crop plants should be studied for their microbiomes so that their better microbiome-based breeding programmes can be introduced accordingly. Thus, exploring the undiscovered microbiome biodiversity of different plants and their successful application in agriculture fields would surely be a step forward towards conservation of biodiversity as well as sustainable development in general and sustainable agriculture in particular.

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Trichoderma and Its Potential Applications

13

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Abstract

Trichoderma are free-living green-spored ascomycetes, ubiquitous inhabitants of soil and aquatic environments present in nearly all types of tropical and temperate regions. *Trichoderma* species are known to maintain a parasitic or symbiotic relationship with plants and animals and are ubiquitous inhabitants of soil and aquatic environments with its diverse applications in the field of agriculture, industries, and bioremediation. It is a well-known biocontrol agent and follows various mechanisms such as competition, mycoparasitism, induced resistance, etc. The following chapter, therefore, briefs the diversity, biology, and various applications of *Trichoderma*.

Keywords

Biocontrol • Biodiversity • Composting • Lignocellulolytic enzymes

13.1 Introduction

Fungi, ubiquitous inhabitants of soil and aquatic environments, are known to maintain a parasitic or symbiotic relationship with plants and animals. These play a pivotal role in nutrient cycling, especially in organic matter decomposition and are major sources for production of biologically active substances. In spite of their varied potentials, their metabolic capabilities are still to be discovered and understood. Each fungal species has an individual role to play, and among them is a species named *Trichoderma*, known throughout the microbial society for its diverse

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applications. *Trichoderma* species was first described by Persoon (1794) and later envisaged into four genera. Further it was recognized and divided into nine aggregates by Rifai (1969) in his monograph on *Trichoderma.* There were several biological species under each aggregate, but Rifai (1969) was unable to define the limits of individual biological species (Samuels et al. 1998). Bisset (1991a, b, c, 1992) elevated Rifai species aggregate to special level and recognized several species within each of five sections of the genus *Trichoderma*.

Trichoderma are green-spored ascomycetes, present in nearly all types of tropical and temperate soils. It is a free-living filamentous fungus commonly present in soil and root ecosystems. This fungus is inhabitant of decaying plant materials and plant rhizosphere (Schuster and Schmoll 2010). Their diverse metabolic competence and belligerently competitive nature help them in comfortably colonizing their habitats (Gams and Bissett 2002). Tulasne brothers first described *Hypocrea*, the teleomorphs of *Trichoderma*, in 1865 (Gams and Bissett 2002). As the number of *Trichoderma* species is increasing, they are being linked to their teleomorphs, for example, *T. virens* is the anamorph of *H. virens*, and *T. harzianum* is the anamorph of *H. lixii*. But some common species like *T. asperellum* have not been linked to any teleomorph and they might be clonal (Samuels 2006).

Trichoderma spp. can be characterized as an opportunistic avirulent plant symbiont. *Trichoderma* is a most prevalent culturable fungus. These are known to form mutualistic endophytic relationships with several plant species (Bae et al. 2011) and release a variety of compounds that induce localized or systemic resistance responses in plants. *Trichoderma* species are highly interactive in soil, root, and foliar environments and therefore considered of great value for the agriculture sector. *Trichoderma* spp. have the ability to produce secondary metabolites that hold vital applications for biocontrol of various plant pathogens (https://www.moldbacteria.com/mold/trichoderma.html). This property has been exploited by various industries for producing formulations against plant pathogens. Apart from disease control, this species also has a pivotal role in plant nutrient management. It readily colonizes plant roots and forms symbiotic associations with plants. It helps in increasing plant root growth and development, crop productivity, resistance to abiotic stresses, and uptake and use of nutrients (Ranasingh et al. 2006). Because of their resistance to most of the chemical pesticides, this fungus can be comfortably applied in fields that have already been incorporated with chemical fungicides. Many species of *Trichoderma* are cellulolytic enzyme producers (i.e., they are capable of degrading hemicelluloses and celluloses since they produce large quantities of the enzyme such as xylanase and cellulase) and therefore can be easily found on cellulosic materials including decaying wood, wood products, textiles, stored cereals, and plant foodstuffs. Because of this reason, many of *Trichoderma* species are also considered as spoilage organisms responsible for causing post-harvest rots of various fruits and vegetables.

This chapter will discuss about the characteristics, morphology, and wide applications of *Trichoderma* spp.

13.2 Biology of *Trichoderma*

Trichoderma produces white- and green-colored conidia from phialids that are present abundantly or scantily on the branches of conidiophores. It can be easily identified in the culture of the fungus. The species-level recognition of the isolates is complicated and perplexing due to the complex and intimately related features of the species. The concept of identification of species is very extensive, and this has resulted in the establishment of different specific and subspecific taxa (Samuels 1996). They are ubiquitous colonizers of cellulosic materials and hence are commonly found wherever there is availability of decomposing plant material (Kubicek et al. 2009; Jaklitsch 2009). They are present in the rhizosphere of plants and induce systemic resistance against various plant pathogens (Harman 2000). Their efficiency of utilizing substrate in addition to their secreting capability for antibiotic metabolites and enzymes reflects that they are very successful colonizers of their habitats (Schuster and Schmoll 2010).

13.3 Biodiversity and Phylogeny of *Trichoderma*

The taxonomy of *Trichoderma* was first illustrated by Persoon in 1794, in his classification of fungi. But his classification of *Trichoderma* was problematic because he incorporated some other fungi and some slime molds, for example, *Puccinia*, *Mucor*, *Ascobolus*, *Physarum*, *Trichia*, and *Stemonitis* (Klein and Eveleigh 2002). In 1865, Tulasne and Tulasne suggested a link to the sexual state of a *Hypocrea* species. In 1939, it was anticipated by Bisby that *Trichoderma* includes only a single species, i.e., *Trichoderma viride*. This led to the misidentification of nearly all strains of *Trichoderma* as "*T. viride*" in literatures prior to 1969 (Druzhinina and Kubicek 2004). Rifai projected the concept of "aggregate" species in 1969, in which he divided *Trichoderma* species into nine "species aggregates," namely, *T. aureoviride* Rifai, *T. hamatum* Bain, *T. harzianum* Rifai, *T. koningii* Oudem, *T. longibrachiatum* Rifai, *T. piluliferum* Rifai, *T. polysporum* Rifai, *T. pseudokoningii* Rifai, and *T. viride*. Subsequently, it led to the discovery of various new species of *Trichoderma*/*Hypocrea*, and by 2006, more than 100 phylogenetically defined species were already incorporated in the genus (Druzhinina et al. 2006a). According to Chaverri and Samuels (2004), Rifai admitted that each species aggregate has more than one morphologically indistinguishable species. Since 1984, Bissett has revised Rifai's aggregate species. In 1991, Bissett conferred the complexity to differentiate *Trichoderma* species on the basis of Rifai's species aggregates, as merely five of Rifai's aggregates species (*T. harzianum*, *T. longibrachiatum*, *T. pseudokoningii*, *T. piluliferum*, and *T. polysporum*) were closely defined, whereas other aggregates were having relatively large number of species (Chaverri and Samuels 2004). In the same year, the genus was subdivided into five sections by Bissett that are *Longibrachiatum*, *Trichoderma*, *Pachybasium*, *Saturnisporum*, and *Hypocreanum* (Druzhinina and Kubicek 2004). The following is the taxonomic information about *Trichoderma*:

Kingdom: Fungi
Division: Ascomycotina
Subdivision: Pezizomycotina
Class: Sordariomycetes
Order: Hypocreales
Family: Hypocreaceae
Genus: *Trichoderma*

In the current years, secure classification of novel species was considerably assisted by advancement of a customized match search tool (TrichoBLAST) and an oligonucleotide barcode (TrichOKEY) (Druzhinina et al. 2005; Kopchinskiy et al. 2005). Phenotype microarray is another functional tool for the categorization of the new isolates of *Trichoderma* species. It allows for the analysis of carbon consumption patterns for 96 carbon sources (Bochner et al. 2001; Kubicek et al. 2003; Druzhinina et al. 2006b). The persistent attempts to explicate geographical incidence and diversity of *Trichoderma/Hypocrea* gave comprehensive credentials of the genus in Europe and worldwide (Samuels et al. 2002a; Chaverri and Samuels 2003; Jaklitsch 2009). Presently, 165 and 471 different names for *Hypocrea* species and *Trichoderma* are recorded in the Index Fungorum database, respectively (http://www.indexfungorum.org/Names/Names.asp). Molecular methods are new tools for classification of this species; however, several names have already been incorporated long before these new methods were identified and worked upon. At the moment, 104 species of the *Trichoderma/Hypocrea* have been listed in International Subcommission (http://www.isth.info/biodiversity/index.php), and these are characterized on the basis of their molecular biology. Seventy-five different species of *Hypocrea* have been recognized in temperate Europe (Jaklitsch 2009). On the other hand, a significant number of alleged *Hypocrea* strains and even more *Trichoderma* strains are still without safe identification, but their sequences have been deposited in GenBank (Druzhinina et al. 2006a) and for which further study is required. A wide range of pigments have been produced by the species of this genus which includes bright greenish yellow to reddish in color, even though many of them are also colorless. In the same way, conidial pigmentation ranges from colorless to several shades of green and occasionally also gray or brown. Except pigmentation, identification of species within the genus is complex as a consequence of the constricted array of distinction of the simple morphology in *Trichoderma* (Gams and Bissett 1998).

13.4 Morphology of *Trichoderma*

13.4.1 Macroscopic Features

Colony characteristics such as rate of growth, development of pustules, pigmentation, and odor are the different characteristics of a species (Gams and Bissett 2002). Optimum conditions for the growth of majority of *Trichoderma* species are between 25 and 30 °C and characteristically do not grow at 35 °C (Samuels et al. 2002a).

However, a few species have the trait of growing well even at high temperature of 35 °C. This trait can serve as significant criteria to differentiate among species of analogous morphology. For instance, *T. harzianum* can be differently identified from morphologically related species such as *T. aggressivum* and *T. atroviride* by growing them at 35 °C. It was observed that after 96 h of incubation, *T. harzianum* grew well at 35 °C, whereas *T. aggressivum* and *T. atroviride* did not have colony radius more than 5 mm (Samuels 2004). Rich source of nutrients such as potato dextrose agar (PDA) shows better observation of mycelia growth and pigmentation. According to Samuels et al. (2002a, b), white and transparent colonies were observed on PDA and cornmeal dextrose agar (CMD), respectively. Spotted blue-green or yellow-green scraps were observed on conidia formation. Sometimes these scraps lead to form concentric rings, whereas the reverse of the colony was observed as pale, tan, or yellowish in color. Additionally, several species of *Trichoderma*, for example, *T. viride*, produce a distinctive sweet smell that resembles to "coconut" odor (Gams and Bissett 2002).

13.4.2 Microscopic Features

Trichoderma generally forms vegetative hyphae that are septated, hyaline, and smooth-walled (Gams and Bissett 2002) with highly branched conidiophores. The side branches emerging from the core branch can be paired. Usually, the emerging angle is at or near 90° with respect to the core branch. A pyramidal structure is formed by the paired branches (Samuels et al. 2002a).

Conidiogenous cells, which are also called phialides, are usually inflated in the center forming a flask-like shape and may be cylindrical or virtually subglobose. They remain in divergent verticils at the end of the conidiophores or in whorls beneath septa along the conidiophores and branches. Sometimes these are irregular, paired, or in solitary (Samuels et al. 2002a; Gams and Bissett 2002).

Conidia are single cell, ellipsoidal, usually green, and occasionally colorless, gray, or brown. They have smooth surfaces; however, rough conidia are found in several species, i.e., *T. viride* (Samuels et al. 2002a; Gams and Bissett 2002). Chlamydospore plays a major part in survival. Generally, they have thick wall and inflated vegetative cells with thick cytoplasm (Lin and Heitman 2005). They are single celled, globose to subglobose, and produced within the hyphae or at the hyphal tips. In general, they are colorless, pale yellow, or green (Samuels et al. 2002a; Gams and Bissett 2002). Chlamydospores are produced profusely in liquid-submerged culture and are also the active propagules in many *Trichoderma*-based formulations. The advantage these propagules is their lower vulnerability to soil fungistasis, resistance to environmental stress, greater inoculum potential compared with conidia, and a longer shelf life (Papavizas 1985).

13.5 Habitat

Trichoderma is a well-studied fungal genus that currently consists of more than 200 molecularly defined species. These are generally considered as cosmopolitan and ubiquitous element of several ecosystems in an extensive variety of climatic zones (Kubicek et al. 2008). Though several are ubiquitous, some are restricted to definite geographical areas (Harman et al. 2004a, b). Some species have parasitic property, while others grow on dead wood and bark, in soil and rhizosphere, in marine sponges (Gal-Hemed et al. 2011), and on woody and herbaceous plants (Jaklitsch 2009; López-Quintero et al. 2013). These are endophytes (Zhang et al. 2007; Mulaw et al. 2010). Hence, this all exemplifies its ability to occupy various ecological niches.

13.6 Life Cycle

The organism grows and ramifies as typical fungal hyphae, 5–10 μm in diameter. Asexual sporulation occurs as single celled and green conidia are released in large numbers. Intercalary resting chlamydospores are also formed, which are generally single celled, although two or more chlamydospores may also be fused together (Schuster and Schmoll 2010).

13.7 Applications of *Trichoderma*

Trichoderma is a comprehensible type of fungus found inhabiting naturally in soil. There are several applications of *Trichoderma* and a few are discussed here.

13.7.1 Disease Control

Trichoderma species are widely used as biocontrol agent. They have a significant role in plant growth by increasing uptake of nutrients from soil and decreasing the activity of the soilborne pathogens (Harman et al. 2004a, b). Among all different species of *Trichoderma*, *T. harzianum* is considered as the most efficient biocontrol agent (Gao et al. 2002, Sharma et al. 2011). *Trichoderma* spp. have evolved numerous mechanisms for biocontrol against the pathogenic fungi simultaneously enhancing plant and root growth. Some of the mechanisms involved are competition for space and nutrients (Elad et al. 1999), mycoparasitism (Haran et al. 1996; Lorito et al. 1996), production of inhibitory substances, inactivation of the pathogenic enzymes (Roco and Perez 2001), and induced resistance (Kapulnik and Chet 2000; Sharma et al. 2012).

13.7.1.1 Competition

Trichoderma is usually considered as being a destructive competitor as it is a very fast-growing fungus and quickly colonizes substrates to prohibit the growth of

pathogens such as *Fusarium* spp. (Papavizas 1985). Rhizosphere competence, subsequent to seed coating, is a vital approach to produce a protection zone in opposition to pathogens (Howell 2003). Application of these species, either by adding in soil or by treating seeds, makes them grow faster along with the development of root system of the treated plants (Ahmad and Baker 1987). Invasion of *Fusarium oxysporum* f.sp. *vasinfectum* and *Fusarium oxysporum* f.sp. *melonis* has been suppressed by soil treatments of *T. harzianum* spores. Competition is an anticipated mechanism, even though it is not verified to be the major activity (Sharma et al. 2012).

13.7.1.2 Mycoparasitism

Chet et al. (1981) have very well acknowledged their mode of hyphal contact and parasitism of *Trichoderma* spp. with a number of soilborne pathogens. They develop tropically in the direction of hyphae of other fungi, entangled around them in a lectin-mediated reaction that leads to the degradation of the cell wall of the pathogen by secreting several lytic enzymes. This course of action limits the growth and activity of plant pathogenic fungi. This contact between pathogen and *Trichoderma* is precise and not simply a contact response. They identify indications from the target fungi that activate coiling and host penetration. Mycoparasitism includes the cell wall-degrading enzymes such as *ß*-1,3-glucanases and chitinases. Endochitinase (42-kDa), chitobiosidase (40-kDa), and N-acetyl-*ß*-D-glucosaminidase (73-kDa) were described to have a considerable hampering effect on the germination of spores and hyphal development of various pathogenic fungi, viz., *Botrytis cinerea*, *Fusarium* spp., *Alternaria* spp., *Ustilago avenae*, *Uncinula necator*, and almost on all fungi that contain chitin as a constituent of their cell wall. Lorito et al. (1998) have studied the characteristics of various enzymes and their capability to hamper the spore germination and hyphal development of the plant pathogenic fungi. It was observed in scanning electron microscopy and fluorescence microscopy that *T. harzianum* and *T. hamatum* were mycoparasites of both *Sclerotium rolfsii* and *Rhizoctonia solani* and produced glucanase and chitinase enzymes to act on the cell wall of the pathogen (Elad et al. 1983; Sharma et al. 2012).

13.7.1.3 Production of Inhibitory Substances

Plant pathogens produce certain enzymes and toxins during infection of host plant. *Trichoderma* species have the ability to produce enzymes that take part in inactivation of the pathogen's enzymes. The enzymes of *Botrytis cinerea*, viz., pectinases, cutinase, glucanase, and chitinase, were suppressed through the action of protease secreted on plant surfaces by T39 (Elad et al. 1999). The in vitro inhibitory biocontrol ability of *T. harzianum* against plant pathogen *Alternaria alternata* (*Alternaria alternata* (Fr.) Keissl.) was investigated in the presence of growth regulators. *A. alternata* is known to secrete enzymes, viz., endopolygalacturonase (endo-PG) and pectate lyase (PL) responsible for the hydrolysis of pectic components of the plant cell wall. Endo-PG secretion by *A. alternata* was decreased to 50% in the presence of *T. harzianum*, and this inhibitory effect was independent of the presence of growth regulators. *Trichoderma* is also known to produce various secondary metabolites (peptaibols, epipoly-thiodioxopiperazines (ETPs), volatile and nonvolatile

Table 13.1 Different species of *Trichoderma* acting against plant pathogens

Organism	Pathogen	Disease	Plant	Disease control	References
T. koningii MTCC 796	*Rhizoctonia solani*	Root rot	Cotton	88.12%	Gajera et al. (2016)
T. viride NBAII Tv23	*Rhizoctonia solani*	Root rot	Cotton	85.34%	Gajera et al. (2016)
Trichoderma sp.	*Fusarium solani*	Root rot	*Phaseolus vulgaris* L	86%	Toghueo et al. (2016)
T. asperellum	*Pythium aphanidermatum*	Seedling damping-off	Tomato	40%	Kipngeno et al. (2015)
T. asperellum	*Phytophthora megakarya*	Black pod	Cacao	90%	Mbarga et al. (2014)
T. asperellum	*Phytophthora ramorum*	Ramorum blight	Oak	Not available	Widmer (2014)
T. harzianum	*Fusarium verticillioides*	Fumonisin contamination	Maize	58%	Ferrigo et al. (2014)

terpenes, pyrones, polyketides, and siderophores) (De Respinis et al. 2010), but in-depth research regarding their ecological roles and genetics of their biosynthesis is still required. A transcription factor Thctf1 regulates the biosynthesis of the volatile antifungal compound6-pentyl-2H-pyran-2-one (6-PP) (Rubio et al. 2009), which plays a major role in inhibiting various pathogens. Phytotoxin named trichodermin has been identified to be produced by a trichothecene-like (TRI) cluster identified in *Trichoderma brevicompactum* (Cardoza et al. 2011). Different species of *Trichoderma* playing a role in biocontrol against pathogenic fungi have been listed in Table 13.1 (Sharma et al. 2012).

13.7.1.4 Induced Resistance

A series of morphological and biochemical reactions are initiated in response to plant defense response, as *Trichoderma* colonizes and penetrates in plant root tissues. This response is termed as induced systemic resistance (Bae et al. 2011). This has been validated by one study in which the roots inoculated with T-203 exhibited high activities of chitinase, β-1, 3-glucanase, cellulase, and peroxidase when compared to an untreated control, 72 h post inoculation (Sharma et al. 2012). Typical fungal structures associated with mycoparasitic interactions of *Trichoderma* spp. were also revealed in scanning electron microscopic studies. Cucumber plants treated with 2,6-dichloroisonicotinic acid, an inducer of the plant defense response, displayed responses that were similar but not identical to those of plants inoculated with *T. harzianum* (Sharma et al. 2012).

13.7.2 Bioremediation

Trichoderma sp. is a producer of organic acids (gluconic acid, fumaric acid, and citric acid) that decrease soil pH and allow dissolution of macro- and micronutrients

Table 13.2 *Trichoderma* sp. used for the bioremediation of different pollutants

Organism	Pollutants	References
Native *Trichoderma* strain	Atrazine	Pelcastre et al. (2013)
T. viride	Pb and Cd	Sahu et al. (2012)
Trichoderma strains	Heavy metals	Kacprzak et al. (2014)
T. harzianum CBMAI 1677	Pentachlorophenol and its main metabolites Pentachloroanisole and 2,3,4,6-tetrachloroanisole	Vacondio et al. (2015)
Trichoderma isolates	Nickel	Nongmaithem et al. (2016)
T. harzianum	Carbamazepine and clarithromycin	Buchicchio et al. (2016)
Trichoderma spp.	Organic solvents	Oros et al. (2011)
T. atroviride	Organophosphate pesticide dichlorvos	Tang et al. (2010)
11 *Trichoderma* strains	Crude oil, naphthalene, phenanthrene, and benzo-α-pyrene	Argumedo-Delira et al. (2012)
T. harzianum	Arsenic tolerance in eucalyptus globule	Arriagada et al. (2009)
T. atroviride	Phytoextraction in Cd-contaminated and Ni-contaminated soils	Cao et al. (2008)
T. harzianum	Aluminum resistance in eucalyptus	Arriagada et al. (2007)
Trichoderma spp.	Pesticide polyresistance	Hatvani et al. (2006)
Trichoderma spp.	Cyanide	Ezzi and lynch (2005)
Trichoderma spp.	Soil and water pollutants	Harman et al. (2004b)
Trichoderma	Diesel contaminated soil	Gestel et al. (2003)
T. atroviride	Heavy metals from sludge	Errasquin and Vazquez (2003)

such as phosphorus, iron, manganese, magnesium, etc., which are necessary for plant metabolism. *Trichoderma* spp. is illustrated by its capability to alter the rhizosphere microflora particularly with strong fungi that are aggressive against plant pathogens by exhaustive colonization of roots (Ociepa 2011; Brotman et al. 2010). It is found to be very resistant to a wide series of toxicants such as heavy metals, organometallic compounds, tannery effluents, and harmful chemicals like cyanide (CN) (Lynch and Moffat 2005). This property makes this fungus a significant fungal genus to be explored as a genetic resource for its employment in bioremediation of toxic pollutants (Hasan 2016). It degrades a number of pollutants mentioned in Table 13.2.

13.7.3 *Trichoderma* for Enzyme Production

Trichoderma sp. has a great potential to produce enzymes, viz., cellulase, xylanase, proteases, and ß-1,3-glucanase (Verma et al. 2007). The potential of this species to produce these enzymes makes it valuable to be used for various applications such as composting and production of these enzymes for industrial purposes by solid-state fermentation.

13.7.3.1 *Trichoderma* in Composting

Composting is a process of degradation of the complex materials present in the waste into simpler ones. The degradation process takes many months, due to which the amount of waste generation keeps on increasing. There is a need to look into some biological measures to manage the waste generated and degrade it in a rapid and eco-friendly manner. Here comes the role of some lignocellulolytic fungi that have the potential to produce enzymes that help in degradation of the celluloses and hemicelluloses present in the waste. *Trichoderma* sp. is a well-known enzyme producer. Many studies have been done on the utilization of this species, alone and in combination with other fungi in waste degradation. Haddadin et al. (2009) used a mix of *T. harzianum* and *Phanerochaete chrysosporium* in composting of olive pomace and got the maturity of the compost within 50 days. Maturity of the compost can be decided when the compost reaches a constant temperature, dark brown and black in color, fine and soft texture, and without any unpleasant odor. Since this fungus is a good cellulose degrader, it has been reported by Haddadin et al. (2009) that *T. harzianum* degraded 59.24% of cellulose inside the olive pomace compost. Lignocellulosics such as cellulose and lignin are the main barriers in degradation process; therefore addition of microorganisms enhances the degradation process (Sharma and Arora 2012). Dayana Amira et al. (2012) showed that the combination of *T. virens* and chicken manure as the source of organic nitrogen gave positive results on the composting of EFB and POME. The application of *Aspergillus* and *Trichoderma* leads to rapid degradation of celluloses and hemicelluloses and showed great potential in shortening the composting period (Biswas and Narayanasamy 2002).

Weltzien (1991) and Hoitink et al. (1997) have stated that composts can be used as an alternative for controlling diseases against fungal pathogens. Compost with *Trichoderma* spp. isolates has proven to be effective when it showed low disease severity in plants treated with compost suggesting that application of extracts produced from well-matured compost fortified with biocontrol agents could be an alternative control strategy (Siddiqui et al. 2008). These act as an enhancer to plant nutrition. Studies reported the extent of *Trichoderma* in promoting the plant growth and protecting the plants from many diseases (Ristaino et al. 1991; Jinantana 1995; De Ceuster and Hoitink 1999; Ibrahim 2005; Srivastava et al. 2010; Bernard et al. 2012).

13.7.3.2 *Trichoderma* for Production of Industrial Enzymes on Nontraditional Substrates

The ability to produce lignocellulolytic enzymes on solid substrates, considered as waste, is a known property of this fungus. There are some important factors that affect the yield of enzyme production, such as the type of strain, culture conditions, nature of the substrate, and availability of nutrients (Pandey et al. 2001). Fermentation process under deficient water conditions is termed as solid-state fermentation (Joshi and Khare 2011). Agro-industrial substrates hold great potential for enzyme production by solid-state fermentation (SSF). The cost of enzyme production by submerged fermentation is higher as compared to SSF. This technology holds great advantages over submerged fermentation (SMF) such as high volumetric productivity, low cost of equipment involved, better yield of product, lesser waste generation, lesser time consuming processes, etc. The most studied species of *Trichoderma* for enzyme production is *T. reesei*. The ability to degrade lignocellulose efficiently is associated with mycelial growth habit that allows the fungus to transport scarce nutrients such as nitrogen and iron to a distance into the nutrient-poor lignocellulosic substrate that constitutes its carbon source. The fungal degradation occurs exocellularly, either in association with outer cell envelope layer or extracellularly, because of the insolubility of lignin, cellulose, and hemicellulose. Table 13.3 specifies the different substrates utilized for enzyme production by different species of *Trichoderma.*

13.8 Conclusion

Building a sustainable economy and protection of our environment has become a dominant topic of today's scenario. In this context, *Trichoderma* spp. possess many qualities and have great potential in the field of agriculture, industries, bioremediation, and waste management. The use of this genus has gained importance worldwide and is now formulated and commercialized for various applications such as biofertilizers, biocontrol, etc. Production of industrially important enzymes is the natural phenomenon of this fungus. The resistance to various heavy metals and toxic chemicals has made this fungus a potent applicant in the field of bioremediation. Managing the waste by production of lignocellulolytic enzymes and degrading the waste are embryonic applications of this fungus. Further, understanding the genetic and physiological abilities is now essential in order to use them in various other applications. With the advancement of molecular techniques, the genetic organization of these fungi can be manipulated, and its abilities can be further enhanced. It has been many years from studying the initial role of *Trichoderma* till the present commercial use of this fungus. The next decade is preparing itself to study and understand this multifunctional genus and their utilization for a wide variety of applications useful for mankind.

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Table 13.3 Enzyme production by *Trichoderma* species on various substrates

Species	Substrate	Enzyme	Activity	References
T. reesei QM9123	Alkali-treated barley straw	Filter paper activity	0.28 IU/ml	Peitersen (1975)
T. reesei	1 and 2% acid-treated corn stover	Cellulases	0.12 and 0.28 IU/ml	Tangnu et al. (1981)
T. reesei (Rut-C30)	2.2% cellulose from washed, steam-treated wood	Cellulase	168 IU/g of cellulose	Chahal et al. (1982)
T. reesei ZU02	Corncob residue	Cellulase	305 IU/g of cellulose	Xia and Cen (1999)
T. harzianum	Sugarcane bagasse	Xylanase	288 U/ml	Rezende et al. (2002)
T. harzianum (TUBF 781).	Wheat bran	Chitinase	3.18 U/gds	Nampoothiri et al. (2004)
T. longibrachiatum IMI 92027 (ATCC 36838)	Wheat bran-crude chitin (9:1 mixture) medium	Chitinase	5.0 IU/g of dry matter of substrate	Kovacs et al. (2004)
T. reesei	Steam-pretreated soybean hulls inoculated	FPU-ds	4 filter paper units/ds	Brijwani and Vadlani (2011)
		β-glucosidase, endocellulase	0.6 IU/gds	
			45 IU/gds	
T. reesei and *Phanerochaete chrysosporium*	Soyabean fiber	Xylanase	757.4 IU/g	Lio and Wang (2012)
T. reesei SAF3	Wheat bran	Xylanase	219 U $(gws)^{-1}$	Kar et al. (2013)

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Potential Microbiological Approaches for the Remediation of Heavy Metal-Contaminated Soils

14

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Abstract

In recent years, due to the geological and anthropogenic activities, metal pollution in soil has been increased drastically. Utilization of microorganisms to remediate the metal-contaminated soil is known as bioremediation. Bioremediation is an important area of research that offers economically effective clean-up technique than the conventional methods. Microorganisms use different mechanisms such as biosorption, bioaccumulation, chelating agents, bioleaching, biomineralization and enzyme-catalysed transformation to convert toxic form of metals to less toxic form. In addition, plants also offer various methods like absorption and accumulation of metals in plant cells and formation of metal-bound compounds. Integrated use of microorganism and plant in bioremediation may ensure an effective clean-up of heavy metals in polluted soils. This chapter summarizes the

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microbial- and plant-microbe-mediated methods for the clean-up of heavy metal-contaminated soil.

Keywords
Heavy Metal • Bioremediation • Phytoremediation • Metal Tolerance • Plant Growth-Promoting Rhizobacteria

14.1 Introduction

Farmlands in many parts of the world are highly contaminated with heavy metals, which are significant environmental pollutants. Heavy metals are the elements which exhibit metallic properties such as malleability, conductivity, cation stability, ductility and ligand specificity. The term heavy metals applies to the group of metals and metalloids which has high density and high relative atomic weight with an atomic number greater than 20 (Raskin et al. 1994). Living organisms require small quantities of heavy metals like Co, Mn, Fe, Cu, Ni, Mo, Zn and V, but the excessive amount of these heavy metals is harmful to the organisms. In addition, heavy metals such as Pb, Cd, Hg and As (metalloid but usually denoted as heavy metal) are not having any beneficial role in biological system and thus are considered as threats to the plants and animals (Chibuike and Obiora 2014). Wang and Chen (2006) grouped the metals into three kinds, which are toxic metals, precious metals and radionuclides. The negative effect of soil heavy metal is long-lasting and significant even after several remediation approaches were taken. Reasons for this may include species of concern, ability to absorb the metal, availability and abundance of metals, soil physiochemical properties, etc. (Rattan et al. 2005). In addition, wider distribution, multiple heavy metal contamination, irreversibility and remediation hardness and strong latency are other important characteristics of heavy metal contamination.

Heavy metals are naturally found in rocks; in addition, man-made inclusion of heavy metals into the biosphere has increased due to industrialization and urbanization. Heavy metals are found higher in soil followed by aquatic ecosystem and atmosphere as particulate or vapour. Soil microorganisms are extremely responsive to low concentration of soil heavy metals but rapidly adjust to any harmful environment. In general higher concentration of toxic metals causes negative impact on the microbial activities and communities in an ecosystem. However, microorganism habitats in the contaminated soils have developed different approaches to resist themselves from metal stress. Metal toxicity also disturbs plant growth; consequently, it affects the plant ecosystem. Plant habitats in the metal-contaminated soils exhibit different metabolic process, reduced growth and reduced biomass. Nonetheless, some plants develop very potential mechanism to combat with metal toxicity.

Bioremediation is a process of using microorganism to remove the pollutants from the soil. Metabolic processes of microorganism are capable of using chemical contaminants as an energy source, transforming highly toxic metal into harmless or

less toxic products. Hence, the bioremediation process provides an eco-friendly tool to remove or reduce the harmful contaminants by biological activity. Phytoremediation is the use of plants to degrade contaminants from soil and also proposed as a cheap, effective and environmentally friendly approach. The integrated use of microorganisms and plants in the clean-up of metal-contaminated soil is an efficient and eco-friendly technique compared to the conventional remediation technologies.

14.2 Heavy Metals

Heavy metal contamination due to intensified agriculture, urbanization, industrialization and mining activities has become a matter of global concern (Krishna et al. 2013). Many of them are toxic, persistent and/or bioaccumulative and are considered as environmentally hazardous (Lodenius 2013). Heavy metals, which are naturally present in soil ecosystem, are described below.

14.2.1 Cadmium (Cd)

Cadmium is distributed in the Earth's crust with the concentration of 0.1 mg/kg. Humans are exposed to Cd mainly by metal industries, eating contaminated food and smoking cigarettes. Other sources include emission from industries such as smelting, mining and manufacturing paints, stabilizers and batteries. Trace amount of Cd is present in foods such as leafy vegetables, potatoes, grains and seeds. The regulatory limit of Cd in agricultural soil is 100 mg/kg. Cadmium is the mobile metal, which can be easily taken up by plants; however, the essential function of Cd in plant is not known. Plants grown in soil containing high level of Cd show various symptoms such as chlorosis, growth inhibition, browning of root tips and death.

14.2.2 Arsenic (As)

Arsenic is a metalloid and it has great environmental concern due to its toxicity and abundance. In countries like India, Bangladesh, China and Hungary, As is found at high concentrations in surface soil. Mainly it occurs as As(V) in AsO_4^{3-}, arsenate, and as As(III) in AsO_2^{-}, arsenite. Smelting and mining process, fabrication and wood preservatives, food additives and agricultural practices are the main sources of As release in the environment. Humans can take up the As in many ways including inhalation, smoking and contaminated water and food. Arsenate is considered to be an analogue of phosphate and competes in the nutrient uptake by the plant root plasmalemma. The number of plants was identified as As tolerance, by suppressing the high-affinity P/As uptake system.

14.2.3 Chromium (Cr)

Chromium occurs as Cr(VI) in the divalent oxyanion chromate and as Cr(III), the trivalent cation. Chromium is considered as one of the most harmful elements in the environment. Chromium naturally occurs in ultramafic and serpentine rocks and in complex with other heavy metals in minerals such as crocoite, bentorite and tarapacaite. Other than the natural sources, man-made sources like electroplating, tanning, paining, pigment manufacture and wood preservation industries also contributing for Cr contamination. Inhalation of Cr compounds by human causes asthma, bronchitis and pneumonitis, and skin contact can produce allergies, dermatitis and necrosis. Even though the Cr is non-essential to the plants, at low concentration, it enhances the plant growth. Nonetheless, at high concentration, it inhibits plant growth, reduces chlorophyll synthesis and causes cell death.

14.2.4 Lead (Pb)

Lead is a universally distributed and abundant toxic element in the soil. Lead is naturally occurring in the bluish-grey metal existing in the Earth's crust. Though Pb occurs naturally in the soil, man-made activities like fossil fuel burning, mining and lead-acid battery manufacturing release Pb into the environment. Pb exposure occurs by inhalation of Pb-contaminated aerosols and taking of Pb-contaminated food and water. Pb is less soluble in water, and its availability to the plants is low, because it precipitates as phosphates and sulphates which are commonly present in plant rhizosphere. It also immobilized in soil when it forms complex with the organic matter. Lead adversely affects seed germination, plant morphology, growth and photosynthesis process.

14.2.5 Mercury (Hg)

Mercury is a heavy metal, which belongs to the transition element series of the periodic table. It exists in nature in three different forms such as elemental, inorganic and organic mercury, with each having its own profile of toxicity. Mercury ranks third on the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) list of materials in terms of risk that it poses to human mortality and morbidity. In the past decades, man-made emission of Hg has risen tremendously, significantly exceeded the emission from natural sources such as volcanic eruption. The Hg is mainly released into the environment by chlor-alkali plants and coal-fired power plants. At high concentration, Hg inhibits mitochondrial activities and induces oxidative stress by generating reactive oxygen species and disturbs the bio-membrane lipids and cellular metabolism of the plants.

14.2.6 Zinc (Zn)

Zinc occurs as the divalent cations Zn^{2+}. About 70 mg of Zn is naturally present in the crustal rocks, but the concentration of Zn is increasing unnaturally, due to the anthropogenic activities. Industrial activities such as mining, coal and waste combustion and steel processing also add Zn to the soil. Zinc is one of the essential micronutrients that affects many metabolic processes in the plants. However, at higher concentration, Zn affects growth, causes senescence and chlorosis and reduces plant growth. In addition, Zn also disturbs biological activities in soil, as it negatively affects micro- and macro-organisms.

14.2.7 Nickel (Ni)

Nickel is a transition element with the atomic number 28 and atomic weight 58.69. In low pH, the metal exists in the form of the nickelous ion Ni (II). The stable compound of nickelous hydroxide $Ni(OH)_2$ is formed when the pH is neutral to slightly alkaline. $Ni(OH)_2$ is highly soluble in acid solution forming Ni(III). In alkaline condition, it forms nickelite ion $HNiO_2$ that is water soluble. Nickel is found in the soils at low concentrations except in ultramafic or serpentinic soils. However, human activities like mining works, burning of coal and oil, phosphate fertilizer and pesticide production lead to increase Ni concentration in soil. Nickel concentration in the natural soil ranges from 10 to 1000 mg/kg of soil; however, the concentration in metal-polluted soil is 20- to 30-fold higher (20–26,000 mg/kg). Plants grown in Ni-contaminated soil showed imbalanced nutrient content, which resulted in reduced plant growth and affects the lipid composition and H-ATPase activity of plant plasma membrane.

14.2.8 Copper (Cu)

Increased industrial and mining activities increased the occurrence of Cu in soil. Copper is considered as an essential micronutrient for the plants and plays important role in photosynthesis and electron transport chain. However, at high concentration, Cu induces injuries to plants, generates oxidative stress and produces reactive oxygen species.

14.2.9 Cobalt (Co)

Cobalt is found mainly in Co^{2+} and Co^{3+} forms, in that Co^{3+} is stable only in complex compound. Cobalt in the form of vitamin B_{12} is essential for human being. At low concentration, cobalt improves plant growth; however, at higher concentration, it affects plant growth.

14.2.10 Manganese (Mn)

Manganese is omnipresent in the environment and present in the Earth's crust. Ocean spray, forest fires, vegetation and volcanic activity are the major natural sources of manganese. Manganese exists in various oxidation states, from Mn(II) to Mn(VII); every state is possible with the Mn^{2+} cation being the predominant form. Excessive accumulation of Mn in plant leaves affects photosynthetic rate. In addition, Mn toxicity causes necrotic brown spotting on leaves, petioles and stems.

14.2.11 Iron (Fe)

Iron is the only macro-bioelement of the heavy metals. In human, high concentration of Fe may cause conjunctivitis, choroiditis and retinitis when comes in contact and remains in the tissues. Iron is an essential metal for plants, which has many important biological roles in the photosynthesis, chloroplast development and chlorophyll biosynthesis. The occurrence of iron toxicity is related to high Fe^{2+} uptake by roots and its transportation to leaves. Excess Fe^{2+} produces free radical that damages cellular structure irreversibly and damages membranes, DNA and proteins.

14.3 Sources of Heavy Metal Contamination

Heavy metal in soil occurs by nature and by human activities. The following list shows the various anthropogenic sources by which huge amount of heavy metals are added to the soil:

1. Mining and uncontrolled smelting of large amount of metals and ores
2. Burning of fossil fuels
3. Improper discharge of agricultural, industrial and domestic waste
4. Discharge from auto exhausts
5. Using pesticides containing heavy metal compounds

14.3.1 Natural Sources

In soils, the parent materials determine heavy metal concentration that are formed from igneous rocks (granite or basalt) or sedimentary rocks (sandstones, shales, limestones, dolomite) or young soils that have been weathered under temperate conditions (Ramussen 2007). During weathering process, the crystalline structures of rock minerals are cleaved and allow the metal ions to be adsorbed in the top soil or carried away in ground or surface water (Parth et al. 2011). Burt et al. (2003) state that mineral content of the parent material is the decisive factor of the amount of metals in the soil. Even though, the metals in the soils are initially derived mainly from parent materials; the biological, physical and chemical properties decide the degree of metal accumulation.

14.3.2 Anthropogenic Sources

14.3.2.1 Wastewater Irrigation

Wastewater is used for irrigation in countries like China, Germany, France and India (Dere et al. 2006; Ingwersen and Streck 2006; Singh and Kumar 2006; Li et al. 2009). It is reported that approximately 20 million ha of land irrigated with municipal wastewater (Hassan et al. 2013). Wastewater is nutritionally rich and provides plant nutrients and enhances soil organic matter content (Liu et al. 2005) and also reduces the fertilizer requirement without yield compensation (Kang et al. 2007).

However, increased application of wastewater leads to accumulation of toxic elements in soil (Singh et al. 2004). It has been reported that even after wastewater treatment, little amount of heavy metals still remains in wastewater which can easily be accumulated in all living forms through the food chain (Muchuweti et al. 2006). Pathak et al. (2010) reported that wastewater irrigation increased concentration of heavy metals in agricultural soil near Bindal River, Dehradun, India. Soil irrigated with treated wastewater at Varanasi, India, observed total concentrations of Zn, Pb, Cd, Cr and Cu as 122.3, 123.5, 3.9, 56.35 and 77.95 mg/kg of soil, respectively, which exceed the Indian Standards (Misra and Tirpathi 2008).

14.3.2.2 Livestock Manures

In parallel to the increase in livestock production, animal manure production has also been increased simultaneously. Livestock manure is a valuable source of organic manure to improve the soil fertility and organic content (Zhang et al. 2012). Livestock are often fed with feed additives to ensure better growth performances. However, the feed additives are reported to contain heavy metals (Cu, Zn, Cd, As) in varying concentrations (Sager 2007). Long-term application of animal manure as organic fertilizer resulted in accumulation of heavy metals in surface soils (He et al. 2009) which leads to the contamination of water through leaching and runoff (Azeez et al. 2009).

14.3.2.3 Fertilizers

In general agrochemicals contain considerable amount of heavy metals which include Cu, Co, Cr, Mo, Sr, Ti, V, Mn, Fe, Ni, Zn, Cd, Pb, Hg, Ba, Sc and As (El-Bahi et al. 2004), while some of them (Cu, Mo, Ni, Zn, Mn, Fe) are plant nutrients at low concentrations (Xu and Tao 2004). Fertilizers generally contain substantial amount of heavy metals as impurities (Tariq and Rashid 2013). Among the inorganic fertilizers, phosphate fertilizers contribute more for heavy metal accumulation in soil, because it contains 0.1–170 mg/kg of total Cd, 1–12 mg/kg of total Co, 7–38 mg/kg of total Ni and 7–225 mg/kg of total Pb. Soil heavy metal contamination through fertilizer application is reported in many countries including China (Luo et al. 2009), Malaysia (Zarcinas et al. 2004), European countries (Nziguheba and Smolders 2008) and Brazil (Zoffoli et al. 2012). Nziguheba and Smolders (2008) reported that application of phosphate fertilizer annually increases 0.3 g/ha of Pb, 1.6 g/ha Cd and As with 7.7 g/ha. Apart from phosphate-based fertilizers, mixed fertilizers act as a source of heavy metal contamination in agricultural soils.

Luo et al. (2009) reported that in China around 5000 tons of Cu and 1200 tons of Zn containing agrochemicals are added to agricultural lands annually. With regard to Fe contamination, various fertilizers added to replenish some deficiency symptoms are considered as being a key contributor (Tariq and Rashid 2013).

14.3.2.4 Pesticides

The term pesticide refers to the substance or mixture of substances, which are used for preventing, destroying, repelling or mitigating any pest; thus, it includes insecticide, nematocide, herbicide, fungicide and molluscicide (Zhang et al. 2011). Even though the pesticides contributed to global agricultural production, the negative impact on environment has drawn major attention. The use of pesticides keeps rising over the years, and at present ~ 2.3 million tons of pesticides are used annually. Apart from highly hazardous organophosphates and organochlorine pesticides including DDT, lindane, endosulfan and chlordane, several other pesticides also contain heavy metals (Wuana and Okieimen 2011). Lead arsenate and several other arsenic-containing compounds have also been used in controlling pests in banana and in fruit orchards (McLaughlin et al. 2000). Consequently, it has been estimated that at least one-third of the agricultural products are dependent vastly on pesticides (Liu et al. 2002).

14.3.2.5 Sewage Sludge Application

Land application of sewage sludge is recognized as an economically viable and environmentally acceptable method of disposal. In China, approximately 10% of the total sewage sludge produced by the country is disposed directly as agricultural land application (Luo et al. 2009). Because of the high contents of N, P, K, Ca and trace elements in sewage sludge, it is considered to be a good fertilizer necessary for plant growth (Wu et al. 2012). However, pathogens, parasites and heavy metals such as Cu, Zn, Cr, Hg, etc. are frequently reported in sludge (Xu et al. 2003). Application of sewage sludge for long run resulted in accumulation of heavy metals in agricultural lands (Bozkurt et al. 2010). Contents of heavy metals such as Cd, Cr, Cu, Ni, Pb and Zn in sewage sludge can vary with a range of 0.5–2% on dry basis (Babel and Dacera 2006).

14.3.2.6 Mining

Though mining brings several socio-economic benefits, it is considered to be one of the most dangerous anthropogenic activities in the world. Metal contamination through mining activities is becoming a topic of high concern (Kien et al. 2010). Heavy metal contamination around the mines is depending upon the geochemical characters and mineralization of tailings (Krishna et al. 2013). Transport and disposal of mine waste without appropriate treatments may spread the toxic metals in agricultural soils (Kien et al. 2010).

14.3.2.7 Fly Ash

Fly ash refers to an airborne fine-grained waste/material with high specific surface area (Ferreira et al. 2003). Fly ash improves soil texture, neutralizes soil acidity,

increases water holding capacity and decreases bulk density. Furthermore, fly ash neutralizes acidity in soils through altering soil pH, improves soil texture, decreases bulk density and increases water and nutrient-holding capacities implying the validity to be used in agriculture. Even though fly ash has wider applicability, its usage is restricted due to the hazardous materials and heavy metals associated with it (Lima et al. 2010). In Denmark, regulations are made for the reuse of fly ash in agricultural field. Fly ash deposits have to be monitored carefully because their granulometric nature, grain morphology and filtration properties could enhance easy contamination of the surroundings (Smołka-Danielowska 2006). Coal fly ash receives much attention, because it contains high concentration of heavy metals such as Cd, Cr, Cu, Ni, Mo, Pb, Se, and Zn and As than other types of fly ashes.

14.4 Effect of Heavy Metal on Soil Microorganisms

Heavy metal toxicity may affect micro and macro-organisms which inhabit on soil, but the degree of toxicity varies based on organism's tolerability. Several researchers reported the microbial population shifts and community structure changes due to heavy metal contamination (Tipayno et al. 2012; Shagol et al. 2014; Krishnamoorthy et al. 2015). The instant toxicity of metals to soil organisms is moderated by metal immobilization by soil colloidal compounds. However, metals may be mobilized by local and global changes in soil condition such as reduction in pH, redox potential and enhanced decomposition of organic matters and changes in soil physicochemical properties. Heavy metals exert toxic effects on soil microorganisms and resulted in changes in diversity and overall activity of soil microbial community (Tipayno et al. 2012). In general, an increased metal concentration affects various metabolic processes of microbial cells (Fig. 14.1) and leads to cell death and reduced growth rate and enzyme activities.

14.4.1 Mechanisms of Microbial Metal Tolerances

At high concentrations, metals react to form toxic compounds in the microbial cells. To exhibit toxic effect in microorganism, heavy metal ions must enter into the cell. Two kinds of metal uptake system exist in microbial cells: the first one is chemiosmotic gradient across the cell membrane, which is not specific, and the second one is more substrate specific, driven by energy from ATP hydrolysis. While the first mechanism does not require energy, it results in an influx of a wider variety of heavy metals; when the heavy metals are present at high concentration inside the cell, it exhibits toxic effect. To survive under metal-stressed conditions, microbial cells developed several active and passive mechanisms (Fig. 14.2). Active mechanisms include the efflux of metal ions outside the cells, accumulation and complexation of the metal ions inside the cells, reduction of the metals to less toxic state, production of metallothionein, etc. (Spain and Alm 2003). Passive mechanisms involve in the binding of metals to the cell wall and the extracellular microbial polymeric substances.

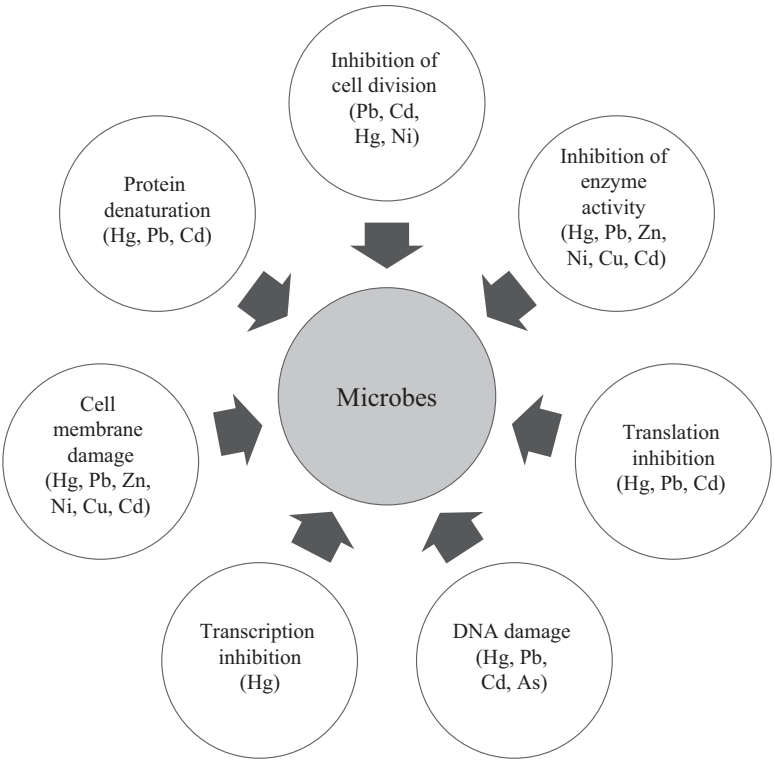

Fig. 14.1 Heavy metals affecting various metabolic activities of soil microorganisms

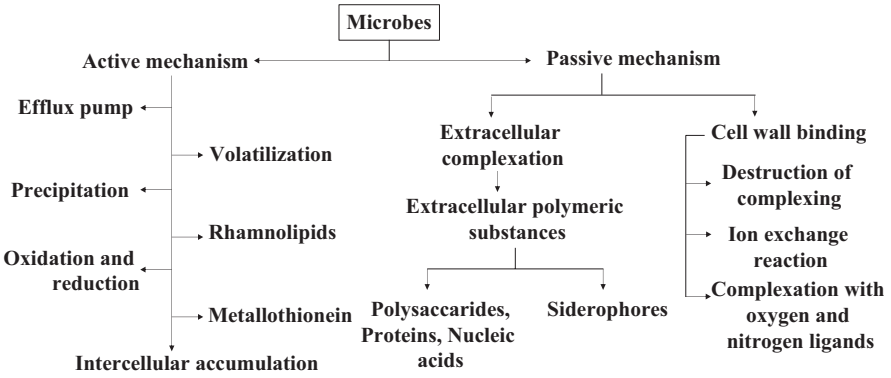

Fig. 14.2 Mechanisms involved in microbial cell metal tolerance

Remediation of heavy metal-contaminated soils can be done by various methods like removal, isolation, incineration, solidification/stabilization, vitrification, thermal treatment, solvent extraction, chemical oxidation, etc. These methods have

some disadvantages such as being very expensive; in some cases, they involve in the movement of contaminated soils, thus adding risk of secondary contamination (Prasad 2004). Nowadays more preferences are being given to in situ methods and microorganisms that are less environmental disruptive and more economical.

14.5 Microbial-Mediated Bioremediation

Heavy metals are not fully degradable either chemically or biologically but can only alter or reduce their toxicity. Microorganisms are not alchemists; no matter how a microorganism acts upon a toxic heavy metal, it is not destroyed (Lovley and Lloyd 2000). That is to say, heavy metals cannot be destroyed by microorganisms but are only transformed from one oxidation state or organic complex to another (Fig. 14.3). As a result of microbial action, the metals may become either (a) inherently less toxic, (b) less water soluble so that it precipitates and then less bioavailable, (c) volatilized and removed from the contaminated soil or (d) more water soluble and be removed by leaching.

14.5.1 Biosorption

Biosorption can be defined as the elimination of heavy metal compounds and particulates from the solution by biological materials (Gadd 1993). Heavy metals can be accumulated by living organisms in two different processes, which are metabolism dependent and metabolism independent. Living and dead biomass as well as

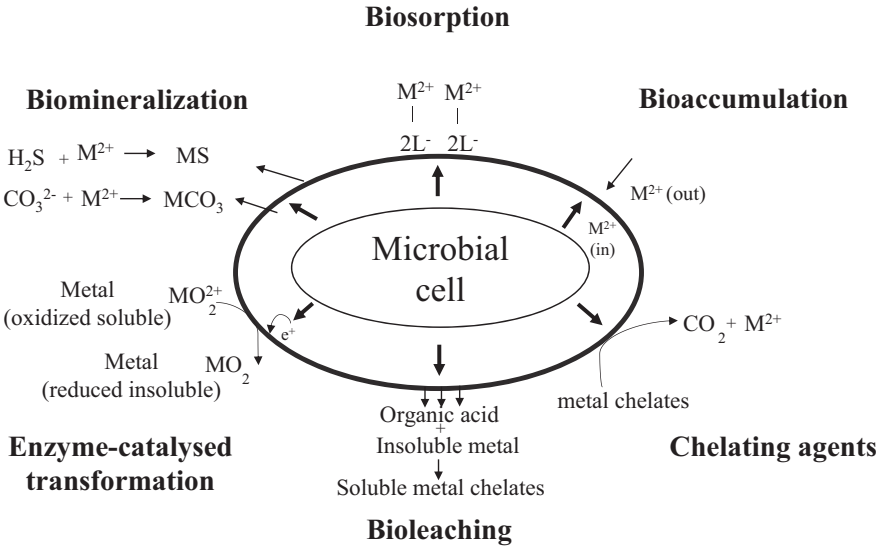

Fig. 14.3 Methods applied by microorganisms in the bioremediation process

the cellular products such as polysaccharides has also been used for heavy metal removal. The metal-sequestering property of an organism can be used to decrease the concentration of heavy metal ions on solution from parts per million (ppm) to parts per billion (ppb). The biosorbents can be classified into the following categories: bacteria (e.g. *Bacillus subtilis*), fungi (e.g. *Rhizopusarrhizus*), yeast (e.g. *Saccharomyces cerevisiae*), algae (*Chlorella sorokiniana*), other polysaccharide materials, etc.

14.5.1.1 Algae

The use of algae as a biosorbent is an economical, attractive and effective proposition. Because algae require low nutrient, do not produce any toxic substances, and are autotrophic, it produces a larger biomass (Das et al. 2008). Biosorption of metals on algal surface depends on ionic charges of metal ions, algal species and chemical composition of the metal ion solution (Gupta et al. 2001).

14.5.1.2 Fungi

Fungi may act as an effective and economically efficient candidate for the metal biosorption from dilute aqueous solutions. The advantage is that the fungi form higher amount of cell wall materials, which have metal-binding properties. Moreover, large quantity of fungal biomass is available from food and antibiotic industries.

14.5.1.3 Bacteria

Due to their small size, ubiquity and capability to grow in a controlled conditions and their resistance against a wide range of environmental condition, bacteria are widely used as a biosorbent. Many bacterial species (e.g. *Bacillus*, *Pseudomonas*, *Streptomyces*, *Escherichia*, etc.) are tested for effective heavy metal biosorption. The metal uptake capacity of bacteria generally ranges between 568 and 0.70 mg g^{-1} (Wang and Chen 2009). Among the reactive compounds associated with bacterial cell walls, the extracellular polymeric substances (EPS) are of particular importance and are well known to have a significant impact on metal adsorption (Comte et al. 2008).

14.5.1.4 Yeast

Among the emerging biosorbents for heavy metal removal, yeast (*Saccharomyces cerevisiae*) has received great attention due to its mediocre capacity for metal uptake. Yeast has been studied in different form such as living cell/dead cell, immobilized cell/free cell, wild type/mutant cell, flocculent/non-flocculent cell, engineered/non-engineered cell and laboratory culture/waste cells in biosorption research (Park et al. 2003). More information on bacterial, fungal, yeast and algal species and their metal biosorption are given in Table 14.1.

Table 14.1 Metal biosorption nature of microorganisms

Metal ions	Microorganism	Biosorption capacity(mg/g)	References
Bacteria			
Pb	*Corynebacterium glutamicum*	567.7	Choi and Yun (2004)
Pb	*Pseudomonas putida*	270.4	Uslu and Tanyol (2006)
Zn	*Bacillus firmus*	418	Salehizadeh and Shojaosadati (2003)
Zn	*Thiobacillus ferrooxidans*	172.4	Liu et al. (2004)
Cu	*Pseudomonas putida*	96.9	Uslu and Tanyol (2006)
Cd	*Pseudomonas* sp.	278.0	Ziagova et al. (2007)
Cr(IV)	*Aeromonas caviae*	284.4	Loukidou et al. (2004)
Ni	*Bacillus thuringiensis*	45.9	Ozturk (2007)
Fungi			
Cd	*Penicillium canescens*	102.7	Say et al. (2003)
As(III)	*Penicillium canescens*	26.4	Say et al. (2003)
Pb	*Penicillium chrysogenum*	96	Skowronski et al. (2001)
Pb	*Aspergillus niger*	93	Spanelova et al. (2003)
Al	*Penicillium* sp.	50	Kapoor and Viraraghavan (1995)
Yeast			
Zn	*Saccharomyces cerevisiae*	3.45	Bakkaloglu et al. (1998)
Ni	*Saccharomyces cerevisiae*	1.47	Bakkaloglu et al. (1998)
Pb	*Saccharomyces cerevisiae*	211.2	Kogej and Pavko (2001)
Cu	*Saccharomyces cerevisiae*	7.11	Donmez and Aksu (1999)
Algae			
Ni	*Fucus vesiculosus*	40	Holan and Volesky (1994)
Pb	*Sargassum natans*	220–270	Holan and Volesky (1994)
Au	*Sargassum natans*	400	Volesky and Kuyucak (1988)
Cu	*Scenedesmus obliquus*	10	Mattuschka et al. (1993)

14.5.2 Bioaccumulation

Bioaccumulation can be defined as uptake of metal by the living organisms and transport into the cell; it is a growth-dependant process which is only mediated by living cells. The biosorption process can be possibly done by both live and dead biomass, but bioaccumulation process can be carried out only by living biomass. Metal accumulation by a microorganism can be studied by metal-binding proteins and peptides (metallothionein and phytochelatins) expressions. In the context of toxic metal exposure, metallothionein and phytochelatins are mediated by hormone- and redox-signalling processes. Genetically modified *Ralstonia eutropha*-expressed mouse metallothionein on the cell surface resulted in decreased toxic effect of the Cd (II) in the contaminated sites (Valls et al. 2000). *Bacillus circulans* and *Bacillus megaterium* were found to accumulate 34.5 and 32.0 mg Cr/gram dry weight, respectively (Srinath et al. 2002). On the other hand, bioaccumulation capacity of the gram-negative bacteria is well established (Tohamy et al. 2006; Noghabi et al.

2007). *Vibrio alginolyticus* isolated from waste of steel factory, Egypt, reported to accumulate 20% of Cd, 31% of Cu^{2+}, 40% of Pb^{2+} and 45% of Zn^{2+} (El-Hendawy et al. 2009). Moreover, bioaccumulation process can be affected by many biotic and abiotic factors. Abiotic factors such as lower temperature, absence of energy source, presence of metabolic inhibitors and toxicity of heavy metals to the microorganisms become important if the metal concentration is above the threshold level. Biotic factors like predator and parasitoids affect the microbial population and the metal bioaccumulation capacity.

14.5.3 Chelating Agents

Microbial low molecular weight organic acids including oxalic, citric, formic, acetic, malic, succinic, malonic, maleic, gluconic, lactic and fumaric acids and siderophores are considered as chelating agents. Microbial chelating agents have special significance because of metal-complexing properties. These chelating substances play a vital role in reducing the detrimental effect of heavy metals. Gluconic acid producing *Enterobacter asburiae* PS13 protects mung bean seeding from Cd toxicity, and this effect was due to the binding of metal ion with the acid ion (Kavita et al. 2008). Organic acids produced by plant-associated microbes facilitate the plant root absorption of metal ions such as Cd, Zn (Li et al. 2010), Pb (Sheng et al. 2008) and Cu (Chen et al. 2005). Since organic acid produced by microbes in the rhizosphere soil may form complexes with heavy metals and inactivate and minimize the cytological impacts of free metal ions (Gao et al. 2010), the heavy metal/organic acid complex is considered as less phytotoxic than the free form of metals (Najeeb et al. 2009).

It also reported that the precipitation of metal oxalates in the intercellular spaces of the endomycorrhizal fungi could reduce the metal availability and toxicity to the plant. The ericoid mycorrhiza *Oidiodendron maius* has been reported to enhance Zn release from insoluble ZnO via excretion of Zn-chelating citric and malic acids (Martino et al. 2003). Organic acid producing *Aspergillus niger* was reported to mobilize Pb (Sayer et al. 1999). Furthermore, they observed that *A. niger* significantly enhanced Pb uptake by *Lolium perenne*. These studies indicate the potential of chelating agents producing microbes in the metal bioremediation process.

14.5.4 Bioleaching

At present bioleaching process is mostly done with *Thiobacillus ferrooxidans*, *Leptospirillum ferrooxidans* and *Thiobacillus thiooxidans*, which converts the heavily soluble metals into water-soluble metals. Microbial leaching process can be defined as the solubilization of metals from solid substances either by the metabolism of leaching bacteria or indirectly by the products of microbial metabolism (Wong and Henry 1988).

Direct mechanism:

$$MS + 2O_2 \rightarrow MSO_4$$

Indirect mechanism:

$$S + H_2O + 3/2O_2 \rightarrow H_2SO_4$$

$$H_2SO_4 + \text{sediment} - M \rightarrow \text{sediment} - 2H + MSO_4$$

where M is a bivalent metal (Chen and Lin 2000). Bioleaching performance by microorganisms is affected by various factors such as microbial strain, quantity and type of substrate, particle size, carbon dioxide and oxygen concentration, pH and redox potential.

The use of fungus in the bioleaching process is more advantageous than the bacterial strains, because fungus can grow well in the acid conditions. The fungus produces weak organic acids that solubilize metals by forming water-soluble complex with metals.

14.5.5 Enzyme-Catalysed Transformation

Microorganisms offer a potentially enormous gene pool to select an enzyme that can help metal remediation. Indeed, microorganisms have developed a wide range of biochemical tricks to protect themselves from potentially toxic metals, and these mechanisms can be useful for the bioremediation applications. Microbial detoxification involves exclusion of metal ions from the cell that in some cases can result in higher local concentrations of metals at the cell surface, where they may react with biological ligands and precipitate (Lloyd 2002). Another mechanism involves redox transformations, for example, the enzyme-catalysed reduction of toxic mercuric ion (Hg^{2+}) to non-toxic elemental mercury [Hg(0)]. This approach is used to treat chlor-alkali waste contaminated with mercury ions; in addition metals that are reduced in this manner include Fe(III), Mn(IV), U(VI), Cr(VI), Se(VI) and As(V).

14.5.6 Biomineralization

Biomineralization can be defined as the formation of crystals intracellularly or in the extracellular matrix of the microorganisms, and this process requires association of living organisms. Microorganisms can precipitate metal through biologically induced and biologically controlled mineralization. When metal cations react with bacterial metabolic products, the metals will form a precipitate and this is called biologically induced precipitation. On the other hand, biologically controlled precipitation occurs as the result of an organism expanding energy to apply a direct control on the precipitation of metal cations. Addition of magnesium and calcium to a carbonate and phosphate medium resulted in the bacterial-mediated precipitation

of carbonate phase through biologically induced mineralization of magnesium and calcium absorption to the bacterial cell wall (Rivadeneyra et al. 2006). The metabolism of the bacteria creates the changes in pH, ionic strength and ionic makeup of the local medium, which in turn creates favourable conditions for magnesium and calcium adsorption to the bacterial cell wall. In addition a number of studies illustrated that the functional groups on the bacterial cell wall can act as a nucleation sites for the non-metabolic precipitation of minerals, leading to another type of biomineralization which is referred to as passive biomineralization.

14.5.7 Designer Microbe Approach

Genetically engineered organisms are the organisms whose genetic material has been altered using recombinant DNA technology to generate an efficient strain for the remediation of soil heavy metal contaminant. In this method, we can construct microbial strains which have the ability to withstand adverse stressful conditions and could be used as a bioremediators for remediation of contaminated soils (Dixit et al. 2015). A list of genetically engineered microorganisms for the removal of heavy metals is presented in Table 14.2. Genetic engineering of endophytic and rhizospheric microorganisms for the degradation of heavy metals is considered to be one of the most hopeful technologies for remediation of metal-contaminated sites.

14.6 Phytoremediation

14.6.1 Effect of Heavy Metal on Plant Growth

Heavy metal induces some biochemical changes in plant; in particular it inhibits the enzyme involved in photosynthesis and other metabolic processes. However, some plants develop tolerance mechanism against heavy metals and grow well in contaminated soils. A wide variety of plant species that grow in metal-polluted soils are

Table 14.2 Genetically altered microorganisms and their metal remediation efficiency

Microorganism	Engineered gene	Metal	Initial concentration (ppm)	Removal efficiency (%)
E. coli strain	Metalloregulatory protein ArsR	As	0.05	100
E. coli strain	SpPCS	Cd^{2+}	–	–
Methylococcus capsulatus	CrR	Cr^{6+}	1.4–1000	100
P. putida strain	Chromate reductase	Cr	–	–
E. coli JM109	Hg^{2+}	Hg	7.4	96
P. fluorescens 4F39	Phytochelatin synthase (PCS)	Ni	145	80

called metallophyte and pseudometallophyte species (Favas et al. 2014). Metallophytes are endemic plant species of natural metal-contaminated soils and, therefore, have developed physiological mechanisms to develop metal stress tolerance (Baker 1981). Pseudometallophyte plants are not native to metal-contaminated soil and have an extensive distribution; because of the selective pressure, the plants develop tolerance mechanisms to survive in metalliferous soils (Becerra-Castro et al. 2012). Chemical compounds involved in plant tolerance to heavy metals are protein which has the capability of making linkage with metals, thereby forming complex biochemical compounds called metal proteins, metallothionein. These metallothioneins are not exactly proteins but peptides.

14.6.2 Plants in Metal Remediation

Phytoremediation involves the use of plant to partially or completely remediate the selected contaminants from soil, sediments, wastewater and sludge. The word 'phytoremediation' is derived from the Greek word 'phyton' (means plant) and Latin word 'remedium' (means to remedy or to correct). Phytoremediation basically comprises six different strategies such as phytodegradation, phytostabilization, phytovolatilization, phytoextraction, phytofiltration and phytostimulation; plants may use more than one strategy concurrently (Fig. 14.4):

1. Phytodegradation: Heavy metals are broken down (metabolized) or mineralized inside plant cells by specific enzymes.
2. Phytostabilization: Heavy metals are precipitated into insoluble forms by direct action of the plant root exudates and subsequently trapped in the soil matrix. Phytostabilization avoids mobilization of contaminant and limits their diffusion in the soil.
3. Phytovolatilization: Heavy metals are converted into non-toxic forms and then released into the atmosphere through the leaf surface. Particularly the metals present in IIB, VA and VIA of the periodic table (Hg, Se and As) are absorbed by some plant species and released into the atmosphere.
4. Phytoextraction: The metal contaminants present in soil are absorbed by the roots followed by translocation and accumulation in the aerial parts. It is mainly applied to the metals such as Cd, Ni, Cu, Zn and Pb but can also be used for other elements (Se and As). This technique preferentially uses plants with the ability to store high concentrations of specific metals in their aerial parts (0.01–1% of the plant dry weight). *Thlaspi caerulescens*, *Alyssum bertolonii*, *Elsholtzia splendens* and *Pteris vittata* are well-known examples of hyperaccumulator plants for Zn/Cd, Ni, Cu and As, respectively (Favas et al. 2014).
5. Phytofiltration: This technique uses the plant to absorb, concentrate and/or precipitate metal contaminants from an aqueous medium through their root system or other submerged organs. Plants with higher root biomass and higher absorption surface with more metal accumulation capacity and tolerance to metals are more preferable. Some examples are *Helianthus annuus*, *Brassica juncea*,

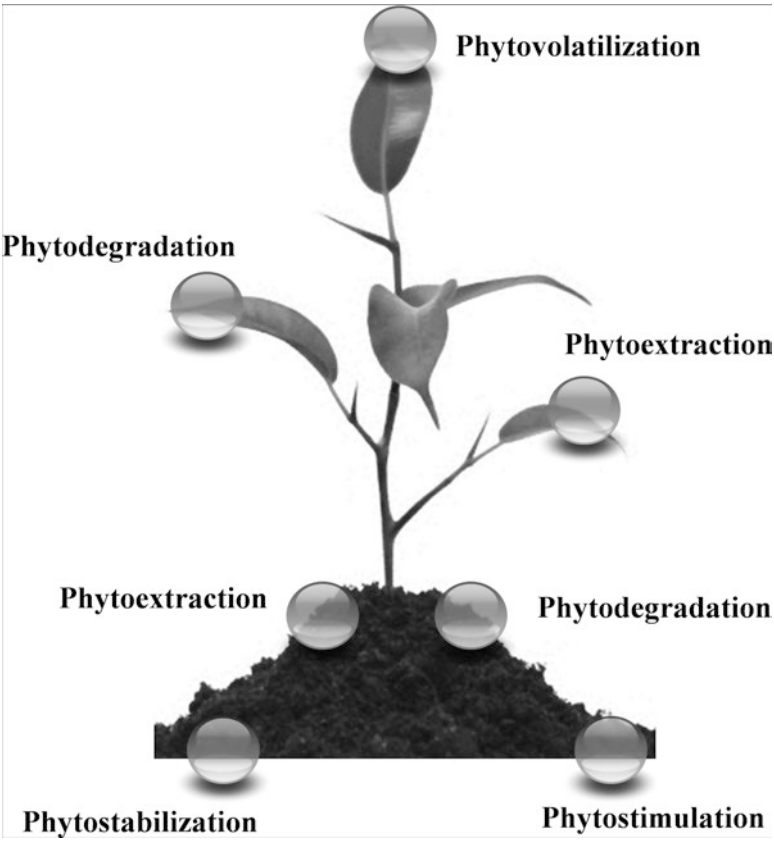

Fig. 14.4 Types of plant-mediated heavy metal remediation

Phragmites australis, *Fontinalis antipyretica*, etc. (Prasad 2004; Favas et al. 2012; Pratas et al. 2012).

6. Phytostimulation: Microorganisms which proliferate in the rhizosphere region utilized the plant root exudates as their carbon and energy source. Microbial population in rhizosphere region is highly heterogeneous due to the variable spatial distribution of nutrients. Bacteria and fungi particularly arbuscular mycorrhizal fungi (AMF) establish the association with plant and play a major role in the removal of metal from the soil.

14.6.3 Microorganisms' Role in Effective Uptake of Metals

The collective use of plant and microorganisms for the remediation of metal-polluted soil results in more efficient and faster clean-up of contaminated sites. Mycorrhizal fungi are widely used in remediation of heavy metal-contaminated soil, and the results revealed that the mycorrhiza employs different strategies for the

remediation. Some studies showed that mycorrhiza enhanced phytoextraction through the accumulation of heavy metals in the plant; others reported the enhanced phytostabilization process through metal immobilization and reduced metal accumulation in plant. Other microorganisms apart from mycorrhiza fungi are also used in conjugation with plant for the remediation of heavy metal-contaminated soils (Chibuike and Obiora 2014).

Hyperaccumulators (*Brassica juncea* and *Brassica napus*) inoculated with *Bacillus* sp. showed higher accumulation of Cd and Ni in plants (Zaidi et al. 2006). However, the report by Madhaiyan et al. (2007) exhibited the reduction in Cd and Ni accumulation in plant when inoculated with *Methylobacterium oryzae* and *Burkholderia* spp. Leung et al. (2006) reported that the addition of mycorrhizal fungi further enhances the plant growth and accumulation of As in *Pteris vittata*. Thus, this indicates the mechanisms employed by microorganisms in the phytoremediation of metal-polluted soils may be dependent on the species of microorganism and the plant involved in the process. There are many reports which showed the effectiveness of remediating metal-contaminated soil with the help of microorganisms and plants (Table 14.3).

14.7 Conclusions

Technological development and industrialization have adverse side effects like accumulating heavy metal in soil, affecting soil ecosystem and degrading soil health. Because of the complexity involved in the conventional methods for the remediation of metal-contaminated soil, the use of microorganisms has emerged as an efficient and time-saver method for bioremediation. Soil is a highly heterogenous environment; so successful bioremediation is highly dependent on interdisciplinary approach such as microbiology, engineering, ecology, geology and chemistry. Improving our knowledge on the ecology, physiology, evolution, biochemistry and genetics of the microorganisms might enhance the efficiency of microbial-mediated metal bioremediation process. In addition, selection of effective microbial strain and manipulation of genes in order to achieve a strain producing biomolecules with high affinity to heavy metal is another way of improving current bioremediation process.

Small-scale trails are representing the feasibility of phytoremediation process. As a matter of fact, phytoremediation appears to be a very promising method for the removal of metals from polluted soils. Phytoremediation can be adopted in larger scale, and it is cost-effective comparative to other methods. Plants, which are tolerant to heavy metals, require low nutrient for their growth; high growth rate and higher biomass productions are the ideal characteristics for the remediation of metal-contaminated soils. Integration of both microorganisms and plant might enhance the efficiency of remediation process. Among the microorganisms, arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria were widely incorporated in various phytoremediation programmes. The success of these combinations highly depends on the species of microorganism, plant and the concentration of heavy metal present in the soil.

Table 14.3 Effects of microorganism inoculation on plant growth and uptake of metal from contaminated soil

Microorganism	Host plant	Metal/ metalloid	Effect/ mechanism	References
Azotobacter chroococcum HKN-5, *Bacillus megaterium* HKP-1	Canola	Pb, Zn	Stimulated plant growth, protected plant from metal toxicity	Wu et al. (2006)
Kluyvera ascorbata SUD165, SUD165/26	Tomato, canola, Indian mustard	Ni, Pb, Zn	Increased biomass, ACC deaminase	Burd et al. (2000)
Pseudomonas putida UW4, *P. putida* HS-2	Canola	Ni	Increased biomass in the field, ACC deaminase	Farwell et al. (2006)
Variovorax parodoxus, *Rhodococcus* sp., *Flavobacterium* sp.	Indian mustard	Cd	Increased root length, IAA, siderophore	Belimov et al. (2005)
Methylobacterium oryzae CBMB20, *Burkholderia* sp. CBMB40	Tomato	Cd, Ni	Increased root length, increased tolerance, ACC deaminase	Madhaiyan et al. (2007)
Glomus mosseae	*Cajanus cajan*	Cd, Pb	Biosorption and dilution effects	Garg and Aggarwal (2011)
G. etunicatum, *G. macrocarpum*	*Glycine max*	Mn	Increased biomass, stimulating the ATP-dependent sequestration of Mn or Mn chelates in the vacuoles, or formation of low-solubility P–Mn complexes	Nogueira et al. (2007)
G. etunicatum	*Calopogonium mucunoides*	Pb	Stimulating plant nutrient acquisition, attenuating the negative effects of Pb on membranes and contributing to the reduction of ROS generation	de Souza et al. (2012)

(continued)

Table 14.3 (continued)

Microorganism	Host plant	Metal/ metalloid	Effect/ mechanism	References
Mixture of *G. clarum*, *Gigaspora margarita* and *Acaulospora* sp.	*Coffea arabica*	Cu, Zn	Improve plant growth, element uptake by AMF hyphae and improve nutrient acquisition	Andrade et al. (2010)
Mycorrhiza and *Aspergillus tubingensis*	*Dendrocalamus strictus*	Al, Fe	Synergetic effect between the organisms, increasing nutrient acquisition, diluting metal effect	Babu and Reddy (2011)
Enterobacter cloacae CAL2	Canola	As	Increased biomass, ACC deaminase	Nie et al. (2002)

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15 Phytoremediation and Rhizoremediation: Uptake, Mobilization and Sequestration of Heavy Metals by Plants

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Abstract

Microorganisms residing over the rhizosphere have the capability to catalyse metal uptake in a symbiotic relationship with the roots. This syntrophic relationship enhances the bioavailability of heavy metals and encourages the root adsorption capacity for vital in addition to non-essential metal. It also changes their chemical properties that ultimately have an effect on metal dissolution. Molecular level understanding of the physiological and evolutionary mechanism along with genetics and biochemistry principles underlying the uptake, transportation, translocation and storage of heavy metals (HMs) in model plant species thus allowing alteration to the HM stress can loan much to our comprehension of the fundamental segments of HM metabolism. A lucid understanding of molecular level changes is necessary for plant biotechnologist, regarding changes provoked in plants because of HM stress. It is also helpful to develop stress-resistant cultivars and species with superior phytoremediation capacity through cell and genetic engineering technologies. We hereby summarize the present understanding of HM uptake by plants and also provide a brief study related to their

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biochemical characteristics of take-up, transport and assortment plus injury and defence mechanism against HM. In this review chapter, we have also focused over the future prospect of research to enhance the discriminate perspective of the basic phytoremediation components specifically rhizoremediation of HMs.

Keywords
Rhizoremediation • Heavy metals • Sequestration • Phytoremediation • Heavy metal detoxification

15.1 Introduction

Rapid industrialization has resulted in startling boost in the release of inorganic as well as organic anthropogenic pollutants into the surroundings. More than 50% of the heavy metal loads have been accounted to the anthropogenic activities. The sources of heavy metal pollution are generally categorized as mineral rock weathering and anthropogenic sources. Heavy metal ores include sulphides, such as Fe, As, Pb, Pb-Zn, Co, Au, Ag and Ni sulphides; oxides such as Al, Mn, Au, Se and Sn and some ores exist both as sulphides and oxides such as Fe, Cu and Co (Duruibe et al. 2007). Ross (1994) has classified anthropogenic generation of heavy metals into five fundamental categories including metalliferous mining and refining (As, CD, Pb, Hg), industrial sources (As, Cd, Cr, Co, Cu, Hg, Ni, Zn), deposition from the atmosphere (As, Cd, Cr, Cu, Pb, Hg, U), agricultural (As, Cd, Cu, Pb, Se, U, Zn) and disposal of waste (As, Cd, Cr, Cu, Pb, Hg, Zn), etc.

15.1.1 Heavy Metal's Impact Over Human Health

Heavy metals seem to be the most insecure element to environment and ecosystem due to their nondegradable and bio-aggregating nature. The European Economic Community (EEC) has grouped these heavy metals into black and grey list, based on their relative toxic effects. Metals included in grey list are potentially less dangerous (Zn, Cu, Ni, Cr, Pb, Co) as compared to those in the black list (Wase and Forster 1997; Sudhakar et al. 1991). The HM pollutants can contaminate potable water and food and thus ultimately affect human health through food chain (Cheng 2003). HMs such as nickel, copper and zinc result in build-up of reactive species of oxygen (ROS) because of indirect oxidative stress brought about in the course of uncoupling of electron transport in photosynthesis and respiration and exhaustion of glutathione (GSH), whereas redox active HMs similar to copper, iron and manganese possess direct capacity to oxidize (Freeman et al. 2005). The well-known and tragic episodes of 'Itai-Itai' and 'Minamata' highlighted the global concern regarding the effect of heavy metal pollution on human beings.

15.1.2 Impacts of HMs on Plants

Some adverse impacts of HM contamination in plants incorporate toxicity owing to modification of essential protein composition or substitution of an essential element thus ending in inactivation of photosystems, chlorosis, stunned growth and root-browning (Gohre and Paszkowski 2006). Panda (2003) reported that HMs toxicity in plants results in the induction of stress (oxidative) in the moss of *Taxithellium* sp. Toxicity in plants because of HM stress is caused due to one or more of the following, viz. altered permeability of plasma membrane, reaction of sulfhydryl groups in proteins with the metal cations, resemblance to phosphate groups and thus reaction with ATP and ADP and substitution of vital ions by HM cations. A broad variety of morphological irregularities, differentiated by uncharacteristic development, together with apical branching or swelling at the tip of the pollen tube were demonstrated by Sawidis (2008) thus being an evidence for the toxic effect of HMs on the pollen as well as pollen tube development.

15.2 Phytoremediation and Rhizoremediation

Phytoremediation includes group of various plant-based innovations, each having an alternate machinery for the remediation of metal-polluted soil, residue or water, for example, rhizofiltration that incorporates the usage of different plant spp. to clean water flows (Dushenkov et al. 1995; Mukhopadhyay and Maiti 2010); phytostabilization (Pivetz 2001), in which plants are utilized to balance out as opposed to clean the polluted soil. Phytovolatilization (Burken and Schnoor 1999; Banuelos et al. 1997) involves the employment of plants to extricate various HMs from soil and afterwards discharge them into the environment through volatilization. In phytoextraction (phytoaccumulation), plants assimilate HMs from belowground parts and transfer them to shoot, branches and leaves where they aggregate (Yoon et al. 2006; Zacchini et al. 2009; Rafati et al. 2011). Phytodegradation (phytotransformation), where plants degrade the pollutants taken up via internal and metabolic reactions, or the degradation of pollutant outside the plant with the help of enzymatic secretions and rhizodegradation (phytostimulation) is where the pollutants are separated in the top soil through microbial movement that is improved by the rhizospheric charisma (Burken and Schnoor 1997; Mukhopadhyay and Maiti 2010).

15.2.1 Phytoaccumulation (Phytoextraction and Phytomining)

Phytoextraction is the capability of plants to retain and store heavy metals starting from the soil in over-the-ground parts (McGrath 1998). Frequently, plants can't differentiate between essential (which are required for development) and non-essential metals, for example, cadmium and zinc or copper. Phytoextraction can be utilized as a part of two routes for soil remediation: (i) by taming regular hyperaccumulators and (ii) by bioengineering plants with certain qualities that permit characteristic

hyperaccumulators to accomplish phytoextraction (Chaney et al. 2005). As indicated by Ghosh and Singh (2005), while developing on HM-contaminated soils, plants carry on in three ways: (i) prevent metals from entering their above-ground parts, i.e. confine HMs in their belowground parts (metal excluders), (ii) effectively gather metals in their aerial tissues (metal indicators) and (iii) move metals in their above-ground parts to levels far surpassing than soils (metal collectors). The two fundamental systems of phytoextraction are (i) chelate-assisted phytoextraction or induced phytoextraction, in which counterfeit chelates are added to build the portability and take-up of metal contaminant, and (ii) continuous phytoextraction, expulsion of metal relies on the frequent ability of plants to remediate (Ghosh and Singh 2005). Continuous phytoextraction is performed by utilising a few plants, known as hyperaccumulators which may occupy uncommonly elevated amounts of contaminants from soil. Their tissues can contain from 1000 to 10,000 ppm of certain heavy metals (Black 1995). To be reasonable for phytoextraction, a plant variety ought to incorporate the attributes enlisted as follows (Miller 1996):

- Ability to collect and endure high concentration of metal in harvestable tissue
- Rapid development rate
- High biomass generation

Hyperaccumulators are the plants wherein the accumulated heavy metal amount > 0.1% plant dry weight and ratio of HM amount accumulated in above ground and root >1 (i.e. high 'TransF', shoot-to-root metal concentration ratio) (Lasat 2002; Pilon-Smits 2005; Violante et al. 2010). Around 400 types of hyperaccumulating plant species have been resolved till now for the most part resembling the families Asteraceae, Brassicaceae, Cyperaceae, Caryophyllaceae, Cunouniaceae, Flacourtiaceae, Fabaceae, Lamiaceae, Violaceae, Poaceae and Euphorbiaceae. A huge number of taxa, i.e. 11 genera and 87 species belonging to Brassicaceae, have been reported to hyperaccumulate different metals (Cd, Co, Cu, Mn, Ni and Zn), metalloids (As) and non-metals (Se) in their shoots (Prasad and Freitas 2003; Freeman et al. 2004). As per Brooks et al. (1998), hyperaccumulators ought to have the accompanying properties:

(i) Concentration is supposed to be 100 folds higher than in ordinary plants for hyperaccumulating metals. In mg/kg, it is supposed to be 100 for Cd, 1000 for As and Ni, 10,000 for Zn and Mn and so forth.
(ii) Bioconcentration component is supposed to be >1 (centralization of the component in plant >amount in soil).
(iii) Translocation factor is supposed to be >1 (i.e. the concentration of element in over-the-ground plant parts > than in roots). Only those species may be suitable for phytoextraction for which both bioconcentration factor and translocation factor is more than 1 (Yoon et al. 2006).

According to Zhuang et al. (2007), bioconcentration factor can be calculated as follows:

$$\text{Bioconcentration factor} = \frac{\text{concentration of target metal in harvestable tissue}}{\text{concentration in soil or other substrate}}$$

Translocation factor is an indicator of the capability of plants to translocate the concentrated HMs from its belowground to above-ground parts. According to Padmavathiamma and Li (2007),

$$\text{Translocation factor} = \frac{\text{concentration in root}}{\text{concentration in shoot}}$$

The process of hyperaccumulation in plants like *T. caerulescens* involves a much more noteworthy influx of HMs in their root, more quick and effective transfer of the consumed HM from root xylem of the shoot and effective storage of absorbed HMs in the shoot (Papoyan and Kochian 2004). Hyperaccumulators store a lower portion of HMs in belowground parts and export higher amounts to the aerial parts (Barcelo and Poschenrieder 2003).

In induced hyperaccumulation, HMs are brought into soil solution in high concentration and root endodermis is disrupted in this manner permitting HMs via soil solution to enter specifically into the xylem of roots through the apoplastic pathway (Robinson et al. 2006). Various studies have reported the application of many chelators like citric acid, ethylenediaminetetraacetic acid, trans-1,2-cyclohex ylenedinitrilotetraacetic acid, diethylenetrinitrilopentaacetic acid, nitrilotriacetic acid, aminopolycarboxylic acids, ethylenediaminedisuccinic acid, hydroxyethyl-ethylenediaminetriacetic acid and hydroxyethyl-iminodiacetic for their capacity to activate metals and increase metal hyperaccumulation in various plant species (Cooper et al. 1999; Barlow et al. 2000; Grcman et al. 2003; Wong et al. 2004; Begonia et al. 2005).

15.2.2 Phytovolatilization

Phytovolatilization is the take-up, transpiration and release of a pollutant by a plant to the atmosphere as same or in an altered form due to its metabolic and transpiration pull (USEPA 1999). Phytovolatilization may be exploited to treat selenium, arsenic, mercury contaminated soils and sediments with *Brassica juncea*, wetland plants and phreatophytes to avail the deep water table (Dietz and Schnoor 2001). Phytovolatilization involves movement of contaminants into the atmosphere, which can then serve as an alternative source of exposure so that health risks may still be linked with the contaminant. In spite of being a promising option, phytoremediation is a time-consuming technique affected by other factors like climate, extent of HM accumulation and distinctiveness of soil environment. Therefore, rhizoremediation is an improved approach to deal with HM contamination problems founded on the effectiveness of plant–microbe symbiosis.

15.2.3 Rhizoremediation/Phytostimulation

Remedies like PGPR (plant growth-promoting rhizobacteria) may be a good option to enhance the biomass and thereby efficiency of the plants for stabilizing, revegetating metal-polluted soil in highly contaminated areas where metal content is far above the ground, adequate to cross the tolerance limit of the plant (Jing et al. 2007).

Plant roots release large quantities of enzymatic and non-enzymatic exudates as proteins, short chain organic acids and phenolics. These exudates influence the bacterial enzyme system inhabiting the rhizosphere soil and also modify rhizospheric soil which is a dwelling place for mycorrhizae fungi. Microbial activity in rhizosphere (in plant roots) may be activated via secretion of nutrients and by facilitating conduits for enhanced water flow as well as diffusion of gases (Karthikeyan and Kulakow 2003). Diverse plant species release different types of bioactive compounds from their roots, called rhizosecretion. Rhizosphere has a fundamental responsibility in phytoremediation of soil contaminated by HMs, in which, microbial populaces are known to influence HM and accessibility to the plant via chelates, fermentation, phosphate solubilization and redox changes, and in this manner, can possibly upgrade the phytoremediation processes (Jing et al. 2007).

Better comprehension of the interactive parts between plant roots, microorganisms and other biota that make up the rhizosphere, their integrative limit in contaminant amassing, regulation, debasement and mineralization can be of great significance in organizing a successful application of rhizoremediation (US DOE 1994). The population of rhizobacteria in soil is very high; its magnitude may be greater than that in the soil having high level of HMs that may affect the population of microbes, community structure and ultimately the microbial activity (Jing et al. 2007).

Belimov et al. (2005) studied 11 cadmium-tolerant bacterial strains from the root zone of *Brassica juncea* L. including *Rhodococcus* sp., *Variovorax paradox* and *Flavobacterium* sp. Seedlings were developed in Cd-supplemented soils in addition to sewage sludge and mining waste having a high concentration of cadmium that were responsible for stimulating root extension in this manner being promising as inoculants to enhance growth of metal collecting plant.

15.2.3.1 Role of Rhizospheric Microbes in Rhizoremediation

Lasat (2002) indicated various features of rhizospheric microorganisms as:

(i) They communicate cooperatively with roots to improve the capability of metal take-up.
(ii) They discharge organic compounds to increase HM bioavailability.
(iii) They encourage ingestion of nutrients plus non-essential metals by roots.
(iv) They specifically affect metal dissolvability by changing their synthetic properties.

15.2.3.2 Importance of PGPR (Plant Growth-Promoting Rhizobacteria) and AMF (Arbuscular Mycorrhizal Fungi)

AMF play an important role in the phytoremediation of HM-contaminated sites (Atimanav and Alok 2004). PGPR and AMF upgrade the plant capacity to sequester HMs. The basic practices engaged in the process may be summarized as follows:

(i) AMF and PGPR help in nutrient recycling, support of soil structure, detoxification of chemicals and control of pest all together altering metal bioavailability ultimately decreasing HM toxicity.
(ii) Rhizobacteria can assist directly in phytoremediation by nitrogen-fixing and solubilising minerals like P, by way of secreting siderophores that sequester Fe from soil, proteins/enzymes and phytohormones.
(iii) PGPR and AMF generate chelating agents known as siderophores, which scavenge ferric ions and considerably enhance bioavailability of soil-bound iron.
(iv) To facilitate the build-up of HMs, AMF also produce small cysteine-rich proteins known as metallothioneins (MTs), which are similar to phytochelatins (PCs) (Cobbett 2000; Denton 2007).

Mycorrhiza could shield the host plants from the phytotoxicity of intemperate copper, zinc and lead by altering its speciation in addition to bioavailability (Jing et al. 2007). Depending on microenvironment of the soil, i.e. bioavailability of the HM, nature of secretion from the roots and level of nutrients, plants and microorganisms can:

(i) Frame particular relationship in which the plant furnishes the microorganisms with a particular carbon source that initiates the microscopic organisms to lessen the phytotoxicity of the polluted soil.
(ii) Frame nonspecific relationship in which ordinary plant processes stimulates the microbial group to deal better with the HM.
(iii) Increase the degradative limit of plants or lessen the phytotoxicity of the polluted soil (Siciliano and Germida 1998).
(iv) Plants can give carbon substrates and supplements and in addition increment contaminant solvency in this manner expanding the degradative action of microorganisms connected with plant roots.

Abou-Shanab et al. (2003) studied the effect of three rhizobacteria: *Microbacterium liquefaciens*, *Sphingomonas macrogoltabidus* and *Microbacterium arabinogalactanolyticum* from the rhizospheric zone of *Alyssum murale* and reported 17%, 24% and 32.4% amplified uptake of nickel, respectively, into the shoot and leaves as compared to uninoculated controls. In *Pteris vittata* L., AMF play an important part in arsenic accumulation due to chemical similarity of phosphorus with arsenic (AMF is recognized to build the take-up of phosphorus by plants) (Al-Agely et al. 2005). Chen et al. (2006) studied the consequences of AMF inoculation on U and As accumulation by *Pteris vittata* and reported noteworthy augmentation of uranium absorption by its roots. Furthermore, rhizoremediation/

phytostimulation may possibly also be helpful in two more processes connected with roots that are phytostabilization and rhizofiltration discussed in the following sections.

15.2.4 Phytostabilization

Phytostabilization may be characterized as (1) restriction of a pollutant in soil in the course of assimilation and aggregation by roots, adsorption onto roots or precipitation within the root region of plants and (2) the deployment of plants as well as plant roots to avoid contaminant movement through wind and water, draining and dispersion of soil (USEPA 1999). The ultimate aim of phytostabilization is stabilization of noxious wastes rather than their removal thus diminishing the hazard to human wellbeing and nature with the intention that the plants play a secondary role to soil amendments (Prasad and Freitas 2003). Contaminants are immobilized by transpiration and root development by decreasing leaching, controlling erosion, making a high-impact environment in root zone and adding natural matter to the substrate that ties metals (Robinson et al. 2006). In phytostabilization, there is aggregation by plant roots or precipitation in rhizosphere by root exudates which immobilises and lessens the accessibility of soil pollutants, inhabit the soil and can likewise serve as a ground cover thus reducing water and wind erosion and direct contact of the contaminants with biota. Phytostabilization may take place via precipitation, sorption, complexation or reduction of metal valence; the main role is to lessen the quantity of water permeating all the way through soil matrix, which may bring about the increase of dangerous leachate and avert soil disintegration and conveyance of the harmful metal to different zones (Ghosh and Singh 2005).

Phytostabilization appears to have strong promise for two toxic elements, chromium and lead. The efficiency of phytostabilization may be enhanced by addition of nutrients such as phosphate and lime to soil. Bluskov et al. (2005) studied the speciation, uptake and distribution of Cr in *Brassica juncea* and reported the majority of metal to be concentrated in plant roots thus proving it suitable for phytostabilization.

15.2.5 Rhizofiltration

Rhizofiltration is adsorption or precipitation of pollutants in solution form onto plant roots or absorption into the roots encompassing the root zone, because of abiotic or biotic forms. Rhizofiltration can be straightforwardly connected to effluents, contaminated waterways or groundwater frameworks. Developing plant roots investigate and infiltrate the three-dimensional volume of contaminated soil, and so the plant vascular framework pulls back water from the deep soil and conveys it to the climate; this transpirational demand at leaf surfaces is the main impetus for the water stream from soil solution to root surface and into and all the way through plant biomass (USEPA 1999). Surface water rhizofiltration might be directed:

1. In situ when either the plants are being developed straightforwardly in the polluted water body or the groundwater is situated inside the rhizosphere (root zone).
2. It may include the pumping of tainted groundwater into troughs loaded with the vast root frameworks of suitable plant species as the huge surface areas of these root systems facilitate effective assimilation of heavy metals from contaminated groundwater into root tissues.
3. Additionally, metals are expelled from groundwater through precipitation brought on by fluids discharged from plant roots, i.e. root exudates (Miller 1996).

Roots of numerous hydroponically developed terrestrial plants, such as *Brassica juncea* (L.), *Helianthus annuus* L. and various grasses, have been reported to be exploited for effectual elimination of noxious metals like copper, cadmium, nickel, zinc and lead from aqueous solutions. Roots of *B. juncea* are shown to concentrate these metals 131–563-fold (on the basis of dry weight) overpreliminary solution concentrations (Dushenkov et al. 1995). Effective exploitation of plants intended for the elimination of heavy metals as of industrial wastewaters and aqueous solutions using phytofiltration technology is significantly based on identification of suitable plant species. It may be a single species or a combination of the aquatic plants including *Ludwigia palustris*, *Scapania undulata*, *Potamogeton lucens*, *Nymphaea spontanea*, *Eichhornia crassipes*, *Azolla filiculoides*, *Lemna minor*, *Lemna gibba*, *Ceratophyllum demersum*, *Nymphaea alba* L., *V. Spiralis*, *Nelumbo nucifera*, *Myriophyllum spicatum*, *Salvinia herzogii*, *Myriophyllum brasiliensis*, *Cabomba* sp., *Myriophyllum aquaticum*, *Mentha aquatic*, afloat *Macrophytes* and *Pistia stratiotes* (Anwar et al. 2008). Phytofiltration (biosorption) technique depends on a number of physical and chemical processes such as ion exchange, chemical adsorption, complexation, surface adsorption, absorption, pore adsorption-complexation, microprecipitation and build-up of hydroxide onto the biosurface. Synthetic change and spectroscopic reviews have demonstrated that functional groups in the biomass include carboxyl, hydroxyl, sulphate, sulfhydryl, phosphate, amino, amide, imine and imidazole moieties as they contain metal restricting properties (Gardea-Torresdey et al. 2004).

However, the facilities and particular equipment in addition to mastery of qualified work force required for the creation of hydroponically developed transplants and the upkeep of effective hydroponic frameworks in the field can increase overhead expenses (Prasad and Freitas 2003). Moreover, the plant mortality due to unfavourable soil conditions, improper management, weather extremes or pest are the factors which should be taken care for the successful establishment of a rhizofiltration system.

15.3 Mechanism of Rhizoremediation: Uptake, Mobilization and Heavy Metal Sequestration by Plants

Though HMs, oxyanions and radionuclides can't be biodegraded, however, their movement in the surroundings can be adjusted through precipitation, redox reactions, adsorption/absorption, complexation and methylation responses intervened

by microorganisms or plants (USEPA 1999). Usually, plants employ two strategies to deal with excessive HM stress which may either be by reduced uptake or increased internal sequestration. A number of mechanisms enable plants to mobilize and uptake essential elements, and these often affect the translocation of other non-essential elements into plants including adsorption by cell walls, chelating agents, redox reactions and pH changes induced by plants; and specialized membrane channels, transporters, pumps and electrochemical gradients assist the elemental movement into roots (USDOE 1994). Effective take-up of contaminant could be accomplished if the metal contaminant behaves as essential nutrients. However, if the properties of HM vary from basic supplements, take-up by plants is less efficient (USEPA 1999). The system of metal take-up, aggregation, rejection, translocation, osmoregulation and compartmentation differ with every plant variety and assume a particular part in phytoremediation (Lone et al. 2008). Phytoremediation includes a complex interaction of different variables and components that can't be found out by a sole, reductionistic elucidation (Fig. 15.1).

The probable sequence of various events taking place during uptake, mobilization and sequestration of heavy metals may be as follows:

(i) Heavy metal solubilization from soil matrix into soil solution
(ii) Metal bioavailability
(iii) Rhizospheric bioactivation of metals

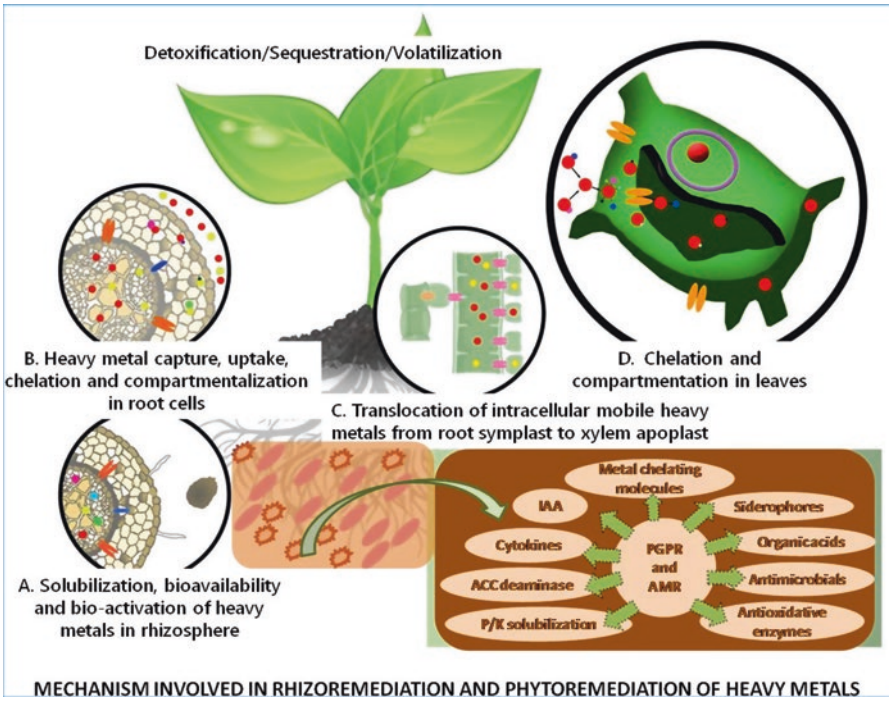

Fig. 15.1 Overall mechanism involved in phytoremediation of heavy metals by green plants

(iv) Metal capturing, uptake and chelation as well as compartmentation by root cells
(v) Translocation of intracellular mobile metal starting from symplast of roots to apoplast of xylem
(vi) Metal translocation to aerial tissues (stem and leaves)
(vii) Chelation as well as compartmentation in leaves
(viii) Detoxification/sequestration/volatilization

15.3.1 Solubilization of Heavy Metals from Soil Matrix Along with Mobilization into Soil Solution

Solubility of HMs is a chemical property that depends on various aspects, for example, dissolved organic matter, clay content and soil pH. For successful phytoremediation, heavy metals must be present in soil solution that is in direct contact with roots (Grcman 2005). Higher plants are exposed to the HMs via soil solution, and metals like Zn, Cu and Cd reach the surface of roots by diffusion and mass flow. Binding and immobilization contained by soil matrix can significantly restrict the potential for metal phytoextraction (Jing et al. 2007). The constraining variables accountable for the dissolvability within soil solution and bioavailability of HMs include complexation with natural and inorganic soil colloids, sorption going on oxides as well as clay, along with precipitation as carbonates, hydroxides and phosphates (Ruby et al. 1999). Uptake of HMs by plant roots depends on moisture content, metal concentration, organic matter and pH of rhizosphere. Availability of metals to the plant roots is enhanced by various secretions such as phytochelatins (PCs), organic acids, amino acids and enzymes (Fig. 15.2).

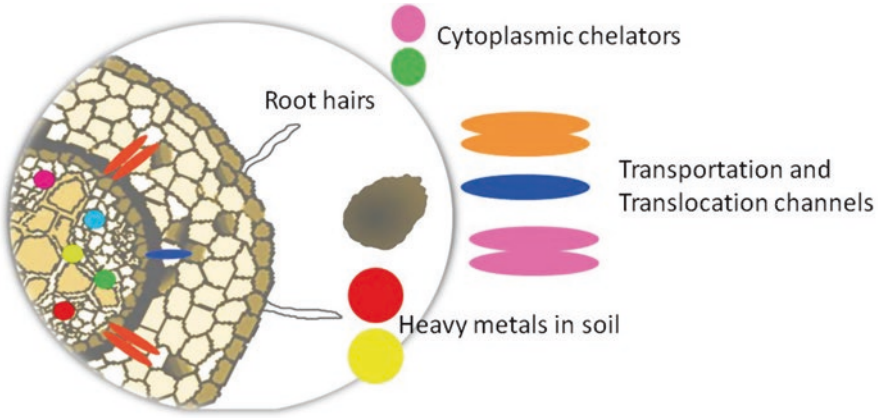

Fig. 15.2 Take-up of heavy metals by roots

15.3.2 Metal Bioavailability

Phytoavailability of metals depends upon aqueous speciation and free ion movement of the HMs; based on mobility in plants, the HMs can be separated into two groupings: (i) trace elements, e.g. Al, Cr, Hg, Pb, Sn and V immobilized in roots plus (ii) mobile, e.g. As, B, Cu, Cd, Mn, Ni, Se and Zn. Bioavailability of metals relies on abiotic variables including physical factors, for example, soil structure and natural substance in pores and also on biotic elements, for example, microbial as well as plant species (Violante et al. 2010). Extraction of HMs by plants is normally constrained by accessibility of heavy metals on the way to plants from the soil (Stanhope et al. 2000). Environmental conditions like, pH, speciation of element, organic substances there in the media and fertilization and plants being used for phytoremediation affect the heavy metals bioavailability (Cheng 2003). Bioavailability of the heavy metal to be extracted can be amplified in the soil by various soil amendments such as chelating agents thus increasing the availability as well as uptake of heavy metals by plants (Huang et al. 1997; Blaylock et al. 1997; Wu et al. 1999; Kirkham 2000; Madrid et al. 2003). High organic matter content (DOC) in the soil and cation exchange capacity (CEC) are two of the leading soil factors that decide the heavy metal availability to plants (Liphadzi and Kirkham 2005). Tests on *Lepidium heterophyllum* for bioavailability of Zn, Cd and Cu demonstrated that the Zn and Cd solubility was controlled for mostly by soil pH and natural carbon content, while the dissolvability of Cu was dependent on pH and DOC, increase in DOC increased the concentration of Cu in soil solution, proton concentration and speciation of the metal additionally impact the Cu^{2+} binding to roots and finally take-up to shoots. Kashem and Singh (2002) carried out studies to examine the effect of nitrogen and potassium on the solvency of Cd, Zn and Ni within soil solution and their accessibility to plants recommended that the cations increased metal take-up by enhancing growth conditions keeping in view the fact that the increase relies upon the plant species. Rhizosphere acidification (proton exudation by roots) may bring the pH down thus increasing the solubility of non-essential metal cations (Robinson et al. 2006). Liberation of HMs in the soil for increased take-up by plants is usually done by adding chelates, acidifying agents and soil amendments such as ammonium thiocyanates, synthetic cross-linked polyacrylates and hydrogels, etc. (Prasad and Freitas 2003) (Fig. 15.3).

15.3.3 Rhizospheric Bioactivation of Trace Metals: Root Microbe Interaction

A number of studies have reported the beneficial interaction amongst plants and microorganisms in rhizosphere (Kuiper et al. 2004). Rhizosphere microorganisms and mycorrhizal fungi can affect the take-up process by modifying assess of plant roots to HMs into the soil solution (Dakora and Phillips 2002). Metal bioavailability in the rhizosphere is firmly influenced by microbial activities, root exudates and root depositions, i.e. mucilage as well as border cells. Mobilization by plant

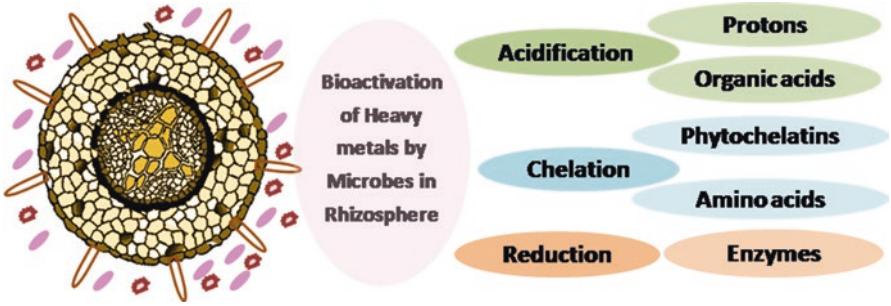

Fig. 15.3 Bioactivation of heavy metals by plant growth-promoting rhizobacteria and arbuscular mycorrhiza

growth-promoting rhizobacteria is due to solubilization of phosphate, siderophore and acid production (Jing et al. 2007). Soil rhizobacteria can likewise specifically impact metal dissolvability by changing speciation of heavy metals in the root zone and metal bioavailability by adjusting their chemical properties, for example, pH, organic matter and redox state, etc. (Jing et al. 2007). Organic acids discharged by many plant roots solubilize metal ions by competing for cation binding sites (Robinson et al. 2006). Ericoid mycorrhizae enhance the capacity of plants to develop on metal-polluted soil by increasing zinc solubility and mobility by discharging natural acids (Giasson et al. 2008; Jing et al. 2007). Endophytes have more intimate associations with their host when contrasted with rhizosphere and phyllosphere microorganisms since they take possession of internal plant tissues (Weyens et al. 2009). Bacterial endophytes are beneficial to their host plant by any of the mechanisms including production of phytohormones, enzymes engaged in growth regulator metabolism, for example, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, ethylene, indole-3-acetic acid (IAA) and siderophores (Rajkumar et al. 2010; Hardoim et al. 2008; Glick et al. 1998;). Moreover, they too can recover plant growth by means of nitrogen fixation (Triplett 1996) and enhance phosphate availability during initial colonization (Kuklinsky-Sobral et al. 2004).

15.3.4 Root Absorption and Compartmentation: Metal Capturing, Uptake, Chelation and Compartmentation by Root Cells

Plants rendered to toxic concentration of heavy metals, tend to avert or lessen uptake into root cells by confining heavy metal ions to the apoplast, fastening them to cell wall or to cellular exudates or by restraining translocation (Manara 2012). Once contained by the cell, metals undergo transportation, chelation, trafficking and lastly sequestration inside the vacuole (Manara 2012). Plants take up essential metals via channels, pores and transporters in the plasma membrane of root cells, yet most vascular plants assimilate harmful HMs through their roots in some cases due to resemblance amongst vital and additional metals. Supplementary heavy metals do

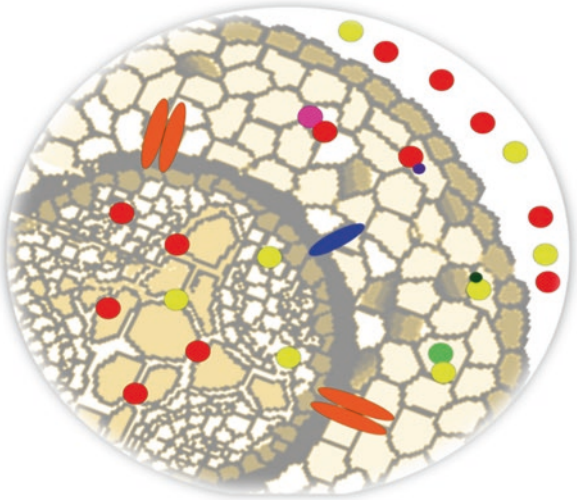

Fig. 15.4 Metal capturing, uptake and chelation as well as compartmentation by root cells

not have specific transporters and can enter the cell by a cotransport or as analogue of an essential element, e.g. selenate behaves as sulphate analogue so is up taken by sulphate transporter and arsenate as phosphate analogue so is transported through the phosphate transporter (Hall and Williams 2003). The machinery of tolerance of arsenate in *Holcus lanatus* involves the adaptive suppression of high-affinity phosphate uptake mechanism and constitutive phytochelatin production (Hartley-Whittaker et al. 2001) (Fig. 15.4).

Transporters are membrane proteins with transport functions, transmembrane transporters receive and transport only specific ions as they possess an extracellular binding domain for the attachment and transportation of ions from the extracellular space into the cell (Lasat 2002). Many metal transport families have been discovered including CDF (Cation Diffusion Facilitator), NRAMP (Natural resistance and macrophage protein), HMA (Heavy metal ATPase), ZIP (the Zrt, Irt-like proteins), CAX (the cation exchanger), CTR (the copper transporters), ABC (the ATP-binding cassette), P-type ATPases and the cation antiporters in tonoplast (Hall and Williams 2003; Milner and Kochian 2008; Plaza et al. 2007; Manara 2012).

In plants, NRAMP metal transporters are reported in both roots and shoot and revealed to convey a broad array of metals, such as manganese, zinc, copper, iron, cadmium, nickel and cobalt, all the way through plasma membrane and the tonoplast (Hall and Williams 2003). The metal transporters are determined by multigene families; Hall and Williams (2003) reported that Arabidopsis has eight heavy metal ATPases and six NRAMPS; *Arabidopsis thaliana* includes around 130 ATP-binding cassette (ABC) proteins contributing to the transportation of various materials including heavy metals (Lee et al. 2005). Yong et al. (2004) reported at least four AtPcrs (Arabidopsis plant cadmium resistance) genes that mediate Cd resistance. Depending on their affinities and differences for a particular metal, a number of metal transporters have reported to be involved in active efflux while others in influx of heavy metal ions (Hall and Williams 2003). The influx of heavy metals like Fe, Zn,

Mn or Cd from out of the cell or from subcellular compartment into the cytoplasm is mediated by ZIP protein family, COPT is occupied in transportation of Cu, P-type ATPases transport small cations and probably phospholipids and Type 1b subfamily proteins (HMA or CPx-ATPases) are involved in the transport of heavy metals; amongst the ZIP genes, AtIRT1 is responsible for Fe acquisition, AtZIP1 and AtZIP3 for the take-up of Zn in roots, AtIRT1 for transportation of divalent metal ions including Cd, Co, Zn and Mn and TcZNT1is responsible for high-affinity Zn uptake in addition to low-affinity Cd uptake (Hanikenne et al. 2005; Plaza et al. 2007).

Thomine et al. (2000) provided evidence that AtNramp genes isolated from Arabidopsis encode multispecific metal transport systems in plants that can transport Fe, Mn and Cd^{2+}. Plaza et al. (2007) described four ZIP genes (TcZNT1, TcZNT5, TcIRT1, TcIRT2) in root tissues with little or no expression in leaves suggesting that they are probably engaged in root uptake or loading of metals in the xylem. The over expression of Zn transporter gene ZNT1 in root and shoot tissue was responsible for increased Zn^{2+} uptake from soil and enhanced uptake into leaf cells (Pence et al. 2000). Salt and Wagner (1993) gave evidences for the transportation of Cd^{2+} via a Cd^{2+}/H^{+} antiport movement across the vacuole membrane thus accounting for vacuolar accumulation of Cd^{2+} and showed the possibility of a nonspecific cation transporter which can as well transport other cations including Ca^{2+}.

HMAs are more selective than other transporters engrossed in metal uptake, e.g. HMA2, HMA3 and HMA4 export Zn and Cd exclusively. Both AtHMA2 (Hossain et al. 2012) and AtHMA4 are located on plasma membrane, and heterologous expression of AtHMA4 in yeast induces tolerance to Zn and Cd toxicity, thus suggesting that this transporter can operate as efflux pump. ABC transporters in addition are engaged in the efflux of metal ions from plasma membrane. For example, AtPDR8 is restricted to the plasma membrane of *Arabidopsis thaliana* root hairs and epidermal cells, conferring both metal tolerance and pathogen resistance. AtPDR8 gets provoked in the presence of cadmium and lead. Transgenic plants overexpressing the protein do not accumulate Cd in the roots or shoots and are tolerant to normally toxic levels of Cd and Pb. In contrast, mutants accumulate elevated levels of cadmium and are sensitive to both metals. Probably, AtPDR8 acts the same as an efflux pump for these heavy metals by plasma membrane. C terminus of the TcHMA4 protein includes abundant promising heavy metal-binding Cys and His recurring residues which participate in HM binding (Papoyan and Kochian 2004).

All the noxious heavy metals should instantly bind to chelators in cytosol for the purpose of detoxification, then sequestered to vacuole. Histidine is accountable for the chelation of nickel in roots thus tolerating it (Ingle et al. 2005). Bluskov and Arocena (2005) revealed the existence of Cr (III) chelated with acetate in epidermal and cortical cells of the roots.

The chelated HMs in plants are sequestered in an intracellular membrane-bound compartment like vacuole. Metal ions penetrating the cytosol are ultimately stored in vacuole thus activating PC synthase to synthesize PC, and hence chelation inactivates the poisonous metal ions before inactivation of the enzymes by them which are required in metabolic routes (Memon et al. 2001). Several plants have been accounted to release phytosiderophores which facilitate uptake, translocation and build-up of Fe and other metal ions.

15.3.5 Translocation of Intracellular Mobile Metal to Xylem Apoplast from Root Symplast: Xylem Transport

The transport of heavy metal ion from root symplast to xylem apoplast requires transporters as metal ions cannot pass through the apoplast of root endodermis because of the existence of casparian strips in the membrane (Pilon-Smits 2005). The two main factors controlling translocation of metal-containing sap are root pressure along with leaf transpiration (Lasat 2002). CDFs catalyse the discharge of transition metal cations like, i.e. Ni^{2+}, Cd^{2+}, Zn^{2+}, Mn^{2+} or Co^{2+} from the cytoplasm to the outer parts of cell or within subcellular cubicles (Hanikenne et al. 2005). Eren and Argüello (2004) successfully cloned HMA2 and reported that Zn^{2+}-dependent ATPase is also stimulated by cadmium, and other divalent heavy metals (Pb^{2+}, Ni^{2+}, Cu^{2+} and Co^{2+}) are also affected to some extent and responsible for Zn^{2+}discharge from cytoplasm of cells. Transporters, i.e. HMA2 and HMA4 in Arabidopsis belonging to divalent heavy metal-transferring subgroup (P-type ATPases), play a fundamental role in Zn transportation system and homeostasis in plants (Hussain et al. 2004). HMA4 or HMA2 might have a very important role in loading of metal into the xylem (Milner and Kochian 2008). P1b-type ATPases are responsible for transportation of heavy metal ions such as Cu, Zn, Cd, Co, etc. across biological membranes (Eren and Argüello 2004). It was reported by Papoyan and Kochian (2004) that TcHMA4 is liable for the loading of metals to xylem instead of being occupied in metal transportation thus making yeast tolerant to a number of HMs like Cd, Zn, Pb and Cu via active efflux from the cell.

Ste'phane et al. (2006) demonstrated Ni transportation as a stable Ni-nicotinamide complex from root to above-ground parts through xylem sap in *T. caerulescens*. In *A. Halleri*, Cd is transported from root to xylem in inorganic forms by an energy-dependent process partially with Zn or Fe transport (Ueno et al. 2008) (Fig. 15.5).

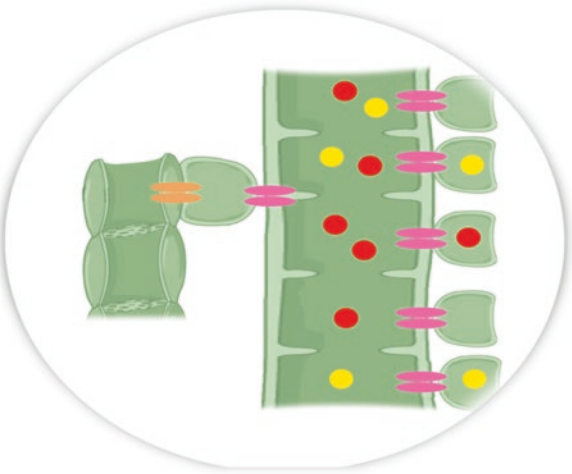

Fig. 15.5 Transfer of heavy metals to xylem apoplast from root symplast through xylem

15.3.6 Translocation of Heavy Metals to Aerial Tissues (Stem and Leaves)

Milner and Kochian (2008) found that Zn was associated with histidine inside the roots and was found as free hydrated Zn^{2+} in xylem sap whose small amount is bound to organic acids. In shoots, Zn was predominantly found in association with organic acids, and only some portions were found in free ionic form or bound with histidine or the cell wall, thus suggesting that from the chelated form in roots, the metal ions are transported towards the xylem in the form of free ions and then again stored in the shoot bound to the chelates.

Non-accumulator plants have a tendency to collect HMs in the root portion, while in hyperaccumulators like *T. caerulescens*, the majority of the absorbed metals were translocated rapidly from root to above-ground parts (Papoyan and Kochian 2004). HMs may be precipitated or stored in the vacuoles as metal complexes but because of limited storage capacity, the surplus heavy metals go through root endodermis and are translocated to shoot. It was observed by Freeman et al. (2004) that cys and o-acetyl-L-serine (OAS) and glutathione concentrations in shoot portion are strongly linked with the hyperaccumulation ability of nickel in numerous *Thlaspi* hyperaccumulators. Histidine is an essential nickel-binding ligand that chelates Ni ion and exports it from roots to above-ground parts in xylem (Ingle et al. 2005). However, a contrasting study of Persans et al. (1999) concluded that in *T. goesingense*, Ni hyperaccumulation was not decided by histidine overproduction in response to Ni. Persans et al. (1999) proposed that higher expression of TgMTP1 is accountable for the improved ability of *T. goesingense* to collect metal ions inside its vacuoles. By functional expression, Eide et al. (1996) cloned a gene (IRT1) of the plant *Arabidopsis thaliana* in a yeast strain defective for iron uptake. HMT1 is the first reported ABC-type transporter which may be involved in tolerance of HMs in certain plants and fungi having transportation ability of both apophytochelatins and phytochelatin-Cd^{2+} complexes (Ortiz et al. 1995). Yong et al. 2004 reported that AtPcr1,a gene found at the plasma membrane diminishes Cd (II) levels in transgenic yeast and Arabidopsis thus conferring a tough Cd-resistant gene at the level of plasma membrane. In an experimental study on the Ni hyperaccumulating plants, *Alyssum lesbiacum*, *A. bertolonii* and *Thlaspigoesingense*, Kupper et al. (2001) showed that bulk of Ni in leaves and stem was set up in the vacuoles in comparison to cell wall; in leaves, the epidermal cells have higher amount of Ni in comparison to the mesophyll and vascular cells.

15.3.7 Chelation Plus Compartmentation in Leaves

During heavy metal stress, organisms adopt different security mechanisms such as compartmentalization, exclusion, complex formation and the binding protein synthesis, for instance, metallothioneins (MTs) or phytochelatins (PCs). Metallothionein formations have lower molecular weight, i.e. 6–7 kDa, cysteine-rich metal-binding proteins established in higher plants, animals, eukaryotic microorganisms and in

some prokaryotes mainly involved in maintaining metal homeostasis and stress responses (Hammer 1986; Yuan et al. 2008). Metallothioneins are largely categorized on the structure basis and cysteine content into three different classes (Kagi 1991):

(i) Class I – Mammalian metallothioneins; usually composed of 60 amino acids (20 cysteine residues)
(ii) Class II – MT found in cyanobacteria, yeast and some higher plants
(iii) Class III – Phytochelatins

Different plants have different organic acids and ligands to bind with heavy metals like Zn, Cd and Cu; elevated concentrations of anionic species of organic acids like citrate, malonate and malate and ligands like metallothioneins and phytochelatins are present in elevated concentrations in the *Alyssum* spp. (ANRCP-1998-3) leaves. Related to the plant adjustment to stressful conditions of noxious heavy metals, glutathione plays a significant role in various biochemical processes via formation of Cd-GSH and Cd-PC complexes that diminishes the free Cd concentration in cytoplasm (Metwally et al. 2005). In plants, ions of different HMs, i.e. Cu, Cd, Hg, Zn and Pb induce the biosynthesis of phytochelatins (Grill et al. 1985; Grill et al. 1989). Phytochelatins are produced from glutathione (GSH) (Grill et al. 1989) having general structure (Y-Glu-Cys) n-Gly where X is Gly, γ-Ala, Ser or Glu and n is generally 2–5 but can be as high as 11 (Robinson et al. 1993; Cobbett 2000). Studies have accounted for isolation of plant MT-like genes from more than a few species of plants, i.e. rice, maize, wheat, soybean, tobacco and *Brassica napus* (Nedkovska and Atanassov 1998). Type I MT genes are expressed predominantly in the root portion, whereas type II MT genes are expressed mainly in the leaves (Nedkovska and Atanassov 1998). The metal basically binds to enzyme, γ-glutamy lcysteinyldipeptidyltranspeptidase (PC synthase), as a result of this activation conversion of glutathione (GSH) to phytochelatin is catalysed (Zenk 1996). The cysteine sulfhydryl residues present in phytochelatins bind to the HM ions thus sequestering them (Zhu et al. 2004). PC-Cd complexes have a major role in tolerance of Cd by the plants thus tolerating Cd by decreasing its free concentration in plants (Baycu 2002).

However, there are reports giving a contrasting role of increased phytochelatin synthesis, e.g. Lee et al. (2003) reported that the improved competence of PC synthesis directs to Cd hypersensitivity. Cr (III) is chelated with oxalic acid in leaves of *Brassica juncea* and stored in spongy mesophyll and epidermal cells (Bluskov and Arocena 2005). It was demonstrated by Bhatia et al. (2005) that the responsibility of malate is the same as a ligand that detoxifies/transports or stores nickel in *Stackhousia tryonii*. The phytoremediated metals are stored in plants in more than a single compartment; cadmium is principally deposited in the less metabolically active parts of leaf (Cosio et al. 2005). The harvesting time and harvesting parts both are influenced by the distribution of metals inside the plants which differs within the different organs and also depends on the age of that organ (Hammer and Keller 2003).

15.3.8 Detoxification/Sequestration/Volatilization

The cellular/molecular mechanism of tolerance along with resistance towards excess heavy metal concentration is specific for particular metal in particular plant sp. and also includes reduced uptake to cytosol by tidying up into the apoplastic region, chelation of HMs within the cytosol with appropriate ligands or discharge from the cytosol either into the apoplast or vacuole (Hall 2002). Detoxification of HM ions inside the plant tissues was done by chelation with suitable ligands, and in the cytosol, the chelate detoxification mechanism may include amino and organic acids (histidine and other amino acids) and thiol-containing compounds, such as phytochelatins, glutathione or metallothioneins (Sharma and Dietz 2006; Grill et al. 1985). Accumulation of HM ions in plants involves mechanism of compartmentation inside the vacuole and cytoplasm chelation (Nascimento and Xing 2006). Cobbett (2000) proposed that the plant reaction towards HM toxicity include immobilization, chelation along with compartmentation of the absorbed HM ions and stress response mechanisms like ethylene and stress proteins.

In *Saccharomyces cerevisiae*, Cu, Ni and Co ions are detoxified by histidine accumulation in vacuole (Milner and Kochian 2008). Organic acids get complexed with HM ions to detoxify them (Prasad and Frietas 2003). The toxic HM ions are detoxified by rapid complexation and compartmentation and hence are made less available for essential metabolic processes (Barcelo and Poschenrieder 2003). The process of free radical formation and reactive oxygen species is stimulated in the plant tissues to overcome the toxicity of HMs, e.g. level of antioxidant enzymes increases against oxidative stress triggered by the increased concentration of arsenic as a secondary defence mechanism (Srivastava et al. 2005). HM stress is also reported to be overcome by the plants by the manufacture of GSH, a precursor to phytochelatins, which acts as an antioxidant directly detoxifying metals by conjugating those (Lee et al. 2003). Phytochelatins have a critical role in the detoxification of HMs, particularly Cd in fungi, plants and *Caenorhabditis elegans* (Vatamaniuk et al. 2005). Glutathione and organic acid metabolism play an imperative role in the tolerance of heavy metals (Prasad and Frietas 2003). Lee et al. (2003) observed and concluded that in conjunction with the enhanced ability of PC production, the enhanced capacity of collecting PC-metal complexes into vacuole is also needed to increase tolerance of Cd in the plants. The damaging oxidative effects of Ni are overcome by various *Thlaspi* hyperaccumulators by enhancing the GSH-dependent antioxidant system (Freeman et al. 2005). Freeman et al. (2005) engineered resistance to Ni (nickel) and cobalt and increased sensitivity to cadmium in *E. coli* by the overexpression of serine acetyltransferase from Ni hyperaccumulating plant *Thlaspi goesingense* thus signifying that glutathione is involved in decreasing the oxidative damage caused because of Ni. Metal-binding proteins 'thiol' are called metallothioneins and are responsible for modulating the internal metal concentrations between undersupplied and noxious levels through closely spaced cysteine thiol groups (Khan et al. 2004). According to Memon et al. (2001), the mechanism for Mn^{2+} detoxification involves the take-up of Mn^{2+} ion by the plasma membrane, binding with malate in cytoplasm along with transportation through vacuole membrane, i.e. tonoplast to vacuole in which Mn separates from malate and gets complexed with oxalate (Fig. 15.6).

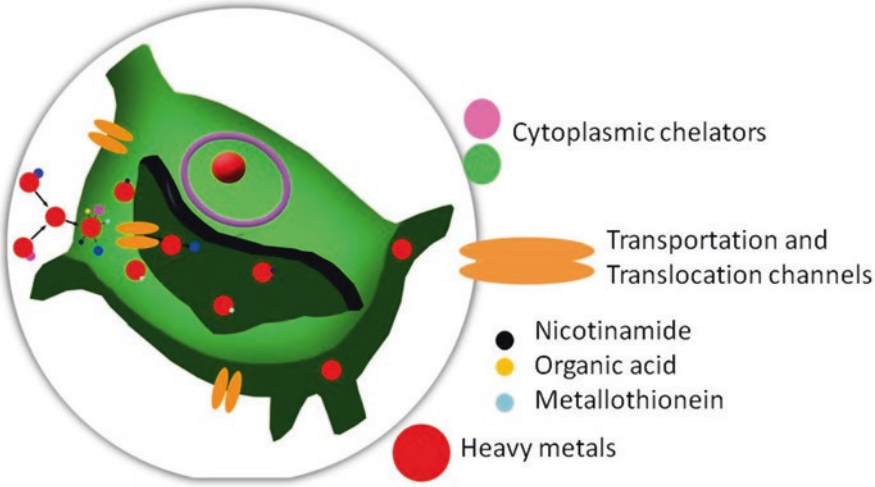

Fig. 15.6 Detoxification/heavy metal sequestration of in the vacuole

15.4 Molecular Basis of Phytoremediation and Rhizoremediation

15.4.1 Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS) and Heavy Metal Tolerance

Stress induced by HMs in plants actuates the immediate or aberrant creation of ROS, (e.g. superoxide radicals, hydrogen peroxide along with hydroxyl radicals) and reactive nitrogen species (RNS) subsequently prompting to oxidative pressure in plants (Anjum et al. 2012; Kruszka et al. 2012). Oxidative stress is a multifarious physiological and chemical process that creates as a consequence of overproduction and amassing of responsive oxygen species (ROS) in reaction to biotic as well as abiotic stresses inside higher plants (Demidchik 2015). ROS accumulation causes oxidative harm en route for nucleic acids, proteins and lipid bilayer. One of the systems of HM resilience is detoxification of ROS mediated by antioxidant compounds and antioxidant enzyme systems (Variyar et al. 2014). Antioxidants form sophisticated intracellular as well as extracellular networks ensuring protection against oxidation and thus shape stress signalling (Demidchik 2015). In plants, oxidative stress is neutralized by secretion of enzymes, for example, superoxide dismutases, catalases and peroxidises in addition to non-enzymatic foraging mechanisms including ascorbate, glutathione, carotenoids, xanthophylls, tocopherol, etc. (Kruszka et al. 2012).

Tripeptide glutathione (GSH, γ-Glu-Cys-Gly) is reported to secure plants against oxidative stress provoked by HMs and metalloids and comprises of both immediate and aberrant control of reactive oxygen species in addition to their reaction products in cells (Anjum et al. 2012). Also, a few GSH-related chemicals, for example, GSH reductase, GSH peroxidases and GSH sulfotransferases frame a productive

framework to ensure plant protection against ROS-incited impacts. Furthermore, glutathione and related catalysts likewise assume noteworthy part in detoxification, chelation and compartmentalization of HMs and metalloids in plants (Anjum et al. 2012). Furthermore, ROS and H_2O_2 are important signalling molecules as they act as agents for the initiation of genes defending against HM-induced stress (Foyer and Noctor 2005; Noctor et al. 2012). Redox signalling molecules allow and induce a series of appropriate physiological processes and produce particular signals incorporated with the action of plant hormones, for example, salicylic acid, ethylene, abscisic acid and jasmonates (Bartoli et al. 2013). SODs (superoxide dismutases) assume principal roles in the stress reaction by changing over the very lethal superoxide radicals (O^{2-}) into less harmful hydrogen peroxide (Kruszka et al. 2012). Nitric oxide (NO) is likewise perceived as a key controller of plant physiological procedures. Several components of signalling pathways have been portrayed that communicate NO effects in plants, including second messengers, protein kinases, phytohormones and target genes, etc. (Astier et al. 2012)**.** Redox and hormone signalling pathways within plants shape an incorporated network of redox-hormones that manages plant development and protection pathways. The focal components of the thiol-disulphide redox administrative centre point of plant cells include glutathione, peroxiredoxins, glutaredoxins, thioredoxins and NADPH-thioredoxin reductases. These are the key controllers for some pathways and reactions related to the signalling of heavy metal stress (Noctor et al. 2012). It has been reported in earlier studies that Cd is maintained and detoxified inside the roots by chelation by way of thiol compounds in addition to subsequent sequestration (Yan and Zhang 2013). Study conducted by Jozefczak et al. (2014) demonstrated a discrepancy in reaction of *Arabidopsis* leaves and roots to Cd. Jozefczak et al. (2014) reported that inside the roots, GSH is responsible for phytochelatin (PC) synthesis meant for Cd chelation. It was followed by activation of numerous antioxidative substances such as ascorbate, superoxide dismutase and catalase to efficiently deal with Cd-induced ROS.

Salicylic acid being a phenolic phytohormone (monohydroxybenzoic acid) is involved in the signal transduction cascades regulating plant defence mechanisms against biotic as well as abiotic stresses. Further, ethylene signalling pathways are also crucial to the survival of unfavourable environment and stress tolerance. Despite other functions, ethylene has been implicated in exposure to heavy metal stress in plants (Sewelam et al. 2014). Mutants with reduced ethylene sensitivity are less sensitive to lithium, which triggers H_2O_2 accumulation (Sewelam et al. 2014). Jasmonates (JAs) exist as a group of compounds called oxylipins that act as signalling compounds in response to plant stress.

15.5 Genomics of Phytoremediation and Rhizoremediation

All the courses that influence plants' susceptibility to heavy metals are subject to both transcriptional and post-translational controls. These processes are controlled by diverse genes. In accordance with this assertion, it has been revealed that the plants tolerance to HMs can be changed significantly using a broad range of genetic,

molecular, along with biotechnological tools (Ovečka and Takáč 2014). Stress induced by heavy metals is responsible to regulate plant genes related to various transcriptional, translational and/or post-translational processes (Kruszka et al. 2012). Plant adjustment to HM stress is coordinated by a coherent, genetically determined signalling arrangement (Hossain et al. 2012). Genes with important roles in uptake, transfer and HM accumulation in plants can be identified by using different 'omics' techniques. The recognition and categorization of gene pools related to the regulation of HM stress bearance in plants including molecules relayed to stress perception, transcription network, metabolic pathway and effector molecules maybe a great help to unveil the machinery of HM tolerance (Hossain et al. 2012). Genes engrossed in homeostasis of plants and HMs belong to diverse families such as ZNT1, ZNT2, NRAMP3 and NRAMP4 coding for metal transporters in vacuole that do not hydrolyse ATP; HMA3 and HMA4 are the metal transporters that hydrolyse ATP; and NAS1, NAS3, NAS4 and MT1B are involved in metal chelation (Visioli and Marmiroli 2012). Using anatomy data, Nguyen et al. (2014) acknowledged 17 root-preferred and 16 shoot-preferred genes at the vegetative stage and 3 pollen, 2 embryos, 2 endosperm, 2 ovary and 1 anther-preferred gene on the reproductive period belonging to ABC transporter protein family in *Oryza sativa*. Furthermore, 47 genes were established to be considerably up-regulated or down-regulated in response to HM stress.

15.6 Conclusions

Plant roots give a rich specialty to microbes to develop at the charge of root exudates; thus microbes perform as biocatalysts to remove contaminants. The versatile nature of plant-microbe mutualism is an energizing zone of research which has demonstrated relentless advance in the most recent decade. Rhizoremediation is a cheap option for the expulsion of pollutants in case where a slow removal is feasible, the level of contamination is not very high and when contaminated areas are large. Notwithstanding the proceedings in the field, particular communications between contaminant removing rhizobacteria and plants are still obscure including the declaration of corruption qualities in rhizosphere, the effect of flat quality move in rhizoremediation and the conceivable outcomes of the determination of particular microscopic organisms by plant rhizosphere. With the usage of high-throughput advances, more data about the microbial groups, root exudates and genomic information have been uncovered. It has casted out to be obvious that plant determination is a significant part of the rhizoremediation procedure and along these lines propels in the information on the subject of particular plant-microbial collaborations are required.

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Beneficial Microbes for Disease Suppression and Plant Growth Promotion

16

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Abstract

Plant growth-promoting microorganisms (PGPMs) constitute the microbes that are intricately associated with the plant system and may consist of rhizospheric bacteria, fungi, mycorrhiza, endophytic fungi, actinomycetes, or those having the mutualistic relationship or nonsymbiotic relationship with plants. One of the most remarkable features of these microbes is the adoption of certain ecological niches or may be occupied with multiple niches at a time in the soil ecosystem that makes way for other species to establish the mutual interactions (physical or biochemical) with other microbes (bipartite) or with plants (tripartite). The plant growth promotion by these microbes involves common mechanisms such as nitrogen fixation, siderophore production, phytohormone production, solubilization of mineral phosphates and secretion of novel secondary metabolites having positive effect on plant health. Some beneficial fungi have been found to promote plant growth through increased photosynthetic rate with improved mineral use efficiency and nutrient uptake, as inoculating these microbes with plants lead into increased chlorophyll content and biomass. These indigenous microbes have been also reported to counteract the different abiotic and biotic stress conditions. The mutual interaction observed between beneficial fungi and pathogenic microbes has been investigated at microscopic level which involves certain physical changes such as coiling of beneficial hyphae around the pathogenic hyphae and some cellular changes such as dissolution of host cytoplasm or secretion of antimicrobial compounds or lytic enzymes in the nearby localities that check the growth and reproduction of pathogenic species. The comprehensive knowledge of the functional mechanism of plant growth promotion by

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these microbes will help to develop strategies against damages covered by various abiotic and biotic stress conditions, and therefore will help in increasing the agricultural production at a global scale.

Keywords
Plant growth-promoting rhizobacteria (PGPR) • Siderophore • Nitrogen fixation • Phosphate solubilization • Phytohormones • Antibiotics

16.1 Introduction

To increase the total agricultural production, the modern agricultural system employs indiscriminate and excessive uses of agrochemicals (fertilizers, pesticides, herbicides and weedicides) that cause greater repercussion on the environment and have severe health hazards. The most drastic and long-term usages of these chemical-based agricultural inputs lead into the declination in the nutritional quality of the soil and thus affect its fertility. Moreover, the excessive usages of these chemicals affect the nontargeted beneficial microbes of the soil and may cause development of resistance among targeted pests and pathogens (Sujatha et al. 2011). Therefore, today there is a need for developing an intensive and eco-friendly strategy to minimize the usages of agrochemical-based food production. During the last few decades, the use of biological resources for plant growth promotion and disease resistance has compensated the huge demand for agrochemical-based fertilizers, and also helped in the maintenance of ecological sustenance and environmental protection to a greater extent. Such efforts have been based on research and development in the area of plant microbe interaction. The indigenous soil microbes and their vast and diverse communities intricately associated with the soils and plants form a complete and an integral part of vegetated agroecosystem (Aislabie and Deslippe 2013). A wide range of ecosystem services is well performed by these soil and rhizosphere microorganisms such as cycling and nutrient immobilization, nitrogen fixation, decomposing organic materials present in the soil, soil aggregation, removing degradable and bioremediating pollutants, plant growth promotion (through direct and indirect mechanisms), disease suppression, disease development on susceptible host and production of greenhouse gases (due to the decomposition of organic matter) (Prasad et al. 2015). However, the speciality of an individual microbe to find in its preferred habitat, in the soil, and its associated ecological niche has been a matter of great interest to the researchers and provided a platform to understand the basis and mechanism of microbial interactions with plant and soil. The long-term study of plant microbe interaction has explored the actual mechanism of plant growth promotion by these microbes and how they resist their opponent or other harmful microbes found in their nearby locality, and, therefore, providing resistance against diseases. The thorough understanding of these microbial interactions at molecular level and the physiochemical changes that occur in the soil due to activities of these potential microbes have invited for developing methods and strategies for their isolation and characterization, to study about their community structure and to explore their probable ecological functions and mechanics of plant growth promotion.

Plant growth-promoting microorganisms (PGPM) include various microbes intricately associated with the plants and have several defined ecological functions. In recent years, many studies have been done on plant growth-promoting microorganisms, and their two-way interaction made this study more enthusiastic and interesting for further comprehensive studies (Bulgarelli et al. 2013). The soil microorganism influences growth of plants in both beneficial and harmful manner. The microorganism which adversely affects the plant growth may be pathogenic or deleterious saprophytic one. On the other hand, the beneficial microbes promote plant growth and productivity through various mechanisms. PGPMs can assist plants in disease suppression, disease resistance, nutrient mobilization, and transport (Lugtenberg and Kamilova 2009). Therefore, the plant microbiome is one of the key determinants of plant health and productivity. These beneficial microorganisms can be grouped into plant growth-promoting bacteria (PGPB), and those associated with plant and found near the rhizospheric region are called plant growth-promoting rhizobacteria (PGPRs) or colonize inside the plant roots to become endophytes, with a number of species having transition in between these two states (Compant et al. 2010). Other PGPMs include cyanobacteria, actinomycetes, protists, certain fungi, mycorrhizal fungi, and nonpathogenic saprophytic fungi.

Plants maintain a complex interaction with their rhizospheric microbial populations which is very crucial for nutrient assimilation, induced systemic resistance, and the development and activation of defense mechanisms. These complex interactions between plant and microorganism are very dynamic, and some diverse microbial communities may perform well in microbial consortia (Hirsch 2004). The ecological association that exists in between plant and other microbial populations can be grouped into some mutualistic interactions including root nodule symbiosis or arbuscular mycorrhizal (AM) symbiosis. In the context of phylogenetic relationship and other ecological parameters, AM symbiosis is probably the most widespread interaction between plants and microbes (Bonfante and Genre 2010). In this interaction both the participating organisms have positive effect on other partner. This two-way beneficial association that exists in between plant and microbes is possible due to the existence of various communicating signaling pathways (Theis et al. 2008). In the microbial consortia, the regulation for maintaining resilience is achieved by the presence of specific functional groups rather than an individual microbial entity and may involve three-way interactions observed between plant, fungi and bacteria (Bonfante and Anca 2009; Dames and Ridsdale 2012). A relatively small number of beneficial plant microbe interactions are well known and have been used extensively for the plant growth promotion and increased agricultural productivity. However, the microbiota of soil ecosystems harbors a very complex group of microorganisms intimately associated with plants. This large number and their interaction with plants are still unexplored, and many of these interactions represent untapped reservoir for optimizing plant growth promotion and production. With a vision for promoting sustainable agriculture, the crops produced needed to be well equipped with disease resistance, salt tolerance, drought tolerance, and heavy metal stress tolerance and of better nutritional value. Moreover, the use of agricultural pesticides should be minimized for providing a clean and green environment.

16.2 Microbes Used in Plant Growth Promotion and Disease Management

Plants get benefited by different microbial populations occupying different habitats surrounding the plant. The most preferable habitat for microbial population is the rhizospheric region of the soil. Some microbes have been observed around the phyllospheric region or around the surface region as epiphytes or locating inside the plants as endophytes. One of the key determinants of microbial existence in the soil is the loss of organic matter from the roots that provides energy for the development of active microbial populations in the rhizosphere. This carbon loss invites many microbial species and mostly saprotrophs or biotrophs such as mycorrhizal fungi to grow in the rhizosphere along with other harmful pathogenic microbes that infect susceptible host and result into disease development. Although the microbes associated with plants are reported from many regions or compartments, the diverse microbial communities are preferably isolated or reported from rhizospheric region in the soil, therefore, the majority of the research on PGPMs have been focused on the rhizosphere. Recently, many research studies done on PGPR mechanics have unraveled the environmental niches occupied by different microbial populations surrounding the rhizospheric regions and have well demonstrated the potential biotechnological applications (Hirsch and Mauchline 2012; Bakker et al. 2013; Mendes et al. 2013).

Microbial communities play an important role in the mitigation of adverse agroecological concerns, disease resistance and stress management, and also reduce the economic losses due to these factors, therefore improve the total agricultural production which affects the economic productivity at a global scale. Recently many studies have been focused on the plant microbiome with respect to plant growth promotion, and health mechanism revealed the microbial populations associated with plants or present in the nearby rhizospheric soil (Berendsen et al. 2012). Among these diverse groups of plant growth-promoting microorganisms, PGPR (plant growth-promoting rhizobacteria) has been reported to be most promising. PGPRs include bacteria that reside around the rhizospheric region of the soil and improve plant health, thus promoting plant growth through plethora of mechanism. Some of the common genera of bacteria that come into PGPR include *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Burkholderia*, *Bradyrhizobium*, *Rhizobium*, *Frankia*, *Serratia*, *Thiobacillus*, *Pseudomonas* and *Bacillus* (Glick et al. 1995; Vessey 2003). Microbial symbionts are frequently capable of conferring stress tolerance to a wide variety of diverse plant hosts including both dicot and monocots (Redman et al. 2002; Zhang et al. 2008). Wakelin et al. (2016) isolated the root-associated *Pseudomonas* and *Burkholderia* species, which shown their plant growth promotion and biocontrol activities. *Streptomyces mutabilis* strains IA1, isolated from a Saharan soil, have shown plant growth-promoting effect on wheat seedlings (Toumatia et al. 2016).

The fungal endophytes show diverse types of relationships with their hosts including symbiotic, mutualistic, commensalistic and parasitic in response to the plant/host genotype, and other environmental factors. The mutualistic relationship of a host plant with their fungal endophytic partner has several benefits as the

endophyte promotes growth and reproductive success of its host by counteracting various abiotic and biotic stresses. The use of fungal endophyte in mitigation of abiotic and biotic stresses may be a promising strategy for enhancing crop production challenged by various stresses. Among various fungal endophytes, *Piriformospora indica*, a root-colonizing endophytic fungus, is well known for its plant growth promotion and explored well for its tolerance to abiotic and biotic stresses (Waller et al. 2005; Schäfer et al. 2007). Many plant growth-promoting fungi are well competent with rhizosphere and assist plants to derive necessary mineral nutrients in an easily available form (Shivanna et al. 1994). Cowan (1979) suggested that some growth-promoting isolates of *Phialophora graminicola* increase the mineral nutrient uptake by plants. *Trichoderma*, a well-known biocontrol agent, is widely employed in its commercial formulations as biofertilizer and biopesticides are a good example of plant growth-promoting fungi (Chet 1987; Verma et al. 2007). The biocontrol activity of this fungus is mainly due to the production of many antimicrobial compounds that work against several pathogens, in addition to their aggressive mode of growth and physiology. It has been reported that 70% of the plant species are harbored by AM fungi as this beneficial interaction plays an important role in their growth and development, mineral absorption, and protection against several abiotic and biotic stresses (Zhang et al. 2016).

The mineral nutrients such as phosphate and nitrogen which are absorbed through extraradical hyphae of AM fungi are directly supplied to plants most probably by using a highly branched structure that is developed inside the root cells and called arbuscules (Harrison 1998; Parniske 2008). These AM fungi also are known to interact with other soil microbial populations residing in the rhizospheric region and therefore work in a complex mutualistic network. Some AM fungi have been reported to interact with phosphate-solubilizing bacteria (PSB), nitrogen-fixing bacteria, and biocontrol agents.

One question that needs to be still addressed is the probable mechanics of the PGPR plant growth promotion and how the association of a specific microbe around a particular rhizospheric region occurs. The mechanism of PGPRs is very crucial to manipulate the rhizospheric flora and in order to maximize the overall activities that strongly promote plant growth and thus enhance productivity (Fig. 16.1). Recently, some of the researchers reported the direct and indirect PGPR mechanics, in which the direct mechanism occurs inside the plant and affects plant metabolism, whereas the indirect mechanism occurs outside the plant (Glick et al. 1995; Vessey 2003; Antoun and Prévost 2006; Siddikee et al. 2010). The direct mechanisms include those that affect the balance of plant growth regulators due to the secretion of various plant growth regulators and growth factors by microbes during their interaction, and absorbed by plants or because the microorganisms function as a sink of plant-released hormones and that induce the plant's metabolism leading to an improvement in its adaptive capacity (Govindasamy et al. 2011; Glick 2014). Some of the most common and well-known direct mechanisms employed by plant growth-promoting microbial species include biological nitrogen fixation, phosphate solubilization, siderophore production and phytohormone production (Fig. 16.2). In contrast, the indirect mechanisms are associated with plant defensive

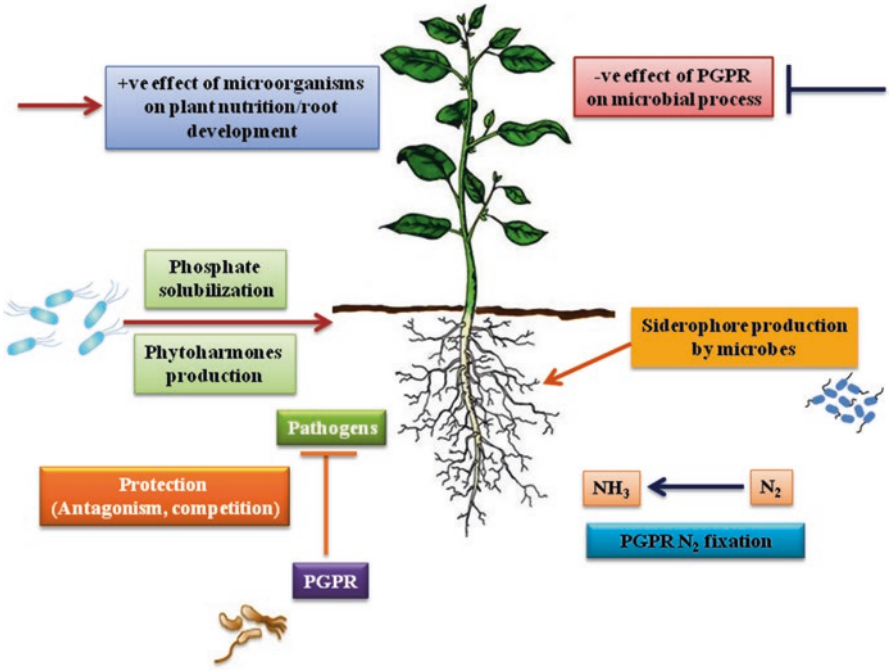

Fig. 16.1 A generalized diagrammatic representation of positive and negative interaction of PGPR-mediated plant growth promotion and disease suppression

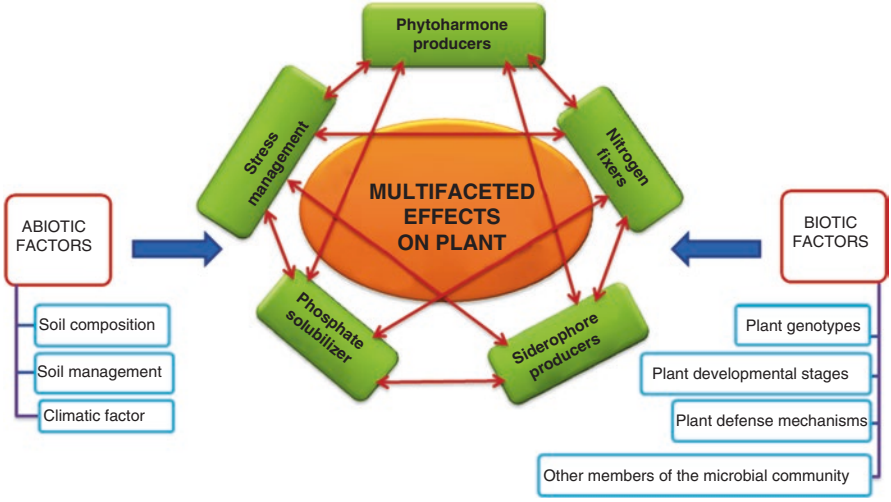

Fig. 16.2 General mechanism and multifaceted route of plant growth-promoting microbes in the soil. The solid arrows indicate the mutual interaction and correlation of each and individual mechanism with other groups. The environmental factors (both abiotic and biotic) affect the whole beneficiary aspects of microbe-mediated growth promotion

metabolic processes, respond to the signaling molecules secreted by bacteria, and thus influence plant growth and metabolic activities in multiple ways. One of the two important mechanisms included in this group is plants' defense against changing or stressed environment (abiotic stress), and other includes biotic stress imposed by pathogens (Jha et al. 2011; Aeron et al. 2011; Glick 2014). The selective enrichment of microbial populations from soil to the rhizospheric region is a matter of great interest. The factors associated with this transition from soil to root or around the regions nearby the roots are well addressed by Bais et al. (2006) and Doornbos et al. (2012). Some explanations in this regard have been put forward and suggested that some carbohydrates and amino acids work as chemi determinants in the rhizosphere (Moe 2013). Additionally, some secondary metabolites specific to plants such as flavonoids were identified as key determinants to establish microbial communities in the rhizospheric region (Weston and Mathesius 2013). The study of the entire microbial community at large scale through genomics, metagenomics, transcriptomics and proteomics approaches would provide the information regarding some novel genes and proteins involved in regulating stress responses. For example, transcriptome analysis of oilseed rape and its symbiont *Stenotrophomonas rhizophila* identified spermidine as a novel PGPM regulator of plant abiotic stress (Alavi et al. 2013). The comparative studies made in such a way will gain the knowledge of stress responsive genes in different microorganisms and their expression pathway under different abiotic and biotic stresses (Fig. 16.1). Moreover, the genomic content of these microorganisms will provide comprehensive mechanisms involved behind the conferred stress tolerances, as well as cultivate them for further experimental investigations (Pope et al. 2011).

16.3 Mechanism of Plant Growth Promotion by PGPM

16.3.1 Tolerance to Abiotic and Biotic Stresses

The plant growth and productivity are constrained by several abiotic and biotic stresses. Among the abiotic stresses, the major one includes high temperature, soil salinity, water stress (drought), mineral deficiency, and heavy metal toxicity, whereas the biotic stresses are those that are imposed by various phytopathogens including bacteria, fungi, viruses and nematodes. The abiotic stress tolerance ability may be conferred and decided by the genome of the plant; relationship with microbes can also provide improved tolerance to or protection from the developed abiotic stresses. The alleviation of stress response (both biotic and abiotic) by the indigenous microbial populations, associated with soil, has attracted the scientific attention, to prefer and employ these over other conventional plant breeding programs for developing varieties having potential for counteracting stress response (Mayak et al. 2004; Tank and Saraf 2010; Marasco et al. 2013). Several plant growth-promoting bacteria elicit severe physiochemical changes in plants relevant to plant defense in the form of induced systemic resistance under biotic stress. Goudjal et al. (2016) characterized the 16 endophytic *Actinobacteria* isolated from roots of native

plants for plant growth promotion and biocontrol activities, and reported that isolated *Streptomyces* sp. SNL2 showed promising plant growth-promoting features, and also showed biocontrol activity against *Fusarium oxysporum* f. sp. *lycopersici*, wilt causing pathogen in tomato. The inoculation of this microbe also resulted into the increase in dry weight of roots and shoots, and the isolate was found to be more similar (99.52%) to *Streptomyces asterosporus* NRRL B-24328^{T}. The antimicrobial metabolites 2,4-diacetylphloroglucinol (DAPG), pyochelin and pyoluteorin are secreted by *Pseudomonas fluorescens* strain CHA0 and contribute in the management of diseases caused by soilborne phytopathogens (Schnider-Keel et al. 2000). In one more study, it was reported that the presence of mineral ions (Cu, Zn and Zn-Cu both) enhanced the production of DAPG by *Pseudomonas fluorescens* 4-92(Pf4-92). Moreover, this isolate was significant in controlling the chickpea wilt pathogen *Fusarium oxysporum* f. sp. *ciceri* in the presence of mineral amendments (Saikia et al. 2009). Recently, two Gram-negative and non-sporulating rhizospheric bacteria *Serratia plymuthica* strains 3Rp8 and 3Re4-18 have been reported to possess strong antagonistic activities toward fungal pathogens *Verticillium dahliae* Kleb, *Rhizoctonia solani* Kühn and *Sclerotinia sclerotiorum,* and had potent plant growth promotion activities (Adam et al. 2016). Wang et al. (2014) reported the efficacy of microbial consortia BBS employing three partners *Bacillus cereus* AR156, *Bacillus subtilis* SM21, and *Serratia* sp. XY21 and in mitigating both abiotic and biotic stress. More recently, Kakar et al. (2016) reported the effect of microbial consortia employing two rhizobacterial strains. *Bacillus amyloliquefaciens* BK7 and *Brevibacillus laterosporus* B4 along with elicitors salicylic acid (SA) and β-amino isobutyric acid and their mixture can be used for stress tolerance against cold and drought conditions in rice. Singh and Jha (2016) demonstrated the plant growth-promoting potential of a bacterial isolate CDP-13, isolated from *Capparis decidua* plant and shown its ameliorative effect against abiotic and biotic stresses. In another study, Chen et al. (2016) demonstrated the salt tolerance capability of *Bacillus amyloliquefaciens* SQR9 in maize. It was reported that *Burkholderia phytofirmans* along with *Enterobacter* sp. FD 17 offered more protective response against drought conditions as in maize as the droughted plants when inoculated with the same had higher shoot and root biomass and leaf area and with greater photosynthetic efficiency when compared to control plant samples. This example explains well the physiological wellness of this microbe against drought conditions and explains the physiological responses to endophytic inoculation which are specific to plant and microbial genotype (Naveed et al. 2014). It has been reported that the inoculation of barley plants with the fungus *Piriformospora indica* has provided the defense against two fungal pathogens, e.g., *Fusarium* and *Blumeria*, and also provided tolerance to plants against high-salt stress conditions (Waller et al. 2005). Similarly, Islam et al. (2016) found that *Bacillus cereus* promoted the growth of *Vigna radiata* under the saline stress conditions by causing significant changes in the antioxidative defense machinery of plants. Recently, one isolate of *Burkholderia phytofirmans* strain PsJN has been reported for providing tolerance against stress conditions. The abiotic stress tolerance provided by strain PsJN, having wide host spectrum range including wheat, maize and grapevine, has been reported in abiotic

stress tolerance. The inoculation of this microbe under the stress conditions resulted into the improved water use efficiency and chlorophyll content with improved photosynthetic rate that leads into increased productivity (Naveed et al. 2014). *Arabidopsis* plants in symbiotic relationship with *Paenibacillus polymyxa* have been reported to counteract both drought response and with improved resistance to pathogen attack (Timmusk and Wagner 1999). Many species coming under the genus *Pseudomonas* have been demonstrated to have potential for ameliorating biotic and abiotic stress tolerance. Tiwari et al. (2016) reported the *Pseudomonas putida* attuned morphophysiological, biochemical and molecular response in *Cicer arietinum* under the water-stressed condition. Sarma and Saikia (2014) reported that *Pseudomonas aeruginosa* strain has improved the growth of *Vigna radiata* (mung beans) plants under drought conditions. The ability of plants in utilizing water for growth depends on their stomatal apertures.

Trichoderma (teleomorph Hypocrea) a fungal genus which is found in many ecosystems. Some strains of *Trichoderma* have been shown to have aggressive behavior for plant pathogens and reduce the severity of plant disease through several common mechanisms such as mycoparasitic coiling and antagonistic activities (Viterbo and Horwitz 2010). In contrast, many rhizocompetent strains have been observed to have direct plant growth-promoting effects such as increased rate of seed germination, nutrient uptake, and fertilizer use efficiency with the stimulation of plant defense against biotic and abiotic damages (Shoresh et al. 2010). Many stress tolerance-associated genes from *Trichoderma* spp. have been identified and transferred to plants for improving biotic and abiotic stress tolerance. For example, the expression of an endochitinase gene from *Trichoderma virens* conferred enhanced tolerance towards Alternaria blight in transgenic *Brassica juncea* (L) (Kamble et al. 2013). Mishra et al. (2016) reported one isolate *Trichoderma harzianum* KSNM (T103) in alleviating to tolerate biotic (root pathogens) and abiotic stresses [high salt (100–1000 mM); heavy metal (chromium, nickel and zinc: 1–10 mM); pesticides, malathion (100–600 ppm); and carbofuran (100–600 ppb)] and promote the growth of plants as well. Recent studies have well demonstrated the role of *Trichoderma* in mitigating various abiotic and biotic stresses. Elad et al. (1998) suggested the prevention of gray mold of cucumber by *T. harzianum* T39 and *Ampelomyces quisqualis* AQ10, well-known antagonistic fungi that work against powdery mildew pathogen of cucurbits. Recently, Zhang et al. (2016) reported the ameliorative effects of *Trichoderma longibrachiatum* T6 against saline stress in wheat through the improvement of antioxidative defense machinery and gene expression. Elsharkawy and Mousa (2015) demonstrated one more fungi *Phoma* spp. isolates (GS8-1, GS8-2 and GS8-3) and non-sporulating fungus (isolate GU21-2) inducing systemic resistance in cucumber against *Colletotrichum orbiculare*. It has been reported that the bacterium *Achromobacter piechaudii*, isolated from dry riverbeds of southern Israel, has eminent potential for counteracting both salt and drought stresses, as observed in pepper and tomato plants (Mayak et al. 2004). The molecular mechanism of abiotic and biotic stress tolerance to their host plants by these microbes is another avenue for the development of transgenics by identifying the targets and therefore preparing crops for manipulating diverse stresses. Many

studies have been done to find out the actual molecular mechanism of stress tolerance by microorganisms. Zhang et al. (2008) reported the salt tolerance induced by *Bacillus subtilis* was shown to be the result of tissue-specific modulation of the expression of the *Arabidopsis* Na^+/K^+ transporter, HKT1. Similarly, Timmusk and Wagner (1999) demonstrated the upregulation of the host gene *ERD15* following the drought resistance by inoculating *Paenibacillus polymyxa.* The molecular entities responsible for counteracting stress response in plant growth-promoting microorganisms may provide some potential targets for developing transgenics with desired modification targeted for improved plant growth and productivity (Nadeem et al. 2014).

16.3.2 Biological Nitrogen Fixation

Biological nitrogen fixation is the mechanism that converts the atmospheric nitrogen (N_2) to ammonia (NH_3 or NH_4^+) which can be easily used by plants. However, the process is restricted to bacteria and archaea, and does not occur in eukaryotes. Nitrogen fixation is carried out by two groups of microbial population. The first group comprises of symbiotic nitrogen fixers and includes root-/legume-associated symbiotic bacteria which possess the specificity and infect the roots to produce nodule, e.g., strains of *Rhizobium*. In other groups of bacteria, there is no symbiotic nitrogen fixation, and nitrogen is fixed without any association or specificity for the crop and so-called free-living nitrogen fixers (Oberson et al. 2013). Examples of such free-living nitrogen fixers include *Azospirillum*, *Azotobacter*, *Burkholderia*, *Herbaspirillum*, *Bacillus* and *Paenibacillus* (Seldin et al. 1984; Berge et al. 2002; von der Weid et al. 2002; Berg et al. 2005; Goswami et al. 2015). In free-living nitrogen fixers, although there is neither any symbiotic association nor they deeply penetrate the plant tissues yet, a somewhat closer intimacy is observed where the fixed nitrogen is taken by plants and the fixers do not use it for their own benefit. This closer intimacy or proximity to the roots enables them to impact plant resource acquisition (nitrogen, phosphorus, and other essential minerals) (Ahemad and Kibret 2014). Nitrogen-fixing PGPRs have been identified and reported among the bacilli, and especially among the proteobacteria. Some of the best studied associative PGPRs belong to the genus *Azospirillum* and are used frequently to improve the fitness of many crops (rice, wheat and maize). The application of *Azotobacter chroococcum* and *Azospirillum brasilense* inoculants in agriculture, especially in cereals, has resulted in notable increases in crop yields (Oberson et al. 2013). Several strains of PGPR fix nitrogen and promote the growth or colonization of many species of diazotrophic bacteria to multiply within plant tissues without damaging defense system. These bacteria, such as *Azoarcus*, *Herbaspirillum* and *Gluconacetobacter*, play an important role in plant growth by producing phytohormones such as indole-3-acetic acid, gibberellic acid and cytokinin. Nitrogen-fixing ability has also been reported from *Bacillus* and *Paenibacillus* as they have been reported to possess *nif* gene cluster which is responsible for encoding nitrogenase enzyme, an important enzyme required in the fixation of nitrogen. *Bacillus azotofixans*, *Bacillus macerans* and *Bacillus polymyxa* were identified as nitrogen fixers,

based on nitrogenase activity (Seldin et al. 1984). In the same way, some species of *Paenibacillus* genus including *Paenibacillus odorifer*, *P. graminis*, *P. peoriae* and *P. brasiliensis* have been described as nitrogen fixers (Heulin et al. 2002; von der Weid et al. 2002). Recently, an endophytic diazotroph *Paenibacillus polymyxa* P2b-2R has been reported for its ability to colonize, fix nitrogen and promote growth of an oilseed crop canola (Puri et al. 2016). These studies well demonstrate the direct role of microorganism in plant growth promotion and development.

16.3.3 Phosphate Solubilization

After nitrogen, phosphorous (P) is the second most limiting nutrient for plants. Although profoundly rich reserve of phosphorous is available in the soil, plants cannot uptake this available form. Plants are only capable to take mono- and dibasic phosphates which are the soluble forms of phosphate (Jha et al. 2012; Jha and Saraf 2015). Microbes mineralize organic phosphorus in soil by solubilizing complex-structured phosphates, viz., tricalcium phosphate, aluminum phosphate, rock phosphate, etc. which convert organic phosphorous to inorganic form finally aiding the phosphate availability to plants. The phosphate-solubilizing bacteria utilize miscellaneous mechanism(s) to solubilize the insoluble forms of the phosphate. The crucial mechanism of phosphate solubilization is depending on organic acid secretion by microbes because of sugar metabolism. Organisms residing in the rhizosphere utilize sugars from root exudates and metabolize it to produce organic acids (Goswami et al. 2014). These acids released by the microorganisms act as excellent chelators of divalent Ca^{2+} cations accompanying the release of phosphates from insoluble phosphatic compounds. Numerous phosphate-solubilizing microbes decrease the pH of the medium by the secretion of organic acids such as lactic, acetic, succinic, tartaric, malic, gluconic, 2-ketogluconic, oxalic and citric acids (Rodríguez and Fraga 1999; Patel et al. 2015), and their detection using high-performance liquid chromatography (HPLC) is also reported (Buch et al. 2008). Among the soil bacterial communities, ecto-rhizospheric (residing on roots and in rhizospheric soil) strains from *Pseudomonas, Bacilli,* and endosymbiotic (residing within the roots/nodules) rhizobia have been described as effective phosphate solubilizers (Goswami et al. 2014). Bacterial strains belonging to genera *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aerobacter*, *Flavobacterium* and *Erwinia* have the ability to solubilize insoluble inorganic phosphate (mineral phosphate) compounds such as tricalcium phosphate, dicalcium phosphate, hydroxyl apatite and rock phosphate (Rodríguez and Fraga 1999; Rodríguez et al. 2006).

Some soil bacteria articulate essential level of acid phosphatases such as *Rhizobium* (Abd-Alla 1994), *Citrobacter*, *Enterobacter*, *Serratia*, *Klebsiella* and *Proteus* (Thaller et al. 1995), as well as some species of *Pseudomonas* (Gügi et al. 1991) and *Bacillus* (Skrary and Cameron 1998). Chen et al. (2006) reported four different strains of phosphate-solubilizing bacteria (PSB), such as *Arthrobacter ureafaciens*, *Phyllobacterium myrsinacearum*, *Rhodococcus erythropolis* and

Delftia sp. following confirming their capability to solubilize significant amounts of tricalcium phosphate in the medium by secreting organic acids. *Bacillus megaterium*, *B. thuringiensis*, *B. circulans*, *B. coagulans*, *B. subtilis*, *B. polymyxa*, *B. sphaericus*, *B. sircalmous*, *B. brevis*, *Xanthomonas maltophilia* and *Pseudomonas striata* could be referred as the most important strains for phosphate solubilization (Kucey et al. 1989; Govindasamy et al. 2011; Goswami et al. 2013). *Pseudomonas fluorescens*, *P. aeruginosa*, *Erwinia herbicola*, *Pseudomonas cepacia* and *Burkholderia cepacia* are demonstrated as competent producers of gluconic acid, which is the main recurrent capable mediator in mineral phosphate solubilization (Rodríguez and Fraga 1999). The symbiotic bacteria *Rhizobium leguminosarum* is reported to produce 2-ketogluconic acid, which aids in the solubilization of phosphate. Other 2-ketogluconic acid producers comprise *Rhizobium meliloti* and some species of *Bacillus* including *Bacillus firmus* (Banik and Dey 1982; Halder et al. 1990; Halder and Chakrabartty 1993). Mixtures of lactic, isovaleric, isobutyric and acetic acids are frequently produced by the strains of *Bacillus licheniformis* and *B. amyloliquefaciens*. Other organic acids, such as glycolic acid, oxalic acid, malonic acid, succinic acid, citric acid and propionic acid, have also been identified among phosphate solubilizers (Banik and Dey 1982; Illmer and Schinner 1992; Chen et al. 2006; Jha and Saraf 2015).

The management of phosphorus solubilization and its utilization for plant beneficiary aspects by the use of phosphate-solubilizing fungi is a promising biotechnological approach. It has been proved that P-solubilization mechanisms not only differ among fungal isolates but are also dependent on the applied P sources (de Oliveira Mendes et al. 2014). There are some fungal strains such as *Aspergillus niger* FS1, *Penicillium canescens* FS23, *Eupenicillium ludwigii* FS27 and *Penicillium islandicum* FS30, which are able to solubilize the P sources (de Oliveira Mendes et al. 2014). Some fungal species belonging to *Aspergillus* and *Penicillium* genera are capable to release P from insoluble inorganic compounds through acidification of the medium and the production of organic acids (Illmer et al. 1995). Recently, Elias et al. (2016) reported the efficacy of mineral phosphate solubilization from rhizospheric fungi of important vegetable crops such as bean, cabbage, tomato and sugarcane, and found that some fungal strains from rhizosphere soil samples such as *Aspergillus* (55.69%), *Penicillium* spp. (23.35%) and some species of *Fusarium* (9.58%) were predominant phosphate solubilizers. However, it has been reported that *Aspergillus* species have greater efficiency in colonization of root and have greater potential for solubilizing the soil phosphates (Nenwani et al. 2010). This can be further demonstrated by the solubilizing index (SI) calculated for measuring the P-solubilizing potential of soil microbes. Recently, Yasser et al. (2014) confirmed that *A. niger*, *Penicillium variable*, and *T. harzianum* showed P-solubilization potential 1.67%, 0.55% and 0.32%, respectively. Similarly, Iman (2008) reported that the SIs of the test phosphate-solubilizing fungal strains (*Penicillium italicum* and *A. niger*) were 2.42 and 3.15, respectively. Conversely, Mahamuni et al. (2012) reported solubilizing index (SI) for various fungal strains (*A. niger* NFCCI 1991, *Aspergillus awamori* NFCCI 1992, *Aspergillus fumigatus* NFCCI 1993, *Alternaria alternata* NFCCI 1994, *Curvularia pallescens* NFCCI

1996, *Penicillium oxalicum* NFCCI 1997, *Penicillium rubrum* NFCCI 1998 and *Trichoderma viride* NFCCI 1999) isolated from sugarcane and sugar beet which ranged from 1.13 to 1.59.

16.3.4 Phytohormone Production

Soil microorganisms promote the plant growth through the production of some important plant growth regulators and phytohormones. These phytohormones can play an important role in various processes such as plant cell enlargement, cell division and extension in symbiotic as well as nonsymbiotic roots (Patten and Glick 1996; Glick 2014). Plant responds to any phytohormone in the rhizosphere that is supplemented on the surface or being fashioned by microbial flora residing in the rhizosphere. The rhizospheric microorganisms particularly PGPRs are the important producers of phytohormones such as auxins, gibberellins, cytokinins, ethylene and abscisic acid (Arshad and Frankenberger 1997; Patten and Glick 1996). Auxin, indole-3-acetic acid (IAA), is a key phytohormone produced by numerous strains of PGPR, and it is distinguished that treatment of IAA-producing rhizobacteria enhances the plant growth (Vessey 2003; Kaymak 2011; Amara et al. 2015). It is well known that IAA stimulates both fast (e.g., enhance cell elongation) and long-term (e.g., differentiation and cell division) responses in plants.

During long-term treatment of IAA, plant has highly developed roots, which allows the plant to absorb nutrients for increasing overall growth of the plant (Aeron et al. 2011). Generally, 80% of the bacterial microbes found in the rhizosphere secrete IAA, which increase the endogenous IAA levels of the plant, and it has significant effect on plant growth. Auxins are notorious to affect the whole plant, but as PGPRs produce IAA in the rhizosphere, plant roots are comparatively more affected by these IAAs (Salisbury 1994). IAA released by rhizobacteria mostly affects the root system by escalating its size and weight, branching number and the surface area in contact with soil. All these changes guide to an increase in its capacity to explore the soil for nutrient exchange, therefore enhancing plant's nutrition pool and growth ability (Gutierrez-Manero et al. 2001; Ramos-Solano et al. 2008). IAA also plays an important role for differentiation of adventitious roots from stem as auxins induce stem tissues to redifferentiate as root tissue. Etesami et al. (2015) concluded that the PGPRs residing in rhizosphere and endophytic niches could generate IAA and maintain plant growth. The ability to synthesize IAA, gibberellins and cytokinins is widespread among soil- and plant-associated bacteria responsible for plant growth promotion, symbiotic associations and also pathogenesis. It is reported that bacterial production of cytokinins and IAA is involved in the virulence of several interactions between microorganisms such as *Agrobacterium*, *Pseudomonas savastanoi*, *Bradyrhizobium japonicum*, *Acetobacter diazotrophicus*, *Herbaspirillum seropedicae* and pathogenic *Erwinia* (Morris, 1986; Litchter et al. 1995; Boiero et al. 2007). Other bacteria that have been reported to be involved in phytohormone production include members of genera *Azospirillum*, *Rhizobium*, *Bradyrhizobium*, *Enterobacter*, *Erwinia* and some species of *Pseudomonas* (Patten

and Glick 1996). Frankenberger and Arshad (1995) demonstrated the role of cytokinins, auxins, gibberellins, abscisic acids and ethylene which, when applied to plants, assist in increasing plant yield and growth. Exogenous application of cytokinin (N6-substituted aminopurines) and IAA enhances the cell division, root development and root hair formation, while it inhibits the root elongation, shoot initiation, or several other physiological processes (Salisbury and Ross 1992; Frankenberger and Arshad 1995; Amara et al. 2015; Jha and Saraf 2015; Maheshwari et al. 2015). Other developmental processes such as the formation of embryo vasculature, nutritional signaling, leaf expansion, branching, chlorophyll production, root growth, promotion of seed germination and delay of senescence are also extremely influenced by cytokinins (Wong et al. 2015). It is reported that the plant pathogenic (*Agrobacterium tumefaciens*, *Pseudomonas syringae* pv. *savastanoi* and *E. herbicola* pv. *gypsophilae*) and nonpathogenic bacteria (*Azotobacter chroococcum*, *Azotobacter beijerinckii*, *P. fluorescens* and *P. putida*) are the most important producer of cytokinins (Sakthivel and Karthikeyan 2012).

In plants, tetracyclic diterpenoid acids like gibberellins (GAs) are involved in a number of developmental and physiological processes (Davies 1995; Crozier et al. 2000). These processes include seed germination, seedling emergence, stem and leaf growth, floral induction, flower and fruit growth, root growth, root hair abundance, regulation of vegetative and reproductive bud dormancy, and delay of senescence in several organs of an array of plant species (Reinoso et al. 2002; King and Evans 2003; Sponsel 2003). There are some bacterial genera which produced gibberellins such as *Pseudomonas*, *Bacillus*, *Azotobacter* and *Azospirillum* (Ludden et al. 1978). Apart from *Azospirillum* sp. and *Rhizobium* sp., production of gibberellin-like substances has also been demonstrated in various bacterial genera (Bottini et al. 2004). Other bacterial species also produced gibberellins, for example, *Acetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *Bacillus* sp., and *Azospirillum* sp. (Bastián et al. 1998; Gutiérrez-Mañero et al. 2001; Bottini et al. 2004). There are some fungal and bacterial species such as *Fusarium moniliforme*, *Gibberella fujikuroi* and *Azospirillum lipoferum* which produced gibberellins for plant growth promotion. In maize, *Azospirillum brasilense* affects the level of gibberellins which deteriorate shoot elongation, growth, and root hair density (Fulchieri et al. 1993) but according to (Cassán et al. 2001) *Azospirillum brasilense and A. lipoferum* both bring deterioration of dwarfism in rice by affecting the level of gibberellins. Some fungal pathogens such as *Ustilago maydis* and *Magnaporthe oryzae* secrete a chorismate mutase and a monooxygenase, respectively, which affect jasmonic acid or salicylic acid homeostasis through infection process (Djamei et al. 2011; Patkar et al. 2015). Chanclud et al. (2016) reported that rice blast fungus *Magnaporthe oryzae* produced cytokinin for the pivotal requirement which affects the plant growth and virulence. In this study they identify a gene (*CKS1*) which is required for CK biosynthesis. The fungus-secreted CKs are expected and professed by the plant in the course of infection, whereas the transcriptional regulation of rice CK-responsive genes is distorted in plants infected with the mutants in which *CSK1* gene was deleted. Similarly, Galuszka et al. (2016) reported that fungal species *Claviceps purpurea* synthesized CK and altered the CK profile of ergot-infected rye

plants by CK biosynthesis genes. Some fungi species such as *Colletotrichum gloeosporioides* and *Fusarium* spp. synthesized auxins as the similar precursor same as used in bacteria (indole-3-acetamide) (Tsavkelova et al. 2012), but other fungal genera, like *Ustilago* (Reineke et al. 2008) and *Rhizoctonia* (Furukawa et al. 1996), can also produce it by indole-3-pyruvate. There are various bacterial and fungal microbes which produce various phytohormones and siderophores which affect the plant growth (Table 16.1).

Table 16.1 Production of plant growth regulators (phytohormones and siderophore) by various microorganisms and their effect on plant morphology and development

Microorganism	Observed effect on plants	References
Auxin productions		
Azospirillum, *Rhizobium*, *Bradyrhizobium*	Decrease of root length, increase of root hair development	Tien et al. (1979), Atzorn et al. (1988), and Badenosch-Jones et al. (1982)
Klebsiella	Increase in root branching and root surface	El-Khawas and Adachi (1999)
Azospirillum, *Gluconacetobacter*, *Herbaspirillum*	Corn seedlings inoculated showed an increase on free active IAA and IBA	Fuentes-Ramírez et al. (1993), Bastián et al. (1998), and Fallik et al. (1989)
Pseudomonas syringae pv. *savastanoi*	Induction of gall and tumor formation	Comai and Kosuge (1980)
Pseudomonas denitrificans, *Pseudomonas rathonis*	All the bacterial strains had been found to increase plant growth of wheat and maize in pot experiments	Egamberdiyeva (2005)
Rhizobacterial isolates	Inoculation with rhizobacterial isolates had significant growth-promoting effects on wheat and rice	Khalid et al. (2001)
Erwinia herbicola pv. *gypsophilae*	Induced the gall formation on *Gypsophila paniculata*	Manulis et al. (1998)
Agrobacterium tumefaciens	Increase host-plant susceptibility	Gohlke and Deeken (2014)
Indole-3-acetic acid		
Kluyvera ascorbata SUD 165	Decreased some plant growth inhibition by heavy metals (nickel, lead, zinc)	Burd et al. (2000)
Rhizobium leguminosarum	Inoculation with *R. leguminosarum* had significant growth-promoting effects on rice seedlings	Biswas et al. (2000) and Dazzo et al. (2000)
	Growth-promoting effects upon inoculation on axenically grown rice seedlings were observed	
Azotobacter sp.	Inoculation with strain efficient in IAA production had significant growth-promoting effects on maize seedlings	Zahir et al. (2000)

(continued)

Table 16.1 (continued)

Microorganism	Observed effect on plants	References
Rhizobacteria	Significant correlation between auxin production by PGPR *in vitro* and growth promotion of inoculated rapeseed seedlings in the modified jar experiments was observed	Arshad and Frankenberger (1997)
Azospirillum brasilense	All the bacterial strains increased rice grain yield over uninoculated control	Thakuria et al. (2004)
Azotobacter sp., *Pseudomonas* sp.	Increasing the concentration of tryptophane from 1 mg ml^{-1} to 5 mg ml^{-1} resulted in decreased growth in both (*Sesbania*, mung bean) crops	Ahmad et al. (2005)
Pseudomonas sp.	A combined bio-inoculation of diacetylphloroglucinol-producing PGPR and AMF, and improved the nutritional quality of wheat grain	Roesti et al. (2006)
Bacillus cereus RC 18, *Bacillus licheniformis* RC08	All bacterial strains were efficient in indole acetic acid (IAA) production, and significantly increased growth of wheat and spinach	Cakmakci et al. (2007)
Mesorhizobium loti MP6	*Mesorhizobium loti* MP6-coated seeds enhanced seed germination, early vegetative growth and grain yield as compared to control	Chandra et al. (2007)
Pseudomonas tolaasii ACC23	PGPR strains protect canola plant against the inhibitory effects of cadmium	Dell'Amico et al. (2008)
Bacillus sp.	The isolate SVPR 30, i.e., strain of *Bacillus* sp., proved to be efficient in promoting a significant increase in the root and shoot parts of rice plants	Beneduzi et al. (2008)
Pisolithus tinctorius	Plant growth promotion	Frankenberger and Poth (1987)
Gibberellins production		
Azospirillum brasilense, Azospirillum lipoferum	Reversion of dwarfism in maize and rice	Cassán et al. (2001)
Azospirillum brasilense	Promotion of shoot elongation, growth, and root hair density	Fulchieri et al. (1993)
Gibberella fujikuroi	"Bakanae" effect in maize, rice, and other plants	Rojas et al. (2001) and Fernández-Martin et al. (1995)
Cytokinins productions		
Azospirillum	Plant growth promotion	Tien et al. (1979)
Pseudomonas syringae pv. *savastanoi*	Induction of gall and tumor formation	Roberto and Kosuge (1987)
Erwinia herbicola	Induced gall formation	Lichter et al. (1995)

(continued)

Table 16.1 (continued)

Microorganism	Observed effect on plants	References
Rhizobium leguminosarum	Plant growth promotion	Noel et al. (1996)
Siderophore production		
Pseudomonas fluorescens	Involvement of ACC deaminase and siderophore production promoted nodulation and yield of groundnut	Dey et al. (2004)
Streptomyces acidiscabies E13	*S. acidiscabies* promoted cowpea growth under nickel stress	Dimkpa et al. (2008)
Kluyvera ascorbata SUD 165	Producing ACC deaminase and siderophore to decreased Ni^{+2} toxicity towards the plants	Burd et al. (2000)
Rhizobium meliloti	Effective in promoting the growth of nonhost, besides inhibiting *Macrophomina phaseolina* causing charcoal rot disease in groundnut	Arora et al. (2001)
Kluyvera ascorbata	Siderophore-producing PGPR and able to plant from heavy metal toxicity	Genrich et al. (1998)
Bradyrhizobium, *Rhizobium*	Decreased plant growth inhibition by heavy metals	Duhan et al. (1998) and Burd et al. (2000)
Bradyrhizobium japonicum	Induced nitrogenase activity of the host plant	Wittenberg et al. (1996)
Pseudomonas putida	Increased iron uptake under iron-stressed conditions and play important role in plant growth	Tripathi et al. (2005)
Rhizobium ciceri	Able to form symbiosis with chickpea produced phenolate-type siderophore in response to iron deficiency, nutritive component of medium	Berraho et al. (1997)
Paenibacillus polymyxa	Suppressed plant diseases and promoting plant growth	Phi et al. (2010)
Proteus vulgaris	Growth promotion and iron transport by producing α-keto acid siderophore	Rani et al. (2009)
Mesorhizobium ciceri, *Azotobacter chroococcum*	Increased plant growth promotion by producing siderophore	Wani et al. (2007)
Bacillus species PSB10	Enhancing plant growth	Wani and Khan (2010)
Pseudomonas aeruginosa, *Ralstonia metallidurans*	Enhance bioaugmentation-assisted phytoextraction	Braud et al. (2009)
Brevibacterium sp.	Growth stimulation	Noordman et al. (2006)
Bacillus sp.	Stimulate plant growth promotion	Rajkumar et al. (2006)

16.3.5 Siderophore Production

Iron is a necessary nutrient for plants and acts as a cofactor for various enzymes and plays a vital role in several physiological processes like photosynthesis, respiration and N_2 fixation, so its scarcity is exhibited in strict metabolic modifications. Iron is relatively plentiful in soils but is often unavailable for plants or soil microorganisms.

Major chemical type, Fe^{+3}, is the oxidized form that reacts to form insoluble oxides and hydroxides which is unreachable to plants and microbes. Plants have developed two types of strategies for proficient iron absorption. The first consists of releasing organic compounds competent of chelating iron, thus exposing it in soluble form where it diffuses toward the plant, gets minimized, and is absorbed in the cell membrane of the plant. The second strategy consists of absorbing the intricate formed by the organic substance and Fe^{+3}, where the iron is compact inside the plant and keenly absorbed. Some rhizosphere bacteria are capable to release iron-chelating substances into the rhizosphere and hence draw iron towards the rhizosphere where it can be fascinated by the plant (Goswami et al. 2016). Siderophores are low-molecular-weight compounds, generally lower than 1 kDa, which have functional groups capable of binding iron in a reversible approach. The most common functional groups are hydroximates and catechols, which are most favorable to bind iron. In the soil, the molar concentration of siderophore is found approximately 10–30 M. Siderophore-producing bacteria frequently belong to the genus *Pseudomonas*, where the most studied organisms are *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* which release pyochelin and pyoverdine type of siderophores (Haas and Défago 2005). Rhizosphere bacteria release these compounds to enlarge their competitive potential, by having an antibiotic activity and recover iron nutrition for the plant (Glick et al. 1995). Siderophore-producing rhizobacteria develop plant health at different levels such as improving iron nutrition, inhibiting the growth of other microbes by the release of their antibiotic molecule and obstructing the growth of pathogens by restrictive iron available for the pathogen, which are not capable to absorb the iron–siderophore complex (Shen et al. 2013).

The most important catecholate-type siderophore is enterobactin, produced by *Escherichia coli* and *Enterobacter*, which are generally linked with plants (Crowley 2006). Ratledge (1987) reported that some soil bacteria also produce different siderophores such as agrobactin (*Agrobacterium tumefaciens*), dihydroxybenzoic acid (*Erwinia* sp. and *Bacillus subtilis*), mycobactins (*Mycobacterium*, *Rhodococcus*, and *Nocardia*) and pyochelins (*Pseudomonas* spp.). Hydroxamates are the second most important group of bacterial siderophores such as schizokinen, aerobactin and ferrioxamines which are produced by *Streptomyces* spp. and *Arthrobacter* spp. (Lee et al. 2012) with hexadentate structure, which is resistant to hydrolysis and enzymatic degradation (Winkelmann 2007). The third group of bacterial siderophores is carboxylates (consist of citrate linked by ornithine), e.g., rhizobactin produced by *Rhizobium* sp. which is an effective source of iron in plants (Hider and Kong 2010) and rhizoferrin produced by *Rhizopus*, *Mucor*, *Phycomyces*, *Chaetostylum*, *Absidia*, *Cokeromyces*, *Cunninghamella*, *Mycotypha* and *Mortierella* (Drechsel et al. 1991; Thieken and Winkelmann 1992). While, *Azotobacter* and *Pseudomonas* spp. produce other different type of bacterial siderophores like pyoverdines, which is a combined form of hydroxamate and carboxylate groups (Cornelis 2010). Some other important siderophore-producing bacteria include *Escherichia coli*, *Salmonella*, *Klebsiella pneumoniae*, *Vibrio cholerae*, *Vibrio anguillarum*, *Aeromonas*, *Aerobacter aerogens*, *Enterobacter*, *Yersinia* and *Mycobacterium* species.

Soil fungi mostly produce four diverse groups of siderophores incorporated with the hydroxamate family, i.e., ferrichromes, coprogens, fusarinines and rhodotorulic acids (Winkelmann 2007). These siderophores play a vital function in the extracellular iron solubilization from minerals or organic substances. The most frequent kind of siderophore is ferrichromes (cyclic hexapeptide) which is produced by soil fungi (Leong and Nielands 1982; Deml et al. 1984). Depending on the side chain of the hydroxamate functional group, ferrichromes are divided into five groups like acetyl (ferrichrome, ferrichrome C, ferricrocin and ferrichrysin), malonyl (malonichrome), trans-b-methylglutaconyl (ferrichrome A), trans-anhydromevalonyl (ferrirubin) and cisanhydromevalonyl (ferrirhodin) (Renshaw et al. 2002; Winkelmann 2007). The ferrichrome-type siderophore is produced by *Ustilago sphaerogena* (Emery 1971) and, similarly, ferricrocin, tetraglycylferrichrome and ferrichrysin produced by *Aspergillus fumigatus* (Wallner et al. 2009), *Neurospora crassa* (Winkelmann 2007), *Cenococcum geophilum* and *Hebeloma crustuliniforme* (Martino and Perotto 2010), respectively. Some other important siderophore-producing fungi include *Aspergillus nidulans*, *A. versicolor*, *Penicillium chrysogenum*, *P. citrinum*, *Mucor*, *Rhizopus*, *Trametes versicolor*, *Ustilago sphaerogena*, *Saccharomyces cerevisiae*, *Rhodotorula minuta* and *Debaromyces species* (Kannahi and Senbagam 2014).

16.3.6 Role in Disease Suppression

Different plant diseases are caused by various plant pathogens such as fungi, bacteria, viruses and nematodes which are controlled by biocontrol agents. Bioagents use different mechanisms of action to suppress the various plant diseases and their pathogens which include direct parasitism of pathogens, inhibition of pathogens by using antibiotics and extracellular cell wall-degrading enzymes, competition for different nutrients (i.e., iron, nitrogen, or carbon) in colonization sites and stimulation, and development of plant defense mechanisms (Howell 2003; Vinale et al. 2014). For the biocontrol of plant pathogens, beneficial microorganisms may use more than one of these mechanisms that may be activated simultaneously. Genetically engineered biocontrol agents can be used for the biocontrol improvement by overexpressing one or more of these traits so that different strains with several antipathogen traits can act together (Glick and Bashan 1997). The combined use of different biocontrol agents can also promote plant growth and suppress the plant fungal diseases (Akhtar et al. 2016).

16.3.6.1 Parasitism

In several studies, it has been reported that *Trichoderma* (free-living fungi) is an opportunistic, avirulent plant symbionts and fungal parasites, which can be used as a biocontrol agent against plant-parasitic nematodes (Windham et al. 1989; Reddy et al. 1996; Rao et al. 1998). Saifullah and Thomas (1996) reported the direct *in vitro* interactions between *T. harzianum* and the potato cyst nematode *Globodera rostochiensis*. In the soil, biocontrol activities of *T. asperellum*-203 and *T. atroviride*

IMI 206040 have been reported against *Meloidogyne javanica* (Sharon et al. 2001). Other *Trichoderma* species have also exhibited considerable biocontrol activity against *M. javanica* in various experiments (Spiegel et al. 2006). Nematode eggs have been also parasitized by *T. asperellum*-203 and *T. atroviride* (Sharon et al. 2001). Further, Sharon et al. (2007) reported the parasitism of *Trichoderma* on *M. javanica* by showing the importance of the gelatinous matrix in the fungal parasitism. The biocontrol mechanisms employed by *Trichoderma* spp. involve the mycoparasitic coiling around the pathogenic hyphae forming appressorium-like structures (Lu et al. 2004) and, therefore, inhibiting the growth and dissemination of many fungal pathogens. The biocontrol activity of *Trichoderma* is well reported against several plant pathogenic fungi such as *Rhizoctonia solani*, *Botrytis cinerea*, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *Pythium* spp. and *Fusarium* spp. (Howell 2003; Gajera et al. 2012). It has been reported that the biocontrol activity of *Trichoderma* employs several other common mechanisms including nutrient competition and secretion of antifungal metabolites (Lorito et al. 1996). However, prior to physical contact between *Trichoderma* and *R. solani*, a diffusible factor is released that causes the activation of transcriptional machinery of *ech42* (endochitinase 42-encoding) genes (Zeilinger et al. 1999). Moreover, some fungi such as arbuscular mycorrhizal fungi (AMF) colonize the endophytic tissues and protect their host against biotic stress such as fungal and bacterial pathogen and plant-parasitic nematode infection (Schouteden et al. 2015). Werner and Zadworny (2002) reported the *in vitro* mycoparasitism in the rhizosphere of *Pinus sylvestris* by the ectomycorrhizal fungus *Laccaria laccata* against *Mucor hiemalis*.

16.3.6.2 Competition

In the soil, competition for resources (nutrients and oxygen) commonly occurs in between soil-inhabiting organisms. Different antagonist directly competes with pathogens for these resources for biocontrol activity. Root-inhabiting microorganisms compete for suitable sites at the root surfaces. It has been demonstrated that the competition for mineral resources particularly, carbon, is the main cause of fungistasis that results into the reduced spore germination in the soil (Alabouvette et al. 2009). Several species of *Pseudomonas* require glucose very efficiently by converting glucose to gluconic acid and 2-ketogluconic acid hence give a competitive advantage over those microorganisms that lack the ability to utilize glucose (Gottschalk 1986). Competition for trace elements, such as iron, copper, zinc, manganese, etc., also occurs in soils. The depletion of soluble and bioavailable form of iron in soil results into strong competition (Loper et al. 1997). However, some microorganisms including biocontrol microbes replenish the required amount of iron through producing siderophores (low-molecular-weight compounds with high iron affinity) under the iron-depleting conditions, which delimiting the growth of other microbes (Loper et al. 1997; Haas and Défago 2005). Suppression of soil-borne plant pathogens by siderophore-producing *Pseudomonads* has been reported in some instances (Loper and Henkels 1999). *Trichoderma* is well known for its competitive saprophytic ability. Different chemical mutagens are known to improve the competitive saprophytic capabilities of *Trichoderma* species (Rashmi et al.

2016). The possible role of competition between *T. harzianum* and *Fusarium oxysporum* was observed by Sivan and Chet (1989) during rhizosphere colonization. AM fungi and soilborne plant pathogens inhabit similar root tissues, and for colonization, there may be direct competition for space (Smith et al. 2010). Davies and Menge (1980) observed localized competition between AM fungi, *Glomus fasciculatus* and *Phytophthora*. They reported reduced development of *Phytophthora* in AM-colonized and adjacent uncolonized root systems. Further, Rosendahl (1985) reported that the *Aphanomyces* was suppressed on pea roots by AM fungi only when these two were present on the same root. Vigo et al. (2000) observed the reduction of infection sites within mycorrhizal root systems and colonization by the AM fungus and showed no effect on the spreading of necrosis.

16.3.6.3 Production of Lytic Enzymes

One of the important mechanisms used by biocontrol agents to control soilborne pathogens involves the production of cell wall-degrading enzymes. Cell wall-degrading enzymes such as β-1,3-glucanase, chitinase, cellulase and protease secreted by biocontrol strains of PGPM exert a direct inhibitory effect on the hyphal growth of fungal pathogens by degrading their cell wall. Chitinase degrades chitin, an insoluble linear polymer of β-1,4-N-acetyl-glucoseamine, which is the major component of the fungal cell wall. The β-1,3-glucanase synthesized by strains of *Paenibacillus* and *Streptomyces* spp. can easily degrade fungal cell walls of pathogenic *F. oxysporum* (Singh et al. 1999). *Streptomyces lydicus* strain A01 isolated from the soil of suburban vegetable field in Beijing, China, is capable of producing natamycin and chitinase and has a significant inhibition effect on *Botrytis cinerea* (Lu et al. 2008; Wu et al. 2013a,b). Li et al. (2015) studied the expression of *Paenibacillus polymyxa* β-1,3 and 1,4-glucanase in *Streptomyces lydicus* A01 and found that it improved its biocontrol effect against *B. cinerea. Bacillus cepacia* also synthesizes β-1,3-glucanase which destroys the cell walls of the different soilborne pathogens such as *R. solani*, *P. ultimum* and *Sclerotium rolfsii* (Fridlender et al. 1993). Different species of *Bacillus* also act as potential biocontrol agents with chitinolytic activities such as *B. licheniformis*, *B. cereus*, *B. circulans*, *B. subtilis* and *B. thuringiensis.* Different Gram-negative bacteria such as *Serratia marcescens*, *Enterobacter agglomerans*, *Pseudomonas aeruginosa* and *P. fluorescens* also have been found to possess chitinolytic activities. Frankowski et al. (2001) observed the production of chitinase by *Serratia plymuthica* C48 which inhibited the spore germination and germ-tube elongation in *Botrytis cinerea.* Mutant strains of *Stenotrophomonas maltophilia* W81 overproduced the extracellular protease which improved the biocontrol activity against *Pythium ultimum* (Dunne and collaborators 2000). Many species of *Trichoderma* and *Streptomyces* mycoparasitize the various phytopathogenic fungi by the secretion of chitinases and glucanases (Whipps 2001). De la Cruz et al. (1992) reported the secretion of different chitinolytic enzymes by *T. harzianum* in liquid culture supplemented with only chitin as a carbon source. *Trichoderma* spp. have been known to produce glucanases, protease, chitinase, and different nonvolatile and volatile antibiotics (Valencia et al. 2011; Meena et al. 2017). Harman et al. (2004) observed the induction of cell wall-degrading enzymes

such as protease and chitinase by *Trichoderma* during parasitic interaction. Naglot et al. (2015) reported the production of chitinase, β-1,3 glucanase, amylase, pectinase, chitinase and protease by *T. viride* strain playing an important role in the biocontrol of potent tea fungal pathogens in Northeast India. The in vitro antagonism of *Trichoderma* and *Gliocladium* sp. with the help of different lytic enzymes controlling some primary and secondary root diseases against certain root pathogens of tea (Baby and Chandramouli 1996; Borthakur and Dutta 1992) and bio-formulation efficacy. Antagonism of *Trichoderma* and *Gliocladium* species have been also controlled some other diseases such as thorny stem blight (Chandramouli and Baby 2002) and *Phomopsis* canker (Ponmurugan et al. 2007).

16.3.6.4 Antibiotic Production

Antibiotic, a bioactive molecule produced by various beneficial microbes, affects the interactions of plant with their pathogens. *Bacillus* genus produces an extensive variety of antibacterial and antifungal antibiotics. Some of these compounds including subtilin, subtilosin A, TasA and sublancin are well known and are derived from ribosomal origin, but others, such as bacilysin, chlorotetain, mycobacillin, rhizocticins, bacillaene, difficidin and lipopeptides belonging to the surfactin, iturin and fengycin families, are formed by non-ribosomal peptide synthetases (NRPSs) and/or polyketide synthases (PKS) (Leclere et al. 2005). *Bacillus subtilis* strains produce a variety of different antifungal metabolites, e.g., zwittermicin A, kanosamine and lipopeptides (Emmert et al. 2004; Ongena et al. 2005). Nine gene clusters (*srf*, *bmy*, *fen*, *nrs*, *dhb*, *bac*, *mln*, *bae* and *dfn*) direct the synthesis of bioactive peptides and polyketides by the enzymes NRPSs and PKS in *B. amyloliquefaciens* FZB42 (Chang et al. 2007). Antibiotics are also produced by different species of *Pseudomonas* such as *Pseudomonas fluorescens* and *Pseudomonas aeruginosa.* Different antibiotics such as phenazine-1-carboxylic acid (PCA), phenazine-1-carboxamide (PCN), pyoluteorin (Plt), pyrrolnitrin (Prn), 2,4-diacetylphloroglucinol (DAPG), oomycin A, viscosinamide, butyrolactones, kanosamine, zwittermicin A, aerugine, rhamnolipids, cepaciamide A, ecomycins, pseudomonic acid, azomycin, antibiotics FR901463, cepafungins, and karalicin are produced by these strains of *Pseudomonas*. Biocontrol fungus also produces a number of antibiotics. Gliovirin, gliotoxin, viridin, pyrones and peptaibols are produced by *Trichoderma* spp. against different fungal pathogens (Howell 2003; Harman et al. 2004). *Trichoderma* and its related genera such as *Emericelopsis* and *Gliocladium* produce peptaibol, a large family of antibiotic peptides (Daniel and Filho 2007). *Trichoderma pseudokoningii* SMF2 produces three major peptaibols, trichokonin VI (TK VI), trichokonin VII and trichokonin VIII (Shi et al. 2012). The peptaibols from *T. pseudokoningii* SMF2 exhibit broad spectrum of antimicrobial activity against Gram-positive bacteria and filamentous fungi including plant fungal pathogens. Some species of mycorrhizal fungus also produce antibiotics. *Leucopaxillus cerealis* var. *piceina* produced an antibiotic, diatretyne nitrile. which inhibited germination of zoospores of *Phytophthora cinnamomi* (Marx 1969). It has been reported that mycorrhiza-associated Streptomycetes inhibit the growth of fungi and bacteria. Schrey et al. (2012) reported the production of some antibiotics such as cycloheximide and

Table 16.2 Antibiotics produced by beneficial rhizospheric microbes

Beneficial microbes	Antibiotics
Pseudomonas spp.	Phenazines, phenazine-1-carboxylic acid, phenazine-1-carboxamide, pyrrolnitrin, pyoluteorin, 2,4-diacetylphloroglucinol, rhamnolipids, oomycin A, cepaciamide A, ecomycins, DDR, viscosinamide, sulfonamide, pyocyanin, butyrolactones, n-butylbenzene, pseudomonic acid, azomycin, FR901463, cepafungins, karalicin
Bacillus spp.	Kanosamine, zwittermicin A, iturin A (cyclopeptide), bacillomycin
Trichoderma spp.	Gliovirin, gliotoxin, viridin, pyrones, peptaibols

actiphenol from mycorrhiza-associated *Streptomyces*. Production of various antibiotics by different biocontrol agents is listed in Table 16.2.

16.3.6.5 Induction of Plant Defenses

Biocontrol strains of PGPM provide an alternate strategy to protect the plant from diseases via induced systematic resistance (ISR). The reduced dissemination of pathogenic propagules or disease severity due to the presence of PGPM-induced resistance mechanisms which are far separated from the pathogens (spatially) is termed as ISR. In addition to the presence of preformed physical and chemical barriers, plants have well-developed immune mechanisms that are able to recognize the structural patterns or conserved motifs solely associated with microbes and absent in plants known as pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs, respectively). Generally, the MAMP-triggered defense responses are very rapid and transient. The early MAMP responses are characterized by the generation of reactive oxygen molecule (ROS), nitric oxide and ethylene signaling which is further terminated by the deposition of callose and synthesis of defense-related antimicrobial molecules (Hermosa et al. 2012). Many MAMPs have been identified for PGPR, such as flagellin or lipopolysaccharides, but also secreted compounds including antibiotics, biosurfactants and volatile organic compounds have been shown to elicit systemic resistance. Rhizospheric microbes can also induce the synthesis of secondary metabolites in plants (Sekar and Kandavel 2010). Roberts and Shuler (1997) reported the accumulation of jasmonic acid and its methyl ester, as a signal transducer after the treatment of suspension cultures of *Rauvolfia canescens* and *Eschscholzia californica* with a yeast elicitor. Ajmalicine, serpentine, picrocrocin, crocetin, hyoscyamine and scopolamine, safranal compounds and tanshinone are recorded as the important metabolites produced by PGPR species in eliciting the physiological and morphological responses in crop plants. A variety of MAMPs are produced by various strains of *Trichoderma*. ET-inducing xylanase (Xyn2/Eix), an effective elicitor of defense response in some specific tobacco and tomato cultivars, was produced by *Trichoderma* as a first recognized MAMP (Rotblat et al. 2002). *Trichoderma*-activated and heat-denatured cellulases also elicit melon defenses through the activation of the SA and ET signaling pathways, respectively (Martinez et al. 2001). Swollenin *TasSwo* gene stimulates defense responses in cucumber roots and leaves (Brotman et al. 2008) and affords local protection against fungi and bacteria, and the endopolygalacturonase ThPG1 generates a response in *Arabidopsis*

similar to the ISR triggered by PGPR (Morán-Diez et al. 2009). The ISR triggered by *Trichoderma* occurs through the JA/ET signaling pathway similarly to PGPR ISR (Shoresh et al. 2005). Cerato-platanin Sm1 is required for *T. virens*-mediated ISR against *Colletotrichum graminicola* in maize (Djonovic et al. 2007). *Trichoderma* treatment of JA/ET-deficient *Arabidopsis* genotypes leads to an enhanced susceptibility to *B. cinerea* (Korolev et al. 2008). ISR triggered by PGPR and *Trichoderma* converges upstream from MYB72, an early key component of the onset of ISR (Segarra et al. 2009). A systemic increase in SA and JA levels was observed after inoculation of high densities of *Trichoderma* (Segarra et al. 2007). In one study, Gallou et al. (2009) reported that the defense signaling against *Rhizoctonia solani* by initial priming of plants (potato) with *T. harzianum* involves JA/ET or SA signaling. The elicitor molecules from bacterial PGPR system or their components induce systemic resistance in many plants such as carnation, radish and *Arabidopsis.* It is considered that some components of microbial system or their parts may act as elicitors for eliciting ISR (Fig. 16.3). The induced systemic resistance by some *Pseudomonads* involves some "O" antigenic side chain of the bacterial outer membrane lipopolysaccharide system that works as inducing determinant. The small

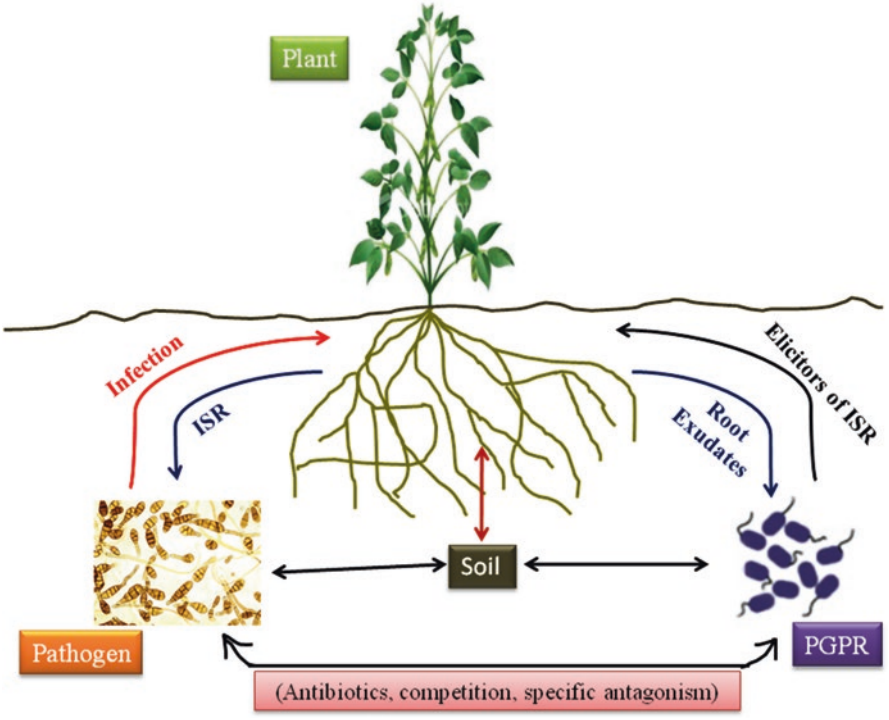

Fig. 16.3 Elicitation of systemic resistance by microbial components or their counterparts. The small molecules or some other chelating complexes may work as elicitors for induction of ISR. The root exudates attract certain beneficial microbes for host colonization and provide initial priming for disease suppression

molecules secreted by PGPR or other beneficial microbial species may also act as elicitors for inducing systemic resistance in plants. Pseudobactin, a common siderophore from *Pseudomonas*, induces systemic resistance in tobacco and *Arabidopsis*. Similarly another siderophore, Pseudomanine produced and secreted by the some *Pseudomonad* spp., has been reported to induce salicylic acid (SA) production in radish and, therefore, plays a critical role in plant defense response (Van Loon and Bakker 2006). The plant growth-promoting effect and defense response shown by certain AM fungi is the response of enhanced specific defense mechanisms that takes place due to the colonization of these fungi. The endophytic association of plants with AM fungi elicits the defense mechanism in such a manner that aggravates the predisposition of early response to attack by a root pathogen (Gianinazzi-Pearson et al. 1994).

16.4 Conclusion

In the present chapter, we have highlighted the potential beneficial aspects of different microorganisms associated with the plants. In order to compensate the world food requirement with increasing population, we need to develop new practices that help to increase the agricultural productivity through sustainable means. The use of soil microorganisms or their commercial formulations as biofertilizers and biopesticides has several advantages over other conventional techniques used for increasing agricultural production. The genetic diversity of microbial populations isolated from different agricultural regimes and their exploitation may provide clues regarding some direct or indirect mechanics for plant growth promotion.

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Rhizoremediation of Environmental Contaminants Using Microbial Communities

17

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Abstract

Over last few decades, the contamination of water and soil has become a major threat to ecosystem and human health. Bioremediation is an attractive tool to overcome the challenges posed by the traditional methods such as incineration and excavation. Recently, phytoremediation has been widely used to remediate the pollutants (such as organic and inorganic) from the environment, but certain compounds and heavy metals tend to inhibit the growth of the plants. In this chapter, we have emphasized on most accepted bioremediation process known as rhizoremediation, which involves the mutualism between microorganisms and plants that degrades the recalcitrant compounds present in the soil and makes eco-friendly environment. Furthermore, we discussed the important factors such as temperature, pH, and organic matter present in the soil, which affects the growth and metabolism of not only the organism but also the plants, interaction between plant and microorganisms, and role of endophytic and rhizobacteria in bioremediation of heavy metals and organic pollutants.

Keywords

Phytoremediation • Rhizoremediation • Endophytic bacteria • Heavy metals • Organic pollutants

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17.1 Introduction

Over the past few centuries, dramatic rise in industrialization has been witnessed leading to enhanced release of anthropogenic compounds into the natural ecosystem. A xenobiotic compound includes petroleum hydrocarbons (PHC), polycyclic aromatic hydrocarbons (PAHs), solvents, metals, pesticides, and salts. These chemicals remain persistent in nature, creating negative effect on ecosystem and human health (Prabhu et al. 2014; Gerhardt et al. 2009; Meagher 2000). Remediation of soil-contaminated sites with the help of conventional techniques such as landfilling and incineration is expensive. Methods such as incineration cause air pollution, while landfilling generates leachates in the form of gases and liquids that can contaminate ground water, and the excavation of soil/land can lead to the generation of toxic air emissions (Kuiper et al. 2004). Hence, there is a need of an hour for alternative methods for restoring the polluted sites that is less expensive, less labor intensive, and eco-friendly. In last few years, bioremediation and phytoremediation have emerged as an alternative method to the previously existing conventional methods. It involves microbes and other biological components to degrade harmful pollutants from the environment (Caplan 1993; Dua et al. 2002). Bioremediation can be applied in situ without the removal and transportation of polluted soil and without causing any disturbance to the soil matrix. Another advantage is that the bacterial degradation of chemicals and pollutants usually results in complete breakdown and mineralization (Heitzer and Sayler 1993).

In situ bioremediation process such as biostimulation, monitored natural attenuation (MNA), bioaugmentation, and phytoremediation (including rhizoremediation) has been used to restore and rehabilitate the contaminated sites. However, one remediation technology is not enough to treat the on-site pollutant as it depends on the contaminant type and source of the contaminant/pollutant (Truu et al. 2015). In recent years, two different approaches for the bioremediation are extensively used to remediate polluted/contaminated soils: microbial-assisted plant remediation (rhizoremediation) and phytoremediation. Phytoremediation is a process which uses the plants to extract, sequestrate, or decontaminate terrestrial and/or aquatic environment, while rhizoremediation utilizes the exudates released by plants which will increase the rhizospheric microorganisms that will help plant growth and the degradation/breakdown of contaminants (Gerhardt et al. 2009). In the present chapter, we discuss about the challenges and potentials of rhizoremediation to remove the persistent chemical and metals from the environment (Fig. 17.1).

17.1.1 Phytoremediation

In phytoremediation process, plants are used to sequester, extract, or detoxify pollutants. This method is cost-effective and eco-friendly since the structural integrity of the soil will be maintained (Khan et al. 2000). In phytoremediation process, genetically engineered or special plants are targeted that have the potential to uptake the pollutants from the environment (Macek et al. 2000). This process is applicable

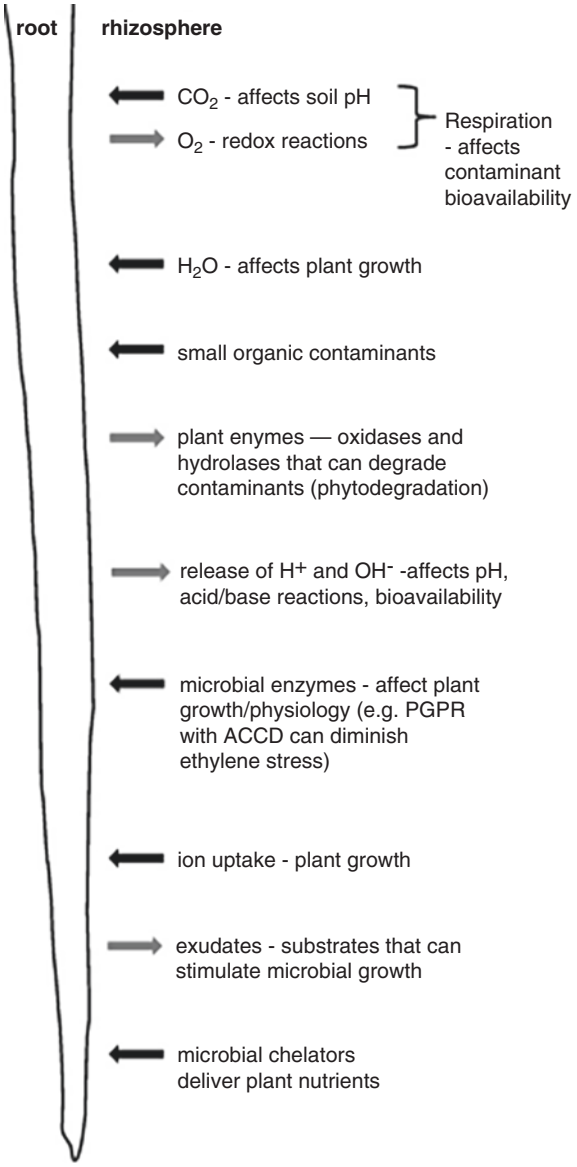

Fig. 17.1 General processes affecting rhizoremediation: plant roots support microbial growth at the root surface and in the rhizosphere. Roots create channels in soil that allow for movement of O_2 and H_2O and that are wide enough for "trapped" contaminants to become accessible to microbes. PGPR, plant growth-promotingrhizobacteria; ACCD, 1-aminocyclopropane-1-carboxylate deaminase (Adapted from Gerhardt et al. 2009)

for organic and inorganic contaminants, which are in solid and liquid form (Salt et al. 1998). Generally, phytoremediation of pollutants by a plant involves the following steps: uptake, translocation, transformation, compartmentalization, and sometimes mineralization (Schnoor et al. 1995). Several extensive research studies were performed in greenhouse laboratory level prior to the field trails. These experiments provided valuable information regarding particular type of phytoremediation mechanism of different organic contaminants. This mechanism includes

transportation of some organic compounds through the plant membranes. Especially, the compounds with low molecular weight habitually removed from the soil and are released via evapotranspiration processes through leaves. This method is also known as phytovolatilization. Some of the nonvolatile compounds can be converted or degraded into nonhazardous entities by catalytic effect of enzymes and chemical sequestration in plants. This is referred as phytodegradation and phytoextraction, respectively. The highly stable compounds in the plants can be degraded along with the biomass during sequestration or incineration (Truu et al. 2015). The uptake of the organic compounds, distribution, and transformation depends not only on physical but also chemical property of the compound (molecular weight, water solubility) and environmental condition (temperature, pH, and soil moisture content) including the plant characteristics (root system and enzymes) (Suresh and Ravishankar 2004). The phytoremediation can be used to target two major kinds of pollutants: elemental pollutants and the organic pollutants (Meagher 2000).

17.1.1.1 Elemental Pollutants

This group of pollutants includes radionuclides and toxic heavy metals, which are very difficult to remediate and only few techniques are available for it. In recent years, plants have become an attractive tool to remediate heavy metals from soil (Clemens et al. 2002; Cobbett and Goldsbrough 2002; Khan et al. 2000). The process of heavy metal removal using plants includes (1) extraction of the contaminants from soil and translocation to aboveground tissues, (2) sequestering of the contaminants in the root system to prevent/stop further spreading and leaching into soil and/or groundwater, or (3) conversion into less harmful and toxic chemicals. For this purpose some of the plants such as sunflower, tobacco, mustard, maize, and sand rocket are used because of their capacity to absorb and hyperaccumulate the pollutant (Meagher 2000). Usually the plants growing in the region enriched with heavy metals have the ability to hyperaccumulate the heavy metals and were thought to have developed a defense mechanism against herbivores. However, plants with such capabilities are rarely available, and hence in modern era, scientists are exploring to develop plant with high metal absorptivity through genetic engineering (Kuiper et al. 2004).

17.1.1.2 Organic Pollutants

This class of pollutants includes organic compounds such as polycyclic aromatic compounds, polychlorinated biphenyls, nitro-aromatics, or linear halogenated hydrocarbons. Plants like willow, alfalfa, and other grasses have the ability to completely mineralize these kinds of compounds. However, the underlying mechanisms of mineralization of these compounds are not clearly understood. Nevertheless, plants have high potential of remediating organic compounds (Kuiper et al. 2004). In addition to several advantages of using phytoremediation, it also possesses some limitations, which includes slow growth rate of the plant, limitation of plant-root penetration in soil, time-consuming, sensitive for some pollutants, and the problem of being part of a food chain, and the process is completely dependent on the climatic changes (Khan et al. 2000).

17.1.2 Rhizoremediation

A combined action of plant and microbial remediation led to a more successful approach to bioremediation of pollutants that particularly belongs to organic compounds. This approach includes bioremediation methods such as phytoremediation and bioaugmentation to remediate the contaminants. Rhizoremediation refers to the use of microbes present in and around the rhizosphere of plants, which are utilized for phytoremediation purposes (Mosa et al. 2016). In recent years, it has popped out as the most effective method to remediate recalcitrant compounds. There will be an interaction between roots, root exudates, rhizosphere soil, and microbes resulting in breakdown of organics to nontoxic or less toxic minerals. The 40% of a plant's photosynthesis is deposited into the soil as organic acids, sugars, and larger organic compounds (Gerhardt et al. 2009). Soil microbes utilize these compounds as carbon, nitrogen, and energy source (Leigh et al. 2002).The rhizosphere of the soil consists of 10–100 times more microbes per gram of soil than un-vegetated soil. In soil containing large volumes of roots, microbial populations can reach titers of 10^{12} cells/g of soil. The plants can gain various benefits by these microbial consortia such as reducing stress hormones in plants, act as a chelators for delivering key plant nutrients, protect plants from pathogens, and reduce the negative effect of recalcitrant compounds on plants by converting/degrading (Hontzeas et al. 2004; Kuiper et al. 2004). The initial study of the rhizosphere is mainly focused on breakdown of herbicides and pesticides. These research studies suggest that the bacteria tend to degrade these compounds and protect plants from negative impact of these compounds (Hoagland et al. 1994; Jacobsen 1997). In the current scenario, many reports are available on breakdown of organic compounds such as TCE (Walton and Anderson 1990), PAHs (Radwan et al. 1995), and PCBs (Brazil et al. 1995). It was observed that grass varieties and leguminous plants, viz., alfalfa, are suitable for rhizoremediation, as these plants can harbor huge number of bacterial consortium on their root system (Kuiper et al. 2004).

The effectiveness of the rhizoremediation depends on the microbes to efficaciously colonize on the growing root. A colonizing process involves multitude of genes from the microbial consortia (Capdevila et al. 2004; Lugtenberg et al. 2001; Silby and Levy 2004; de Weert et al. 2002). These genes include production of biotin and thiamine, synthesis of amino acid synthesis, O-antigen of lipopolysaccharide, and an efflux pump induced by isoflavonoids. Although the chemotactic response can be evoked by different compounds depending on the colonizing species, the key factor for successful root colonization is the chemotaxis, which is specific toward root exudate compounds (Capdevila et al. 2004; Kuiper et al. 2004; de Weert et al. 2002). Among the compounds that influence the colonization complex includes aromatic compounds such as coumarins and flavonoids which plays a key role. The accumulations of these compounds are very low as these compounds are degraded by microbial consortia and used as the carbon and nitrogen sources, respectively (Leigh et al. 2002). It is fortuitous that these aromatic compounds are similar to many organic contaminants structurally, viz., polychlorinated biphenyls (PCBs), PHC, and PAHs, thereby providing means to exploit natural processes in the rhizosphere for the bioremediation of contaminants (Jacobsen 1997).

17.1.3 Microbe-Plant Interactions in Phytoremediation

The investigation of plant-microbe interactions has been under investigation for over 50 years, but these studies were mainly focused on plant-pathogen interactions. Over the decades, the ecology of microbes in the rhizosphere was focused toward many kinds of decontamination processes. The group of organisms acquainted in the rhizosphere is associated with plants and aids in its metabolism. They were found to be in synergism with plant roots and are known as rhizosphere microorganisms. In the early twentieth century, Hiltner defined the term rhizosphere, as the volume/amount of the soil that is influenced by the roots of plants (Kavamura and Esposito 2010).

In general, the microbial consortia of rhizosphere are stimulated by the plant roots while providing proper aeration, releasing of exoenzymes, and excreting a root exudate compounds which not only provide nutrients but also provide surface for colonization, niches to protect bacteria against desiccation, and other biotic and abiotic stresses (Kuiper et al. 2004). In return, the rhizospheric microorganism boosts plant growth by nutrient mobilization, nitrogen fixation, decreasing the level of plant stress hormone, production of plant growth regulators, and degradation of pollutants before they negatively impact the plant (Fig. 17.2) (Chaudhry et al. 2005; Segura and Ramos 2013). This mutualism between plant and microbes known as rhizosphere effect results in increased number, diversity, and degradative capability of the microbes (Kent and Triplett 2002; Ramos et al. 2000). In most of the cases, the microbial consortia are responsible for biodegradation process. In rhizoremediation, the amount and composition of root exudates will be plant specific. These exudates are majorly composed of organic acids (lactate, oxalate, acetate, malate, succinate, fumarate, and citrate), amino acids, and sugars along with some secondary metabolites (viz., isoprenoids, alkaloids, and flavonoids). These are released into the soil as the rhizo-deposits; among them majority of organic acid secreted exudates are dissociated anions (carboxylates) (Jones 1998; Martin et al. 2014; Singer et al. 2003; Singh et al. 2004). Rhizo-deposition results over 10–44% of the fixed carbon (Bais et al. 2006). The exudates of the roots can be utilized by the microbial consortia as the carbon source (Singer et al. 2003). Many secondary metabolites possess a similar structure as that of contaminants thus inducing the expression of specific catabolic genes of microbial consortia, which are necessary for the degradation of the contaminant. Some of the secondary metabolites like salicylate induce the microbial degradation of PAHs (naphthalene, fluoranthene, pyrene, chrysene) and PCB (Chen and Aitken 1999; Master and Mohn 2001; Singer et al. 2000), while terpenes aid in breakdown of toluene, phenol, and TCE (Truu et al. 2015). In some cases, the metabolites cannot be used as sole carbon sources. Hence, the microbes utilize easily degradable root-exuded compounds which serve as co-metabolites (i.e., aerobic biodegradation of trichloroethylene). The interaction between rhizospheric bacteria and plant roots excretes some biosurfactants that enhance the bioavailability and uptake of pollutants (Schwitzguébel et al. 2002; Wenzel 2009). In aged soil, this process may be beneficial as they contain low contaminant (Dams et al. 2007; Gunderson et al. 2007).

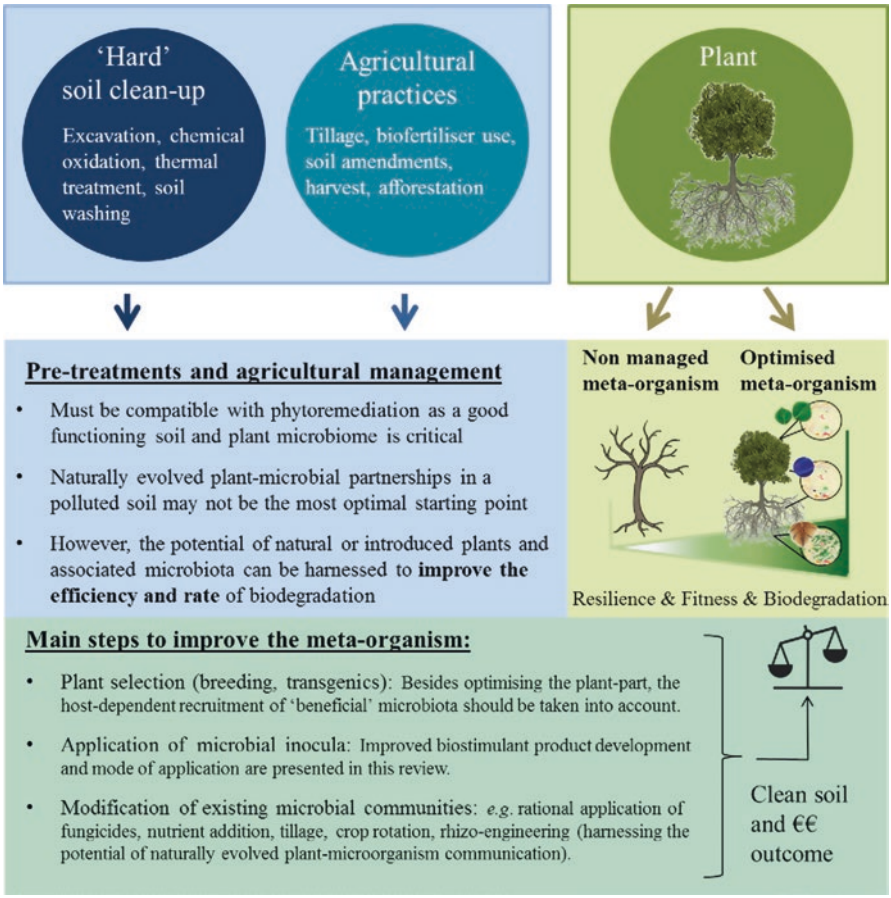

Fig. 17.2 Schematic diagram showing the integration of phytoremediation in soil cleanup treatment strains and optimization of the plant microbiome. Identification of the limiting factors to natural attenuation and overview of different approaches (e.g., rational plant selection and microbiome engineering) to turn the plant from a potential low-productivity state to a high-productivity, diverse, and resilient state with high phytoremediation activity (Thijs et al. 2017)

This microbial-assisted phytoremediation was investigated with both indigenous microbes and intentionally stimulated microbes through seed inoculation in the laboratory, greenhouse, and field. A wide range of enzymes has been found in plants, root-colonizing bacteria, endophytic bacteria, and fungi that can effectively degrade the contaminants. These include dehalogenases, dioxygenases, laccases, phosphatases, P450 monooxygenases, nitrilases, peroxidases, and nitro-reductases (Table 17.1).

Table 17.1 Plant and microbial enzymes with a role in degradation of organic compounds

Enzyme family	Catalytic action	Examples of known sources
Various plant enzymes for uptake, transport, sequestration, and degradation	General uptake and degradation	All plants
Dehalogenase	Hydrolyzes chlorine and fluorine from halogenated, aliphatic hydrocarbons (e.g., trichloroethylene), and aromatic hydrocarbons (e.g., PCBs, DDT)	*Xanthobacter autotrophicus* (B), Hybrid poplar (*Populus* spp.), *Sphingobium chlorophenolicum* (B)
Laccase	Degradation of various aromatic compounds	Alfalfa (*Medicago sativa*), *Trametes versicolor* (F), *Coriolopsis polyzona* (F)
Dioxygenase	Degradation of various aromatic compounds	*Pseudomonas* sp. (B), *Mycobacterium* sp. (B)
Peroxidase	Degradation of various aromatic compounds; reductive, dehalogenation of aliphatic hydrocarbons	Horseradish (*Armoracia rusticana*), *Phanerochaete chrysosporidium* (F), *Phanerochaetelaevis* (F), Alfalfa (*Medicago sativa*)
Nitrilase	Cleaves cyanide groups from aromatic and aliphatic nitriles	Willow (*Salix* spp.), *Aspergillus niger* (F)
Nitroreductase	Reduces nitro groups on nitro-aromatic compounds (e.g., 2,4,6-trinitrotoluene); removes N from ring structures	*Comamonas* sp. (B), *Pseudomonas putida* (B), Hybrid poplar (*Populus* spp.)
Phosphatase	Cleaves phosphate groups from organophosphates (e.g., pesticides)	Giant duckweed (*Spirodela polyrhiza*)
Cytochrome P450 monooxygenase	Hydroxylation of aromatic and aliphatic hydrocarbons	Most aerobic bacteria, all fungi, and all plants

Microbial sources are designated (B) for bacterium or (F) for fungus. All fungi except for *Aspergillus* are white-rot fungi (Gerhardt et al. 2009)

17.2 Factors Affecting Rhizoremediation

Rhizoremediation is mainly affected by various physical, chemical, and biological properties/compositions of the root-associated soil. Many studies were carried out to interpret the effects of soil moisture, pH, temperature, aeration, and organic matter composition on the breakdown of pesticides (Charnay et al. 2005; Rasmussen and Olsen 2004). Factors such as accessibility of mineral nutrients, the age of plants, and presence of contaminants affect the quantity and quality of exudates secreted. Since the rhizoremediation is majorly dependent on the nature and quality of the root exudates. The root exudates mediate the acquirement of minerals by plants, thus stimulating the microbial growth and activities in the rhizosphere, in addition to changing of some physicochemical conditions. Under stress condition, plants

respond by varying the composition of root exudates, in turn controlling the metabolic profile and activities of rhizosphere microorganisms (Chaudhry et al. 2005).

17.2.1 Soil Conditions

The physicochemical nature/composition of the soil plays a crucial role in the success of bioremediation. The microbial metabolic activity and chemical diffusion in soil depends on factors, viz., moisture, redox conditions, temperature, pH, organic matter, nutrients and nature, and amount of clay. The aerobic microbial mineralization/degradation of selected pesticides (benzolin-ethyl, isoproturon, and glyphosphate) in different types of soil at different moisture content was evaluated by Schroll et al. (2006). They found a linear correlation ($p < 0.0001$) while increasing soil moisture content (within a soil water potential range of −20 and −0.015 MPa), which increases the relative pesticide mineralization/degradation.

17.2.2 Temperature

Temperature plays a vital role in biodegradation of recalcitrant chemical compounds by microbial consortia since majority of the biochemical reactions and metabolic activity of microbes depends on thermal thermodynamics. The cell membrane permeability and cell physiology-altering proteins are majorly impacted by temperature (Alberty 2006; Mastronicolis et al. 1998).

17.2.3 pH

Most of the putrefaction of compounds are due to the enzymes secreted by the plant-microbe interactions. The catalytic activities of these enzymes are pH dependent; the optimal bacterial growth was observed at the optimal pH 6.5 and 7.5 for most of the organisms. Siddique et al. (2002) noticed that the *Pandoraea sp.* isolated from an enrichment culture degrade the HCH isomer in the pH range of 4–9. They also observed that the growth and biodegradation of α- and γ-isomers of HCH seem to be optimal when pH of the soil slurry is 9. Similar observation was made by Singh et al. (2004) while studying the putrefaction of organophosphate pesticides in the soil. They understood that the degradation was slow at acidic pH compared to that of neutral or alkaline pH.

17.2.4 Soil Organic Matter

The organic matter in soil affects the adsorption/desorption process of pesticides in the soil including the nutrients for cell growth. Perrin-Ganier et al. (2001) monitored putrefaction of isoproturon (herbicide) by introducing phosphorus (P),

nitrogen (N), and sewage sludge separately, thus observed that P and N had the greatest effect on the process of isoproturon degradation.

17.3 Role of Endophytes in Rhizoremediation

In recent few years, much is focused on the utilization of endophytic microbes/bacteria in phytoremediation to degrade xenobiotic compounds from the environment. These bacteria are nonpathogenic and find its existence in most if not all higher plant species. Some of these species such as *Pseudomonas*, *Burkholderia*, *Bacillus*, and *Azospirillum*are found most abundantly in soil (Lodewyckx et al. 2002; Moore et al. 2006). The endophytes possess plant growth-promoting ability and also pathogen controlling capability (Berg et al. 2005; Ryan et al. 2008). The major advantage of employing endophytes over other rhizospheric bacteria in phytoremediation is that, in rhizospheric bacteria, there will be huge competition among the strains. This reduces the number of desired strains, and it is very difficult to control these organisms. Conversely, endophytic bacteria are acquainted in the internal membranes/tissues of plants thus reducing the problem of competition between bacterial strains (Doty 2008; McGuinness and Dowling 2009).

Genetic modification strategies of these endophytes have gained more attention in phytoremediation process. Barac et al. (2004) reported that introduction of toluene degradation plasmid (pTOM) from *B. cepacia* G4 into a natural endophyte such as yellow lupine is capable of degrading toluene up to 50–70%. While Germaine et al. (2006) reported that interaction of natural endophytes with a genetically modified endophyte possessed the capability of degrading 2,4-dichlorophenoxyacetic acid. The same group has also reported 40% higher degradation of 2,4-dichlorophenoxyacetic acid by using *Pseudomonas putida* VM1441(pNAH7). Weyens et al. (2009a, b) showed the co-culture of genetically modified TCE-degrading strain (i.e., *P. putida* W619-TCE) along with natural TCE strain of tree growing on TCE-contaminated soil showed 90% reduction of TCE evapotranspiration under the field conditions.

The genetic engineered endophytes were used to improvise the phytoremediation of organic/inorganicpollutants and toxic metals. The incorporation of modified yellow lupine which was inoculated with pTOM-Bu61 plasmid (encoding for trichloroethylene degradation constitutively) and ncc-nre (Ni resistance/sequestration in *B. cepacia* VM1468), along with the natural yellow lupine showed significant reduction in TCE and Ni phytotoxicity. This also promoted 30% enhancement in root biomass and 50% decrease in the enzyme activities involved in antioxidative defense in the roots. In addition, to the decreasing trend in TCE evapotranspiration, it showed about a fivefold higher Ni uptake after inoculation of two types of yellow lupine plants together (Weyens et al. 2010). The bioaugmentation of two grass species (*FestucaarundinaceaSchreb*. and *FestucapratensisHuds*) along with the endophytic fungi (*Neotyphodiumcoenophialum* and *Neotyphodiumuncinatum*) showed 80–84% and 64–72% of PAH and TPH reduction compared to that of control plants, which showed only 30% removal(Soleimani et al. 2010). Apart from the

rhizosphereendophytes, the culturable endophytes in aquatic plants showed enhancement in phytoremediation(Chen et al. 2012). It was shown that genetically engineered endophyticbacteria possess much easier in application than genetic plants because it has the ability to colonize multiple plants, and it also benefits plants by reducing stress hormones, nitrogen fixation, and phosphate solubilization (Dimkpa et al. 2009; Doty et al. 2009; Gai et al. 2009).

17.4 Polycyclic Aromatic Hydrocarbons (PAHs)

Rhizoremediation is a process which uses effect of both microbial degradation and plant growth for the breakdown of toxic compounds to less toxic/volatile compounds (Song et al. 2004; Tang et al. 2010). Tang et al. (2010) conducted the pilot plant experiments to analyze the outcome of bioaugmentation and environmental factors for rhizoremediation of petroleum-contaminated soils using different plant species. Among the tested sources, ryegrass resulted in 5% total petroleum hydrocarbons (TPH) degradation in soil. They observed that with different microbial species and plant growth-promoting rhizobacteria (PGPR), the TPH degradation increased in the following order: cotton +PGPR > cotton + EMA > cotton + PGPR > cotton > control. They suggested that rhizoremediation can be increased with proper optimization of the factors like plant growth and EMA microbial community in soil (Tang et al. 2010; Tyagi et al. 2011). Huang et al. (2005) developed a technique known as multiprocess phytoremediation system (MPPS) which consists of contaminant-degrading bacteria, land farming (aeration and light exposure), plant growth-promoting rhizobacteria (PGPR), and growth of the contaminant-tolerant plant, i.e., tall fescue (*Festucaarundinacea*). Using the MPPS, they were able to remove 90% of all fractions of TPHs from soil. Figure 17.2 clearly shows the combined strategies for phytoremediation.

17.5 Petroleum Hydrocarbons (PHCs)

Petroleum hydrocarbons (PHCs) are organic compounds comprised of carbon and hydrogen atoms arranged in varying structural configurations. They are classified in two main categories, namely, diesel range organics (DROs) and gasoline range organics (GROs). GROs include mono-aromatic hydrocarbons such as toluene, benzene, xylenes (BTEX), ethylbenzene, and short-chain alkanes (C6–C10) with low boiling points (60–170 °C) such as 2,3-dimethyl butane, isopentane, n-butane, and pentane. DROs consist of long-chain alkanes (C10–C40) and hydrophobic chemicals like polycyclic aromatic hydrocarbons (PAH) (Gkorezis et al. 2016; Kamath et al. 2004). Petroleum hydrocarbons (PHCs) are biodegradable and bio- and phytoremediable (Gkorezis et al. 2016). The plant-associated bacteria include phyllospheric, endophytic, and rhizospheric bacteria. The mutualism between these host plants and the bacteria allows for greater survivability and treatment of polluted soils by mutual benefitting both the organisms (Weyens et al. 2009b, 2015). Possible

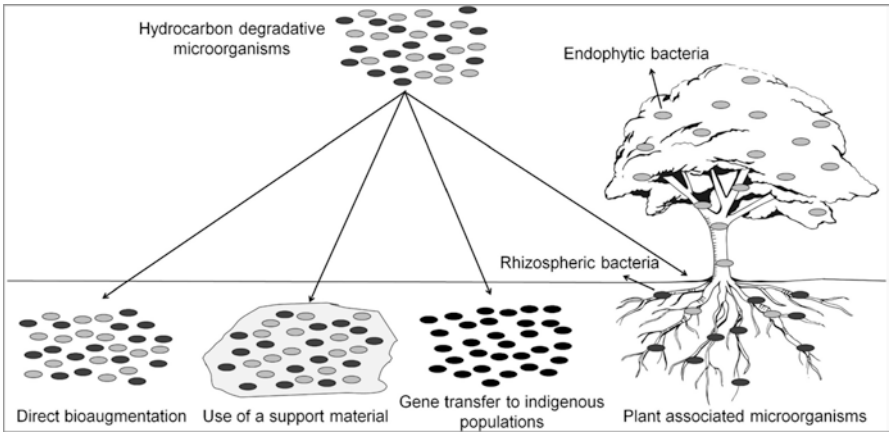

Fig. 17.3 Possible strategies for the bioremediation of PHC-contaminated sites (Gkorezis et al. 2016)

mechanism for the bioremediation/rhizoremediation of PHC-contaminated sites is shown in Fig. 17.3.

The capability of the microbes to breakdown PHCs is greatly contributed to the presence of catabolic genes and enzymes that helps them to use PHCs as energy source (Das and Chandran 2010). Table 17.2 shows the interaction of microbes and plant species for breakdown of PHC component. The advantages and disadvantages of phytoremediation over traditional technologies are shown in Table 17.3.

17.6 Rhizoremediation of Heavy Metals

Rhizoremediation, a special case of phytoremediation, is a process, which exploits the microbial species present in the rhizosphere of plants. These microbes share a symbiotic/mutualistic relationship with the roots of plants and aid in retrieving soils polluted with heavy metals (Fig. 17.4). These heavy metals not only possess a serious threat to the surrounding ecosystem but also are more probable to get absorbed by plants through roots and enter the food chain. Subsequently, it reaches the animal kingdom from Kingdom Plantae (Ganesan 2012). Heavy metals are classified from their traditional analogs in the sense that these metals have density greater than 5 g/cm^3 (Kareem et al. 2016). It is renowned that heavy metals are present ubiquitously in soil in trace amounts. However, from the advent of industrialization and urbanization over the past few centuries, it has been a customary habit for humans to release heavy metals and other harmful pollutants into the environment. Apart from natural occurrences, the main sources of heavy metals include industrial wastes, fertilizers, and petroleum byproducts. These heavy metals act as genotoxic substances and interfere with protein synthesis, respiration, and carbohydrate metabolism (Khan et al. 2009). Consequently, they result in poor growth and low

Table 17.2 Selected paradigms of successful rhizodegradation of PHCs (Gkorezis et al. 2016)

Plant species	Microorganisms	PHC component	References
Zea mays	*Pseudomonas* sp. strain UG14Lr, *Pseudomonas putida* strain MUB1	Phenanthrene/ pyrene	Chouychai et al. (2009, 2012)
Lolium perenne	*Pantoea* sp. strain BTRH79	Diesel oil	Afzal et al. (2012)
Lotus corniculatus	*Pantoea* sp. strain BTRH79	Diesel oil	Yousaf et al. (2010)
Medicago sativa	*Rhizobium meliloti* strain ACCC17519	Various PAHs	Teng et al. (2015)
Zea mays	*Gordonia* sp. strain S2RP-17	Diesel oil	Hong et al. (2011)
Lolium multiflorum	*Acinetobacter* sp.	Various PAHs	Yu et al. (2011)
Secalecereale, *Medicago sativa*	*Azospirillum brasilense* strain SR80	Crude oil	Muratova et al. (2010)
Lolium multiflorum	*Rhodococcus* sp. strain ITRH43	Diesel oil	Andria et al. (2009)
Sorghum bicolor	*Sinorhizobium meliloti* strain P221	Phenanthrene	Muratova et al. (2009)
Hordeum vulgare	*Mycobacterium* sp. Strain KMS	Pyrene	Child et al. (2007a, b)
Triticum aestivum	*Pseudomonas* sp. strain GF3	Phenanthrene	Sheng and Gong (2006)
Trifolium repens	*Rhizobiumleguminosarum*	Chrysene	Johnson et al. (2004)
Hordeum vulgare	*Pseudomonasfluorescens*, *Pseudomonas aureofaciens*	Phenanthrene	Anokhina et al. (2004)
Lolium multiflorum	*Pseudomonas putida* strain PCL1444	Various PAHs	Kuiper et al. (2001)
Hordeum vulgare	*Pseudomonas putida* strain KT2440	Various PAHs	Child et al. (2007a, b)

Table 17.3 Advantages and disadvantages of phytoremediation over traditional technologies (Das and Chandran 2010; Stępniewska and Kuźniar 2013)

Advantages	Disadvantages
Relatively low cost	Longer remediation times
Easily implemented and maintained	Climate dependent
Several mechanisms for removal	Effects to food web might be unknown
Environmentally friendly	Ultimate contaminant fates might be unknown
Aesthetically pleasing	Results are variable
Reduces landfilled wastes	
Harvestable plant material	

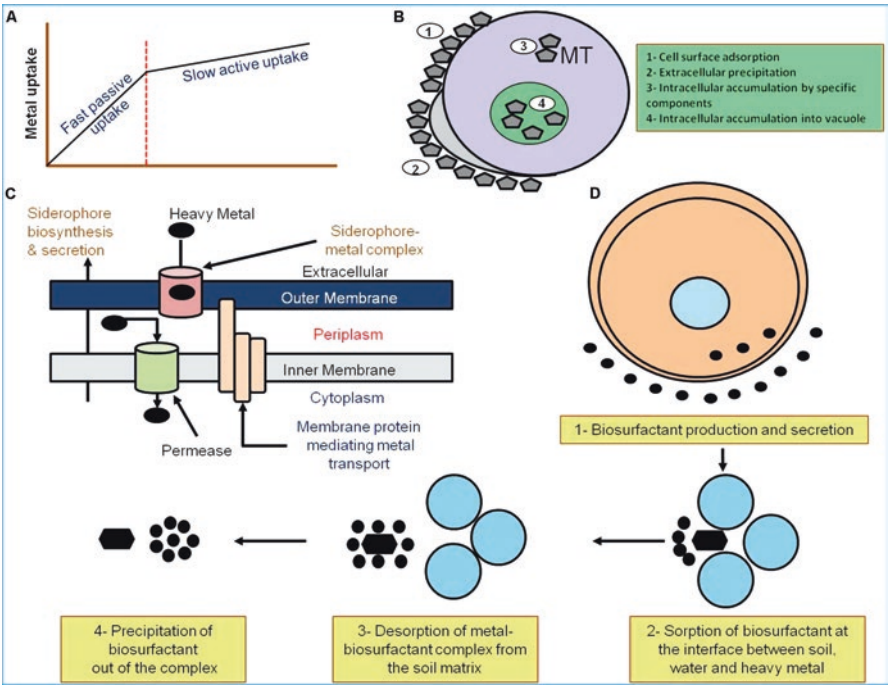

Fig. 17.4 Mechanism of microbial remediation. (**a**) Passive and active heavy metal uptake by biological materials. (**b**) Mechanisms of heavy metal biosorption by bacterial cells. Bacterial biosorption of heavy metals through (1) cell surface adsorption, (2) extracellular precipitation, (3) intracellular accumulation through special components, such as metallothioneins (MT), or (4) intracellular accumulation into vacuoles. (**c**) Heavy metal remediation via siderophore formation. (**d**) Mechanism of bacterial heavy metal remediation through biosurfactant production (Adapted from Kareem et al. 2016)

yield of crops. Different origins of heavy metals, their density, and toxicity to living beings are shown in Table 17.4 (adapted from Seshadri et al. 2015).

Many biological agents have entangled themselves in removing these hazardous entities and preventing the plants from getting being damaged. The microbial population in the rhizosphere tends to act alone or as a part of community in eliminating these metals. Plants and their mutually associated microbial allies are tabulated in Table 17.5 (adapted from Kamaludeen and Ramasamy 2008). This bacterium present around the roots tends to reduce/increase the absorption of metals by plants, stabilizes the metals by forming organo-complexes, and diminishes the heavy metal accumulation/aggregation in the rhizosphere. *Pseudomonas putida*, a gram-negative bacterium, was found to show high tolerance against heavy metals such as cobalt, zinc, cadmium, copper, nickel, and lead (Uslu and Tanyol 2006). *P. putida* TPHK-1 was found to be a highly efficient and unique strain especially in breaking down the diesel oil in the presence of heavy metals. Tolerance toward heavy metals tied together with celerity in deprivation of hydrocarbons from soil, even at high

Table 17.4 Sources of heavy metals in soils and their expected ionic species in soil solution

Metal	Density (g/cm^3)	Ionic species in soil solution	Contaminant sources	Toxicity[a]
Arsenic (As)	5.73	As(III): $As(OH)_3$, AsO_3^{3-}, As(V): $H_2As_4^-$, $HAsO_4^{2-}$	Timber treatment, paints, pesticides, geothermal	Toxic to plants, humans, and animals
Cadmium(Cd)	8.64	Cd^{2+}, $CdOH^+$, $CdCl^-$, $CdHCO_3^+$	Electroplating, batteries, fertilizers	Toxic to plants, humans, and animals
Chromium(Cr)	7.81	Cr(III): Cr^{3+}, CrO_2^-, $CrOH^{2+}$, $Cr(OH)_4^-$, Cr(VI): $Cr_2O_7^{2-}$	Timber treatment, leather tanning, pesticides, dyes	Cr(VI) toxic to plants, humans, and animals[b]
Copper (Cu)	8.96	Cu^{2+}(II), Cu^{2+}(III)	Fungicides, electrical, paints, pigments, timber treatment, fertilizers, mine tailings	Toxic to plants, humans, and animals
Lead(Pb)	11.35	Pb^{2+}, $PbOH^+$, $PbCl^-$, $PbHCO_3^-$, $PbSO_4$	Batteries, metal products, preservatives, petrol additives	Toxic to plants, humans, and animals
Manganese (Mn)	7.21	Mn^{2+}, $MnOH^+$, $MnCl^-$, $MnCO_3$	Fertilizer	Toxic to plants
Mercury (Hg)	13.55	Hg^{2+}, $HgOH^+$, $HgCl_2$, CH_3Hg^+, $Hg(OH)_2$	Instruments, fumigants, geothermal	Toxic to humans and animals
Molybdenum (Mo)	10.2	MoO_4^{2-}, $HMoO_4^-$, H_2MoO_4	Fertilizer	Toxic to animals
Nickel (Ni)	8.9	Ni^{2+}, $NiSO_4$, $NiHCO_3^+$, $NiCO_3$	Alloys, batteries, mine tailings	Toxic to plants and animals
Zinc (Zn)	7.13	Zn^{2+}, $ZnSO_4$, $ZnCl^+$, $ZnHCO_3^+$, $ZnCO_3$	Galvanizing, dyes, paints, timber treatment, fertilizers, mine tailings	Toxic to plants

Adapted from Seshadri et al. (2015)

[a]Most likely to observe at elevated concentrations in soils and water

[b]While Cr(VI) is very mobile and highly toxic, Cr(III) is essential in animal and human nutrition and generally immobile in the environment

concentrations, indicates that *P. putida* TPHK-1 is a promising strain in remediating both hydrocarbons and heavy metals simultaneously (Ramadass et al. 2016). Siderophores were found to be iron-chelating agents present in microbes such as *Pseudomonas fluorescens-putida* group and increased the yield of crops up to 144% (Joseph et al. 1980).

Plant growth-promoting rhizobacteria (PGPR) is the title given to the group of bacteria, which helps in growth of plants by remediating the soil. However, different routes are exploited by different bacteria in remediating the soil, which are contaminated with heavy metals as depicted in B, C, and D in Fig. 17.1 (Adapted from Kareem et al. 2016). The rate at which the metal is taken up can either be passive (fast) or active (slow). Similarly, other mechanisms like direct biosorption,

Table 17.5 Microbes and their communities associated with plants in metal rich soils

Plants	Microbe/Microbial communities and their characteristics	Soil nature
Thlaspi goesingense	*Holophaga/Acidobacterium division* and *α- proteobacteria*, *Methylobacteriummesophilicum*, *Sphingomonas*	Ni-rich serpentine soils
T. caerulescens	Ni-resistant bacteria predominant in rhizosphere than bulk soils	
Alyssum murale	Ni-resistant, siderophore, and acid producing bacteria more in rhizosphere than bulk soils	
	Sphingomonas macrogoltabidus, *Microbacterium liquefaciens*, *M. arabinogalactanolyticum*	
A. bertolonii	Gram-positive α-proteobacteria	
Rinorea bengalensis, *Dichapelatum gelonioidesssp. andamanicum*	*Pseudomonas*, *Bacillus*, *Cupriavidus sp.*	
Agrostis tenuis	*Arthrobacter*, *Ochrobactrum*, *Bacillus*, *Serratia* sp., and AM fungi – *Acaulospora*, *Gigaspora*	
Pteris vittata	*Pseudomonas*	As-contaminated cattle dip sites
Phragmites sp.	Cu-tolerant, exopolymer producing bacterial communities, predominantly, *Bacillus*	As-contaminated soils

Adapted from Kamaludeen and Ramasamy (2008)

siderophore formation, and remediation through biosurfactants are most common among microbes.

17.7 Conclusion

From the existing literature, it is imminent that phytoremediation is an attractive and potent tool for remediating the toxic pollutants present in the environment. Rhizoremediation, a special phytoremediation technique that involves both plants and microbes, elucidates their usage in removing hazardous components. However, with the exponential increase of population and ever-increasing pollution, the progress made in remediating is gloomy. On the other hand, it is promising to note that the allocation of assets and awareness in the society toward such eminent concerns is augmenting day by day. In conclusion, the near future holds more hope on a larger scale toward such promising maneuvers than the contemporaneous.

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Microbial Pesticides: Development, Prospects and Popularization in India

18

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Abstract

India has a vast potential for microbial pesticides, as our economy is agriculture based; however, its adoption needs education for their maximum gains. The scientists should also explore all the possibilities for popularization and constraints in this emerging field. Extensive and inappropriate pesticide use has caused pest resistance to major groups of pesticides, resurgence of secondary pests, high pesticide residue in the produce and decimation of natural enemies. Their excessive use has caused adverse effects on human beings and environment. An eco-friendly alternative to chemical pesticides is biopesticides, which falls into three classes. These include microbial pesticides, plant-incorporated protectants (PIPs) and biochemical pesticides. The microbial pesticides comprise of bacteria, fungi, protozoans and viruses. This chapter also includes the genetic improvement of microbial pesticides, use of microbial pesticides in India, role of microbial pesticides in bio-intensive integrated pest management (IPM) and their advantages and disadvantages.

Keywords

Biopesticides • Microbial pesticides • *Bacillus thuringiensis* • *Trichoderma* • *Metarhizium* sp

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18.1 Introduction

In the field of agriculture, there are numerous problems of pests like insects, fungi and weeds from the ancient period resulting in decrease in yield as well as productivity of crops. Pests are being introduced/shifted continuously to the new areas either naturally or accidentally or introduced with new crops or global warming. In some cases, organisms introduced intentionally become pests. Global trade has caused an increased numbers of invasive non-native pest species being introduced to new areas. For control these invasive species requires an unparalleled challenge the world over. For this reason, the challenges are to secure high-quality yield and to make agriculture profitable and environmentally compatible. Chemical means of plant protection occupy an important place as regards to their volume/quantity of application in integrated pest and disease management of plants. Due to their residual effect, they cause toxicity to humans and other animals.

Despite many years of effective control by using conventional agrochemical insecticides, the continuous use of these chemicals has threatened their effectiveness. It includes development of insecticide resistance and use cancellation or deregistration of insecticide(s) due to human health and environmental problems. Therefore, an eco-friendly alternative is the need of the hour. Improvement in pest control strategies includes higher quality and greater quantity of agricultural products. Therefore, there is a great need to develop effective, biodegradable and environment-friendly biopesticides. Priority should be given to biopesticide use against pests to avoid adverse impact of chemical insecticides (Mazid et al. 2011).

18.2 Biological Pesticides

The biopesticides are certain types of pesticides derived from natural materials such as microorganisms, plants, animals and certain minerals. For example, canola oil, neem oil and baking soda have pesticidal applications and are considered biopesticides. By 2001, there were about 195 registered biopesticide active ingredients and 780 products. These are biochemical pesticides, which are naturally occurring substances for control pests by nontoxic mechanisms. They are living organisms (natural enemies) or their products (phytochemicals/botanical pesticides, microbial products) or byproducts, which can be used for the management of pests that are injurious to crop plants. Biopesticides have a vital role in crop protection, although most commonly in combination with other tools including chemical pesticides as part of bio-intensive integrated pest management (IPM). They are biological pesticides based on pathogenic microorganisms, which are specific to a target pest; they offer an ecologically beneficial and effective control to pest problems. They are safer to the environment and to human health as they are degradable, and there is no residue effect on human beings. Commonly used microbial pesticides are microorganisms, which have pathogenic effect on pest of interest. These include bioinsecticides (*Bacillus thuringiensis*, *B. sphaericus*), biofungicides (*Trichoderma*) and

bioherbicides (*Phytophthora*). The benefits to agriculture through the use of biopesticides are considerable (Gupta and Dikshit 2010). The biopesticides are often effective in very small quantity and degrade quickly which results in lower exposures, avoiding the pollution problems.

18.3 Types of Biological Pesticides

18.3.1 Microbial Pesticides

Microbial pesticides are naturally occurring or genetically altered bacteria, fungi, algae, protozoans or viruses. These can be effective and used as alternatives to chemical insecticides. Microbial toxins are biological toxin material derived from microorganisms, viz. bacterium or fungus. Pathogenic effect of those microorganisms on the target pests is species specific. The effect of microbial entomopathogens occurs due to invasion through the integument or gut of the insect, which results in multiplication of the pathogen causing killing of the host, e.g. insects. The pathogens produce insecticidal toxin important in pathogenesis. Most of the toxins produced by microbial pathogens are identified as peptides, but they vary greatly in terms of structure, toxicity and specificity (Burges 1981).

These microbial pesticides offer an alternative to chemical insecticides having target specificity and ecological safe due to which that they are used either alone or in combination with other pest management programmes. One definition for integrated pest management (IPM) which is most relevant to this practice comes from Flint and van den Bosch (1981): 'It is an ecologically based pest control strategy which relies on natural mortality factors and control devices that disrupt these factors as little as possible. An integrated pest management program considers all available pest control actions, and evaluates the potential interaction among various control practices, cultural operations, weather, other pests, and the crop to be protected' (Flint and van den Bosch 1981).

They have efficiency and safety for humans and other non-target organisms. They leave less or no residue in food. They are ecologically safe, so that other natural enemies are free of their threats, leading to preservation of other natural enemies, and increased biodiversity in managed ecosystem. So, microbial agents are highly specific against target pests so they facilitate the survival of beneficial insects in treated crops. This is the reason that microbial insecticides are being developed as biological control agents during the last three decades. Microorganism, e.g. a bacterium, fungus, virus or protozoan as the active ingredient can control different pests, although each active ingredient is relatively specific for its target pest. For example, there are some fungi that control certain weeds, and others kill specific insects. One bacterial species like *Bacillus thuringiensis* may be more effective on *Aedes aegypti*, while another *B. sphaericus* strain can be effective on different types of mosquitoes like *Culex quinquefasciatus* (Lacey et al. 2001).

18.3.2 Plant-Incorporated Protectants (PIPs)

Plant-incorporated protectants (PIPs) are pesticidal substances produced from plant genetic material that has been incorporated to the plant. For example, scientists take the gene for the *Bt* pesticidal protein and introduce it into the plant genome. Now, GM plant, instead of the *Bt* bacterium, produces the substance that kills pest. Both the protein and its genetic material are regulated by EPA (Environmental Protection Agency); the plant itself is not regulated (Mazid et al. 2011).

18.3.3 Biochemical Pesticides

Biochemical pesticides are naturally occurring substances, for example, plant extracts, fatty acids or pheromones, controlling pests by nontoxic mechanisms. Conventional pesticides are synthetic materials that usually kill or inactivate the pest. Biochemical pesticides consist of substances that interfere with growth or mating, PGRs (plant growth regulators) or substances that repel or attract pests (pheromones). It is sometimes difficult to determine the mode of action of natural pesticides, EPA has established a committee to determine whether a pesticide meets the criteria for a biochemical pesticide (Mazid et al. 2011).

18.4 Microbial Pesticides

18.4.1 Bacteria

Several efforts have been made to establish microbial insecticides, like *Bt*, which is in use commercially over 40 years (Gelernter and Schwab 1993). Commercial *Bacillus* species such as *Bacillus thuringiensis israelensis* Bti and *Bacillus sphaericus* 2362 (Bs) were found particularly effective against mosquito (Revathi et al. 2013) and other dipteran larvae. Bti was discovered in 1975 to increase toxicity against mosquito larvae (Goldberg and Margalit 1977). Various bacterial species and subspecies, especially *Bacillus*, *Pseudomonas*, etc., have been established as microbial pesticides which control insect pest and plant diseases. The most salient among these are insecticides based on several subspecies of *Bacillus thuringiensis* Berliner. These include *B. thuringiensis* sp. *kurstaki* and *B. thuringiensis* sp. *aizawai*, which are highly toxic to lepidopteran larval species, and *B. thuringiensis israelensis*, with activity against mosquito larvae, black fly (simuliid) and fungus gnats. Other examples are *B. thuringiensis tenebrionis* having activity against coleopteran adults and larvae, most notably the Colorado potato beetle (*Leptinotarsa decemlineata*), and *B. thuringiensis japonensis* strain *Buibui*, with activity against soil-inhabiting beetles (Carlton 1993; Copping and Menn 2000). *Bt* produces crystalline protein that kills specific target insect pests like lepidopteran species. *Bt* crystalline proteins binding with gut receptor determine the target insect pest (Kumar 2012).

Toxicity of *Bti* and some other toxic strains is mainly due to the parasporal inclusion bodies (δ-endotoxins) produced during sporulation time. These endotoxins are assimilated by the larvae resulting in high toxicity. *Bt* and their subspecies produce different insecticidal crystal proteins (δ-endotoxins), and these toxins, when ingested by the larvae, damage the gut tissues resulting in gut paralysis (Chilcott et al. 1983; Aronson and Shai 2001). After that, the infected larvae stop feeding, and finally they die from the combined effects of starvation and midgut epithelium impairment (Betz et al. 2000; Zhu et al. 2000; Darboux et al. 2001). Other microbial pesticides act by outcompeting insect pest organisms. Microbial pesticides should be continuously supervised so that they do not cause injury to non-target organisms, including humans (Mazid et al. 2011). Gray et al. (2006) reported *Bt* toxins (bacteriocin) produced by plant growth-promoting rhizobacteria, having insecticidal attributes. *Bt* is marketed the world over for the control of several important plant pests, mainly caterpillars, mosquito larvae and black flies. Commercial *Bt*-based products include powders containing a combination of dried spores and crystal toxins. They are applied at site of feeding of larvae on leaves or other environments. *Bt* toxin genes have been genetically engineered into several crops.

Seed bacterization of clusterbean (*Cyamopsis tetragonoloba*) with *Pseudomonas maltophilia* controlled root rot up to 40.8% when co-inoculated with *Rhizoctonia bataticola*, *R. solani*, *Fusarium oxysporum* and *Sclerotinia sclerotiorum* under screen house conditions (Yadav et al. 2007). *P maltophilia* also served as plant growth-promoting rhizobacteria along with biocontrol agent when tested alone or in combination with bioinoculants in chickpea under field conditions as it increased seed yield and reduced crop mortality (Pathak et al. 2007a and b).

18.4.2 Fungi

The pathogenic fungi are also an important group of microbial pest management organisms (Khachatourians 2009) that grow in both terrestrial and aquatic habitats and when specifically associated with insects are known as entomopathogenic fungi. These may be obligate or facultative, commensals or symbionts of insects. The pathogenic action depends on contact, and they infect and/or kill sucking insect pests, viz. aphids, thrips, mealy bugs, whiteflies, scale insects, mosquitoes and all types of mites (Barbara and Clewes 2003; Pineda et al. 2007). Entomopathogenic fungi are promising microbial biopesticides that have a multiplicity of mechanisms for pathogenesis. Biopesticides are covered into 12 classes within 6 phyla and belong to 4 major groups such as *Laboulbeniales*, *Pyrenomycetes*, *Hyphomycetes* and *Zygomycetes*. Common microbial biopesticides species include *Beauveria bassiana*, *Metarhizium anisopliae*, *Nomuraea rileyi*, *Paecilomyces farinosus* and *Verticillium lecanii*. Many of them have been commercialized globally (Table 18.1). These fungi attack the host via the integument or gut epithelium and establish their conidia in joints and integument (Pekrul and Grula 1979). Some species such as *B. bassiana* and *M. anisopliae* cause muscardine insect disease, and after killing the host, cadavers become mummified or covered by mycelial growth (Miranpuri and

Table 18.1 List of some important microbial pesticides

Entomopathogenic bacteria	
Lepidoptera	*Bacillus thuringiensis* subspecies kurstaki, *B. thuringiensis* subspecies aizawai
Coleoptera: Scarabaeidae, *Popillia japonica*	*B. thuringiensis* subspecies japonensis, *Paenibacillus popilliae*
Entomopathogenic fungi	
Coleoptera (Scarabaeidae)	*Beauveria brongniartii*
Hemiptera, Thysanoptera	*Conidiobolus thromboides* Acari
Hemiptera	*Aschersonia aleyrodis*, *Lecanicillium longisporum*
Coleoptera, Diptera, Hemiptera, Isoptera	*Metarhizium anisopliae* sensu lato
Lepidoptera	*Nomuraea rileyi*

Source: Pekrul and Grula (1979)

Khachatourians 1995). Some fungi, primarily *Streptomycetes*, also produce toxins that act against insects (Dowd 2002). There are about 50 compounds, which have been reported as active against various insects belonging to Lepidoptera, Homoptera and Coleoptera, Orthoptera and mites (Cole and Robinson 1972). The most active toxins are cycloheximide, actinomycin A and novobiocin. Spinosyns are commercially available biopesticidal compounds originally isolated from the actinomycete *Saccharopolyspora spinosa* (Sparks et al. 1999) and are active against dipterans, hymenopterans, siphonapterans and thysanopterans but are not much active against coleopterans, aphids and nematodes (Sparks et al. 1999). The entomopathogenic fungi have relatively broad host range and are amenable for mass production. The fungi penetrate through the insect cuticle and sporulate on the dried insects, which provide the way for epizootics (Pathak and Kumar 2016).

18.4.3 Viruses

Insect-specific viruses can be highly effective for natural control of several caterpillar pests. Epizootics generally kill populations of some pests, particularly when insect numbers are high. Insect viruses are to be eaten by an insect to cause infection but may also spread from insect to insect during egg laying or mating. Baculoviruses are target-specific rod-shaped viruses, which can destroy and infect a number of important plant pests. Their large-scale production poses certain difficulties, so their use has been limited to small areas. Nuclear polyhedrosis viruses and granulosis viruses are available to control some caterpillar pests (Suman and Dikshit 2010). Viral products for codling moth, *Heliothis zea* and beet armyworm nuclear polyhedrosis virus have been registered for control of pest Lepidoptera, such as the cotton bollworm and cotton budworm (Arthurs and Lacey 2004; Arthurs et al. 2005). Baculoviruses are effective against lepidopterous pests of cotton, rice and vegetables. They are produced by some IPM centres but not available commercially in India.

18.4.4 Protozoan

Although protozoans infect a wide range of pests naturally and induce chronic and debilitating effects that reduce the target pest populations, the use of protozoan pathogens as biopesticide agents has not been very successful. Taxonomically, protozoans are subdivided into several phyla, some of which contain entomogenous species. Microsporan protozoans have been investigated extensively as possible components of integrated pest management programmes. Microsporidia are ubiquitous, obligatory intracellular parasites that are disease agents for several insect species. Two genera, *Nosema* and *Vairimorpha*, have potential as they attack lepidopteran and orthopteran insects and cause killing of hoppers more than any other insect (Lewis 2002). Spores in infected midgut cells are sloughed into the gut lumen, and they are eliminated along with faeces to the maize plant. The spores remain viable and are consumed during larval feeding, and the infection cycle is repeated for next generation. There is vertical transmission, when a female larva (*Nosema*) is infected and passed to the filial generation. As the infected larva matures to form an adult, the ovarial tissue and developing oocytes become infected with *N. pyrausta*. The embryo is infected within the yolk, and when larvae hatch, they are infected with *N. pyrausta* resulting in horizontal as well as vertical transmissions in natural populations of European corn borer. *N. pyrausta* suppresses populations of European corn borer by reducing oviposition, percentage hatch and survival of infected neonate larvae (Bidochka and Khachatourians 1991). The only protozoan which has been registered for use as a biopesticide is the microsporidian *Nosema locustae.* This organism is most effective when ingested by nymphal stages of grasshoppers and kills them within 3 to 6 weeks postinfection (Bidochka and Khachatourians 1991). However, not all infected grasshoppers are killed by this protozoan infection.

18.5 Microbial Products as Biopesticides

In addition to the proteinaceous toxins, microorganisms are also known to produce anti-pest chemical compounds. Fermentation provides a readily screenable source of bioactivity against target organisms of agricultural interest. Antinsectan compounds derived from nonfilamentous bacteria (e.g. thiolutin, aminolevulinic acid, thuringiensin, xenorhabdins), actinomycetes and some fungi (e.g. actinomycin A, aplasmomycin, avermectins, citromycin, piericidins, spinosyns, milbemycins, nikkomycin, cyclic peptides, etc.) are well known as toxins, antifeedants, growth inhibitors and physiological disrupters against a variety of pests (Dowd 2002; Koul and Dhaliwal 2002; Kirst 2010). Some of these compounds have been commercialized, such as avermectins and spinosyns.

18.6 Genetic Improvement

18.6.1 Bacteria

The genetic improvement of microbial pesticides aims to make them more effective by increasing their rate of reproduction, speed of transmission and infective ability or increasing the quantity of toxin produced. For example, genetic transformation of *B. thuringiensis* has produced a strain that displays insecticidal activity against both coleopteran and lepidopteran insects (Lereclus et al. 1992). The activity of *B. thuringiensis* on crop or soil can be enhanced by genetic manipulation. *B. thuringiensis* crystal proteins of the *Cry34* and *Cry35* classes function as binary toxins which show activity on the western corn rootworm, *Diabrotica virgifera virgifera*. *Cry34A/Cry35A* pairs are more active than the *Cry34B/Cry35B* pairs. The binary *Cry34/Cry35 B. thuringiensis* crystal proteins are closely related to each other, are environmentally ubiquitous and share sequence similarities consistent with activity through membrane disruption in target organisms. Modified Cry35 proteins whose segments, domains and motifs have been exchanged with other proteins enhance insecticidal activity against the test pathogen and rootworms (Schnepf et al. 2007). Similarly, toxin polypeptide (Cry8Bb1) from *B. thuringiensis* has been engineered to contain a proteolytic protection site, making it insensitive to a plant protease, helping to protect the toxin from any proteolytic inactivation. Modified Cry8Bb1 has been used for control of corn rootworms, boll weevils, wireworms, Colorado potato beetles and the alfalfa weevils (Abad et al. 2008). *B. cereus* group genomes have a *Bacillus* enhancin-like (*bel*) gene, which increases the insecticidal activity of *B. thuringiensis*-based biopesticides and transgenic crops based on *B. thuringiensis* genes (Fang et al. 2009). *Bel* genes encode peptides, which have 20–30% similarity with viral enhancin protein. These proteins enhance viral infections as they degrade the peritrophic matrix of insect midguts. The combination of *Bel* and *Cry1Ac* increased the mortality rate 2.2-fold (Fang et al. 2009).

Bacillus thuringiensis is widely used as biopesticide globally. It is a pathogen of lepidopterous pest like the American bollworm in cotton and stem borer in rice. *B. thuringiensis*-based biopesticides are effective tools against secondary lepidopterans. For instance, the cabbage head caterpillar is quite susceptible to most of the Cry1A toxins such as Cry1Aa, Cry1Ab and Cry1Ac (Srinivasan and Hsu 2008). Legume pod borer (*Maruca vitrata*) was found to be highly susceptible to Cry1Ab and Cry1Cabin in Taiwan and West Africa (Machuka 2002; Srinivasan 2012).

18.6.2 Fungi

Two commonly used entomopathogenic fungi, *Metarhizium anisopliae* and *B. bassiana*, have been extensively studied for elucidation of pathogenic processes and manipulation of the genes of fungi to improve biocontrol performance (St. Leger and Wang 2010). Additional copies of the gene encoding the regulating cuticle-degrading protease Pr1 were inserted into the genome of *M. anisopliae* and

overexpressed. The mutant reduced survival time in tobacco hornworm (*M. sexta*) by 25% when compared with the parent wild-type strain (St. Leger et al. 1996). Scorpion toxin (AaIT) expressed in the *M. anisopliae* strain ARSEF 549 showed maximum virulence. The modified fungus gave the same mortality rates in *M. sexta* at 22-fold lower spore doses than the wild type, and it reduced survival time by 40% (Wang and Leger 2007).

Trichoderma is effective against soil-borne diseases root rot of dry land crops such as groundnut, black gram and chickpea. While, *Trichogramma* lays eggs in the eggs of various lepidopteran pests of sugarcane inter node borer, pink boll worm, spotted bollworm in cotton and stem borer in rice. They are also used against vegetable and fruit pests.

18.7 Research and Development of Microbial Pesticides

Over the past decade, rapid development has occurred in the field of molecular biology, protein engineering and genetic engineering, all gradually improving the microbial pesticide production. This field had developed substantial application prospects, with extensive social and economic benefits. The superior characteristics of microbial pesticides attracted have made them a hot spot of research in biotechnology institutions and companies. The research and application of microbial pesticides are gradually replacing the highly toxic pesticides in the market. Chemical pesticides' production has declined by 2% per year (Cheng et al. 2010), while microbial pesticides' production is increasing at the rate of 20% annually. Bailey et al. (2010) reported that in 1972–2008 in Canada, Pest Management Regulatory Agency approved registration of 24 microbial active substances with 83 formulations. The majority of the registrations (55/83) occurred up to 2000, and in 2008 alone, there were ten new products (a combination of new active substances, strains, formulations and uses) under regulatory evaluation (Table 18.2). The main varieties are *Bt* pesticides, viral pesticides (*Heliothis armigera nuclear polyhedrosis viruses* (NPV), etc.) and fungal pesticides (*Trichoderma*, etc.). The

Table 18.2 Various biopesticides registered under Insecticides Act of 1968

S. no.	Name of biopesticide
1	*Bacillus thuringiensis var. kurstaki*
2	*Bacillus thuringiensis var. israelensis*
3	*Bacillus thuringiensis var. galleriae*
4	*Bacillus sphaericus*
5	*Trichoderma viride*
6	*Pseudomonas fluorescens*
7	*Trichoderma harzianum*
8	NPV of *Helicoverpa armigera*
9	NPV of *Spodoptera litura*
10	*Beauveria bassiana*
11	*Neem based pesticides*
12	*Cymbopogon*

sales of *Bt* products was $ 984 million in 1997 and went up to $ 3.6 billion in 2005. In 2006, the global leading species of microbial pesticides were as follows: *Bt CryF1*, *NRRL21882* (*Aspergillus flavus*), *Bacillus licheniformis* strain *SB3086*, etc. (Wang 2006; Xu 2008).

In India, by 2006, 194 substances had been registered as chemical pesticides, but only 12 biopesticides (such as *Bt*, *Trichoderma*, *Pseudomonas* and *Beauveria* species) were registered. The new developed and registered microbial pesticides are increasing at a rate of 4% each year; however, the market share of biopesticides has risen to 30% (Gupta 2006).

18.7.1 The Use of Microbial Pesticides in India

Various success stories about utilization of microbial pesticides and biocontrol agents in Indian agriculture have been reported (Kalra and Khanuja 2007). *Bacillus thuringiensis* controls diamond back moths and *Helicoverpa* on cotton, pigeon pea and tomato; *Beauveria* controls mango hoppers, mango mealy bugs and coffee pod borer; NPV controls *Helicoverpa* on gram; *Trichogramma* controls sugarcane borer; and *Trichogramma*-based products control rots and wilts in various crops.

Various microbial pathogens have shown resistance to *B. thuringiensis*. Within the last few years, at least 16 insect species have been identified that exhibit resistance to *B. thuringiensis.* δ-Endotoxins under laboratory conditions and field-evolved resistance have been documented in noctuids such as *Spodoptera frugiperda*, *Busseola fusca* and *H. zea* (Tabashnik et al. 2009). Reports of resistance development in field populations of *Plutella xylostella* are essentially from the countries where *Bacillus thuringiensis* is extensively used, i.e. China, Japan, the Philippines, India, Malaysia and North America. To avoid this resistance problem, genetic engineering was considered as a useful tool where microbial genes from *B. thuringiensis* were transferred to form transgenic plants, and today we have *B. thuringiensis* cotton (*Bt* cotton) and *B. thuringiensis* maize available in 13 and 9 countries, respectively. They are grown on 42.1 million ha of land (Shelton et al. 2008). The development of such transgenics was seen as a panacea in terms of microbial control of pests; however, field resistance in *H. zea* as a result of an increase in the frequency of resistance alleles is alarming (Tabashnik et al. 2008). Factors associated with field resistance are the failure to use high-dose *B. thuringiensis* cultivars and lack of a sufficient refuge. While implementation of the high-dose/refuge insect resistance management strategy has been successful in delaying field resistance to *Bt* crops (Huang et al. 2011), gene pyramiding is another tool used to address the emerging resistance problem (Zhao et al. 2003; Manyangariwa et al. 2006). Pyramiding means the stacking of multiple genes so that more than one toxin is expressed in the transgenic plant.

The management of plant diseases, viz. dry root rot of chickpea and cotton (*Rhizoctonia bataticola*), *Sclerotinia* stem rot of mustard (*Sclerotinia sclerotiorum*), *Phytophthora* rot/gummosis (*Phytophthora* spp.), canker (*Xanthomonas axonopodis pv. citri*) and fruit dropping and postharvest fruit decay in Kinnow, was

a formulation of fungal bioagent *Trichoderma* (2×10^7 cfu g^{-1}), bacterial bioagent *Pseudomonas fluorescens* (1×10^9 cfu g^{-1}) or yeast bioagent *Sporidiobolus pararoseus* (10^9 cfu ml^{-1}) (Gaur and Sharma 2012).

Despite several advantages of biopesticides, the rate of their consumption is not up to mark as compared to chemical pesticides. The main reasons are short shelf life, susceptibility to environmental conditions, expensive production systems and efficacy problems.

18.7.2 Role of Microbial Pesticides in IPM

Crop protection has relied basically on synthetic chemical pesticides in the past, but their use is now declining as a result of new laws and legislations and the evolution in the process of insect resistance. Therefore, it is necessary to replace the pest management strategy. Microbial pesticide is the best alternative to synthetic chemical pesticides based on living microorganisms or natural products. In the European Union, there are new opportunities for development of microbial pesticides in combination with integrated pest management, ecological science and postgenomic technologies (Chandler et al. 2011). In this regard, the use of microbial pesticides and bioagents has assumed significance as an important component of IPM due to their economic viability and eco-friendly nature instead of chemical synthetic pesticides (Birch 2011). Microbial pesticide application as a component of IPM programmes can play important role in overcoming disadvantage of chemical insecticides that have some important characteristics such as biodegradable, self-perpetuating and less harmful on beneficial pests, mostly host specific and less shelf life (Matyjaszczyk 2015).

18.7.3 Advantages of Microbial Pesticides

The main advantages of microbial pesticides are as follows and are also listed in Table 18.3:

- Microbial insecticide organisms are nontoxic and nonpathogenic to humans, wildlife and other organisms not closely related to the target pest. The greatest strength of microbial pesticides is their safety.
- Microbial insecticides have toxic mode having specificity to a single group or species of insects. Most of the microbial insecticides generally do not directly affect beneficial insects (including predators or parasites of pests) in treated areas.
- Microbial insecticides can be used in conjunction with the chemical insecticides as in most cases they are not deactivated or damaged by residues of conventional insecticides.
- Residues of microbial insecticides have no hazards to humans or other beneficial organisms. So these can be applied even at the time when a crop is almost ready for harvest.

Table 18.3 Benefits of biopesticides

Advantages	Characterization
Cost-effectiveness	Costlier but with less number of applications
Persistence and residue effect	Mostly biodegradable and self-propagating
Knockdown effect	Delayed
Handling and bulkiness	Bulky: carrier based using talc powder
	Easy: liquid formulations
Pest resurgence	Less
Resistance	Less prone
Effect on beneficial flora	Less harmful on beneficial insects (pest specific)
Waiting time	Almost nil
Nature of control	Preventive as well as killing of pests
Shelf life	Less/dependent on storage conditions

- In some cases, the microbial pesticides become established in a pest population or in its habitat providing control during subsequent pest generations or seasons.
- They encourage the beneficial soil microflora and enhance root and plant growth. In this way they take a part in the increase of crop yield.
- Various examples are biofungicides (*Trichoderma*), bioherbicides (*Phytophthora*) and bioinsecticides (*Bacillus thuringiensis*). The interest on microbial pesticides is based on the advantages associated with such products which are (1) inherently less harmful and lead to less environmental load, (2) designed to affect only few target organisms or in some cases one specific pest only and (3) often effective in very small quantities decomposing quickly and thus avoiding the pollution problems used alone or as an integral component of integrated pest management (IPM) programmes.

18.7.4 Disadvantages of Microbial Pesticides

Naturally, there are also limitations, which are listed below, but advantages overcome the disadvantages. These factors just provide users to choose effective microbial products.

Because a single microbial insecticide is specifically used for target species or group of insects, so it may control only specific pests present in a field and garden. Other types of pests which are present in the treated area remain continue to cause damage. Similar limitations are applied to conventional insecticides because they too are not equally effective against all pests. This is because of selectivity indeed, and this negative aspect is often more noticeable between general predators, chemicals and microbials. Sometimes, predators and chemicals may also be dangerous to other beneficial insects in threatened area.

- Heat, moisture unavailability or exposure to ultraviolet radiation/bright sunlight reduces the effectiveness of several types of microbial insecticides. Consequently, proper timing/storage conditions and method of application are especially important for some products.

- Special formulation and storage procedures are required for microbial pesticides.
- As several microbial insecticides are pest specific or target specific, the potential market for these products may be limited. Consequently, some products are not commonly available or are relatively expensive (several insect viruses). Although, biopesticides are used as alternative pest management strategies, several constraints such as developing stable formulations, standardizing appropriate delivery methods, lack of biopesticides/microbial pesticides based pathogenic microorganisms specific to a target pest.

18.7.5 Future Prospects

Management of pest populations such as plant pathogens and insects is attracting global attention as safer strategy while posing less risk to human beings and the environment. In the USA, Environmental Protection Agency monitors the microbial pesticides which also supports their registration based on the findings of 'no unreasonable adverse effects' to humans and the environment to permit their sale and distribution under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) as well as ensures a 'reasonable certainty of no harm' under the Federal Food, Drug, and Cosmetic Act (FFDCA) to provide pesticide residue-free food and feed (Leahy et al. 2014). Most of the times, farmers are affected by the problems of pesticide resistance and withdrawal of plant protection products, and yet they are 'policy takers' rather than 'policy makers'. Hence, a public-private partnership (PPP) approach to the development, manufacturing and sale of environment-friendly alternatives to chemical pesticides for developing countries is the need of the day.

Research in production, formulation and delivery may greatly assist in commercialization of microbial pesticides. More emphasis should be given towards integrating biological agents into production system and the use of biopesticides in conjunction with chemical pesticides having integrated approach. At the same time, it is also required to encourage public-funded programmes, commercial investors and pesticide companies to take up microbial pesticide enterprises. Constitution of strict regulatory mechanisms to maintain their quality and availability at affordable cost in the developing countries is equally important. Thus, various aspects of microbial pesticides covering the current status, constraints, prospects and regulatory network towards their effective utilization for the benefit of human kind need to be reviewed regularly.

Increased adoption mainly depends upon:

1. Concentrate efficacy of microbial pesticides to control crop damage and resultant increase in crop yield and quality.
2. Prices should be affordable.
3. Strengthening of extension system and supply chain management to increase the usage of microbial pesticides. For it an effective delivery system is quite essential.

18.8 Conclusion

Microbial pesticides/biopesticides can be an alternative to the chemical pesticides as indiscriminate pesticide use is detrimental to the environment and human health and also increases insect resistance to pesticides. The biopesticides are being used as alternative pest management strategies; several constraints such as developing stable formulations, standardizing appropriate delivery methods, lack of registration procedures, etc. are associated with their introduction and promotion in most of the developing world. The demand for microbial pesticides is rising steadily in all parts of the world. When used in integrated pest management systems, microbial pesticides' efficacy can be equal to or better than conventional products. By combining performance and safety, biopesticides perform efficaciously with minimum application restrictions along with human and environmental safety benefits. It is likely that in the future, their role will be more fruitful in agriculture and horticulture.

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Potential of Beneficial Bacteria as Eco-friendly Options for Chemical-Free Alternative Agriculture

19

Ch. Srinivasarao and M. Manjunath

Abstract

Plant-microbial interactions are important determinants of crop and soil health. Microbial inoculants, viz. *Azotobacter*, *Azospirillum*, *Rhizobium*, *Pseudomonas* and *Bacillus*, have been commonly employed for increasing plant growth and crop yields and improving soil health in rice, wheat, legumes, vegetables and other cropping systems. Around the world, different bacterial isolates have proven their abilities to improve plant growth through colonization of roots, production of plant hormones (such as indole acetic acid, cytokinins), biological nitrogen fixation, organic matter decomposition, solubilization, transformation and mobilization of nutrients and improve fertility of soil, besides controlling plant diseases. This compilation critically analyses the advantages of such biological inputs particularly bacteria, emphasizing their roles and the need to augment the incorporation of such biological inputs by gradually restricting the use of chemical inputs by employing suitable combinations of useful microbes for chemical-free sustainable agricultural production.

Keywords

Plant-microbe interactions • PGPR • Nutrient enrichment • Soil fertility

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19.1 Introduction

There is an urgent necessity to increase and sustain the agricultural production from decreasing land and changing climatic conditions. Modern agricultural methods with high inputs of harmful agrochemicals are leading to the slow decline of our natural resources and pollution of soil, water and air. Alternatives to these harmful chemicals are required to sustain the agricultural production the world over (Farrar et al. 2014). Microorganisms have enormous ability to augment the yield and productivity in various cropping systems (Pereg and McMillan 2015; Ahemad and Kirbet 2014). Their significance in plant health management is widely recognized (Hardoim et al. 2015). They are the potential alternatives to harmful agrochemicals and have very important roles to play in developing sustainable agricultural technologies (Horrigan et al. 2002; Oteino et al. 2015). With increasing emphasis and rising global concern for environmental and food quality, the utilization of useful bacteria for reducing the use of agrochemicals in agriculture is a potentially promising option. They are useful environment-friendly supplements, which can remarkably reduce the application of chemicals. More recently, there is a revival of interest in environmental friendly, sustainable agricultural practices, which has accelerated research efforts on biological agents (Esitken et al. 2005; Mitter et al. 2016). Bacteria are applied to various crops, viz. cereals, legumes, fruits and vegetables, to enhance growth, seed emergence and crop yield, and several products/formulations have been developed and validated across crops in different regions (Minorsky 2008; Kumar et al. 2013; Prasanna et al. 2013a, b, c). The intricacy of the soil ecosystem is dependent upon interactions among its physical, chemical and biological factors, as regulated by the existing environmental situations (Buscot 2005). The functional characteristics of diverse microorganisms have a considerable influence on soil functions (Nannipieri et al. 2003). Several interactions of microorganisms microbial are controlled by specific molecules/signals (Pace 1997) and are associated with nutrient cycling and maintenance of health of soil and plants (Barea et al. 2004). Soil microorganisms interact with roots (Glick 1995; Barea et al. 2002), and their interactions lead to the occurrence of a vibrant environment referred to as the rhizosphere, where microorganisms communicate with each other, microfauna and the plants (Barea 2005). The variation in physical, chemical and biological characteristics of the rhizospheric soil vis-à-vis that of bulk soil is the determinant of activity and diversity of microorganisms that subsequently results in increased crop productivity (Kennedy 1998). The use of beneficial microbes as sustainable agricultural option has spread tremendously in various parts of the world, due to a significant increase observed in plant growth and yield of important crops (Amara and Dahdoh 1997; Biswas et al. 2000; Asghar et al. 2002; Chen et al. 1994; Hilali et al. 2001; Nain et al. 2010; Prasanna et al. 2013a, b, c; Manjunath et al. 2015, 2016). This compilation gives an overview of the promising role of plant beneficial bacteria as environment-friendly options for sustaining agricultural productivity.

19.2 Plant Beneficial Bacteria

Plant-associated microbes reside in the phyllosphere, rhizosphere and inside tissues of plants (Klopper et al. 1999). They can be used as biofertilizers and biopesticides. They help the plants by several mechanisms.

19.2.1 Plant Growth-Promoting Rhizobacteria (PGPR)

Interactions between microorganisms and plants in the rhizosphere are considered as the determinants of soil fertility and plant productivity (de Souza et al. 2015). The rhizosphere harbours different kinds of microorganisms, which may show useful, neutral or damaging effects on plant. Bacteria are extremely enormous; a gramme of rhizospheric soil contains 10^{10} cells with 10,000 different species (Reid and Greene 2012). Rhizosphere interactions play a very important role in transformation, solubilization, mobilization of nutrients in the soil and nutrient uptake by the plants to reap full genetic capabilities of the crop and combat diseases and pests (Fatima et al. 2009; Kennedy 1998).

The need of the day is to increase the efficiency of the limited amount of external inputs by utilizing the suitable combinations of useful microbes for sustainable agricultural production. Beneficial bacteria that dwell on plant roots and improve plant growth are referred as plant growth-promoting rhizobacteria (Frankenberger and Arshad 1995; Arshad and Frankenberger 1998). Rhizobacteria derive nutrients from plant root exudates and in return help growth and development of plants by different mechanisms.

Species of *Rhizobium*, *Bradyrhizobium*, *Azotobacter*, *Bacillus*, *Streptomyces*, *Pseudomonas*, *Azospirillum*, *Herbaspirillum*, *Thiobacillus*, *Klebsiella*, *Enterobacter* and *Serratia* have established their role in enhancing crop productivity (Oberson et al. 2013). Such bacteria positively influence plant growth by improving nutrient cycling or producing growth-promoting substances such as auxins and other plant hormones and vitamins, besides indirectly influencing by suppressing pathogens through the synthesis of antibiotics (Hardarson 1993; Gupta et al. 2015; Ramadan et al. 2016). The growth-promoting capability of certain bacterial species may be very specific to particular plant species, genotypes and cultivar (Nowak 1998). For example, *Azotobacter* strains derived from *Cucurbita maxima*, jute and wheat rhizospheric soils were crop specific (Poi and Kabi 1979). Apart from improving root development through the provision of nutrients, they also indirectly influence the microfauna and flora through modifications of root exudates.

19.2.2 Endophytes

Endophytic microbes live inside the healthy tissues of plants without negatively affecting them (Schulz and Boyle 2006). They are most commonly found in the intercellular spaces. Endophytes have tremendous potential to improve productivity

of various crops (Brader et al. 2014; Antoun et al. 1998). Every single plant that is present on this planet earth harbours endophytic microorganisms, but only few species have been studied (Strobel et al. 2004). They employ different indirect and direct mechanisms to stimulate plant growth (Chaturvedi et al. 2016). Since the endophytes live in similar place as that of plant pathogens, they have enormous potential to suppress them by synthesizing new metabolites (Berg et al. 2005). Extensive and systematic research on endophytes will lead to identification of new metabolites/drugs for successful management of diseases in plants and humans (Strobel et al. 2004). They also help in seedling emergence, elicit plant growth under stress environment by osmotic and stomatal regulation (Chanway 1997; Compant et al. 2005a, b) and improve crop growth by phosphorus solubilization, nutrients uptake, root development and production of IAA, vitamins and siderophore (Bent and Chanway 1998; Wakelin et al. 2004; Lee et al. 2004; Costa and Loper 1994; Pirttila et al. 2004; Compant et al. 2005a, b; Xinxian et al. 2011). Nowadays, endophytes especially bacteria are more elaborately used in forest regeneration and bioremediation of contaminated soils. Yang et al. (2011) isolated 72 endophytic bacteria from healthy tomato leaves and stems of field-grown plants and found that the strain W4 inhibited the *Botrytis cinerea* Pers, up to 78% in dual culture test and 100% using fermentation filtrate diluted 20 times. An endophytic bacterium *Azospirillum lipoferum* minimizes the ill effects of drought by producing gibberellins and abscisic acid in maize (Cohen et al. 2009). The *Enterobacter* sp. and *Klebsiella* sp. improved the growth of *Piper nigrum* (Jasim et al. 2013).

19.3 Mechanisms of Plant Beneficial Bacteria

Beneficial bacteria stimulate plant growth by different mechanisms, and the interrelationships between these mechanisms have not been well characterized (Glick 1995; Kloepper 1993). The direct mechanisms include biological nitrogen fixation; solubilization of phosphorus, potassium and zinc; and synthesis of plant hormones, antibiotics, vitamins and siderophores (Bowen and Rovira 1999; Glick 1995; Lalande et al. 1989 Bloemberg and Lugtenberg 2001; Glick 1995; Persello-Cartieaux et al. 2003; Prasanna et al. 2013a).

19.3.1 Improving Nutrient Availability to Plants

The potential option to avoid environmental degradation is the utilization of bioinoculants (Bashan and Levanony 1991, Kennedy et al. 2004; Welbaum et al. 2004), which, through their extensive interactions with plant roots, lead to dynamic changes in the status of nutrients, water and gases. Novel and innovative ways of plant growth improvement are required to relieve the burden imposed on our environment and other resources by modern agriculture. The extensive application of useful bacteria to crop plants as inoculants is the need of the hour as they substantially reduce requirement of chemical fertilizers and pesticides, which often contaminate the

environment (Bhardwaj et al. 2014). The microorganisms have key roles in chemical transformations, including mobilization and solubilization of fixed nutrients in soil. The microorganisms, which are responsible for improving the soil fertility and plant growth, are referred to as biofertilizers. The most capable nitrogen fixers are *Rhizobium*, *Mesorhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium* and *Allorhizobium* (Graham and Vance 2000). These bacteria fix atmospheric nitrogen through symbiosis with specific legume plants by the formation of root/stem nodules via intrinsic signalling systems. Non-symbiotic/free-living nitrogen-fixing bacteria include *Azotobacter*, *Azospirillum*, *Herbaspirillum*, *Acetobacter* and *Azoarcus* (Steenhoudt and Vanderleyden 2000). They employ nitrogenase enzyme complex that works under limited oxygen conditions, *Azospirillum* predominantly found in the rhizosphere, whereas the other bacteria live as endophytes inside the plant parts like leaves, stems and roots. In comparison with synthetic fertilizers, formulations containing useful microbes help plant in several ways by providing an eco-friendly environment for sustaining soil and crop productivity (O′ Connell 1992).

19.3.1.1 Nitrogen

Nitrogen is the very important macronutrient required by the plants. Bacterial biofertilizers can help in minimizing the use of chemical nitrogenous fertilizers. A range of diazotrophs such as *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Azospirillum*, *Azotobacter*, *Azorhizobium*, *Bacillus*, *Herbaspirillum* and *Klebsiella* can supplement the nitrogen by biological nitrogen fixation (BNF) (Hegazi et al. 1998; Kennedy 1998; Kennedy and Islam 2001; Okon and Labandera-Gonzalez 1994). The estimated amount of nitrogen fixation by *Rhizobium*-legumes and cereal-bacterial associations is between 20 and 100 kg N/ha and 10–30 kg N/ha for each crop, respectively (Kennedy and Islam 2001; IARI 2014). They increase yield of about10–60% in case of legumes and 10–20% in cereals and vegetables (IARI 2014). Both greenhouse and field studies have shown the capability of *Azospirillum* to increase yield in the range 5–30% (Okon and Labandera-Gonzalez 1994). Inoculation of seeds with isolates of PGPR significantly promoted growth followed by grain yield (13.7%) and straw yield (12.4%) of wheat cv. Pasban 90 under unsterile conditions (Khalid et al. 2004). A study conducted in the eastern part of Turkey (Erzurum and Ispir) using two nitrogen-fixing and phosphorus-solubilizing PGPR strains separately and together (OSU-142 + M-13 + *Azospirillum* sp.245) improved nutrient content and yield of wheat in comparison with recommended and half doses of nitrogenous fertilizer application (Turan et al. 2010). Bacterial inoculations including the consortia notably enhanced the plant uptake of nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, iron, manganese, zinc and copper. The treatment of seeds with OSU-142 + M-13 + *Azospirillum* sp.245 may be able to supplement up to 80–90% of N and P fertilizer requirements in wheat production (Turan et al. 2010). Two rhizobacterial strains *Pseudomonas fluorescens* (ACC50) and *P. fluorescens* biotype F (ACC73) with ACC deaminase activity, chitinase activity, phosphorus solubilization, auxins production led to superior root colonization improved root weight and straw yield in wheat. The strain *P. fluorescens* (ACC50) was more promising than *P. fluorescens* biotype F, as it considerably improved the

root weight by 43%, 40%, 31%, 24% and 19% over controls at 0%, 25%, 50%, 75% and 100% fertilizers, respectively (Shaharoona et al. 2008). Kloepper et al. (1989) showed up to 30% and 43% yield increase with *Azotobacter* and *Bacillus* inoculation in wheat. The cultures of *Pseudomonas putida* and *Pseudomonas fluorescens* increased shoot and root length in wheat and potato. Bacterial and cyanobacterial strains (bacteria-cyanobacteria - Bacillus sp. + *Brevundimonas diminuta* + *Anabaena* sp.; *Bacillus* sp. + *Calothrix* sp. + *Anabaena* sp.) in wheat crop showed the synergistic effects on crop growth and productivity in addition to enhanced soil fertility (Nain et al. 2010).

19.3.1.2 Phosphorus

Phosphorus (P) is the second most important essential plant nutrient. It is applied to soils as phosphatic fertilizers. However, large part of applied inorganic fertilizers rapidly gets immobilized and becomes nonavailable to plants. Hence, P availability to plants is a very important issue particularly in areas with high fixation and immobilization problems (Manske et al. 2001; Salimpour et al. 2010; Shaharoona et al. 2008; Vig and Singh 1983). Soil microbes have abilities to augment the phosphorus availability by mineralizing organic phosphorus (Abd-Alla 1994; Bishop et al. 1994) or solubilizing inorganic phosphorus by producing organic acids like carboxylic, which reduces soil pH (Kucey et al. 1989; Salimpour et al. 2010; Fankem et al. 2008; Puente et al. 2004; Rodriguez et al. 2006). Rhizosphere of different crops harbours numerous phosphorus-solubilizing microorganisms (Ghosh et al. 2003). Bacterial potential of phosphate solubilization is a key attribute of plant growth-promoting rhizobacteria (PGPR) to make available improved phosphorus for plant uptake (Afzal et al. 2005). A pot experiment with *Pseudomonas putida* 108 strain considerably increased root development (86% increase), grain yield (59%) and straw yield (46%) at different phosphorus fertilization levels in comparison with the uninoculated control. In field experiments, *Pseudomonas putida* 108 significantly increased number of tillers/plant and height of plant. *Pseudomonas fluorescens* 153 considerably improved the yield and 1000 grain weight (Zabihi et al. 2011). Naiman et al. (2009) reported an increase of 17, 14 and 19% grain yield on treating with *Azospirillum* 1, *Azospirillum* 2 and *Pseudomonas fluorescens*, respectively, in comparison with the uninoculated control. The aerial biomass of plants inoculated with *Azospirillum 2* increased by 26%, whereas those treated with *Pseudomonas fluorescens* increased by 23% over the controls without urea. *Pseudomonas* strain *P. putida* 108 significantly improved the nitrogen, phosphorus and potassium uptake of wheat in comparison with the uninoculated control (Zabihi et al. 2011).

19.4 Phytostimulation

Phytostimulators positively affect the plant growth by synthesizing plant hormones or facilitate the uptake of certain nutrients by plants from the environment or play a role as signalling molecules or help in the biocontrol of phytopathogenic organisms (Kloepper 1993; Glick 1995). Phytohormones play a key role as signals and

regulators of plant growth. They also coordinate several physiological reactions in plants, such as branching, tillering, fruit ripening and seed germination mediated through their effects on root formation and proliferation resulting in the efficient usage of nutrients and water. They increase plant resistance to biotic and abiotic stresses and regulate the expression of genes, synthesis of enzymes, pigments and metabolites (Zakharychev 1999; Haahtela et al. 1990; Tien et al. 1979). Auxins are the most widely and abundantly synthesized plant hormones of microbial origin (Arshad and Frankenberger 1998). Rhizospheric microorganisms of several crops have the abilities to synthesize auxins by utilizing the various substrates available in the root exudates (Strzelczyk and Pokojska-Burdziej 1984). Around 80% of rhizospheric bacteria synthesize indole-3-acetic acid (IAA) (Loper and Schroth 1986). IAA plays key role as a regulator of plant growth and development. Free-living PGPR, e.g. *Alcaligenes faecalis* and *Acetobacter diazotrophicus*, several species of *Pseudomonas* and *Azospirillum* and symbionts like *Rhizobium* spp. and *Bradyrhizobium japonicum* stimulate root growth by producing low levels of IAA (Patten and Glick 1996). The level of auxin production differs among species, different strains of the same species, and is also affected by culture condition, growth stage and availability of the nutrients (Shahab et al. 2009). There is a common consensus for auxin production that it is the key factor responsible for the stimulation of rooting and improving growth of plants (Bloemberg and Lugtenberg 2001). Microbial phytohormones exert beneficial effects when plant seeds are treated with cultures and/or suspensions of producer microorganisms. Seed treatment of *Azospirillum*, *Beijerinckia*, *Rhizobium*, *Agrobacterium*, *Bacillus*, *Pseudomonas*, *Mycobacterium*, *Arthrobacter*, *Methylovorus* and *Flavobacterium* strongly stimulates the germination capacity besides increasing the growth and productivity of crops (Polyanskaya et al. 2002). Beneficial bacteria and cyanobacteria applied individually or as consortia improved plant growth and yield of okra. Microbial treatments had a significant effect on root weight and yield. The yield of okra varied from 444.6 to 478.4 g/plant; highest values were recorded in *Azotobacter* sp., followed by *Anabaena* sp.-*Providencia* sp. and *Anabaena* sp.- *Azotobacter* sp.; and a positive correlation (0.69) was observed between yield and root weight (Manjunath et al. 2016).

19.4.1 Synthesis of Plant Growth Regulators by Microorganisms

19.4.1.1 Indole-3-Acetic Acid (IAA)

Auxins are important for plant and rhizobacterial interactions (Katsy 2005). Plant growth-promoting rhizobacteria (PGPR), which represent universal symbionts of higher plants, display a set of positive features; auxin synthesis is also one among them (Belimov et al. 1999). Auxins modulate plant growth at different stages such as biosynthesis of various metabolites, photosynthesis, flowering, fruiting, pigment formation and plant resistance to adverse biotic and abiotic factors (Ahmed and Kirbet 2014). Indole-3-acetic acid is a thoroughly studied auxin with regard to its genetic, biochemical and physiological parameters (Sergeeva et al. 2002). IAA is

involved in various cellular and developmental activities including cell division, differentiation, root formation and tropisms (Napier and Venis 1995). The synthesis of IAA is most common among soil- and plant-associated prokaryotes (Costacurta and Vanderleyden 1995). Microbial plant growth regulators, including IAA, enhance the growth and development of plants (Bano and Musarrat 2004; Donnell et al. 2003; Lambrecht et al. 2000). Rhizospheric bacteria showed relatively greater potential for IAA synthesis than phyllosphere and histoplane microbes. Some examples of epiphytic and rhizospheric bacteria include *Rhizobium* spp., *Bradyrhizobium* spp., *Azospirillum* spp., *Azotobacter* spp., *Alcaligenes* spp., *Enterobacter* spp., *Erwinia* spp., *Acetobacter* spp., *Agrobacterium* spp., *Herbaspirillum* spp., *Pseudomonas*, *Bacillus* and *Xanthomonas* (Datta and Basu 2000; Park et al. 2005). Both *in planta* IAA and microbially synthesized IAA act together to enhance cell growth, extensive root development and subsequently augment the plant nutrient uptake from soil. Two IAA producing bacterial (*Providencia* sp. and *Alcaligenes* sp.) and cyanobacterial (*Anabaena oscillarioides* and *Anabaena torulosa*) strains used individually and as consortia with optimum dose of chemical fertilizers improved the plant growth and yield of wheat (variety PBW343). The treatment with *Providencia* sp. inoculation showed significantly higher values for various plant growth parameters recorded, in comparison with control (Manjunath et al. 2011).

19.4.1.2 Cytokinins

Cytokinins constitute a set of plant hormones that stimulate cell division and callus growth in cells that otherwise might have become multinuclear. The equilibrium between cytokinin and auxin levels regulates cell differentiation and organogenesis, and increased ratio of auxin of cytokinin leads to shoot development and root formation (George et al. 2008). Rhizospheric microorganisms associated with plants are known to produce cytokinins. Arkhipova et al. (2005, 2007) reported that cytokinin-producing *Bacillus subtilis* enhanced the growth of *Lactuca sativa* L. after inoculation even when they were grown under water stress. Apart from producing auxins and cytokinins, microbes also produce gibberellins. Several reports have shown that cytokinin and gibberellic acids help in alleviating the adverse effects of salt stress on various crops (Xiong and Zhu 2002).

19.5 Fighting Biotic Stress

Beneficial bacterial inoculants are increasingly being used for the control of plant diseases as an eco-friendly option to reduce the application of harmful agrochemicals (Bach et al. 2016). These bacteria employ the following mechanisms for plant pathogen control: competition for nutrients and space nutrients at the infection site, parasitism and production of cell wall-hydrolysing enzymes, synthesis of antibiotics and induced systemic resistance in the plants. It is expected that several mechanisms of action be at work in many biocontrol agents (Lugtenberg and Kamilova 2009).

Rhizobacteria present in suppressive soils manage plant diseases inflicted by various plant pathogens.

The biocontrol agent that is best characterized by molecular techniques is the genus *Pseudomonas*. *Pseudomonas* strains are able to synthesize several antifungal metabolites, out of which phenazines, pyrrolnitrin, 2, 4-diacetylphloroglucinol (DAPG) and pyoluteorin are the most frequently detected classes. Different strains of *Bacillus*, viz. *B. amyloliquefaciens*, *B. pumilus*, *B. subtilis*, *B. licheniformis*, etc., also produce various secondary metabolites which help in controlling plant diseases and enhancing plant growth (Fendrihan et al. 2016).

19.5.1 Rhizosphere Competence

The primary factor that determines the effectiveness of microorganisms is their establishment in soil or colonization on roots. This area is gaining a lot of attention, especially, in relation to plant-microbe interactions. Widely accepted research findings across world are emphasizing the beneficial effects of root-associated microorganisms on crop health and productivity. They help the plants to overcome undesirable effects of environmental stresses and enhance the yield (Lareen et al. 2016). Inoculant bacteria are often coated on seeds. Establishment of inoculant bacteria at population densities sufficient to exert a beneficial effect in rhizosphere is very important (Negi et al. 2008). Survival of inoculant bacteria in rhizosphere, making use of nutrients exuded by the roots, efficiently colonizing the entire root system and competing with other microorganisms are important in getting desired effects (Bloemberg and Lugtenberg 2001). As per the research findings, organic acids are nutritional basis of rhizosphere colonization (Negi et al. 2008). Lugtenberg et al. (1999) reported that a deficiency in the consumption of organic acids by the inoculants led to reduce colonization of tomato rhizosphere.

Although the effective colonization by inoculated bacteria is governed by a large number of factors, including those of plant and microbe and their compatibility mediated by signalling, availability of micro−/macronutrients can be critical. Production of siderophores makes the organisms more competitive for iron (Chin-A-Woeng et al. 1997). Some of the genes of *Pseudomonas* species were identified which can be induced or repressed by the presence/absence of phytopathogenic fungi. The existence of *Phytophtora parasitica* induces many genes in P. putida, including those encoding diacylglycerol kinase, ABC transporters and outer membrane porins. This can be ascertained by in vitro expression technique (Lee and Cooksey 2000). In contrast, two ribosomal RNA operons of *P. fluorescens* were found to be repressed by *Pythium ultimum* (Smith et al. 1999).

19.5.2 Induced Systemic Resistance

Interactions with bacteria can bring two types of defence reactions that give protection against further infection (Wu and Baldwin 2009). The enhancement of plant's

resistance capacity after infection by a plant pathogen is termed as systemic acquired resistance (SAR) (Hammerschmidt and Kuc 1995; Van Loon et al. 1998; Ramamoorthy et al. 2001), where as beneficial rhizospheric and endophytic bacteria can activate a defence mechanism that is called as induced systemic resistance (ISR) (Pieterse et al. 2014). ISR acts through a salicylic acid-independent pathway and systemic acquired resistance (SAR) through salicylic acid-dependent pathway. SAR sometimes causes necrosis on plants when it is expressed to a highest level by the inducer (Cameron et al. 1994), whereas beneficial bacteria-induced ISR usually produce no necrotic symptoms (Van Loon et al. 1998). These two resistant mechanisms are exhibited after infection or challenge inoculation with disease-causing organisms (Van Loon 1997). Various beneficial rhizospheric *Pseudomonas* species induce systemic resistance mechanism in plants, which provides protection against wide range of phytopathogenic organisms including fungi, bacteria and viruses (Bakker et al. 2007). Seeds or seedlings treatment with rhizobacterial strains has resulted in enhanced resistance in the treated plants (Kloepper et al. 1999). The number of rhizobacterial *Pseudomonas* species capable of inducing ISR is increasing day by day. Bacterial factors that are probably responsible for ISRs include salicylic acid, siderophores and the O-antigen of lipopolysaccharide. The salicylic acid causes an ISR when present even in nanograms (Demeyer et al. 1999). PGPR produces certain metabolites that act against plant disease-causing organisms (Minorsky 2008).

19.5.3 Secondary Metabolites

Making use of microbial antagonists to manage plant pathogens in crops has been proposed as a substitute to chemical pesticides. Bacillus species and fluorescent pseudomonads play an important role in containment of plant pathogens (Kell et al. 1992). The bacterial antagonists inhibit plant pathogens by releasing extracellular metabolites, which are active at very low concentration (Beneduzi et al. 2012). Antibiotics synthesized by PGPR include phenazine-1-carboxyclic acid, phenazine-1-carboxamide, pyoluteorin, 2,4 diacetylphloroglucinol, pyrrolnitrin, oomycinA, kanosamine, zwittermicin-A, butyrolactones, aerugine, cepaciamide A, rhamnolipids, viscosinamide, ecomycins, pseudomonic acid, azomycin, antitumor antibiotics FR901463, cepafungins and antiviral antibiotic karalicin (Fernando et al. 2005). These antibiotics possess antimicrobial, antihelminthic, insect antifeedant, phytotoxic, cytotoxic, antioxidant, antitumour and plant growth-promoting activities. *Bacillus subtilis* produces several cyclic lipopeptides such as surfactins, fengycins and iturins (Fernando et al. 2005). These compounds protect the plants from different pathogens. They activate ISR in host plants and also cause holes in the cytoplasmic membranes of fungal and bacterial pathogens (Falardeau et al. 2013). *Bacillus subtilis* UMAF6639 efficiently controls the *Podosphaera fusca*, powdery mildew fungus of cucurbit, by producing antifungal compounds iturin and fengycin also by induced systemic resistance (ISR) through the elicitation of jasmonate- and salicylic acid-dependent pathways (Garcia-Gutierrez et al. 2013).

19.6 Rainfed Agriculture

Drought is the major limiting factor especially in arid and semiarid areas causing the most fatal economic losses in agriculture (Gagné-Bourque et al. 2016). Drought stress coupled with high temperatures (>45oC) lasting >8–10 weeks has become a regular feature of rainfed agriculture in recent times (Srinivasarao et al. 2013a, b). This type of abiotic stress alter the water relations at cellular and entire plant level resulting in both specific and non-specific reactions and damages (Beck et al. 2007). The climate change with delayed rainfall and extended dry spells during the crop growth period is making already nutrient hungry and low organic carbon content of dryland soils vulnerable. Provision of nutrients through chemical fertilizers is scarce in drylands, and nutrients by and large come from the mineralization of organic matter (Lal et al. 1997; Lal 2003; Singh et al. 1999). Even if chemical fertilizer is used, they have to be synchronized with rainfall or soil moisture conditions to get the desired benefits, and this is becoming difficult due to monsoon variability and deficit rainfall in the cropping season. Under these circumstances, the use of beneficial microorganisms is a potential option to address these problems. To reap full potential of beneficial microbes, the presence of adequate amount of soil organic matter is essential as it is paramount important for their survival. Therefore, improving the soil organic matter content especially in drylands is very important. To keep adequate soil organic carbon (SOC) concentration, incorporation of huge amount of organic residues such as crop residues, green leaf manures, farmyard manure, etc. is necessary in drylands as decomposition rate is faster (Srinivasarao et al. 2012a, 2013a, b). Crop rotations with legumes, incorporation of crop residues, farmyard manure, groundnut shells and green leaf manuring resulted in improving SOC concentration and yield in different crops in diverse agroecosystems of India (Srinivasarao et al. 2013a, b, 2015). Leaving of soybean/safflower and rice (*Oryza sativa* L.)/lentil (*Lens esculenta* Moench) crop residues in the field and incorporation of FYM 6 t/ha led to the improvement in SOC stock (Srinivasarao et al. 2012b, c). Various long-term experiments with incorporation of FYM and other organic nutrients resulted in improving microbial population, enzyme activities and organic matter content of soil in groundnut, sorghum, finger millet, soybean and other production systems in different regions of India (Srinivasarao et al. 2009). As per the study, every 1 Mg enhancement in profile SOC stock has led to the increase of yields of groundnut pod, finger millet, sorghum, pearl millet, soybean and castor by 13, 124, 90,170, 145 and 150 kg ha^{-1} $yr.^{-1}$, respectively (Srinivasarao et al. 2012a, 2013a, b). Treatment of plants with native beneficial microbes enhances the drought tolerance in arid/semiarid regions (Maurhofer et al. 1994). Many studies have reported the effectiveness of beneficial rhizobacteria in protecting the plants from undesirable effects of environmental stresses (Enebak et al. 1997; Glick et al. 1997; Timmusk et al. 1999). They are helping the plants to tolerate abiotic stresses by different mechanisms even by altering the plant responses at gene level (Bashan and Holguin 1998; Srivastava et al. 2008; Timmusk et al. 2014; Ngumbi and Kloepper 2016). *Bacillus megaterium* and *Pseudomonas putida* promote plant growth under drought conditions (Marulanda et al. 2007). Kasim et al. (2013) reported that seed

priming of wheat seeds with *Bacillus amyloliquefaciens* 5113 and *Azospirillum brasilense* NO40 significantly helped in overcoming drought stress condition by upregulation of genes (APX1, SAMS1 and HSP17.8, APX1 and SAMS1) in the leaves and enhanced activity of enzymes responsible for plant ascorbate glutathione redox cycle. Priming of *Bacillus thuringiensis* AZP2 on wheat resulted in improving drought stress tolerance; 80% of plants survived drought stress of about 6 days (Timmusk et al. 2014). Treatment with *Paenibacillus polymyxa* helps in providing drought tolerance characteristics in *Arabidopsis thaliana* by inducing ERD15 (early response to dehydration 15) drought responsive gene Timmusk and Wagner (1999). Under salt stress, beneficial bacteria improved germination rate, plant growth and tolerance to drought and yield. They enhance the root growth and increase root surface area, and this in turn improves the nutrient and water uptake (Klopper et al. 2004; Timmusk et al. 2014). Proline is a very important osmolyte and its synthesis increases in plants that are under drought stress (Huang et al. 2014). It protects the plants from detrimental effects of drought by removing free radicals and maintaining cellular redox potential (Hayat et al. 2012). Increased abscisic acid (ABA) level helps crop plants to overcome drought stress, and PGPR improve the ABA content that in turn improves growth and development of plants (Ngumbi and JKloepper 2016). Under drought stress conditions, inoculation of *Azospirillum brasilense* Sp245 resulted in a better water status, grain yield and mineral quality in wheat (Creus et al. 2004). Exopolysaccharide synthesizing bacteria confer increased ability of plants to overcome water stress (Bensalim et al. 1998). *Pseudomonas putida* GAP-P45 improves the drought tolerance of sunflower seedlings by producing EPS biofilm on the root surface, improving soil aggregation and increasing relative water content in the leaves (Sandhya et al. 2010).

Ethylene has several physiological effects on crop growth and development and influences its responses to environmental stresses. Bacteria like *Pseudomonas* spp., *Achromobacter piechaudii* and *Burkholderia caryophylli* lowers the ethylene concentration in plants by producing an enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (Wu and Baldwin 2009). These bacteria protect the plants from stress conditions like drought, flood and salinity. They improve the nodulation in legumes and enhance the root initiation of cuttings (Gamalero and Glick 2015). Inoculation with ACC deaminase-producing bacteria on plants led to increased root growth and improved tolerance of salt and water stress (Mayak et al. 2004a, b); such effects were recorded in axenic conditions and more recently in field conditions.

19.7 Genetic Interventions to Improve the Functionality of Beneficial Bacteria

Screening of useful bacteria for identifying the genes, which exert beneficial effects on crop growth and health, would help in further improving the performance through genetic modification (Negi et al. 2008). Some of the genes, which are already identified, include nifD, nifH and nifK (nitrogen fixation); ipdC and ppdC (Auxin synthesis); pqqB, pqqC, pqqD and pqqE (phosphate solubilization); phlA, phlB, phlC and

phlD (2,4 diacetylphloroglucinol synthesis); and acdS (ACC deamination) (Bruto et al. 2014). A mini-Tn5 vector with the biosynthetic operon for the phenazine-1-carboxylic acid (PCA) has been transferred to *P. fluorescens* strains, which helped in improving their rhizosphere competence and capability to control fungal diseases (Timms-Wilson et al. 2000). Similarly, the biocontrol capacity of *Pseudomonas* strains producing PCA was extended after the incorporation of the phzH gene from *P. chlororaphis* PCL1391. The transfer of this gene led to the synthesis of phenazine-1-carboxamide (PCN) by these strains and in their capacity to biocontrol tomato root and foot rot (Chin-A-Woeng et al. 2001). Further, thorough, understanding of plant microbial interactions and organisms implicated in the process to get even more beneficial effects on crop growth and development is required; the work in this direction is progressing in different regions of the world (Mitter et al. 2016).

19.8 Conclusions

Soil-plant-microbe interactions are multifaceted, and they influence the crop health and productivity by numerous ways. Microorganisms have a huge role to play in determining soil fertility as they are the drivers of decomposition of organic matter entering the soil and therefore in the recycling of nutrients in soil. Beneficial microorganisms provide an alternative to the use of chemicals for crop growth and development. Research has established that beneficial bacteria could play a key role in agriculture, horticulture, forestry in improving productivity and also in environmental restoration processes. Beneficial bacteria have shown very positive effects when applied correctly to the precise crop and environmental situation. They also help in substantive reduction in cost of production. From the point of sustainability of agriculture and good eco-environment establishment, there is a necessity to apply organic and microbial fertilizers in a balance and coherent way to keep high and stable yield.

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Bacterial Rhizoremediation of Petroleum Hydrocarbons (PHC)

20

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Abstract

Crude oil-based products majorly diesel and petrol are one of the major sources of energy today, and their transport across the world frequently results in spillage, contaminating the soil and water. So, it has become a necessity now to go for in situ technologies that can efficiently remediate persistent contaminants from soil in a cost-effective and environmentally friendly method. Currently the chapter gives an idea about rhizoremediation, which is slowly becoming a very promising technique to detoxify the pollutants. Moreover to this the other aspects of rhizoremediation like root exudates and microbial abundance in rhizosphere, effects of weather, time, irrigation, and oxygen requirement on rhizoremediation and finally looking into some soil amendment techniques to improve the process are also discussed.

Keywords

Petroleum hydrocarbons • PHC • Rhizoremediation • Rhizosphere • Exudates

20.1 Introduction

Crude oil and petroleum products are of specific concern in pollution studies due to their structural complexity, slow biodegradability, biomagnification potential, and above all the serious health hazards associated with their release into the environment. Plants are used successfully in the rhizoremediation of a wide range of contaminated soils, due to favorable conditions for microbial degradation surrounding

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the roots. Organic pollutants differ in charge and solubility. Nonpolar compounds such as hydrocarbons are poorly soluble in water and sorb readily to hydrophobic soil particles like soil organic matter. Organic contaminants include petroleum hydrocarbons, often introduced into the environment via the oil and gas explorations. Crude oil is a complex mixture of hydrocarbons consisting of aliphatic, aromatic, heterocyclic, and asphaltene hydrocarbons, ranging in size from C_1 to $>C_{50}$. The proportion of these hydrocarbons is highly variable and ranges from as much as 97% by weight in the lighter oils to as little as 50% in the heavier oils and bitumens. The hydrocarbons in crude oil are mostly alkanes (C_nH_{2n+2}), cycloalkanes, or naphthenes (C_nH_{2n}) in addition to various aromatic hydrocarbons or asphaltenes (C_nH_n), while the other organic compounds contain nitrogen, oxygen, and sulfur along with trace amounts of metals such as iron, nickel, copper, and vanadium. The major products of petroleum distillates are fuels which include methane, ethane, and other short-chain alkanes, diesel fuel (petrodiesel), fuel oils, gasoline (petrol), jet fuel, kerosene, and liquefied petroleum gas (LPG). All these petroleum hydrocarbons are environmental pollutants if present in land and aquatic ecosystem. Rhizoremediation is an efficient remedy to remove these pollutants. It progresses by two ways, either by a mutual relationship of plants and the indigenous microbial population living in the rhizosphere or by planting hydrocarbon-tolerant plants at the contaminated soils. Plants or rhizosphere provides a unique environment for hydrocarbon-degrading microorganisms to multiply and help in detoxification.

20.2 Petroleum Hydrocarbons (PHC): Pollutants in Environment

PHCs are one of the major environmental pollutants (Okoh 2006). They lead to environmental damage and are seen as the most significant contamination sources on the globe (Snape et al. 2001).

20.2.1 Sources

The development of petroleum industry has led to inevitable spillages that occur during routine operations. There are many records of such acute accidents during transportation, which leads to PHC pollution. Other causes of oil contamination include leakage of pipelines and storage tanks, fuel leakage from transport vehicles, land disposal of petroleum wastes, and seepage from natural deposits (Balba et al. 1998). The accidents with tankers, pipelines, and oil wells release huge quantities of petroleum into the land and marine ecosystems. The largest oil spills in history by volume were in the range of 140–800 thousand tons, and most of them were due to tanker accidents. Among petroleum products, diesel has been widely used in various industries. Due to its relatively high mobility, the possibility of contamination of surface waters and groundwaters as well as soils is high (Gallego et al. 2001).

20.2.2 Indian Perspective

India is a big market for consumption of petroleum products, and a significant part of them are imported from other countries. Petroleum products rank second after coal as a major source of energy in India. Oil constitutes over 35% of the primary energy consumption in India. Crude oil reserves in India are estimated at 757 million metric tons (MMT). Crude oil production during 2009–2010 was 33.69 MMT, which has increased by 11.8%–37.46 MMT in 2014–2015. The consumption of petroleum products during 2009–2010 was 138.19 MMT which increased to 184.67 MMT during 2015–2016 (Indian Petroleum and Natural Gas Statistics 2015–2016). Thus demand is far more than supply. India imported 202.85 MMT of crude oil in 2015–2016 which was 159.25 MMT during 2009–2010 (Indian Petroleum and Natural Gas Statistics 2015–2016). The rise in automobile industry has increased the demand for petroleum products and is expected to rise to more than 240 MMT by 2021–2022 which will further increase by 51.61% in 2031–2032 (Garg 2012). Indian government has established many new refineries and also has increased the refining capacity of existing refineries. Thus, India is a major petroleum-consuming country thereby predisposing the environment to the drastic effects associated with its exploration and exploitation. Recently two ships collided off the Mumbai coast leaking more than 2000 tons of oil into the sea. The large oil spill in the open ocean may do less harm to marine ecosystem than the relatively small spill near the shore. The consequences of these oil spills include widespread, long-term, and serious damage to human health, natural resources, marine ecosystems, and terrestrial life.

20.3 Effect of PHC Pollution in Soils

PHCs can be found in any one of the following forms: (a) adsorbed on the surface of organic soil constituents, (b) fixed within the soil pores, (c) found in mobile form, or (d) as a continuous layer on the soil surface (Trofimov and Rozanova 2003). Crude oil creates anaerobic condition in the soil and also leads to water logging and accumulation of acidic metabolites, which results in high concentration of aluminum and manganese ions, which are toxic to plant growth. Other physical parameters such as the mineral and organic matter content, redox properties, cation exchange capacity, and pH value get affected. Hydrophobic nature of PHCs creates a lower water holding capacity, moisture content, and hydraulic conductivity as compared to unpolluted soils (Nwaoguikpe 2011). Oil pollution leads to substantial increase in the organic carbon content in the soil. Under the impact of oil pollution, soil humus becomes enriched in humic acids, whereas the degree of humification of soil organic matter decreases (Trofimov and Rozanova 2003). These changes in soil properties greatly affect or cause toxicity to the biotic components of the ecosystem.

20.3.1 Toxicity of PHC

The toxic effects of the petroleum hydrocarbon spill mainly depend on the composition and concentration of the polluting petroleum product. For instance, n-alkanes are less toxic and persistent than aromatic compounds. In particular, the polyaromatic hydrocarbons (PAHs) (e.g., naphthacene, benzo(a)pyrene) are carcinogenic and have been implicated in many a wide range of human health problems and also disease problems with aquatic organisms (Grimmer et al. 1991).

20.3.2 Toxicity to Plants

PHC pollution causes both the decrease in plant growth and crop productivity. The following authors have reported a slower rate of seed germination in PHC-contaminated soil: Adam and Duncan (2002) screened different plant species including grasses, legumes, herbs, and commercial crops for their ability to germinate in 25 and 50 g/kg diesel fuel-contaminated soil. They suggested that volatile compounds can lead to delayed and decreased seed germination as they are capable of entering easily through the plant cell walls. Also, PHCs may form a thin layer on the seed, preventing the entry of oxygen and water. Sharifi et al. (2007) observed the effect of 25, 50, 75, and 100 g/kg of spent oil on seed germination, shoot height, and biomass of six herbaceous plant species including one species of Fabaceae (*Medicago truncatula*), four species of Gramineae (*Bromous mermis*, *Secal seral*, *Triticum sativa*, and *Agropyron desertorum*), and one species of Linaceae (*Linum usitatissimum*) and reported dose-dependent responses to the contaminated soils by all species. Only 16.2, 15, and 2.7% germination was seen in *A. desertorum*, *B. mermis*, and *L. usitatissimum*, respectively, while 63.5% germination was observed in *M. truncatula*. Reduction in seedling height and biomass was also observed in all plant species. Similarly Ogbo (2009) studied effects of 1, 2, 3, 4, and 5% of diesel contamination on four crop plants *Arachis hypogaea*, *Vigna unguiculata*, *Sorghum bicolor*, and *Zea mays*. They observed that all the test plants tolerated diesel fuel contamination at 1–3% levels of contamination as seed germination was between 89 and 33%. In the presence of 1% diesel, 89% of seed germination was reported in *Z. mays* and *S. bicolor*, but only 77 and 68% seeds germinated in *A. hypogaea* and *V. unguiculata*, respectively. Table 20.1 tells us more about the toxic effect of hydrocarbons on various plants.

20.3.3 Toxicity to Animals/Birds/Humans

PHC constitutes mainly the aromatic compounds, which are toxic, mutagenic, or carcinogenic (Balba et al. 1998). Birds and animals if they ingest the oil from the environment may face problems such as congestion, pneumonia, emphysema, and even death by breathing in droplets of oil or oil fumes or gas. Ingestion may also lead to decreased absorption of nutrients and finally result in death of these birds and animals due to severe liver damage and anemia. Symptoms of crude oil toxicity

Table 20.1 Studies on crude oil impact on plant growth

Common name of plant	Scientific name of plant	Research findings	References
Corn	*Zea mays*	Low germination of seeds	Gallegos-Martinez et al. (2000)
Jew's mallow	*Corchorus olitorius* L.	Shorter length of seedlings	Adenipekun et al. (2008)
Vetch	*Vicia sativa* L.	Late development of eophylls in seedlings	Chupakhina and Maslennikov (2004)
Millet	*Panicum miliaceum* L.		
Barley	*Hordeum vulgare* L.		
Jew's mallow	*Corchorus olitorius* L.	Lower number of leaves and reduced leaf area	Adenipekun et al. (2009)
Smooth bromegrass	*Medicago truncatula*	Reduction in germination, aboveground height, and biomass for all species	Sharifi et al. (2007)
	Bromus mermis		
Wheat	*Secale cereale*		
Flax	*Triticum aestivum*		
Castor	*Ricinus communis* L.	Stimulation of growth parameters – plant height, stem girth, leaf area, fresh and dry weights, and root length	Vwioko and Fashemi (2005)
Slim amaranth	*Amaranthus hybridus* L.	Decrease in the total chlorophyll and protein levels	Odjegba and Sadiq (2002)
Cowpea	*Vigna unguiculata* L. Walp	Reduction in germination time, increase in germination percentage, plant height, leaf number, and total biomass	Ataga and Adedokun (2007)
Alfalfa	*Medicago sativa* L.	Light crude oil at 1–10% prevents normal growth and germination of Alfalfa in soil	Dariush Minai-Tehrani et al. (2007)
Maize	*Zea mays*	Inhibited germination and growth at high concentrations	Ogboghodo et al. (2004)
Prairie turnip	*Lathyrus venosus* Muhl	Lowest seedling mass in contaminated soil	Robson et al. (2004)
Crested wheatgrass	*Agropyron pectiniforme* R. & S.		
Yellow sweet clover	*Melilotus officinalis* (L.) Lam.		
Guinea corn	*Sorghum bicolor*	Inhibition of germination and growth in guinea corn, serves as a bioindicator of crude oil-polluted areas	Akaninwor et al. (2007)

(continued)

Table 20.1 (continued)

Common name of plant	Scientific name of plant	Research findings	References
Maize	*Zea mays*	*Z. mays* and *G. max* seedlings possess greater potential to enhance remediation based upon percent emergence and plant biomass production in contaminated soil	Issoufi et al. (2006)
Alfalfa	*Medicago sativa*		
Ryegrass	*Lolium perenne*		
Wheat	*Triticum aestivum*		
Soybean	*Glycine max*		
Winter vetch	*Vicia villosa*		
Cowpea	*Vigna unguiculata*	Total amylase, starch phosphorylase, and mitotic activities were inhibited	Achuba (2006)
Broad beans	*Vicia faba*	Co-inoculation of *V. faba* plant roots with nodule bacteria and PGPR-enhanced plant growth and nitrogen fixation	Radwan et al. (2005)
Okra	*Abelmoschus esculentus*	Combination of crude oil, microbes and fertilizers stimulated germination, growth, biomass, microbial population, and rate of degradation	Ogbonna et al. (2007)

include liver necrosis, blocking of the liver, fat disintegration, and dissociation of hepatocytes (Sathishkumar et al. 2008). Eventually food chain is also affected.

20.3.4 Toxicity to Microorganisms

Very little attention has been given to the effects of crude oil on natural microbial populations. Stevens et al. (2003) demonstrated how some polycyclic aromatic hydrocarbons negatively affect soil microbial community composition and function. Ebuehi et al. (2005) reported the effects of crude oil on hydrocarbon-utilizing bacteria and total heterotrophic bacteria in the soil. According to his results, the hydrocarbon-utilizing bacterial population increased, while the population of heterotrophic bacteria decreased as a result of the crude oil contamination. Gill and Ratledge (1972) reported that alkanes are toxic to microorganisms, whereas other aromatics like pinene, limonene, camphene, and isobornyl acetate also showed microbial toxicity according to Andrews et al. (1980). Walker et al. (1975) demonstrated the toxicity of crude and refined oil to natural bacterial populations from pristine sediments with refined oil being more toxic. Even for bacteria surviving in the presence of dissolved aromatic hydrocarbons like naphthalene, increase in lag phase and decreased growth rate were observed (Calder and Lader 1976). Sikkema et al. (1995) reviewed the mechanism of membrane toxicity of cyclic hydrocarbons. The accumulation of these compounds in the membrane of microorganisms has considerable effects on the structural and functional properties. Hydrocarbon alters the membrane structure by changing fluidity and protein conformations which

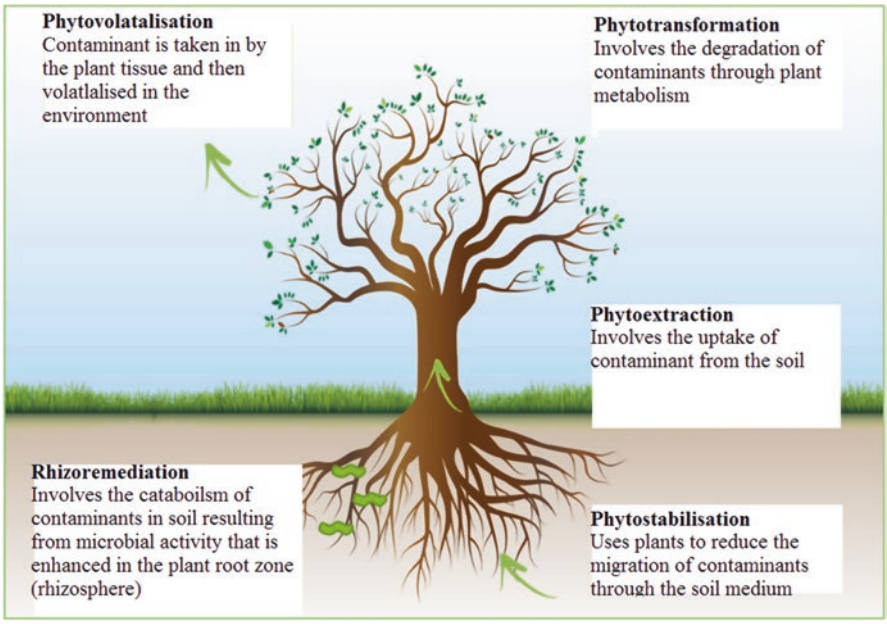

Fig. 20.1 Mechanisms involved in phytoremediation

results in disruption of the barrier and energy transduction functions while affecting membrane-bound and embedded enzyme activity (Van Hamme et al. 2003).

20.4 Treatment of Contaminated Soil

There are various physical, chemical, and biological methods to treat the contaminated environment. But the most cost-effective treatment is phytoremediation, which can be further classified into rhizoremediation and other process like phytovolatilization, phytotransformation, phytostabilization, and phytoextraction (Fig. 20.1).

20.4.1 Biological Method: Phytoremediation/Rhizoremediation

Phytoremediation uses plants for in situ remediation of contaminated soil, sludge, sediment, and groundwater through any of the mechanisms: extraction, filtration, stabilization, degradation, and evapotranspiration. Many plants, which have extensive fibrous roots such as common grasses, wheat, corn, soya bean, peas, and beans, were studied for their rhizoremediation potential (Glick 2003). Rhizoremediation is the degradation of recalcitrant pollutants by bacteria in the rhizosphere. Rhizosphere provides a large surface area for a significant population of bacteria to remediate the pollutants even 10–20 m deep in the soil. It has proven to be an effective and

affordable "green technology" for remediating soils contaminated with petroleum hydrocarbons. The roots act as mediators in supplying both the nutrients (amino acids, carbohydrates, and organic acids) and bacteria with cofactors required for the activation of bacterial enzymes which nullifies the need to supply exogenous carbon source.

The objective of rhizoremediation is increased microbial numbers and activity, and the exploitation of that increased microbial activity to enhance biotreatment. It is the primary mechanism responsible for hydrocarbon degradation in phytoremediation efforts (Frick et al. 1999; Hutchinson et al. 2003). Microbial densities in the rhizosphere are suggested to be 1–4 orders of magnitude higher than in bulk soil. Consequently, rhizoremediation appears to be an economically favorable, with minimal maintenance, in situ treatment for pollutants in surface soils. Rhizoremediation is also very efficient in the use of wild-type bacteria in their native environments to degrade a variety of pollutants (Pilon 2005).

20.5 Rhizosphere: Exudates, Nutrients, and Root Colonization

The rhizosphere of most plants harbors a wealth of microorganisms that can contribute significantly to the degradation of petroleum hydrocarbons during rhizoremediation. Thus, a plant if not directly removing these contaminants can influence the microbial community within its root zone to a great extent. Rhizosphere microorganisms constantly look for nutrients in the surrounding environments. The exudation of nutrients by plant roots creates a nutrient-rich environment in which microbial activity is stimulated. Plant root exudates contain sugars, organic acids, and amino acids as main components (Ling et al. 2015; Yang et al. 2001; Yoshitomi and Shann 2001; Miya and Firestone 2001; Gao et al. 2010; Xie et al. 2012; LeFevre et al. 2013; White et al. 2003; Zhao et al. 2006; Ling et al. 2009; Yi and Crowley 2007; Techer et al. 2011). A list of compounds found in these exudates is shown in Table 20.2. Root exudates fall into two specific categories: (a) low molecular weight compounds (LMWCs: amino and organic acids, sugars, phenolic compounds, and other secondary metabolites) and (b) high molecular weight compounds (HMWCs: polysaccharides and proteins). The characteristics (quantity and quality) of root exudates are determined by the cultivar, plant species, developmental stage, various physicochemical factors (soil type, pH, temperature, nutrient availability), and the presence of microorganisms (Badri and Vivanco 2009; Neumann 2007; Rangel et al. 2007; Gransee and Wittenmayer 2000; Hutsch et al. 2002; Leigh et al. 2002; Shukla et al. 2011; Xue et al. 2013). Most of the exudates occur at the root tips and at sites of lateral branching, decreasing with increasing distance from the root surface (Martin et al. 2014; Gao et al. 2011; Neumann 2007; Marschner et al. 2011). Also the mucigel secreted by root cells provides nutrients (Lynch 1990). Rhizosphere is known to be dominated by gram-negative rods such as *Pseudomonas* species (Kuiper et al. 2004).

Plants export and secrete compounds in the rhizosphere through many ways (Weston et al. 2012; Badri and Vivanco 2009). Both passive and active pathways help in release of root exudates (Bertin et al. 2003; Weston et al. 2012; Huang et al.

Table 20.2 List of compounds found in root exudates

Compounds	Example of compounds	References
Carbohydrates	Glucose, fructose, sucrose, maltose, galactose, xylose, arabinose, mannitol	Vancura and Hovadik (1965), Bertin et al. (2003), Badri and Vivanco (2009), Bais et al. (2006), Ryan et al. (2001), Haichar et al. (2014), Compant et al. (2010), Haldar and Sengupta (2015), Hejl and Koster (2004), Shukla et al. (2011), Huang et al. (2014), Somers et al. (2004), Neal et al. (2012), Badri et al. (2013), Badri et al. (2012), and Zhang et al. (2014)
Flavonoids and flavonols	Naringenin, kaempferol, quercetin, myricetin, rutin, genistein, strigolactone	
Amino acids	All proteinogenic amino acids	
Organic acids	Acetic acid, propionic acid, citric acid, butyric acid, succinic acid, chorismic acid, sinapic acid, caffeic acid	
Volatile compounds	Ethanol, methanol, formaldehyde, acetone, acetaldehyde, propionaldehyde	
Vitamins	Thiamine, biotin, niacin, riboflavin, pyridoxine	
Aromatics	Phenols, 1-carvone, p-cymene, limonene	
Enzymes	Phosphatase, dehydrogenase, peroxidase, dehalogenase	
Lignin derivatives	Catechol, benzoic acid, nicotinic acid, phloroglucinol	

2014). Majority of organic low molecular weight compounds (LMWC) are released through a passive transport (i.e., not requiring energy), which permits passage of an ion or a molecule across a membrane without energy intake (Huang et al. 2014; Ryan et al. 2001). Active transport requires energy and refers to the passage of an ion or molecule through a membrane against its concentration gradient. Plants due to their stationary lifestyle require many adaptive strategies to cope up with the environment, suggesting that the number of compounds produced by plants may require a large number of transporters (Dixon 2001). Weston et al. (2012) and Battey and Blackbourn (1993) have found the involvement of transport vesicles to facilitate the movement of high molecular weight organic compounds secreted by the roots.

Plant roots enhance microbial biodegradation of petroleum hydrocarbons via physical processes such as nutrient and pollutant transport, microbial attachment sites, and soil aeration (Fig. 20.2) (Martin et al. 2014). Exudates supply nutrients to the microorganisms which help in the improvement of petroleum hydrocarbon degradation (Walton et al. 1995; Kuiper et al. 2004). Plants can improve degradation via the root exudation of enzymes, such as peroxidases, laccases, and phenol oxidases, which catalyze the oxidation of various hydrocarbons (Martin et al. 2014; Gao et al. 2011). However, enzymatic breakdown by bacterial enzymes is considered to be the

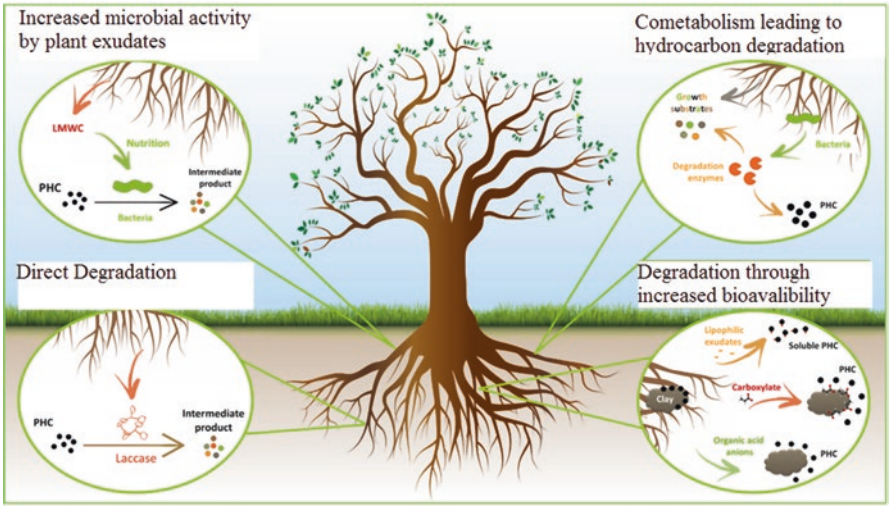

Fig. 20.2 Improvement of petroleum hydrocarbon degradation by root exudates. *PHC* petroleum hydrocarbon, *LMWC* low molecular weight compounds

primary mechanism for petroleum hydrocarbons degradation (Martin et al. 2014). Also many secondary metabolites exuded by roots such as flavonoids are structurally similar to aromatic hydrocarbons (Singer 2006; Bais et al. 2008). This structural analogy leads to co-metabolism. Bioavailability can often be a limiting factor for hydrocarbon degradation because soil solids easily absorb polycyclic aromatic hydrocarbons after entering the soil (Gao et al. 2011).

20.6 Effects of the Rhizosphere Bacterial Population on Plants

Plant roots have a direct effect on the surrounding microbial populations; likewise microorganisms in the rhizosphere also have a marked influence on the growth of plants. Inappropriate microbial populations in the rhizosphere cause plant growth impairment (Atlas and Bartha 1998). Microbes in the rhizosphere benefit the plant by many ways like increasing recycling and dissolving of mineral nutrients and synthesis of amino acids, vitamins, and plant hormones that stimulate plant growth (Atlas and Bartha 1998). In turn microorganisms release antagonistic substances in the rhizosphere that allows the plant to enter an amensal relationship with other plants. Table 20.3 shows the number of bacterial and archaeal taxa identified in the rhizosphere microbiome.

Table 20.3 Occurrence of bacterial and archaeal taxa identified in the rhizosphere microbiome

Host	Main findings related to rhizosphere microbiome composition	References
Maize crop	*Azospirillum*, *Bradyrhizobium*, and *Ideonella* were the most abundant genera found in the rhizosphere, comprising *c*. 5%, 21%, and 11% of the clones, respectively	Roesch et al. (2007)
Oat	A total of 1917 taxa were detected, and the community was dominated by *Proteobacteria* and *Firmicutes*. Less expected rhizosphere-competent phyla were also detected, including *Actinobacteria*, *Verrucomicrobia*, and *Nitrospira*	DeAngelis et al. (2009)
Oak in a forest soil	The predominant phyla were *Proteobacteria* (38%), *Acidobacteria* (24%), and *Actinobacteria* (11%)	Uroz et al. (2010)
Sugar beet in agricultural soil	The community was dominated by *Proteobacteria* (39%), *Firmicutes* (20%), and *Actinobacteria* (9%). The *Gamma-* and *Betaproteobacteria* and *Firmicutes* were identified as the most dynamic taxa associated with disease suppression	Mendes et al. (2011)
Potato in field soil	Most of the genera belonged to *Proteobacteria* (46%), followed by *Firmicutes* (18%), *Actinobacteria* (11%), *Bacteroidetes* (7%), and *Acidobacteria* (3%). The bacterial families *Streptomycetaceae*, *Micromonosporaceae*, and *Pseudomonadaceae* showed the strongest response at the potato cultivar level	Weinert et al. (2011)
Rhizophora mangle and *Laguncularia racemosa* in mangrove	Four classes were found: *Halobacteria*, *Methanobacteria*, *Methanomicrobia*, and *Thermoprotei*	Pires et al. (2012)
Potato in field soil	*Actinobacteria* and *Alphaproteobacteria* were the most abundant groups, followed by *Gammaproteobacteria*, *Betaproteobacteria*, *Acidobacteria*, *Gemmatimonadetes*, *Firmicutes*, *Verrucomicrobia*, *Deltaproteobacteria*, *Cyanobacteria*, and *Bacteroidetes*	Inceoglu et al. (2011)
Mammillaria carnea (cactus) in semiarid environment	Dominant bacterial groups were *Acidobacteria*, *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes*	Torres-Cortes et al. (2012)
Arabidopsis thaliana	The rhizosphere was dominated by *Acidobacteria*, *Proteobacteria*, *Planctomycetes*, and *Actinobacteria*. *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* were found as dominant phyla in root bacterial communities and significantly enriched compared with soil and rhizosphere	Bulgarelli et al. (2012)

20.7 Rhizoremediation and Depth of Contamination

Rhizoremediation is most effective at sites with shallow (i.e., root accessible) contaminated soils where contaminants can be treated in the rhizosphere. Both grasses and trees are widely used in rhizoremediation but with limitations. Two of the ideal common perennial grasses used for rhizoremediation are tall fescue (*Festuca arundinacea* Schreb.) and perennial ryegrass (*Lolium perenne* L.) because of their deep and extensive root systems, robust growth after establishment, and tolerance to drought, acidic soils, and cold temperatures (Nedunuri et al. 2000; Parrish et al. 2004; Muratova et al. 2008). While grasses have more fibrous root systems, they typically do not root as deeply as trees. Herbaceous plants have an average maximum rooting depth of 2.4 ± 0.1 m, except for prairie grasses that can reach depths of 4–6 m. Hawaiian plants, Milo and Kou, which were used to remediate saline soils contaminated with TPHs, rooted to a depth of more than 1.5 m by growing through the brackish water table into a zone of concentrated contaminants (US Army Corps 2003). If contaminants occur at depths greater than roots, then remediation with grasses may be limited. Advantage of using grasses is that they provide more uniform coverage of the soil surface, diminishing surface runoff and erosion (Collins 2007).

Roots of some trees can be expected to grow at least 3 m into a soil profile, and it is possible to encourage rooting to a depth of 5 m or more using the tree-in-a-well concept. Studies reveal that planting trees, particularly willows (*Salix* spp.) and hybrid poplars (*Populus* spp.), accelerates rhizoremediation due to many reasons like easy propagation, fast growth, deep and sometimes phreatophytic roots that extend to the water table, high water uptake rates, high absorption surface areas, perennial growth, and tolerance to contaminants and flooding (Newman and Reynolds 2004; Barac et al. 2009; Widdowson et al. 2005; Aitchison et al. 2000). Trees are typically chosen for sites with contaminated groundwater to prevent offsite migration (Collins 2007).

20.8 Effects of Weather Conditions

Rhizoremediation might be best suited for tropical countries where plant growth occurs all year round. In temperate climates, the active contribution of rhizoremediation is restricted to the growing period only as in winters plants are deleafed, transformation and uptake ceases, and soil water is no longer transpired (Huxtable et al. 1997).

20.9 Time Scale of Cleanup

Degradation of organics may be limited by mass transfer, i.e., desorption and mass transport of chemicals from soil particles to the aqueous phase may become the rate determining step. Therefore, rhizoremediation may require more time to achieve cleanup standards than other more costly alternatives such as excavation or ex situ treatment, especially for hydrophobic pollutants that are tightly bound to soil particles.

20.9.1 Effects of Irrigation

Irrigation can enhance bioremediation of certain diesel components. For terrestrial rhizoremediation applications, it is often desirable to include irrigation costs on the order of 10–20 inches of water per year. Drip irrigation is more efficient than step irrigation as it encourages the growth of weeds that compete for nutrients with plants and hinder their delivery to the contaminated zone. Irrigation is especially important during the start of the project.

20.9.2 Importance of Oxygen

The importance of oxygen in the biological remediation of petroleum contaminants, especially saturated aliphatics (i.e., diesel), is well documented (Frick et al. 1999; Olson et al. 2003). Plants may enhance the oxygenation of contaminated soils improving remediation potential. Roots can act as physical channels, which transport oxygen to the root zone, enhancing aerobic conditions for biological degradation. Roots also increase the soil porosity allowing increased diffusion of atmospheric oxygen (Rentz et al. 2004). Vegetation cover can also moderate temperature and moisture conditions, which influences availability of oxygen (Gunther et al. 1996). Plant root systems may increase the moisture content of soil by promoting an effective circuit for water movement (Jing et al. 2008). Jing et al. (2008) showed that soil moisture content increased by 5% in petroleum-polluted soil planted with grasses.

20.9.3 Cost

Rhizoremediation is usually less costly than other alternatives of bioremediation such as soil excavation, soil venting, soil washing, or enhanced extraction, and many more. Costs involved are installation of vegetation at the site, expenditure on design, site preparation, reporting, monitoring, and operation and maintenance. It would be useful to include preliminary greenhouse experiments along with agronomic soil testing during the design phase to ensure vigorous plant growth at the field site.

20.10 Plants Used in Rhizoremediation of PHC-Contaminated Soils

Despite the fact that remediation of PHC-contaminated soil with weed plants has shown significant potential, rhizoremediation is still in its infancy. The use of living weeds alone is generally considered to be a restrictive factor for rhizoremediation. A large number of the latest studies have paid more attention to relative technologies used to enhance rhizoremediation efficacy at the laboratory scale (Table 20.4).

Table 20.4 Studies on rhizoremediation of PHC contaminants in soil

Common name of plant	Scientific name of plant	Research findings	References
Switch grass	*Panicum virgatum*	57 % degradation of PAHs	Pradhan et al. (1998)
Little blue stem grass	*Schizachyrium scoparium*	47 % degradation of PAHs	
Alfalfa	*Medicago sativa*	72 % degradation of PAHs	Wiltse et al. (1998)
Perennial ryegrass	*Lolium perenne*	87.7 % of TPH degradation	Omotayo et al. (2014)
Fescue	*Lolium arundinaceum*	Degradation of 2–4 ring alkylated PAHs in crude soil-contaminated site	White et al. (2006)
Ryegrass	*Lolium multiflorum* L.		
Bermuda grass	*Cynodon dactylon* L.		
Cowpea	*Vigna unguiculata*	Reduction of 54% hydrocarbons in crude oil-contaminated sites	Tanee and Akonye (2009)
Cinchona	*Cinchona robusta*	Degradation of n-hexadecane	Vega-Jarquin et al. (2001)
Yam	*Dioscorea composita*		
Broad bean	*Vicia faba*	30% reduction in TPH	Diab (2008)
Maize	*Zea mays*	16.8% reduction in TPH	
Wheat	*Triticum aestivum*	13.7% reduction in TPH	
Cocksfoot	*Dactylis glomerata*	20% degradation of naphthalene, degradation rate decreased with an increase in molecular weight of hydrocarbon	Smith et al. (2006)
Tall fescue	*Festuca arundinacea*		
Red fescue	*Festuca rubra*		
Ryegrass	*Lolium perenne*		
Bird's-foot trefoil	*Lotus corniculatus*		
Red clover	*Trifolium pratense*		
White clover	*Trifolium repens*		
Annual ryegrass	*Lolium multiflorum*	Maturity of plant root contributes to reduction in the bioavailability of target PAHs	Parrish et al. (2005)
Tall fescue	*Festuca arundinacea* Schreb.		
Yellow sweet clover	*Melilotus officinalis* Lam.		
Sunflower	*Helianthus annuus* L.	Vegetation increases total numbers of beneficial fungi and bacteria in contaminated soil	Olson and Fletcher (2000)
Bermuda grass	*Cynodon dactylon L.*		
Southern crabgrass	*Digitaria ciliaris* (Retz.) Koeler.		
Maize	*Zea mays* L.	Increase in hydrocarbon bioavailability, stimulates bacterial population	Radwan et al. (1995) and Chaineau et al. (2000)

(continued)

Table 20.4 (continued)

Common name of plant	Scientific name of plant	Research findings	References
Rice	*Oryza sativa* L.cv.	Significant decrease in TPH concentration under vegetated conditions	Kaimi et al. (2007)
Naked spinach	*Spinacia oleracea* L.cv.		
Devil's beggartick	*Ohrai. Pueraria lobata* (wild)		
	Ohwi Bidens frondosa L.		
Slender oat	*Avena barbata*	A large phenanthrene degrader population in rhizosphere is related to root debris and soil exudates	Miya and Firestone (2001)
Tall fescue	*Festuca arundinacea*	Greater total bacterial numbers and PAH-degrading bacteria in rhizosphere soil	Ho and Banks (2006)
Alfalfa	*Medicago sativa* L.	Rhizosphere microflora of alfalfa was less inhibited by hydrocarbon contamination with higher degradative potential compared to reed	Muratova et al. (2003)
Reed	*Phragmites australis*		

20.11 Soil Amendment for Enhancing Rhizoremediation

The application of soil amendment seems to be a valuable choice for the rhizoremediation of PHC-contaminated soil. It enables excellent vegetative coverage and also increases the rate of PHC removal in soil. Addition of compost to soil helps reduce the negative effects of PHCs on ryegrass growth and accelerates PHC removal from the soil (Vouillamoz and Milke 2001). Palmroth et al. (2006) confirmed that in soil amended with NPK fertilizer, more than 65% of hydrocarbons were removed and the addition of municipal biowaste compost removed 60% of hydrocarbons over 39 months; hydrocarbons failed to considerably decline in non-amended soil. Adding *Jatropha curcas* amended with organic wastes (BSG) to soil greatly enhances the removal of waste lubricating oil to 89.6% and 96.6% in soil contaminated with 2.5% and 1.0% oil, respectively. A loss of 56.6% and 67.3% was recorded in the corresponding planted soils without organic change over189 days (Agamuthu et al. 2010). Though typical amendments such as NPK have contributed to plant productivity and effective degradation of PHC pollutants, when overused the soil-remaining fertilizers not taken up by the plants sometimes "burn" the plants and may even cause environmental issues (Kang et al. 2010). Naturally produced biosurfactants (rhamnolipids), which have no phytotoxicity to plants and may increase PHC bioavailability, have been tried to enhance PHC degradation (Zhang et al. 1997). Previous studies have shown that rhamnolipids can enhance the uptake of PAHs by ryegrass roots and the degradation of PAHs by alfalfa (Zhang et al. 2010; Zhu and Rock 2008).

20.12 Plant Growth-Promoting Rhizobacteria (PGPR) for Enhancing Rhizoremediation

Plant growth-promoting rhizobacteria (PGPR) are microscopic organisms fit for advancing plant development by colonizing the plant root surface and the adjacent soil interface (Berendsen et al. 2012; Lugtenberg and Kamilova 2009; Van Hamme et al. 2003. PGPR strains can improve the grass germination recurrence and at the same time invigorate grasses to grow better in acute contamination. Soil contamination accelerates ethylene production in plants, leading to retarded plant growth. Catalyst ACC deaminase can devour ACC, the antecedent of ethylene into 2-oxobutanoate and alkali (Glick 2005). Diminished ethylene levels permit plants utilized as a part of phytoremediation to develop and survive better in intensely contaminated soils. Additionally, PGPR strains can go about as biocontrol specialists, shielding the rhizosphere from pathogenic organisms (Compant et al. 2005; Whipps 2001). The involvement of PGPR strains in rhizoremediation can make the plant more resistant to contaminants than using plants alone (Huang et al. 2004b; Kang et al. 2010; Koo et al. 2010). Thus, PGPR can help quicken detoxification of contaminants. As indicated by Huang et al. (2004a, b), amid a nursery analysis, the germination recurrence for wild rye expanded by 61% with PGPR at 0.5 g kg^{-1} of creosote. For tall fescue, plant germination recurrence expanded by 40% with PGPR at 3 g kg^{-1} of creosote. Additionally, the presence of PGPR incredibly upgraded the PHC (polycyclic hydrocarbons) and creosote expulsion when contrasted with phytoremediation alone. PGPR strains can improve the grass germination recurrence and invigorate grasses to develop better in vigorously tainted soils, in this manner advancing cleaning of PHCs.

20.13 Inoculation of Plants with Microbes for Enhancing Rhizoremediation

Weeds with an extensive root framework, for example, grasses are favored for rhizoremediation because of their substantial root surface zone, which can help build up dynamic microbial action (Aprill and Sims 1990). Euliss et al. (2008) recommended that distinctive plants may improve rhizosphere remediation by specifically selecting microbial groups. Along these lines, inoculation of plants with microorganisms in rhizosphere may not just shield plant roots from toxin harmfulness (Robert et al. 2008) but additionally improve rhizoremediation adequacy. As of late, more reviews have been given to improve rhizodegradation effectiveness by inoculating organisms, especially indigenous microorganisms. Autochthonous microorganisms are more perfect with nearby local areas than allochthonous organisms, which do not possess a practical specialty (Atlas and Bartha 1998). *Cyperus laxus* Lam., a local plant developing in marshes, inoculated with autochthonous microbial strains isolated from *C. laxus* rhizosphere degraded PHCs two times higher than non-inoculated plants following 60 days in culture. Besides, the root biomass of *C. laxus* was 1.6 times more than non-inoculated plants (Escalante et al. 2005).

Proficient hydrocarbon-degrading bacterial strains that can compete with the native habitat and are firmly connected to plants encourage rhizoremediation. For example, Italian ryegrass (*Lolium multiflorum* var. Taurus) when treated with an alkane-degrading strain (BTRH79) demonstrated higher hydrocarbon degradation than that in other treatments (Yousaf et al. 2010).

20.14 Genetic Engineering for Enhancing Rhizoremediation

The usage of plants for the cleanup of harmful compound in soils is constrained by the moderate development rate of the plants, which means quite a while it is regularly required for the reclamation of polluted sites. The productivity of utilizing plants can be significantly enhanced through hereditary building advancements (Bennett et al. 2003; Kawahigashi 2009). It is also desirable to construct recombinant bacterial strains containing different traits, such as the degradation of contaminants together with the production of biosurfactants, good colonization abilities, and the capacity to promote plant growth. Genetically engineered plant microbial systems have been established to improve rhizoremediation for example expressing degradative enzymes like orthomonooxygenase in root colonizing baceteria (*P. fluorescens*) for toluene degradation (Francova et al. 2003). Even plant-endophytic bacteria have been manipulated to improve the remediation of organic pollutants (Mastretta et al. 2006).

Barac et al. (2004) studied on toluene phytoremediation using engineered endophytic bacteria. The authors transferred the pTOM plasmid, which encodes the toluene degradation genes, via conjugation from *B. cepacia* G4 to *B. cepacia* L.S.2.4, a natural endophyte of yellow lupine. Although the recombinant strain was not maintained in the endophytic community, there was a horizontal gene transfer of the *tom* (toluene monooxygenase) operon to different members of the endogenous endophytic community (Taghavi et al. 2005), demonstrating new avenues for introducing desirable traits into the community. Still, the release of recombinant organisms in the field is restricted in many countries, and these legal limitations, together with some well-sustained scientific concerns, may limit the development of this field. Notwithstanding these discoveries, little research has concentrated on utilizing transgenic weeds for phytoremediation of PHC-contaminated soils. The usage of transgenic plants, particularly transgenic weeds, requires additionally examining and keeping in mind the end goal to expand the productivity of phytoremediation.

20.15 Combined Approaches for Enhancing Phytoremediation

In many cases, remediation technology using plants and one enhancement approach and plants may still be inefficient. For a rhizoremediation framework to be more successful, plant resilience and TPH debasement should be enhanced by utilization

of a blend of the methodologies sited above. A multi-handle phytoremediation framework (MPPS) has been proposed to join agronomic treatment, inoculation with contaminant degrading microscopic organisms, and the development of the contaminant-tolerant plants, for example, tall fescue (*Festuca arundinacea*) with plant growth-promoting rhizobacteria (PGPR). Huang et al. (2004a, 2005) have demonstrated that amid the initial 4 months in culture, the evacuation of TPHs and 16 need PAHs by MPSS was twice that of agronomic treatment, half more than vaccination with organisms, and 45% more than phytoremediation alone. A consolidated approach comprising of phytoremediation, surfactant flushing, and microbial corruption adequately disseminates oil poisons from loess soil and is suggested for rebuilding of PHC-sullied destinations (Zhu et al. 2010). Zhang et al. (2010) have presented a multi-procedure phytoremediation framework comprising of mycorrhizal organisms, sweet-smelling hydrocarbon debasing microscopic organisms (ARDB), and rhamnolipids for the bioremediation of PAHs. Following 90 days, the aggregate PAH expulsion by the multi-strategy phytoremediation framework was 251.83% more noteworthy than that of phytoremediation alone. These reviews demonstrate that applying one approach alone is not extremely productive, but rather joining different procedures can cure abandons. Along these lines, phytoremediation in conjunction with different methodologies might be an ideal answer for upgrading PHC expulsion.

20.16 Conclusions and Future Prospects

Rhizoremediation is an economic and environmentally sustainable remediation alternative for the degradation of hydrocarbon contaminants from the soil. In this review, we discussed about the root exudates as one of the most important mediators, and the extent to which biodegradation is achieved is highly variable among plant species. The process restores the biological as well as the physicochemical properties of degraded soils, thus making them fit for crop production. Although microorganisms and plants can be used independently for the cleanup of polluted sites, combining these two groups of organisms (microorganisms and rhizomediators) increases the remediation efficiency. There is a need for more research on the various microorganisms and plants that could be combined for remediation of PHC-polluted soils. An understanding of the plant-microorganism-soil interaction in polluted environments is also essential for effective bioremediation. Finally, more studies about the impact of using recombinant microorganisms over indigenous microbial communities are needed to meet with safety requirements, especially with the increasing need for recombinant microbes to deal with highly toxic chemicals, such as dioxins and PCBs.

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Biotechnology of Commercial Microbial Products

21

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Abstract

Industrial biotechnology has revolutionized the conventional manufacturing of chemicals through engineering of microbes, especially in recent years largely owing to reengineering of cellular metabolism. Metabolic engineering has been widely used to overproduce indigenously synthesized metabolites in *E. coli*, *S. cerevisiae*, and other hosts. Plant secondary metabolites are low molecular weight compounds which not only help the plant in its defense mechanism but also are helpful to humans in curing a wide variety of ailments/diseases. The amount of secondary metabolite production is very less using traditional techniques, in comparison with conventional methods where they are produced in large quantities from different microbes using metabolic and cellular engineering. In this chapter, we have focused on various plant secondary metabolites produced through metabolic engineering from microbes such as *E. coli* and *S. cerevisiae*.

Keywords

Plant secondary metabolites • Metabolic engineering • *E. coli* • *S. cerevisiae* • Flavonoids

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21.1 Introduction

Plant secondary metabolites are organic compounds typically with molecular weight of less than 3 KDa and are less necessary for a cell's immediate sustenance. However, it serves in plethoric roles which aids in organism's survival and reproduction stages (Williams et al. 1989). In plants, these compounds play an important and necessary role in defense against pests, microbial pathogens, and herbivores (SchÃfer and Wink 2009). When plants do not produce enough products, engineering of microbes is substantive for the commercial production of plant products. These compounds not only protect the plants against UV irradiation but also play a crucial role in allelopathy and tritrophic interactions and also in attracting seed-dispersing birds, animals, and other pollinators (Paul Christou and Harry Klee 2004; SchÃfer and Wink 2009). Secondary metabolites such as nicotine from tobacco and caffeine from coffee beans have shown potent pharmacological effects on humans. For these reasons, many plant secondary metabolites are used in traditional folk medicines and/or recreational drugs (Gómez-Galera et al. 2007; Miralpeix et al. 2013; Newman and Cragg 2007; Wilson and Roberts 2012). The schematic overview of biosynthetic pathways and precursors leading to the formation of major classes of plant secondary metabolites is represented in Fig. 21.1 (Marienhagen and Bott 2013). Over the past several decades, apart from traditional method, the recombinant technology also gained a lot of attention for large-scale production of useful secondary metabolites (Miralpeix et al. 2013).

21.2 Bottlenecks of Traditional Production of Plant Secondary Metabolites

Traditionally more than 50,000 medicinal plants are used by humans, of which two-thirds are from the wild, which is raising concerns regarding local extinction, diminishing populations, habitat abasement, and loss of genetic diversity. Some plant species are used profligately (e.g., *Glycyrrhiza glabra* (licorice), *Piper methysticum* (kava), and *Arctostaphylos uva-ursi* (bearberry) (Miralpeix et al. 2013).

Among all, only 10% of the medicinal plant varieties are cultivated depending on specific environmental factors and conditions (Canter et al. 2005). When medicinal plants are grown on a large scale, the valuable secondary metabolites can be extracted and isolated easily. However, when the seeds are difficult to germinate and unable to thrive at low altitudes as in the case of *Picrorhiza kurroa*, the extraction/purification of metabolites becomes a tedious job (Bhat et al. 2012). The extraction and isolation of the useful metabolites is an expensive process (Table 21.1) (Kolewe et al. 2008). Anticancer drugs such as paclitaxel (Taxol®) from *Taxus brevifolia* require 340 tons of bark which means 25 kg per year and is equivalent to 38,000 trees (Rischer et al. 2013; Wilson and Roberts 2012).

Another disadvantage in the extraction or production of secondary metabolites is the interference of undesirable compounds or toxins as in the case of *Ginkgo biloba* that contains 24–27% flavonoid glycosides, 6–7% terpene lactones, and the toxic

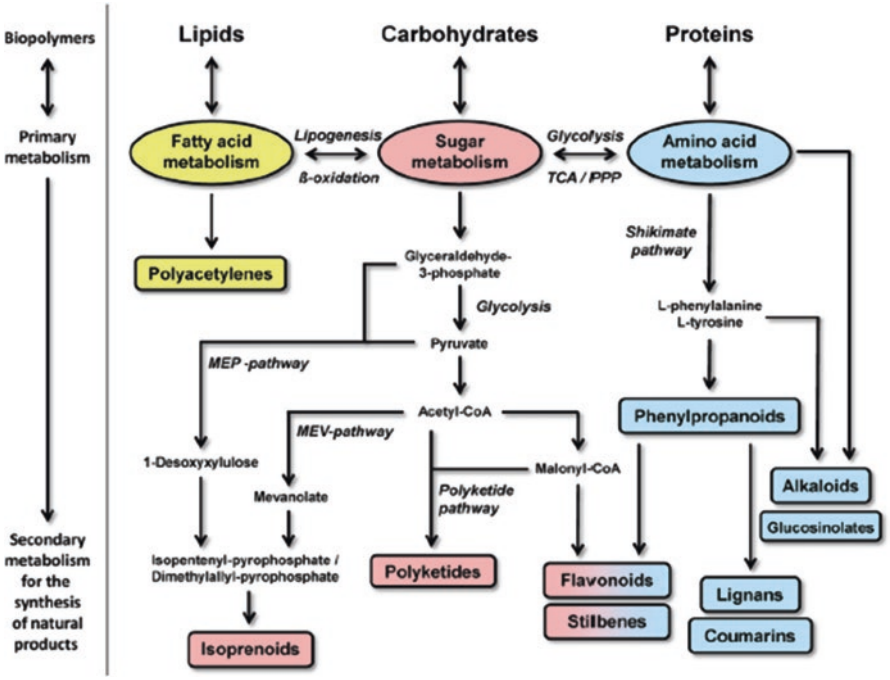

Fig. 21.1 Schematic overview of biosynthetic routes and precursors of the primary metabolism leading to formation of the major classes of the plant natural products (PNPs). Individual modules of the primary metabolism and the PNP classes are color-coded to illustrate their respective origin from fatty acid, sugar, or amino acid metabolism (Source: Marienhagen and Bott 2013)

Table 21.1 Plant-derived products with commercial relevance

Product	Use	Plant species	Cost (US$ per kilogram)
Ajmalicine	Antihypertensive	*C. roseus*	37,000
Artemisinin	Antimalarial	*A. annua*	400
Ajmaline	Antihypertensive	*R. serpentina*	75,000
Berberine	Intestinal ailment	*C. japonica*	3250
Camptothecin	Antitumor	*C. acuminata*	432,000
Capsaicin	Counterirritant	*C. frutescens*	750
Codeine	Sedative	*P. somniferum*	17,000
Colchicine	Antitumor	*C. autumnale*	35,000
Digoxin	Heart stimulant	*D. lanata*	3000
Diosgenin	Steroidal precursor	*D. deltoidea*	1000
Ellipticine	Antitumor	*O. elliptica*	240,000
Emetine	Anti-amoebic	*C. ipecacuanha*	1500
Morphine	Sedative	*P. somniferum*	340,000
Quinine	Antimalarial	*C. ledgeriana*	500
Sanguinarine	Antiplaque	*S. canadensis*	4800
Shikonin	Antibacterial	*L. erythrorhizon*	4500

Source: Rao and Ravishankar (2002)

component ginkgolic acid. The target with desired metabolites can be achieved during the extraction process, but the selection of genotypes that yield these extracts close to the desired chemical profiles makes processing much more tedious (Canter et al. 2005). Alternatively, chemical synthesis is more preferred over traditional method for synthesis of secondary metabolites that are not economically feasible in production and extraction. The widely used flavor compound vanillin is extracted from natural source *Vanilla planifolia*, commonly called as vanilla orchid that is synthesized using chemical synthesis. Interestingly, less than only 0.1% of this compound is obtained from plant sources (Wilson and Roberts 2012).

When the natural sources are limited and chemical synthesis is unfeasible, the best alternative and yet effective tool for production of secondary metabolites is engineering of metabolic pathways at genome-scale level, codon optimization, and translational level (Wang et al. 2011a; Yadav et al. 2012). Complex metabolic pathways are compartmentalized with different reactions taking place in specialized and differentiated cell types combined with shuttling of intermediates through signaling between cells to regulate the entire metabolic pathways (Hilliou et al. 1999; Julsing et al. 2006; Pickens et al. 2011).

21.3 Ecological Importance of Plant-Derived Secondary Metabolite Products

Since time immemorial, human beings are dependent on plants not only for food but also for secondary metabolites, which are used in a wide variety of applications such as pharmaceuticals, perfumes, insecticides, drugs, agrochemicals, and food flavoring agents. Chemically plant secondary metabolites are classified into glycosides (steroids, phenolics), terpenoids, alkaloids, etc. According to WHO survey, nearly 80% of the world's population depend more on natural plant products than synthetic products. According to recent reports by global nutraceutical market in 2014, the value of metabolites exceeded by $171.8 billion on a global scale and by about $75.9 billion in US market alone. It is estimated to reach up to $241.1 billion by 2019 (Jain and Ramawat 2013; Wang et al. 2016b). Various uses and effects of plant secondary metabolites are listed in Tables 21.1 and 21.2.

21.4 Microbial Metabolic Engineering

Metabolic engineering is the targeted modification of metabolic pathway or pathways in organisms to produce desirable products, chemical transformation, and supramolecular assembly (Lessard 1996). It can be applied for directed improvement of specific biochemical reactions and/or introduction of new pathway genes through rDNA technology (Stephanopoulos 1999). The main goal of metabolic engineering is systematic analysis of metabolic and other pathways with molecular biology techniques to enhance cellular properties for the product improvement by designing rational genetic modifications (Kumar and Prasad 2011). Microbial

Table 21.2 Various applications and uses of plant secondary metabolites

Product	Plant species	Uses	References
Shikonin	*Lithospermum erythrorhizon*	Dye, pharmaceutical	Fujita et al. (1981)
Codeine, morphine	*P. somniferum*	Analgesic	Kamo et al. 1982)
Quinine	*Cinchona officinalis*	Antimalarial, Antimicrobial	Rajan and Bagai (2013) and Rojas et al. (2006)
Atropine	*Atropa belladonna*	Muscle relaxant	Mintzer and Burns (2000) and Rajput (2014)
Digoxin	*D. lanata*	Cardiovascular disorders	Batterman and De Graff (1947) and Katz et al. (2016)
Reserpine	*Rauwolfia serpentina*	Hypotensive	Schlittler et al. (1954) and Wilkins et al. (1954)
Diosgenin	*D. deltoidea*	Antifertility	Allaw et al. (2016) and Nie et al. (2016)
Vanillin	*Vanilla* sp.	Vanilla	Walton et al. (2003)
Jasmine	*Jasminum* sp.	Perfume	Hongratanaworakit (2010) and Rath et al. (2008)
Vinblastine, ajmalicine, vincristine	*Catharanthus roseus*	Anticancer	Idrees et al. (2010)
Taxol	*Taxus brevifolia*	Anticancer	Rao (1993) and Weaver (2014)
Baccharine	*Baccharis megapotamica*	Anticancer	Dos Reis Lívero et al. (2016), Jarvis and Mazzola (1982), and Kupchan et al. (1977)
Cesaline	*Caesalpinia gilliesii*	Anticancer	Montgomery and Yamauchi (1977) and Ulubelen et al. (1967)
Fagaronine	*Fagara zanthoxyloides*	Anticancer	Larsen et al. (1993), Ouchani et al. (2015), and Vavrecková et al. (1994)
Maytansine	*Maytenus buchananii*	Anticancer	Blum and Kahlert (1978) and Widdison et al. (2006)
Harringtonine	*Cephalotaxus harringtonia*	Anticancer	Komoto et al. (2015) and Liu et al. (2016b)
Thalicarpine	*Thalictrum dasycarpum*	Anticancer	Creasey (1976), Creaven et al. (1974), and Kupchan et al. (1978)
Ellipticine, 3-deoxycolchine	*Ochrosia moorei*	Anticancer	Bournique et al. (1972), Fang et al. (2016), and Rizza et al. (2016)
Pyrethrins	*Tagetes erecta*, *Chrysanthemum cinerariaefolium*	Insecticide	Galardo et al. (2015), Madden and Lindquist (1946), and Pal et al. (1953)
Rotenoids	*Derris elliptica*, *Tephrosia* sp.	Insecticide, antimicrobial	Dohutia et al. (2015), Khan et al. (2006), and Sae-Yun et al. (2006)

(continued)

Table 21.2 (continued)

Product	Plant species	Uses	References
Nicotine	*N. tabacum*, *N. rustica*	Insecticide	Wang et al. (2015a)
Saffron	*Crocus sativus*	Food color and flavoring agent	D'Archivio and Maggi (2017)
Stevioside	*Stevia rebaudiana*	Sweetener	Pavlíček and Tůma (2017) and Torri et al. (2016)
Thaumatin	*Thaumatococcus daniellii*	Sweetener	Kaneko and Kitabatake (2001) and Liu et al. (2010)
Capsaicin	*C. frutescens*	Chili	Liu et al. (2016a), and Pubchem; Wang et al. (2016a)
Rosmarinic acid	*Coleus blumei*	Spice, antioxidant	Corral-Lugo et al. (2016), Petersen and Simmonds (2003), and Venkatachalam et al. (2016)
Anthraquinones	*Morinda citrifolia*	Laxative, dye, antitumor activity	Chien et al. (2015), Huang et al. (2007), Tarasiuk et al. (1998), and Yen et al. (2000)
Berberine	*Coptis japonica*	Antibacterial	Peng et al. (2015)
Sarcoplasmine (hyoscine)	*Datura stramonium*	Treatment of nausea	Soni et al. (2012)

fermentation goes back to the ancient process in feed and food applications. In order to make the process, cost-effective strain improvement and development are of main focus for industrial production (Yang et al. 1998).

Metabolic engineering involves the application of engineering principles of design and analysis of metabolic pathways in order to achieve a particular goal (Shams Yazdani and Gonzalez 2008). Metabolic engineering became an area of vast interest when the strains were improved for the better performance and expression of useful metabolites (Lee et al. 2010). Microbes are useful for expression of many valuable products and secondary metabolites. Thus, engineering of microbes can be used to achieve the need of the hour goals such as (Fig. 21.2) (Anesiadis et al. 2008; Yadav et al. 2012):

(i) Increase in substrate uptake and product formation/yield.
(ii) Enhance the process performance and to speed up the process.
(iii) Save energy and improve cellular properties.
(iv) Reduce and/or stop by-product formation.
(v) Develop the strains which are resistant to environmental stress, etc.

The process of microbial metabolic engineering comprises of three main steps:

1. Studying metabolic pathways which involves reconstruction and detailed study of biochemical pathways (Anesiadis et al. 2008)

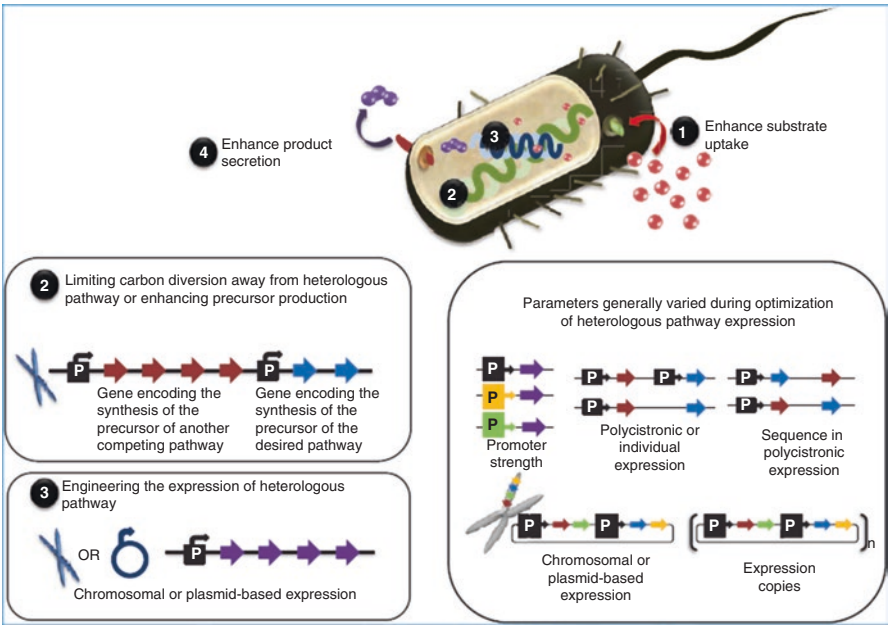

Fig. 21.2 A holistic view of metabolic and cellular engineering in microbes. (1) Enhancement in the rate of substrate uptake, (2) reduction of flux to undesirable by products and enhancement of precursor and cofactor flux, (3) introduction of the heterologous pathway and optimization of the activity of its constituent enzymes, and (4) export of the product to the extracellular medium in order to shift equilibrium toward product formation (Source: Yadav et al. 2012)

2. Use of computational techniques to develop in silico models to design a particular metabolic pathway
3. Applying computational suggested designing at experimental levels using different genetic engineering approaches (Kumar and Prasad 2011)

Several advances are used for the improvement of secondary metabolites produced by certain bacteria in metabolic engineering as listed below (Kumar and Prasad 2011; Lee et al. 2012):

(A) Heterologous expression of entire gene clusters
(B) Engineering regulatory networks
(C) Gene insertion and deletion
(D) Redirecting metabolic pathway
(E) Stimulation by precursors
(F) Genetic knockout of loci
(G) Quorum sensing

21.5 Metabolic Engineering of *E. coli* for the Production of Different Plant Secondary Metabolites

21.5.1 Flavonoid Production in Genetically Engineered *E. coli*

E. coli is considered as the workhorse for the expression of different heterologous proteins. In recent years, many studies have been reported on the production of flavonoids in *E. coli* (Fowler et al. 2009; Leonard et al. 2005, 2006, 2007; Santos et al. 2011). Due to a wide range of health benefits from flavonoids, great interest has popped out on their biosynthesis/production using microbial hosts. Leonard et al. (2006) engineered an *E. coli* strain, for the production of plant-specific flavonol derivatives such as kaempferol, quercetin, and myricetin by expression of a soluble flavonoid 3′,5′-hydroxylase (F3′5′H) chimera, along with a flavonol biosynthetic fusion protein (comprising of chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3*β*-hydroxylase (FHT), and flavonol synthase (FLS)), together with 4-coumaroyl-CoA ligase (4CL), the enzyme that activates phenylpropanoid acids to the corresponding coenzyme moiety. In order to overcome the expression of cinnamate-4-hydroxylase (C4H), which requires specific cytochrome reductase in bacteria, Hwang et al. (2003) constructed an artificial gene cluster which contained three genes of heterologous origins under the control of T7 promoter (P_{T7})-PAL from the yeast *Rhodotorula rubra*, 4CL from the actinomycete *Streptomyces coelicolor* A3(2), and CHS from the licorice plant *Glycyrrhiza echinata.* This engineered strain successfully produced pinocembrin chalcone (751 μg/l) from naringenin chalcone and phenylalanine (452.6 μg/l) from tyrosine, respectively.

Subsequently, Watts et al. (2004) constructed gene cluster TAL from *Rhodobacter sphaeroides* with 4CL and CHS from *Arabidopsis thaliana* in *E. coli* and observed high-level production of naringenin (20.8 mg/l) by exogenous feeding of 4-coumaric acid. Highest production of (+)-catechin (8.8 mg/l) was obtained from flavanones by expressing three-gene cluster consisting of flavanone 3β-hydroxylase [F3H], DFR, and leucoanthocyanidin reductase [LAR] of the flavonoid biosynthetic pathway in *E. coli* along with the optimization of various bioprocessing conditions (Chemler et al. 2007). In an attempt to overcome the major drawbacks of media supplementation and phenylpropanoic precursors, Santos et al. (2011) developed an economical process by constructing four-enzyme heterologous pathway (consisting of CHS and codon-optimized TAL, 4CL, and CHI) which was assembled into two different *E. coli* strains that have been engineered for high L-tyrosine production. The above engineered strains were able to produce naringenin (29 mg/l) using glucose as substrate and up to 84 mg/l of naringenin using glucose along with the suppression of fatty acid biosynthetic enzymes.

The production of flavonoids is greatly limited by low availability of free intracellular precursors such as malonyl-CoA in *E. coli* (Miyahisa et al. 2005; Zha et al. 2009). In order to increase the malonyl-CoA levels, acetyl-CoA carboxylase gene, which is responsible for the catalytic conversion of acetyl-CoA to malonyl-CoA, was engineered to overexpress itself which leads to an augmentation in malonyl-CoA levels (Miyahisa et al. 2005). Engineering various central metabolic pathways

in E. coli, which resulted in BirAPl strain, further enhanced flavonoid production. Co-expression of intracellular malonyl coenzyme A (malonyl-CoA) through organized overexpression of four acetyl-CoA carboxylase (ACC) subunits from *Photorhabdus luminescens* (PlACC) under a constitutive promoter leads to 5.8-fold increase in flavanone production. The co-expression of PlACC along with biotin ligase in above engineered strain ($BirA_{Pl}$) further increased the flavanone synthesis by 11.7-fold. Furthermore, amplification of pathways for the increase in acetyl-CoA and malonyl-CoA led to 429 mg/l flavanone production, which represents approximately a 14-fold increase compared to the parental strains (Leonard et al. 2007). Additionally, redirection of the carbon flux to pathways responsible for generation of malonyl-CoA and overexpression of ACC resulted in a threefold increase in cellular malonyl-CoA concentration. Thus, the engineered/modified strain showed a nearly fourfold higher flavonoid expression than parenteral strain (Zha et al. 2009).

At the genome level, Fowler et al. (2009) formulated a cipher of evolutionary design (CiED) in an attempt to divert the metabolic flux toward the expression of malonyl coenzyme A (CoA) and other cofactors by identifying the pathways for gene deletions and other pathway modifications which enhance flavonoid production capacity. As a result, the naringenin and eriodictyol expression was increased up to 6.6- and 4.2-fold, respectively. The overexpression of both malonyl-CoA synthetase (matB) and malonate carrier protein (matC) and inhibiting fatty acidsynthesis using cerulenin have enhanced the intracellular levels of malonyl-CoA, thus increasing the flavanone expression levels dramatically up to 710 mg/l (Leonard et al. 2008).

In an another study by Chemler et al. (2010), enhanced production of leucocyanidin (817 mg/l) and (+)-catechin (39 mg/l) was observed. They are NADPH-dependent enzymes by utilizing a stoichiometric-based modeling approach to identify combinations of gene knockouts and to redirect the metabolic flux of pools toward the generation of NADPH. The modular pathway engineering was done combinatorially, i.e., by modifying/enhancing plasmid gene copy number and promoter strength to explore balanced pathway which can direct the utilized glucose toward L-tyrosine production then to naringenin. Thus, the optimized strain was capable of producing 100.64 mg/L (2S)-naringenin directly from glucose (Wu et al. 2014).

Tyrosine yield was increased by engineering tyrosine biosynthesis pathway and by introducing flavonoid biosynthetic genes in *E. coli* to produce approximately 40 mg/L of two bioactive O-methylated flavonoids (i.e., sakuranetin and ponciretin) (Kim et al. 2013). For the first time, the production of 7-*O*-methyl aromadendrin (7-OMA), one of the important flavonoid glycosides from its precursor, and *p*-coumaric acid in *E. coli* was reported by Malla et al. (2012). The expression of naringenin (flavanone) was attained by feeding *p*-coumaric acid and then reconstructing the plant biosynthetic pathway by introducing the genes from different species such as: chalcone synthase from *Petunia hybrida*, 4-coumarate–coenzyme A (CoA) ligase from *Petroselinum crispum*, and chalcone isomerase from *Medicago sativa*. In order to enhance the availability pool of malonyl-CoA, genes of acyl-CoA carboxylase, biotin ligase, and acetyl-CoA synthetase from *Nocardia farcinica* were

also introduced. The modified strain was able to express 30 mg/liter (99.2 μM) 7-OMA from 500 μM naringenin within 24 h.

Luteolin-7-*O*-glucuronide and quercetin-3-*O*-glucuronide were biosynthesized to levels of 300 and 687 mg/L, respectively, by enabling nucleotide biosynthetic genes, which enhance their substrate production in *E. coli*. They have deleted the *araA* gene encoding UDP-4-deoxy-4-formamido-L-arabinose formyl transferase/UDP-glucuronic acid C-4 decarboxylase and overexpressed the gene UDP-glucose dehydrogenase (*ugd*) (He et al. 2008; Kim et al. 2014). All the above findings suggest that insufficient supply of free intracellular malonyl-CoA and cofactors such as NADPH is a limiting reaction for the high-level production of flavonoids in *E. coli.* The genome-wide analysis for rational redesign to reconstruct metabolic pathways, which will direct the metabolic flux toward the malonyl-CoA and NADPH, helps to overcome the problem.

21.5.2 Anthocyanin Expression in Genetically Engineered *E. coli*

Anthocyanins are natural plant pigments and are used for wide varieties of applications such as dye, edible pigment, and antioxidant activity (Brouillard 1982; Markakis 2012). Its biosynthetic pathway has gained attention of many scientists who interpreted them and reported results. The biosynthetic pathway of anthocyanin is one of the best understood pathway (Honda et al. 2002; Nakajima et al. 2001). For the first time, Yan et al. (2005a) produced stable, glycosylated anthocyanins from flavanones such as eriodictyol and naringenin. The metabolic pathway was constructed such that it contained genes of heterologous origins from plants: flavanone 3β-hydroxylase and anthocyanidin synthase (ANS) from *Malus domestica*, dihydroflavonol 4-reductase from *Anthurium andraeanum*, and UDP-glucose:flavonoid 3-O-glucosyltransferase from *Petunia hybrida* and expressed plant-specific anthocyanin. Through traditional metabolic engineering techniques, Leonard et al. (2008) developed *E. coli* strains which are capable of high-level flavonoid synthesis, by incorporating alternative carbon assimilation pathway and inhibiting of competitive reaction pathways, to increase intracellular flavonoid precursors and cofactors for the expression of anthocyanins (113 mg/l).

21.5.3 Polyphenolic Compound Expression in Genetically Engineered *E. coli*

Polyphenolic compounds are phytochemicals used as antioxidants and food ingredients. They are derived from tyrosine or phenylalanine by condensation of several malonyl-CoA molecules (Marienhagen and Bott 2013). Under the control of the T7 promoter, Miyahisa et al. (2005) have cloned the flavone synthase I gene from *Petroselinum crispum* and the synthetic ribosome-binding sequence in pACYC-Duet-1, which caused the *E. coli* cells to produce flavones such as apigenin (13 mg/l) from tyrosine and chrysin (9.4 mg/l) from phenylalanine. Pinocembrin (40.2 mg/l)

(flavanone) expression from engineered/modified *E. coli* cells using glucose as precursor was established by Wu et al. (2013a). Expression of eriodictyol (107 mg/l) using tyrosine as fed precursor was established by Zhu et al. (2014). A total of 18 pathways from homologous enzymes was reconstructed for efficient expression of catechin (910.9 mg/l) from engineered *E. coli* using 1.0 g/L of eriodictyol as a substrate in batch culture with minimal media (Zhao et al. 2015).

Co-incubation of genetically engineered *Saccharomyces cerevisiae* and *E. coli* cells for the production of genistein (6 mg/L) (flavanone) from tyrosine was studied by Katsuyama et al. (2006). Multivariate modular metabolic engineering was employed to assess and alleviate pathway bottlenecks in the production of resveratrol. An *E. coli* strain was engineered such that it contains genes of tyrosine ammonia lyase (TAL), 4-coumarate:CoA ligase (4CL), stilbene synthase (STS), malonate synthetase, and malonate carrier protein and was found to produce resveratrol (35.02 mg/L) from L-tyrosine (Wu et al. 2013b). Summeren-Wesenhagen and Marienhagen (2015) constructed three-step biosynthetic pathway from two different enzymatic steps for the production of pinosylvin. Further in order to overcome the bottlenecks such as low levels of malonyl coenzyme A (malonyl-CoA) and low stilbene synthase activity, addition of cerulenin during the production of intracellular malonyl-CoA pools improved the activity in *E. coli*, and in vivo evolution of the stilbene synthase from *Pinus strobus* elevated pinosylvin titers of 70 mg/liter from glucose as substrate and further increased to 91 mg/liter by the addition of L-phenylalanine.

About seven biosynthetic genes from different bacteria and plants were used to reconstruct the complete biosynthetic pathway using eight biosynthetic bricks in *E. coli*. The modified strain was able to produce three bioactive natural stilbenoids (resveratrol, piceatannol, and pinosylvin), four phenyl propanoid acids (cinnamic acid, p-coumaric acid, caffeic acid, and ferulic acid), and three natural curcuminoids (curcumin, bisdemethoxycurcumin, and dicinnamoylmethane) (Wang et al. 2015b). Huang et al. (2013) exploited the catalytic potential of 4HPA3H in the whole-cell bioconversion and produced 3.82 g/L (461.12 mg/L/OD) caffeic acid from p-coumaric acid. Further, de novo production of caffeic acid reached 766.68 mg/L when phenylalanine over-producer was engineered into a tyrosine over-producer and introduced into the artificial pathway of *E. coli.* The first report of caffeic acid production from tyrosine was studied by Rodrigues et al. (2015). The codon optimization of the gene and different combinations of plasmids were used to engineer pathway that involves the conversion of tyrosine to p-coumaric acid and then from p-coumaric acid to caffeic acid, by introducing tyrosine ammonia lyase (TAL) from *Rhodotorula glutinis* and 4-coumarate 3-hydroxylase (C3H) from *Saccharothrix espanaensis* or cytochrome P450 CYP199A2 from *Rhodopseudomonas palustris* (Rodrigues et al. 2015).

21.5.4 Alkaloid Expression in Genetically Engineered *E. coli*

Nakagawa et al. (2011) constructed a tailor-made biosynthetic pathway in *E. coli* which can use simple and economical carbon sources such as glycerol as a substrate at low cost for the expression of 46 mg/l of (S)-reticuline (benzylisoquinoline alkaloid). The production of (S)-reticuline from dopamine by improving production efficiency and by combining both in vivo tetrahydropapaveroline expression *in E. coli* and in vitro enzymatic synthesis of (S)-reticuline resulted in 593 mg L^{-1} from 1 L of the reaction mixture (Matsumura et al. 2016).

21.5.5 Terpenoid Expression in Genetically Engineered *E. coli*

Terpenoids are the largest class of phytonutrients present in green foods, soy plants, and cereals and serve as anti-inflammatory, anti-infectious, and anticancer agents (Jain and Ramawat 2013; Mora-Pale et al. 2013). Alper et al. (2005) used systematic and combinatorial methods to identify the knockout genes in *E. coli* and screened 64 knockout strains for the overproduction of lycopene. ATP and NADPH are important cofactors for the synthesis of terpenoids. In order to increase the β-carotene production, Zhao et al. (2013) optimized β-carotene biosynthetic pathway and engineered five different central metabolic modules to enhance ATP and NADPH. This engineered strain was able to produce 2.1 g/L β-carotene with a yield of 60 mg/g DCW.

Li et al. (2014b) compared two different approaches for zeaxanthin production in *E. coli*. They found that tunable intergenic regions approach is much more efficient than using fusion protein-mediated substrate channeling. After the elimination of rate-limiting step (i.e., reaction catalyzed by CrtZ), they were able to produce 11.95 ± 0.21 mg g^{-1} DCW of zeaxanthin using glucose as substrate. Chromosomal integration approach was applied for the expression of astaxanthin from engineered *E. coli* strain. Xanthophyll biosynthetic genes from *Nostoc punctiforme* and *Pantoea ananatis* were integrated into the chromosome as individual expression cassettes for the expression of the isoprenoid precursor, i.e., isopentenyl diphosphate (IPP) which produces β-carotene. The expression of crtEBIY along with the β-carotene ketolase gene crtW148 (NpF4798) and the β-carotene-hydroxylase gene (crtZ) under controlled conditions redirected the pathway toward the astaxanthin production utilizing glucose as substrate (1.4 mg/g cdw) (Lemuth et al. 2011).

21.6 Metabolic Engineering of Yeast for the Production of Plant Secondary Metabolites

Yeast species is one of the important microbes commonly used to host diverse secondary metabolite pathways. The budding yeast, *Saccharomyces cerevisiae*, is also a common industrial microorganism used extensively in food and beverage

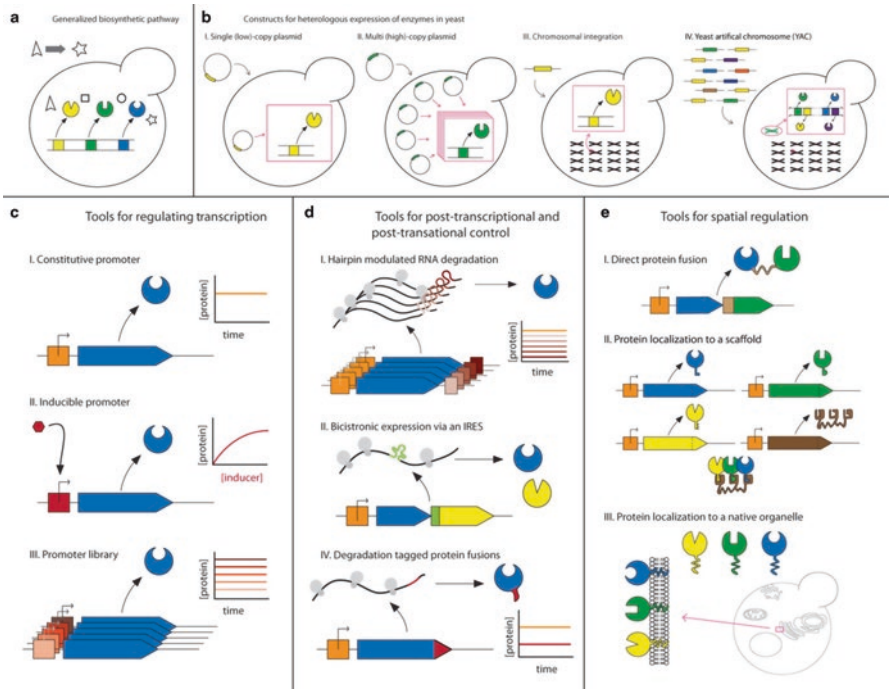

Fig. 21.3 Tools for controlling enzyme expression in yeast. (**a**) Metabolic engineering efforts in yeast utilize an array of tools for the expression and regulation of heterologous genes in *Saccharomyces cerevisiae*. Tools enabling heterologous enzyme expression (**b**), transcriptional regulation (**c**), posttranscriptional and posttranslational regulation (**d**), and spatial regulation (**e**) in yeast are illustrated (Siddiqui et al. 2012)

production (Siddiqui et al. 2012).The *S. cerevisiae* expression system provides a number of advantages over *E. coli*. Firstly, as a eukaryote, yeast has compartments of cells similar to plant and has capacity to modify eukaryotic proteins posttranslationally. Secondly, yeast has an endoplasmic reticulum system which confers the ability to support functional expression of membrane-bound cytochrome such as P450s enzymes (Pompon et al. 1996). *S. cerevisiae* is also a key model organism for fundamental molecular biology research, and it was the first eukaryotic organism to have its genome completely sequenced (Goffeau et al. 1996). This fundamental knowledge helped in developing number of tools for pathway engineering and the genetic stability of foreign genes (Mumberg et al. 1994, 1995). The goal of reaching maximum productivity depends on a wide variety of factors including optimizing the metabolic flux, reducing toxic intermediates and balancing stress on the host cell. Various tools for optimizing the expression of heterologous genes by *S. cerevisiae* are shown in Fig. 21.3 which includes increasing gene copy number, transcriptional activity, and/or posttranslational processing (Siddiqui et al. 2012).

21.6.1 Metabolic Engineering of Flavonoids in Yeast

The first study of enzymes involved in flavonoid pathway in yeast was reported by introducing PAL and C4H genes to deaminate phenylalanine into cinnamic acid with further hydroxylation to yield 4-coumaric acid. It was observed that biochemical coupling of PAL and C4H is sufficient/enough to drive the flux toward phenylpropanoid pathway. Subsequently, the modified yeast strain was able to produce approximately 0.8 mg/l of pinocembrin and 7 mg/l of naringenin by expression of PAL, 4CL, and CHS without inserting cinnamate 4-hydroxylase (C4H) (Jiang et al. 2005). Yan et al. (2005b) reconstructed both the flavonoid pathway by co-expressing CHI with either flavanone 3β-hydroxylase (F3H) or flavone synthase II (FNSII) and the isoflavonoid pathway. While using 4-coumaric acid as a precursor, they were able to produce 28.3 mg/l of naringenin in the culture. Ralston et al. (2005) developed a yeast expression platform by partial reconstruction of flavonoid and isoflavonoid biosynthesis using soybean type I and type II chalcone isomerases. Becker et al. (2003) reconstructed biochemical pathway by co-expression of coenzyme A ligase gene (4CL216) from hybrid poplar and resveratrol synthase gene (vstl). This engineered *S. cerevisiae* strain was able to express resveratrol by feeding just 4-coumaric acid as a substrate. Zhang et al. (2006) were successfully able to increase resveratrol production in yeast up to 15-fold by constructing a fusion protein of 4CL and STS (4CL::STS). Alternatively, Feng et al. (2006) studied that fusion protein might have brought the active sites of 4CL and STS into close proximity, thus reducing the diffusion capability of intermediates and increasing the catalytic efficiency (Feng et al. 2006). The other factors that contribute to enhance resveratrol flux in yeast systems were codon optimization and heterologous expression of related transporters. The codon optimization of TAL enhanced translation and improved p-coumaric acid and also resveratrol biosynthesis drastically. The low affinity with high capacity of bacterial *araE* transporter was able to enhance resveratrol accumulation. It was observed that modified yeast strain carrying the *araE* gene was capable of producing up to 2.44-fold higher resveratrol than control cells (Wang et al. 2011b). Flavonoid derivative such as genistein was successfully cloned and produced in yeast strain (Li et al. 2014a).

21.6.2 Production of Terpenoids in Yeast

The yeast strain was engineered by expressing linalool synthase and geraniol synthase genes to express monoterpenoid alcohols by using internal geranyl pyrophosphate as precursor (Oswald et al. 2007). Ro et al. (2006) have produced high amount of artemisinic acid using an engineered mevalonate pathway, amorphadiene synthase and a novel cytochrome P450monooxygenase from *Artemisia annua*. Furthermore, the biosynthetic pathway of farnesyl pyrophosphate (*FPP*) was also modified to produce more FPP and the usage of FPP to steroid was also blocked to increase the FPP flux into artemisinic acid. The engineered yeast cell was capable of yielding 115 mg/L of artemisinic acid (Ro et al. 2008). Günel et al. (2006)

successfully redirected carbon flow from the terpenoid pathway to ergosterol formation and toward the production of carotenoid by cloning a gene encoding geranyl pyrophosphate synthase from bell pepper (*C. annum*) in *S. pombe*. Zhuang and Chappell (2015) clearly explained the building platforms for the terpene in yeast.

21.7 Conclusion: Promises and Perils

It is evident from the existing literature that a number of plant secondary metabolites are produced using microbes as hosts. It is logically sound to understand that the fast growing, highly selective microbes to be more appropriate for producing such beneficial entities that we require every day. The associated burden of low productivity and purity is substantially reduced with the advent in technology and better understanding of the micro level hosts. It is eminent that *E. coli* is the best available prokaryotic host for its minimalism with complexities compared with other prokaryotic hosts. However, to address the posttranslational modification problems associated with the products, simple and proficient eukaryotic hosts such as *S. cerevisiae* are preferred. With the engineering of metabolic pathways on the rise, the productivity of metabolites has shot up particularly in the last decade. However, other than few frequently used products like vanillin, most of the secondary metabolite production from microbes on a large scale is underdevelopment. With the growing investments in the field of biotechnology, specifically in developed and developing countries, it is no doubt that the much-awaited milestone in the production of metabolites on a large scale is not far. Ever-growing research on the global level definitely would result in commercial and economic production of plant products in microbial cells.

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Beneficial Plant-Microbes Interactions: Biodiversity of Microbes from Diverse Extreme Environments and Its Impact for Crop Improvement

22

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Abstract

Microbes are capable of colonizing the rhizosphere and phyllosphere as well as living inside the plant tissues as endophytes. The microbiomes associated with the crops have the ability to produce phytohormones (indoleacetic acid and gibberellic acid); solubilize (phosphorus, potassium and zinc) and bind nutrients, besides eliciting plant defence reactions against pathogens; and also help in plant growth under harsh environments. The biodiversity of plant growth-promoting (PGP) microbes have been illustrated by different genera and species and their mechanisms of action for the following different phyla of domain Archaea, Bacteria and Eukarya: *Actinobacteria*, *Ascomycota*, *Bacteroidetes*, *Basidiomycota*, *Crenarchaeota*, *Euryarchaeota*, *Firmicutes* and *Proteobacteria* ($\alpha/\beta/\gamma/\delta$). This book chapter intends to present research results obtained so far concerning the application of beneficial microbes as PGP microbes and their potential biotechnological application to increase the plant growth and yields and soil health. The diverse range of activities as well as the number of microbes sorted out in different culture collections around the world, may provide an important resource to rationalize the use of chemical fertilizers in agriculture. There are many microbial species that act as PGP microbes, described in the literature as successful for improving plant growth and health. However, there is a gap between the mode of action/mechanism of the PGP microbes for plant

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growth and the role of the PGP microbes as biofertilizers. Hence, this book chapter bridges the gap mentioned and summarizes the mechanism of PGP microbes as biofertilizers for agricultural sustainability.

Keywords
Biodiversity • Biofertilizers • Extreme environments • Microbiome • Plant growth promotion

22.1 Introduction

The principal sources of fluctuations in global food production in developing countries have been affected by climate variability. The important risks of increasing global warming are variable and untimely rainfall events, unstable winter/summer seasons, more disease occurrences and crop failures (Adger et al. 2005). However, many research outputs indicate that prolonged short growth season collectively with higher growth temperatures can provide new opportunities for agriculture in many agroecological zones (Uleberg et al. 2014). These climate change impacts on agricultural outputs in different continents are expected to differ and hence require customized adaptive strategies (Tscharntke et al. 2012; Uleberg et al. 2014). These strategies should include those factors which affect agriculture in a great deal such as land use and soil properties, local climate, local and regional market forces, agriculture management strategies and agricultural tradition composed of coherent traditional wisdom and agricultural practices (Reidsma et al. 2010; Tripathi and Singh, 2013). Extreme environments represent unique ecosystems which harbour novel biodiversity of different groups of microbes including acidophilic, alkaliphilic, halophilic, psychrophilic, thermophilic and xerophilic (Yadav et al. 2015d; Saxena et al. 2016; Yadav et al. 2017). Microbial communities associated with plant growing in most diverse conditions, including extremes of acidity, alkalinity, salinity, and temperature and water deficiency, have been sorted out and characterized for potential biotechnological application in agricultures, medical, industry and environments. These extremophilic microbiomes associated with plant microbes have developed adaptive features which permit them to grow and survive under such extreme environmental conditions. These extremophiles can grow optimally in some of the earth's most hostile environments of temperature (−2–20°C, psychrophiles; 60–115°C, thermophiles), salinity (10–30% NaCl, halophiles) and pH (<4, acidophiles and >9, alkaliphiles) (Yadav et al. 2015c, d).

The study of biophysical and biogeochemical processes has identified changes in the abiotic components of the system, mainly due to the ability of the microorganisms to carry out metabolic processes unimaginable outside the rhizosphere without the genetic contribution of plants and microorganisms that inhabit abiotic stresses. In this case, nutrient mobilization, such as iron and phosphorus, or metabolic activities related to the immobilization of heavy metals or biodegradation of xenobiotic pollutants such as pesticides, polyaromatic hydrocarbons and other compounds are

highly stable and dangerous for human health. Therefore, the rhizosphere constitutes a system especially suitable for obtaining culturable beneficial microorganisms or genes with a great biotechnological application oriented to nutrient mobilization and bioremediation. Today, it is a widely accepted fact that certain strains of rhizospheric microbiome, referred to as plant PGP microbes, stimulate plant growth, plant fitness, adaptation to extreme conditions and soil health. Thanks to the knowledge of communication signals between microbiomes of plants, we are able to understand, at least partially, the PGP mechanisms. In the 1990s, the interaction of microbes with plants was simply thought of as being an effect, but today it is recognized as a process with a high level of complexity in which at least two genomes share information without sharing the same spaces from a cellular perspective. It is already a widely known fact that PGP microbes, broadly speaking, can improve plant fitness and soil health for sustainable agriculture. Beyond their effect on nutrition or their biocontrol capacity, some PGP microbial strains are able to effectively protect crops/plants from pathogens, triggering a response in the plant that makes it resistant to further pathogen attack.

Microbial diversity associated with crops is considered important for maintaining the sustainability of agriculture production systems. A microbe helps plant for growth, yield and adaptation to extreme conditions. The microbiome of plants could be classified into three categories, e.g. rhizospheric, phyllospheric and endophytic. The rhizosphere is the zone of soil influenced by plant roots through the release of different substrates that affect microbial function and its stability. A number of microbial species belonging to genera *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Aspergillus*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Flavobacterium*, *Haloarcula*, *Halobacterium*, *Halococcus*, *Haloferax*, *Methylobacterium*, *Paenibacillus*, *Penicillium*, *Piriformospora*, *Pseudomonas*, *Rhizobium* and *Serratia* have been sorted out associated with the plant rhizosphere (Xie et al. 1996; Lavania et al. 2006; Chaiharn and Lumyong 2011; Yadav et al. 2011, 2014, 2017a 2016c, 2017a, b, c, d; Meena et al. 2012; Kumar et al. 2016; Suman et al. 2016b; Shah et al. 2017). The microbiome which colonizes in the interior of the plant parts, *viz.* root, stem or seeds, without causing any harmful effect on host plant is referred to as endophytic microbes. These microbes have been isolated from a variety of crops/plants including wheat (Coombs and Franco 2003; Jha and Kumar 2009; Verma et al. 2013, 2014, 2015a, b, 2016a, b), rice (Mano and Morisaki 2007; Naik et al. 2009; Piromyou et al. 2015), maize (Araújo et al. 2000; Montanez et al. 2012; Thanh and Diep 2014), soybean (Hung and Annapurna 2004; Mingma et al. 2014), pea (Narula et al. 2013; Tariq et al. 2014), bean (Suyal et al. 2015) and chickpea (Saini et al. 2015). A large number of endophytic microbial species belonging to different genera including *Achromobacter*, *Aspergillus*, *Azoarcus*, *Burkholderia*, *Enterobacter*, *Gluconacetobacter*, *Herbaspirillum*, *Klebsiella*, *Microbispora*, *Micromonospora*, *Nocardioides*, *Pantoea,* *Planomonospora*, *Pseudomonas*, *Penicillium*, *Piriformospora*, *Serratia*, *Streptomyces* and *Thermomonospora* have been reported from different host plants and characterized for PGP attributes (Hallmann et al. 1997; Ryan et al. 2008; Verma et al. 2014, 2015b). The phyllosphere is common niche for synergism between microbes and plants. Microbiomes of leaf surface are the most

adapted microbes as they can tolerate high temperature (40–55°C) and UV radiation. Many microbes such as *Agrobacterium*, *Methylobacterium*, *Pantoea*, *Penicillium* and *Pseudomonas* have been reported in the phyllosphere of different crops (Verma et al. 2013, 2014, 2015a, b). Microbes associated with crops are able to promote the plant growth under different abiotic stress conditions. Several microbes have been reported that they can promote plant growth either directly by N_2-fixation; solubilization of minerals such as phosphorus (P), potassium (K) and zinc (Zn); and production of different groups of siderophores, indoleacetic acids, gibberellic acids and cytokinin or indirectly via production of antagonistic substances, antibiotics and lytic enzymes (Glick et al. 1999b; Tilak et al. 2005).

Biotechnology has opened up new possibilities for potential applications of beneficial microbiomes to the soil for the PGP and biocontrol of soilborne pathogens. The nutritional and environmental requirements of these microbes are very diverse. The microbial inoculation has a much better stimulatory effect on plant growth in nutrient-deficient soil as well as in nutrient-rich soil. An understanding of microbial diversity and its potential applications in agriculture is important and useful to arrive at measures that can act as indicators of plant growth, yield and soil health. The present book chapter describes the beneficial interaction between microbes and plants. The method of isolation of microbiomes plant growing in extreme conditions and role of microbes in crop improvement have been discussed here.

22.2 Isolation and Characterization of Microbiomes of Crops

The microbes associated with plant can be enumerated using different growth media such as DSMZ-97, DSMZ-823 and DSMZ-1184; OS media for halophilic archaea, Jensen's agar for N_2-fixing bacteria, King's B agar for *Pseudomonads*, Luria Bertani agar for endophytic bacteria, nutrient agar for heterotrophic, soil extract agar for soil-specific microbes, trypticase soya agar for *Arthrobacter* and yeast extract mannitol agar for *Rhizobium* (Table 22.1). Medium compositions and conditions for isolation of microbes associated with plant, growing under different extreme and normal habitat, are provided in Table 22.2. The culturable microbes from soil and rhizosphere soil can be isolated through enrichment using the standard serial dilution plating technique (Fig. 22.1). The culturable bacteria from rhizospheric soil can be isolated through enrichment technique using the standard serial dilution plating technique. Heat enrichment technique with serial dilution method can be used for isolation of *Bacillus* and *Bacillus*-derived genera (BBDG). A selective enrichment technique using 0.25 M and 0.75 M sodium acetate buffer with LB broth and T_3 agar can be employed for isolation of *Bacillus thuringiensis* (Yadav et al. 2015d). For isolation of endophytic bacteria, the roots should be washed in running water to remove adhering soil and surface sterilized by dipping in 0.1% of mercuric chloride for 5 min following 2% of sodium hypochlorite for 10 min. The roots and stem of selected crops can be cut into 1 cm pieces and place onto selective and complex growth medium. Epiphytic bacteria can be isolated form phyllosphere of selected plants. Plant leaves (3 g) can be agitated at 150 rpm at ambient temperature for 2 h

Table 22.1 Media employed for isolation of plant-associated microbes

S.N.	Growth media	Microbes	References
1.	Ammonium mineral salt	Methylotrophs	Holland et al. (2000)
2.	Congo red yeast mannitol	*Rhizobium*	Yumoto et al. (2002)
3.	DSMZ-97, DSMZ-823, DSMZ-1184; OS	Halophilic archaea	Yadav et al. (2015c)
4.	Jensen agar	N_2-fixing bacteria	Jensen (1965)
5.	King's Bagar	*Pseudomonas* sp.	Mishra et al. (2009)
6.	Luria Bertani agar	Endophytic bacteria	Ventosa et al. (1982)
7.	Nutrient agar	Heterotrophic bacteria	Ramesh and Lonsane (1987)
8.	Potato dextrose agar	Fungus	Sehgal and Gibbons (1960)
9.	Soil extract agar	Soil-specific microbes	Shivaji et al. (1988)
10.	Tryptic soy agar	*Arthrobacter*	Shivaji et al. (1989)

in 500 mL Erlenmeyer flasks containing 25 g of glass beads and 50 mL of phosphate buffer. After agitation, appropriate dilutions of the flask contents can be plated onto different medium. Imprint method was also used to isolate epiphytic bacteria (Holland et al. 2000).

For identification of microbes, genomic DNA can be isolated using Zymo Research Fungal/Bacterial DNA MicroPrep™ following the standard protocol prescribed by the manufacturer. Different primers can be used for amplification of 16S rRNA gene for archaea and bacteria while 18S rRNA gene for fungi. PCR-amplified 16S/18S rRNA genes have to purified and sequenced. The partial 16S or 18S rRNA gene sequences should be compared with sequences available in the NCBI database. To know the taxonomical affiliation, the neighbour joining (NJ) method in the program MEGA 4.0.2 can be used to construct phylogenetic tree of different microbes (Fig. 22.2).

22.3 Plant-Microbe Interactions and Biodiversity of Microbes

The different groups of microbes have been reported as plant associated such as archaea (*Euryarchaeota*), bacteria (*Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Deinococcus-Thermus*, *Firmicutes* and *Proteobacteria*) and fungi (*Ascomycota* and *Basidiomycota*) (Fig. 22.2). The *Proteobacteria* were further grouped as *α*-, *β*-, *γ*- and *δ-Proteobacteria*. Overall the distribution of microbes varied in all bacterial phyla; *Proteobacteria* were the most dominant followed by *Actinobacteria*. The least number of microbes was reported from phyla *Deinococcus-Thermus* and *Acidobacteria* followed by *Bacteroidetes*. There was the first report on archaea that to be identified as endophytes associated with rice by the culture-independent approach. *Methanospirillum* sp. and *Candidatus Methanoregula boonei* have been reported as endophytic archaea from rice (Sun et al. 2008). The archaea isolated from phylum *Euryarchaeota* belonged to different genera such as *Haloferax*, *Methanobacterium*, *Methanosaeta, Methanospirillum* and *Thermoplasma* (Chelius and Triplett 2001).

Table 22.2 Media and the conditions employed for isolated microbes associated with crops growing in diverse extreme habitat

Category	Media and conditions
Acidophilic	Ammonium minerals salt: 0.70 g K_2HPO_4; 0.54 g KH_2PO_4; 1 g $MgSO_4.7H_2O$; 0.2 g $CaCl_2$. $2H_2O$; 4.0 mg $FeSO_4$.$7H_2O$; 0.5 g NH_4Cl; $ZnSO_4$. $7H_2O$; 30 µg $MnCl_2.4H_2O$; 300 µg H_3BO_3; 10 µg $CuCl_2$. $2H_2O$; 200 µg $CoCl_2$. $6H_2O$; 20 µg $NiCl_2.6H_2O$; 60 µg $Na_2MoO_4.2H_2O$
	Jensen's agar (JA): 20 g sucrose;1 g K_2HPO_4; 0.5 g Mg_2SO_4; 0.5 g NaCl; 0.001 g Na_2MoO_4; 0.01 g $FeSO_4$; 2 g $CaCO_3$
	King's B agar (KB): 20 g protease peptone; 1.5 g K_2HPO_4; 1.5 g $MgSO_4.7H_2O$; 10 ml glycerol
	Nutrient agar: 5 g peptone; 5 g NaCl; 3 g beef extract
	Soil extract agar: 2 g glucose; 1 g yeast extract; 0.5 g K_2HPO_4; 100 ml soil extract (250 g soil from sampling site +1 L H_20, autoclave and filter)
	T_3 agar: 3 g tryptone; 2 g tryptose; 1.5 g yeast extract; 0.005 g $MnCl_2$; 0.05 g sodium phosphate
	Tryptic soy agar: 17 g tryptone; 3 g soya meal; 2.5 g dextrose; 5 g NaCl; 2.5 g K_2HPO_4; 20 g agar
	Yeast extract mannitol agar: 1 g yeast extract; 10 g mannitol; 0.5 g $K_2HPO_4.H_2O$; 0.002 g $MgSO_4.7H_2O$; 0.1 g NaCl
	@all media with 3–5 pH and incubation at 30°C for 1–5 days
Alkaliphilic	Ammonium minerals salt: Jensen's agar; King's B agar; nutrient agar; soil extract agar; T_3 agar; tryptic soy agar; yeast extract mannitol agar
	Horikoshi agar: 10 g glucose; 5 g polypeptone; 5 g yeast extract; 1 g K_2HPO_4; 0.2 g $MgSO_4.7H_2O$; 20 g agar and after autoclaving, aseptically add 100.0 ml of sterile 10% Na_2CO_3 to the medium
	@all media with 8–11 pH and incubation at 30°C for 1–7 days
Halophilic	Ammonium minerals salt: Jensen's agar; King's B agar; nutrient agar; soil extract agar; T_3 agar; tryptic soy agar; yeast extract mannitol agar
	Chemically defined medium: 5 g casamino acids; 5 g yeast extract; 1 g sodium glutamate; 3 g tri-sodium citrate; 20 g $MgSO_4$; 2 g KCl; 100 g NaCl; 36 mg $FeCl_2$; 0.36 mg $MgCl_2$
	Halophilic medium: 100 g NaCl; 2 g KCl; 1 g $MgSO_4{\cdot}7H_2O$; 0.36 g $CaCl_2{\cdot}2H_2O$; 0.23 g NaBr; 0.06 g $NaHCO_3$; 5 g protease peptone; 10 g yeast extract; 1 g glucose; trace $FeCl_3$
	@all media with 5, 7.5, 10, 15% NaCl, 7.0–7.2 pH and incubation at 30°C for 1–7 days
Psychrophilic	Ammonium minerals salt: Jensen's agar; King's B agar; nutrient agar; soil extract agar; T_3 agar; tryptic soy agar; yeast extract mannitol agar
	All media diluted 10, 50 and 100 times; pH-7.0–7.2 and incubation at 5°C for 7–15 days
Thermophilic	Ammonium minerals salt: Jensen's agar; King's B agar; nutrient agar; soil extract agar; T_3 agar; tryptic soy agar; yeast extract mannitol agar
	Thermus medium: 4 g yeast extract; 8 g polypeptone peptone; 2 g NaCl
	@all media with 7.0–7.2 pH and incubation at 45–60°C for 1–7 days
Xerophilic	Ammonium minerals salt: Jensen's agar; King's B agar; nutrient agar; soil extract agar; T_3 agar; tryptic soy agar; yeast extract mannitol agar
	@all media with 5–15% PEG-8000, pH 7.2–7.4 and incubation at 30–45°C for 3–7 days

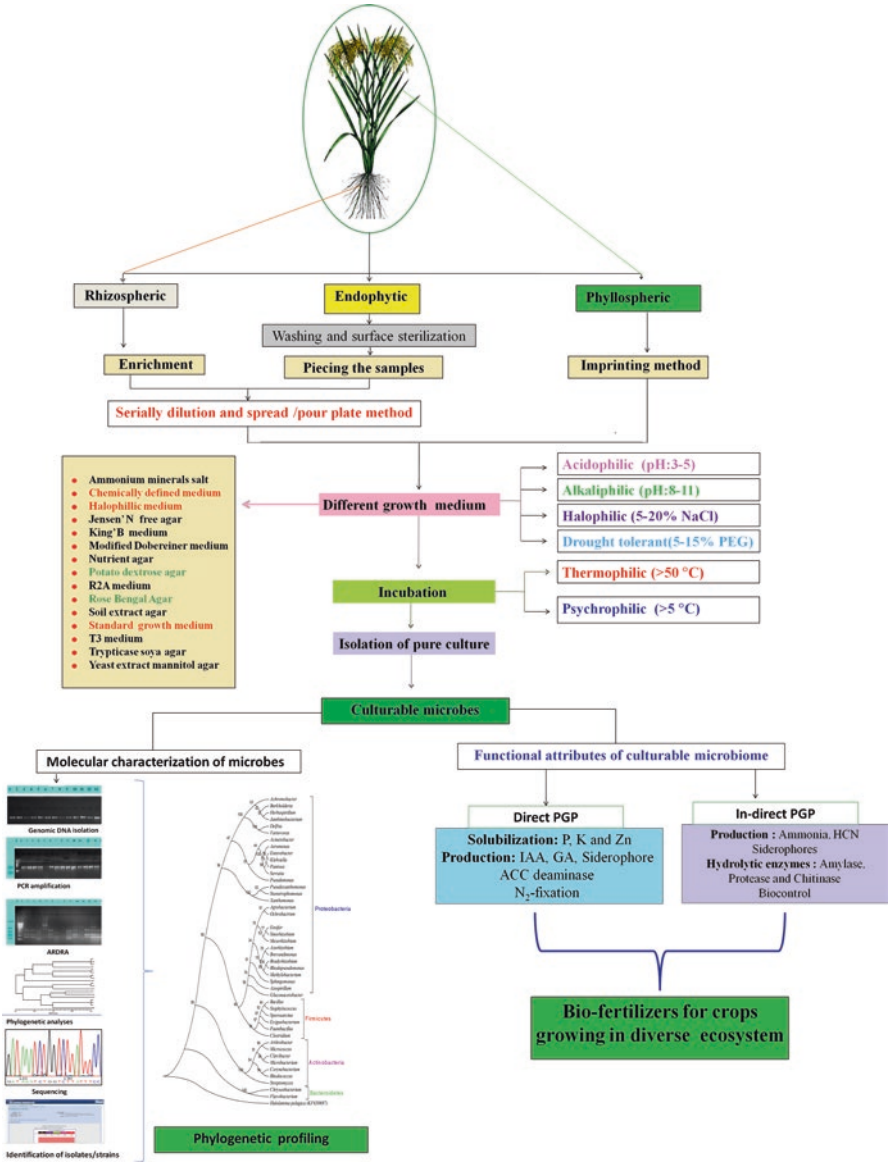

Fig. 22.1 A schematic representation of the isolation, characterization, identification and potential application of culturable and un-culturable microbiomes of crops

Most studies on the occurrence of microbiomes of crops have been performed using culture-dependent approaches. The members of BBDG are associated with different plants and show different plant growth-promoting attributes such as solubilization of P, K and Zn; production of phytohormones and biocontrol against different pathogens and have been consistently described as culturable microbes which

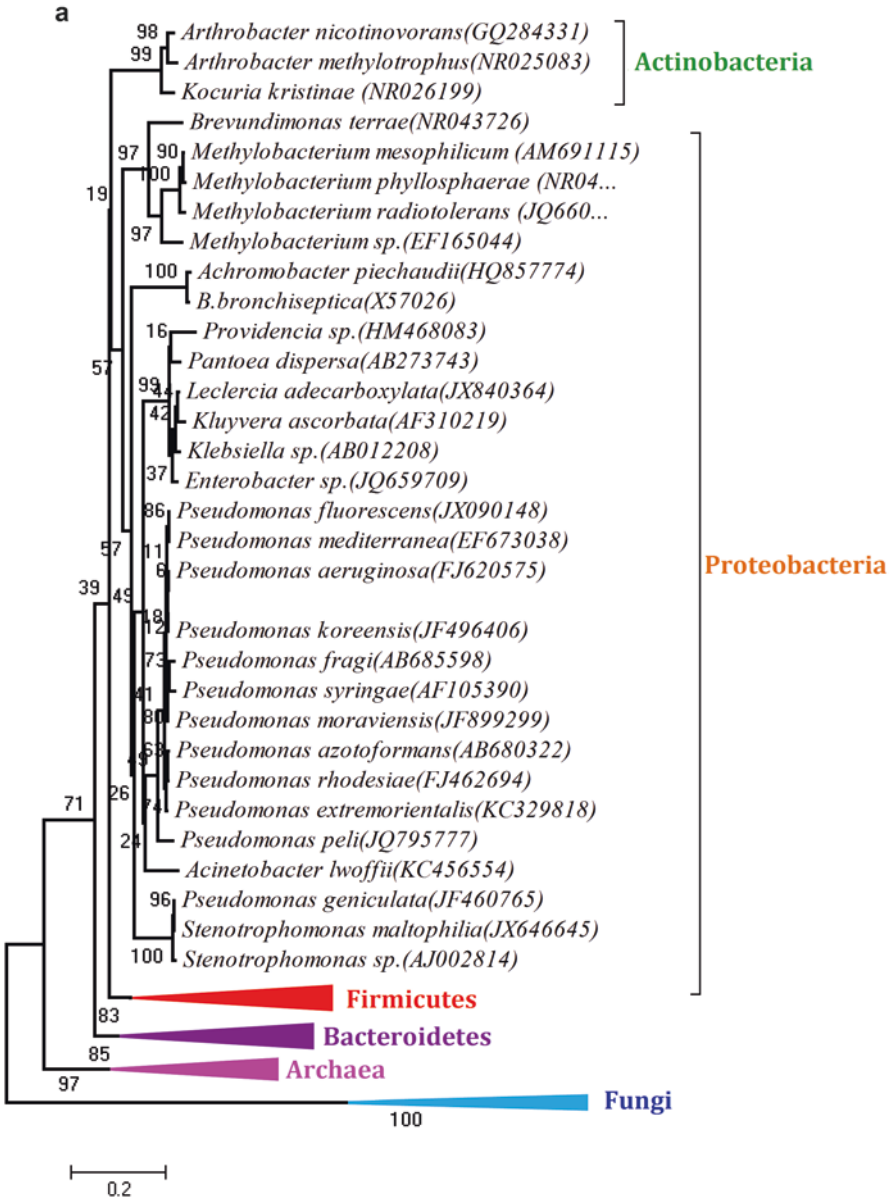

Fig. 22.2 (**a** and **b**) Phylogenetic tree showing the relationship among different groups of microbes associated with crops growing in diverse extreme environments

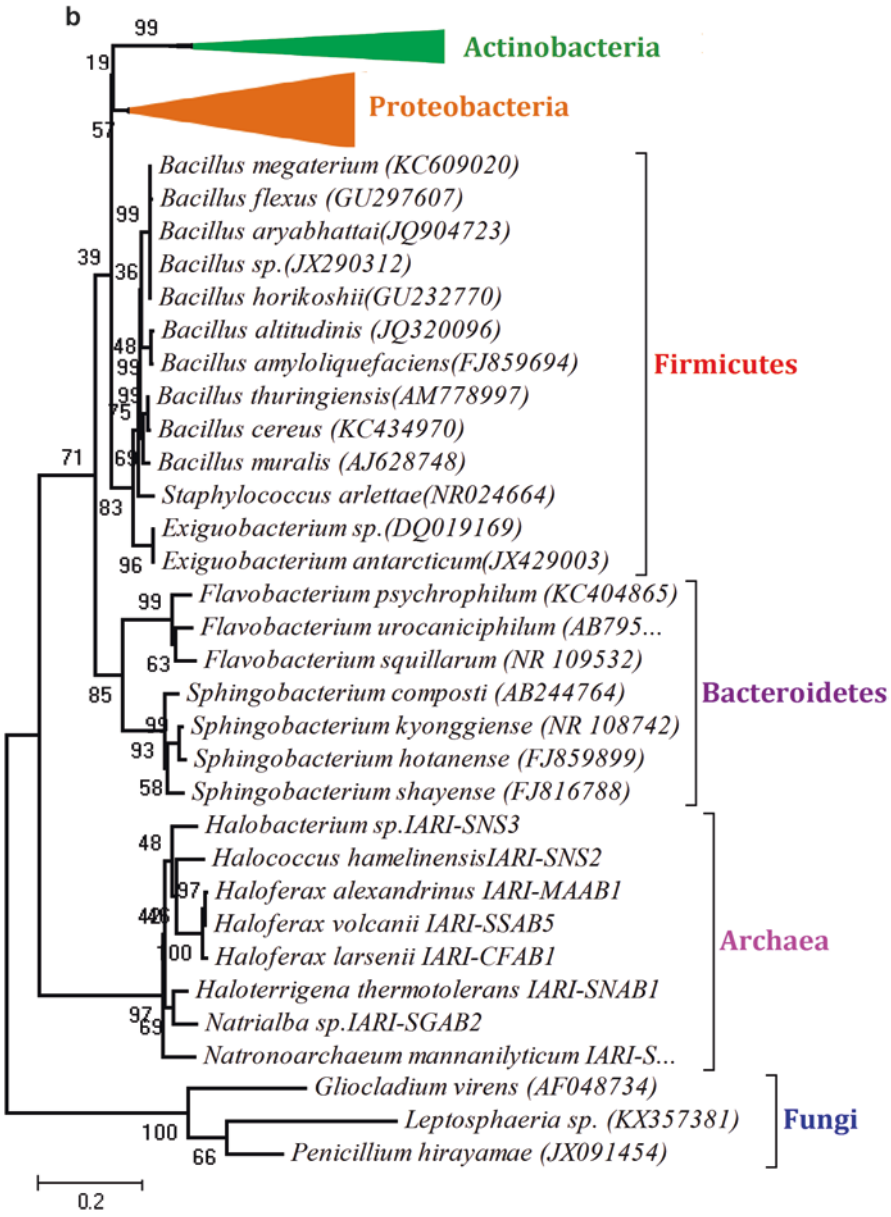

Fig. 22.2 (continued)

can colonize different crops such as wheat (Verma 2015; Verma et al. 2013, 2014; 2015a; 2016a, b), rice (Sun et al. 2008), maize (Liu et al. 2015; Kämpfer et al. 2016), soybean (Hung and Annapurna 2004) and chickpea (Saini et al. 2015).

The bacterial species belong to phylum *Proteobacteria* are ubiquitous in nature. Among *Proteobacteria*, the members of *γ-Proteobacteria* are the most dominant and have been reported from different crops such as wheat (Verma et al. 2014; 2015a, b, c), rice (Sun et al. 2008), maize (Szilagyi-Zecchin et al. 2014; Thanh and Diep 2014) and millet (Gupta et al. 2013). The pink-pigmented facultative methylotrophs (PPFMs) have been reported from diverse host plants *viz.* wheat (Verma et al. 2015a, b, c), rice (Dourado et al. 2015) and common bean (de Oliveira Costa et al. 2012). In plant colonization, the frequency and distribution may be influenced by plant genotype or by interactions with other associated microorganisms, which may result in increasing plant fitness. The different species of *Pantoea* have been described as cosmopolitan associated with wheat (Verma et al. 2014), rice (Rangjaroen et al. 2014) and maize (Ikeda et al. 2013). Members of *Pantoea* are ubiquitous in plant tissue; they are able to influence plant growth through the production of auxins or cytokinins and induce systemic resistance against diseases. There are few reports for niche/plant-specific microbes, whereas there were many reports on niche-specific microbiomes from different extreme environments (Kumar et al. 2014a, b; Pandey et al. 2013; Verma et al. 2014, 2015b, 2016a; Yadav et al. 2015a, c, d).

22.3.1 Archaea

Archaea is one of the most abundant microbes reported from extreme environments. There are very few reports of archaea as associated with crops such as maize, rice and halophytic crops (*Abutilon*, *Cressa*, *Sporobolus*, *Suaeda nudiflora*) (Chelius and Triplett 2001; Sun et al. 2008; Wang et al. 2009; Saxena et al. 2015a; Yadav et al. 2015c; 2017a). Archaea have been reported as un-culturable from maize (Chelius and Triplett 2001) and rice (Sun et al. 2008), whereas culturable archaea have been reported from *Abutilon*, *Cressa*, *Sporobolus* and *Suaeda nudiflora* (Yadav et al. 2015c). Chelius and Triplett (2001) reported un-culturable archaea associated with maize roots (*Zea mays* L.). The diversity within the archaeal domain was low in comparisons with bacteria and fungi. Sun et al. (2008) have reported archaea as endophytes from rice. That study was first reported on endophytic archaea (*Methanospirillum* sp.) as associated with rice by the culture-independent approach.

Saxena et al. (2015a) reported culturable haloarchaea from different halophytic plants growing in extreme hypersaline region of Rann of Kutch, India. Archaea are known to inhabit extreme environments and have never been studied with perspectives to understand their interactions with eubacteria and to sustain vegetation in extremes of salinity, moisture stress and temperature. Many species of haloarchaea have been isolated from hypersaline regions of Rann of Kutch including *Haloarcula*, *Halobacterium*, *Halococcus* and *Haloferax*. There are very few reports on characterization of halophilic archaea for their plant growth-promoting attributes so as to help the vegetation to survive better in these extreme environments characterized by

nutrient-deficient milieu. Soil microorganisms play an important role in soil processes that determine plant productivity. In study by Saxena et al. (2015a), it has been found that different halophilic genera of archaea solubilize phosphorus under abiotic stress of salinity. These halophilic archaea have been identified using 16S rRNA gene sequencing and BLAST analysis as *Haloarcula*, *Halobacterium*, *Halococcus*, *Haloferax*, *Halolamina*, *Haloterrigena*, *Natrialba* and *Natrinema* (Table 22.3).

22.3.2 Actinobacteria

Actinobacteria is a phylum of Gram-positive bacteria. The members of bacteria belonging to phylum *Actinobacteria* are classified into six classes, namely, *Acidimicrobiia*, *Actinobacteria*, *Coriobacteriia*, *Nitriliruptoria*, *Rubrobacteria* and *Thermoleophilia*. Among six different classes, members of the class *Actinobacteria* are the most dominant and contain one of the largest bacterial genera, *Streptomyces* (961 species). Members of the phylum *Actinobacteria* are ubiquitous in nature and have been isolated from different extreme environments (extreme temperatures, pH, salinities, pressure and drought) and are associated with plant growing in different habitats. The rhizospheric actinobacteria are the most dominant in nature, and they are of great economic importance in agriculture, medicine, industry and environments. The rhizospheric actinobacteria have been reported, biochemically characterized and identified using 16S rRNA gene sequencing. Based on a comprehensive literature analysis, members of the phylum *Actinobacteria* have been reported from different genera such as *Acidimicrobium*, *Actinomyces*, *Arthrobacter*, *Bifidobacterium*, *Cellulomonas*, *Clavibacter*, *Corynebacterium*, *Frankia*, *Microbacterium*, *Micrococcus*, *Mycobacterium*, *Nocardia*, *Propionibacterium*, *Pseudonocardia*, *Rhodococcus*, *Sanguibacter* and *Streptomyces* (Conn and Franco 2004; Verma et al. 2013, 2014, 2015b, 2016b). *Actinobacteria* with multifarious PGP attributes can be utilized as biofertilizers with replacement of chemical fertilizers for sustainable agriculture as they can enhance plant growth and yield by direct or indirect plant growth. The actinobacteria are important in biotechnological applications in different industrial and agricultural processes.

22.3.3 Bacilli

The members of BBDG are a heterogeneous collection of aerobic or facultative anaerobic endospore-forming bacteria that are ubiquitous in many extreme environments, and they have been reported as associated with different plants, showing different PGP attributes such as solubilization of nutrients and production of IAA, GA, and siderophores and N_2-fixation. Members of the families *Bacillaceae* and *Paenibacillaceae* are widely used in agriculture as plant growth-promoting and disease-suppressing agents, besides their use in industry as a source of enzymes and in medicine. Garbeva et al. (2003) showed that the majority (95%) of Gram-positive bacteria in soils under different types of management regimes were alleged as

Table 22.3 Plant growth-promoting attributes of different microbes

Microbes	PGP attributes	Host/association	References
Archaea			
Halobacterium sp., *Halococcus hamelinensis*, *Haloferax alexandrinus*, *Haloterrigena thermotolerans*, *Methanobacterium bryantii*, *Methanosarcina*, *Methanospirillum* sp., *Natrialba* sp., *Natronoarchaeum mannanilyticum*, *Nitrosomonas communis*	P-solubilization, IAA, siderophore, nitrogen fixation	*Abutilon*, *Cressa*, maize, rice, *Sporobolus*, *Suaeda nudiflora*,	Chelius and Triplett (2001), Sun et al. (2008), Wang et al. (2009), Saxena et al. (2015a), Yadav et al. (2015c) and Gaba et al. (2017)
Actinobacteria			
Arthrobacter humicola, *A. methylotrophus*, *Arthrobacter* sp., *Cellulosimicrobium* sp., *Kocuria*, *Micrococcus luteus*, *Streptomyces*	P-solubilization, IAA, biocontrol	Cowpea, millet, mustard, wheat	Dimkpa et al. (2008) and Tiwari et al. (2011); Verma (2015) and Verma et al. (2013, 2015b)
Bacteroidetes			
Flavobacterium psychrophilum, *Flavobacterium* sp., *Sphingobacterium* sp.	P-solubilization, K-solubilization	Barley, millet, wheat	Verma et al. (2014, 2016b) and Rana et al. (2016a)
Proteobacteria			
Achromobacter piechaudii, *Acinetobacter* sp., *Advenella* sp., *Agrobacterium larrymoorei*, *Alcaligenes* sp., *Azotobacter tropicalis*, *Bradyrhizobium* sp., *Enterobacter* sp., *Methylobacterium phyllosphaerae*, *M. radiotolerans*, *Nitrinicola lacisaponensis*, *Pantoea agglomerans* sp., *Providencia rustigianii*, *Pseudomonas cedrina*, *P. fluorescens*, *P. gessardii*, *P. putida*, *P. rhodesiae*, *P. thivervalensis*, *Serratia marcescens*, *Tetrathiobacter* sp., *Variovorax*	Multifunction PGP attributes including solubilization of P, K, Zn; production of ammonia, HCN, siderophore; and biocontrol	Amaranth, barley, buckwheat, cotton, cowpea, gram, maize, millet, mustard, oat, rice, sunflower, tomato, wheat	Forchetti et al. (2007), Deepa et al. (2010), Verma et al. (2013), Marag et al. (2015), Verma (2015), Yadav (2015a; 2017b) and Rana et al. (2016a, b)

(continued)

Table 22.3 (continued)

Microbes	PGP attributes	Host/association	References
Firmicutes			
Bacillus aerophilus, *B. alcalophilus*, *B. altitudinis*, *B. amyloliquefaciens*, *B. cereus*, *B. circulans*, *B. endophyticus*, *B. flexus*, *B. fusiformis*, *B. licheniformis*, *B. megaterium*, *B. methylotrophicus*, *B. mojavensis*, *B. pumilus*, *B. solisalsi*, *B. sphaericus*, *B. tequilensis*, *B. thuringiensis*, *Exiguobacterium acetylicum*, *Lysinibacillus*, *Paenibacillus alvei*, *P. dendritiformis*, *P. polymyxa*, *P. xylanexedens*, *Planococcus salinarum*, *Staphylococcus*	Multifunction PGP attributes	Amaranth, apple, barley, buckwheat, maize, mustard, oat, pepper, rice, *sorghum*, sunflower, tomato, wheat	Forchetti et al. (2007), Karlidag et al. (2007), Beneduzi et al. (2008), Hariprasad and Niranjana (2009), Hassen and Labuschagne (2010), Yu et al. (2011), Kumar et al. (2013), Yadav et al. (2015b, 2016c) and Suman et al. (2016a, b))
Fungi			
Gliocladium, *Leptosphaeria*, *Metarhizium*, *Penicillium*, *Piriformospora indica*, *Sporotrichum thermophile*, *Trichoderma*, *T. longibrachiatum*, *Williopsis saturnus*	IAA, siderophore, P solubilization; biocontrol	Amaranth, barley, buckwheat cotton, maize, oat, rice, *sorghum*, soybean, wheat	Nassar et al. (2005), Khan et al. (2008), Pham et al. (2008), Singh and Satyanarayana (2010), Khan et al. (2012), Gill et al. (2016), Hajieghrari and Mohammadi (2016), Rana et al. (2016a), Rawat et al. (2016), Zhang et al. (2016), and Yuan et al. (2017)

Bacillus species; *B. mycoides*, *B. pumilus*, *B. megaterium* and *B. thuringiensis* as well as derived genera such as *Paenibacillus* were frequently identified by sequencing. The members of BBDG are widely dispersed in nature and easy to multiply, have a long shelf life when sporulated and are nonpathogenic. Among BBDG, *B. subtilis*, *B. mycoides*, *B. pumilus*, *B. megaterium*, *B. thuringiensis* and *B. firmus* are ubiquitous in rhizosphere soil (Garbeva et al. 2003; Saxena et al. 2016; Verma et al. 2016a; Yadav et al. 2017b, c, e, f). The genus *Bacillus* consists of a heterogenic group of endospores forming Gram-positive rods, which survive for extended periods under adverse environmental conditions due to endospore.

Jetiyanon et al. (2003) observed that a PGPR mixture containing *B. amyloliquefaciens* strain IN937a and *B. pumilus* strain IN937b induced systemic resistance against

southern blight of tomato (*Lycopersicon esculentum*) caused by *Sclerotium rolfsii*, anthracnose of long cayenne pepper (*Capsicum annuum* var. *acuminatum*) caused by *Colletotrichum gloeosporioides* and mosaic disease of cucumber (*Cucumis sativus*) caused by cucumber mosaic virus (CMV) under field conditions in Thailand.

Verma et al. (2015c) investigated 41 endophytic bacteria from roots and culms of wheat, growing in north-western Indian Himalayas. These bacteria possess multifarious PGP attributes such as N_2-fixation and PK and Zn solubilization; GA, cytokinin, and auxin production; and ACC deaminase activity and biocontrol against different fungal pathogen under the extreme low temperatures condition. Among all, *Bacillus amyloliquefaciens* IARI-HHS2-30 exhibited appreciable level of K solubilization at low temperature condition. Endophytic nature and plant growth-promoting ability of IARI-HHS2-30 were tested qualitatively and followed by inoculation onto wheat seedlings in low temperature conditions. *Bacillus amyloliquefaciens* IARI-HHS2-30 increases in root/shoot length, fresh weight and chlorophyll a content after 30 days of inoculation. PGP attributes coupled with psychrophilic ability suggest that this endophytic bacterium may be exploited as bio-inoculants for various crops in low temperature and high altitude condition.

Yadav et al. (2016c) reported and characterized psychrotrophic *Bacilli* from different sites in NW Indian Himalayas. A total of 247 bacteria have been isolated and characterized for different plant growth-promoting attributes. On the basis of 16S rRNA gene sequencing and BLAST analysis, these bacteria were identified as *Exiguobacterium*, *Lysinibacillus*, *Paenibacillus*, *Pontibacillus*, *Sporosarcina*, *Staphylococcus* and *Virgibacillus*. Looking the PGP attributes of these strains *Bacillus licheniformis*, *Bacillus muralis*, *Desemzia incerta*, *Paenibacillus tylopili* and *Sporosarcina globispora*, biofertilizers can be developed for crops growing at low temperature conditions.

Verma et al. (2016a) investigated culturable BBDG associated with wheat growing in diverse extreme environments of pH, salinity, drought and temperature. A total of 395 BBDG have been isolated and characterized for molecular identification using 16S rRNA gene sequencing and PGP attributes under different abiotic stress conditions of pH, salinity, drought and temperature. All BBDG belong to families *Bacillaceae*, *Paenibacillaceae*, *Planococcaceae*, *Staphylococcaceae* and *Bacillales incertae sedis*, with eight different genera *Bacillus*, *Exiguobacterium*, *Lysinibacillus*, *Paenibacillus*, *Planococcus*, *Planomicrobium*, *Sporosarcina* and *Staphylococcus.* The study by Verma et al. (2016a) was the first report for the presence of different novels and efficient multifunctional PGP species of *Bacillus endophyticus*, *Paenibacillus xylanexedens*, *Planococcus citreus*, *Planomicrobium okeanokoites*, *Sporosarcina* sp. and *Staphylococcus succinus* in wheat microbiome. These niche-specific and abiotic stress-tolerant BBDG could be used as bio-inoculants for crops growing under stressed conditions of pH, salinity, drought and temperature.

22.3.4 Proteobacteria

The phylum *Proteobacteria* are Gram-negative bacteria which included *α/β/γ/δ-Proteobacteria*, which has been reported from most of the studied crops. Among *Proteobacteria*, *α-Proteobacteria* grows at very low levels of nutrients and includes agriculturally imperative bacteria capable of inducing *Azospirillum* and N_2-fixation in symbiosis with plants. The *β-Proteobacteria* is highly metabolically diverse and contains chemolithoautotrophs, heterotrophs and photoautotrophs, while the *γ-Proteobacteria* is the largest class in terms of species *Pseudomonas* and *Azotobacter*. The species of genus *Azospirillum* are microaerophilic, free-living, non-symbiotic, nitrogen-fixing bacteria. Groups of bacteria established a close association with various crops such as maize, sorghum, sugarcane, ray grass and *Amaranthus*. This microbe fixes atmospheric N_2 in asymbiotic manner and makes it available to crops (Steenhoudt and Vanderleyden 2000). The members of *Azospirillum* species grow in the rhizosphere of the crops or infrequently penetrate into the root tissues but are not able to produce any visible nodule but grow intracellularly (Saikia et al. 2007). Iron-chelating compounds (siderophores) have been secretes by the members of *Azospirillum* that help in the sequestering of iron sufficient for plant growth and developments. Free-living N_2-fixing bacteria were for the first time reported by Beijerinck in 1925 under the name of *Spirillum lipoferum*, and later on this organism was renamed as *Azospirillum* (nitrogen-fixing *Spirillum*) in 1978. The species of genus *Azospirillum* is one of the most dominant PGP microbes which are able to fix about >10–40 kgN/ha. Many species of *Azospirillum* have been identified using 16S rRNA gene sequencing and named as *A. lipoferum*, *A. brasilense*, *A. amazonense* and *A. iraquense*.

Azotobacter is Gram-negative, aerobic, heterotrophic, rod-shaped N_2-fixing bacteria present in normal as well as alkaline soils. The species of genus *Azotobacter* are free living as well as associated with different plants and are endophytic (Martyniuk and Martyniuk 2003; Jiménez et al. 2011; Lenart 2012; Czaban and Wróblewska 2017). Among different members of *Azotobacter*, the most predominant are *A. agilis*, *A. chroococcum*, *A. beijerinckii*, *A. vinelandii* and *A. ingrinis* which have been reported from different crops. The *Azotobacter chroococcum* is the most dominant present in and associated with different crops and has exhibited ability to fix atmospheric N_2, 20–40 Kg N/ha. It can also produce various growth-promoting substances, *viz.* auxins, gibberellins, cytokinins and IAA including vitamins and antibiotics, which control plant pathogens and help to maintain soil fertility. Among the different bacterial genera of phylum *Proteobacteria*, the most dominant PGP genus belongs to *Pseudomonas. Pseudomonas* is a genus of Gram-negative, aerobic *γ-Proteobacteria*, belonging to the family *Pseudomonadaceae* and containing 191 validly described species. The biocontrol properties of *P. protegens* and *P. fluorescens* are currently best understood. Other prominent *Pseudomonas* species with biocontrol properties include *P. chlororaphis*, which produces antibiotic (phenazine-type), which is an active agent against certain fungal plant pathogens (Chin-A-Woeng et al. 2000).

Verma et al. (2013) reported 135 wheat-associated PGP bacteria from acidic soil; among all isolates *Pseudomonas chlororaphis* IARI-THD-13, *Pseudomonas fluorescens* IARI-THD-21, *Pseudomonas rhodesiae* IARI-THD-11 and *Pseudomonas rhodesiae* IARI-THD-28 exhibited direct and indirect PGP attributes such as solubilization of nutrients (P, K and Zn); production of NH_3, HCN, auxins and siderophores; and biological nitrogen fixation at low pH (3–5). These PGP bacteria also inhibited different fungal pathogens at abiotic stress of low pH. Acidotolerant microbes with multifarious PGP attributes could be applied as biofertilizers at place of chemical fertilizers under acidic conditions. In another investigation by Verma et al. (2014), 348 isolates from wheat growing under high temperature condition have been isolated which belonged to three phyla, namely, *Actinobacteria*, *Firmicutes* and *Proteobacteria*. All isolated microbes have been molecularly characterized for its identification and screened for different PGP attributes. Among the isolated microbes, BBDG and *Pseudomonas* were predominant in rhizosphere, *Pseudomonas fuscovaginae* IARI-IIWP-29, *Pseudomonas lini* IARI-IIWP-33, *Pseudomonas monteilii* IARI-IIWP-27, *Pseudomonas stutzeri* IARI-IHD-4 and *Pseudomonas thivervalensis* IARI-IHD-3 from internal tissues and *Methylobacterium* from phyllosphere. These thermotolerant PGP bacteria could be used for different crops and soil fertility under the high temperature conditions.

The wheat-associated psychrotrophic microbiomes have been isolated and characterized for PGP under the low temperature conditions (Verma et al. 2015b). Wheat growing in Indian Himalayas region has been selected, and using different isolation techniques, edophytic, rhizospheric and phyllospheric microbes have been isolated. All 247 isolates have been identified using 16S rRNA gene sequencing, and it was found that all isolates belonged to four phyla *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes* including different genera *Achromobacter*, *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Brevundimonas*, *Enterobacter*, *Exiguobacterium, Flavobacterium*, *Klebsiella*, *Kluyvera*, *Kocuria*, *Leclercia*, *Methylobacterium*, *Pantoea*, *Planococcus*, *Providencia*, *Pseudomonas*, *Staphylococcus* and *Stenotrophomonas.* Among all identified microbes, BBDG were predominant followed by members of *Pseudomonas* species. The all isolates were screened for tolerance to low temperature as well as for direct and indirect PGP attributes under the low temperature conditions. The cold-adapted microbes with PGP attributes such as solubilization of nitrogen, phosphorus and potassium could be applied as microbial consortium for crops growing under low temperature and in hill area, which will be the best replacement for NPK chemical fertilizers.

The wheat microbiomes (epiphytic, endophytic and rhizospheric) have been deciphered from wheat growing in peninsular zone of India (Verma et al. 2016b). A total of 264 microbial isolates have been sorted out using standard isolation techniques of serial dilution and imprinting method with the help of 11 different selective and complex media. To reduce the number of bacteria, the technique used was amplified ribosomal DNA restriction analysis (ARDRA), with three restriction enzymes *Alu* I, *Msp* I and *Hae* III. On the basis of phylogenetic analysis, it was found that all the bacteria can be grouped into 12–16 (70) with >75% similarity index. In this study the wheat-associated microbiomes belonged to three different phyla *Actinobacteria*, *Firmicutes*, and *Proteobacteria*, with different genera such as

Arthrobacter, *Bacillus*, *Delftia*, *Enterobacter*, *Exiguobacterium*, *Klebsiella*, *Methylobacterium*, *Micrococcus*, *Paenibacillus*, *Pseudomonas*, *Rhodobacter*, *Salmonella* and *Staphylococcus.* All the identified microbes have been screened for PGP attributes; under high temperature conditions, it was found that ten different species, e.g. *Alcaligenes faecalis*, *Arthrobacter* sp., *Bacillus siamensis*, *B. subtilis*, *Delftia acidovorans*, *Methylobacterium* sp., *M. mesophilicum*, *Pseudomonas poae*, *P. putida* and *P. stutzeri* exhibited more than six different PGP activities. These thermotolerant bacterial isolates may be used as bio-inoculants for plant growth promotion and biocontrol agents for crops growing at high temperature condition.

22.4 Microbes Associated with Crops Growing in Diverse Extreme Habitat

22.4.1 Saline Environments

Soil salinity is one of the imperative limiting factors for agricultural crops especially in arid/semiarid regions and high hypersaline regions of the world. There are many technologies along with PGP microbe-mediated plant tolerance against abiotic stress of salt that have been described (Rabie and Almadini 2005; Jiang et al. 2007; Paul and Nair 2008; Jalili et al. 2009).

Yang et al. (2009) reported the tomato seedlings under high salinity by application of *Achromobacter piechaudii*, which exhibited PGP attributes of ACC deaminase. *Azospirillum piechaudii*, with PGP attributes of ACC, increased the growth of tomato seedlings under saline condition by >66% in comparison with control. PGP microbe-mediated plant tolerance against salt stress has been intensively studied, showing that inoculation with microbes can alleviate the effects of salt stress in different plant species. High K^+/Na^+ ratios were found in salt-stressed maize in which selectivity for Na^+, K^+ and Ca^{2+} was altered upon inoculation with *Azospirillum* (Hamdia et al. 2004). The co-inoculation of plants with different microbial species may contribute to relieve abiotic stress of salt, e.g. salt stress has also been shown to effect when two microbes *Azospirillum* and *Rhizobium* have been inoculated together (Dardanelli et al. 2008).

Halophilic archaea (haloarchaea) thrive in environments with salt concentrations approaching saturation. Many species of haloarchaea have been isolated from hypersaline environments including *Haloarcula argentinensis*, *Halobacterium* sp., *Halococcus hamelinensis*, *Haloferax alexandrines*, *Haloferax larsenii*, *Haloferax volcanii*, *Halolamina pelagic*, *Halostagnicola kamekurae*, *Haloterrigena thermotolerans*, *Natrinema* sp. and *Natronoarchaeum mannanilyticum*. These haloarchaea have been isolated and characterized from the rhizosphere of plant species growing in saline and hypersaline environments (Oren 2002a, b; Yadav et al. 2015c; de la Vega et al. 2016; Gaba et al. 2017). Saxena et al. (2015a) reported that archaea are known to inhabit extreme environments and have never been studied with perspectives to understand their interactions with eubacteria and to sustain vegetation in extremes of salinity, moisture stress and temperature. Many haloarchaea of *Halobacteriaceae* family have been isolated and characterized for PGP attributes

under abiotic stress of saline, e.g. *Haloarcula*, *Halobacterium*, *Halococcus* and *Haloferax*. The archaeal community was profiled using 16S rRNA gene sequencing and phylogenetic analysis.

Yadav et al. (2015c) deciphered 157 halophilic archaea (*Haloarcula*, *Halobacterium*, *Halococcus*, *Haloferax*, *Halolamina*, *Halosarcina*, *Haloterrigena and Natronoarchaeum*) associated with different salt-tolerant plants (*Abutilon*, *Cenchrus*, *Dichanthium*, *Sporobolus* and *Suaeda nudiflora*) using standard serial dilution method with different selective and complex growth media. First time an archaea solubilizing media have been formulated as Haloarchaea P solubilization (HPS) medium, which show the P-solubilization as well as growth of different halophilic archaea. Among the screened archaea for P-solubilization, one archaea *Natrinema* sp. strain IARI-WRAB2 solubilized P (134.61 mg/L), which has almost higher solubilization than other microbes. The mechanisms of P-solubilization have been explained by lowing in pH and production of different organic acids (gluconic acid, citric acid, formic acid, fumaric acid succinic acid, propionic acid and tartaric acid). These P-solubilizing haloarchaea could be applied in co-inoculation with other microbes, and it may play a role in P nutrition to vegetation growing in these hypersaline soils.

22.4.2 High Temperature

Microbes associated with crops have a high potential for sustainable agriculture because they can improve plant growth, under abiotic stress conditions of temperatures. PGP microbes can directly or indirectly facilitate the growth of plant by production of IAA, GA and cytokinin; solubilization of P, K and ZN; and production of NH_3, HCN and different groups of iron-chelating compounds (siderophores) (Tilak et al. 2005; Verma et al. 2016b). There are considerable populations of P- or K-solubilizing microbes in soil which are associated with epiphytic, endophytic and rhizospheric plant. P-solubilizing microbes have the ability to solubilize inorganic phosphate compounds, by production of different organic acids (Vyas et al. 2009; Yadav et al. 2015e). PGP attributes such as P-solubilization have been exhibited by many genera such as *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Delftia*, *Haloarcula*, *Halobacterium*, *Halococcus*, *Haloferax*, *Halolamina*, *Halosarcina*, *Haloterrigena*, *Methylobacterium*, *Natronoarchaeum*, *Pseudomonas*, *Rhodobacter*, *Salmonella*, *Staphylococcus and Streptomyces.* K-solubilizing microbes were found to resolve K, Si and Al from insoluble minerals found in soils. K-solubilizing microbes solubilized K, by production of different organic acids such as gluconic acid, citric acid, formic acid, fumaric acid, succinic acid, propionic acid, tartaric acid etc. The K-solubilizing microbes may be used potassium bio-inoculant for the amelioration of K-deficient soil in agriculture at high temperatures. Different groups of microbes have been solubilized by K at high temperature conditions, e.g. *Achromobacter*, *Alcaligenes*, *Bacillus*, *Delftia*, *Methylobacterium*, *Providencia*, *Pseudomonas*, *Rhodobacter and Salmonella* (Verma et al. 2014, 2015b, 2016a).

22.4.3 Low Temperature

The microorganisms from extreme cold environments are of particular importance in global ecology. The majority of aquatic ecosystems are permanently or seasonally covered to cold temperatures (Yadav et al. 2015a, b, c, d, e; 2017c, e). Microbes capable of coping with low temperatures are widespread in these natural environments. Psychrophilic/psychrotrophic microbes are adapted to thrive well at low temperatures. Microbial activity of psychrophiles has even been reported at subzero temperatures. In general, psychrophilic microbes exhibit higher growth yield and microbial activity at low temperatures compared to temperatures close to the maximum temperature of growth.

In the past few years, the diversity of microbiomes inhabiting cold environments has been extensively investigated with a focus on culture-dependent and culture-independent techniques. Cold-adapted psychrophilic/psychrotrophic microbes have been reported from Antarctic subglacial, glaciers, ice cap cores, ice-covered lakes, snow and regions under low temperature conditions. The cold-adapted microbes could play an important role in agriculture, medicine, industry and environments. Cold-adapted microbes have potential biotechnology application in cryosurgery as they produced ant-freezing compound under freezing conditions. They could be used in agriculture as bio-inoculants for crops growing under low temperature and in hilly regions as they have the ability to solubilize different nutrients, fix atmospheric nitrogen as well as produce IAA, GA, cytokinin and iron-chelating compounds. Many PGP and cold-active enzymes producing microbes have been isolated from low temperature environments including *Arthrobacter*, *Bacillus*, *Brevundimonas*, *Burkholderia*, *Pseudomonas*, *Citricoccus*, *Exiguobacterium*, *Flavobacterium*, *Janthinobacterium*, *Kocuria*, *Lysinibacillus*, *Methylobacterium*, *Microbacterium*, *Paenibacillus*, *Providencia* and *Serratia* (Mishra et al. 2011b; Selvakumar et al. 2013; Verma et al. 2015b; Yadav et al. 2014, 2015a, b, d, e, 2016c, d, 2017c; Singh et al. 2016; Shukla et al. 2016).

Prospecting the cold habitats has led to the isolation of a great diversity of psychrotrophic microorganisms. The cold-adapted microbes have potential biotechnological applications in agriculture, medicine and industry. The microbial diversity from the cold environment could serve as a database for selection of bio-inoculants with PGP ability and could be used for improving the growth and yield of crops grown at high altitudes with prevailing low temperatures (Kumar et al. 2013; Yadav et al. 2014; Kumar et al. 2016).Cold-adapted psychrotrophic PGP microbes have been shown to promote plant growth either directly by atmospheric N_2-fixation, production of iron-chelating compounds (siderophores) and solubilization of P, K and Zn or indirectly via production of antagonistic substances, antibiotics and lytic enzymes (chitinase, cellulase, lipase, xylanase, and pectinase) (Verma et al. 2015b, c, 2016a; Yadav et al. 2016c). Psychrotrophic PGP microbes have been reported from different genera including *Arthrobacter*, *Bacillus*, *Brevundimonas*, *Burkholderia*, *Pseudomonas*, *Citricoccus*, *Exiguobacterium*, *Flavobacterium*, *Janthinobacterium*, *Kocuria*, *Lysinibacillus*, *Methylobacterium*, *Microbacterium*, *Paenibacillus*, *Providencia* and *Serratia* (Saxena et al. 2015b; Verma et al. 2015b; Yadav et al.

2016c). Among different taxa, *Bacillus*, *Pseudomonas* and *Exiguobacterium* have been the best characterized for plant growth promotion at low temperatures (Mishra et al. 2011a; Selvakumar et al. 2011; Yadav et al. 2015e, 2016c). Psychrophilic/psychrotrophic microbes as biofertilizers, biocontrol agents and bioremediators would be of great use in agriculture under cold habitat and in hilly regions. Psychrophilic/psychrotolerant microbes are important for many reasons, particularly because they produce cold-adapted enzyme, which provide opportunities to study the adaptation of life to low temperature (Saxena et al. 2016; Yadav et al. 2016c).

Yadav et al. (2016c) investigated PGP psychrotrophic *Bacilli* from different sites in NW Indian Himalayas. A total of 247 microbes have been isolated and characterized for PGP attributes at low temperatures. Using 16S rRNA gene sequencing, it has been found that these psychrotrophic microbes belong to 11 different genera, viz. *Desemzia*, *Exiguobacterium*, *Jeotgalicoccus*, *Lysinibacillus*, *Paenibacillus*, *Planococcus*, *Pontibacillus*, *Sinobaca*, *Sporosarcina*, *Staphylococcus* and *Virgibacillus*. Among the strains, variations were observed for production of ammonia, gibberellic acid, indole-3-acetic acid, siderophores and solubilization of phosphate. Among all the strains, five bacteria *Bacillus licheniformis*, *B. muralis*, *Desemzia incerta*, *Paenibacillus tylopili* and *Sporosarcina globispora* possess multiple PGP attributes, and hence these could be used as inoculants at low temperature.

Rana et al. (2017) deciphered the endophytic microbiomes from maize growing in Indian Himalayan regions. For the diversity of endophytic microbes from maize, the plant samples were collected from the Indian Himalayan regions. The total 66 distinct morphotypes were isolated using specific medium from sterilized and macerated root and stem of maize. These microbes belonged to different genera such as *Achromobacter*, *Acinetobacter*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Brevundimonas*, *Enterobacter*, *Exiguobacterium*, *Flavobacterium*, *Klebsiella*, *Kocuria*, *Methylobacterium*, *Paenibacillus*, *Pantoea*, *Planococcus*, *Providencia*, *Pseudomonas*, *Rhizobium*, *Staphylococcus* and *Stenotrophomonas.* The growth parameters of bacteria were studied at pH (4–9), temperature (5–50°C) and salinity (5–15%). The microbial isolates were screened for their ability to exhibit plant growth-promoting attributes in vitro. The percentages of isolates positive for siderophores, ammonia, phytase and pectinase production are 18, 33, 33 and 34, respectively. Solubilization of phosphorous, potassium and zinc was shown by 53%, 42% and 34% isolates, respectively. About 15% of isolates shows cellulase activity, while about 53% and 24% of isolates possessed the amylase and xylanase activity. Endophytes can also be beneficial to their host by producing a range of natural products that could be harnessed for potential use in medicine, agriculture or industry.

22.4.4 Drought Environments

The extreme habitat is limiting factor for plant growth and yield. Among different extreme environments, drought stress limits the growth and productivity of crops (Shanker et al. 2014). Lim and Kim (2013) reported that *Bacillus licheniformis* K11 can increase growth and productivity of pepper under drought stress. Under

dought stress conditions, leaf transpiration and leaf conductance decrease, and the water use efficiency rises; this mechanism promotes plant gwoth under water-deficient environments (Aroca and Ruiz-Lozano, 2009). Plant responses to drought to increase in abscisic acid levels that cause stomatal closure to minimize water loss; these events involve production of activated oxygen species (Cho et al. 2008). Other plant signalling compounds are involved in regulating stomatal closure, such as methyl jasmonate, salicylic acid and ethylene. On the whole, the beneficial effects of PGP microbes on plant, drought tolerance are caused by changes in hormonal contents, mainly that of ABA, ethylene and cytokinins. *Azospirillum lipoferum* has been inoculated in wheat seedlings under drought stress and alleviated the plant drought stress, increasing wheat growth and yield (Arzanesh et al. 2011). Drought increases the vulnerability to nutrient losses from the rooting zone through erosion. Under drought situations, roots are known to extend their length, increase their surface area and alter their architecture in an effort to capture less mobile nutrients such as phosphorus. Drought also disrupts root-microbe associations that play a major role in plant nutrient acquisition.

Verma et al. (2014) deciphered the 348 drought-tolerant microbiomes of wheat growing in different regions of central zone of India. Isolated microbes have been characterized for drought stress, 5–15% PEG-8000 and PGP attributes such as solubilization of P and K; production of iron-chelating compounds (siderophores), IAA, GA, cytokinin; and biocontrol against three different fungal pathogens. All the PGP bacteria have been identified using 16S rRNA gene sequencing and BLAST analysis. On the basis of phylogenetic profiling, it was found that all the isolates belonged to three phyla *Actinobacteria*, *Firmicutes* and *Proteobacteria*. Among the identified bacteria two bacteria, *Bacillus* and *Pseudomonas*, were predominant in the rhizosphere, while *Methylobacterium* was in the phyllosphere. Phosphate solubilization and siderophore production are the predominant traits exhibited by these microbes. Many species of genera *Bacillus*, *Exiguobacterium*, *Micrococcus*, *Pseudomonas* and *Psychrobacter* showed antagonistic properties against fungal pathogens. These promising drought-tolerant bacteria showing a range of useful PGP attributes insist to be explored for agricultural applications under rainfed conditions.

Kour et al. (2017) evaluated the microbial diversity from crops growing under drought stress, which is one of the major abiotic stresses affecting yield of dry land crops. Microbial populations of drought soils have adapted and are tolerant to drought stress and can be screened for isolation of efficient drought-tolerant plant growth-promoting microbial strains that can be used as bio-inoculants for crops grown under rainfed conditions. Microbes with drought-tolerant and phosphorus-solubilizing attributes could be suitable bio-inoculants for crops grown in stressed ecosystem. A total of 180 microbes have been isolated on different growth media such as ammonium minerals salt, Jensen's agar, King's B agar, modified Dobereiner medium agar, nutrient agar, R_2 agar, soil extract agar, T_3 agar, trypticase soy agar and yeast extract mannitol agar. All the isolated microbes have been screened for drought tolerance on PEG-infused plates with water potential of −0.5 Mpa. Of 180 isolates 91, 38, 17 and 12 isolates were found to be tolerant to 5, 6, 7 and 8% PEG-8000, respectively. The selected drought-tolerant P-solubilizing microbes have been

screened for other plant growth-promoting attributes such as production of siderophore, IAA, HCN, and ammonia, ACC deaminase activity and antifungal activity against fungal pathogens like *Fusarium graminearum*, *Rhizoctonia solani* and *Macrophomina phaseolina.* The P-solubilizing microbes with multiple plant growth-promoting attributes have been evaluated for plant growth under controlled conditions of drought stress. These microbes belonged to different genera such as *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Methylobacterium*, *Paenibacillus*, *Pantoea*, *Providencia* and *Pseudomonas*. Drought-adapted P-solubilizing microbes may be applied as bio-inoculants and biocontrol agents in crops growing under rainfed conditions.

22.4.5 Alkaline/Acidic Environments

Alkaline/acidic environments are hot spots for microbial diversity with PGP attributes. Many acidotolerant bacterial genera have been sorted out and characterized for PGP attributes at low pH conditions from acidic environments including *Azotobacter*, *Bacillus*, *Flavobacterium*, *Pseudomonas* and *Serratia* (Yadav et al. 2011, 2013; Florentino et al. 2016; Feliatra et al. 2016). The species of genus *Bacillus*, *Enterobacter*, *Pseudomonas* (Yadav et al. 2013), *Lysinibacillus* and *Methylobacterium* (Holland and Polacco, 1994; Wellner et al. 2011) have been discovered as the most predominant genera from rhizopsheric soil and reported worldwide. Quantitative and qualitative variations in these traits allow these microbes to inhabit diverse niche in agroecosystem. Microbiomes of wheat are considered important for maintaining the sustainability of agricultural production systems. Epiphytic, endophytic and rhizospheric microbes have been shown to promote plant growth under acidic and alkaline conditions. PGP microbes are used as biological control agents for the suppression of soilborne pathogens. The PGP microbes with multifarous PGP attributes may be used as bio-inoculants which would be suitable for a long-term sustainable agricultural system. A number of bacterial species are associated with the plant rhizosphere belonging to the genera *Azospirillum*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Methylobacterium*, *Pseudomonas*, *Rhizobium* and *Serratia* (Yadav et al. 2011, 2013, 2015d; Meena et al. 2012; Lavania et al. 2006).

Nautiyal et al. (2000) have isolated and characterized four P-solubilizing microbes NBRI0603, NBRI2601, NBRI3246 and NBRI4003 from the rhizosphere of chickpea growing in alkaline soils. In vitro conditions all the P-solubilizing bacteria produced acid, which may have contributed to phosphate solubilization. Among the four strains, NBRI2601 was the most efficient strain in terms of its capability to solubilize phosphorus in the presence of 10% salt, pH 12 or 45°C. Verma et al. (2013) elucidated the microbiomes of wheat growing in acidic soil in southern hills zone of India. Bacterial diversity has been analysed by ARDRA using three restriction enzymes *Alu* I, *Hae* III and *Msp* I. All the 135 bacteria isolates have been identified using 16S rRNA gene sequencing, which can be grouped into four phyla, namely, *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria*. Acidotolerant

isolates may be used as inoculants for plant growth promotion and biocontrol agents for crops growing under acidic condition.

22.5 Beneficial Role of Microbes in Crop Improvements

Plants play an imperative role in selecting and enriching the types of microbes by the constituents of their root exudates. Thus, depending on the nature and concentrations of organic constituents of exudates, beneficial interactions between plant and microbes in form of epiphytic/endophytic/rhizospheric have been developed. Microbiomes of different crops are of agricultural importance as they can enhance plant growth and improve plant nutrition through biological N_2-fixation and other mechanisms. Microbes may increase crop yields, remove contaminants, inhibit pathogens and produce fixed nitrogen or novel substances (Quadt-Hallmann et al. 1997). The growth stimulation by microbes can be a consequence of biological N_2-fixation (de Bruijn et al. 1997; Suman et al. 2001; Iniguez et al. 2004; Taulé et al. 2012; Pankievicz et al. 2015; Verma et al. 2016a, b); production of phytohormones, such as IAA and cytokines (Rashid et al. 2012; Lin and Xu 2013; Verma 2015); and biocontrol of phytopathogens through the production of antifungal or antibacterial agents (Raaijmakers et al. 2002; Errakhi et al. 2016), siderophores (Leong 1986; Verma et al. 2014, 2015a, b, c, 2016a; Ellis 2017), and nutrients (Bach et al. 2016); and induction of acquired host resistance (Van Loon et al. 1998; Pal and Gardener 2006) or enhancing the bioavailability of minerals (Haas and Défago 2005) (Table 22.3).

22.5.1 Biological N_2-Fixation

Nitrogen is the major limiting factor for plant growth; the application of N_2-fixing microbes as biofertilizers has emerged as one of the most efficient and environmentally sustainable methods for increasing the growth and yield of crop plants. Biological nitrogen fixation (BNF) is one of the possible biological alternatives to N-fertilizers and could lead to more productive and sustainable agriculture without harming the environment. Many associative and endophytic bacteria are now known to fix atmospheric nitrogen and supply it to the associated host plants. A variety of nitrogen-fixing microbes like *Arthrobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Gluconacetobacter*, *Herbaspirillum*, *Klebsiella*, *Pseudomonas* and *Serratia* have been isolated from the rhizosphere of various crops, which contribute fixed nitrogen to the associated plants (Elbeltagy et al. 2001; Giller 2001; Boddey et al. 2003; Wei et al. 2014; Reis and Teixeira 2015; Suman et al. 2016a).

Choudhury and Kennedy (2004) reported that co-inoculums of *Azolla* and cyanobacteria can supplement the nitrogen requirements of plants, replacing 30–50% of the required urea-N in rice production. N_2-fixation by different microbes such as *Azotobacter*, *Clostridium*, *Azospirillum*, *Herbaspirillum* and *Burkholderia* can

substitute for urea-N, while *Rhizobium* can promote the growth physiology or improve the root morphology of the rice plant. Pham et al. (2017) have isolated rhizospheric and endophytic and rhizospheric *Pseudomonas stutzeri* A15 from rice. This strain showed PGP effect and the potential contribution of biological nitrogen fixation in rice. *P. stutzeri* A15 induced significant growth promotion compared to uninoculated rice seedlings. Furthermore, inoculation with strain A15 performed significantly better than chemical nitrogen fertilization, clearly pointing to the potential of this bacterium as biofertilizer.

22.5.2 Phytohormones Production

Plant-associated microbes typically produce plant growth hormones such as auxins and gibberellins. Gibberellin production is the most typical for the root-associated microbes, and auxin production is common to all plant-associated microbes including epiphytic, endophytic as well as rhizospheric. Auxins are a group of indole derivatives that have various growth-promoting functions in plants, such as promotion of root formation, regulation of fruit ripening and stimulation of cell division, extension and differentiation. Indoleacetic acid (IAA) is the most well-known auxin. The production of such growth regulators by microbes provides numerous benefits to the host plant including the facilitation of root system expansion, which enhances the absorption of water and nutrients and improves plant survival. The ability to synthesize these phytohormones is widely distributed among plant-associated microbes, and IAA may potentially be used to promote plant growth or suppress weed growth. Diverse microbial species possess the ability to produce the auxin phytohormone IAA.

Thanh and Diep (2014) reported 301 endophytic microbes from maize plant cultivated on acrisols of the eastern part of South Vietnam. The bacterial isolates have been isolated and screened for different PGP attributes. It has been found that all isolates have the ability for N_2-fixation and P-solubilization along with auxin production. These PG endophytic microbes were identified as A*zotobacter*, *Bacillus* and *Enterobacter.* Both pathogenic and beneficial plant-associated microbial species are capable of synthesizing cytokinins. Among plant-associated methylotrophs, species such as *Methylovorusmays* and *Methylobacterium mesophilicum* JCM2829 synthesize and excrete cytokinins (Ivanova et al. 2001; Ivanova et al. 2008).

Verma et al. (2014) have isolated the wheat microbiomes (epiphytic, endophytic and rhizospheric) from five different locations in agroecological zone (central zone) in India. A total of 222 rhizospheric bacteria were isolated and identified as *Acinetobacter*, *Bacillus*, *Duganella*, *Exiguobacterium*, *Kocuria*, *Lysinibacillus*, *Micrococcus*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Serratia* and *Stenotrophomonas*. Among 222, 89 bacteria isolated from the phyllosphere belong to different genera of *Arthrobacter*, *Bacillus*, *Corynebacterium*, *Methylobacterium*, *Paenibacillus*, *Pseudomonas* and *Psychrobacter*, and 37 endophytic bacteria were isolated and identified belonging to genera of *Delftia*, *Micrococcus*, *Pseudomonas* and *Stenotrophomonas*. Among total isolates, 12% isolates produced IAA.

The biodiversity of wheat-associated bacteria from the northern hills zone of India was deciphered (Verma et al. 2015b). A total of 247 bacteria were isolated from five different sites. Analysis of these bacteria by amplified ribosomal DNA restriction analysis (ARDRA) using three restriction enzymes led to the grouping of these isolates into 19–33 clusters for the different sites. Among all isolated bacteria, 14% showed IAA production in which strain IARI-HHS1-3 showed the highest IAA production (70.8 ± 1.5 μg mg^{-1} protein day^{-1}) followed by IARI-HHS1-8 (69.1 ± 0.5 μg mg^{-1} protein day^{-1}). Tabatabaei et al. (2016) have reported *Pseudomonas* was isolated from wheat. An in vitro experiment was conducted to observe the effect of the inoculation of four indole-3-acetic acid (IAA)-producing *Pseudomonas* isolates and exogenous IAA on seed germination traits and α-amylase activity of durum wheat. A significant relationship between concentrations of bacterial IAA and the germination inhibition percent in durum wheat seeds by different bacteria strains was observed.

22.5.3 Solubilization of Phosphorus, Potassium and Zinc

Phosphorus (P) is the major essential macronutrient for biological growth and development. Microorganisms offer a biological rescue system capable of solubilizing the insoluble inorganic P of soil and make it available to the plants. The ability of some microorganisms to convert insoluble phosphorus (P) to an accessible form, like orthophosphate, is an important trait in PGP microbes for increasing plant yields. The rhizospheric phosphate-utilizing bacteria could be a promising source for plant growth-promoting agent in agriculture.

Phosphate solubilization is a common trait among microbes associated with different crops. For instance, the majority of microbial populations from wheat, rice, maize and legumes were able to solubilize mineral phosphates in plate assays, and a vast number of PGP microbes with phosphate-solubilizing property have been reported which include members belonging to *Burkholderia*, *Enterobacter*, *Halolamina*, *Pantoea*, *Pseudomonas*, *Citrobacter* and *Azotobacter* (Forchetti et al. 2007; Verma et al. 2014, 2016a; Singh et al. 2016; Yadav et al. 2016c; Gaba et al. 2017; Kumar et al. 2017). Possible mechanisms for solubilization from organic bound phosphate involve enzymes, namely, C-P lyase, non-specific phosphatases and phytases. However, most of the bacterial genera solubilize phosphate through the production of organic acids such as gluconate, ketogluconate, acetate, lactate, oxalate, tartrate, succinate, citrate and glycolate (Khan et al. 2009; Stella and Halimi 2015; Yadav 2015; 2016a). The type of organic acid produced for P-solubilization may depend upon the carbon source utilized as substrate. The highest P-solubilization has been observed when glucose, sucrose or galactose has been used as the sole carbon source in the medium (Khan et al. 2009; Vyas and Gulati 2009; Park et al. 2010).

Yadav et al. (2015c) characterized and screened the archaea for phosphate solubilization using a newly designed Haloarchaea P solubilization (HPS) medium. The medium supported the growth and P-solubilization activity of archaea. Employing the HPS medium, 20 isolates showed the P-solubilization. Phosphate-solubilizing

archaea were identified as 17 distinct species of 11 genera, namely, *Haloarcula*, *Halobacterium*, *Halococcus*, *Haloferax*, *Halolamina*, *Halosarcina*, *Halostagnicola*, *Haloterrigena*, *Natrialba*, *Natrinema* and *Natronoarchaeum*. *Natrinema* sp. strain IARI-WRAB2 was identified as the most efficient P-solubilizer (134.61 mg/L) followed by *Halococcus hamelinensis* strain IARI-SNS2 (112.56 mg/L). Zinc is a nutrient at low concentration but toxic at higher concentration. Zinc solubilization by bacteria has an immense importance in zinc nutrition to plants. K-solubilizing bacteria (KSB) were found to resolve potassium, silicon and aluminium from insoluble minerals. BBDG were best characterized for K-solubilization (Sheng et al. 2008; Verma et al. 2015b). The K-solubilizing bacteria may have use in the amelioration of K-deficient soil in agriculture. There are only few reports on K-solubilization by endophytic bacteria isolated from wheat (Verma et al. 2014, 2015c, 2016b).

Verma et al. (2016a) have reported 395 *Bacilli* from wheat, and these bacteria have been screened for direct and indirect PGP traits, and result has been represented by 55 representative *Bacilli*. Of 55 representatives, 39, 18 and 40 strains exhibited solubilization of phosphorus, potassium and zinc, respectively. Among P-, K- and Zn-solubilizers, *Paenibacillus polymyxa* BNW6 solubilized the highest amount of phosphorus, 95.6 ± 1.0 mg L^{-1}, followed by *Sporosarcina* sp. BNW4, 75.6 ± 1.0 mg L^{-1}. *Planococcus salinarum* BSH13 (46.9 ± 1.2 mg L^{-1}) and *Bacillus pumilus* BCZ15 (7.5 ± 0.5 mg L^{-1}) solubilized the highest amount of potassium and zinc, respectively. Among plant growth-promoting bacteria, ammonia-producing *Bacilli* were the highest (79.0%), when compared to P-solubilizer (73.9%), Zn-solubilizers (67.1%), protease producers (56.7%), IAA producers (55.2%), siderophore producers (49.1%), biocontrol agents (47.8%), K-solubilizers (39.2%), N_2-fixers (31.4%), HCN producers (27.3%) and gibberellic acid producers (24.8%).

22.5.4 ACC Deaminase Activity

Ethylene is a stress-induced plant hormone that can inhibit plant growth. Some microbes can lower the level of ethylene in the plant by cleaving the plant-produced ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC). Inoculation of such microbes can mitigate the effect of various stressors by sustaining plant growth in the face of ethylene. ACC deaminase-producing microbes may play a role in regulating ethylene levels after such bursts, ensuring that ethylene levels stay below the point where growth is impaired (Glick 1995; 1999a). Ethylene is a key regulator of the colonization of plant tissue by bacteria which in turn suggests that the ethylene inhibiting effects of ACC deaminase may be a microbial colonization strategy. Generally, ethylene is an essential metabolite for the normal growth and development of plants (Khalid et al. 2004, 2006). This plant growth hormone is produced endogenously by approximately all plants and is also produced by different biotic and abiotic processes in soils and is important in inducing multifarious physiological changes in plants. Apart from being a plant growth regulator, ethylene has also been established as a stress hormone. Under stress conditions like those generated

by salinity, drought, water logging, heavy metals and pathogenicity, the endogenous level of ethylene is significantly increased which negatively affects the overall plant growth. PGP microbes which possess the enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, facilitate plant growth and development by decreasing ethylene levels, inducing salt tolerance and reducing drought stress in plants. Microbial strains exhibiting ACC deaminase activity have been identified in a wide range of genera such as *Acinetobacter*, *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, *Serratia*, *Rhizobium*, etc. (Khalid et al. 2006; Srivastava et al. 2014; Xu et al. 2014; Verma et al. 2015c, 2016a).

Verma et al. (2014, 2015b) reported psychrotolerant and drought-tolerant microbes from wheat showing ACC deaminase activity by different genera of *Arthrobacter*, *Flavobacterium*, *Bacillus*, *Methylobacterium*, *Providencia*, *Pseudomonas*, *Stenotrophomonas* and *Enterobacter.* These bacteria also possess attributes such as solubilization of phosphorus, potassium and zinc and production of IAA, siderophore, HCN and ammonia and showed antifungal activity against plant pathogens.

22.5.5 Biocontrol

The indirect mechanism of plant growth occurs when bacteria lessen or prevent the detrimental effects of pathogens on plants by production of inhibitory substances or by increasing the natural resistance of the host. Phytopathogenic microbes can be control by the release of siderophores, chitinases, antibiotics, and fluorescent pigment or cyanide production. Biocontrol systems are eco-friendly and cost-efficient and involved in improving the soil consistency and maintenance of natural soil flora. To act efficiently, the biocontrol agent should remain active under a large range of conditions, viz. varying pH, temperature and concentrations of different ions. Biocontrol agents limit growth of pathogen as well as few nematodes and insects. Biocontrol microbes can limit pathogens directly by production of antagonistic substances, competition for iron and detoxification or degradation of virulence factors or indirectly by inducing systemic resistance (ISR) in plants against certain diseases, signal interference, competition for nutrients and niches and interference with activity, survival, germination and sporulation of the pathogen. Recent studies have indicated that biological control of bacterial wilt disease could be achieved using antagonistic bacteria. Different bacterial species, namely, *Alcaligenes*, *Bacillus*, *Clavibacter*, *Curtobacterium*, *Flavobacterium*, *Kluyvera*, *Microbacterium* and *Pseudomonas*, have been reported as inhibitors to plant pathogens (Inderiati and Franco 2008; Ramesh et al. 2009; Nagendran et al. 2013; Gholami et al. 2014; Purnawati 2014; Yadav et al. 2015e; 2016b; Verma et al. 2017).

Iron is a necessary cofactor for many enzymatic reactions and is an essential nutrient for virtually all organisms. In aerobic conditions, iron exists predominantly in its ferric state (Fe^{3+}) and reacts to form highly insoluble hydroxides and

oxyhydroxides that are largely unavailable to plants and microorganisms. To acquire sufficient iron, siderophores produced by bacteria can bind Fe^{3+} with a high affinity to solubilize this metal for its efficient uptake. Bacterial siderophores are low-molecular-weight compounds with high Fe^{3+} chelating affinities responsible for the solubilization and transport of this element into bacterial cells. Some bacteria produce hydroxamate-type siderophores, and others produce catecholate-types. In a state of iron limitation, the siderophore-producing microorganisms are also able to bind and transport the iron-siderophore complex by the expression of specific proteins. The production of siderophores by microorganisms is beneficial to plants because it can inhibit the growth of plant pathogens. Siderophores have been implicated for both direct and indirect enhancement of plant growth by plant growth-promoting microbes.

22.6 Conclusions and Future Prospect

The need of today's world is high output yield and enhanced production of the crop as well as fertility of soil to get in an eco-friendly manner. Hence, the research has to be focused on the new concept of microbial engineering based on favourable partitioning of the exotic biomolecules, which creates a unique setting for the interaction between plant and microbes. Future research in microbes will rely on the development of molecular and biotechnological approaches to increase our knowledge of microbes and to achieve an integrated management of microbial populations of endophytic, epiphytic and rhizospheric.

In the course of the past few decades, the human population has doubled. Food production has similarly increased. Use of man-made fertilizers has enabled much of the increase in the crop production. Concurrent with the escalating use of commercial fertilizers, the intensity of agricultural practices has increased, and a wide variety of fungicides, bactericides and pesticides are utilized in large-scale crop production. Because of their close interaction with plants, attention has been focused on endophytes and their potential use in sustainable agriculture. An increasing number of researchers are attempting to elucidate the mechanisms of plant growth promotion, biological control and bioremediation mediated by endophytes by examining species and conditions that lead to greater plant benefits. Research in this field is clearly very promising and will have significant economic and environmental impacts in the future.

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Crop Genetic Engineering: An Approach to Improve Fungal Resistance in Plant System

23

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Abstract

Fungal disease in crop plants from the past two decades has seen to be increasing which is recognized as a serious threat to food security worldwide. It is difficult for plant to survive under these unfavorable conditions which cause an unprecedented number of fungal and fungal-like diseases which are the most common kind of plant disease. Various approaches such as use of chemical pesticides and other synthetic molecules have been used to control the fungal infections in crop plants. Different transgenic plants have been developed by introducing various genes responsible for resistance in opposition to fungal pathogens. Genes of the enzymes responsible for cell wall degradation are frequently applied to generate transgenic plants for fungal resistance. This chapter mainly emphasizes on how transgenic approach helps to confer plant resistance toward fungal diseases.

Keywords

Food security • Disease • Pesticides • Transgenic • Fungal resistance • Enzymes

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23.1 Introduction

The population of the world is increasing rapidly, and it is estimated that it will increase by approximately 40% to 8.04 billion by 2025 (UN 1996). The developing nations are expected to share approximately 90% of population increase worldwide. Due to the continued urbanization in developing nations, food demands and their pattern are also changing. Therefore, there is continued demand to increase food production.

Damage caused by pests and crop diseases is one of the most important factors and challenges which reduce the yield of the crop and crop productivity globally. Worldwide, it has been estimated that 16 and 18% yield loss takes place due to pathogens and animal pests, respectively. A wide array of changes in the agricultural ecosystem encourages pest outbreaks that result in heavy loss of crops due to pests. Among pests, fungi are the major factors affecting crop production in agricultural ecosystem.

The diseases caused by fungi in crop plants are seriously threatening the world's food supply, as fungi are the most common cause of plant diseases. More than 10,000 kinds of fungi among 100,000 known species can cause disease in plants. Epidemic and persistent outbreaks of fungal infection in wheat (rust caused by *Puccinia graminis*), potatoes (late blight caused by *Phytophthora infestans*), rice (rice blast caused by *Magnaporthe oryzae*), maize (smut caused by *Ustilago maydis*), and soybean (rust caused by *Phakopsora pachyrhizi*) cause heavy loss of crop, although varying regionally but posing a growing threat to food security (Pennisi 2010) (Table 23.1).

Fungi are placed in eukaryotic group of organism, which also includes yeast, mushrooms, and molds. Fungi are non-chlorophytic, spore-forming organism; the true fungi are mostly found in filamentous and branched form. Most of the fungal species are saprophyte, but approximately 20,000 fungal species are parasites, which cause disease in crop and plants. All plants are attacked by at least one species of fungi or another of phytopathogenic fungi. One or different kinds of plants can be attacked by individual species of fungi. (Schultz et al. 2007).

Table 23.1 List of fungal diseases in major crops

Fungal pathogens	Disease	Crops
Alternaria triticina	Leaf blight	Wheat
Fusarium oxysporum f. sp. *lycopersici*	Fusarium wilt	Tomato
Rhizoctonia solani	Sheath blight	Rice
Sclerotium rolfsii	Wilt	Potato
Pythiumaphani dermatum	Damping off	Tomato
Sclerotinia sclerotiorum	Head rot	Sunflower
Alternaria solani	Early blight	tomato, potato
Alternaria alternate f. sp. *lycopersici*	Early blight	Tomato
Ceratocystis paradoxa	Pine apple disease	Sugarcane
Phytophthora infestans	Late blight	Potato

Several approaches have been used to control fungal infection in crop plants since long time, but despite of using several strategies to control the infection, farmers are mainly dependent on using chemical pesticides. But this crop protection strategy has been proved unfriendly to the public health as well as for environment, because fungicidal residue gets accumulated in vegetables and crop as well as in environment that leads to environmental risk as well as health-related issues (Bakhsh 2013).

With the advancement in genetic engineering technology, fungal resistance transgenic plants have been developed, which increase the plant protection, reducing the yield loss, and also there is significant decrease in utilization of pesticides. Transgenic technology is becoming key components of integrated pest management (IPM) across the world (Kos et al. 2009).

23.2 Nature of Diseases, Signs and Symptoms of Fungal Infections

Fungi can cause general or localized signs and/or symptoms. The easily identifiable and most distinctive features of infections caused by fungi are signs of physical appearance of fungal pathogens on plants. Signs may include the presence of mycelia, hyphae, spore, and fruiting bodies of the fungal pathogens. The proper identification and diagnosis of disease are mostly based on presence of these signs. Different kinds of fruiting bodies, along with the mycelium and spores, in majority of cases lead to the real and accurate identification of fungal diseases.

The symptoms of the fungal diseases can be visibly detected in the plants that may include change in function, color, or shape in plant as it responds to the fungal pathogens. In most cases, fungal infections lead to the appearance of local necrosis in the host tissues in plants, often causing distortions, stunting, and abnormal changes in plant tissues and organs (Moore and Vodopich 1998).

23.3 Impact on Economy Due to Fungal Pests

The economic impact of crop disease caused by different fungi can be complex; the actual impact varies and depends on factors such as considerable crop losses caused by individual fungal infection worldwide. Diseases caused by fungal infection presently destroy at least 125 million tons of the major crops such as rice, wheat, maize, and potatoes annually. The major crop losses and damage due to fungi cost approximately $60 billion annually in global agriculture (Source: Agriculture Today: The National Agriculture Magazine 2012). More than three million people can be fed, if some of the crop loss could be saved from pests and fungal diseases.

23.3.1 Major Fungal Pathogens of Crops

23.3.1.1 *Fusarium oxysporum* f. sp. *lycopersici*

Fusarium oxysporum f. sp. *lycopersici*, a soilborne plant pathogen that belongs to class *Sordariomycetes*, causes *Fusarium* wilt on tomato, which is the most economically important vegetable crop and used worldwide (Sudhamoy et al. 2009). At least 100 kinds of *Fusarium* vascular wilt diseases are known worldwide affecting productivity of the tomato (Burgess et al. 2008). *Fusarium* comes in the plant via its root tips (Sally et al. 2006) and persists viably in the soil for very long period (Thangavelu et al. 2003). The mycelium starts growing in the xylem vessels very fast, where they cut off water supply that results into wilting (Stephen and Andre 2003). The appearance of yellowed and wilted leaves is the characteristic symptom of the disease. This disease may induce 30–40% yield loss, and under favorable weather condition, the yield loss may reach to 80% (Kiran et al. 2008; Kapoor 1988). 25–55% *Fusarium* wilt incidences have been recorded from various parts of India (Pandey and Gupta 2013; Asha et al. 2011).

23.3.1.2 *Alternaria alternata*

Alternaria alternata, which is the most common and destructive fungus of crop and vegetable plants especially tomato plants, belongs to the class *Dothideomycetes*. It causes early blight disease in tomato as well as in other crop plants. The spores of fungi are almost found everywhere: air, soil, and water. The colonies are generally gray, green, or black in color and able to grow thick. The fungus is responsible for infection on foliage (leaf blight), stem (collar rot), and fruit which causes harsh damage throughout all period of plant development (Sabriye et al. 2011).

23.3.1.3 *Alternaria solani*

Alternaria solani which causes early blight of potato and tomato belongs to the class *Dothideomycetes*. This pathogen showed capability to produce in a large variety of temperatures (Pound 1951) and is reported to infect potato and other cultivated plants of the *Solanaceae*, particularly tomato (*Lycopersicon esculentum*) and eggplant (*Solanum melongena* L.) under both dry and wet conditions (Waggoner and Horsfall 1969), and its propagules survive between crops as mycelia or conidia in soil, plant debris, and seed (Sherf and MacNab 1986).

Disease on potatoes like the early blight which is caused by *Alternaria solani* is a major serious biotic threat to potato production worldwide causing severe crop yield losses (Fiers et al. 2012).

23.3.1.4 *Alternaria triticina*

Alternaria triticina is the causative agent of the disease *Alternaria* leaf blight in wheat, and it belongs to the class *Dothideomycetes*. It was first illustrated as a species in India by Prasada and Prabhu (1962) who distinguished the species as *Alternaria*, and it is found specifically on wheat and forms short chains of spores. The major hosts of *A. triticina* are durum wheat (*Triticum turgidum* subsp. durum) and bread wheat (*Triticum aestivum*). As compared to bread wheat, attack of the pathogens was more severe on durum wheat (Prabhu and Prasada 1966; Prasada and Prabhu 1962; Singh et al. 1999).

23.3.1.5 *Rhizoctonia solani*

Rhizoctonia solani is placed under the class *Agaricomycetes* and which is a soil-borne pathogen causing a large diversity of diseases in economically significant plant species including almost all cultivated crops. It is one of the fungi responsible for crop losses that may range from slight to heavy each year, depending on the weather, the plant growth stage when infection occurs, the extent of infection, and the rice varieties grown (Parmeter 1970). *Rhizoctonia solani* is a part of the multinucleate group of *Rhizoctonia* (Carling 1996), which are genetically diverse responsible for rice sheath blight in a number of developing countries. Since two decades *Rhizoctonia solani* resulted in major restraint in the production of rice (Zheng et al. 2013; Rinehart et al. 2004).

23.3.1.6 *Sclerotinia sclerotiorum*

The fungus *Sclerotinia sclerotiorum* is among the most nonspecific plant pathogens infecting mostly herbaceous plants of diverse phylogenetic backgrounds, over 400 species in 75 plant families worldwide. Cultivated crops susceptible to *S. sclerotiorum* include soybean, pea, bean, potato, carrot, sunflower, cucurbits, cucurbits, lettuce, and mustards (Boland and Hall 1994). The fungus belongs to the class *Leotiomycetes* and is both soil- and airborne. Sclerotia provide an endurance arrangement inside soil, whereas ascospores are responsible for the aerial scattering. Since *S. sclerotiorum* poses huge host choice, there are no sole indications that correspond to all plants being infected by this fungus. Water-soaked lesions appear on the leaves after infection that expand rapidly and move down the petiole into the stem. Dark lesions develop on the stems of some infected plants infected with *S. sclerotiorum*.

23.4 Different Strategies for Controlling the Fungal Infections

23.4.1 Use of Fungicides

Over the past several years, diverse attempts have been made to fend off fungal invaders and control fungal diseases through the development of chemical pesticides. Use of chemical pesticides is generally considered as the most common and efficient pest management strategy and also the key component in controlling plant diseases caused by fungi that threaten crop productivity. However, the excessive use of chemical fungicides has negative impact on beneficial organism and severely affects the human health (neurological, tumor, cancer) and the environment. For achieving high potential yield by preventing crop losses, farmers mainly rely on use of fungicides, and therefore excessive dose of fungicides is used to control the fungal diseases (Devine and Furlong 2007). Heavy and repeated use of fungicides results into exertion of selection pressure on the fungal population, and there is high risk of emergence of resistant strains of fungus. Heavy use of fungicides selectively restrains the sensitive population while allowing the development and multiplication of resistance strains.

Chemical fungicides have several drawbacks and pose huge negative impact on public discourse; it is encouraged to develop or isolate a compound which is more target oriented and has low perseverance in the environment. In order to minimize the risks to the animal and human health and environment like increasing use of chemical fungicides, substitute products have been tested with fruitful results for controlling fungal diseases.

23.4.2 Use of Synthetic Compounds

As alternative to chemical fungicides, various synthetic inducers such as hydrogen peroxide and salicylic acid were effective as antimicrobial agents (Abdel-Monaim 2013). Such compounds were tested in a number of plant pathogens like *R. solani* which is present in potato and faba bean, *M. phaseolina* in water melon (Saleh et al. 2009), *F. oxysporum* in tomato (Abdel-Monaim et al. 2012), and *F. solani* in lentil (Morsy 2005).

23.4.3 Biofungicides and Natural Products

Biological control has been extensively applied for disease control, which consists of biocontrol agents like *Trichoderma* and *Bacillus* (Schoneberg et al. 2015; Dunlap et al. 2011), some hormones (Petti et al. 2012), algal extracts, and chitosan (Pagnussatt et al. 2014; Forrer et al. 2014). Approximately 60% of the fungus-based biofungicides were developed from *Trichoderma* (Verma et al. 2007). Several isolates of *T. harzianum*, *T. atroviride*, *T. viride*, and *T. polysporum* have been commercially prepared against fungi (Yang et al. 1999; Verma et al. 2007). Biological control methods have been extensively applied for disease control, which consists of biocontrol agents like Trichoderma and Bacillus (Schoneberg et al. 2015; Dunlap et al. 2011), some hormones (Petti et al. 2012), algal extracts, and chitosan (Pagnussatt et al. 2014; Forrer et al. 2014). In present time, naturally derived antifungal agents like those of plant-based extracts and essential oils (Bajpai and Kang 2012), enzymes (Hammami et al. 2013) and peptide (Hammami et al. 2011) which established thriving and a successful approach for controlling various fungal diseases in plants.

23.4.4 Fungicides Based on Nanoparticles

In recent years, antimicrobial agents based on nanoparticle (NP) are budding as a hopeful substitute to conventional chemical-based antimicrobial due to their multidimensional physicochemical properties and biological compatibility (Hajipour et al. 2012; Li et al. 2008). It was observed from studies, in most cases, that microorganisms tend to develop resistance to the traditional chemical-based antimicrobial agents after a certain time (Raffi et al. 2010). In contradictory, there are much lower chances of developing resistance in the case of NP-based agents (Kim et al. 2007).

In recent times, antimicrobial properties of several NPs (Ag, Cu, CuO, Fe_2O_3, ZnO, TiO_2, etc.) have been explored, and these are effective as well as inexpensive. Being a nontoxic, TiO_2 is drawing considerable attention as an eco-friendly, chemically stable, and clean phytocatalyst and may be a potential candidate for other novel application (Yasa et al. 2012; Hoffmann et al. 1995). Ag doped hollow TiO_2 nanoparticles were reported as effective fungicides in opposition to *Venturia inaequalis* and the *Fusarium solani*, which are both phytopathogens (Boxi et al. 2016).

23.4.5 Transgenic Approach

For the last 30 years, it was observed that the perspective of research has shifted toward crop genetic engineering to increase the possibility of rapid achievement of goal for acquiring resistance against pests in crop plants. The genetic engineering technique allows to alter genome of the higher plants that changes their metabolism and physiology that leads to plants for better resistance toward pests with improve yield quality (Mullet 1990). This technique allows using vast array of useful genes and also introduction of different desirable genes in single vents in plants. Genetic engineering practices have been utilized to manipulate plants to modify the plants genetically for enhancement of nutritional value, to improve quality of the crop, and to increase resistance against different pests (Lemaux 2008). Several genes from different sources have been used to transform the crop plants for fungal resistance. Synthesis of the enzymes which are accomplished by demeaning of the cell wall of pathogenic fungi is used as defense mechanism by the plants in response to the fungal invasion. To improve this defense mechanism of plants, fungal resistance transgenic plants have been developed to increase the disease resistance. In many studies transgenic plants developed with chitinase genes have shown resistance against fungal infections; it is because of the degradation of chitin cell wall of fungus by chitinase. Chitinase gene (*chi1*) from *Rhizopus oligosporus* in tobacco (Terakawa et al. 1997) and chitinase gene (*RCC2*) of rice in chrysanthemum resistant to gray mold (Takatsu et al. 1999) have shown resistance against fungus. Chitinase gene (*RCC2*) was introduced in grapevine (Yamamoto et al. 2000) and cucumber (Kishimoto et al. 2002) and has shown enhanced resistance. Chitinase gene (*Chi*) from tobacco was used to develop a fungal resistant peanut (Rohini and Rao 2001). Cultivars of rice which are resistant to fungus were successfully developed by Datta et al. (2001) via introduction of a rice chitinase gene (*RC7*) from *R. solani*-infected rice plants. Further, Kumar et al. (2003) used another rice chitinase (*chi*11) to develop *R. solani* resistant rice.

The glucanase gene has also been used to develop transgenic plants against fungal diseases. Introduction of *β*-1,3 and 1,4-glucanase gene (*Gns1*) in rice has enhanced the disease resistance ability against blast disease (Nishizawa et al. 2003). β-1,3-glucanase gene (*βglu*) was introduced from potato to flax for enhancing resistance against *Fusarium* infection (Wrobel-Kwiatkowska et al. 2004). Indian mustard was developed with glucanase gene to overcome *Alternaria* leaf spot sickness responsible by *Alternaria brassicae* (Mondal et al. 2007).

Apart from these, a number of antimicrobial compounds such as proteins and peptides were also introduced in different transgenic plants which are effective in conferring disease resistance. Three different antifungal genes, the trichosanthin gene (TCS) (Xiaotian et al. 2000), afp gene of Aspergillus giganteus encodes for an antifungal protein (Coca et al. 2004) and artificially prepared antifungal genes like Ap-CecA and ER-CecA (Coca et al. 2006), were introduced in rice for providing antifungal resistance. Chitinase and glucanase are antifungal genes that have shown efficacy for controlling fungal diseases in transgenic crop plants. These genes should be utilized to develop more fungal resistant crop plants in future.

23.5 Conclusions and Future Perspective

During lifetime, plants are exposed to numerous kinds of pests that result in huge crop losses. More than 10,000 kinds of fungi infest crop and can cause disease in plants resulting into significant loss in crop productivity. Protection of crop plants from fungal pathogens and increase in yield productivity is the need of the hour. Various control measures have been applied such as use of chemical pesticides and biocontrol agents. Use of nanoparticles approach is another idea which is one step ahead of the other. Scientists have been trying to strengthen the plant defense mechanism by introducing various fungicidal genes in them through plant genetic engineering approach. Various kinds of fungicidal genes from different sources have been introduced in crop plants to provide fungal resistance. The genes responsible for synthesis of enzymes which are capable of degrading fungal cell wall are encouraged to be introduced in plants (Terakawa et al. 1997). Chitinase and glucanase are antifungal genes, and they have been established as fruitful candidate genes for successful management of fungal diseases in transgenic crop plants. These genes should be properly utilized to generate more fungal-resistant crop plants in the future. This will greatly help to significantly increase the agricultural productivity by protecting the crop plants from fungal diseases. Apart from this, the introduction of novel molecules further strengthens the idea of developing transgenic plants to control fungal diseases and therefore may form part of integrated pest management (IPM).

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Fungi: An Effective Tool for Bioremediation

24

Sachin Gupta, Anshu Wali, Moni Gupta, and Sudheer Kumar Annepu

Abstract

Pesticides which are hydrophobic in nature are often adsorbed as well as retained by the soil particles and organic matter, whereas, the water soluble pesticides enter the surface and ground water bodies and can enter drinking water wells causing health problems by entering food chain directly. Currently, one of the most effective and common remediation practices is incineration, but it is associated with a number of disadvantages. One promising alternative treatment strategy to incineration is bioremediation which is to exploit the ability of microorganisms for removing pollutants from contaminated sites. Fungi are among the potential candidates of bioremediation as they are natural decomposers of waste matter and secrete several extracellular enzymes capable of decomposing lignin and cellulose, the two essential components of plant fiber. It is necessary to correctly identify and select the fungal species to target a particular pollutant to achieve a successful mycoremediation. White-rot fungi possess a number of advantages in relation to degradation of insoluble chemicals and toxic environmental pollutants that can be exploited in bioremediation systems. The accessibility and bioavail-

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ability of the pollutants serve as a limitation in bioremediation including fungal-mediated bioremediation of pesticides. As a future perspective, there is a need not only to isolate and characterize the novel pesticide mineralizing fungal strains but also to characterize the chemistry, toxicity, and environmental fates of the metabolites produced during fungal biodegradation of pesticides.

Keywords
Pollution • Pesticide • Bioremediation • White-rot fungi • Lignin

24.1 Introduction

Pesticides are used in agriculture to ensure the quality as well as yield of the crops by managing different pests including insects, pathogens, weeds, etc. which are responsible for causing huge losses to the crops (Liu et al. 2001). Injudicious use of synthetic organic compound pesticides has led to the major problem of environmental pollution worldwide. Many of these novel compounds introduced to the nature as synthetic pesticides are termed xenobiotics, and many of them are not easily degraded by the indigenous microflora and fauna (Sullia 2004). India is the largest consumer of pesticides in South Asian countries (Agnihotri 1999).

It has been reported that only about 5% of the total applied pesticides is able to hit the target pests, while the rest enters into soil and water resources (Kookana et al. 1998; Nawaz et al. 2011). Hydrophobic pesticides are often adsorbed and retained by the soil particles and organic matter (Xiao et al. 2011; Bhalerao 2012), whereas, the water soluble pesticides shows a tendency to enter the surface and ground water bodies (Casara et al. 2012). Once in the groundwater, these pollutants can enter drinking water wells causing health problems by directly entering the food chain (Strandberg et al. 1998; Bavcon et al. 2002) The main reasons for persistence of these compounds in nature are that the conditions as well as the microorganisms capable of biodegrading these toxic compounds may not be present at the contaminated site (Frazar 2000). Even if the necessary microorganisms are present, some limiting factor, such as shortage of nutrients, creates unfavorable conditions for the biodegradation of contaminants. The other possibility being the compound could be recalcitrant or resistant to biodegradation (Field et al. 1993). However, some microorganisms survive in pesticide-contaminated sites, and the metabolic processes of these organisms are capable of using chemical contaminants as a source of energy, rendering the contaminants harmless or less toxic products in majority of the cases (Kirk and Farrell 1987; Hatakka 2001).

Several classes of chemicals including polycyclic aromatic hydrocarbons, pentachlorophenols, polychlorinated biphenyls, 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane, benzene, toluene, ethylbenzene xylene, and trinitrotoluene have been targeted by the United States Environmental Protection Agency (USEPA) as priority pollutants due to their toxic effects on the human health as well as to the

environment. Polycyclic aromatic hydrocarbons (PAH) are recalcitrant environmental contaminants generated from the burning of fossil fuels, coal mining, oil drilling, and wood burning (Lau et al. 2003; Verdin et al. 2004). All of these chemical compounds pose a significant threat to the health and vitality of the earth system. The elimination of wide ranges of pollutants and wastes from the environment is therefore an absolute requirement for promotion of a sustainable development of our society with minimum possible environmental impact.

Environmental contamination due to pesticides affects not only the ecosystem services of soil and water resources but also the health of animals, plants, microorganisms, and human beings. Hence, it is the need of the hour to devise the environmental friendly suitable strategies for remediating the pesticides from the contaminated environments. Due to the magnitude and severity of the problem and the lack of a reasonable solution, a rapid cost-effective ecologically responsible method of cleanup is urgently needed (Hamman 2004). Currently, incineration is the most effective and common remediation practice, but this is extremely costly, in monetary and energy terms. One promising alternative treatment strategy that is effective, minimally hazardous, economical, versatile, and environment-friendly, therefore, is to exploit the ability of microorganisms to remove pollutants from contaminated sites (Finley et al. 2010).

24.2 Bioremediation

Heavy metals, pesticides, and phenolics contamination due to natural and anthropogenic sources is presently a global environment concern. Release of heavy metals without proper treatment poses a significant threat to public health because of its persistence, biomagnification, and accumulation in food chain. Microbial metal bioremediation is an efficient strategy due to the involvement of low cost, high efficiency, and eco-friendly nature (Rajendran et al. 2003; Wasi et al. 2011). Bioremediation provides a reliable and a low-cost alternative. The microorganisms have the capacity to remove, immobilize, or detoxify metals and radionuclide through various mechanisms (Ji and Silver 1995). Detoxification of metals by microbes has been attributed to a number of processes including oxidation-reduction, complexation, methylation, and reactions involving biosurfactants (bioemulsifiers) and siderophores. In response to metal toxicity, many microorganisms have developed unique intracellular or extracellular mechanisms to resist and detoxify harmful metals. The mechanisms may be specific to a particular metal or a general mechanism for a variety of metals (Wasi et al. 2008).

Biodegradation is a complex process occurring in the environment and involves abiotic and biotic biotransformations performed by microorganisms or plants (Fenner et al. 2013). It involves the complete rupture of an organic compound into its inorganic constituents. Microorganisms hold the potential of potent bioremediation agents because of their ubiquity, large numbers and biomass relative to other living organisms, wider diversity, ability to function even under anaerobic and other

extreme conditions, and capabilities in their catalytic mechanisms. Understanding their genetics and biochemistry, and developing methods for their application as bioremediation agents in the field, has become a topic of great research (Megharaj et al. 2011). Probably, there is more diversity in microbial members and their capabilities to synthesize or degrade organic compounds than the existence of diversity in sources and chemical complexities of organic pollutants (Ramakrishnan et al. 2011).

These biodegradation reactions depend on the structure of pesticides and environmental conditions and are often the main mechanism of decontamination and modifying toxicological properties of pesticides (Hussain et al. 2009). Thus, biodegradation is considered to be the most promising, relatively efficient, and cost-effective technology. There are essentially two approaches involving bioremediation. In situ methods involve the treatment of contaminated material on-site, whereas the physical removal of contaminated material to treat it elsewhere is referred to as ex situ. To excavate and remove contaminated soil using chemical methods or by incineration is a relatively costly procedure. In contrast, the overall expense is far less if the soil is left where it is and decontaminated on the site. Moreover, in washing or extracting toxic materials from the soil, contamination simply moves from one place to another and is not eradicated, while incineration may cause problems of being energy intensive and dioxin formation.

Methods of bioremediation offer means to degrade toxic organic materials from pesticides, industrial waste, oil spills, etc. converting them to more innocuous compounds. Whole mineralization of contaminants, i.e., their transformation to CO_2, H_2O, N_2, HCl, etc., is the ultimate goal of bioremediation. Although heavy metals and radioactive cations cannot be decomposed but can be rendered into forms of low solubility, making them less harmful in the ground, they might be physically removed by phytoremediation or mycoremediation which involves the harvesting of the entire plant or fungus (Singh et al. 2014). Microorganisms provide a potential wealth in biodegradation. The use of bioremediation is typically less expensive than the physicochemical methods. This technology offers the potential to treat contaminated soil and groundwater in situ (Kearney and Wauchope 1998); hence, it requires little energy input and preserves the soil structure as such (Höhener et al. 1998). Reduced impact on the natural ecosystem is perhaps the most attractive feature of bioremediation (Zhang and Chiao 2002).

In soils, microbial metabolism is perhaps the most important pesticide degradative process (Kearney and Wauchope 1998) as the degrading microorganisms obtain C, N, or energy from the pesticide molecules (Gan and Koskinen 1998). The goal of bioremediation is to reduce pollutant levels to undetectable, nontoxic, or acceptable levels, i.e., within the limits set by regulatory agencies (Pointing 2001). They ideally completely mineralize organopollutants to carbon dioxide. From an environmental point of view, this total mineralization is desirable as it represents complete detoxification (Gan and Koskinen 1998). Microbial processes leading to elimination of organic environmental contamination assume great importance. Progress in the biotechnology of biodegradation relies upon the underlying sciences of environmental microbiology and analytical geochemistry. New analytical and molecular

tools have deepened our insights into the mechanisms, the occurrence, and the identity of active players effecting biodegradation of environmental pollutants (Jeon and Madsen 2012).

Nature plays an important role in modulating the microbial activity which has been shown to be greatly influenced by environmental factors. The purpose of bioremediation, therefore, is to minimize these environmental pollutants (Paul et al. 2005). It is an important consideration that any biodegradation operation must compete economically and functionally with various physicochemical processes available for the removal of pollutants (Wasi et al. 2008). For soils, these methods include thermal treatment (incineration) and solvent or detergent washing technique, etc. (Amend and Lederman 1992). Application of individual microbes and microbial communities for large-scale treatment of domestic and industrial wastes is well documented (Gadd 2010). The ability of these organisms to reduce the concentration of xenobiotics is directly linked to their long-term adaptation to environments where these compounds exist. The process of biodegradation depends on the metabolic potential of microorganisms to detoxify or transform the pollutant molecule (Ramakrishnan et al. 2011). The microbial populations of soil or aquatic environments are diverse and may be synergistic or antagonistic communities.

In natural environments, biodegradation involves metabolic cooperation that refers to transferring the substrates and products within a well-coordinated microbial community (Abraham et al. 2002). Microorganisms have the ability to interact chemically as well as physically, with substances leading to either complete degradation of the target molecule or may involve certain structural changes. The complexity of microbial mechanisms for degradation of organopollutants and the time period before microbial degradation starts, requiring weeks to months, have made the technology slow to emerge as a viable method of remediation (Nerud et al. 2003). Detailed studies of the principles of biodegradation and the development of efficient methods of decontamination are apparent (Nerud et al. 2003).

24.3 Bioremediation Using Fungi

Fungi feature among nature's most vigorous agents for the decomposition of waste matter and are an essential component of the soil food web (Rhodes 2012). The key organism for breaking down the leaf litter is fungus. Indeed, fungi are the only organisms on Earth capable of decomposing wood. The mycelium of fungi exudes powerful extracellular enzymes and acids capable of decomposing lignin and cellulose, the two essential components of plant fiber. A rich material called humus is formed as the fungus breaks down wood and leaves. In the natural ecosystem of soils, a realm of organisms from different kingdoms make their assault on different substrates, and the rate of degradation becomes maximal on ample supply of N, P, K, and other essential inorganic elements (Rhodes 2013). While they often function together with bacteria and an array of microorganisms, it is the fungi that handle breaking down some of the largest molecules present in nature (Gilbert-Lopez et al. 2010). Aspergillus and other molds are highly efficient in decomposing starches,

hemicelluloses, celluloses, pectins, and other sugar polymers, and some of them can even degrade intractable substrates such as fats, oils, chitin, and keratin.

Substrates such as paper and textiles (cotton, jute, and linen) are readily degraded by these molds, and the process is often referred to as biodeterioration. To achieve a successful mycoremediation, a simple screening procedure is essential to select the correct fungal species to target a particular pollutant (Matsubara et al. 2006). Many fungi including *Aspergillus niger*, *Aspergillus terreus*, *Cladosporium oxysporum*, *Mucor thermohyalospora*, *Fusarium ventricosum*, *Phanerochaete chrysosporium*, *and Trichoderma harzianum* have been tested for their ability to degrade endosulfan (Bhalerao and Puranik 2007). Some of the fungal strains have also been observed to perform esterification, dehydrogenation, hydroxylation, and dioxygenation during the transformation of different pesticides (Pinto et al. 2012; Deng et al. 2015). 3-Phenoxybenzoic acid has also been found to be hydroxylated into 3-hydroxy-5-phenoxybenzoic acid which is dioxygenated into gallic acid and phenol (Deng et al. 2015). León -Santiesteban et al. (2016)) reported that a fungal strain *Rhizopus oryzae* CDBB-H-1877 has the potential for biosorption of pentachlorophenol through methylation and dechlorination. A few fungi belonging to the class zygomycetes and *Aspergillus* spp. have been demonstrated to decolorize and detoxify textile wastewaters. Some of the well-known fungi like *Penicillium chrysogenum*, *Scedosporium apiospermum*, *Penicillium digitatum*, *and Fusarium solani* are also reported for degradation capabilities of polychlorinated biphenyls (PCB). These fungi show the involvement of non-ligninolytic enzymes for degradation of PCBs (Tigini et al. 2009).

24.4 Biodegrading Capacities of White-Rot Fungi

Presently, prokaryotes are being used for bioremediation conducted on a commercial scale, and comparatively few recent attempts to use white-rot fungi have been made. However, these filamentous fungi offer advantages over bacteria in the diversity of compounds they are able to oxidize (Pointing 2001). In addition, as compared to bacteria, they are robust organisms and generally more tolerant to high concentrations of polluting chemicals (Evans and Hedger 2001). Therefore, white-rot fungi represent a powerful and prospective tool in soil bioremediation (Sasek 2003). Fungi generally biotransform pesticides and other xenobiotics by introducing minor structural changes to the molecule, rendering it nontoxic. The biotransformed pesticide is released into the environment, where it becomes susceptible to further degradation by bacteria (Diez 2010). Furthermore, there is a minimum pollutant concentration level below which the enzymes are not expressed in bacteria, thus limiting the technology (Adenipekun and Lawal 2012). White-rot fungi digest lignin through the secretion of enzymes giving a bleached appearance to wood from undissolved cellulose, whereas, brown-rot fungi degrade cellulose, leaving lignin as brownish deposit.

These fungi cause checkered, cubical cracking and shrinking in wood, which is apparent on felled conifer trees (Stamets 2005). A number of other white-rot fungi

also can degrade persistent xenobiotic compounds, e.g., *Pleurotus ostreatus*, *Trametes versicolor*, *Bjerkandera adusta*, *Lentinula edodes*, *Irpex lacteus*, *Agaricus bisporus*, *Pleurotus tuber regium*, and *Pleurotus pulmonarius* (Singh 2006). Other toxic materials that have been reported to be successfully degraded using white-rot fungi include polychlorinated biphenyls and dioxins, pesticides, phenols, chlorophenols, effluents from pulp and paper mills, dyestuffs, and heavy metals (Singh 2006).

Application of fungal technology for the cleanup of contaminants has shown promise since *Phanerochaete chrysosporium* was found to be able to metabolize a number of important environmental pollutants and to degrade and mineralize a wide variety of industrial and agricultural pollutants (Sasek 2003). It is an ideal model for bioremediation by fungi presenting simultaneous oxidative and reductive mechanisms which permit its use in multifarious situations. Enzymes involved in degradation of pollutants in *P. chrysosporium* are found to be lignin peroxidases (LiP) and manganese-dependent peroxidases (MnP), which have also been shown to facilitate both reductive and lipid peroxidation-mediated degradation of environmental pollutants. It is capable of degrading several chlorinated xenobiotics under conditions which do not favor the production of lignin peroxidases (LiP) and manganese-dependent peroxidases (MnP) (Kullman and Matsumura 1996).

White-rot fungi are able to degrade a wide variety of environmental pollutants to carbon dioxide, including a number of chlorinated pollutants such as DDT, lindane, chlordane, polychlorinated biphenyls, and 3,4 dichloroaniline (Arisoy 1998). The main reason that white-rot fungi are active to such a wide range of compounds is their release of extracellular lignin-modifying enzymes including lignin peroxidase (LiP), manganese peroxidase (MnP), various H_2O_2-producing enzymes, and laccase which can act upon various molecules that are broadly similar to lignin (Kirk and Farrell 1987; Adenipekun and Lawal 2012). White-rot fungi can degrade insoluble chemicals such as lignin or an extremely diverse range of very persistent or toxic environmental pollutants (Barr and Aust 1994). The mycelial growth habit is also advantageous as it allows rapid colonization of substrates, and hyphal extension enables penetration of soil reaching pollutants in ways that other organisms cannot do (Reddy and Mathew 2001), thus maximizing physical, mechanical, and enzymatic contact with the surrounding environment. In addition, these fungi use inexpensive and abundant lignocellulosic materials as a nutrient source and can tolerate a wide range of environmental conditions (Maloney 2001) and, moreover, do not require pre-conditioning to a particular pollutant, because their degradative system is induced by nutrient deprivation (Barr and Aust 1994).

Basidiomycetous, ascomycetous, and hyphomycetous fungi isolated from marine environments are reported to have capabilities of degradation of effluent from textile industries. Nwachukwu and Osuji (2007) conducted a study for determining the ability of white-rot fungus, *Lentinus subnudus*, to degrade atrazine, heptachlor, and metolachlor and observed that up to 94% of both metolachlor and heptachlor were degraded, whereas 78% degradation of atrazine was achieved after 25 days. Purnomo et al. (2014) characterized white-rot fungi, *P. ostreatus*, for biodegradation of heptachlor and heptachlor epoxide, and this strain showed the potential to remove

about 89% and 32% of initially added heptachlor and heptachlor epoxide, respectively, over 28 days of incubation. Nyakundi et al. (2011) reported the potential of five white-rot fungi cultures and their mixture for degradation of diazinon and methomyl pesticides and observed that mixture of various white-rot fungi and pure cultures degraded diazinon and methomyl; however, the fungal consortium was more effective than pure cultures. Similarly, Xiao et al. (2011) reported the ability of *Phlebia acanthocystis*, *Phlebia brevispora*, and *Phlebia aurea* to degrade aldrin and dieldrin pesticides. Kamei et al. (2011) characterized a white-rot fungus, *Trametes hirsuta*, for biodegradation of endosulfan and found that this strain had the potential to use and degrade endosulfan sulfate produced during the biodegradation of endosulfan.

24.5 Role of Enzymes in Biodegradation

Fungi and bacteria are considered as the extracellular enzyme-producing microorganisms for excellence. Enzymes are central to the biology of many pesticides and are activated in situ by enzymatic action, and many pesticides function by targeting particular enzymes with essential physiological roles (Riya and Jagatpati 2012). These are involved in the degradation of pesticide compounds through intrinsic detoxification mechanisms and evolved metabolic resistance via biodegradation by soil and water microorganisms (Scott et al. 2008). Applying enzymes to degrade or transform pesticides is an innovative technique for the removal of these chemicals from polluted environments. Enzyme-catalyzed degradation of a pesticide may be more effective than existing chemical methods. White-rot fungi have been proposed as promising bioremediation agents, especially for compounds that are not readily degraded by bacteria because of their ability to produce extracellular enzymes that act on a broad array of organic compounds. Some of these extracellular enzymes involved in lignin degradation are lignin peroxidase, manganese peroxidase, laccase, and oxidases. The three main enzyme families implicated in degradation are esterases, glutathione S-transferases (GSTs), and cytochrome P450 (Bass and Field 2011). A number of recent studies have shown the involvement of peroxidase and laccase enzymes in the biodegradation of different pesticides (Donoso et al. 2008; Pizzul et al. 2009; Kadimaliev et al. 2011). Donoso et al. (2008) documented the involvement of peroxidase and laccase activity in the degradation of tribromophenol (TBP) by *Trametes versicolor*. Similarly, Kadimaliev et al. (2011) measured the biodegradation of phenol through *Lentinus tigrinus* in liquid medium with the help of laccase and peroxidase enzymes. In all these processes, fungi and bacteria are involved in producing intracellular or extracellular enzymes including hydrolytic enzymes, peroxidases, oxygenases, etc. (Van et al. 2003). Due to the diverse chemistry used in pesticides, the biochemistry of pesticide bioremediation requires a wide range of catalytic mechanisms and therefore a wide range of enzyme classes.

24.6 Practical Implementation of Mycoremediation Using White-Rot Fungi

Knowledge of fields such as fungal physiology, biochemistry, enzymology, ecology, genetics, molecular biology, engineering, etc. is essential for using white-rot fungi successfully for bioremediation. A four-phase strategy including bench-scale treatability, on-site pilot testing, production of inoculum, and finally full-scale application has been advocated (Lamar and White 2001). Fungal inocula coated with alginate, gelatin, agarose, carrageenan, chitosan, etc. in the form of pellets may offer a better outcome than with inocula produced using bulk substrates which is termed encapsulation and is derived from the mushroom spawn industry and preserves the viability of the inoculum and contributes nutrients to maximally support the degradation of pollutants. Substrates such as wood chips, wheat straw, peat, corncobs, sawdust, a nutrient-fortified mixture of grain and sawdust, bark, rice, annual plant stems and wood, fish oil, alfalfa, spent mushroom compost, sugarcane bagasse, coffee pulp, sugar beet pulp, okra, canola meal, cyclodextrins, and surfactants can be used in inoculum production both off-site or on-site or as mixed with contaminated soils to improve the processes of degradation (Singh 2006). It is critical to attain the correct C:N ratio in the substrates used for avoiding any impeding effect on the efficiency of the fungi in the bioremediation process. Native microbial populations also provide a potential competition to the mycoremediation process, but there is, as yet, a lack of defined protocols to eliminate such influences. There are some patents available which refer to the subject of remediation using white-rot fungi (Singh 2006).

24.7 Limitations of Using Fungi as Tools of Bioremediation

Although several fungal strains have been isolated and characterized for biodegradation and bioremediation of different organic compounds including pesticides, still there are few drawbacks which limit their wider application. Fungal biodegradation of organic compounds including pesticides has normally been observed as a relatively slower process, and, sometimes, it does also not lead toward complete removal of the contaminants (Sasec and Cajthaml 2014).

Moreover, it has also been observed that fungi requires more time for adaptation to the contaminated environment as well as removal of the pollutants (Kulshreshtha et al. 2014). Under field conditions, this might be due to the mass transfer limitations because of variations and fluctuations in physicochemical and climatic conditions of the soils/ fields (Boopathy 2000). Similarly, accessibility and bioavailability of the pollutants also serve as a limitation in bioremediation including fungal-mediated bioremediation of pesticides. Hence, there is a rising need that bioremediation processes including fungal-mediated remediation must be tailored to the site-specific conditions. However, there is need to understand the processes and mechanisms underlying this regulation in fungal biodegradation of pesticides resulting from changes in physicochemical characteristics of the environment. There is

need to know whether such changes affect the chemistry of the pesticides or the physiology of the degrading fungi. Another drawback associated with fungal biodegradation is partial degradation of the organic compounds including pesticides leading toward the accumulation of secondary metabolites which might be harmful or harmless depending upon their chemical nature (Badawi et al. 2009; Pinto et al. 2012). It has been observed that, sometimes, these secondary metabolites have been found to be even more harmful as compared to their parent compounds (Boopathy 2000).

Only few fungal strains have been found to mineralize the pesticides. Moreover, there is very limited information regarding the environmental fate of such metabolites produced during fungal biodegradation. This problem can be solved by the use of microbial consortium including both fungal and bacterial strains. Another strategy is to add a specific gene that can confer specific degradation capability to indigenous microorganism. The addition of degradative genes relies on the delivery and uptake of genetic material by an indigenous microorganism. The two possible approaches that can be taken include the use of microbial cells to deliver gene via conjugation and to add naked gene in soil and allow its uptake via transformation (Singh 2008).

24.8 Future Perspectives

Bioremediation is the most effective management tool to manage the polluted environment and recover contaminated soil and water because it is less harmful and affects only a few target organisms and a specific pest. Moreover, it is very effective in small quantities and often decomposes quickly. The fungal biodegradation of the pesticides does not seem to be conserved to any specific genus or species of fungi as bacterial biodegradation of some pesticides has also been reported. Despite that some of the pesticides presented were observed to be degraded by the fungal strains belonging to a single genus, this might be due to the fact that only few strains have been isolated for biodegradation of those pesticides. Moreover, it has been observed that the potential of bacteria and fungi to completely degrade the pesticides enhances significantly when they were used in co-culture with each other. In order to achieve better bioavailability and biodegradation of pesticides, such fungal/microbial consortia needs to be tested for their relation and interaction with each other and with pesticides as well as environmental conditions.

As a future perspective, there is a need not only to isolate and characterize the novel pesticide mineralizing fungal strains but also to characterize the chemistry, toxicity, and environmental fates of the metabolites produced during fungal biodegradation of pesticides. Although fungal biodegradation has been considerably studied under in vitro conditions in liquid cultures, there are no many studies on such degradation in soil, sediment, and sludge under field conditions. Hence, studies on large scale and more in-depth evaluation of fungal biodegradation of pesticides are needed. Some of the enzymatic activities, metabolites, and processes involved in the fungal biodegradation of some pesticides have been discovered, and metabolic

pathways for such pesticide transformations have been proposed. Further studies need to be conducted to discover the enzymatic and genetic basis of the fungal biodegradation of pesticides by applying the advanced omics-based approaches to identify the genes, enzymes, and metabolites involved during fungal biodegradation of pesticides. Such genetic characterization will further serve as baseline information for genetic engineering of the pesticide-degrading fungal strains which might enable us to enhance their capability and potential.

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Commercial Microbial Products: Exploiting Beneficial Plant-Microbe Interaction

25

Pallavi, Dinesh Chandra, and A.K. Sharma

Abstract

Plants and microbes are known to interact with each other since ancient times. The plant growth-promoting microbes have the ability to facilitate nutrient uptake, modulating plant growth and imparting abiotic and biotic stress tolerance to the plants. These microbes along with proper carrier form the basis of commercial microbial inoculants, which slowly but steadily are gaining acknowledgment in the market due to the drawbacks associated with their counterpart agrochemicals like reduced soil fertility, food toxicity, or increasing cost and diminishing profits. The nitrogen fixers and phosphate and zinc solubilizers are the foremost microbial categories that are presently exploited on a commercial level. The success of microbial inoculants in the field relies on the carrier material used in the formulation. Many carriers are explored for this purpose; peat, perlite, clay, vermiculite, alginate, agricultural waste products, and biochar are among the leading options.

Keywords

Sustainable agriculture • Phosphate solubilizers • Microbial inoculants • Carriers • Formulation

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25.1 Introduction

The 1960s was the decade of green revolution in India. It saved us from the hand of food scarcity and hunger. However, it was the result of increased cultivated area by cutting down the forest cover, development of new varieties, and input of inorganic fertilizers to support these varieties. Later on, it was termed as "exploitive agriculture" because of its dependence on the extensive use of fertilizers, pesticides, bactericides, and fungicides. All these agrochemicals are widely known to harm the environmental and human health, raising the demand for organic products among the consumers (Noble and Ruaysoongnern 2010). To meet these requirements, innovative, sustainable, and cost-effective approaches for crop protection and enhancement need to be developed. Plants and microbes live in an intricately balanced relationship which includes either synergistic or antagonistic behavior. Synergistic interactions show promising prospects in improving the current agriculture scenario by developing microbial inoculants which are equivalent to their counterpart agrochemicals in increasing crop productivity and providing environmental stress resistance without causing any imbalance to the ecosystem. Commercialization of these microbial inoculants can result in solving many problems which farmers and environmentalist are facing all around the world.

Microbial inoculants/biofertilizers are the preparation that contains live or latent cells of efficient microbial strains capable of nitrogen fixation, phosphate and zinc solubilization, or cellulolytic activity. They heighten the pace of microbial processes which augment the availability of nutrients that can be well absorbed by plants. Inoculant is the means to deliver living bacteria from the factory and introduce them onto plants (Tittabutr et al. 2007). They additionally stimulate the phytohormones providing better nutrient uptake and enhanced stress tolerance while maintaining soil health and environment. Administration of even a small quantity of biofertilizer is enough to get sought after results as its each gram contains at least 10 million viable cells of the selected strain (Anandaraj and Delapierre 2010).

Earliest bacterial inoculants that came into practice were the cultures of *Rhizobium* spp. in legumes (Fages 1992; Nehra and Choudhary 2015) followed by *Azotobacter*, blue-green algae (BGA), and other diverse group of microorganisms (Bashan 1998). *Azospirillum* and *vesicular-arbuscular mycorrhizae* (VAM) are reasonably recent discoveries. In the late 1970s, *Pseudomonas* spp. (Glick 1995; Glick and Bashan 1997) showed growth-enhancing potential in the nonlegume plants (Döbereiner and Day 1976). *Acetobacter*, *Bacillus*, and *Flavobacterium* are some other microorganisms with plant growth-promoting potential (Tang and Yang 1997). In India, Joshi (1920) led the legume-*Rhizobium* symbiosis studies, and by 1956 its commercial production began. The Ministry of Agriculture of India has started the National Project on Development and Use of Biofertilizers (NPDB) under their ninth plan to encourage biofertilizer application.

25.2 Need of Microbial Inoculants

There is a significant lacuna between the need and supply of food in the world. The problem is further intensified by the lack of ecologically and economically congruent agriculture techniques that are capable of providing adequate nourishment that can satiate the increasing human population while keeping the quality and quantity of agricultural products. Presently, the strategies which are being used include the application of chemical fertilizers, pesticides, production of genetically modified crops, and another controversial solution for improving the crop productivity. The drawbacks of using chemicals for enhancing the agricultural productivity are well documented; they not only cause damage to the ecosystem by causing soil deterioration and water pollution but also are not an economical choice. Phosphate fertilizers need phosphoric acid for their production, and India imports 95% of its rock phosphate making the production a costly affair along with causing a strain on Indian foreign exchange (Sharma et al. 2013). In light of the nuisance associated with the agrochemicals, it is important to use alternative sources, which are environment-friendly plant nutrients.

The prices of chemical inputs required to meet the food demands are getting higher with passing time. To make agriculture a profitable venture, the cost of production needs to be brought down, and cheaply produced microbial inoculants can help in this regard. Microbial inoculants for agriculture have grabbed the attention of researchers and manufacturing companies throughout the world because they hold the potential of providing an ecological and inexpensive substitute to chemical applications. These products include improved *Rhizobium* inoculants, cyanobacterial fertilizers, *Bacillus thuringiensis*, virus-based insecticides, frost protectant, plant growth regulators, fungicides, and waste processing treatments.

25.3 Plant-Microbe Interactions

Drawbacks of extensive usage of agrochemicals forced the researchers to look for a healthier approach toward raising the crop productivity. This led them to the vast world of microorganisms like bacteria, fungi, and algae which interact with plants on two levels: rhizospheric and endophytic. The rhizospheric microbes reside in the rhizosphere, root surface, and superficial intercellular species (Vessey 2003), while endophytic microbes live in the apoplastic spaces, rarely invading the intracellular spaces of the host plant. It is a well-proven fact now that many microbes are capable of improving growth of their host plants along with imparting resistance to diseases, pests, and abiotic stress. Among the well-studied examples are *Azospirillum*, *Rhizobium*, *Bacillus*, *Serratia*, *Stenotrophomonas*, *Streptomyces*, *Pseudomonas*, and the fungal genera *Ampelomyces*, *Coniothyrium*, and *Trichoderma* (Berg 2009).

Glick et al. (1999) described two categories of microbes based on plant growth promotion: indirect and direct facilitation. The former includes conferring resistance against pathogen attack (Lugtenberg and Kamilova 2009) by secretion of antifungal metabolites such as HCN, 2,4-diacetylphloroglucinol, phenazines,

pyoluteorin, pyrrolnitrin, viscosinamide, and tensin (Bhattacharyya and Jha 2012). Some other compounds like lipopolysaccharides (LPS), siderophores, cyclic lipopeptides, homoserine lactones, and volatiles like dimethyl sulfide (DMDS), acetoin, 2,3-butanediol and benzaldehyde are also responsible for imparting pathogen resistance in plants (Rudrappa et al. 2010; D'Alessandro et al. 2014). This is called induced systemic resistance (ISR) (Lugtenberg and Kamilova 2009). Jasmonate and ethylene signaling involved in ISR further stimulate the host plant's defense responses to reduce the harmful effect of phytopathogens (Glick 2012).

The direct plant growth promotion entails facilitating the acquisition of nutrient resources from the environment including fixed nitrogen, iron, and phosphate (Dobbelaere and Okon 2007). Bacterial siderophores and other organic acids can solubilize inorganic nutrients with low solubility and make them available to the plant (Katiyar and Goel 2004). Recently, de Werra et al. (2009) demonstrated that *Pseudomonas fluorescens* CHA0 produced gluconic acid causes acidification of its environment resulting insolubilization of mineral phosphate. Another means of modulating plant growth is by modifying plant hormone levels, such as auxin, cytokinin, and ethylene. For example, *B. amyloliquefaciens* synthesize indole-3-acetic acid (IAA), and *B. subtilis* and *B. megaterium* synthesize cytokinin, while *B. pumilus* produces gibberellins, abscisic acid, and jasmonic acid (Arkhipova et al. 2007; Mohite 2013; Kudoyarova et al. 2015).

25.4 Array of Microbial Products

Microbial inoculants are the products containing living microbes which, on application, colonize the rhizosphere or the interior of the plant and are used in agriculture to boost plant productivity, protection from pests and diseases, and improved soil fertility. These products offer vast potential to deliver sustainable, cost-effective approach that will help farmers to improve crop growth and productivity while limiting environmental impacts. Hellriegel and Wilfarth (1888) reported that soil from legume field is nitrogen enriched and the addition of this soil can enhance the fertility of nutrient deficient soil. This report led to the first patent filed (US 570813) in the field of bioinoculants using the pure culture of rhizobia by Nobbe and Hiltner in 1896. The patent was later commercialized by the name of "Nitragin" (Nobbe and Hiltner 1896). After that, some other microbial species were found to be beneficial for plant growth, and researchers all around the world started working in this direction. The main classes of microbes that are commercially exploited are the following.

25.4.1 Nitrogen-Fixing Microbial Inoculants

Nitrogen (N_2) is an essential key element for crop growth and productivity. The atmosphere contains 78% N_2, yet it remains inaccessible to growing plants, as organisms can solely utilize the ammonia (NH_3) form of nitrogen to synthesize nucleic acids, amino acids, proteins, and other compounds necessary for life. The

conversion of atmospheric N_2 into ammonia occurs with the help of nitrogen-fixing microorganisms using nitrogenase enzyme system (Kim and Rees 1994). Biological nitrogen fixation (BNF) represents 67% of the nitrogen fixed globally, while the rest is synthesized industrially by the Haber-Bosch method (Rubio and Ludden 2008). BNF can be used as a sustainable substitute for chemical fertilizers (Ladha et al. 1997; Ahemad and Kibret 2014).

BNF is a process mediated in nature solely by diazotrophs; they provide benefits to plant while living in strong association with the plant and also after death by releasing nitrogen to the surroundings. In leguminous and few nonleguminous plants, the N_2-fixing bacteria live inside small swollen growths on the roots called nodules. These nodules are the site of nitrogen fixation; NH_3 produced during the process is assimilated by the plant. Nitrogen fixation by legumes is a symbiotic relationship between a plant and a bacteria. However, other forms of nitrogen fixation are also present in nature including blue-green algae, free-living soil bacteria, and lichens. These types of nitrogen fixation add a significant amount of NH_3 to natural ecosystems, but this is not true for most cropping systems, except paddy rice. Their contribution is less than 5 pounds of nitrogen/acre/year. On the other hand, nitrogen fixation by leguminous plants can be in the magnitude of 25–75 pounds of nitrogen/acre/year in a natural ecosystem and up to several hundred pounds in a cropping system (Vessey 2003). BNF is restricted only to bacteria and archaea (e.g., *Methanococcus voltae*). Till date, no plant species (eukaryotic organisms) is reported to fix atmospheric N_2 into ammonia and use it directly for its growth. One of the major mechanisms utilized by PGPR through which plants get benefited is the enrichment of the soil in the form of ammonia (Young 1992). The most studied PGPR in N_2 fixation is rhizobia (including *Azorhizobium*, *Bradyrhizobium*, *Allorhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium*) for their ability to fix N_2 in their legume hosts (Gualtieri and Bisseling 2000).

Two forms of nitrogen-fixing bacteria are known. The first form, the free-living bacteria (nonsymbiotic), includes the cyanobacteria (*Anabaena* and *Nostoc*), *Azoarcus*, *Azotobacter*, *Beijerinckia*, *Clostridium*, and *Gluconacetobacter diazotrophicus* (Bhattacharyya and Jha 2012). The second form encompasses the mutualistic (symbiotic) bacteria that include *Rhizobium* with leguminous plants, *Azospirillum* species associated with cereal grasses, and *Frankia* associated with certain dicotyledonous species (Ahemad and Khan 2012). The symbiotic nitrogen-fixing bacteria penetrate the root hairs of host plants, where they proliferate and stimulate the establishment of root nodules and enlargements of plant cells. In nitrogen deficient soils lacking the required bacterium, seeds are treated with commercial cultures of *Rhizobium* species for efficient nodule formation and optimum growth promotion of leguminous plant.

25.4.2 Phosphate-Solubilizing Microbial Inoculants

Phosphorus (P) is an indispensable element in the nutrition of plants, after nitrogen, and plays a crucial role in different aspects of cellular machinery from energy

metabolism to the structure of the genetic material. It is a typical limiting factor behind the lacking growth and productivity of terrestrial plants. Phosphorus is absorbed by the plants from the soil as two soluble forms, the monobasic ($H_2PO_4^-$) and the dibasic (HPO_4^{2-}) phosphate anions (Glass 1989). Depending on the quality of the soil, reactive phosphate anions are trapped by precipitation with cations like Mg^{2+}, Ca^{2+}, Al^{3+}, and Fe^{3+}. Most of the applied phosphate fertilizers get firmly bound to the soil. Thus, soils may have a vast reservoir of total P, but the amount accessible to plants is usually a tiny proportion of this reservoir unless it is solubilized by the activity of microbes (Stevenson and Cole 1999; Chandra et al. 2015).

Phosphate-solubilizing bacteria (PSB) solubilize insoluble inorganic phosphate compounds (such as dicalcium and tricalcium phosphate (TCP), rock phosphate, hydroxyapatite) and solubilize through the production of organic acids such as acetic acid, citric acid, gluconic acid, 2-ketogluconic acid, lactic acid, propionic acid, isovaleric acid, isobutyric acid, etc. *Pseudomonas*, *Bacillus*, *Rhizobium*, *Alcaligenes*, *Acinetobacter*, *Arthrobacter*, *Azospirillum*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, and *Serratia* are the common PSB isolated from the rhizosphere (Kim et al. 1998; Bhattacharyya and Jha 2012). The solubilization of P in the vicinity of roots is the most common mode of action of PGPR that ensures nutrient availability to host plants (Richardson 2001). Many studies endorse the benefits associated with the PSB such as *Enterobacter agglomerans* with tomato (Kim et al. 1998), *Bacillus* sp. with five crop species (Pal 1998), *Rhizobium* sp. and *Bradyrhizobium japonicum* with radish (Antoun et al. 1998), *Rhizobium leguminosarum* bv. *phaseoli* with maize (Chabot et al. 1998), *Bacillus circulans* and *Cladosporium herbarum* with wheat (Singh and Kapoor 1999), *Pseudomonas chlororaphis* and *P. putida* with soybean (Cattelan et al. 1999), and *Azotobacter chroococcum* with wheat (Kumar and Narula 1999).

25.4.3 Zinc-Solubilizing Bacterial Inoculants

Micronutrients are essential for the optimal growth and productivity of the plants and are required in relatively small concentrations (5–100 mg kg^{-1}). Among the micronutrients, Zn plays a major role in the growth and productivity of the plants. It influences plant life processes such as resistance to abiotic and biotic stresses, N_2 metabolism and its uptake, protein quality, chlorophyll synthesis, photosynthesis, and protection against oxidative damage (Potarzycki and Grzebisz 2009; Sunithakumari et al. 2014). In case of reduced Zn availability, crop yields and the quality of production will be adversely affected. Zinc deficiency in plants leads to reduced synthesis of cytochromes, carbohydrates, auxins, chlorophyll, and nucleotides and membrane integrity resulting in susceptibility to heat stress (Singh et al. 2005). Therefore, for proper function of crop plants, a certain minimum amount of Zn supply is indispensable. The process of zinc solubilization depends on the soil pH and moisture; therefore, the arid and semiarid areas of Indian agroecosystems

are zinc deficient. In India, around 50% of the soils are deficient in zinc, and this remains the most important nutritional disorder affecting the majority of the crop production. Increased application of chemical fertilizers, intensive agriculture, and poor irrigation system are the reasons that contribute to the decrease of zinc content (Das and Green 2013). Zinc deficiency is expected to increase from 42 to 63% by the year 2025 due to the continuous diminution of soil fertility (Singh 2009).

An extensive literature report indicates that Zn concentration in the grain is naturally very low, particularly when grown on Zn-deficient soils. The primary cause for the widespread occurrence of Zn deficiency problems in crop plants is due to its low solubility in soils rather than a low total amount of Zn (Cakmak 2008). Supplementation of zinc in the form of chemical fertilizer is deemed unsuccessful due to its unavailability to plants, and dilemma can be prevented by the identification of rhizospheric microorganisms which has the potential to transform various inaccessible forms of the metal to accessible forms.

Numerous genera of rhizobacteria belonging to *Bacillus* spp., *Pseudomonas* spp., and *Xanthomonas* spp. have the ability to transform the complex unavailable form ($Zn_3(PO_4)_2$, $ZnCO_3$, and ZnO) of metal into available form. The secretion of organic acids is the functional mechanism involved in metal solubilization (Cunningham and Kuiack 1992). Gluconic acid is believed to be the major organic acid involved in the solubilization of insoluble minerals (Henri et al. 2008). Organic acids secreted by root-associated microorganism increase soil Zn availability by sequestering cations and by reducing rhizospheric pH. The cell surface and membranes of these microbes have protons, chelating ligands, and oxidoreductive systems which help in Zn solubilization (Wakatsuki 1995). These bacteria also exhibit other plant growth-promoting traits which are beneficial to plants, such as the production of phytohormones (indole acetic acid, gibberellins, cytokinin) siderophores, hydrogen cyanide, vitamins, antibiotics, and antifungal substances (Rodriguez and Fraga 1999).

25.5 Inoculum Production Technology

The commercialization of microbial inoculants starts with a screening of new and more efficient microorganisms followed by the optimization of fermentation procedure for mass production and in the end developing a formulation (Singleton et al. 2002; Vassilev et al. 2016). The final product is called bioformulation, containing one or more beneficial microbial strains or their metabolites in its biologically active form supported by easy-to-use, nontoxic, inert, and economical carrier materials for maintaining the viability and efficiency of cells or metabolites and to increase their shelf life (Arora et al. 2010; Bashan et al. 2014). A well-formulated microbial preparation is the key to its optimum performance and commercial success. The focus, while preparing a bioformulation, should be on keeping the production cost low while maintaining the quality.

25.5.1 Inoculant Formulation

The most critical step in commercializing microbial inoculants is developing its formulation for ensuring success in the market. Viable bacterial strains are introduced in a proper carrier along with additives for protection and stabilization of microbial cell. The formulation should also be easy to process so that it is delivered to target in most apt condition and form.

25.5.2 Carrier for Inoculant Formulation

The success of a bioinoculant depends on the selection of the carrier material as it is responsible for maintaining the viability of the bacteria during transportation, storage, and even after its application in the field. Bashan (1998) postulated the desirable characteristics for a suitable carrier as follows:

(i) It should allow the addition of bacterial nutrients.
(ii) It must have a high water-holding capacity.
(iii) It should allow easy sterilization and temperature resistant.
(iv) It must have a proper pH buffering capability.
(v) It must be non-pollutant and biodegradable.
(vi) It must allow easy handling by the farmer.

It is hard to find a natural product exhibiting all these properties. However, new technologies are currently heading toward the development of novel carriers with better characteristics. Carriers can be divided into four categories (Bashan 1998), listed in Table 25.1.

25.5.2.1 Conventional and Nonconventional Carriers

Fine ground peat is a traditional carrier for legume inoculant production (Burton 1976; Roughley 1970). However, there are considerable dilemmas associated with peat like availability (Strijdom and Deschodt 1976) and incompatibility with some *Rhizobium* strains (Brockwell et al. 1985). Also, excessive extraction of peat from a wetland is threatening to the ecosystem. Several potential substrates including a variety of coals, bentonite, corn oil, mineral soils, and vermiculite have been tested as a replacement of peat (Crawford and Berryhill 1983; Kremer and Peterson 1982; Chao and Alexander 1984; Sparrow and Ham 1983a; Graham-Weiss et al. 1987). Perlite also known as hydrated aluminum silicate and obtained from volcanic stone is another conceivable carrier. It is easy to sterilize with no prospect of producing toxic substances (Sparrow and Ham 1983a). Clays are conventionally used in the agricultural formulation as granules, suspension, or powder. It prolongs the life of microbial inoculants by acting as desiccant due to larger pore size distribution, total porosity, and surface area. It provides protective microhabitat to the bacteria but not to pathogens (Kotb and Angle 1986).

Table 25.1 Categories of carriers

Category	Types	References
1. Soils	Peat, coal	Singh and Sharma (1973)
	Clays and inorganic soil	Chao and Alexander (1984), and Kotb and Angle (1986)
2. Waste materials	Composts farmyard manure soybean and peanut oil	Kremer and Peterson (1982)
	Wheat bran	Jackson et al. (1991)
	Sawdust	Arora et al. (2008)
	Spent mushroom compost	Bahl and Jauhri (1986)
	Plant debris	Richter et al. (1989)
	Poultry manure and banana waste	del Carmen Rivera-Cruz et al. (2008)
	Earthworm casts	Sekar and Karmegam (2010)
	Pine wood biochar	Hale et al. (2014); Sun et al. (2016)
3. Inert materials	Vermiculite	Paau (1988), and Sparrow and Ham (1983a, b)
	Perlite, ground rock phosphate, calcium sulfate, polyacrylamide gels	Dommergues et al. (1979), and Sparrow and Ham (1983b)
	Alginate beads	Aino et al. (1997), and Trivedi and Pandey (2008)
	Carboxymethyl cellulose	Da Silva et al. (2012)
4. Plain lyophilized microbial cultures and oil dried bacteria	–	Mohammadi (1994), Mohammadi and Lahdenperä (1994), and Johnston (1962)

Some of the alternative carriers that are being explored include exopolysaccharides (EPS) of microbial origin (Bashan et al. 2014). Rhizobia are among the well-known EPS producers (Nwodo et al. 2012). EPS act as a shield for nitrogenase enzyme from high oxygen concentration in the nodules and function as molecular signals during the process of preinfection and infection that occurs in the rhizosphere, thus facilitating the process. These benefits make them a viable substitute for peat (Vu et al. 2009; Freitas et al. 2011).

Another potential substitute is biochar produced from woody feedstocks by low-oxygen pyrolysis. They serve as effective carriers and provide additional benefits as compared to other carriers (Saranya et al. 2011; Lehmann and Joseph 2015). Biochar is presterilized during its production by pyrolysis, thus facilitating the inoculum production process. The raw materials for biochar production include agroforestry residues, compost, and manures, many of which are considered waste products and require disposal fees. Part of organic and inorganic nutrients of the feedstock are retained in the biochar thus act as a source of nutrition for the plants. The porous internal structure provides a protective shelter for the inoculum while excluding predators. Biochar may also absorb nutrient from root exudates thus can provide additional support for inoculum growth after its introduction into the soil (Zimmerman et al. 2011).

25.5.2.2 Polymer Entrapped Inoculants Formulation

Diverse polymer-based formulations have been developed and are being used in inoculant industry. They have exhibited potential as viable carriers for microbial inoculants (Jung et al. 1982) that offered superior results over peat. They encapsulate and protect microorganisms against environmental stresses and slowly release them into soil, where later on polymers are degraded by soil microorganisms. They offer prolonged shelf life with consistent batch quality and amiable atmosphere for the bacteria which can be manipulated easily according to the requirement of specific bacteria. However, a major constraint about polymers is that they require more processing by the industry making them expensive compared to peat-based inoculants (Fages 1992).

Alginate is a commonly used polymer for encapsulation of microorganisms and is naturally occurring, composed of β-1, 4-linked D-mannuronic acid, and L-glucuronic acid. It is extracted from many macroalgae and bacteria (Smidsrod and Skjak-Braek 1990). Recently, alginate cost has dropped because of its increased mass production in the Far East, making it potentially more attractive to the inoculant industry. The main advantages associated with alginate-based products are nontoxicity, biodegradability, and their ability to slowly release microorganisms into the soil (Fages 1992; Kitamikado et al. 1990). This technology was used to successfully encapsulate the plant-beneficial bacteria *A. Brasilense* and *P. fluorescens* (Fages 1992) for inoculating wheat plants under field conditions. The bacterial survival in the field and their population in case of alginate were as good as the survival of bacterial population from other carrier-based inoculants (Bashan et al. 1987). The life expectancy of bacteria in the field can be further increased by the addition of clay and skim milk to the alginate beads. Alginate mixed with perlite was used to entrap *Rhizobium*, and it was observed that microbial colonization of cells released from the beads was far better than attained by direct soil inoculation of wheat roots (Hegde and Brahmaprakash 1992).

25.5.2.3 Liquid Inoculants

Liquid inoculants are not as the name suggests that is simple broth culture. Instead, they consist of desired microorganisms and their nutrients along with special cell protectant and additives to promote cell survival in a package and after application to seed or soil. Additives used in the preparation of liquid inoculants have been selected based on their ability to protect bacterial cells in the package and on seeds in extreme conditions such as high temperature, desiccation, and toxic condition of seeds and seed chemicals. Most of the additives are high molecular weight polymers of complex chemical nature which are nontoxic, highly water soluble (Deaker et al. 2004), can limit heat transfer, and possess significant viscoelastic properties (Mugnier and Jung 1985). Some of the polymers which are presently used in the preparation of liquid inoculants include polyvinyl pyrrolidone (PVP), methyl cellulose, trehalose, glycerol, Fe-EDTA, sodium alginate, tapioca flour, etc. (Tittabutr et al. 2007; Singleton et al. 2002). PVP binds to toxic seed exudates mobilized during seed germination. It has a high water-binding capacity which delays drying of inoculants after application. PVP solution tends to coalesce into ridges on their seed

coat as it dries, perhaps providing a thicker layer of protection than some other compounds. Its viscous nature facilitates inoculants adherence to seeds (Singleton et al. 2002). Supplementing growth medium with PVP protected both fast- and slow-growing *Rhizobium* (Bushby and Marshall 1977). Glycerol shows similar properties and protects cells from desiccation. Its flow characteristics appear to promote rapid and even coating on seeds (Mary et al. 1985; Al-Rashidi et al. 1982). Trehalose reportedly improves cell tolerance to desiccation, osmotic, and temperature stress. It is a compatible osmolyte thus helps in stabilizing both enzymes and cell membranes and readily manufactured by *Bradyrhizobium* under ideal conditions (Lippert and Galinski 1992; Streeter 1985). Vincent et al. (1962) reported the addition of maltose and montmorillonite clay to *Rhizobium* culture could protect it against high temperature and desiccation. Polymers with high solubility are convenient for batch processing of inoculants and make seed application a simpler process for farmers. Liquid *Rhizobium* inoculants containing PVP as an osmoprotectant performed better than lignite-based inoculants resulting in enhanced shelf life, nodulation, and nitrogen fixation in cowpea (Girisha et al. 2006).

25.5.2.4 The Use of Nanotechnology in Bioformulation Development

Nanotechnology is an emerging field, with lots of potential application in the area of agriculture especially in the preparation of nanocides and nanomaterial-assisted fertilizers which are capable of controlled release in soil. Nano-formulations show the better stability of living microbial cells in the field (Kim et al. 2006; Bailey et al. 2010; Ghormade et al. 2011). Usually, bioformulations can be costly because large volumes are required for optimum performance in agricultural fields. However, with the help of nanotechnology, large surface area is available with much smaller volume thus increasing the concentration of the preparation and reducing the consumer cost (Ghormade et al. 2011). Due to smaller particle size, nanomaterial-based formulations can efficiently overcome the limitations like susceptibility to desiccation, heat, UV radiation, and fluctuating environmental conditions that are faced in application, delivery, and storage of ordinary bioformulations resulting in highly stable, effective, and eco-friendly pest management practices which can beat the existing pesticide industry (Sasson et al. 2007).

25.6 Status of Bioinoculants Production Around the World

According to a report by markets and markets (http://www.marketsandmarkets.com/Publishing Date: October 2016) titled "Biofertilizers Market by Type (Nitrogen-Fixing, Phosphate-Solubilizing, Potash-Mobilizing), Microorganism (*Rhizobium*, *Azotobacter*, *Azospirillum*, Cyanobacteria, P-Solubilizer), Mode of Application, Crop Type, Form, and Region – Global Forecast to 2022," the market value of biofertilizers was estimated USD 946.6 million in 2015; and its projected compound annual growth rate (CAGR) is 14.08% during the forecast period, to reach a value of USD 2.31 billion by 2022.

García-Fraile et al. (2015) reviewed the current scenario of biofertilizer application across the globe. In the USA apart from the application of rhizobial strains in legume crops, farmers avoid the use of biofertilizers in other major crops like wheat, corn, soybean, cotton, and forage crops as these are relatively low-value products. Nevertheless, several companies are placing some microbial-based fertility products on the markets, which are raising acceptance. Studies carried out by the manufacturer and the University of Minnesota in 2010 indicate increase in corn and soybean production in fields supplied with these products. In Canada, there are more than 150 microbe-based biofertilizers with contents ranging between 10^6 and 10^9 CFU per gram. Here also the most accepted biofertilizers are rhizobial strains for legume crops. Other microbes which are being commercialized here are *B. subtilis*, *Bradyrhizobium japonicum*, *Delftia acidovorans*, and *Lactobacillus helveticus*. According to the studies carried out by the International Plant Nutrition Institute, consumption of biofertilizers is around 60,000–70,000 tons per year in Brazil. It was also revealed that in the South American cone (Argentina, Paraguay, Bolivia, and Uruguay), more than 30 million hectares of soybean crops are sown every year, and, of those, more than 70% are inoculated with *Bradyrhizobium* sp. Moreover, *Azospirillum* and *Pseudomonas* are being used in plantations of wheat and maize. Europe is one of the regions of the planet which fully support the expansion of biofertilizer market. Economy reports estimate that in Europe the biofertilizer market will reach a value of more than 4500 million dollars by 2017. In Asia, the growth of biofertilizer market is determined by government efforts to promote a more sustainable form of agriculture. In China, from 1996 to 2006, the number of registered microbial products had reached 511. It has been estimated that in India the money spent on biofertilizers and biopesticides is around USD 1.5 billion. Organic agriculture in the country occupies a surface greater than 100,000 hectares and is expanding, and decrease in chemical products can already be noted (Sekar et al. 2016). Moreover, there are over 100 biofertilizer producers in the country. Some of the commercial microbial products available in India are summarized in Table 25.2.

25.7 Problems Associated with Microbial Inoculants

Despite being the center of interest for many research centers, agricultural departments, and industrial producer, the use of biofertilizer is limited. Their production faces the challenge of screening and formulation development for the optimum result. On the consumer's side, farmers are not satisfied with the inconsistent quality of biofertilizers leading to a lack of acceptance. Overall the poor performance of biofertilizers can be attributed to inefficient production by selection of strains which are susceptible to adverse environmental conditions, techniques used for sterilization, fermentation, carriers, contamination of the final product due to poor storage and transportation facilities, and last but not the least to the lack of knowledge transfer to the farm producers about the correct way of biofertilizer application.The global acceptance of biofertilizers requires reduction of these gaps between their production and implementation, and only then the expansion of their market can be achieved.

Table 25.2 List of commonly used commercial microbial inoculants in India

Trade name	Microbes	Category	Mechanism	Suitable for
Gmax Phosphomax, KisanPSB, Astha PSB	*Bacillus megaterium*, *Pseudomonas striata*	Phospho-bacteria	Ensures the ability of culture to solubilize insoluble source of phosphates under field conditions	All crops
GmaxTricon, SKS TV	*Trichoderma virid*	Antagonistic fungi	Biocontrol of soil-borne plant-pathogenic fungi	All season all crops
Gmax FYTON, Astha PF, SKS PF	*Pseudomonas Fluorescens*	Antagonistic bacteria	Biocontrol agent against various fungal and bacterial diseases such as *Pythium* spp., *Phytophtora* spp., *Rhizoctonia solani*, *Fusarium* spp., etc	Tomatoes, chili, cut flowers, orchards, vineyards ornamentals, potato, cucumbers, and eggplant
GmaxSugarmax	*Gluconacetobacter diazotrophicus*	Diazotrophs	Nitrogen fixation	Sugar-containing plants like sugarcane, sweet sorghum and not suitable for other crops
GmaxNitromax	Combined product of *Azospirillum* and *Azotobacter*	Nitrogen fixer	Nitrogen fixation	All crops
Kisan Azotobacter, Astha azo, Sanjivini- N2, Nitrofix, BIO N MORE	*Azotobacter*	Nitrogen fixer	Nitrogen fixation	All crops
Rhizobia, Sanjivini-N1,Astharhizo	*Rhizobium* sp.	Nitrogen fixer	Symbiotic nitrogen fixation	Legumes

(continued)

Table 25.2 (continued)

Trade name	Microbes	Category	Mechanism	Suitable for
UPAJ- K, eco-potash	*Bacillus mucilaginosus*	Potassium-solubilizing bacteria	Mobilize the available potash in the soil and make it available to the root zone/plant system,	All crops
UPAJ- Z, BioZinc, zinc-cure	*Bacillus* spp., *Pseudomonas* spp., *Xanthomonas* spp.	Zinc-solubilizing bacteria (ZSB)	Organic acids (e.g., gluconic acid) secreted by ZSB solubilize the unavailable Zn and make it available to crops	All crops
Agri VAM, bio e rich	*Glomus* sp.	Arbuscular mycorrhiza	Phosphate mobilization	All crops
SKS VL	*Verticillium lecanii*	Entomopathogenic fungi	Checks spread of green scales, aphid, whiteflies, thrips, mealy bugs, and red spider mite	All crops
SKS BB	*Beauveria bassiana*, *B. brongniartii*	Entomopathogenic fungi	Effective against several lepidopteron crop pests	Sugarcane, sweet potato, groundnut, coconut, plantation crops
SKS MA	*Metarhizium anisopliae*	Entomopathogenic fungi	Control of grubs of rhinoceros beetle, sugarcane root grubs and pyrilla, rice leaf folder, termites, mango hoppers, and *Helicoverpa armigera*	Sugarcane, rice, mango

25.8 Conclusion

Biofertilizer development needs a consolidated approach from research centers all across the country. Presently, very few strains of *Rhizobium*, *Azotobacter*, and *Azospirillum* are being commercialized. It is necessary that along with improving the already known strains by genetic manipulation techniques, new and superior strains get recognized. Selection of inappropriate carrier is a major limitation for development of carriers as the quality and shelf life of biofertilizers depend on its effectiveness. Further, technologies suitable for large-scale production of biofertilizers are also not up to the mark and need attention. While promotional efforts are necessary, their success can only be assured by the availability of biofertilizers of high and consistent quality. A system by which the quality is monitored by the central and state level authorities may be devised and enforced.

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Microbe-Mediated Biotic Stress Management in Plants

26

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Abstract

Biotic stress factors have a major impact on plants and cause extensive losses to crop production. Plants possess a range of defenses that can be actively expressed in response to pathogens. The timely activation of these defense responses is important and determines whether plant is able to cope or succumb to the challenge of a pathogen. Plant defense mechanisms which are involved in biotic stress management are classified as innate and induced plant response. Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two forms of induced resistance; in both types of resistance, prior infection or treatment preconditions plant defenses leading to resistance (or tolerance) against further challenge by a pathogen. Identification of a number of biological and chemical elicitors has to a great extent helped in unraveling the understanding of the biochemical and physiological basis of ISR and SAR. Combining SAR and ISR can provide protection against a number of pathogens including the pathogens that resist through both pathways. The use of pesticides for the control of crop diseases and pests is however inefficient and not eco-friendly. Genetic engineering has enabled the cloning of genes and their insertion into the crop plants to make them resistant to different biotic stresses.

Keywords

Innate resistance • Acquired resistance • Biotic stress

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26.1 Introduction

Can we envision a place on Earth where an organism remains free from infectious disease and is unlikely to become infected even in presence of pathogens? Natural suppressive soils are one such habitat. In these soils, fungi, bacteria, and nematodes are not able to infect the roots of crop plants. Activation of myriad defense mechanisms which work in coordination imparts resistance to a disease. Infected plants exhibit specific symptoms, including blight, rotting, wilting, vein clearing, leaf chlorosis, and water-soaked leaf lesions that are due to the pathogen which produces mycelium and toxins (Knogge 1996; Dong et al. 2014).

Numerous biotic stresses such as the potato blight in Ireland, coffee rust in Brazil, maize leaf blight (*Cochliobolus heterostrophus*) in the United States, and the great Bengal famine in 1943 are of historical importance (Hussain 2015). These diseases entirely destroyed the crops leading to millions of human deaths and migration to other countries in the past.

Crop production is further threatened by new pathogen races and insect biotypes (Sanghera et al. 2011). Globally about 15% losses in food production are caused by pathogens and pose a major challenge in breeding disease-resistant crops. Genetic polymorphism in pathogens and insect populations is influenced/modified by climatic factors resulting in the emergence of virulent strains or biotypes (Anderson et al. 2004) that are likely to change the outcome of host-pathogen interaction. Thus, food production losses are likely to continue to be caused by disease or insect pest outbreaks or the pests and pathogens may spread to the areas where they were not present before (Ijaz and Khan 2012).

26.2 Plant Defense Mechanisms

Plants respond to pathogens through dynamic and intricate and defense system. The mechanism of defense has been classified as innate and systemic plant response. Figure 26.1 represents an overview of plant defense responses. Plant exhibits an innate defense in two ways, viz., specific (cultivar/pathogen race specific) and nonspecific (nonhost or general resistance) (Monaghan and Zipfel 2012). The molecular mechanism basis of nonhost resistance is not yet fully clear. It is probably based on constitutive barriers and inducible responses that comprise different proteins and other organic molecules produced before infection or during pathogen attack (Kiraly et al. 2007; Jones and Dangl 2006).

Constitutive defenses comprise morphological and structural barriers (epidermis layer, trichomes, thorns, cell walls, etc.), chemical compounds (phenolics, nitrogen compounds, metabolites, glucosinolates, steroids terpenoids, and saponins), and enzymes and proteins (Ferreira et al. 2007; Dahal et al. 2009). These compounds make the plants tolerant or resistant to biotic stresses, protect them from invasion, and also provide rigidity and strength. Inducible defenses such as toxic chemical production, pathogen-degrading enzymes, e.g., chitinases and glucanases, and deliberate cell suicide are sparingly used by plants. Their production and

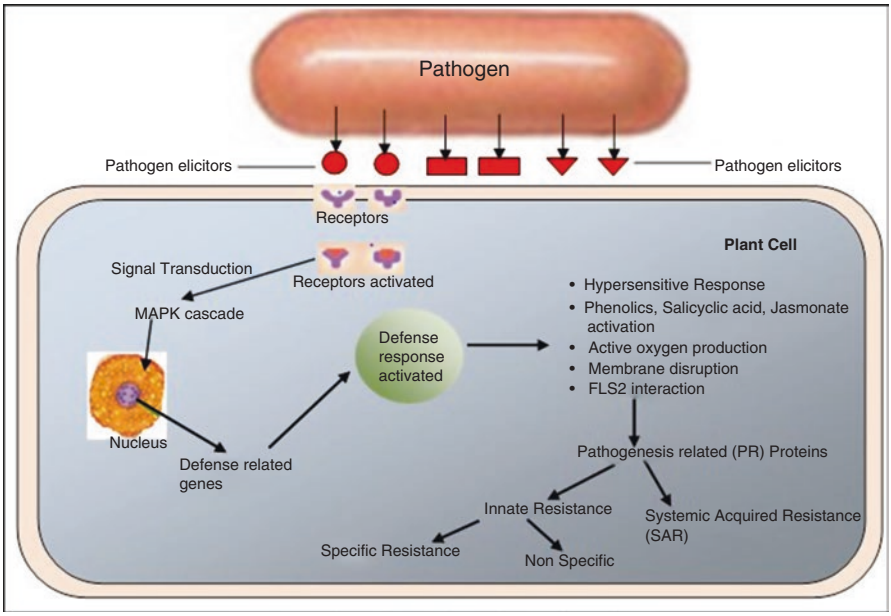

Fig. 26.1 Overview of cellular mechanisms of biotic stress response leading to innate immunity and systemic acquired resistance

maintenance involve high energy and nutrient requirements. These compounds may exist in their biologically active forms or stored as inactive precursors. These are converted to their active forms in response to tissue damage or pathogen attack (Onaga and Wydra 2016) (Fig. 26.1).

26.3 Innate Immunity

Innate immunity in plants is of two types, namely, effector-triggered immunity (ETI) and microbial-associated molecular-pattern-triggered immunity (MTI; also called PTI). Callose deposition induction, reactive oxygen species production, salicylic acid (SA) accumulation, and expression of pathogenesis-related (PR) genes take place in PAMP-triggered immunity (PTI) (Yang and Huang 2014). However, successful pathogens produce protein effectors to suppress PTI, leading to effector-triggered susceptibility (ETS) (Dou and Zhou 2012; Feng and Zhou 2012). To counter the pathogen, plants have evolved a secondary immune response, called as effector-triggered immunity (ETI). Resistance (R) proteins trigger ETI, and these proteins can recognize specific pathogen effectors and suppress them. R proteins trigger hypersensitive response (HR). Death of cells at infection site to limit pathogen growth is mediated by HR (Huang et al. 2016).

Microbe−/pathogen-associated molecular patterns (MAMPs/PAMPs) are molecular signatures typical of whole classes of microbes. The recognition of these

signatures plays a key role in innate immunity. Fungal chitin, xylanase or bacterial flagellin, lipopolysaccharides, and peptidoglycans are examples of PAMP. Damage-associated molecular patterns (DAMPs) respond to a compromised "self" and are recognized as endogenous elicitors (Boller and Felix 2009), and the other that responds to a compromised "self" (Malinovsky et al. 2014) is recognized by plants (Zvereva and Pooggin 2012). Transmembrane pattern recognition receptors (PRRs) are involved in PAMPs and DAMPs recognition (Onaga and Wydra 2016).

26.3.1 Pathogen or Microbe-Associated Molecular-Pattern (PAMP/MAMP)-Triggered Immunity (PTI)

Plants fulfill particular needs of many microorganisms. Communication between plants and microbes takes place by using different signaling molecules during their interaction. Many microbes can be harmful to plants affecting growth and survival. Plants recognize certain compounds released by microbes and mount inducible defense PTI (formerly called horizontal or basal disease resistance). PTI is the first line of active plant defense and plays a role in plant-microbe interactions (Schwessinger and Zipfel 2008). In PTI recognition of conserved, microbial elicitors known as PAMPs are recognized by PRRs. PRRs are membrane-bound extracellular receptors of either the receptor-like proteins (RLPs) or receptor-like kinase (RLK) families. RLPs have a resemblance with the extracellular domains of RLKs but lack the cytosolic signaling domain, whereas RLKs possess both extracellular and intracellular kinase domains (Nurnberger and Kemmerling 2009).

Plants recognize pathogens directly or indirectly. In direct recognition, plants can detect extracellular molecules referred to as pathogen-associated molecular patterns or microbe-associated molecular patterns (PAMPs/MAMPs), e.g., bacterial flagellin, Ef-TU proteins, lipopolysaccharides, and peptidoglycans (Boller and Felix 2009; Freeman and Beattie 2008), and/or intracellular effector proteins, e.g., Avr3a, Avrk, and Avra10 proteins or tissue damage using pattern recognition receptor (PRR) proteins located on the cell surface or intracellularly (Allen et al. 2004; Rivas and Thomas 2005; Boller and Felix 2009). Some of the examples of PTI that have been shown to contribute to resistance in plants are mentioned in the following section.

26.3.1.1 Flagellin-Induced Resistance

Bacterial flagellum is composed of flagellin and is so far the best characterized PAMP in plants. The N-terminal part of the flagellin of *Pseudomonas syringae* has 22 amino acid (flg22) peptide-spanning region in the N-terminal part. This region elicits typical immune responses in a broad variety of plants (Felix et al. 1999). Flagellin perception in the model plant *Arabidopsis thaliana* is due to the leucine-rich repeat receptor-like kinase (LRR-RLK) FLAGELLIN-SENSING 2 (FLS2) PRR. In some species of plants, flagellin appears to be recognized by other means. For example, in rice, the PRR activation is not allowed by flg22 epitope, but flagellin causes cell death (Takai et al. 2008). The glycosylation status of flagellin

proteins determines adapted and nonadapted bacteria by Solanaceae plants, such as tobacco and tomato (Takeuchi et al. 2003; Taguchi et al. 2006). Another flagellin, flgII-28, has been identified in Solanaceae (Cai et al. 2011), though the corresponding PRR is yet to be identified. A stretch of 33 amino acid residues physically links both flg22 and flgII-28, an indication that detection of both molecules is brought about by the same receptor, FLS2 (Clarke et al. 2013).

26.3.1.2 Elongation Factor Tu (EF-Tu)-Induced Resistance

Elongation factor Tu (EF-Tu) is the most prevalent bacterial protein. It was first isolated from *Escherichia coli*. It plays the role of PAMP in Brassicaceae family including *A. thaliana* (Kunze et al. 2004). Defense responses in plants are triggered by the conserved N-acetylated epitope elf18 (first 18 amino acids of the protein). As an elicitor the shorter peptide, elf12 (first 12 N-terminal amino acids), comprising the acetyl group is inactive but acts as a specific antagonist for EF-Tu-related elicitors. EF-Tu is recognized by the LRR-RLK EF-TU RECEPTOR (EFR) of the same subfamily (LRRXII) as FLS2 (Zipfel et al. 2006).

26.3.1.3 Plant Perception of PAMPs from Fungi and Oomycetes

The major constituent of fungal cell walls is chitin which is a homopolymer of (1,4)-linked N-acetylglucosamine (GlcNAc) unit and is a classical PAMP (Dodds and Rathjen 2010). Plants do not have glucosamine polymers; therefore, during plant defense responses, chitin becomes an ideal point of attack. Breakdown of microbial chitin polymers by plant chitinases (hydrolytic enzymes) takes place when pathogen comes in contact with the host. Different plants employ mechanisms that have common factors to perceive chitin, and this could be the possible reason for the evolution of pathogen countermeasures, e.g., in the fungal pathogen *Cladosporium fulvum*, a biotroph (Jashni et al. 2015). In this regard, the reaction of tomato with the induction of defense-related, signal transduction and transcription genes to external chitin application supports the role of the described mechanisms (Kiirika et al. 2013).

The lysine motif (LysM)-RLP was the first chitin-binding PRR that was identified in rice and was named chitin elicitor-binding protein (CEBiP) (Shimizu et al. 2010). CEBiP is a glycoprotein that is localized in the plasma membrane. After binding with chitin, CEBiP homodimerizes, and there is formation of a hetero-oligomeric complex with the chitin elicitor receptor kinase 1 (OsCERK1), the rice ortholog of Arabidopsis AtCERK1. A sandwich-type receptor system for chitin is formed due to binding (Hayafune et al. 2014). The mechanism of perception, however, varies between plant species.

26.3.2 Infection Self-Perception DAMPs

Damage-associated molecular patterns are self-molecules of plants. Plants can sense these molecules, and they are available for recognition only after cell/tissue damage. DAMP perception in plants bears striking similarities to DAMP perception in animals, and these similarities have been reviewed (Lotze et al. 2007). A perfect

example is the *Arabidopsis* plasma membrane LRR receptor kinase (LRR-RK), designated *PEPR1/PEPR2*, which perceives *AtPep* peptides derived from propeptide (ProPEPs) encoded by a seven-member multigenic family (*Pep1-Pep7*). It has been reported that treatment with methyl jasmonate, Pep peptides, wounding, and pathogen-associated molecular patterns cause transcriptional induction of both PEPR1 and PEPR2 (Yamaguchi et al. 2010; Gaulin et al. 2006). Cell wall components derived from the enzymatic activity of highly specific microbial homogalacturonan (HGA) is another good example of DAMPs (Liu et al. 2014a, b). The enhanced production of oligogalacturonic acid (OGA) fragments from plant cell walls potentially acts as DAMP, which are perceived by receptors such as *RLK THESEUS1 (THE1)*, *ER*, and *WAK1*. Thus, a good approach to understanding and having a strategy to improve plant protection is to study the expression of endogenous molecules and microbial cell wall-degrading enzymes and their inhibitors, e.g., polygalacturonases (PGs) and polygalacturonase-inhibiting proteins (PGIPs) (Schacht et al. 2011).

26.4 Inducible Defense (IR, SAR, and ISR)

Plants have the ability to induce both local and systemic resistance to subsequent attack by the same or different pathogens (Walters et al. 2005; Hammerschmidt 2007). This induced resistance (IR) may control the pathogens or damaging factors, completely or partially (Kuc 1982; Chen et al. 2014). Studies have revealed that genes expressed during IR responses produce proteins with chitinase, glucanase, and other enzymatic activities that are involved in defense reactions to a wide array of pathogens (Van Loon et al. 2006). There are different ways to manage the activation of defense mechanism in the plant. The two common ways are called induced systemic resistance (ISR) and systemic acquired resistance (SAR) (Pieterse et al. 2012).

Induced systemic resistance (ISR) and systemic acquired resistance (SAR) are different phenomena, but they are active plant defense responses to plant-pathogen attack. ISR is akin to hypersensitive response, while SAR is alike inherent immunity of plant system. Ross in Ross 1961 coined the terms while working on interactions between tobacco and its mosaic virus (TMV). Nonpathogenic plant growth-promoting rhizobacteria (PGPR) cause induction of ISR. However, the trigger for SAR is infection of a pathogen.

26.4.1 Systemic Acquired Resistance and Induced Systemic Resistance

Induced resistance refers to a state of "enhanced defensive capacity" developed in a plant due to environmental stimuli, whereby the plant's innate defenses are activated against subsequent biotic challenges. This resistance is effective against a large number of pathogens and parasites including fungi, bacteria, viruses,

nematodes, parasitic plants, and even insect herbivores (Benhamou and Nicole 1999; Hammerschmidt and Kuc 1995; Kessler and Baldwin 2002; McDowell and Dangl 2000; Sticher et al. 1997; Van Loon et al. 1998; Walling 2000; Vallad and Goodman 2004). The systemic acquired resistance (SAR) and induced systemic resistance (ISR) are the two types of induced resistance. There differentiation is done on the basis of regulatory pathways involved and the nature of the elicitor as demonstrated in model plant system (Knoester et al. 1999; Maleck et al. 2000; Pieterse et al. 1996, 1998; Schenk et al. 2000; Uknes et al. 1992; Ward et al. 1991, Yan et al. 2002).

Plant exposure to virulent, avirulent, and nonpathogenic microbes can trigger SAR. Pathogenesis-related proteins (chitinase and glucanase) and salicylic acid are accumulated in SAR, and the time required for this accumulation depends on the plant and elicitors. ISR is potentiated by plant growth-promoting rhizobacteria (PGPR), such as strains belonging to genus *Pseudomonas* that cause no apparent damage to the plant's root system (Van Loon and Glick 2004). Unlike SAR, ISR does not involve the accumulation of salicylic acid or pathogenesis-related proteins but jasmonate and ethylene signaling molecules (Fig. 26.2) (Pieterse et al. 2002; Yan et al. 2002).

26.4.2 Mechanisms Involved in Systemic Acquired Resistance (SAR)

26.4.2.1 Mechanical Plant Defense Mechanisms

The pathogen invades the host by penetration directly through the cell wall and intrusion through natural opening such as stomata. Therefore, epidermal cell walls quality and stomatal structure may be enumerated as hindering to infection. The wax or hairs add indirectly in resistance to penetration. The entrance of pathogen may directly be impeded due to thickness or toughness of cell walls. Epidermal membrane is a mechanical barrier to pathogen attack. There is static resistance to spread which is present before occurrence of infection and dynamic defense reactions that come into play after infection occurs (Akai 2012).

Epidermal structures like stomata and trichomes are produced by the first layer of the epidermis. Epidermal cell progenies are not pushed into the core of the plant tissue as a result of divisions. Hence, there is preservation of any changes due to mutations, and such changes are passed down to the cell lineage that constitutes the plant's outer skin. The quick response of stomata to environmental changes is in terms of reduction in their dimensions and areas (Mehri et al. 2009; Çelik et al. 2014). Many abiotic and biotic factors regulate stomatal movements, including radiation and the plant hormone ethylene (Jansen and Van Den Noort 2000; Acharya and Assmann 2009; Wilkinson and Davies 2010). There are studies that show that abiotic stress treatments do not affect stomatal density (Rodiyati et al. 2004; Inamullah and Isoda 2005).

Zeng et al. (2010) reported that stoma is a main route for pathogen invasion, and closure of stomata seems to be part of an immune response of plant. Fungal toxins

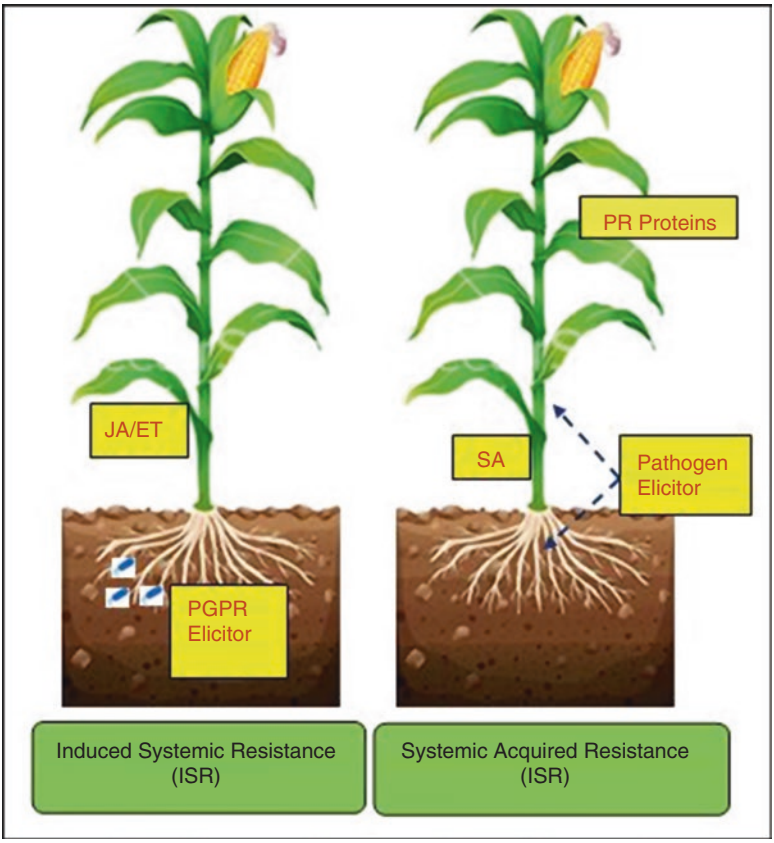

Fig. 26.2 Two forms of induced defense in plants. Systemic acquired resistance (*SAR*), induced by exposure of below- or aboveground tissues to biotic or abiotic elicitors, dependent on salicylic acid (*SA*) signaling and resulting in accumulation of pathogenesis-related proteins (*PR* proteins). Induced systemic resistance (*ISR*), induced (primed) by exposure of roots to plant growth-promoting rhizobacteria (*PGPR*) and dependent on jasmonic acid (*JA*) and ethylene (*ET*) signaling

cause decrease in stomatal conductance resulting in strong stomatal closure in plants (Dehgahi et al. 2015a, b). Initial stomatal closure can possibly be induced by some defense-related phenomena (Blatt et al. 1999; Chaerle et al. 2001). A probable mechanism was the selective inhibition of K+ uptake by guard cells. However, some fungal toxins promote stomatal opening primarily by activating an H+−pump in the plasma membrane via stimulation of H+−ATPase, which in turn creates an electrochemical gradient to drive K+ influx into guard cells (Wang et al. 2013).

An important regulatory role in plant-environment interactions is believed to be played by stomata (Pei et al. 2000; Garcıa-Mata and Lamattina 2001; Desikan et al. 2002). Abiotic factors such as water status and solar radiation affect stomata conductance, but plants are also affected by foliar pathogens that penetrate them through stomata. Under light conditions opening in plants is impaired by pathogen

infection, and there is significant reduction in stomatal aperture average width (Melotto et al. 2006). Pathogen infection showed plant reducing transpiration rate which indicated closure of stomata. Opening and closing of stomata are controlled by environmental factors including humidity, light, and CO_2 concentration (Fan et al. 2004; Dehgahi et al. 2015a, b).

26.4.2.2 Biochemical Plant Defense Mechanisms

Effect of Pathogen on Plant Protein Contents

PR proteins are a category of plant proteins which are produced in plants in the event of a pathogen attack. Seventeen families of PR proteins have been discovered and classified as PR-1 to PR-17 (Swarupa et al. 2014). Pathogen identification receptors (PRRs) are the most deliberated recognition proteins. These are cell exterior receptors and resistance genes (R-genes). Some of these proteins are cell-surface receptors, but many of them are cytoplasmic proteins of the nucleotide-binding leucine-rich rado (Swarupa et al. 2014).

Several factors reduce the protein content of a plant under stress. The protein synthesis process is affected by various stresses. Probably the nitrogen meant to be used for protein synthesis is consumed by the infectious agent (Weintraub and Jones 2010; Siddique et al. 2014). Oxidative stress is created and biomolecules are affected. Due to stress, biomolecules undergo conformational changes, oxidation, rupture of covalent bonds, and formation of free radicals such as the hydroxyl and superoxide anion (Variyar et al. 2004). Molecular properties of proteins are modified by free radicals resulting in oxidative modifications of the proteins (Wilkinson and Gould 1996). Stress causes RNA synthesis failure and subsequent protein synthesis collapses (Bajaj 1970). Chemical nature of the protein, its physical state, and the irradiation condition determine these changes (Moon and Song 2001). In particular, the quaternary structure of proteins and their concentration and the oxygen presence seem to determine the effect of stress on protein conformation (Garrison 1987; Kiong et al. 2008). In general, covalent bonds of polypeptide chains are broken due to stress, and this brings abut irreversible changes in conformation of protein at the molecular levels (Kume and Matsuda 1995). Stress accelerates free radical generation in living systems causing oxidative injury. The primary damage due to stress is modification of enzymatic repair processes (Alikamanoğlu et al. 2007). Stress causes altered gene expression, leading to qualitative and quantitative changes in protein content (Corthals et al. 2000). These proteins might be involved in stress situations and are essential to a plant's function and growth (Gygi et al. 1999). Some authors reported increasing concentrations of protein in pathogen-infected plants. Synthesis of several different proteins by the host cell after pathogen infection could be the reason for this (Langham and Glover 2005). A possible reason for the increase in protein concentration could be activation of some genes which confer resistance. The activation of the host defense mechanisms and also pathogen attack mechanisms leads to a higher amount of protein content in infected resistant plants (Agrios 1997; Siddique et al. 2014).

Effect of Pathogen on Plant Antioxidants Including Peroxidase Activity

Identification of plants resistant to diseases can be carried out by detecting changes in metabolites produced by plants and changes in enzyme activities once they are exposed to any stressor (Krishna et al. 2013). For instance, research has demonstrated that antioxidative enzymes contribute to conferring resistance to plants in response to biotic stresses (Mittler 2002). Many plants are known to produce little molecular antioxidants, for example, phenolic compounds, ascorbate, glutathione, and tocopherols for cellular protection (Margesin et al. 2007). Antioxidant enzymes are effective and efficient against various oxidative stresses (Mittler 2002; Shohael et al. 2006). Under normal conditions, there is regulation of scavenging process and the production of both enzymes and antioxidants (Yordanova et al. 2004). Oxidative stress occurs when ROS might be in surplus of antioxidant scavenging volume. This happens when plants are faced with biotic and abiotic stresses. Plants have evolved a cellular strategy that involves activation of various enzymatic antioxidants to combat against pathogen toxicity. There is an enzymatic system which operates according to the sequential and simultaneous actions of a number of enzymes including peroxidase (Kovacs and Keresztes 2002). Antioxidant system modulation could reflect a defense response to the cellular damage provoked by pathogen toxins (Singh and Upadhyay 2014). Plant-pathogen interactions are affected by peroxidase, and it interferes with growth of plant cells (Passardi et al. 2004). Peroxidase in the plants is affected by special in vitro conditions including limited space, metabolic waste products, limited exchange of gases, and the medium nutritive substances content (Svábová et al. 2011). Indeed, peroxidase plays a key protective role against oxidative stress and is the primary indicator of cellular damage (Hameed et al. 2008).

26.4.2.3 Effect of Pathogen on Plant Photosynthesis

Photosynthesis comprising of the "dark" and "light" reactions is a primary metabolic pathway in plants (Benson and Bremner 2004). The "light" reaction occurs when photosynthetic pigments (chlorophylls a and b) capture light energy and transfer it through an electron transport chain that generates energy in the form of adenosine triphosphate (ATP) and decreasing the power through it in the form of diminished NADPH (Stephenson 2011). Infection by species of *Fusarium* adversely affects light as well as dark reaction of photosynthesis (Ayres et al. 1996; Pshibytko et al. 2006). Necrosis and leaf wilting were observed due to reduction in the chlorophyll content. Concentration of chlorophyll was higher than chlorophyll b in untreated plants. However, fungal-attacked plants showed higher concentrations of chlorophyll b compared to chlorophyll a (Dehgahi et al. 2015a, b). Infectious agent also consumes fixed carbon which could have been used for plant growth (Ayres et al. 1996). A drop in the uptake of minerals (e.g., magnesium) required for chlorophyll synthesis will indirectly reduce chlorophyll content in pathogen-infected plants and interfere with the photosynthesis reaction (Giri and Mukerji 2004; Murkute et al. 2006; Sheng et al. 2008). Activity of enzymes involved in carbon assimilation including ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) determines the efficiency of photosynthetic activity (Matsumoto et al. 2005; Ruiz-Lozano et al. 2012) which may be damaged by pathogen infection (Dehgahi et al. 2015a, b).

26.4.2.4 Effect of Pathogen on Plant Cell Structure

Inoculation with fungal toxin or culture filtrate causes plant cells to appear abnormal, shrunk, and irregular with broken cell walls compared to untreated plant cells which displayed intact cells with uniform polyhedral shapes and small nucleus and lesser cytoplasm density (Yao et al. 2001; Dehgahi et al. 2014). Fungal-attacked cells showed symptoms of plasmolysis, denser cytoplasm density, shrinkage, and cell wall rupture.

Plant cells attacked by fungi show the presence of storage materials which may contain protein and starch reserves around the nucleus (Pearce 2001; Das et al. 2008). The plant cells are ruptured, and there is spillage of cytoplasmic components into the intercellular space. As a result of fungal toxin dose increase, there is more accumulation of mass homogenous cell population and denser cytoplasm in treated cells as compared to cells that are untreated (Dehgahi et al. 2015a, b).

26.4.2.5 Effect of Pathogen on Plant Cell Organelles

In fungal toxin-treated plant cells, the chloroplasts, mitochondria, vacuoles, cell walls, and plasma membranes structure appear damaged. Meanwhile, untreated control plant cells had intact cell walls and undamaged organelles (Wang et al. 2014; Dehgahi et al. 2015a, b). Fungal-infected cells showed damaged plasma membrane and distorted chloroplasts. Fungal attack makes the chloroplast outer membrane to swell and finally rupture (Dehgahi et al. 2014) and caused shrinkage or damage of the cell membrane, condensation of the cytoplasm, and fragmentation of the nucleus. In fungal-infected plants, cytoplasm was disarranged, mitochondria had diffuse central zone, and plastids were disordered. The plant cells attacked with fungi had swollen chloroplasts and had plastoglobuli on them. Larger plastoglobuli were found in the stromal regions. Starch grains were also prominent in chloroplasts following high fungal inoculation. There is separation of plasma membrane from the cell wall, and numerous small vacuoles are formed in the cytoplasm of the fungal-attacked cells. Cell death is caused due to increase of vacuole number and later clearance of cytoplasm (Jiao et al. 2013).

26.5 New Approaches to Overcome Biotic Stress in Plants by Microbial Interactions

With the burgeoning global population and the erosion of agricultural land, it becomes important to look for ways to improve food production. Bacteria, fungi, oomycetes, viruses, and insect pests pose threats to food growth and transport. Globally, pre- and post-harvest losses in crops are to the tune of 30% (Oerke 2006; Flood 2010; Bebber and Gurr 2015). Pathogens have arsenal of effectors to induce effector-triggered susceptibility (ETS), while plants have evolved new resistance (R) proteins to recognize the new effectors. This interplay of defense and counter-defense between pathogen and host has resulted in different types of pathogen effectors and resistance genes (Tsuda and Katagiri 2010; Liu et al. 2014a, b; Bigeard et al. 2015; Huang et al. 2016).

26.5.1 The Role of Plant Growth-Promoting Bacteria (PGPBs)

Plants are able to acquire induced systemic resistance (ISR) to pathogens after inoculation with PGPBs. PGPBs, in association with plant roots, can prime the innate immune system of and confer resistance to a wide array of pathogens with a minimal impact on yield and growth (Van Hulten et al. 2006; Zelicourt et al. 2013). The plant hormones salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) mediate a signaling network resulting in molecular recognition between the plant and microbe.

Several PGPBs, including *Acinetobacter lwoffii*, *Azospirillum brasilense*, *Bacillus pumilus*, *Chryseobacterium balustinum*, *Paenibacillus alvei*, *Pseudomonas putida*, *Pseudomonas fluorescens*, and *Serratia marcescens* colonize roots and provide protection to different plant species of crops including vegetables and trees against foliar diseases field and greenhouse trials (Van Loon 2007).

26.5.2 The Role of Beneficial Fungus in Plant Defense

Besides the classic mycorrhizal fungi, many other fungi such as *Trichoderma* spp. and *Piriformospora indica* suppress plant diseases leading to plant growth stimulation (Van Wees et al. 2008). These microorganisms are able to form endophytic associations and interact with other microbes in the rhizosphere, thereby influencing disease protection, plant growth, and yield.

For example *Trichoderma* genomes have revealed mycotrophy and mycoparasitism as ancestral lifestyles of species of this genus. Some *Trichoderma* strains have become established in the plant rhizosphere and evolved as intercellular root colonizers. As a result, they stimulate plant growth and defenses against pathogens. Like other beneficial microbes, *Trichoderma* elicits ISR by JA/ET-dependent pathways and triggers priming responses in the plant. The *Trichoderma*-plant cross talk is a dynamic process. There may be overlapping of expression of defense-related genes of the JA/ET and/or SA pathways. This depends on the *Trichoderma* strains, their concentrations, the plant material, the stage of the plant, and the timing of the interaction. The phytohormones ET and IAA produced by *Trichoderma* play roles in interconnecting plant development and defense responses. The expression of *Trichoderma* genes in plants has beneficial results, mainly in the control of plant diseases and resistance to adverse environmental conditions (Hermosa et al. 2012).

Piriformospora indica confers disease resistance systemically. *P. indica* colonizes the roots of many plant species and stimulates growth, biomass, and seed production of the hosts. The host colonization by the fungus stimulates it to produce phosphatidic acid, which triggers the OXI1 pathway (Camehl et al. 2011). This pathway is activated when there is pathogen attack and triggers host defense (Rentel et al. 2004).

26.5.3 RNAi-Mediated Plant Defense

Gene expression in eukaryotes is regulated by 20 to 30 nucleotide (nt)-long noncoding RNA molecules called small RNAs (sRNAs) (Zamore and Haley 2005; Chapman and Carrington 2007). They are differentiated by their biogenesis pathway and precursor structure, and in plants, RNAs are of two types: microRNA (miRNA) and small interfering RNA (siRNA). miRNAs are derived from RNAs with imperfectly base-paired hairpin structures and are usually 21–24 nt long (Chen 2009). The formation of siRNAs takes place from perfectly complementary long double-stranded RNAs (dsRNAs) and may need RNA-dependent RNA polymerases (RDRs) (Bartel 2009; Katiyar-Agarwal and Jin 2010). The subclasses of siRNA present in plants include heterochromatic siRNAs (hc-siRNAs), trans-acting siRNAs (ta-siRNAs), natural antisense transcript-derived siRNAs (nat-siRNAs), and long siRNAs (lsiRNAs). The regulation of gene in pathogens or hosts is induced by sRNAs by post-transcriptional gene silencing (PTGS) or transcriptional gene silencing (TGS). Both miRNAs and siRNAs can induce PTGS by messenger RNA (mRNA) cleavage/degradation or translational inhibition via a RNA-induced silencing complex (RISC), while TGS is usually mediated by siRNAs and some specific miRNAs which results in either DNA methylation, histone modification, or chromatin modification (Baulcombe 1996; Chellappan et al. 2004; Vaucheret 2006; Wu et al. 2010; Cui and Cao 2014). Different sRNAs can have species-specific and complicated biogenesis pathways, and while there are some common steps, many steps are unique to certain sRNAs.

After plant cell wall penetration, localization of oomycetes, fungi, and bacteria for amplification in the intercellular space takes place. Oomycetes and fungi also enter into the cells in the later infection stages. The host PTI response is activated immediately by entry of these microbes. miRNAs and siRNAs act as key fine-tuning regulators of plant hormones including auxin, abscisic acid (ABA), SA, and jasmonic acid (JA) and are required for PTI (Zhang et al. 2011). Several RNAi strategies have shown success in plant improvement against biotic stresses. The first miRNA identified to be involved in PTI is *Arabidopsis* miR393. miR393 was induced in response to *Pseudomonas syringae* attacked by a flagellin-derived peptide, flg22. Auxin signaling is suppressed by miR393 by negatively regulating mRNAs of auxin receptors, transport inhibitor response 1 (TIR1), AFB2, and AFB3. The plants are allowed to prioritize defense signaling over plant growth, and a series of defense responses are triggered (Navarro et al. 2006). SA provides defense against biotrophic pathogens, while glucosinolates are antimicrobial molecules that contribute to plant defense against pests and diseases.

26.5.4 Transgenic Approach for Crop Designing

Transgenic plants have been produced with genes involved in different pathways to enhance disease resistance against fungal pathogens. For controlling diseases, an apt approach would be expression of pathogenesis-related genes and defensins.

Some proteins, called defensins, are small cysteine-rich peptides and have antimicrobial activity. The transgenic expression of plant defensins protects vegetative tissues against pathogen attack (Sanghera et al. 2011).

Enhanced resistance in tobacco plants against *Rhizoctonia solani* has been shown by the chit1 gene from the entomopathogenic fungus *Metarhizium anisopliae*, encoding the endochitinase Chit42 (Kern et al. 2010). Three genes, ech42, nag70, and gluc78, encoding hydrolytic enzymes from a biocontrol fungus *Trichoderma atroviride* were introduced in rice. Gluc78-overexpressing transgenic plants showed enhanced resistance to *Magnaporthe grisea* (Sanghera et al. 2011) (Table 26.1).

Rizhsky and Mittler (2001) used the *Halobacterium halobium* bacterio-opsin (bO) gene under the control of the wound-inducible promoter Pin2 to develop transgenic tobacco plants resistant to *Pseudomonas syringae* pv. *tabaci* via *Agrobacterium*-mediated transformation.

Bacterio-opsin activates the self-defense mechanisms in plants by enhancing proton pumping across the cell membrane (Mittler et al. 1995). Transgenic tobacco plants produced hypersensitive response (HR) due to expression of the bO gene, and there was enhanced expression of different types of defense-related proteins such as

Table 26.1 Transgenic engineering of crops to enhanced resistance against fungal pathogens

Crop	Gene donor fungus	Target fungal pathogen	Gene/gene product inserted in plant
Tobacco	*Phytophthora cryptogea*	*Phytophthora parasitica*	β-Cryptogein elicitor
Tobacco	*Phytolacca americana*	Broad-spectrum resistance to viral and fungal pathogens	PAPII
Potato	*Trichoderma harzianum*	Foliar and soilborne fungal pathogen	Endochitinase
Carrot	*Pseudomonas fluorescence*	*Alternaria dauci*, *Alternaria radicina*, and *Botrytis cinerea*	Microbial factor 3 (*MF3*)
Tobacco	*Erwinia amylovora*	*Botrytis cinerea*	*hrp N*
Tobacco	*W. japonica*	*B. cinerea*	PR1
Rice	*T. viride*	*R. solani*	PR3
Grape	*Trichoderma harzianum*	*Botrytis cinerea*	Endochitinase
Tobacco	*Baculovirus*	*Alternaria alternate*	Chitinase
Tobacco	*Pseudomonas*	*Colletotrichum destructivum*	Chloroperoxidase
Rice	*Trichoderma atroviride*	*Magnaporthe grisea*	*ech*42, *nag* 70, *gluc*78
Rice	*Streptomyces griseus*	*Magnaporthe grisea*	*Chitinase C* (Chi C)
Apple	*Trichoderma harzianum*	*Venturia inaequalis*	Endochitinase, exochitinase
Pearl millet	*Aspergillus giganteus*	Rust and downy mildew	*Afp*
Rice	Fungal gene	Multiple pathogen	*Glucose oxidase gene*
Rice	*Aspergillus giganteus*	*Magnaporthe grisea*	*AFP*

chitinase, glucanase, and salicylic acid. The transgenic tobacco plants expressing the bO gene, when challenged with *P. syringae* pv. *tabaci*, slowed down the pathogen growth (Sanghera et al. 2011).

26.6 Conclusion

Environmentally friendly strategies such as organic cultivation are necessary for crop production in the future. Methodologies for crop protection in organic productions are scarce throughout the world. Biocontrol is a tool with a potentially broad range of stress control and potential to improve crop production without the negative environmental impact associated with chemical pesticides. Plant resistance to biotic stresses is jointly controlled by the plants' anatomy, physiology, biochemistry, genetics, development, and evolution. A lot of data have been generated on quantitative trait loci (QTLs), candidate genes, proteins, and metabolites associated with plant defenses. Various signaling pathways tracking and regulating the pathogens ingress are involved in complex phenomenon of plant-pathogen interaction. Activation of both innate and systemic acquired resistance are involved in the interactions leading to effective protection and need direct and indirect pathways to quickly limit the entry or spread of biotic agents in the plant. Newer and more effective elicitors of SAR and ISR will surely be developed, perhaps due to our growing understanding of the underlying mechanisms of these pathways within the plant. Application of elicitor cocktails that induce a balance of defenses regulated by salicylic acid, jasmonic acid, ethylene, and other undefined regulators may be possible against specific pests or complexes of threats in future. However, this needs a paradigm shift in conventional agriculture that relies on pesticides to control solve pests and a concerted effort to manage pests as opposed to eliminating them.

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Microbial Interactions and Perspectives for Bioremediation of Pesticides in the Soils

27

Ratna Prabha, D.P. Singh, and M.K. Verma

Abstract

Microbes with uncountable number of species represent the most abundant organisms on earth. Microorganism plays vital role in the pesticide bioremediation. Pesticide biodegradation capacity exhibited by soil microbes is among the major factor limiting contamination and preserving the resilience of soil. Numerous studies are dedicated over bioremediation of pesticides through different microbial species. The biotransformations in natural system is a common process and many times necessary for the survival of microorganisms, leading to biological degradation of applied pesticides. Microbial evolution and bioremediation exhibits a natural balance between them. Bioremediation through microbes reflects numerous benefits, for instance, there is least possibility of environmental disturbance, economical, and lesser likelihood of secondary exposure along with no disturbance to the ecosystem. Owing to these reasons, the isolation and characterization of microbial species with the capability of pesticide bioremediation are gaining attention of scientists from last many years.

The present chapter includes information about different microbial species, including bacteria, cyanobacteria, and fungi employed in the bioremediation of pesticides. Furthermore, an attempt is taken to cover different metagenomics studies where researchers aimed to uncover the bioremediation potential linked with unculturable microbial communities.

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Bioremediation • Bacteria • Cyanobacteria • Fungi • Metagenomics • Pesticides • Biodegradation

27.1 Introduction

Over the last few years, highly toxic organic compounds have been synthesized and applied in the environment for direct or indirect applications. Pesticides, polychlorinated biphenyls (PCBs), chlorophenols, polycyclic aromatic hydrocarbons (PAHs), fuels, and dyes are a few of these chemical compounds (Diez 2010). Certain synthetic chemicals are very much resistant toward biodegradation via native flora (Rochkind-Dubinsky et al. 1987; Chikere 2013) rather than the naturally occurring organic compounds which are easily degraded when exposed to the environment. Thus, hazardous wastes and chemicals are now occupying the status of one of the greatest problems worldwide (Chikere 2013).

Agriculture sector is not untouched with this scenario. From the last few decades, there is development of intensive agricultural practices, and this has led to the emergence of the large-scale agrochemical industries (Burrows et al. 2002). Current agricultural system heavily relies over a large range of synthetic chemicals like insecticides, fungicides, herbicides, etc. Continuous use and secretion of such synthetic chemicals had become regular practice, and it is now leading to the problem of environmental pollution. Though, apart from their complex nature, many pesticides, like herbicide 2,4-dichlorophenoxyacetic acid (2,4-D), are easily degraded by soil and water microbes; other herbicides like 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) are not so easily degraded and survive in soils for longer time (Mwangi et al. 2010; Chauhan and Singh 2015).

Application of pesticides has given enormous benefits to the society via improvement of quantity and quality of world's food production. Thus, the application of pesticides had become an integral component of the current agricultural system. Since the pest pathogen problems are continuously persisting, use of pesticides is indispensable in near future. Large-scale application of these chemicals has led to severe environmental pollution and human health problems. The presence of pesticides in food and water led to many possible chronic effects over human health, e.g., carcinogenesis, neurotoxicity, and effects on reproduction and cell development, specifically in the early stages of life (Burrows et al. 2002; Prüss-Ustün et al. 2011; Damalas and Eleftherohorinos 2011; Myers et al. 2016). Therefore, looking into the perspectives of sustainable agriculture, it now becomes pertinent to put efforts to find out biological solutions for the degradation of pesticides, and microbes are among the most viable option for this purpose.

27.2 Pesticides: An Introduction and Overview

A pesticide may be defined as a substance or mixture of substances applied for killing, preventing, and controlling different pests (any unwanted species of plants or animals) which imposes harmful impact over the production, processing, storage, and even marketing of different agricultural products including food, wood and wood products, or animal feedstuffs (WHO/UNEP 1990). In simple terms, pesticides are the chemical compounds applied against crop pests and disease vectors. Currently, up to two million tons per year of pesticide is used in the world, of which 45% is used by Europe followed by the USA (24%), while the rest of the world occupies only 25% share. In Asia, the pesticide consumption is increasing at an alarming rate. Currently, China is the largest consumer of pesticides in Asia followed by Korea, Japan, and India. In India, pesticide use is approximately ~0.5 kg/hectare, and large contribution is of organochlorine pesticides owing to the warm humid climatic conditions (Kandpal 2014). Pesticides are extremely toxic and stable in nature and possess less soluble active ingredients. These compounds and their degradation products impose severe toxic effects over environment and human health. They move across the atmosphere, soils, and rivers, resulting in the accumulation of toxic substances. In addition, pesticides also cause direct health impacts like poisoning, neurological, cardiopulmonary, fetal deformities, and skin problems, etc. Continuous application of synthetic pesticides leads to the extinction of beneficial soil organisms including microflora. Furthermore, pesticides are also harmful for other advantageous organisms like earthworms, spiders, bees, etc. Certain pesticides like DDT, chlororganic compounds, and endosulfan possess the tendency to accumulate in living tissues and lead to serious diseases to humans. They also exert damaging effect over the soil flora and fauna.

In general, "pesticide" is a common term that covers herbicides, insecticides, fungicides, rodenticides, and wood preservatives that are applied for removal of pests. Different classification are provided for the pesticides, depending on their mode of action, targeted pests, chemical composition, etc. On the basis of mode of action, pesticides are classified as nonsystemic and systemic pesticides. Nonsystemic or contact pesticides do not penetrate plant tissues and thus are not transported within the plant vascular system and carried out the desired effect. They are also named as contact pesticides as they will affect by coming in contact with the targeted pest, e.g., paraquat and diquat dibromide. Systemic pesticides are able to penetrate the plant tissues and move through the plant vascular system for the desired effect, e.g., 2,4-D and glyphosate (Buchel 1983). On the basis of targeted pests, pesticides are termed as insecticides (pyrethroids, organophosphorus, carbamates, etc.), fungicides (thiocarbamates, dithiocarbamates, triazoles, etc.), fumigants (methyl bromide, ethylene dibromide), herbicides (chlorophenoxy, acetanilides, etc.), insect repellents (diethyltoluamide), rodenticides (indandiones, warfarins), etc. (Zacharia and Margarita 2011). On the basis of constituents, pesticides are classified as organochlorine, organophosphate, carbamate, synthetic pyrethroids, and inorganic pesticides (Odukkathil and Vasudevan 2013). Organochlorines are organic compounds with five or more chlorine atoms and exert a long-term residual effect

on the environment owing to their resistance toward most chemical and microbial degradations, e.g., DDT, lindane, endosulfan, aldrin, etc. Organophosphorous (parathion, malathion, diazinon) contains a phosphate group as their basic structural framework and are toxic to vertebrates and invertebrates as cholinesterase inhibitors. Carbamates are organic pesticides derived from carbamic acid; they are cholinesterase inhibitors. Synthetic pyrethroids are synthetic analogs of the naturally occurring pyrethrins, a product of pyrethrum plant flowers. They exhibit fast knocking-down effect against insect pests, low mammalian toxicity, and facile biodegradation; some of the examples are permethrin, cypermethrin, and deltamethrin. Apart from these, pesticides are also classified on the basis of their mode of action (broad and narrow spectrum where broad spectrum is able to kill wide range of pests, while narrow-spectrum pesticides are lethal only to a particular group of pests). The World Health Organization (WHO) has grouped pesticides in four classes on the basis of their potential risks to human health on accidental contact with human being. These classes are class Ia (extremely hazardous), class Ib (highly hazardous), class II (moderately hazardous), class III (slightly hazardous), and class IV (products unlikely to cause acute hazard in normal use).

27.3 Degradation of Pesticides and Bioremediation

Overall, three phases are involved in pesticide degradation (Hatzios 1995; Shimabukuro 1985; Palanisami et al. 2009). In the phase I, oxidation, reduction, or hydrolysis occurs which converts the parent compound into a more water-soluble and less toxic degradation product. Oxygenation is the most common initial step in the biotransformation of pesticides. Maximum of these reactions are carried out by different oxidative enzymes like cytochrome P450s, peroxidase, and polyphenol oxidases. Phase II involves conjugation of a pesticide or pesticide metabolites to a sugar, amino acid, or glutathione, which further increases water solubility and reduces the toxicity than the parent compound. In general, the products/metabolites from phase II possess little or even no toxicity and can be accumulated in cellular organelles. Glutathione S-transferase is the enzyme with key role in phase II and is a homo or heterodimer multifunctional enzyme present in the cytosol (Armstrong 1994; Marrs 1996; Rushmore and Pickett 1993; Palanisami et al. 2009). Ultimately, in the third phase, metabolites from phase II are further converted into nontoxic secondary conjugates (Shimabukuro 1985; Palanisami et al. 2009).

Degradation of pesticides follows diverse metabolic pathway on the basis of the nature of pesticide, environmental circumstances, and nature of microbes. In general, this process involves:

1. Oxidative transformation carried out by oxidative enzymes (cytochrome p450, peroxidases, and polyphenol oxidases).
2. Hydrolytic transformation through hydrolytic (hydrolases) which cleaves bonds of the substrate through addition of hydrogen or hydroxyl group from water molecules.

3. Reductive transformation via reductive enzymes (nitroreductase) which involves removal of anion through reduction.
4. Conjugation reaction in which exogenous or endogenous natural compound is added to pesticide allowing mineralization. This process involves existing enzymes, and thus it is a co-metabolic procedure which includes different reactions like xyloxylation, alkylation, acylation, and nitrosylation. This type of biotransformation happens in fungal biodegradation of pesticides.
5. Reductive dehalogenation occurs by reductive dehydrohalogenase enzyme.

During the process, for ATP production, organohalide is involved as a terminal electron acceptor (Odukkathil and Vasudevan 2013).

Biodegradation can be stated as the biologically catalyzed reduction in complexity of chemicals (Karigar and Rao 2011; Joutey et al. 2013; Kehinde and Isaac 2016). Pesticide degradation in a soil is a combined action of numerous factors comprising population densities and activity of pesticide-degrading microbes, pesticide bioavailability, and soil parameters (e.g., pH, soil water content, and temperature) (Swarcewicz and Gregorczyk 2012; Dechesne et al. 2014). Biotic and abiotic pathways are available for the degradation of pesticides in soil and water, though microbe-mediated degradation is the primary mechanism of pesticide breakdown and detoxification in various soils (Singh and Walker 2006; Akbar and Sultan 2016). Microbes exert significant impact over persistence of maximum pesticides in soils. Pesticide biodegradation capacity owned by soil microbes is a main factor restricting contamination and maintaining the resilience of soil (Javaid et al. 2016). Certain studies were carried out on pesticide-degrading bacteria though much detail is not available regarding metabolic pathways for degradation of these molecules (Ladino-Orjuela et al. 2016). Therefore, isolation of pesticide-degrading microbes and characterization of produced metabolites intermediates are essential for a better knowledge of fate of pesticides in the environment. Continuous efforts are required in this way, and presently a number of bacteria with pesticide-degrading abilities are isolated from the natural environment. Furthermore, catabolic genes accountable for the degradation of numerous xenobiotics, along with pesticides, are recognized, isolated, and cloned into a variety of different organisms like *Streptomyces*, algae, fungi, etc. (Kumar et al. 1996; Chen et al. 2012). Soil microbes specifically the members from the genus *Pseudomonas* exert nutritional versatility and possess the ability of the degradation of a wide variety of complex, naturally occurring aromatic and aliphatic compounds (Seo et al. 2009; Das and Chandran 2011).

27.4 Bioremediation of Pesticides: Importance of Microorganisms

Bioremediation is a promising procedure that employs living organisms for remediation of polluted sites. It is a very eco-friendly and cost-effective approach where biological agents like bacteria, cyanobacteria, or fungi are applied for the degradation or removal of the harmful contaminants from the polluted sites. Bioremediation

means application of microbes to remediate or degrade the immobilize pollutant from environment (Shanahan 2004). Natural bioremediation is in application by civilizations for the treatment of waste water, though, its use in a planned way for removal of hazardous waste is relatively recent development. Modern bioremediation and application of microbes for consumption of pollutants is basically started by George Robinson. He applied microorganisms for removal of oil spill across the coast of Santa Barbara, California, in the Tale 1960 (Uqab et al. 2016). Bioremediation utilizes the efficiency of microbial degradation for providing a cost-effective and reliable method for removal of pesticides and other pollutants from source. Numerous soils and water bodies are successfully remediated from pesticide contamination through the microorganisms capable of degrading the pollutants (Singh and Walker 2006; Akbar and Sultan 2016). For, a successful bioremediation technology, an efficient bacterial strain is required that can degrade largest pollutants to minimum level (Singh 2008).

Microbial communities owe a significant role in complete mineralization or transformation or degradation of pesticides (Alvarez et al. 2012). Actually, combined microbial communities carry out the entire mineralization of any compound, if a readily available carbon and energy source is provided, specifically through a co-metabolic pathway (Hamme et al. 2003; Arora et al. 2015; Leewis et al. 2016). Among the microbial population, bacteria and fungi occupy a major role in the degradation of pesticide and their breakdown products (McGuinness and Dowling 2009; Porto et al. 2011). Numerous microbes are thoroughly studied for their ability to degrade pesticides and associated environmental pollutants. Till now, major research in the area of pesticide degradation by microbes is focused over bacteria; very little is known about other microbes such as fungi, actinomycetes, cyanobacteria, etc. The foremost reasons for this lie in the fact that bacteria are easy to culture with quick growth; thus, they are more adaptable to genetic manipulations, and the probability of mutants reverting back is very little (Kumar et al. 1996; Das and Chandran 2011). Numerous literature focuses over the xenobiotics degradation by microbes. These studies basically have two aims, first to identify the basic understanding of biodegradation activities, its initiation and transfer among other soil microbes. This information provides the basis of the environmental fate of a crude array of compounds. Second aim is to decipher bioremediation strategies for removal or detoxification of dangerously high concentrations of pesticide residues (Kumar et al. 1996; Seo et al. 2009; Sinha et al. 2009; Abdel-Shafy and Mansour 2016).

Isolation and identification of pesticide degrading microbes is significant for three basic reasons, (1) for identification of the mechanism of the intrinsic procedure of microbial metabolism, (2) for a better understanding of the mechanisms of gene/enzyme evolution, and (3) for application of these microorganisms for the detoxification and decontamination of polluted aquatic and terrestrial environments, i.e., for bioremediation purposes. Numerous microbes were isolated with ability to use pesticides as an energy source, e.g., fungi like *Trametes hirsutus*, *Phanerochaete chrysosporium*, *Phanerochaete sordia*, and *Cyathus bulleri* are capable to degrade lindane and other pesticides (Singh and Walker 2006; Singh and Kuhad 1999; Singh and Kuhad 2000; Singh et al. 1999) (Table 27.1).

Table 27.1 Details of different microorganisms with pesticide-degrading capacity

Organisms	Pesticide/herbicide/insecticide	References
Bacteria		
Acinetobacter calcoaceticus	Bifenthrin	Tingting et al. (2012)
Acinetobacter johnsonii (MA-19) strain	Organophosphate pesticides	Xie et al. (2009)
Acinetobacter	Esbiothrin	Ha et al. (2009)
Acidomonas sp.	Allethrin	Paingankar et al. (2005)
Azospirillum and *Pseudomonas*	Ethion	Zhang et al. (2007)
A. xylosoxidans JCp4 and *Ochrobactrum* sp. FCp1	Chlorpyrifos	Akbar and Sultan (2016)
Bacillus thuringiensis	Malathion	Zeinat et al. (2008)
Burkholderia cepacia strain CH-9	Imidacloprid and metribuzin	Madhuban et al. (2011)
Bacillus sp. and *Chryseobacterium joostei*	Lindane, methyl parathion, and carbofuran	Foster et al. (2004)
Enterobacter aerogenes	Bifenthrin, cypermethrin	Lio and Xie (2009)
Escherichia coli	BHC, DDT, endosulfan, HCH isomers, and 2,4-D	Qiao et al. (2003), Gupta (2005), Shun-Peng et al. (2005), Chaudhary et al. (2006), Santacruz et al. (2005), and Xue-Dong et al. (2003)
Ochrobactrum	Triazophos	Shunpeng and Shen (2005)
Photosynthetic bacterium (GJ-22)	Cypermethrin (CMP)	Yin et al. (2012)
Paracoccus sp. strain	Pyridine	Qiao and Wang (2010)
Pseudomonas	Endosulfan, atrazine	Prabakaran and Peterson (2006) and Wyss et al. (2006)
Pseudomonas putida and *Pseudomonas mendocina*	Permethrin and cypermethrin pesticides	Mendoza et al. (2011)
Pseudomonas and *Alcaligenes* sp.	Herbicide 2,4-D, endosulfan, lindane, chlorpyrifos	Mulbry and Kearney (1991), Jayashree and Vasudevan (2007a, b), Gupta et al. (2001), and Yang et al. (2005)
Rhodococcus bacteria	Para-nitrophenol	Zhang et al. (2009)
Rhodobacter sphaeroides	Chlorinated pesticides, herbicides, and fungicides	Harada et al. (2006)
Sphingobium japonicum	Hexachlorocyclohexane	Liu et al. (2007)

(continued)

Table 27.1 (continued)

Organisms	Pesticide/herbicide/insecticide	References
Stenotrophomonas maltophilia	DDT and endosulfan	Barragán-Huerta et al. (2007)
Sphingomonas	DDT	Shunpeng and Mingxing (2006)
Sphingobacterium sp.,	DDT	Fang et al. (2010)
Sphingobium sp. JQL4-5	Fenpropathrin	Yuanfan et al. (2010)
Sphingomonas yanoikuyae	Carbamate and pyrethrin	Ouyang et al. (2008)
Vibrio and *Shewanella*	Methyl parathion	Liu et al. (2006)
Cyanobacteria		
A. oryzae	Malathion	Ibrahim and Essa (2010)
Anabaena PD-1	Polychlorinated biphenyls (PCBs)	Zhang et al. (2015)
Aulosira fertilissima ARM 68 and *Nostoc muscorum* ARM 221	Monocrotophos and malathion	Subramanian et al. (1994)
Anabaena sp. strain PCC 7120 and *Nostoc ellipsosporum*	Lindane (γ-hexachlorocyclohexane)	Kuritz and Wolk (1995)
A. oryzae, *N. muscorum*, and *S. platensis*	Malathion	Ibrahim et al. (2014)
Anabaena sp., *Arthrospira fusiformis*, *Leptolyngbya boryana*, *Microcystis aeruginosa*, *Nostoc punctiforme*, *Spirulina platensis*	Glyphosate	Forlani et al. (2008) and Lipok et al. (2009)
Microcystis novacekii	Methyl parathion	Fioravante et al. (2010)
Nostoc sp. MM1, *Nostoc* sp. MM2, *Nostoc* sp. MM3, *Nostoc* muscorum and *Anabaena* sp.	Fenamiphos	Cáceres et al. (2008)
Phormidium valderianum BDU 20041	Chlorpyrifos	Palanisami et al. (2009)
Synechococcus elongatus, *Anacystis nidulans*, and *Microcystis aeruginosa*	Organophosphorus and organochlorine insecticides	Vijayakumar (2012)
Spirulina sp.	Glyphosate	Lipok et al. (2007, 2009)
Synechocystis sp.	Aniliofos	Singh et al. (2016)
Fungi		
Aspergillus	Endosulfan	Bhalerao and Puranik (2007) and Javaid et al. (2016)
C. elegans	DEET, an insecticide	Seo et al. (2005)

(continued)

Table 27.1 (continued)

Organisms	Pesticide/herbicide/insecticide	References
Fusarium oxysporum, *Lentinula edodes*, *Penicillium brevicompactum*, and *Lecanicillium saksenae*	Terbuthylazine, difenoconazole, and pendimethalin	Hai et al. (2012)
Fusarium verticillioides	Lindane	Guillén-Jiménez et al. (2012) and Pinto et al. (2012)
Mortierella sp. strains W8 and Cm1- 45	Endosulfan	Kataoka et al. (2010)
Trichoderma viride and *T. harzianum*	Pirimicarb	Romeh (2001)
Rot fungi	Methomyl and diazinon	Sagar and Singh (2011)
White-rot fungi	Aldrin, aldicarb, alachlor, atrazine, chlordane, diuron, DDT, dieldrin, gamma-hexachlorocyclohexane (γ-HCH), heptachlor, lindane, mirex, metalaxyl, terbuthylazine	Das and Chandran (2011) and Nyakundi et al. (2011)

In the last few decades, there had been a considerable rise in different studies focusing on bioremediation of pollutants (both organic and inorganic) contaminated soils. Across different organic contaminants, bioremediation of pesticides and polyaromatic hydrocarbons from contaminated soils is of major research interest across the world (Odukkathil and Vasudevan 2013). Majority of these studies focused over the innate capacity of microorganisms and certain plants for detoxification of the environmental pollutants. Though, most studies suggested soil bacteria as the major factor contributing to the improved biodegradation (Singh and Walker 2006; Walker and Roberts 1993). A number of pure bacterial isolates capable of utilizing particular pesticides as a sole source of carbon, nitrogen, or phosphorus were identified (Singh and Kuhad 1999, 2000; Singh and Walker 2006) (Table 27.1). Microbes commonly identified for pesticide bioremediation are *Pseudomonas* sp., *Bacillus* sp., *Klebsiella* sp., *Pandoraea* sp., *Phanerochaete chrysosporium*, and *Mycobacterium* sp. (Odukkathil and Vasudevan 2013). The capability of microorganisms to diminish the xenobiotics levels is directly associated with their long-term adaptation to contaminated environments. Furthermore, genetic engineering can be applied for improvement of the efficiency of microbes with biodegradation or bioremediation properties (Schroll et al. 2004; Porto et al. 2011). *Pseudomonas striata*, *Achromobacter* sp., *Aspergillus ustus*, *Aspergillus versicolor*, *Fusarium oxysporum*, *Fusarium solani*, *Penicillium chrysogenum*, *Penicillium janthinellu*, *Penicillium rugulosum*, and *Trichoderma viride* were evaluated for their ability to degrade different pesticides (Porto et al. 2011).

27.5 Bacteria for Pesticide Biodegradation

Degradation of pesticides led to the release of carbon dioxide (CO_2) and water (H_2O) via oxidation of original compounds. Microorganisms utilize the energy through the degradation of pesticides. Though, efficiency of degradation depends on various parameters like temperature, soil pH, moisture content, etc.; biodegradation of pesticides through microbes has a significant impact over the fertility of agricultural soils. Apart from this, microbes owe other vital advantages also like, diversity, broad distribution, and adaptation of various metabolic pathways. Different advance approaches like genetic manipulation and creation of genetically engineering bacteria have been also employed for degradation of pesticides (Cui et al. 2012).

Most of the bacterial species with pesticide degradation ability are from genera *Flavobacterium*, *Arthrobacter*, *Azotobacter*, *Burkholderia*, and *Pseudomonas*. The kind of degradation varies with species and the target compounds. *Pseudomonas* sp. and *Klebsiella pneumoniae* possess ability of degradation of s-triazine herbicides, e.g., atrazine. *Pseudomonas* and *Alcaligenes* sp. also possess the ability to degrade herbicide 2,4-D (Mulbry and Kearney 1991) organochlorine pesticide like endosulfan (Jayashree and Vasudevan 2007a, b), lindane (Gupta et al. 2001), organophosphorus insecticide chlorpyrifos (Yang et al. 2005), etc. Generally microbial consortia are more efficient compared to single strains for pesticide degradation.

Sphingobium japonicum is another microbial strain identified for the degradation of chlorinated pesticides (hexachlorocyclohexane) (Liu et al. 2007). Another bacteria *Burkholderia cepacia* strain CH-9 is involved in the degradation of imidacloprid and metribuzin (Madhuban et al. 2011). *Acinetobacter calcoaceticus* degraded bifenthrin, i.e., a synthetic pesticide (Tingting et al. 2012). Photosynthetic bacterium (GJ-22) is observed with the capability of degrading cypermethrin (CMP) (Yin et al. 2012). In another study, *Pseudomonas putida* and *Pseudomonas mendocina* show very high ability of permethrin and cypermethrin pesticide biodegradation (Mendoza et al. 2011). *Paracoccus* sp. strain is studied for its ability to degrade pyridine (Qiao and Wang 2010). Strain of *Enterobacter aerogenes* is reported to degrade different pesticides, i.e., bifenthrin, cypermethrin, etc. (Lio and Xie 2009). *Acinetobacter johnsonii* (MA-19) strain is studied for the degradation of organophosphate pesticides (Xie et al. 2009). Zhang et al. (2009) studied efficiency of Rhodococcus bacteria for para-nitrophenol degradation (Zhang et al. 2009). Likewise, different strains of *Bacillus* and L-proteobacteria were evaluated for organophosphate pesticide degradation (Sabdono and Radjasa 2008). *Bacillus thuringiensis* is effective for malathion degradation (Zeinat et al. 2008). *Acinetobacter* degrades esbiothrin (Ha et al. 2009). *Stenotrophomonas maltophilia* possess the ability to degrade DDT and endosulfan (Barragán-Huerta et al. 2007). *Pseudomonas* breaks endosulfan (Prabakaran and Peterson 2006) along with atrazine (Wyss et al. 2006). *Sphingomonas* is another gram-negative bacterial strain which is highly effective for DDT degradation (Shunpeng and Mingxing 2006). *Rhodobacter sphaeroides* efficiently degraded various pesticides, e.g., chlorinated pesticides, herbicides, fungicides, etc., by the fermentation process (Harada et al. 2006). *Vibrio* and *Shewanella* bacteria are effective methyl parathion degraders (Liu

et al. 2006). Photosynthetic bacteria are reported with capability to degrade different pesticides (chlorpyrifos, phoxim, and triazophos) (Zhang et al. 2005). *Ochrobactrum* is reported to oxidize triazophos and degrade the compound up to 95% (Shunpeng and Shen 2005). *Acidomonas* sp. is reported to degrade allethrin, a pyrethroid insecticide (Paingankar et al. 2005). Anaerobic degradation of aldrin (an organochlorine insecticide) is also reported by microorganisms (Guohui 2004). Ethion is anaerobically degraded by mesophilic bacteria, *Azospirillum* and *Pseudomonas* (Zhang et al. 2007). Scientists also applied bacterial consortium, e.g., *Bacillus* sp. and *Chryseobacterium joostei* for biodegradation of lindane, methyl parathion, and carbofuran (Foster et al. 2004). Me-parathion is degraded by psychrotrophic bacterium (Krishna and Philip 2009). Six bacterial genera, including *Micrococcus* and *Pseudomonas*, were found effective for degradation of organochloride pesticides, i.e., endosulfan (Li et al. 2004). *Escherichia coli* is efficient in degrading organochlorine insecticide and various pesticides like BHC, DDT, endosulfan, HCH isomers, and 2,4-D (Qiao et al. 2003; Gupta 2005; Shun-Peng et al. 2005; Chaudhary et al. 2006; Santacruz et al. 2005; Xue-Dong et al. 2003). Another efficient degrader of pesticides is DLL-1 bacterial strain (Yu-Suo et al. 2003). Different bacterial strains are also isolated with the ability to degrade diazinon and profenofos (Abo-Amer 2011; Malghani et al. 2009). In another study conducted by Ortiz-Hernandez and Sanchez-Salinas (2010), a bacterial consortium of six pure strain was isolated from agricultural soil with tetrachlorvinphos and organophosphate pesticide degradation capacity. Lactic acid bacteria also possess organophosphorous insecticides degradation capability (Kye et al. 2009). Matsumura et al. (1968) studied degradation of dieldrin by a *Pseudomonas* sp. Further, it was identified that bacteria with the ability to degrade dieldrin also carried out the biodegradation of aldrin, endrin, and DDT (Patil et al. 1970). Microbes from the genera *Bacillus*, *Pseudomonas*, *Arthrobacter*, and *Micrococcus* are specifically involved in organochlorine degradation (Langlois et al. 1970). *Sphingobacterium* sp., possess the capacity of biodegradation of DDT (Fang et al. 2010). Different bacteria with methyl parathion degradation capability were isolated worldwide (Liu et al. 2003; Hong et al. 2005). *Sphingobium* sp. JQL4-5 possesses the ability to degrade fenpropathrin (Yuanfan et al. 2010). *Pseudomonas putida* and *Acinetobacter rhizosphaerae* were able to degrade the organophosphate fenamiphos. Different microbes were isolated with the ability of biodegradation of carbamate pesticides. Different strains of *Pseudomonas*, *Flavobacterium*, *Achromobacterium*, *Sphingomonas*, and *Arthrobacter* are capable of degrading carbofuran. Chlorpyrifos is a common pesticide applied for pest control in vegetable and cotton fields, though it is toxic compounds and led to soil and water contamination. A bacterial consortium of six bacterial strains, i.e., *Stenotrophomonas maltophilia*, *Proteus vulgaris*, *Vibrio metschinkouii*, *Serratia ficaria*, *Serratia* spp., and *Yersinia enterocolitica*, possess tetrachlorvinphos degradation capacity (Ortiz-Hernández and Sánchez-Salinas 2010). Two different bacteria, *A. xylosoxidans* JCp4 and *Ochrobactrum* sp. FCp1, were identified to degrade chlorpyrifos (Akbar and Sultan 2016). Different strains of *Streptomycetes* were known with potential application in the degradation of chlorpyrifos (CP) pesticide (Briceño et al. 2012). Different actinomycetes strains

with ability to degrade carbamate pesticides have been also isolated (De Schrijver and De Mot 1999). Actinomycete strain HP-S-01 was isolated with the ability to break down deltamethrin along with degradation of bifenthrin, fenvalerate, and fenpropathrin (Chen et al. 2011). *Sphingomonas yanoikuyae* was able to degrade carbamate and pyrethrin (OPs) (Ouyang et al. 2008).

27.6 Cyanobacteria for Pesticide Biodegradation

Cyanobacteria exhibit numerous advantages over other microbes as bioremediators, owing to their photoautotrophic character and nitrogen fixation capacity due to which they are self-sufficient for growth and capable to survive in polluted environments (Sokhoh et al. 1992). These organisms had already proven potential for removal of different environmental contaminants, e.g., pesticides (Megharaj et al. 1994), naphthalene (Cerniglia et al. 1980a, b), phenanthrene (Narro et al. 1992), crude oil (Sokhoh et al. 1992; Al-Hasan et al. 1998, 2001), heavy metals (Singh et al. 2011b), and xenobiotics (Megharaj et al. 1987) etc.

Cyanobacteria accumulate relatively high concentration of pesticides (Vijayakumar 2012). Different species like *Synechococcus elongatus*, *Anacystis nidulans*, and *Microcystis aeruginosa* are effective degraders of different organophosphorus and organochlorine insecticides (Vijayakumar 2012). Species from genera *Oscillatoria*, *Synechococcus*, *Nodularia*, *Nostoc*, *Microcystis*, and *Anabaena* possess lindane residue degradation capability (El-Bestawy et al. 2007). *Spirulina* sp. can degrade the glyphosate herbicide (Lipok et al. 2007, 2009). Furthermore, *Synechocystis* sp. possesses aniliofos (herbicide) degradation efficiency (Singh et al. 2016). In another study, six cyanobacterial strains (*Anabaena* sp., *Arthrospira fusiformis*, *Leptolyngbya boryana*, *Microcystis aeruginosa*, *Nostoc punctiforme*, *Spirulina platensis*) were evaluated for their resistance toward glyphosate; one of the extensively studied organophosphonate (Forlani et al. 2008; Lipok et al. 2009) and all of the strains exhibit remarkable tolerance (Arunakumara et al. 2013). Five different species of cyanobacteria, *Nostoc* sp. MM1, *Nostoc* sp. MM2, *Nostoc* sp. MM3, *Nostoc muscorum*, and *Anabaena* sp., are able to degradate fenamiphos and convert fenamiphos to its primary oxidation product, fenamiphos sulfoxide (FSO) (Cáceres et al. 2008). In another study, three cyanobacterial strains *A. oryzae*, *N. muscorum*, and *S. platensis* were evaluated for their capacity to degrade malathion (Ibrahim et al. 2014). Palanisami et al. (2009) studied the biodegradation capacity of marine cyanobacterium *Phormidium valderianum* BDU 20041 for chlorpyrifos. *Anabaena* sp. strain PCC 7120 and *Nostoc ellipsosporum* are able to degrade lindane (g-hexachlorocyclohexane), a highly chlorinated aliphatic pesticide (Kuritz and Wolk 1995). Ibrahim and Essa (2010) conducted a study to evaluate the effect of malathion, an organophosphorus insecticide over different cyanobacterial species (*Anabaena oryzae*, *Nostoc ellipsosporum*, *Calothrix castellii*, *Tolypothrix ceytonica*, and *Synechococcus* sp.), and they observed that *A. oryzae* possesses the

capability to biodegrade malathion. *Microcystis novacekii* possess the capacity to degrade methyl parathion (Fioravante et al. 2010). *Anabaena* PD-1 reflects significant resistance toward polychlorinated biphenyls (PCBs) (Zhang et al. 2015). *Aulosira fertilissima* ARM 68 and *Nostoc muscorum* ARM 221 exhibit tolerance against organophosphorus pesticides, monocrotophos, and malathion (Subramanian et al. 1994).

27.7 Fungi for Pesticide Biodegradation

Different fungal species from natural sources have also been assessed for their efficiency for biodegradation of different toxic organic chemicals. Researchers had studied the bioremediation activity of different fungal species. Fungal strains, *Fusarium oxysporum*, *Lentinula edodes*, *Penicillium brevicompactum*, and *Lecanicillium saksenae*, were highly efficient for the biodegradation of different pesticides (terbuthylazine, difenoconazole, and pendimethalin) (Hai et al. 2012). *Fusarium verticillioides* is capable to degrade lindane and utilize it as a carbon and energy source (Guillén-Jiménez et al. 2012; Pinto et al. 2012). In another study, non-acclimated mixed culture of bacteria and white-rot fungus exhibited biodegradation activity against aldicarb, atrazine, and alachlor (Nyakundi et al. 2011). Rot fungi isolated from contaminated soils were able to degrade methomyl and diazinon (pesticides) (Sagar and Singh 2011). Endosulfan (pesticide) can be degraded by strains of *Aspergillus* (Bhalerao and Puranik 2007; Javaid et al. 2016). Various fungal strains also possess DDD pesticide degradation ability (Ortega et al. 2011). *Mortierella* sp. strains W8 and Cm1- 45 are endosulfan-degrading, aerobic fungal strains which were efficient for the bioremediation of soil contaminated with organochlorine pesticides and improved the soil fertility (Kataoka et al. 2010). Members of the genus *Gliocladium* were found very effective for biodegradation of carbofuran (Slaoui et al. 2007). DEET, an insecticide, is effectively degraded by *C. elegans* (Seo et al. 2005). *Trichoderma viride* and *T. harzianum* possess very high potential for degradation of pirimicarb (Romeh 2001). *Phanerochaete chrysoporium* also degraded various kinds of pesticides (Odukkathil and Vasudevan 2013). Different fungal species, *Agrocybe semiorbicularis*, *Auricularia auricula*, *Avatha discolor*, *Coriolus versicolor*, *Dichomitus squalens*, *Flammulina velutipes*, *Hypholoma fasciculare*, *Pleurotus ostreatus*, and *Stereum hirsutum*, exhibited biodegradation capability for different pesticides (phenylamide, dicarboximide, phenylurea, triazine, chlorinated, and organophosphorus compounds) (Bending et al. 2002; Rani and Dhania 2014). Similarly, Quintero et al. (2007) stated that white-rot fungi are able to degrade various pesticides like aldrin, atrazine, chlordane, diuron, DDT, dieldrin, gamma-hexachlorocyclohexane (γ-HCH), heptachlor, lindane, mirex, metalaxyl, terbuthylazine, etc. up to different levels (Das and Chandran 2011).

27.8 Metagenomics: Assessment of Unidentified Microbial Communities for Bioremediation Potential

A vast majority of Earth's microbes are still uncharacterized owing to the inability of their isolation and cultivation on proper media. Though, cultivation techniques are continuously improving, still culture of these microbes in laboratory condition is not feasible (Leadbetter 2003). This is specifically true in complex biological systems such as soils, where, in spite of a huge bacterial diversity (up to 10^{10} bacteria and possibly thousands of diverse species per gram of soil) (Rosselló-Mora and Amann 2001), only less than 1% of bacteria are cultured till now (Torsvik and Qvreas 2002). Numerous other molecular approaches independent of cultivation have been evolved for exploration of microbes, both culturable and unculturable. These approaches facilitate the exploration of amazing taxonomic and functional variety of microbes, simultaneously. Metagenomics is the culture-independent genomic analysis of whole microbial communities (Schloss and Handelsman 2003). In other terms, metagenomics facilitates access to the pool of genomes of a particular environment. The metagenomics libraries are composed of the environmental genome, and clones are screened either for a particular trait (function-driven perspective) or sequence (sequence-driven perspective) (Schloss and Handelsman 2003). These molecular techniques would improve numerous features of environmental biotechnology, starting from environmental monitoring (Guschin et al. 1997) to biodegradation and bioremediation (Dennis et al. 2003). Metagenomics libraries can be assessed for both functional and genetic diversity. New catabolic genes for the degradation of xenobiotics are identified through the functional metagenomics. Clones are screened for a particular attribute on suitable media. For instance, haloaromatic compounds could be applied as sole electron acceptors as it is already identified that bacteria use them as metabolic source (El-Fantroussi et al. 1998; Ojo 2007). Thus, metagenomics propose considerable assurance for prediction of the in situ microbial responses, activities, and dynamics along with bioremediation of hydrocarbon and pollutants (Chikere 2013).

27.9 Metagenomics Studies for Bioremediation of Pesticides

Soils represent complicated ecological niche possessing one of the major reservoirs of diverse microorganisms. Though, majority of the microbes in nature are inaccessible owing to their uncultivable nature. Metagenomics approaches facilitate assessment of novel valuable genetic resources including novel enzymes and development of numerous biotechnological applications (Li et al. 2008). We describe different case studies employing metagenomics approaches for the bioremediation of different pesticides.

Li et al. (2008) successfully cloned pyrethroid-hydrolyzing esterase gene using metagenomics DNA coupled with activity-based functional screening from soil for the degradation of insecticides, pyrethroids, and pyrethrins, which impose severe environmental issues and human exposure. They created a metagenomics library

from vegetable soil for the isolation through functional expression screening of plasmid clones having esterase activity (Li et al. 2008). Another study was conducted by Manickam et al. (2010), for the assessment of microbial diversity in three different chlorinated pesticide contaminated sites and evaluation of dehydrodechlorinase (linA) gene variants occupied in gamma-hexachlorocyclohexane (c-HCH, lindane) degradation. With the application of metagenomics approaches, they were able to identify the presence of biodegradative genes like linA reflecting biodegradation capacity of persistent pesticide HCH by the complex and diverse microbial communities present at the sites. The study facilitates assessment of different catabolic genes and their bioremediation potential for chlorinated pesticides (Manickam et al. 2010). Fan et al. (2012) conducted a study for identification of appropriate enzymes including hydrolases for degradation of pyrethroid pesticides, which present an effective solution for biodegradation of this broadly applied pest control agent. Though numerous pyrethroid esterases are already purified and characterized from different sources including metagenomes, the thermostable pyrethroid esterases were not identified. In the study, Fan et al. (2012) isolated a novel pyrethroid-hydrolyzing enzyme Sys410 belonging to family V esterases/lipases from Turban Basin metagenomics library through activity-based functional screening. The enzyme effectively carried out the degradation of cyhalothrin, cypermethrin, sumicidin, and deltamethrin (Fan et al. 2012). Researchers also take attempt for the evaluation of microbial community accountable for the in situ bioremediation of hexachlorocyclohexane (HCH) through shotgun metagenomics sequencing approaches for different sets of soil samples (Sangwan et al. 2012). They identified that certain genera were dominant in the samples from the HCH-dumpsite. Such genera included *Chromohalobacter*, *Marinimicrobium*, *Idiomarina*, *Salinosphaera*, *Halomonas*, *Sphingopyxis*, *Novosphingobium*, *Sphingomonas*, and *Pseudomonas* (bacteria); *Halobacterium*, *Haloarcula*, and *Halorhabdus* (archaea); and *Fusarium* (fungi). HCH degradation genes (lin genes) were also abundantly present (Sangwan et al. 2012). Fang et al. (2014) analyzed six different datasets from freshwater and marine sediments for the assessment of abundance and diversity of biodegradation genes and prominent degradation pathways of dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexane (HCH), and atrazine (ATZ). In results, it was observed that the abundance and diversity of genes for biodegradation of DDT, HCH, and ATZ varied according to the sample source and locations. For degradation of organic pollutants, lip and mnp genes (encoding for peroxidase) and the carA gene (encoding for laccase) were identified as the dominant genes. The hdt, hdg, and atzB genes encoding for hydratase, dehalogenase, and ethylaminohydrolase were identified as the most abundant genes concerned with DDT, HCH, and ATZ degradation, respectively. Almost complete biodegradation pathways for DDT and ATZ were mapped, while limited HCH degradation pathways were identified (Fang et al. 2014). For understanding the impact of fenitrothion or MEP, an organophosphorus insecticide over the microbial diversity of the soil, Itoh et al. (2014) conducted study and observed significant difference among the microbial communities after the application of fenitrothion. MEP-degrading microbes like *Burkholderia* (bacteria) more quickly increased in the soils reflecting the succession and

adaptation strategies of microbial communities under insecticide application (Itoh et al. 2014). Chaussonnerie et al. (2016) reported two new *Citrobacter* isolates with the ability of reproducing chlordecone transformation, an organochlorine insecticide, through metagenomics studies of soils contaminated by chlordecone or other organochlorines and from sludge of a wastewater treatment plant (Chaussonnerie et al. 2016).

27.10 Conclusion

Decontamination of polluted areas owing to continuous use of pesticides is requirement of current time. Conventional approaches for the degradation of toxic chemicals are not very proficient. These approaches are expensive and are not eco-friendly. For the pesticide degradation and final decontamination of polluted areas, biodegradation is emerging as the best choice, rather than other remediation approaches like incineration, land farming, thermal disposition, etc. Microbe-mediated bioremediation is a superior approach for development and employment of technology for reduction/removal of pesticides and other contaminants from the polluted soils. Application of biological agents (bacteria, fungi, and enzymes) for the removal of harmful chemicals from environment is most proficient method owing to their cost-effective and eco-friendly nature. The natural ability of different soil microorganisms to degrade pesticides has been extensively studied, though a lot of microbial diversity responsible for pesticide bioremediation is still left for exploration. There is need of in-depth study over the mechanisms of microbes and their enzymes involved in degradation process. In addition, study of different microbial consortia would serve as good models for understanding the current bacterial co-metabolism relationships involved in pesticide degradation.

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Role of Plant Growth-Promoting Rhizobacteria for Improving Crop Productivity in Sustainable Agriculture

28

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Abstract

The plant growth-promoting rhizobacteria (or PGPR) are the beneficial microorganism that colonizes rhizosphere and help in promoting plant growth, protecting from biotic and abiotic stresses, and significantly increasing soil fertility. For the effective ways of developing sustainable agriculture for improving crop productivity with a minimal disturbance to the environment is the exploration of plant growth-promoting rhizobacteria and some other microbe-based symbioses in plants. For increasing crop yields, the use of PGPR has been well proven for its eco-friendly sound by promoting plant growth either direct or indirect mechanism. The mechanisms of plant growth-promoting rhizobacteria include resistance against plant pathogens, solubilizing nutrients for easy uptake, and maintaining the plant growth regulator hormone. This chapter emphasizes an eco-friendly approach to increase crop production and health, the development of sustainable agriculture, the mechanism of PGPR for agricultural sustainability, and the role in different major crop plant varieties along with their mechanism of action.

Keywords

Plant growth-promoting rhizobacteria (PGPR) • Phytopathogens • Sustainable agriculture • Biofertilizer • Crop yields

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28.1 Introduction

Agriculture is the basic human activity to meet the demand of food for population that significantly increases the use of synthetic chemical fertilizers and pesticides, which causes further environmental damage with potential risks to soil, animal, and human health. Chemical pollutant like nitrous oxide (N_2O) is produced by the excessive use of nitrogen fertilizers and is a main source of greenhouse gases affecting global warming. It also reduces biological nitrogen fixation in the soil. Farmers apply high doses of nitrogen fertilizers in the form of ammonium nitrate to fertilize their soil. Farmers apply a high concentration of nitrogen fertilizers in the form of ammonium nitrate to fertilize their soil to grow crops.

However, on the influx of ammonium, plants no longer need the symbiotic microbes to provide nitrogen, and this diminished the degree of symbiosis. Moreover, some of the nitrifying bacteria also take benefit of this excess ammonium and utilize it to produce nitrate. The higher concentration of nitrate is then utilized by some of denitrifying bacteria to produce N_2O, and additional nitrate leaches into the groundwater (Galloway et al. 2008). Natural production of nitrous oxide increases by the microbial processes of nitrification and denitrification. The process where nitrogen oxide is reduced to gaseous products by microorganisms and released back into the atmosphere is known as denitrification, whereas nitrification is a two-step process of ammonium (NH_4) being converted to nitrate (NO_3) by soil bacteria (Butterbach et al. 2013). Toward a sustainable agricultural vision, crop plants are essential to be equipped with resistance in disease, drought tolerance, heavy metal stress tolerance, and salt tolerance and produced with better nutritional value. To fulfill the above properties of desired crop, one possibility is to use soil microorganisms such as bacteria, fungi, algae, etc. that enhance the nutrient use efficiency, uptake and translocation of mineral nutrients, and water-use efficiency (Armada et al. 2014). Among potential soil microorganisms, rhizosphere bacteria known as plant growth-promoting rhizobacteria (PGPR) are the most promising and widely used. It is a group of bacteria that can be mainly found in the rhizosphere (Ahmad et al. 2008).

PGPR are generally used to enhance plant health and promote plant growth without any environmental contamination (Calvo et al. 2014). The term plant growth-promoting bacteria belongs to bacteria that colonize the rhizosphere and promote plant growth. These bacteria can play an essential role in helping plants to establish and grow in nutrient-deficient conditions. Rhizosphere is the soil environment in close vicinity to the plant roots and is a zone of maximum microbial activity where maximum interactions were observed. PGPR helps plants directly or indirectly to increase plant growth-promoting attributes such as increase in seedling emergence, effective nodulation as well as nodule functioning, enhanced, increased indigenous plant hormones, root hair proliferation, root hair deformation and branching, early mineral and water uptake, promote accumulation of carbohydrates and increasing the yield (Podile and Kishore 2006). In the last two decades, the use of PGPR for sustainable crop production increased enormously on global basis. Various researchers reported about the increase in plant growth and yield of important crops in

response to inoculation with these organisms (Seldin 1984; Zhang et al. 1996; Amara and Dahdoh 1997; Chanway 1998; Pan et al. 1999; Bin et al. 2000; Gupta et al. 2000; Mariano and Kloepper 2000; Biswas et al. 2000; Asghar et al. 2002; Vessey 2003; Gray and Smith 2005; Silva et al. 2006; Figueiredo et al. 2008; Araujo 2008). Some of the studies revealed that the plant growth-promoting ability of some bacteria may be vastly specific to certain plant species, cultivar, and genotype and are exploited for a specific cause (Bashan 1998; Lucy et al. 2004).

28.2 Rhizosphere

The rhizosphere is the interface between plant roots and soil where interactions among a myriad of microorganisms and invertebrates affect biogeochemical cycling, plant growth, and tolerance to biotic and abiotic stress. The rhizosphere is the thin region of soil that is directly influenced by root secretions and associated soil microorganism. This zone is rich in nutrients as compared with the loose soil (non-rhizosphere soil) due to the accumulation of plant exudates such as sugars, amino acids, phenolic, flavonoids, vitamins, enzymes, and hormones providing a source of nutrients and energy for bacteria (Gray and Smith 2005). The zone increases the 10–100-fold greater microbial population, enhancing violent competition for nutrients as well as the existence of species which show a variety of functional diversity and metabolic versatility (Sinha et al. 2001). The rhizosphere itself can be divided into (a) endorhizosphere, it is the internal root area up to cortical region which harbors large population of bacteria with diverse functions, (b) rhizoplane, and (c) ectorhizosphere (Lynch 1990). Glick (1995) reported that rhizospheric soil contains various types of bacterial genera which exhibit different beneficial effects on plant growth. The microbes colonizing rhizosphere include bacteria, fungi, actinomycetes, protozoa, algae, etc. However, bacteria are the most profuse microorganisms present in the rhizosphere (Kaymak 2010). The role in the promotion of plant growth by the application of these microbes is known and well proven (Saharan and Nehra 2011; Bhattacharyya and Jha 2012). Root exudates are rich in various chemical compounds that favor PGPR population buildup in rhizosphere (Table 28.1).

28.3 Plant Growth-Promoting Rhizobacteria (PGPR)

The term plant growth-promoting rhizobacteria was introduced by Kloepper and Schroth in 1978. PGPR are not only associated with the root and rhizosphere to exert beneficial effects on plant growth but also have positive effects on controlling plant pathogens in various crop plants (Kloepper et al. 1980; and Son et al. 2014). Therefore, PGPR serve as one of the active ingredients in biofertilizer formulation. The concept of PGPR was given by several workers after Kloepper. Bashan and Holguin (1997) studied about the division of PGPR into two classes, biocontrol PGPB (plant growth-promoting bacteria) and PGPB. This division may comprise beneficial bacteria that are not rhizosphere bacteria, but it does not seem to have

Table 28.1 Constituents of root exudates found in different plant species

Amino acids	Nucleosides	Enzymes	Vitamins	Inorganic ions	Organic acids
Leucine, lysine, methionine, serine, threonine, proline, valine, α-alanine, β-alanine, asparagines, aspartate, cysteine, cystine, glutamate, glycine, isoleucine, tryptophan, ornithine, histidine, arginine, homoserine, phenylalanine	Adenine, guanine, cytidine, uridine	Acid/ alkaline phosphatase, invertase, amylase, protease	Biotin, thiamin, pantothenate, riboflavin, niacin	OH, H^+, CO_2, H_2, HCO^{3-}	Citric acid, oxalic acid, malic acid, fumaric acid, succinic acid, acetic acid, butyric acid, valeric acid, glycolic acid, piscidic acid, formic acid, aconitic acid, lactic acid, pyruvic acid, glutaric acid, malonic acid, and erythronic acid and sugars such as glucose, fructose, galactose, ribose, xylose, rhamnose, arabinose, desoxyribose, oligosaccharides, raffinose, and maltose

been widely accepted. Vessey (2003) documented that various species of soil bacteria which curl in the plant rhizosphere, but which can grow on, in, or around tissues of plant, and provoke plant growth by an excess of mechanisms are collectively known as PGPR, Gray and Smith (2005) also studied that the PGPR associations range in the degree of bacterial proximity to the root and intimacy of association. In overall, PGPR can be divided into extracellular PGPR (ePGPR), existing in the rhizosphere, on the rhizoplane, and in the spaces between cells of the root cortex, and intracellular PGPR (iPGPR), which exist inside root cells, usually in particular nodular structures. Currently, there are numerous PGPR inoculants commercialized that appear to promote plant growth over at least one mechanism, suppression of plant pathogens (bioprotectants), improved nutrient acquisition and translocation (biofertilizers), and phytohormone production (biostimulants). The genera of bacteria belonging to *Bacillus*, *Streptomyces*, *Pseudomonas*, *Burkholderia*, and *Agrobacterium* are the biological control agents mostly studied and commercially marketed. They suppress plant disease through at least one mechanism, antibiotic production of siderophores, and induction of systemic resistance (Tenuta 2003). For decades, a numerous of PGPR have been documented, and some of them have been commercialized including the species *Bacillus*, *Enterobacter*, *Pseudomonas*, *Azotobacter*, *Klebsiella*, *Azospirillum*, *Serratia*, and *Variovorax* (Glick 2012). Moreover, the exploitation of PGPR in the agriculture field and allied sectors

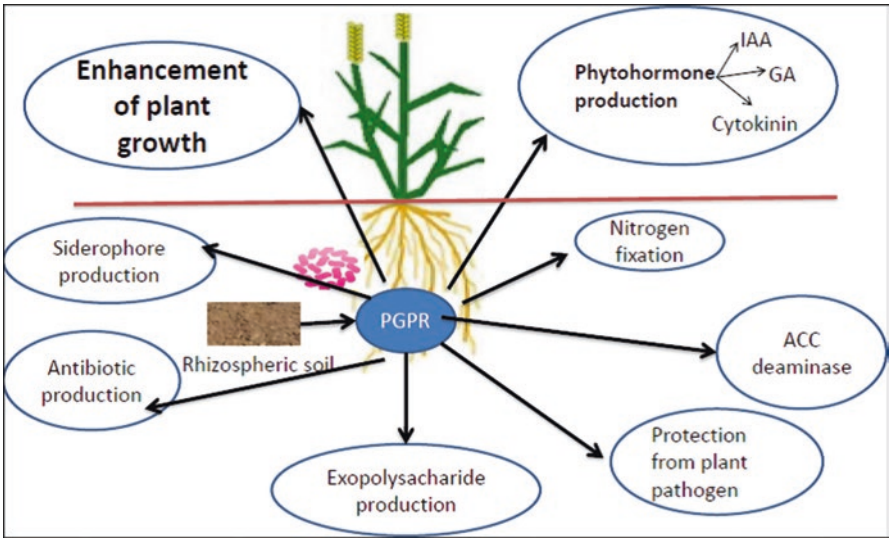

Fig. 28.1 Role of PGPR in rhizosphere

signifies only a small fraction of agricultural practice globally (Bashan et al. 2014). This may be due to the inconsistent properties of the inoculated PGPR in a diverse environment, which could influence crop production. The successful utilization of PGPR depends on its survival in soil, ecological fitness, compatibility with the host, rhizosphere competitiveness, and other environmental factors including biotic and abiotic factors prevailing in the microenvironment (Martinez-Viveros et al. 2010).

Another challenge is modes of action of PGPR, which is diverse in nature and varies with environmental conditions (Dey et al. 2004; Choudhary et al. 2011). These disadvantages limit the application and popularity of PGPR. Therefore, the competition between commercial chemical fertilizers and PGPR as a biofertilizer is deemed redundant in the face of the global agricultural productivity needed to feed the booming world's population, which is predicted to escalate to eight billion people by 2025 and nine billion by 2050 (Fig. 28.1).

28.4 Functional Attributes of PGPR

28.4.1 Abiotic Stress Tolerance in Plants

The significant reductions of agricultural yield are mainly from abiotic stresses in plants. However, the intensity of abiotic stress varies depending on the plant factors like physiological disorders and type of soil (deficiency of hormonal and nutritional imbalances) (Nadeem et al. 2010). The PGPR could be reduced by the effect of toxic cadmium pollution on barley plants due to the ability of the bacteria to bind with cadmium (Cd) ions from the soil by binding mechanisms, thereby decreasing the availability of cadmium in the soil (Pishchik et al. 2002).

Moreover, Nautiyal et al. (2008) reported that the *Bacillus lentimorbus* strain increased the antioxidant capacity of the edible parts of spinach, carrots, and lettuce, as well as increasing growth. The results produced are important, mainly to improve the nutrient content of these crops. Another major effect of PGPR on plants under abiotic stress conditions is the enhancement of leaf water status, especially under drought stress and salinity (Naveed et al. 2014). Sarma and Saikia (2014) demonstrated that *Pseudomonas aeruginosa* improved the growth of *Vigna radiata* plants under drought conditions. These bioagents may alter the ability of plants in utilizing water for growth under drought condition by means of opening and closing behavior of stomatal apertures. The treated plants may behave in such a way to maintain the balance between water content in leaf and water uptake by the roots. The stomatal conductance (water vapor exiting through the stomata) of plant leaf was higher in PGPR-inoculated plants than non-PGPR-inoculated ones under drought conditions. The finding from both studies demonstrates that PGPR-inoculated plants tend to enhance the water-use efficiency of plants. This finding could be helpful to the environment in terms of dropping excessive usage of water.

28.4.2 Siderophore Production

Siderophores, low molecular weight iron binding protein, are involved in the process of chelating ferric iron ($Fe3^{+}$) from the root rhizosphere. Iron is a vital nutrient (bulk minerals) for all forms of life present on the earth, yet it is unavailable/less available in the soil for plants. All microorganisms essentially require iron with exception to certain lactobacilli (Neilands 1995). Iron is commonly present in nature in the form of $Fe3^{+}$, which is highly insoluble and unavailable to plants. PGPR secrete siderophores which chelate Fe under Fe deficit condition. When Fe is limited, microbial siderophores provide plants with Fe and improve their growth. Here, plants sequester iron by utilizing siderophores secreted by the PGPR. In both Gram-negative and Gram-positive rhizobacteria, iron is chelated in the form of $Fe3^{+}$ siderophore complex on bacterial membrane and is reduced to $Fe2^{+}$ which is further released into the cell from the siderophore. During the process of reduction, the siderophore may be destroyed or recycled (Neilands 1995; Rajkumar et al. 2010). Therefore, it acts as solubilizing agents for iron from minerals and/or organic compounds under iron-deficit conditions (Indiragandhi et al. 2008). Siderophores usually form stable complexes not only in iron but also in other heavy metals which are of concern with environment such as Pb, Zn Al, Cu, Cd, In, and Ga, as well as with some radionuclides including Np and U (Neubauer et al. 2000). While siderophore binds to a metal, it increases the soluble metal concentration (Rajkumar et al. 2010). Hence, bacterial siderophores benefit to improve the stresses imposed on plants by high heavy metal pollution in the soil.

28.4.3 Plant Growth Regulators (PGRs)

PGPR synthesize and export phytohormones (PGRs) also called as plant growth regulators that may be produced in distinct organs of plant and can be translocated to other sites of action, where these pledge specific physiological, biochemical, and morphological role leading to plant growth and development (Hayat et al. 2010). Phytohormones are organic in nature, regulate physiological processes of plants even at low concentrations, and also take part in the development of tissues where they are produced. Among different plant growth regulators, auxins, cytokinins, gibberellins, abscisic acid, and ethylene are common, and their mechanisms are well documented. Indole-3-acetic acid (IAA), most active and common auxin present in plants, regulates many aspects of plant growth and development throughout the plant life and plays a role in cell cycle, cell division, apical dominance, flowering, cell elongation and differentiation to root initiation, fruit ripening, tropistic responses, senescence, and stimulation of plant growth. Guilfoyle et al. (1998) reported that the regulation of these processes by auxin is believed to involve auxin-induced changes in gene expression. In addition to IAA, some bacteria such as *Azospirillum* and *Paenibacillus polymyxa* also release indole-3-butyric acid (IBA), Trp and tryptophol or indole-3-ethanol (TOL), and other compounds in rhizosphere that could indirectly contribute to plant growth. Cytokinins are important phytohormones usually present in mere amounts in biological samples (Vessey 2003). These promote cell division, root development, and root hair formation and are also involved in the processes such as photosynthesis or chloroplast differentiation (Frankenberger and Arshad 1995). They are also known to hide auxin-induced apical dominance, encourage opening of stomata, and retard senescence organs of plants, particularly in leaves (Crozier et al. 2001). Cytokinin-producing bacteria, *Azotobacter chroococcum*, and cytokinin precursor's adenine (ADE) and isopentyl alcohol (IA) were verified on maize crop under controlled and field conditions (Nieto and Frankenberger 1991).

More than 30 growth-promoting compounds of cytokinin group have been reported to be produced by plant-associated PGPR. They induced improvement in crop growth. PGPR also produce phytohormones, namely, gibberellic acid (GA) and gibberellins (GAs). Nearly 89 gibberellins are known for stem elongation (Dobbelaere et al. 2003). GAs also affect reproductive processes in a wide range of plants (Crozier et al. 2001). PGPR like *Pseudomonas* sp. and *Azospirillum* sp. produce gibberellins (gibberellic acid). Ethylene, a gaseous phytohormone, is a plant growth regulator synthesized by almost all species of bacteria (Primrose 1979). Ethylene is a ripening hormone, promotes adventitious roots and root hair formation, stimulates germination, promotes plant growth, and breaks dormancy of seeds, development, and senescence. If ethylene concentration remains high after germination, root elongation, as well as symbiotic N_2 fixation in leguminous plants, is inhibited. PGPR produces enzyme like 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which hydrolyzes ACC and lowers the level of ethylene in crop rhizosphere. The hydrolysis products, ammonia and α-ketobutyrate, can be used by the bacterium as a source of carbon and nitrogen for growth and development. In this way,

the bacterium acts as a sink for ACC and thus drops the ethylene level in plants, avoiding some of the potentially harmful consequences of high concentrations of ethylene (Glick et al. 1998).

PGPR with ACC deaminase characteristics enhances crop growth and yield and may be included in biofertilizer biotechnology (Shaharoona et al. 2006). The role of PGPR in the production of β-glucanase, phosphatase, dehydrogenase, and antibiotics has also been recognized. Another recently recognized mechanism for plant growth promotion is due to the production of volatiles by PGPR. Ryu et al. (2004) reported the role of bacterial volatiles in plant growth promotion in vitro. PGPR release different volatile blends, and the differences in these volatile blends stimulate the plant growth. Volatile compounds like 2,3-butanediol and acetoin (3-hydroxy-2-butanone) produced by *Bacillus amyloliquefaciens* and *B. subtilis* stimulated the growth of *Arabidopsis thaliana* in in vitro experiments. The volatile-mediated growth promotion by PGPR is by the activation of cytokinin-signaling pathways.

28.4.4 Nitrogen Fixation

The most important limiting plant nutrient is nitrogen for plant growth (Havlin et al. 1999). Some rhizobacteria have the ability to fix nitrogen into organic forms which could be utilized by the plants. PGPR also form associations of symbiotic and non-symbiotic microbes with plants to fix atmospheric nitrogen changing it into functional form ammonia. The symbiotic nitrogen fixers form a mutualistic relationship with the plant. The microbes form nodules on the root surfaces where nitrogen fixation takes place. Nonsymbiotic nitrogen-fixing microbes live freely. *Rhizobium* is a symbiotic nitrogen fixer, while as *Cyanobacteria*, *Acetobacter* fix nitrogen freely. Numerous studies have shown greater nitrogen fixation activities in inoculated plants as compared to uninoculated plants (Boddey and Dobereiner 1984).

28.4.5 Role of PGPR in Crop Production

Globally, there are three chief staple food crops, viz., rice, wheat, and maize. A variety of PGPR participated in the interaction with both C3 and C4 plants and can significantly enhance their yield (Kennedy et al. 2004a, b). Angus (2001) reported that rice crop removes around 16–17 kg N to produce 1 ton dry weight of rice including straw and wheat crop requires about 26–28 kg N to produce 1 ton of grain-containing straw. Kennedy et al. (2004a, b) reported that maize crops require 9–11 kg N to produce 1 ton biomass. The nitrogen necessity of cereals is normally met with chemical urea that is applied at the rate depending on soil fertility (Scharf 2001). PGPR biofertilizers supplement and reduce the use of urea-nitrogen (Kennedy et al. 2004a, b). Those closely associated with rice rhizosphere are *Burkholderia*, *Herbaspirillum*, and *Azospirillum*. A free-living heterotrophic diazotroph like *Azotobacter vinelandii* and *A. chroococcum* uses carbon from sugar as

energy source (Kennedy and Tchan 1992). Some obligatory anaerobic heterotrophs like *Clostridia* are capable of fixing N_2 in anaerobic condition (Kennedy et al. 2004a, b) and are usually isolated from rice fields. Their activity in rice may be improved with the addition of organic source like straw (Kanungo et al. 1997), wherein cellulose is broken down into cellobiose and glucose. The yield of rice can be improved with the application of *Azotobacter* and *Azospirillum* spp. (Reis et al. 2000). Similarly, Tran Van et al. (2000) reported that *Burkholderia* sp. increases rice grain yields significantly up to 8 t ha^{-1} by supplementing 25–30 kg N ha^{-1} as synthetic fertilizer. Diazotrophs such as *Klebsiella*, *Enterobacter*, *Citrobacter*, and *Pseudomonas* spp. in family *Enterobacteriaceae* showing plant growth-promoting traits have been isolated from rice rhizosphere (Kennedy et al. 2004a, b). *Herbaspirillum seropedicae*, a rice endophyte (James et al. 2000), can fix up to 45% of total plant N in rice from the atmosphere (Baldani et al. 2000).

The nitrogen fixation range by *Herbaspirillum* was assessed to be up to 58 mg per tube under aseptic conditions (Reis et al. 2000). Multi-strain biofertilizer containing three different PGPR like *Pseudomonas* (*P. putida*, *P. fluorescens*), *Klebsiella pneumonia*, and *Citrobacter freundii* isolated from rice rhizospheric soil of Hanoi, Vietnam (Nguyen et al. 2003), increased rice grain yield significantly in Vietnam and Australia (Williams and Kennedy 2002). The use of urea-N in wheat production can be supplemented by many strains of *Azotobacter*, *Bacillus*, *Azospirillum*, *Azorhizobium*, *Herbaspirillum*, and *Klebsiella* either by BNF or growth promotion (Kennedy and Islam 2001). Due to higher grain protein content, the N requirement of wheat is higher than that for rice. Wheat yields vary widely from 1 to 7 ton ha^{-1} depending on inherent soil fertility, amount of applied fertilizer, diseases, wheat variety, other management practices, and environmental conditions (Angus 2001). Thus, the amount of N estimated to be removed by wheat crop varies between 26 and 200 kg N ha^{-1}, depending on the yield (Reeves et al. 2002).

For maximizing wheat yields from N-deficient soils, chemical fertilizers such as urea are used to enhance N supply. Biofertilizers are also being used to supplement the use of urea worldwide. Kennedy and Islam (2001) reported that the estimated amount of BNF by such wheat-bacterial associations was between 10 and 30 kg N ha^{-1} and about 10% of their total N requirement. Kuzyakov and Domanski (2000) reported that wheat converted about 30% of carbon assimilates into the process of rhizo-deposition and part of this belowground translocated carbon is incorporated by rhizosphere microorganisms. Studies indicate that PGPR may act as natural elicitor for improving the growth and production of wheat. Important PGPR which can increase wheat yield across the world are *Pseudomonas* sp., *Bacillus cereus*, *Azospirillum brasilense*, *A. lipoferum*, and *Herbaspirillum*. Common PGPR species found in rhizosphere of maize are *Enterobacter* sp., *Rahnella aquatilis*, *Herbaspirillum seropedicae*, *Paenibacillus azotofixans*, *Klebsiella* sp., *Bacillus circulans*, and *Azospirillum* spp. (Chelius and Triplett 2000). The positive effects of *Azospirillum* on maize growth are mainly due to physiological changes of the inoculated plant roots by enhancing water and mineral nutrient uptake (Okan and Kapulnik 1986). Two bacterial species *Azospirillum irakense* and *Azospirillum brasilense* are used as inoculant biofertilizer for maize. Other species of *Azospirillum*

capable of increasing the yield of maize are *A. lipoferum* and *A. indigens*. Riggs et al. (2001) reported that *Azorhizobium caulinodans* is also capable of giving such beneficial effects. *Herbaspirillum seropedicae* can enhance the N use efficiency of maize plant, and the yield increase is up to 19.5% (Riggs et al. 2001).

PGPR strains *Burkholderia cepacia*, *Pseudomonas fluorescens*, *Serratia proteamaculans*, *Rhizobium* sp., and *Sinorhizobium* sp. increase corn growth, grain yield, and plant height of maize in different agroecological zones. Kennedy and Islam (2001) reported that *Acetobacter* also known as *Gluconacetobacter* (*A. diazotrophicus*), *Azospirillum* (*A. brasilense*, *A. lipoferum*, *A. amazonense*), *Burkholderia* (*B. brasiliensis*, *B. tropicalis*), and *Herbaspirillum* sp. are the diazotrophs present in sugarcane plants. It takes approximately 1.45 kg N ha^{-1} to produce 1 ton moist biomass of sugarcane (Bhuiyan 1995) or about 7 kg N ha^{-1} for 1 t of dry cane. Depending on soil fertility, genotype, and targeted yield, 150–250 kg urea-N ha^{-1} is applied for sugarcane cultivation. Boddey et al. (1991) documented that more than 70% of sugarcane N (200 kg N/ha/y) was derived from biological fixed N_2 by *Azospirillum diazotrophicus*. Similarly, *Acetobacter* (with nif H+) sugarcane system has also been well established (Lee et al. 2002). *Klebsiella mobilis* and *Azotobacter* sp. have been reported to improve potato yield. Similarly, *Achromobacter piechaudii* and *Pseudomonas fluorescens* increase tomato yield.

Positive effect of PGPR (*Pseudomonas putida*, *Pseudomonas fluorescens*, *Bacillus cereus*, *Serratia liquefaciens*, *Escherichia coli*, *Arthrobacter citreus*, *Delftia acidovorans*, and *Mesorhizobium loti*) inoculation on the growth and yield of rapeseed has been reported by many researchers. Chiarini et al. (1998) documented that *Burkholderia cepacia* alone or in combination with *Enterobacter* sp. and *Pseudomonas fluorescens* have also been evaluated for their ability to promote growth of *Sorghum bicolor*. Anjum et al. (2007) also conducted a study that inoculation with effective bacterial strains (*Pseudomonas denitrificans*, *Pseudomonas alcaligenes*, *Bacillus polymyxa*, *Mycobacterium phlei*, and *Azospirillum brasilense*) increases the root and shoot growth of cotton. Çakmakci et al. (2007) documented on barley under greenhouse conditions in order to investigate seed inoculation with five different nitrogen-fixing (*Bacillus licheniformis*, *Paenibacillus polymyxa*, *Rhodobacter capsulatus*, *Pseudomonas putida*, and *Bacillus* spp.) and two different phosphate-solubilizing (*Bacillus megaterium* and *Bacillus* spp.) M-13 bacteria in comparison to control and mineral fertilizer (N and P) application.

PGPR strains, from a range of genera, enhance legume growth, nodulation, and nitrogen fixation when co-inoculated with their effective rhizobia. Examples of these are *Azospirillum lipoferum*, *Azospirillum brasilense*, *Azotobacter chroococcum*, *Bacillus cereus*, *Bacillus endophyticus*, *Bacillus pumilus*, *Bacillus subtilis*, *Bacillus firmus*, *Bacillus megaterium*, *Paenibacillus lautus*, *Paenibacillus macerans*, *Paenibacillus polymyxa*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Serratia liquefaciens*, *Serratia proteamaculans*, *Aeromonas hydrophila*, and *Streptomyces* (Pal et al. 2004; Kishore et al. 2005; Figueiredo et al. 2007). All these bacteria including cyanobacteria can supplement urea-N by BNF but only if conditions for the expression of nitrogen-fixing activity and subsequent transfer of N to plants are favorable.

In general, it is supposed that PGPR are more effective in promoting plant growth under limited supply of nutrients; however, present scenario does not allow to compromise on actual potential of crop productivity by reducing the use of chemical fertilizers. Hence, it is of great importance to isolate those PGPR strains which could be effective in nutrient-deficient conditions. Ligero et al. (1999) described that ethylene biosynthesis in plant roots is significantly affected by NO_3 concentration existing nearly around the roots.

Glick et al. (1998) documented that higher levels of NO_3 in rooting medium excite ACC oxidase activity which lead to significant increased ethylene production and are usually believed to be root growth inhibitory. Major nitrogenous fertilizer supplied in ammonical form is readily oxidized to NO_3 under aerobic conditions. It may possible that NO_3 present in the root vicinity diminishes the efficiency of PGPR by inducing ethylene synthesis, though PGPR containing ACC deaminase reduces the NO_3-induced ethylene synthesis.

28.4.6 Phosphorus Solubilization

Phosphate-solubilizing bacteria (PSB) play an important role in providing phosphate to plants in a more environment-friendly and sustainable manner. The naturally abundant phosphate-solubilizing bacteria solubilize Ca-bound phosphatic compounds in an alkaline soil environment and convert the insoluble phosphatic compounds into soluble forms and make them available to crop plants. These bacteria are generally applied in agronomic practices for the significant increase in crop productivity and also maintaining the health of soils. Çakmakçi et al. (2006) reported that the beneficial effects of phosphate bacteria on plant growth vary significantly depending on environmental conditions, bacterial strains, plants, and soil conditions. Banerjee et al. (2006) studied various bacterial species in the genera *Rhizobium* and *Bacillus* and found *Pseudomonas* has the most powerful phosphate-solubilizing bacteria. Many researchers also report on phosphate solubilization by nonsymbiotic nitrogen fixer, *Azotobacter* (Kumar et al. 2001). The production of 2-ketogluconic acid is associated to phosphate-solubilizing activity of *Rhizobium* which indicates that phosphate-solubilizing activity of the organism is totally due to its ability to decrease medium pH (Halder and Chakrabarty 1993). The phosphate-solubilizing ability also depends on the nature of nitrogen source used in the media, with greater solubilization in the presence of ammonium salts than when nitrate is used as nitrogen source. This has been attributed to extrusion of protons to compensate for ammonium uptake, leading to a decreased extracellular pH (Roos 1984).

Reyes et al. (1999) reported that ammonium can lead to decrease in phosphorus solubilization. Several strains of P-solubilizers such as *Bacillus megaterium*, *B. brevis*, *B. thuringiensis*, *B. polymyxa*, *B. sphaericus*, and *Xanthomonas maltophilia* have been identified in vitro (de Freitas et al. 1997). Van Veen et al. (1997) studied phosphate-solubilizing bacteria of *Bacillus* and *Paenibacillus* sp. which have been applied to soils to successfully improve the phosphorus status of plants. Most annual crops often do not get promoted by the direct application of phosphate rock in a

short time. The inorganic phosphate-solubilizing activity by phosphate bacteria ranges between 25 and 42 mg phosphate mL^{-1}, whereas the organic phosphate mineralization may occur between 8 and 18 mg P mL^{-1} (Tao et al. 2008).

Sundara et al. (2002) reported that the application of phosphate fertilizers can be reduced by 25 and 50%, by using PSB inoculum along with single super phosphate and rock phosphate, respectively. Ghaderi et al. (2008) found that 29–62% P can be released by *Pseudomonas fluorescens* and *P. putida* along with the highest value of 0.74 mg P per 50 ml from Fe_2O_3. *Pseudomonas fluorescens* is very effective and can solubilize 100 mg P L^{-1} containing $Ca_3(PO_4)_2$ or 92 and 51 mg P L^{-1} containing $AlPO_4$ and $FePO_4$, respectively (Henri et al. 2008). Zaidi 1999 documented that rock phosphatic minerals are often insoluble to provide sufficient P for plant uptake. However, phosphate bacteria can release phosphate from the fixed insoluble minerals and thus help to increase crop yields. Ponmurugan and Gopi (2006) documented that PGPR not only improve BNF but also contribute in increasing the availability of soluble P and, thus, improve plant growth. Sharma et al. (2007) documented that PSB improved seedling length of *Cicer arietinum* and improve sugarcane yield (Sundara et al. 2002). Co-inoculation of PGPR and PSB can diminish the application of phosphate fertilizers by 50% without affecting the production of corn yield (Yazdani et al. 2009). With the application of inoculation along with P fertilizers against sole phosphate by fertilization, grain yield of wheat increased 20–40% (Afzal and Bano 2008). Plant's available P increases by the activity of PSB especially belonging to the genera *Pseudomonas*, *Bacillus*, and *Enterobacter*. Hence, PSB has enormous potential to be used in biofertilizer formulations.

28.4.7 Production of Enzymes

Enzyme production through PGPR also helps in producing protection enzymes, and their mode of action could be labeled that of biopesticides. PGPR also produce metabolites that contribute to antibiosis, plant growth through the control of phytopathogenic agents, and antifungal properties used as defense systems by involving the hydrolytic enzyme production such as chitinase and glucanase. The cell wall of the fungus is mainly composed of chitin and α-glucan; thus, chitinase- and β-glucanase-producing bacteria would prevent fungal growth. The *Sinorhizobium fredii* KCC5 and *Pseudomonas fluorescens* LPK2 produce chitinase and β-glucanases and control the fusarium wilt produced by *Fusarium udum* (Kumar et al. 2010). Apart from exhibiting the production of chitinase and β-glucanases, *Pseudomonas* spp. prevent *Phytophthora capsici* and *Rhizoctonia solani*, two of the most destructive crop pathogens in the world (Arora et al. 2008).

28.4.8 Biocontrol Agents

Some of studies demonstrated that a biofertilizer prepared by combining PGPR with compost could improve growth-promoting effects and biocontrol of plants

(Chen et al. 2011). Indirect plant growth promotion comprises the inhibition of the harmful effects of phytopathogenic organisms. PGPR have also been shown to produce various antagonistic metabolites that are involved in the direct inhibition of plant pathogens (El-Akhal et al. 2013; Silo-Suh et al. 1994). It comprises antibiosis, i.e., the inhibition of microbial growth by diffusible antibiotics, volatile organic compounds, toxins, biosurfactants, and parasitism that may involve the production of extracellular cell wall-degrading enzymes such as chitinases and β-1,3-glucanase (Beneduzi et al. 2012; Dobereiner 1961). *Bacillus* spp. (Gong et al. 2006) and *Pseudomonas* spp. (Leonardo et al. 2006) are two PGPR that have been reported to be effective biocontrol agents. Among these bacterial species, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Bacillus cereus* are the most effective species at controlling plant diseases through various mechanisms (Francis et al. 2010).

More recently, *Pseudomonas* and *Bacillus* species have been implicated in biocontrol due to their effective competitive interactions with bacteria, fungi, oomycetes, protozoa, and nematodes (Radzki et al. 2013; Simonet et al. 1990; Munoz-Rojas and Caballero-Mellado 2003).

28.4.9 Role of Nanotechnology in Biofertilizers for Agricultural Sustainability

The application of modern technologies such as nano-biotechnology has remarkable potential to revolutionize the agricultural industry. Nano-agriculture, which currently emphasizes on target farming, involves the use of nano-sized particles such as nano-fertilizer and offers exclusive tools for improving the productivity of the crop plants through efficient nutrient uptake (Tarafdar et al. 2013). The distinctive properties of nano-sized particles with respect to their biological, chemical, and physical properties compared to those at a larger scale provide the potential to protect plants, detect plant diseases, enhance food quality, monitor plant growth, increase food production, and reduce waste. The enormous efficiency of nano-fertilizers related to ordinary fertilizers has been proven as they reduce nitrogen loss due to leaching, emissions, and long-term incorporation by soil microorganisms (Liu et al. 2006). Furthermore, Suman et al. (2010) have demonstrated the advantage of using nano-fertilizers by showing that controlled release fertilizers may also improve the soil by reducing the toxic effects associated with the overapplication of traditional chemical fertilizers.

PGPR usage as fertilizer by conventional methods is not effective as 90% are lost to the air during application, they are intolerant to the environment (UV radiation, heat, etc.), and, as runoff, they affect application costs to the farmer. Some technologies like nano-encapsulation technology could be used as a versatile tool to protect PGPR, enhancing their service life and dispersion in fertilizer formulation and allowing the controlled release of the plant growth-promoting rhizobacteria.

28.5 Beneficial Aspects of Plant Growth-Promoting Rhizobacteria

In the last few decades, biofertilizers are becoming a crucial and important aspect of the organic farming and a major player for the economy on a global scale. Biofertilizers are defined as products that contain living microorganisms, when applied to seeds, plant surfaces, or soil; they colonize the rhizosphere or interior of the plant and promote plant growth by increasing the availability and supply of primary nutrients to the crop plants (Vessey 2003). Also, biofertilizer can be a mixture of live or latent cells, promoting nitrogen-fixing, phosphate-solubilizing, and cellulolytic microorganisms used for applications to soil, roots, seed, or composting areas with the purpose of enhancing the quantity of those mutualistic beneficial microorganisms and accelerating microbial processes, which improve the availability of nutrients that can then be easily assimilated and absorbed by the plants (Mishra et al. 2013).

Malusa and Vassilev (2014) reported that biofertilizer is the formulated product containing one or more microorganisms that improve the growth of the plants by either replacing soil nutrients or by making nutrients more available to plants. Biofertilizer products are generally based on the plant growth-promoting microorganisms (PGPM), which can be classified into three dominant groups of microorganisms: arbuscular mycorrhizal fungi (AMF) (Jeffries et al. 2003), plant growth-promoting rhizobacteria, and nitrogen-fixing rhizobia (Franche et al. 2009), which are believed to be beneficial to plant nutrition and growth. PGPR can be classified as biofertilizers when they act as a plant nourishment and enrichment source that would fill the nutrient cycle between the soil, plant roots, and microorganisms present. However, it has been described that PGPR have been used worldwide as biofertilizers, contributing to enhance crop yields and soil fertility.

Hence, with the prospective contribution of the PGPR, this leads to forestry and sustained agriculture (Khalid et al. 2009). Some of studies demonstrated that a biofertilizer prepared by combining PGPR with composts could improve growth-promoting effects and biocontrol aspect of plant disease control (Chen et al. 2011). The ability to form endospores by bacteria allows PGPR, especially *Bacillus* spp., to survive in a wide range of environmental conditions, thus facilitating the effective formulation of biofertilizer (Perez-Garcia et al. 2011). Sufficient densities of PGPR in biofertilizer provide a beneficial role in producing a proper rhizosphere for plant growth and converting nutritionally important elements through biological process. For example, the availability of N, P, and K increases as well as preventing growth of pathogen and its development in the natural environment (Vessey 2003; Waddington 1998). The high availability of N, P, and K can improve soil fertility, increase plant health, and extend microorganisms' survival rates in soil (Yang et al. 2011).

28.6 Concluding Remarks

PGPR is an important alternative to some of the traditional agricultural techniques, and it is now widely in practice. PGPR that live in association with plant roots offer enhanced plant growth by various strategies (direct as well as indirect). It increases soil fertility and plant growth in eco-friendly manner. Soil fertility and plant health can be enhanced through PGPR and crop rhizosphere interactions. PGPR biofertilizers not only promote crop growth but also enhance the resistance against pathogens. A better understanding of the basic principles of the rhizosphere ecology, comprising the function and its diversity of residing microorganisms, is on the way, but advance study is needed to enhance microbial-based technology to benefit plant growth and development in the natural environment. Combined use of PGPR along with organic and inorganic nutrient sources delivers a sustainable agricultural production. There is a necessity of planning systematic methodologies to utilize all the beneficial aspects of PGPR helping their development as consistent components in the management of sustainable agricultural systems and reducing the hazardous impact of chemical fertilizers and pesticides.

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Belowground Microbial Crosstalk and Rhizosphere Biology

29

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Abstract

Microorganisms in the soils and rhizosphere take part in biogeochemical cycles and improve the soil fertility. Diverse chemical substances in root exudates released through root systems also influence the rhizosphere biology. Moreover, structural and physical heterogeneity of soils affects the interactions of rhizosphere microorganisms with plants. The crosstalk in the rhizosphere among different kinds of microorganisms and plants is undertaken through various biochemical mechanisms. Different signaling molecules involved in crosstalk/communications are secreted for the beneficial and/or harmful interactions in the rhizosphere. The initial signal exchange in plant and microbes occurs through the release of root exudates. Enhanced microbial population in the rhizosphere improves the growth of plants by the recycling of nutrients and production of hormones. Moreover, certain microorganisms provide resistance to microbial diseases and tolerance to toxic compounds and abiotic stresses. Thus, crosstalk/communication in the roots of plant and microbes is very much essential for improving crop productivity. This chapter describes microbial communities in the rhizosphere and the associated biological processes these communities perform to sustain chemical communications. With the discovery and characterization of different kinds of secreted compounds in the rhizosphere and by the use of genomic, transcriptomic, proteomic, and metabolomic techniques, our understanding of the signal exchange between microbes and plants has expanded enormously.

Keywords

Microorganisms • Rhizosphere • Root exudates • Signalling molecules • Root microbiome • Biological processes • Hormones

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29.1 Introduction

A close coordination of cell division, cell expansion, and cell differentiation is involved for plant growth and development. The signal communications in the root and shoot system is countered by biotic and abiotic factors. Organic compounds secreted by plants (sugars, organic acids, phenolic compounds, and vitamins) act as nutrients or signals by the microbial populations. The phytohormones or volatile compounds released by microorganisms may activate plant immunity or regulate plant growth and morphogenesis (Ortíz-Castro et al. 2009). Plants interact and compete with other plants, herbivores, and microorganisms for space, water, and mineral nutrients (Ryan and Delhaize 2001; Barea et al. 2005; Bais et al. 2006; Baetz and Martinoia 2014). These interactions of endophytes, mycorrhizal fungi, and nitrogen-fixing bacteria with roots may be beneficial, but the interactions with parasitic plants, bacterial and fungal pathogens, and insects may harm to the plant (Walker et al. 2003).

Microbial cell count up to 10^{11} per gram of soil in the rhizosphere has been reported. The microbial population contains about 30,000 prokaryotic species (Egamberdieva et al. 2008; Badri and Vivanco 2009). These microbial populations in rhizosphere affect interactions of plants and soil environment (Mendes et al. 2013). Plants roots are populated by microbial communities largely known as root microbiome (Hacquard et al. 2015). This root microbiome is selected from different kind of microorganisms inhabiting bulk soil outside the rhizosphere. Some plants shape rhizosphere microbiome with the recruitment of beneficial bacteria or fungi (Berendsen et al. 2012), and host genotype has also been found to influence the pattern and composition of microbial communities (Badri et al. 2013; Bulgarelli et al. 2012, 2015). Moreover, edaphic and environmental factors also affect the composition of microbiome (Chaparro et al. 2012; Hacquard et al. 2015). Interactions of plant roots with rhizosphere microbes influence the plant growth and development (Berendsen et al. 2012; Panke-Buisse et al. 2015) because of their role in nutrient cycling and acquisition of mineralized nutrients (Mishra et al. 2012; Bulgarelli et al. 2013). The communities around the roots also have significant impacts on plant through stress tolerance in fields (Yang et al. 2008; Mendes et al. 2011; Panke-Buisse et al. 2015).

Besides, providing water/nutrients, roots also synthesize and secrete a diverse range of compounds (Flores et al. 1999). Exudates from roots work as attractants and/or repellants in the rhizosphere (Estabrook and Yoder 1998; Bais et al. 2001). Roots influence communities directly in their close space by the release of compounds that may encourage beneficial symbioses and suppress competing species (Nardi et al. 2000). A major part of photosynthetically fixed carbon (5–21%) is reportedly transferred to the rhizosphere via root exudation (Marschner 1995). In natural ecosystems, equilibrium between utilization of metabolites in root exudates is affected by the seasonal variations of the environment (Whipps and Lynch 1986). Therefore, identification of microbial communities is very pertinent to the recent effects of climate change on agricultural productivity (Alexandratos and Bruinsma 2012).

Microorganisms produce diverse chemical signals like quorum-sensing (QS) molecules, e.g., N-acyl homoserine lactones (AHLs) and diffusible signal factor (DSF), antibiotics, phytohormones, and volatile organic compounds (VOCs). These

molecules usually function as inter-kingdom signals that involve interactions with plant hormonal signaling via salicylic acid, jasmonic acid, and ethylene. Recruitment of plant-beneficial rhizosphere microorganisms may result in the symbioses with mycorrhiza and rhizobia, which are initiated by the exchange of specific plant signals (strigolactones and flavonoids, respectively) and microbial signals (Myc and Nod factors, respectively). Plants also use inhibition strategies (e.g., QS mimicry and quenching) to ward off harmful microorganisms. Molecular and genomic tools are applied to uncover complex defense mechanisms and signal cascades that were evolved in plants and microbes over the time (Pieterse and Dicke 2007). Thus, interspecies or inter-kingdom crosstalk during plant–microbe or plant–plant interactions is essential for the proper functioning, health, stability, and ecosystem function (Bais et al. 2004b; Pellegrino and Bedini 2014).

29.2 Rhizosphere Biology

Plant genotypes differentially influence and recruit microbial population of their choice in the rhizosphere. These differences are due to variations in root exudates of the plant species or genotypes (Mukerji et al. 2002) which are affected by the environment, genetic composition, and developmental stage that attracts plant microbiota (Bakker et al. 2012; Hardoim et al. 2015). Plants usually recruit their own microbiome that influences plant competitiveness against stresses and productivity (Berg et al. 2014). However, it is yet to decipher at length which mechanisms invite selections (Agler et al. 2016). Plant roots usually select growth-promoting bacteria (the PGPRs) from the genera *Acinetobacter*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Pseudomonas*, *Rhizobium*, and *Serratia*. These rhizobacteria produce plant growth regulators, solubilize phosphorus, fix the nitrogen, and elicit tolerance against abiotic and biotic stresses (Yang et al. 2008; Bhattacharyya and Jha 2012; Bhardwaj et al. 2014; Pérez-Montaño et al. 2014). Biofertilizers have been found to promote plant growth by supplying fixed nitrogen and mineralized phosphorus to the host (Vessey 2003; Badar and Qureshi 2012; Sindhu et al. 2014). On the other hand, phytostimulators produce phytohormones such as indole acetic acid (IAA), gibberellins (GA_3), and cytokinins that influence root architecture to stimulate plant growth (Spaepen et al. 2007; Duca et al. 2014). The species of *Bacillus*, *Pseudomonas*, *Azospirillum*, *Enterobacter*, *Azotobacter*, *Pantoea*, *Streptomyces*, and *Rhizobium* produce different hormones. *Pseudomonas* spp. (e.g., *P. fluorescens*), *Streptomyces* spp., and *Bacillus* spp. have been found to inhibit the proliferation of the pathogens (Radja Commare et al. 2002; Bhattacharyya and Jha 2012; Sindhu et al. 2016).

PGPRs like *Paenibacillus polymyxa*, *Achromobacter piechaudii*, and *Rhizobium tropici* induce tolerance during drought stress in *Arabidopsis*, tomato (*Solanum lycopersicum*), and common bean (*Phaseolus vulgaris*), respectively, due to degradation of reactive oxygen species and ACC (1-aminocyclopropane-1-carboxylate) (Timmusk and Wagner 1999; Mayak et al. 2004b; Figueiredo et al. 2008; Yang et al. 2008). Salinity tolerance in plants is protected by the inoculation of *Achromobacter piechaudii* and *B. subtilis* (Mayak et al. 2004a; Zhang et al. 2008; Choudhary and

Sindhu 2016). Many endophytic bacteria scavenge nutrients, fix nitrogen, antagonize pathogen, and promote plant growth of staple crop crops (rice, wheat, maize, and millet) ((Montanez et al. 2012; Sessitsch et al. 2012; Gupta et al. 2013; Gond et al. 2015). Diazotrophic endophytes *Klebsiella*, *Enterobacter*, *Bradyrhizobium*, *Alcaligenes*, *Azospirillum*, *Herbaspirillum*, *Ideonella*, *Acetobacter*, and *Acinetobacter* were isolated from wild rice (*Oryza alta*) plants to provide nitrogen to their hosts (Baldani et al. 2000; Chaudhary et al. 2012).

Profiling of root exudates and microbial communities in two *Arabidopsis* mutants having different jasmonate pathway, namely, myc2 and med25, is reported (Carvalhais et al. 2015b). Both mutants showed distinct exudation patterns in comparison to the wild plants. Studies suggest that root exudates modulate changes in microbial communities. Sugar exudation in 28 plant species as determined by regressions of exuded sugars against root mass was demonstrated through periodic samplings of plants (Okubo et al. 2016). Results demonstrated that root symbiotic associations have great impacts on the rate of sugar exudation and free-living microbial communities that inhabit the rhizosphere of legume plants.

Infection with *Pseudomonas syringae* pv. *tomato* (Pst DC3000) selectively recruits rhizobacterium *Bacillus subtilis* FB17 by *Arabidopsis thaliana* (Rudrappa et al. 2008). With Pst DC3000, the secretion of L-malic acid by roots was shown to recruit the rhizobacterium. Transcriptomics deciphered that *B. subtilis* FB17 interaction causes alteration in the expression of *Arabidopsis* genes that involves regulation of auxin production, metabolism, defense, and stress responses and also caused modifications in the cell wall (Lakshmanan et al. 2012). *B. subtilis* population increased in response to aphid attack of foliage in *Capsicum annuum* and correlate with declining pathogen *Ralstonia solanacearum* population (Lee et al. 2012). Such communication strategies may be highjacked by parasitic organisms (Morris et al. 1998; Subramanian et al. 2007; Cameron et al. 2013) as is the case of pathogen *Phytophthora sojae*. The parasitic weed *Striga* perceives strigolactones for colonization on wheat host plants (Scholes and Press 2008). Therefore, understanding of microbe–microbe interactions and their effects on the population of microbial communities is essential to identify the microbial determinants, which shape microbial communities.

Soil pH, CO_2 concentration, air temperature, and nutrients, e.g., carbon, nitrogen, and phosphate, affect pathogenic abundance and beneficial microbe distribution (Duffy et al. 1997; Lacey and Wilson 2001; Dumbrell et al. 2010; Liu et al. 2017). The hormones involved in plant immunity shape the root microbiome (Lebeis et al. 2015) and enhance the availability of small molecule chemicals in rhizosphere (Lynch and Whipps 1990; Bardgett et al. 1998; Bever et al. 2012; Miransari 2013). Microbial communities with high bacterial cells in the root tip and root hair zone were observed in wild oat roots than the in bulk soils (DeAngelis et al. 2009). The association of different microbial communities with plant roots enriches specific microbes in the rhizosphere (Berendsen et al. 2012).

The root exudate composition in plant species and genotypes varies with the plant age and stress exposure also (Haichar et al. 2008; Compant et al. 2010; Bever et al. 2012; Perez-Jaramillo et al. 2015), and certain specific exudates released by plants may attract or repel specific microbial communities (Grayston et al. 1998; Bertin et al. 2003;

Kumar et al. 2007; Marschner et al. 2011). Root exudates from certain plants attract symbiotic microbes that help improve their nutrient supply and uptake (Parniske 2008; Marschner et al. 2011; Oldroyd 2013). Many others produce siderophores to increase the soluble iron uptake and availability of iron for the plants through their roots (Hartmann et al. 2009; Carvalhais et al. 2013). Some plant roots release strigolactones to attract mycorrhiza for improving phosphate and nitrogen supply (Akiyama et al. 2005). Specific flavonoids from legumes influence symbiosis with N-fixing rhizobia (Morris et al. 1998; Bertin et al. 2003; Hassan and Mathesius 2011). Climatic change is going to influence rhizosphere biology by modifying the root exudation pattern, rate, composition, and biogeochemical cycling (Liu et al. 2017). Therefore, understanding the rhizosphere biology in context to climatic change and reflections thereon is greatly needed to harness beneficial microbial interactions as a low-input biotechnology to secure agricultural sustainability (Dubey et al. 2016).

29.2.1 Effect of Beneficial Microorganisms on Suppression of Pathogens

Beneficial microbial communities in agricultural soils are known to influence population of pathogenic microbes negatively and maintain suppression of the growth of pathogens through secretion of metabolites (Doornbos and van Loon 2012). Certain bacteria *Bacillus amyloliquefaciens* and *Pseudomonas fluorescens* contain large gene clusters that are involved to detoxify and produce/release of antibiotics and siderophores (Paulsen et al. 2005; Chen et al. 2007). Different antibiotic compounds secreted by antagonistic microbes include 2,4-diacetylphloroglucinol (DAPG), phenazine carboxylic acid (PCA), and oomycin A (van Loon and Bakker 2006). Antibiotics limit pathogenic growth in soils (Raaijmakers and Mazzola 2012). In addition, microbes also produce other secondary metabolites and hydrolytic enzymes that alter the plant signaling and metabolism (Constacurta and Vanderleyden 1995; Kim et al. 2011a, b).

Root exudation may be altered after microbial inoculation/infection, and the release of specific exudates may attract selective enrichment of microbial composition (Prikyrl et al. 1985; Bulgarelli et al. 2013). Secretion of antibiotic phenazime-1-carboxylic acid and 2,4-DAPG by the *Pseudomonas* spp. caused suppression of *Rhizoctonia solani* (Mendes et al. 2011; Raaijmakers et al. 1997), while the production of lipoproteins by *Pseudomonas* and *Bacillus* spp. suppressed a wide range of plant pathogens (Raaijmakers et al. 2010; Watrous et al. 2012; Zachow et al. 2015). 2,4-DAPG-producing *Pseudomonas* spp. limited the growth of *Gaeumannomyces graminis* var. *tritici* causing take-all disease (TAD) by impairing ATP synthesis (Weller et al. 2002). The 2,4-DAPG concentration by the producing bacteria and the take-all disease severity were inversely proportional (Raaijmakers and Weller 1998). Thus, microbes that produce secondary metabolites, i.e., antibiotics, toxins, lytic enzymes, and siderophores, may outcompete pathogens at various levels of disease development process (Thomashow and Weller 1988; Bais et al. 2006; van Loon and Bakker 2006; Kim et al. 2011a).

Species of *Pseudomonas*, including *Pseudomonas cepacia*, *P. fluorescens*, *P. syringae*, *P. aeruginosa*, and *P. aureofaciens*, produce hydrogen cyanide (HCN), 2,4-diacetylphloroglucinol, pyrrolnitrin, phenazine, oomycin A, and other compounds that protect plants against diseases (Burkhead et al. 1994; Ligon et al. 2000; Haas and Keel 2003; Mansfield et al. 2012). The production of these compounds depends on different factors (Duffy and Défago 1999). The production of hydrogen cyanide is affected by light and temperature (Vickery et al. 1987), while acidic pH facilitates production of pyrrolnitrin (Hwang et al. 2002). Therefore, changes in soil conditions coupled with the climate changes affect antibiotic production in beneficial microbes (Davidson and Janssens 2006; Frey et al. 2013).

29.2.2 Establishment and Performance of Introduced Microbial Inoculants

Growth-promoting *Azospirillum* species improve growth and biomass of host plants and is used for biofertilization (Bashan and Holguin 1997; Veresoglou and Menexes 2010). Increased grain yield and oil content were obtained in rapeseed (*Brassica napus*) with application of composite inoculum of *Azospirillum* spp. and *Azotobacter* spp. (Namvar and Khandan 2015). The effects were attributed to indole acetic acid, gibberellins, a variety of polyamines and amino acids produced by bacteria, and increased nutrient availability to plants (Thuler et al. 2003; Bashan and de Bashan 2010; Veresoglou and Menexes 2010). Entomopathogenic *Bacillus thuringiensis* is applied to protect crops from specific pests of foliage crops (Sanchis and Bourguet 2008). A biomix of PGPR strains consisting of *Bacillus pumilus*, *Bacillus subtilis*, and *Curtobacterium flaccumfaciens* to cucumber seeds enhanced the biological control of several cucumber pathogens and also increased the plant growth (Raupach and Kloepper 1998).

The importance of arbuscular mycorrhizal fungi (AMF) is also recognized for their ability to increase host access to mineral nutrients, predominantly phosphate. Kourtev et al. (2002) reported a higher abundance of AMF associated with invasive plant species (Japanese barberry and Japanese stilt grass) in comparison to the co-occurring native blueberry plant. The plants under invasion alter the soil microflora for their own benefit, e.g., by stimulating their own association with AMF (Callaway et al. 2004). On the other hand, invasive *Brassicaceae* weeds such as garlic mustard (*Alliaria petiolata*) produced glucosinolates, which led to a decline in the abundance and function of AMF (Roberts and Anderson 2001). The presence of AMF has been associated with reduced bacterial pathogens (Parniske 2008). The coinoculation of the prairie legume *Amorpha canescens* with AMF and rhizobial bacteria increases plant biomass (Larimer et al. 2014) suggesting a synergistic effect of rhizobia with AMF on *A. canescens* to increase productivity (Heijden et al. 2015).

T. harzianum is a commercially used biofungicide. Application of *T. harzianum* as biofertilizer in tomato reduced the need of chemical fertilizers by 25% without affecting the yield (Cai et al. 2014). Its application was also associated with the reductions in disease severity caused by *Fusarium* wilt (Chen et al. 2012). Usually composite PGPR strains were more effective than individual strains in suppressing disease or improving plant growth (Perez-Piqueres et al. 2006; Berg and Smalla 2009; Ahemad and Khan 2011; Yang et al. 2011). The coinoculation of *Cicer arietinum* (chickpea) with *P. indica* and *Pseudomonas striata* caused increase of *P. striata* in the rhizosphere (Meena et al. 2010). Plant-beneficial effects were also observed; synergistic effect increases in *P. striata* population. The inoculation of *C. arietinum* with the *Glomus intraradices*, *Pseudomonas alcaligenes*, and *Bacillus pumilus* reduced impact of *M. phaseolina* (root-rot fungus) and *M. incognita* (root-knot nematode) in comparison to the individual- and dual-strain inoculants and control indicating synergy in AMF and bacterial treatment for controlling *Macrophomina phaseolina* and *Meloidogyne incognita* in *C. arietinum* (Akhtar and Siddiqui 2008).

Similarly, composite inoculum of *Pseudomonas putida* with nodule-inducing *Sinorhizobium meliloti* in the legume *Medicago sativa* caused increased nodulation and plant biomass (Guinazu et al. 2009). Coinoculation of two PGPR strains in tomato showed higher impacts in composite PGPR application under 75% fertilizer dose as compared to a control with full fertilizer dose having no PGPR inoculants (Hernandez and Chailloux 2004). Treatments with PGPR, mycorrhizal fungi and fertilizer (half dose) exhibited greater yield in field in comparison to the full dose of fertilizer as control (Hernandez and Chailloux 2004). Formulation of compost and beneficial bacteria also suppressed plant pathogens (Pugliese et al. 2011; Yang et al. 2011). Four strains of PGPRs produced siderophore, HCN, and IAA and showed protease and β-1,3-glucanase activities, whereas *S. meliloti* strain that produced IAA and solubilized insoluble phosphate was tested (Sarhan and Shehata 2014). Inoculation of *Bradyrhizobium* strain GSA11 and *Rhizobium* strain GSA110 caused 141.94 and 151.43% increase in dry shoot weight, respectively, under chillum jar conditions after 60 days of plant growth (Choudhary and Sindhu 2016).

Endophytes are proved as for their role in herbicide tolerance in plants, and several bacterial endophytes were found to degrade various herbicides (Tetard-Jones and Edwards 2016). The endophyte strain *Pseudomonas putida* POPHV6 from poplar tree stem showed degradation of 2,4-D and led to lower herbicide accumulation in aerial tissues (Germaine et al. 2006). Similarly, plant-associated bacteria were identified to degrade and detoxify the atrazine or glyphosate herbicides (Kuklinsky-Sobral et al. 2005; Ngigi et al. 2012). Kong et al. (2017) determined the PGP trait in nodule endophytic *Pseudomonas brassicacearum* Zy-2-1 being a coinoculant of *Medicago lupulina* with *Sinorhizobium meliloti* under copper (Cu) stress condition. Coinoculation of *S. meliloti* with Zy-2-1 increased root weight, plant dry weight, nodules, nodule weight, and N-content of *M. lupulina* plant at a concentration of 100 or 300 mg/kg Cu^{2+}.

29.3 Signal Molecule-Mediated Communication Between Microorganisms and Plants

Roots are key sites for microbial interactions with plants and with its surroundings (Bais et al. 2006). Diverse compounds such as amino acids (AAs), organic acids (OAs), phenolics, alkaloids, flavonols, glucosinolates, indole compounds, fatty acids, polysaccharides, and proteins that act as signals or perform other activities are secreted by roots in the rhizosphere soil (Weston and Mathesius 2013; Li et al. 2014; Zhang et al. 2014). Microbial diversity in the soil can benefit plant health and crop productivity, and root-exuded compounds may prevent the growth of harmful microbes (Bais et al. 2006; Dutta et al. 2013; Li et al. 2013). Root exudates also favor control of abiotic and biotic stresses, and phytochemicals collected from the root exudates of *Arabidopsis thaliana* showed the modulation of microbial composition (Badri et al. 2013). Legumes release flavonoids to attract rhizobia for symbiosis for to provide fixed nitrogen (Zhang et al. 2009) and synthesize and secrete nodulation factor needed for symbiosis (Berge et al. 2009). Such symbiotic association adds up to 50–70 × 10^6 tons of fixed nitrogen annually into the soils (Herridge et al. 2008) reducing the need for N-fertilizers. Similarly, arbuscular mycorrhizal fungi (AMF) benefit plants spreading their complicated and long hyphal networks into the soils for the acquisition of nutrients, especially phosphorous which is made available to their hosts (Klironomos et al. 2000; Jeffries et al. 2003). Improved understanding on fundamental mechanisms and their ecological balances can lead to better exploitation and manipulation of these signals for crop production and protection (Rasmann and Turlings 2016).

29.3.1 Signals Involved in Communication in Bacterial Cells

Signal molecule-mediated crosstalk is reported to be associated with change in metabolism (An et al. 2014), virulence-associated control (Sperandio et al. 2002; Chu et al. 2011), and propagation (Rocha et al. 2012). Regulation in bacterial behavior is linked with the population density, the stimuli of which clubbed with the response is quorum sensing (QS) (Miller and Bassler 2001; An et al. 2014). Signal may be intercepted and responded by non-related organisms that may use this for competitive advantage by altering the behavior of unrelated preceptor (Atkinson and Williams 2009). Signals may get degraded by other microorganisms in the niche also (Dong et al. 2000; Molina et al. 2003; Newton and Fray 2004). Many of communication systems utilized by microorganisms differ in the type of chemical compounds produced as signal molecules and the molecular machinery used to receive and respond.

29.3.1.1 Quorum Sensing and Performance of Bacterial Population

Quorum-sensing (QS) process allows microbes to interact with other microbes in the environment and work at population-wide scale for changes accordingly to the species present in the communities (Waters and Bassler 2005). Various physiological mechanisms and regulations work in a QS-dependent way by autoinducer molecules N-acyl

homoserine lactones (AHLs) in Gram-negative bacteria (Li and Tian 2012), and this has benefitted bacterial population to communicate as multicellular organisms during adaptation to the changing environments, colonization, biofilm formation, and defense against competitors (Waters and Bassler 2005). N-acyl homoserine lactones (AHLs) are identified as the principal signal molecules for QS and cell-to-cell communication in bacteria (Dong and Zhang 2005). There are several other chemicals and enzymes also that target various components of the bacterial QS system and disrupt the process which is called quorum quenching (QQ) (Hong et al. 2012; Koul and Kalia 2017). The first QQ enzyme, an AHL lactonase, was isolated from *Bacillus* sp. 240B1 (Dong et al. 2000, 2001). Thus, by targeting of the QS signals either by inhibition of AHL biosynthesis or by degradation of AHLs, these QQ molecules could be developed for agricultural applications as a strategy to avoid bacterial pathogenicity. *Bacillus cereus* U92 is one of the most efficient quenching strains that can be used as a biocontrol agent in crops like tomato and potato (Zamani et al. 2013).

Besides AHLs, there exist several other chemically distinct classes of QS molecules in Gram-negative bacteria (Table 29.1) (Sperandio et al. 2003; Vendeville

Table 29.1 Different effects on plants after application of pure AHL molecules or inoculation with AHL-producing bacteria

AHL produced	Effect on plant growth	Plant	References
C4-HSL, C6-HSL, C8-HSL	Primary root growth	*Arabidopsis*	von Rad et al. (2008) Liu et al. (2012)
Serratia liquefaciens (C4-HSL, C6-HSL)	Resistance against necrotrophic pathogens, SA levels, defense-gene regulation	Tomato	Schuhegger et al. (2006)
Oxo-C6-HSL	Primary root growth, calmodulin signaling	*Arabidopsis*	Zhao et al. (2015)
C6-HSL	Herbivore susceptibility	*N. attenuata*	Heidel et al. (2010)
C6-HSL, C8-HSL	Root growth, plant biomass increase	*Arabidopsis*	Schenk et al. (2012)
Oxo-C8-HSL	Primary root growth promotion, ethylene level	*Arabidopsis*	Palmer et al. (2014)
Oxo-C10-HSL	Auxin-induced adventitious root formation	Mung beans	Bai et al. (2012)
C10-HSL, C12-HSL	Inhibition of primary root growth	*Arabidopsis*	Zhao et al. (2015)
Sinorhizobium meliloti (oxo-C14-HSL)	Enhanced nodulation in roots	*Medicago truncatula*	Veliz-Vallejos et al. (2014)
Oxo-C14-HSL	SA-/oxylipin-related defense against biotrophic pathogens	*Arabidopsis*	Schenk et al. (2014)
Sinorhizobium meliloti (oxo-C14-HSL)	Resistance against biotrophic pathogens	Tomato, barley, wheat, *Arabidopsis*	Hernandez-Reyes et al. (2014)

et al. 2005; Williams 2007). AHL-mediated QS is extensively reported in bacteria *Vibrio* (Milton 2006), *Pseudomonas* (Williams and Cámara 2009), *Rhizobium* (Sanchez-Contreras et al. 2007), *Erwinia* (Barnard and Salmond 2007), *Agrobacterium* (White and Winans 2007), and *Yersinia* (Atkinson et al. 2006). The (AI-2)/LuxS QS is reported to be shared by both Gram-negative and Gram-positive bacteria (Winzer et al. 2002; Vendeville et al. 2005).

Different genera/species that produce similar AHL may be capable of crosstalk. *P. aeruginosa*, *Serratia liquefaciens*, and *Aeromonas hydrophila* produce the N-butanoyl-homoserine lactone (C4-HSL) (Winson et al. 1995; Swift et al. 1997), but other bacteria *C. violaceum* respond to short chain AHL-producing bacteria by producing the purple pigment violacein (McClean 1997). Quorum sensing in bacteria such as *C. violaceum* and *A. hydrophila* is antagonized by long-chain AHLs, i.e., the latter inhibit violacein and exoenzyme production, respectively (McClean 1997; Swift et al. 1999). In contrast, *P. aeruginosa* produces both long (3-oxo-C12-HSL)- and short (e.g., C4-HSL)-chain AHLs. However, 3-oxo-C12-HSL does not antagonize the C4-HSL-dependent activation of RhlR in *P. aeruginosa*, whereas RhlR is inhibited by 3-oxo-C12-HSL and its expression in *E. coli* (Winzer et al. 2000). These observations indicated that there must be some compartmentalization of the hierarchical *las* and *rhl* QS systems.

Cha et al. (1998) reported that AHLs are produced by all 106 plant-associated bacteria from seven genera excluding *Xanthomonas campestris* which does not employ AHLs for cell-to-cell communication system but causes black rot in cruciferous crops like cabbages (Onsando 1992) by producing extracellular enzymes proteases, pectinases, and cellulases (Dow and Daniels 1994). The expression of exoenzymes and the control of biofilm dispersal, resistance to toxin, and survival (He et al. 2006) depend on a small diffusible signal factor (DSF) (Slater et al. 2000) that is characterized as cis-11-methyl-2-dodecenoic acid (Wang et al. 2004). Therefore, DSF-mediated intercellular signaling is clubbed to intracellular signaling via c-di-GMP (Ryan et al. 2006). DSF is also produced by *Stenotrophomonas maltophilia* together with seven other structurally related C12–C14 fatty acids (Fouhy et al. 2007; Boon et al. 2008). The *S. maltophilia rpf* locus showed high homology to that of *X. campestris*, and the DSF signal was associated in regulating virulence-associated phenotypes including swimming motility, extracellular protease, and lipopolysaccharide along with antibiotic resistance. An *rpf*F homologue from *Burkholderia cenocepacia* was highly conserved in this genus and regulates synthesis of DSF-related signal, cis-2-dodecenoic acid.

The DSF family comprises cis-2-unsaturated fatty acids of differing length and branching (Deng et al. 2011; Ryan et al. 2015). Besides cis-11-methyl-2-dodecenoic acid from *Xanthomonas campestris* pv. *campestris* (Xcc) (Barber et al. 1997; Wang et al. 2004), other members of the family were reported in *Pseudomonas aeruginosa* (cis-2-decenoic acid), *Xanthomonas oryzae* (cis, cis-11 methyldodeca-2,5-dienoic acid; CDSF), *Burkholderia cenocepacia* (cis-2-dodecenoic acid; BDSF), and *Xylella fastidiosa* (cis-2-tetradecenoic acid; XfDSF; cis-2-hexadecenoic acid; XfDSF2) (Beaulieu et al. 2013; Ionescu et al. 2016). Many bacteria except *P. aeruginosa* produce multiple signals from DSF family, and each genus seems

responsive to the major signal (Ionescu et al. 2013, 2016). *P. aeruginosa* is capable of sensing DSF or BDSF molecules for bacterial behavior like altered biofilm and increased antibiotic tolerance (Ryan et al. 2008). Moreover, *B. cenocepacia* and *P. aeruginosa* have additional QS systems mediated by N-acyl homoserine lactones and alkyl quinolones, and there is evidence of regulatory interplay between these different systems (Schmid et al. 2012; Udine et al. 2013). Cis-2-decenoic acid induces biofilm dispersal in Gram-negative and Gram-positive bacteria that do not produce DSF family signals (Marques et al. 2015) and improve the efficacy of antibiotic action in a number of organisms. DSF can induce defense-related responses in plants also (Kakkar et al. 2015).

Fray et al. (1999) introduced *Yersinia enterocolitica* AHL synthase gene *yen*I into tobacco plants to determine whether AHLs could be made in planta. YenI directed the synthesis of a 1:1 mixture of 3-oxo-C6-HSL and C6-HSL (Throup et al. 1995). Transgenic tobacco plants synthesized the same two AHLs in a similar ratio. The YenI protein was directed to the chloroplasts because the AHL precursors were abundantly present. Similarly, the *P. aeruginosa* AHL synthase gene *las*I (synthesizes 3-oxo-C12-HSL) was introduced in tobacco individually and in combination with *yen*I (Scott et al. 2006). Transgenic plants produced physiologically significant levels of 3-oxo-C12-HSL and double transformant produced both the long- and short-chain AHLs. AHLs were also detected in root exudates and in the rhizosphere and non-rhizosphere soil collected from transgenically grown plants (Scott et al. 2006). Similarly, *yen*I has also been introduced into potato (Toth et al. 2004) and transgenic potatoes produced both 3-oxo-C6-HSL and C6-HSL. The stems contained higher AHL levels than the tubers.

29.3.2 Crosstalk Between Microorganisms and Plants

In signal molecules produced by microorganisms, intra-domain signal-mediated bacterial communications may also act on the plants. The effect was observed in some bacterial and fungal strains from maize and bean rhizosphere (Prikyrl et al. 1985). The process may stimulate carbohydrate release from the plant cell wall (Kim et al. 2011a). Similarly, production and release of indole compounds (bacterial signals) also cause cooperative activities like production of virulence factors, formation of biofilms, and manipulation of plant root development through interference with auxin signaling (Bailly et al. 2014). The early stage of legume–*Rhizobium* symbiosis and mycorrhiza formation by AMF indicates inter-domain communication for successful establishment (Kosuta et al. 2003; Olah et al. 2005; Janczarek et al. 2015; Sun et al. 2015). Likewise, volatile organic compounds (VOCs) produced by PGPRs promoted growth in *Arabidopsis thaliana* (Ryu et al. 2003) and ISR in plants, stimulating expression of defense genes effective against fungi, bacteria, oomycetes, and viruses (Heil and Bostock 2002; Zhang et al. 2002).

Exposure to AHL from *Serratia liquefaciens* MG1 and *Pseudomonas putida* IsoF increased resistance in tomato against fungal pathogen *Alternaria alternata* by inducing ethylene and SA-dependent defense genes (Schuhegger et al. 2006). The

AHL N-3-oxo-tetradecanoyl-L-homoserine lactone also supported pathogen defense in *Arabidopsis* due to several prominent mechanisms in response to *Pseudomonas syringae* (Schenk et al. 2014). There are reports that bacteria-derived signals may modulate fungal development under specific conditions. Secondary metabolites from *Pseudomonas aeruginosa* facilitated *Aspergillus fumigatus* development (Zheng et al. 2015).

29.3.2.1 Signal Molecules Released by Plants

Plant root exudates include ions, enzymes, mucilage, and a diverse type of carbon-containing primary and secondary metabolites (Bertin et al. 2003) secreted in rhizosphere (Fig. 29.1). Low-molecular-weight compounds (amino acids, organic acids, sugars, flavonoids, aliphatic acids, fatty acids, and small molecule secondary metabolites) are prevalent, while high-molecular-weight compounds (mucilage, polysaccharides, peptides, and proteins) are less diverse but often compose a larger proportion of the root exudates (Marschner 1995).

Secretion of different compounds from different plants (Rovira 1969), ecotypes (Micallef et al. 2009), and even in distinct portion of roots within a plant (Uren 2007) has been reported. These compounds attract microbes in rhizosphere and initiate symbiotic and pathogenic interactions (Bais et al. 2006). Root exudate composition and concentration also vary (De-la-Pena et al. 2010) with biotic and abiotic factors (Tang et al. 1995; Flores et al. 1999). The diverse compounds released in root exudates create a unique nutrient-rich environment in the rhizosphere (Badri et al. 2009b). These compounds may be used as nutrient or growth substrates by soil microorganisms (Vandenkoornhuyse et al. 2007) or act as antimicrobials (Bais et al. 2006; Perry et al. 2007). Some bacteria secrete antimicrobial metabolites cyclic lipopeptide surfactin and iturin A that shield roots against pathogenic fungi like *Rhizoctonia* spp. or pathogenic Gram-negative bacteria *P. syringae* (Asaka and Shoda 1996; Bais et al. 2004a). Therefore, the population of microbes that inhabit the rhizosphere also changes with the composition of the exudates (Badri et al. 2009a).

Legumes release specific flavonoids to chemically attract and initiate symbiotic relationships with rhizobia (Zhang et al. 2009). Maize (*Zea mays*) also secretes a benzoxazinoid 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one to attract the rhizobacterium *Pseudomonas putida* KT2440 that repels other pathogenic microbes in the maize rhizosphere (Neal et al. 2012). Likewise, *Arabidopsis* infection by *Pseudomonas syringae* pv. *tomato* DC3000 caused the expression of L-malic acid (MA) transporter and increased the secretion of malic acid (MA) by roots (Rudrappa et al. 2008; Lakshmanan et al. 2012). MA abundance in rhizosphere recruits beneficial rhizobacterium *B. subtilis* FB17 and promotes biofilm formation by *B. subtilis* FB17 on *Arabidopsis* roots (Lakshmanan et al. 2013). It further produces systemic resistance responses against the pathogen.

Phenolics are released in the root exudates and are involved in pathogen suppression (Bais et al. 2005; Lanoue et al. 2009; Badri et al. 2013). This antagonistic function may be direct or indirect (Ling et al. 2013). Phenolic compounds can affect the pathogen indirectly through modulation of expression of antibiotic-related genes (de Werra et al. 2011). Thus, rhizosphere is enriched with mutualistic microbes to

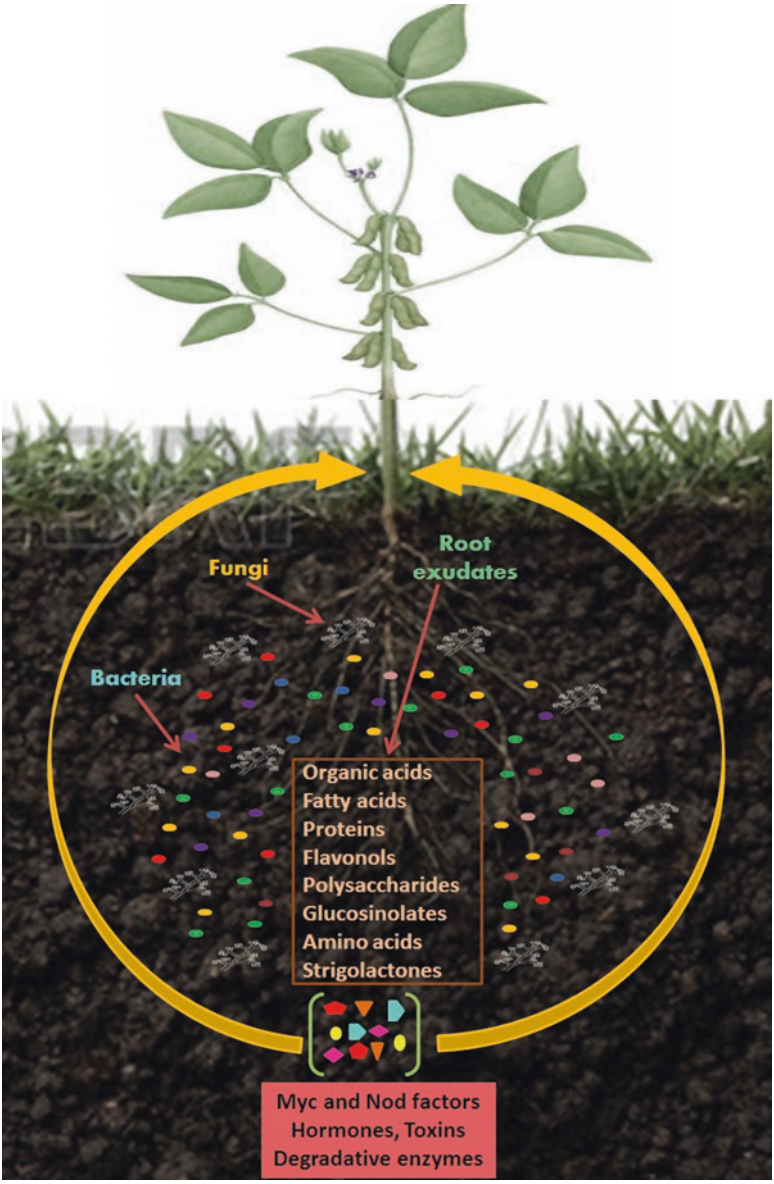

Fig. 29.1 Overview of interactions between plants, fungi, and bacteria in the rhizosphere. Microbial communities in the rhizosphere communicate with each other and the plant roots using a variety of mechanisms. Microorganisms in the soil are chemoattracted by diverse compounds released in root exudates. Some microbes synthesize Nod factors and Myc factors as signal molecule for nodule development and mycorrhiza formation. Some microorganisms produce hormones (auxins, salicylic acid, jasmonic acid, and ethylene) or toxins that control growth and plant defense responses. The interaction of microbial communities leads to improved plant health when plant roots establish beneficial interactions with root microbes

protect plants against pathogens (Qiu et al. 2013; Li et al. 2015) enriching it with antimicrobial compounds and lytic enzymes as weapon against pathogens (Berendsen et al. 2012; Yu et al. 2014).

29.3.2.2 Hormones

Hormones produced or released by microorganisms or plants play important roles in plant growth and signaling toward plant responses to biotic and abiotic stresses (Bari and Jones 2009). Auxin, brassinosteroid, and gibberellins are key hormones for the regulation of plant growth. Others, e.g., abscisic acid (ABA), cytokinin (CK), and peptides are also implicated as plant defense signals. Among 50 bacteria isolated from plant rhizosphere, 86, 58, and 90% isolates produced auxin-, gibberellin-, and kinetin-like substances, respectively (Barea et al. 1976). Hormones like salicylic acid (SA), jasmonates (JA), and ethylene (ET) are produced under the conditions of biotic and abiotic stresses. There exists hormone-mediated crosstalk between different pathways in plants (Depuydt and Hardtke 2011) suggesting that auxin, gibberellin, and brassinosteroid signaling arose during terrestrial evolution of plants and organisms.

Auxins Production

The production of indole-3-acetic acid (IAA) in specifically defined culture media is reported in PGPR strains including *Azotobacter chroococcum* (Muller et al. 1989), *Azospirillum* (Bar and Okon 1992; Remans et al. 2008), *Rhizobium* species (Hirsch and Fang 1994), *Bacillus polymyxa* (Holl et al. 1988), *Pseudomonas fluorescens* (Dubeikovsky et al. 1993), and *Pseudomonas putida* (Taghavi et al. 2009). IAA affects plant growth and pathogenesis (Spaepen et al. 2007; Park et al. 2015). Bacterial secretion of IAA is reported to stimulate root growth (Spaepen et al. 2007) that may enhance uptake of nutrients by the associated plants (Lifshitz et al. 1987). The effect of IAA on plants is inversely concentration dependent, and low concentrations can promote root growth, whereas high concentration can inhibit root growth (Arshad and Frankenberger 1992). The inoculation response of *A. brasilense* Sp245 to common bean (*Phaseolus vulgaris* L.) is related to bacterial auxin produced (Remans et al. 2008). Plant growth promotion after inoculation with PGPRs is attributed to secretion of IAA by *Azospirillum brasilense* (Okon and Vanderleyden 1997), *Rhizobium* species (Hirsch and Fang 1994), and *Xanthomonas* and *Pseudomonas* (Patten and Glick 1996; Zhang et al. 1997). Different biosynthesis pathways were identified for IAA biosynthesis among plant-associated bacterial species (Spaepen et al. 2007).

Strains of *Rhodopseudomonas* spp. KL9 and BL6 show growth enhancement of tomato seedlings under axenic conditions along with the IAA and aminolevulinic acid (ALA) production (Hyun and Song 2007). Seed bacterization of chickpea cultivar C235 with *Pseudomonas* producing IAA shows stunting effect on the root and shoot at 5 and 10 days of seedling growth (Malik and Sindhu 2011). Increased nodule number and nodule biomass were observed on coinoculation of chickpea with IAA-producing *Pseudomonas* strains and *Mesorhizobium* sp. *Cicer* strain Ca181. Bacteria *Bacillus subtilis*, *B. flexus*, *B. cereus*, *B. megaterium*, and *B.*

endophyticus produced IAA up to 4.0–24.3 μg ml^{-1} (Pena-Yam et al. 2016). Inoculation of chili pepper (*Capsicum annuum* L. cv. Jalapeño) with *B. subtilis* strain ITC-N67 showed an increase in stem diameter and root volume.

However, deleterious effects of IAA-producing rhizobacteria are recorded by many strains including *Enterobacter taylorae*, *Klebsiella planticola*, *Alcaligenes faecalis*, *Xanthomonas maltophilia*, *Pseudomonas* sp., and *Flavobacterium* sp. (Sarwar and Kremmer 1995; Suzuki et al. 2003). Mutants of *Pseudomonas putida* producing high amount of IAA inhibit root growth in canola seedlings (*Brassica campestris*) (Xie et al. 1996) showing ambiguity about the effects of IAA on growth of root, shoot, and rate of seedling emergence (de Freitas and Germida 1990; Sarwar and Kremmer 1995; Barazani and Friedman 1999). Inoculation of IAA producer *Enterobacter* sp. I-3 in lettuce and radish seedlings showed reduced biomass production (Park et al. 2015). Under *in vitro* studies, addition of tryptophan to the culture exudate significantly reduced the root length, leaf width, leaf length, and lateral roots. Growth retardation effects on the root and shoot of *Phalaris minor* seedlings were noticed after inoculation of SYB101 and CPS67 with varied IAA and ALA production capacity (Phour 2012). Cuttings of sour cherry (*Prunus cerasus*) and black currant (*Ribes nigrum*) were inoculated with a recombinant strain of *P. fluorescens* which produce high IAA (Dubeikovsky et al. 1993). High bacterial density on roots of cherry cuttings inhibited root growth, but low densities on black currant promoted the growth. Sarwar and Kremer (1995) showed that an *Enterobacter taylorae* strain with high auxin-producing capacity (72 μg ml^{-1}) inhibited growth of *Convolvulus arvensis*. Mejri et al. (2010) reported that IAA from *Pseudomonas trivialis* X33d suppressed growth of great brome weed but promoted growth of durum wheat. Thus, effective IAA-producing rhizobacterial strains can be subsequently applied as biofertilizers and bioherbicides (Harding and Raizada 2015; Hernandez-Leon et al. 2015).

In *A. tumefaciens*, IAA inhibits virulence (*vir*) gene expression by competing with induced phenolics acetosyringone interacting with VirA (Liu and Nester 2006). Thus, *vir* gene inhibition by IAA may be a putative negative feedback system upon increased IAA production in transformed plant cells. In *P. syringae* pv. *syringae*, IAA involves the expression of syringomycin synthesis that is required for total virulence of *P. syringae* pv. *syringae* on stone fruits (Xu and Gross 1988b). IAA^- mutants of *P. syringae* pv. *syringae* were significantly reduced in syringomycin production (Mazzola and White 1994). The use of an *iaa*M deletion mutant of *Erwinia chrysanthemi* 3937 showed positive role of IAA on Type III secretion system (TTSS) and exoenzymes through Gac-Rsm posttranscriptional regulatory pathway. The expression level of oligogalacturonate lyase (ogl) and three endo-pectate lyases, *pel*D, *pel*I, and *pel*L, was reduced in the *iaa*M mutant as compared to wild-type *E. chrysanthemi* strain 3937. Moreover, transcription of TTSS genes, *dsp*E (a putative TTSS effector) and *hrp*N (TTSS hairpin), is reduced in *iaa*M mutant of *E. chrysanthemi* 3937 (Yang et al. 2007). IAA may enhance protection against damage by adverse conditions in *E. coli* (Bianco et al. 2006a) and induce the expression of genes related to survival under stress conditions. The molecule can interact with cell wall peroxidases and induce reactive oxygen

species (ROS) formation in the cell wall (Kawano et al. 2001). Genes of tricarboxylic acid cycle (TCA), glyoxylate shunt, and amino acid biosynthesis (leucine, isoleucine, valine, and proline) in the central metabolic pathways were upregulated by IAA in *E. coli* (Bianco et al. 2006b). Liu and Nester (2006) showed that IAA at 200 mM reduced growth of various plant-associated bacteria but not of that which occupy other ecological niches.

Addition of IAA to the culture medium provokes invasive growth and expression of genes in unicellular *Saccharomyces cerevisiae* (Prusty et al. 2004). A gene involved in adhesion, FLO11 was also induced by IAA suggesting that FLO11 activation by elevated concentrations of IAA which occur at plant wound might be crucial for infecting wound sites in plants (Verstrepen and Klis 2006). For cyanobacteria, IAA triggers differentiation of cyanobacterial hormogonia (Bunt 1961). Overall, IAA plays a key role in modulating level of the alarmone guanosine 5′-diphosphate 3′-diphosphate (ppGpp) in plant chloroplasts. In bacteria, ppGpp mediates the "stringent control" upon stress conditions (Braeken et al. 2006). Takahashi et al. (2004) for the first time detected ppGpp in chloroplasts of plant cells.

Cytokinins

Cytokinins produced by microorganisms (Persello-Cartieaux et al. 2003) are N6-substituted aminopurines that influence physiological and developmental processes (Salisbury and Ross 1992; Maheshwari et al. 2015). Plant responds to exogenous applications of cytokinin that stimulate cell division, root development, root hair formation, inhibit root elongation, shoot initiation, and/or certain other physiological parameters (Amara et al. 2015; Jha and Saraf 2015). Cytokinin production is well characterized in plant-associated microorganisms (Kado 1984) which belong to *Pseudomonas*, *Azospirillum*, and *Bacillus* and are isolated from a range of species, e.g., barley, canola, bean, and *Arabidopsis* (Alexandre et al. 1996; Persello-Cartieaux et al. 2001). The growth of *A. thaliana* and *P. vulgaris* seedlings was enhanced by a *Bacillus megaterium* that produced cytokinins (Ortíz-Castro et al. 2008a; Ortíz-Castro et al. 2008b). Other bacterial genera producing cytokinins are *Proteus*, *Klebsiella*, *Escherichia*, *Pseudomonas*, and *Xanthomonas* (Maheshwari et al. 2015).

The production of cytokinin-like substance (zeatin) was reported in culture filtrates of *Rhizobium leguminosarum* and *Bradyrhizobium japonicum* 61A68 (Phillips and Torrey 1972) in which isopentenyl adenine (IPA) and zeatin (Z) was reported in two *Rhizobium* strains ANU240 and IC3342 (Upadhyaya et al. 1991). Growth-promoting effect of *Pseudomonas* G20-18 on wheat and radish plants by production and release of cytokinin is reported by (García de Salamone et al. 2001). The production of IPA, dihydroxyzeatin riboside (DHZR), and zeatin riboside (ZR) by *Pseudomonas fluorescens* AK1 and *Pseudomonas aeruginosa* AK2 and the impact of their inoculation on plant growth in rice seedling are reported (Karnwal and Kaushik 2011). Most of the microorganisms are capable of producing and secreting cytokinins in various capacities under *in vitro* conditions (Amara et al. 2015; Maheshwari et al. 2015).

Gibberellins

Gibberellins (GAs) influence developmental processes in higher plants including seed germination, stem elongation, flowering, and fruit setting (Hedden and Phillips 2000). Currently, 136 GAs from 128 plant species are known out of which 28 are produced by seven fungal species and only five GAs (GA1, GA3, GA4, GA9, and GA20) are produced by seven bacterial species (MacMillan 2001). Gibberellin was first characterization in bacteria using physicochemical methods (Atzorn et al. 1988), who demonstrated GA1, GA4, GA9, and GA20 in gnotobiotic cultures of *Rhizobium meliloti*. Further, using gas chromatography-mass spectroscopy (GC-MS), production of gibberellins was observed in *Acetobacter diazotrophicus*, *Herbaspirillum seropedicae* (Bastián et al. 1998), *Bacillus* sp. (Gutierrez-Manero et al. 2001), and *Azospirillum* sp. Only two *Bacillus* strains, i.e., *B. pumilus* and *B. licheniformis* are known to produce gibberellins (Gutierrez-Manero et al. 2001).

PGPR strains that produce GAs promote plant growth (Atzorn et al. 1988; Bastián et al. 1998; Gutierrez-Manero et al. 2001). The hormone translocates from roots to aerial parts of the plant and affects aerial part significantly if the bacteria producing GA also produce auxins for enhancing nutrient supply (Wong et al. 2015). GA-producing fungi that act as phytopathogens can cause diseases on plants (Malonek et al. 2005). The pathogenic fungus *Gibberella fujikuroi* suggests that pathogenic fungi produce GAs (Kudoyarova et al. 2015). Studies on *Arabidopsis* DELLA proteins revealed its role in mediating GA-, SA-, and JA-/ET-mediated defense signaling pathways in plant immune response (Navarro et al. 2008).

Strigolactones

Strigolactones are phytohormones with multiple functions including modulation of lateral root (LR) development. These compounds are produced by a many monocot and dicot plants at concentrations as minimum as 10^{-10} M (Humphrey and Beale 2006). (+)-strigol is derived from the carotenoid biosynthetic pathway (Matusova et al. 2005). Strigolactones are partially synthesized in the plastids and translocated to the cytosol (Humphrey and Beale 2006). LR initiation was not affected by strigolactone analog GR24 but is negatively influenced by LR priming and emergence, especially near the root–shoot junction (Jiang et al. 2016). The effect of GR24 on LR development depends on the hormonal balance with auxins and cytokinins, two other main players in LR development.

Strigolactones activate metabolism of AM fungus and promote the growth of fungus toward the plant roots (Gutjahr 2014). In the AM symbiosis, strigolactones are the key signaling molecules (Bonfante and Genre 2015). *A. thaliana* exudates contain lower amounts of the signal molecule (Westwood 2000). Presence of strigolactones is co-opted for the secondary function AM fungi (Brewer et al. 2013). Host-derived compounds evolved into signaling molecules are used to actively select mycorrhizal fungi (Bonfante and Genre 2015).

Strigolactones act as environmental signaling with microbial recruitment to mediate root architecture and plant productivity (Czarnecki et al. 2013). The production of strigolactones by red clover is stimulated by low P conditions (Yoneyama et al. 2001) which also favor AM development. Soil inoculation with *Glomus*

clarum and *G. margarita* suppressed emergence of *Striga* in maize and more than half in sorghum under field (Lendzemo et al. 2005) suggesting that exudation of strigolactones in mycorrhizal roots is higher than in non-mycorrhizal roots.

Ethylene

Ethylene increases in plants upon their exposure to biotic and abiotic stress conditions. Infection by bacterial and fungal pathogens in plants also initiates synthesis of ethylene (Robinson et al. 2001). Therefore, a decrease in ethylene production should indirectly promote root elongation as well as suppression of the disease. Thus, plant growth-promoting rhizosphere bacteria facilitate plant growth by lowering of plant's ethylene concentration through action of the enzyme ACC deaminase (Glick et al. 1999; Glick 2004). Hynes et al. (2008) screened 563 bacteria from the roots of pea, lentil, and chickpea for the ACC deaminase activity, suppression of legume fungal pathogens and promotion of plant growth.

Response to the ethylene due to the environmental changes depends on plants and environmental conditions (Stepanova et al. 2008). A small family of genes has been found to mediate tissue-specific responses to ethylene. The phytohormone regulates development of plants by altering the properties of DELLA protein nuclear growth repressors (Achard et al. 2003). A transcriptional factor, ethylene response factor 1 (ERF1), is known in *Arabidopsis thaliana* to regulate resistance in plants against necrotrophic fungi *Botrytis cinerea* and *Plectosphaerella cucumerina.* ERF1 confers resistance in *Arabidopsis* against *Fusarium oxysporum* sp. *conglutinans* and *F. oxysporum* f. sp. *lycopersici* (Berrocal-Lobo and Molina 2004).

Plant hormones interact at different levels in network mode to reflect signaling pathways under interactions (Fig. 29.2). Ethylene and jasmonic acid regulate the

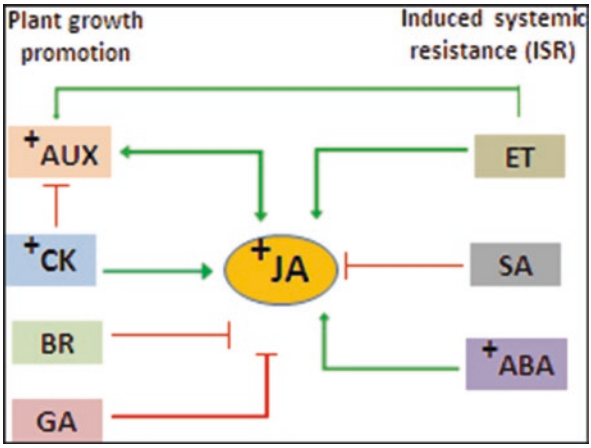

Fig. 29.2 Schematic representation of the interactions between phytohormones. The abbreviations used are: *Aux* auxin, *CK* cytokinin, *BR* brassinosteroids, *GA* gibberellic acid, *ET* ethylene, *JA* jasmonic acid, *SA* salicylic acid, *ABA* abscisic acid. The arrows indicate activation or positive interaction, blocked lines indicate repression or negative interaction, and + sign indicates mobile signal

plant response toward biotic stimuli, and ethylene also negatively regulates plant response toward rhizobial bacterial signal. This regulation comes during the Nod factor signal transduction pathway. Sun et al. (2006) showed jasmonic acid-mediated inhibition of plant's responses against *Rhizobium* due to Nod factor-induced calcium spiking. The fast effect of ethylene and jasmonic acid on Nod factor signaling reflects direct crosstalk between these three transduction pathways to coordinate diverse plant responses.

Martelella endophytica YC6887 causes increase in the number of lateral roots and plant biomass in *Arabidopsis* by producing phenylacetic acid (Khan et al. 2016). Results indicated that *Arabidopsis* root system development upon *M. endophytica* YC6887 colonization was dependent on auxin signaling and was not dependent on ethylene and jasmonic acid signaling.

29.3.3 Beneficial Interactions: Exchange of Signals During Symbiosis

In legumes, the formation of N-fixing root nodules and lateral roots (LRs) determine root system architecture. Plant cells that either make root nodule or are involved in LR formation are located closely at root apical meristem (RAM) (Sargent et al. 1987; Herrbach et al. 2014). Symbiosis results in protein-rich food, oil, fiber, and feed to the agroecosystems (Herridge et al. 2008; Jensen et al. 2012). The improvement of LR deployment in crops may maximize water, nutrient, and fertilizer acquisition that lead to proper nutrient uptake (Gamuyao et al. 2012). LRs and nodule formation are induced by environmental conditions like low N-availability (Ruffel et al. 2008; Jin et al. 2012). Recently, carboxy-terminally encoded peptides (CEP) signaling molecules were deciphered to control root developmental aspects (Delay et al. 2013; Tabata et al. 2014; Djordjevic et al. 2015). CEP peptides regulate nodulation positively but deregulate lateral root (LR) in various legumes (Mohd-Radzman et al. 2015). Thus, the understanding of signaling molecules, receptors, and downstream pathways that regulate nodule establishment and LR development in legumes under varying environmental conditions is needed for improved nutrient acquisition in the soil and root systems.

29.3.3.1 *Rhizobium*-Legume Symbiosis: Nodule Development on Legume Roots

N-fixing associations in plants of *Fabaceae* family and the soil bacteria *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Rhizobium* (rhizobia) largely contribute to crop productivity. The population density of rhizobia seems low if legumes are not in large population (Woomer et al. 1988; Kucey and Hynes 1989) suggesting that the symbiotic state is critical to the saprophytic population of rhizobia. Populations of naturalized rhizobia and introduced inoculant rhizobia differ in their tolerance to major environmental factors that affect the persistence and survival of individual species in the soil (Vidor and Miller 1980).

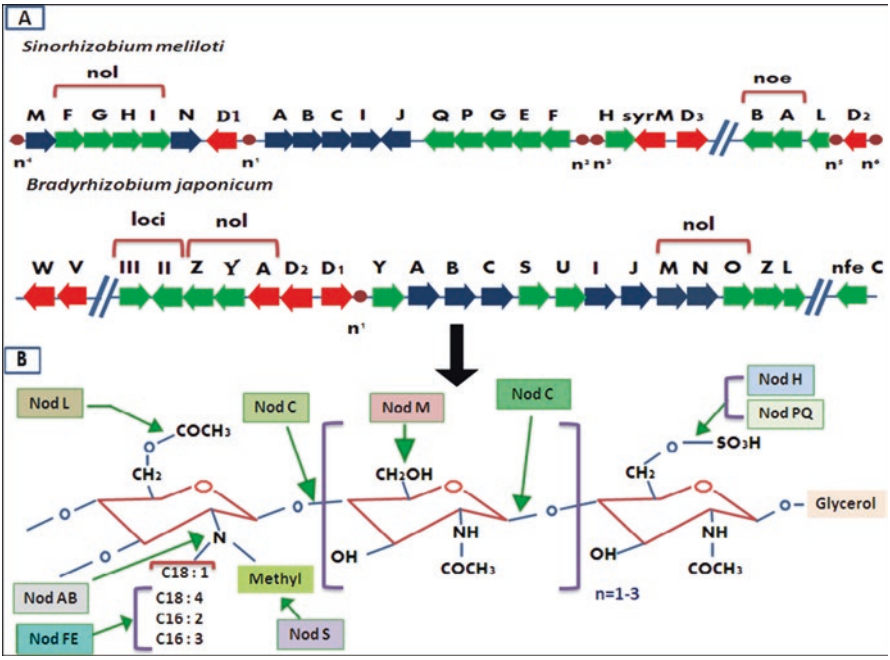

Fig. 29.3 (**A**) Nodulation genes (*nod*, *nol*, *noe*) of *Sinorhizobium meliloti* and *Bradyrhizobium japonicum*. Regulatory genes are shown in red, common genes in blue, and host-specific nodulation genes in green. Regulatory *nod*D product interacts with specific flavonoids, binds with *nod* boxes (*n′*), and activates transcription of other nodulation genes. (**B**) Various enzymes coded by nodulation genes make Nod factor (lipochitooligosaccharide) which causes nodule organogenesis

Flavonoids from the root exudates attract rhizobial population toward the plant roots for colonization in the vicinity of root hairs in the soil. The nodulation (*nod*) genes of rhizobia are common, host specific, and regulatory (Fig. 29.3). The common *nod* genes are involved in the production of basic lipochitin-oligosaccharide molecule (Perret et al. 2000; Sindhu and Dadarwal 2001). The mutations in the host-specific *nod* genes may cause a delay in nodulation or changed host range (Denarie et al. 1992). The recognition of effector proteins by R genes present in certain plant varieties limits host range and causes transcriptional activation of *nod* genes (Downie 1994; Russelle 2008). The products of different nodulation genes are required for the biosynthesis of Nod factors (NFs) or lipochitin oligosaccharides (LCO) (Perret et al. 2000).

Flavonoid signals are perceived by bacterial NodD regulatory proteins to induce the synthesis of lipochitooligosaccharidic NFs (Oldroyd and Downie 2008). Besides flavonoids, other compounds, e.g., betaines, jasmonate, xanthones, vanillin, etc., may also trigger *nod* gene expression but generally at higher concentrations than flavonoids (Cooper 2007). The synthesis of Nod factor backbone is controlled by the *nod*ABC genes that are present in all rhizobia. The strain-specific combinations of nodulation genes (*nod*, *nol*, or *noe*) cause various strain-specific alterations to the

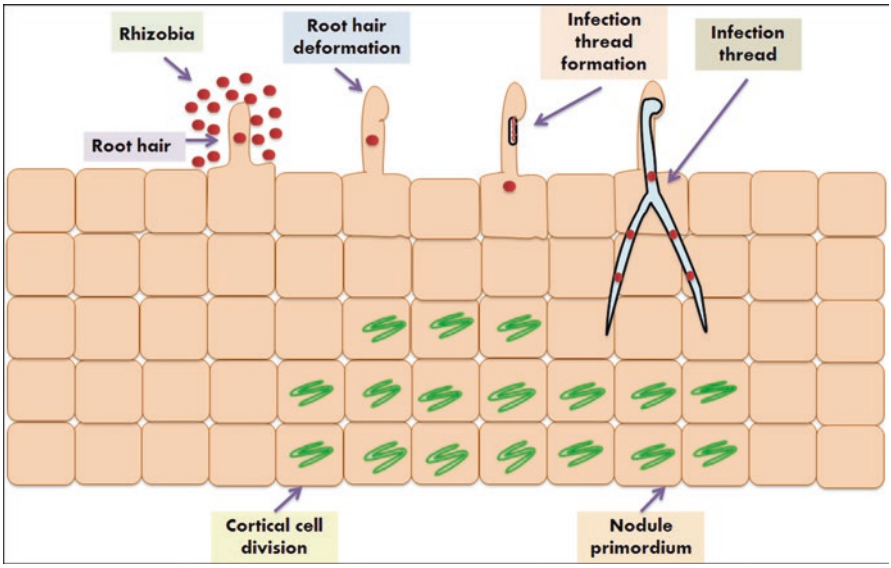

Fig. 29.4 Rhizobia are chemoattracted by root exudates and flavonoids toward the root hairs. Nod factor produced by rhizobia causes deformation of root hairs, and an infection thread is formed. Rhizobia travel toward nodule primordium and release bacteria in cortical cells. Nodule organogenesis occurs and N_2-fixing bacteroids fix nitrogen for the legume plant

core structure. In nodulation, rhizobia attach to root hairs at the infecting root zone just behind the apical meristem (Fig. 29.4). Rhizobia attaching at the surface of root hair either employ acidic extracellular polysaccharide or specific calcium-dependent protein, rhicadhesin, cellulose fibrils (Mateos et al. 1995; Smit et al. 1987), and legume root lectin (Kijne et al. 1988). The lipooligosaccharides (Nod factors) produced by infecting rhizobia cause curling and deformations of root hair and cortical cell divisions in compatible host (Lerouge et al. 1990; Broughton et al. 2000).

Nodule formation in alfalfa is linked to Nod factor of *Sinorhizobium meliloti* (Lerouge et al. 1990). *S. meliloti* induces two parallel occurring nodule-specific processes for forming indeterminate nodules adjacent to root protoxylem pole (Timmers et al. 1999; Xiao et al. 2014; Djordjevic et al. 2015). Rhizobial NFs may trigger transcriptionally activated key symbiotic (SYM) genes (Levy et al. 2004; Miwa et al. 2006) that initiate signal pathway1 and 2 (MtNSP1 and 2) (Smit et al. 2005) and MtCLV3/ESR-related12 and 13 (MtCLE12 and 13) (Mortier et al. 2010; Saur et al. 2011). Nodulation is further controlled by complex interactions among hormones and peptides (Gonzalez-Rizzo et al. 2006; Mortier et al. 2012; van Zeijl et al. 2015). Overall, with the NF/SYM pathway, the signals determine the formation of nodules on the root system (Penmetsa and Cook 1997; Oldroyd et al. 2013).

The rhizobia in the infection thread are embedded in a mucigel of cell wall polysaccharides, plant-derived matrix glycoprotein, and rhizobial exopolysaccharides (Callaham and Torrey 1981; Broughton et al. 2000). The growth of the infection thread proceeds by the induction of mitosis in the root cortex (Dudley et al. 1987).

Nodules may have one or more rhizobial strains which can be either determinant or indeterminate (Russelle 2008). Infections may be stopped owing to a breakdown in host–rhizobial communication to regulate a number of nodules (Djordjevic et al. 1986). The regulation of nodule number during symbiosis reflects strict regulation of the process mediated by ethylene- and CLE-related pathways (Penmetsa and Cook 1997; Kassaw et al. 2015).

Rhizobia from infection droplets are released into nodule tissue cells by resembling endocytosis process (Roth and Stacey 1989a). It then occupies an organelle-like cytoplasmic compartment, termed the "symbiosome," that is bounded by a peribacteroid membrane (Roth and Stacey 1989b). The peribacteroid membrane-enclosed bacteria continue to divide until the cytoplasm of each infected plant cell contains thousands of rhizobial cells. The infected cells are completely filled with bacteria at late infection phase and differentiated into the pleomorphic endosymbiotic bacteroids (Brewin 1991). These bacteroids specifically express genes involved in nitrogen fixation and reduce atmospheric nitrogen by the action of the nitrogenase enzyme.

The programmed senescence of nitrogen-fixing bacteroids is integral part of the development of indeterminate nodules (Vasse et al. 1990). Recently, several papain-like and legumain-like cysteine proteases called vacuolar processing enzymes (VPEs) were identified. They are strongly expressed during nodule senescence (Van Wyk et al. 2014). In nodules, papain-like cysteine proteases have known functions in the regulation of bacterial symbiosis, N-fixation, and leghemoglobin synthesis (Vande Velde et al. 2006; Li et al. 2008). With their caspase-like activity, they further play an important role in programmed cell death (Hara-Nishimura et al. 2005; Roberts et al. 2012). Many of the rhizobial cells may be destroyed along with the plant cells at nodule senescence (Pladys et al. 1991), and thus, the differentiated bacteroids may fail to switch from biotrophic to saprotrophic life conditions in the soil (Quispel 1988).

Interaction of Flavonoids with NodD

To initiate legume–rhizobial symbiosis, legumes prominently secrete phenolics, especially flavonoids that diffuse across the bacterial membrane. Flavonoids comprising chalcones, flavones, flavonols, flavandiols, anthocyanins, and proanthocyanidins are natural products (Winkel-Shirley 2001, 2006). Many other plants also synthesize isoflavonoids (Yu and Mcgonigle 2005; Du et al. 2010; Wang 2011). Flavonoid signal perception by a nodule-causing bacterium activates bacterial nodulation genes that encode various enzymes for synthesizing bacterial Nod factors (Long 1996; Oldroyd et al. 2013). NodDs bind to conserved DNA sequences, known as nod boxes located in the promoter regions of different nodulation gene operons (Peck et al. 2006). Differential responses of NodDs from different rhizobial species to different flavonoids are reported (Broughton et al. 2000; Peck et al. 2006). Point mutations in *nod*D from *Rhizobium leguminosarum* bv. *trifolii* managed for extended host range due to the induction of *nod* gene expression by different flavonoid inducers that normally remain inactive (McIver et al. 1989). Likewise, *nod*D1

transfer of the broad-host-range *Rhizobium* sp. NGR234 enabled engineered rhizobia to nodulate with non-legume *Parasponia* (Bender et al. 1988).

Flavonoid-deficient roots in transgenic plants produced by RNA interference of chalcone synthase failed to initiate nodule formation (Wasson et al. 2006). In addition, these flavonoid-deficient roots also had increased auxin transport at the site of nodulation (Wasson et al. 2006). Isoflavone-mediated auxin transports inhibition no more important for soybean to nodulate (Subramanian et al. 2006). Silencing of *M. truncatula* flavonoid-biosynthesis enzymes demonstrated that flavones and flavonols can play as internal inducers of rhizobial nodulation genes and regulators of auxin transport by *Sinorhizobium meliloti*, respectively (Zhang et al. 2009).

Regulation of Nodulation by Crosstalk Between Ethylene, JA, and Nod Factor

A lipochitooligosaccharide signal (Nod factor) produced by rhizobia can initiate nodule formation. Nod factors induce a series of rapid responses in cells of the legume root (calcium spiking) (Oldroyd and Downie 2004). The mutants devoid of Nod factor signaling failed in the activation of spiking (Walker et al. 2000; Harris et al. 2003). A calcium-/calmodulin-dependent protein kinase is essentially required for Nod factor signaling (Levy et al. 2004; Mitra et al. 2004). Nodule initiation may be regulated by auxin: cytokinin signaling (Cooper and Long 1994; Hirsch et al. 1989, 1997), abscisic acid (Suzuki et al. 2004), brassinosteroids and gibberellins (Ferguson et al. 2005), light signaling (Nishimura et al. 2002a, b), and nutrients, e.g., nitrate (Carroll and Gresshoff 1983). Ethylene has a strong effect on nodule development, but its formation inhibited when ethylene levels are very high. The ethylene-insensitive mutant, sickle (skl), of *Medicago truncatula* showed more than tenfold increase in the nodules formed (Penmetsa and Cook 1997). Ethylene also regulates multiple steps during nodulation and Nod factor-induced gene expression leading to calcium spiking (Penmetsa and Cook 1997; Oldroyd et al. 2001).

A novel role for jasmonic acid in regulation of N-fixing nodules lies in its role as a negative regulator of nodulation and involvement in the suppression of plant genes expression. Although, JA and ethylene play similar roles in maintaining spiking and their response to concentration of Nod factor, they have reverse impact on spike period. Jasmonic acid increases spike period but ethylene shortens it. The skl mutant showed greater sensitivity to JA for the effect on calcium spike frequencyindicating that both antagonistic and synergistic interactions are operative between the ethylene and JA pathways.

Cytokinins Involved in Nodulation

Nodulating rhizobia produce cytokinins (Frugier et al. 2008) that mimic the effect of the Nod factor (Cooper and Long 1994; Oldroyd 2007). Positive correlations in concentration of CK in plants and nodulation are reported in some legumes (Lorteau et al. 2001). CKs appear at the infection site rapidly (Lohar et al. 2004), and the genes for CK signaling become upregulated after rhizobial inoculation (Frugier et al. 2008). Moreover, low accumulation of CK with perception blocks nodulation (Murray et al. 2007). In the LHK1 CK receptor mutants, nodules develop

spontaneously in the absence of rhizobia demonstrating that CK signaling is needed to activate cell division in cortical tissues and nodule organogenesis (Tirichine et al. 2007). These results indicate that cytokinins are the key differentiation signal for nodule organogenesis and genes homologous to the *A. tumefaciens*' *ipt* gene have been observed in *Sinorhizobium meliloti* and *Mesorhizobium loti*.

29.3.3.2 Mycorrhizal Symbiosis

Arbuscular mycorrhizal fungi (AMF) belonging to *Glomeromycota* form mutual symbioses with more than 80% of the higher plants. AM development normally begins with the germination of asexual fungal spore in the soil. The fungal hypha profusely branch in response to the signal molecules synthesized by the plant and differentiate into appressoria on the root surface. The branching fungal hypha penetrates between or through the epidermal cells and growth of fungi occurs either intercellular or intracellular in the cortical tissue. Intracellular hypha differentiates into highly branched arbuscules that are involved in nutrient transfer between plant and fungus. The arbuscule life span is between 4 and 10 days, and they are completely degraded after collapsing. By the use of genomic techniques and genetics of model plants, several aspects of chemical communication have been elucidated during the pre-symbiotic stage, intracellular accommodation and intraradical colonization processes.

Crosstalk in Plant and Fungi at the Pre-symbiotic Stage

Plants produce signal molecules termed as "branching factors" (BFs) that are needed for morphogenesis and differentiation of fungal hypha (Giovannetti et al. 1994). In root exudates from P-deficient plants, flavonoids affect spore germination, arbuscular mycorrhizal fungi growth (Nair et al. 1991), fungal differentiation, and root colonization (Siqueira et al. 1991; Kape et al. 1993). BFs were purified from the root exudates of *Lotus japonicus* grown in P-limiting condition. Purified lipophilic compound induced branching of *G. margarita* hypha (Akiyama et al. 2005). The purified BF was chemically related to strigolactones, a sesquiterpene lactone, that induce seed germination of the parasitic plants *Striga* and *Orobanche*. BF was identified as a 5-deoxy-strigol by chemical synthesis. Akiyama et al. (2005) demonstrated that 5-deoxy-strigol, sorgolactone, strigol, and GR24 (synthetic analogue) induced hyphal branching of *G. margarita* at very low concentrations. A molecule of less than 500 Da present in the *Ocimum basilicum* root exudates induced hyphal branching in *Glomus mosseae* (Giovannetti et al. 1996). BF was noticed in all host root exudates but not in the nonhost root exudates (Nagahashi and Douds 2000; Buee et al. 2000). Exudates of plants grown under P-limiting condition actively activate hyphal branching than the exudate of plants gown in P-sufficiency conditions (Nagahashi and Douds 2000), suggesting that the synthesis rate or activity of the BF under P-limiting condition remains high (Nair et al. 1991).

A host plant is primed by the signal molecules of the AM fungi for colonization (Bonfante and Genre 2015; Conn et al. 2015). Pathogenic oomycetes utilize mycorrhizal signal constituents to identify plant surfaces and promote infection structures (Wang et al. 2012). AM fungi may use signals to initiate colonization if their network is supported and facilitated by the host plant (Veiga et al. 2013). Since fungal

spores can germinate without the hosts, their hyphae can connect to compatible fungal networks easily (Denison and Kiers 2011).

Fungal Signals Involved in Activation of Plant Responses

AMF release signal molecules after germination to induce expression of different plant genes (Mukherjee and Ane 2010; Chabaud et al. 2011; Maillet et al. 2011). The exudates of germinating spores contain a mixture of different N-acetylglucosamine oligosaccharides (chitooligosaccharides) including tetra- or penta-chitooligosaccharides (Genre et al. 2013) and lipochitooligosaccharides (Maillet et al. 2011). Signaling induced by chitooligosaccharides depends on symbiosis genes DMI1, DMI2, and DMI3 required for both AMF and rhizobia (Maillet et al. 2011; Genre et al. 2013). A plant receptor for fungal chitin derivatives is identified in *Parasponia andersonii* (Op den Camp et al. 2011), but silencing of the LysM receptor kinase abolished both nodulation and AM formation (Op den Camp et al. 2011).

Other active fungal molecules that induced the expression of several plant genes at early stages of AM proliferation include defense-related proteins like early nodulins and some putative proteins with predicted functions for signal transduction (Lambais and Mehdy 1995; Weidmann et al. 2004). An *in vitro* system for the study of early stages of AM proliferation in *Agrobacterium rhizogenes*-transformed *M. truncatula* roots containing a *gus*A fusion under the control of the *MtENOD11* promoter was developed (Chabaud et al. 2002). The activation of *MtENOD11* transcription was further assessed in reaction to the purified Nod factors and *Sinorhizobium meliloti* infection (Journet et al. 2001). The system demonstrated that the hypha from germinating spores of *G. rosea*, G. *gigantea*, *G. margarita*, and *G. intraradices* produced diffusible factor that induced the expression of *MtENOD11*, whereas this response was not observed in co-cultures with pathogenic fungi (Kosuta et al. 2003). These results indicated that AMF secretes specific diffusible factors that can induce the expression of an early nodulin gene in the host roots.

Eleven genes were involved in signal transduction, transcription, and translation, with induced expression during appressorium differentiation in *M. truncatula–G. mosseae* interaction in the absence of direct contact of the roots of myc$^+$ plants with the AMF and dependent on DMI3 (Weidmann et al. 2004). Akiyama (2006) showed that methanol extracts of germinating *G. margarita* spores induced expression of the AM-inducible *L. japonicus* Cbp1 (calcium-binding protein 1) promoter at the infection site in the *L. japonicas* T90B transgenic line and confirmed a lipophilic nonpolar signal molecule different in the chemical nature from the Nod factor.

Role for Cytokinins and Gibberellins in AM Symbiosis

Cytokinin levels were raised in AM-infected plants (Allen et al. 1980; Shaul-Keinan et al. 2002), although the origin of cytokinin was unclear from being fungal or plant side (Barker and Tagu 2000). The development of AM symbiosis in cytokinin-insensitive *Medicago truncatula cre*1 mutant suggested that cytokinins may not regulate mycorrhizal development significantly (Plet et al. 2011). On the basis of the GAs role in nodulation of *Pisum sativum* (Ferguson et al. 2011), GA-related gene expression was examined in *M. truncatula* (Ortu et al. 2012), and upregulation

of GA-biosynthetic genes in tomato was reported (García-Garrido et al. 2010). Furthermore, Shaul-Keinan et al. (2002) reported high levels of bioactive GA1 and its deactivation product GA8 in roots of AM-colonized plants using gas chromatography-mass spectrometry (GC-MS). In pea susceptible to AM, GA synthesis and GA response mutants may be explored further for the role of plant-derived GAs in the development of AM (Ross et al. 2011). The na-1 mutation located in kaurenoic acid oxidase gene is expressed in vegetative tissues of the plant (Ingram and Reid 1987; Davidson et al. 2004). DELLA proteins 1A and CRY were found as important regulators of GA synthesis and root growth in pea, and alleles la and cry-s were considered as null mutations in two DELLA genes of pea (Potts et al. 1985; Weston et al. 2008, 2009). DELLA proteins negatively regulate GA signaling and are degraded in the presence of bioactive GAs (Harberd et al. 2009). Further, colonization of AM in brassinosteroid-deficient *lkb* from a leaky mutation in the gene involved campesterol production during biosynthesis of brassinosteroid (Nomura et al. 1999; Schultz et al. 2001).

29.3.4 Harmful Interactions: Disease Development on Plants

Plant diseases are of ecological and economic importance. Microbial diseases cause malfunction in plants and result in the reduced capability of the plant to survive and maintain their ecological niche. Plant diseases caused due to pathogenic microorganisms influence the growth and destroy plant tissues to reduce crop yields from 25 to 100% (Choudhary and Sindhu 2015). Plant pathogens usually enter the plant through wounds or natural openings such as stomata. Some plant pathogens penetrate the plant directly, and such penetration of the plant involves attachment of the pathogen to the plant surface through the cuticle and the cell wall. Plant tissues may be attacked by the enzymes produced by the pathogen, and these enzymes softens the plant tissue in the vicinity of penetration. After entry into plant tissue, microbial pathogens disrupt normal plant function by producing toxins, degradative enzymes, and growth regulators. Plant pathogens produce pectinases, cellulases, and hemicellulases that result in degeneration of the plant structure, producing soft rots and other lesions. Destruction of the plant growth regulators by plant pathogens results in dwarfism, whereas microbial production of IAA, gibberellins, and cytokinins by some plant pathogens results in gall formation and excessive elongation of plant stems. Plants develop diverse morphological or metabolic abnormalities as a result of microbial infections and develop various kinds of diseases such as necrosis (rots), wilt, chlorosis, hypoplasia, hyperplasia, gall, scab, canker, and blight.

29.3.4.1 Pathogenesis-Related Signaling

Biochemical signaling plays a significant role in pathogen–host specificity, host defense response induction, and antagonism between pathogens and biocontrol microorganisms. Many disease symptoms depend on plant hormones, and some plant hormones also act as plant defense signals (Naseem and Dandekar 2012). Plant-triggered mitogen-activated protein kinase (MAPK) cascade percepts the

pathogens or their associated signals by specific receptors, and thus hormone (jasmonates and ethylene)-dependent and hormone-independent signaling is activated resulting in a defense mechanism in plant against invading pathogen. The hormones actively coordinate the MAPK signaling cascades that integrate different aspects of multiphasic defense responses in plants (Pandey et al. 2016). The elaborated defense system in plants involves various local or systemic reactions and signaling pathways that activate a multilateral pathogen resistance mechanism (Grant and Mansfield 1999; McDowell and Dangl 2000). Defense responses generate pathogen-related (PR) proteins in plants that are induced under pathological conditions (Antoniw et al. 1980). The major PR protein families include 11 different classes on the basis of their amino acid sequences (van Loon et al. 1994). Many PR proteins were antimicrobial in nature. In different *in vitro* studies, chitinases (PR-3 class) and β-1,3-glucanases (PR-2 class) inhibited fungal growth (Mauch et al. 1988; Sela-Buurlage et al. 1993) and presumably hydrolytically degraded fungal cell walls. Transgenic studies suggested constitutive upregulation of the expression of PR proteins like chitinases, β-1,3-glucanases, tobacco PR-1, and type I barley ribosome-inactivating protein (Alexander et al. 1993; Zhu et al. 1994; Jach et al. 1995) that decreased disease severity.

Inducible plant defense strategies have been evolved on the basis of pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) in which the pathogen-associated molecular patterns (PAMPs) are expressed by pattern recognition receptors (PRRs) in the host. The Ca^{2+} and mitogen-activated protein (MAP) kinase signaling cascades and transcriptome activation are activated by PAMPs (Boller and Felix 2009) and lead to defense responses like oxidative bursts, production of ethylene, and modification of plant cell walls (Asai et al. 2002). In contrast, effector-triggered immunity (ETI) ultimately triggers HR cell death in plants (Liu et al. 2007). The induction of defense signaling is mediated by jasmonic acid, ethylene, and salicylic acid also (Broekaert et al. 2006; Meng and Zhang 2013) that act as secondary signal in signaling networks by PTI and ETI in the plants (Jones and Dangl 2006; Meng and Zhang 2013). Host innate immunity to *Pythium* is conferred by the JA and ET signal pathway and includes cell wall components of the pathogen, metabolites, and protein effectors (Okubara et al. 2016).

Following pathogen attack, PR-10 proteins are activated in many plant species including asparagus (Warner et al. 1993), parsley (Somssich et al. 1986), pea (Barral and Clark 1991), potato (Matton and Brisson 1989), soybean (Crowell et al. 1992), and sorghum (Lo et al. 1999). PR-10 proteins are homologous to ribonuclease (RNase) from phosphate-starved ginseng cells (Moiseyev et al. 1994) suggesting similar activity. Agarwal and Agarwal (2016) pointed out a pathogenesis-related gene, JcPR-10a, from the biofuel crop *Jatropha curcas* L. toward stress/defense tolerance. The JcPR-10a recombinant protein exhibited RNase and DNase activity, and the protein also possessed antifungal activity against collar rot causing fungus *Macrophomina phaseolina*. Therefore, *JcPR-10a* gene can be a candidate gene to engineer stress tolerance in *Jatropha* and other plants that are susceptible to collar rot by *Macrophomina.*

29.3.4.2 Effect of Hormones on Defense Signaling

Auxins and cytokinins act in defense responses either dependent on SA or JA or independent of any of these (Naseem and Dandekar 2012). The function of auxins and cytokinins in defense and immunity depends on differential synthesis by plant parts itself, and some auxin- and cytokinin-like molecules are synthesized by root pathogens also (Estruch et al. 1991; Argueso et al. 2009; Chen et al. 2014). *Agrobacterium tumefaciens* and *A. rhizogenes*, the soil pathogenic bacteria (Costantino et al. 1980), carry a plasmid containing transfer DNA (T-DNA) region (Liu and Kado 1979; Lee et al. 2009) which encodes two transcripts, named *iaaH* and *iaaM*, for auxin biosynthetic enzymes (Wood et al. 2001). It also encodes for trans-zeatin synthesizing (*tzs*) gene engaged in cytokinin biosynthesis (Akiyoshi et al. 1987; Hwang et al. 2010). Thus, the control of auxin and its signaling pathway significantly contributes to the defense network in plants (Ludwig-Muller 2015).

Plants activate different types of induced resistance, depending on the organism that interacts with the plant. The example of induced resistance is SAR (systemic acquired resistance) triggered by pathogens causing limited infections (Durrant and Dong 2004). Induced systemic resistance (ISR) is activated by nonpathogenic rhizobacteria when it colonizes with plant roots (van Loon et al. 1998), and wound-induced resistance is typically elicited upon tissue damage such as that caused by insect feeding (Kessler and Baldwin 2002; Howe 2004). Salicylic acid, jasmonic acid, and ethylene regulate the signaling pathways (Pozo et al. 2004; Lorenzo and Solano 2005; Von Dahl and Baldwin 2007), and other plant hormones including ABA (Mauch-Mani and Mauch 2005), brassinosteroids (Nakashita et al. 2003), and auxin (Navarro et al. 2006; Wang et al. 2007) also implicate plant defense.

Insect attack on plant roots and leaves imposes diverse pressure on plants and after recognition; plants initiate their metabolism through phytohormone cascade (Johnson et al. 2016). Jasmonates are the major regulators of plant responses toward insect attack (Erb et al. 2012; Lu et al. 2015), but salicylic acid (SA) signaling can counter JA response (Gilardoni et al. 2011). Rice roots upon insect attack do not enhance levels of ABA and ET (Lu et al. 2015), the two major synergistic signal molecules in the wound responses of leaves. Specific strains of *B. amyloliquefaciens*, *B. subtilis*, *B. pasteurii*, *B. cereus*, *B. pumilus*, *B. mycoides*, and *B. sphaericus* reduced the incidence/severity of different diseases in many hosts (Kloepper et al. 2004). These strains induced systemic resistance (ISR) in many crop plants including tomato, muskmelon, watermelon, cucumber, sugar beet, tobacco, *Arabidopsis* sp., loblolly pine, and tropical crops. Moreover, ISR induced by *Bacillus* spp. provided protection against leaf-spotting fungal and bacterial diseases like root knot, blight, mold, and damping off.

Salicylic Acid

Salicylic acid (SA) is a small phenolic molecule that plays important regulatory role in plant immune response. Characterization of genes for SA biosynthesis, conjugation, accumulation, signaling and crosstalk with various other hormones has been worked out in detail (An and Mou 2011). SA is also a precursor in production of SA-containing siderophore like pseudomonine in *P. fluorescens* WCS374 and pyochelin in *P. aeruginosa* 7NSK2 (Audenaert et al. 2002). A mutant of 7NSK2 lacking

SA and pyochelin production failed to induce resistance. A treatment with the mixture of the two mutants expressed significant suppressiveness of *B. cinerea* (Audenaert et al. 2002). Salicylic acid is also important in providing basal defense to *Solanum tuberosum* against *Phytophthora infestans* (Halim et al. 2007).

A large set of genes were differentially transcribed in the roots of *Arabidopsis* following root colonization by *P. fluorescens* WCS417r (Verhagen et al. 2004). MYB72 transcription factor gene was found to be upregulated by WCS417r and a *myb72* knockout mutants of *Arabidopsis* that no longer expressed WCS417r-mediated ISR. The volatile compound 2,3-butanediol triggered *Bacillus*-mediated ISR in *Arabidopsis* (Kloepper et al. 2004). However, the signaling pathway activated in *Bacillus* was found dependent on ethylene, but it was found independent of SA and JA signaling (Ryu et al. 2004). Induced ET biosynthesis and following intracellular signaling was shown to induce expression of transcription factors that constituted primary EIN3-like regulators and downstream ERF-like transcription factors (Broekaert et al. 2006).

Transduction of the SA signal leads to activation of genes encoding pathogenesis-related (PR) proteins (van Loon et al. 2006). Mutations in NPR1 gene rendered the plant unresponsive to pathogen-induced production of SA (Dong 2004). NPR1 gene interacts with TGA transcription factors for the activation of SA-responsive genes coding PR proteins (Dong 2004). Suppression of JA-inducible gene expression due to SA was restricted in *npr*1 mutants to demonstrate crucial role for NPR1 in the crosstalk between SA/JA (Spoel et al. 2003, 2007). Spoel et al. (2003) showed that nuclear localization of NPR1 is not required for SA-mediated suppression of the JA response. Similar function of NPR1 in crosstalk is noted for rice (*Oryza sativa*) (Yuan et al. 2007). NPR1-silenced wild tobacco (*N. attenuata*) plants accumulated increased levels of SA upon insect herbivory and were highly susceptible to herbivore attack (Rayapuram and Baldwin 2007).

Many plant pathogens manipulate host auxins to interfere with the host development (Chen et al. 2007). Plants that overproduced the defense signal molecule SA frequently show phenotypes reminiscent of auxin-deficient or auxin-insensitive mutants (Wang et al. 2007). SA application caused global repression of auxin-related genes that result in stabilization of Aux/IAA repressor proteins and inhibition of auxin (Wang et al. 2007). Application of exogenous ABA prevented SA accumulation and suppressed resistance to *P. syringae* in *Arabidopsis* (Mohr and Cahill 2003). A loss-of-function mutation in the *Arabidopsis MPK*4 gene, which encodes a mitogen-activated kinase, was found to impair JA signaling and simultaneously conferred enhanced resistance against bacterial and oomycete pathogens due to constitutive activation of SA signaling (Petersen et al. 2000). Similar to *mpk4*, *ssi2* plants exhibit impaired JA signaling constitutive expression of SA-mediated defense and disease resistance (Kachroo et al. 2003).

Most wilt-causing pathogenic strains of the *R. solanacearum* degrade SA via gentisic acid. *R. solanacearum* strain GMI1000 expressed SA-degraded pathway during tomato pathogenesis (Lowe-Power et al. 2016). The results showed that *R. solanacearum* degrades plant SA for its own protection and for enhancing virulence on plant hosts like tobacco that can use SA as a defense signal (Lowe-Power et al. 2016).

Jasmonic Acid

Jasmonic acid (JA) is a lipid-derived molecule having a key role in modulating various physiological processes. It is a key cellular signal for the activation of immune responses against most insect herbivores and necrotrophic microorganisms (Glazebrook 2005). Cyclic precursors of JA are known to function as potent signals of plant defense responses (Farmer and Ryan 1992). Likewise, volatile derivatives of JA, e.g., methyl jasmonate (meJA) and cis-jasmone, are airborne signals to stimulate plant defense (Birkett et al. 2000). JA and ethylene are needed for defense against necrotrophic pathogens (Thomma et al. 2001) and associated gene expression (Xu et al. 1994; Lorenzo et al. 2003). The transcription factor ethylene response factor1 (ERF1) works to act as in synergistic signaling of JA/ethylene (Lorenzo et al. 2003).

Root exudates modulate changes in microbial communities (Carvalhais et al. 2015a). Disruption of the pathogen JA pathway can alter the root exudation patterns and change rhizosphere microbial communities (Carvalhais et al. 2015a) in *Arabidopsis thaliana*, which correlates with the exudation as an integral part of plant response to pathogens. Thus, composition of soil microbiome can be changed via shifts in root exudation profile (Chaparro et al. 2013). Pathogenic fungus *Fusarium graminearum* in barley rhizosphere triggers the exudation of phenolics that prevented spore germination (Lanoue et al. 2009). Similarly, alterations of phenolics exudates in barley plants infected with *Pythium ultimum* caused induction of antibiotic-related genes in *Pseudomonas protegens* (Jousset et al. 2011). Increase in the abundance of *Streptomyces*, *Bacillus*, and *Lysinibacillus* taxa in the med25 rhizosphere and an *Enterobacteriaceae* population in myc2 rhizosphere is reported (Carvalhais et al. 2015b). The amendment of biochar was also found to induce defense responses and resulted in about 50% reduction in *Botrytis cinerea* disease severity in tomato genotypes (Mehari et al. 2015). Biochar amendment induced priming of early as well as late-acting defense responses particularly in the induction of genes *Pti*5 (ET-related) and *Pi*2 (JA-related), which are known to be crucial in resistance against *B. cinerea*.

Several MAP kinases are implicated in defense signaling of plants by transferring information from sensors to cellular responses in all eukaryotes (Menke et al. 2004; Nakagami et al. 2005). Mitogen-activated protein kinase 4 (MPK4) as a negative regulator of SA signaling and a positive regulator of JA signaling in *Arabidopsis* has been identified by Petersen et al. (2000). Inactivation of MPK4 in *mpk*4 mutant plants resulted in elevated SA levels and constitutive expression of SA-responsive PR genes, suppression of JA-responsive genes, and enhanced susceptibility to the necrotroph *A. brassicicola*. Interestingly, the *mpk*4 mutation blocked JA-responsive gene expression independently of SA accumulation, as SA-nonaccumulating *mpk*4/*Nah*G transgenics still exhibited increased susceptibility to *A. brassicicola* and suppression of MeJA-induced PDF1.2 expression (Petersen et al. 2000; Brodersen et al. 2006).

29.4 Conclusion

Microorganisms present in the rhizosphere of plants are involved in many biogeochemical processes, which affect agricultural productivity, nutrient recycling, disease control, and degradation of pollutants. Present agricultural practices negatively

impact the population of soil microorganisms by reducing organic matter content in the soils and thus causing contamination of groundwater. Besides inoculation with beneficial microbes, edaphic factors and plant root exudates have been demonstrated to play important role in the formation of root microbiome. The multipartite interactions that lead to assembly and maintenance of the root and rhizosphere microbiome are highly complex, deterministic, and dynamic, and different kinds of signaling molecules are responsible for such communication into the rhizosphere. Many of these interactions are mediated by photoassimilates that are excreted by plant roots and constitute the initial signaling event between plants and microorganisms. The root exudates serve numerous functions, which range from changing the physicochemical soil properties, inhibiting the growth of weed plants, combatting the herbivores and regulating the microbial community (Rasmann and Turlings 2016).

Many microorganisms develop mutualistic interactions with plants. These mutually benefiting plant–microbe interactions based on specific plant-/microbe-mediated signaling phenomenon are of great value from agronomical perspective. Studies suggest many commonalities but differences too, in the subject area leading to exploring the defense strategies employed by roots and foliar tissues during pathogen attack (De Coninck et al. 2015). Therefore, a good understanding of the interaction of plant roots with the microorganisms in the rhizosphere would be important to engineer resistance against root pathogens without negatively altering root-beneficial microbe interactions. Farming methods that support recruitment and maintenance of beneficial microbial communities in the rhizosphere will benefit the agriculture in the form of enhanced crop yields and disease suppression. The understanding and exploitation of the signals between plant and microorganisms could become the basis for crop improvement and protection.

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Plant Interaction with Methylotrophic Communities

30

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Abstract

Methylotrophs are a diverse group of bacterial community utilizing a number of C1 carbon compounds as a source of carbon and energy. This peculiar group of microorganisms has capability to enhance plant growth by solubilizing phosphates, by producing siderophores, by inhibiting ethylene accumulation in plants in adverse conditions, by fixing atmospheric nitrogen, by producing phytohormones such as auxins and cytokinins, and by degrading various harmful and toxic compounds. The plant roots are colonized by different types of methylotrophic bacteria, and solubilized essential elements are provided to the plants making them healthier and strong. There are a number of beneficial biological interactions of methylotrophs with the plants. Interaction of methanotrophs with plants leads to the reduction in greenhouse effects in the environment. The interaction of methylotrophic bacteria with plants as endophytes, epiphytes, plant colonizers, phytohormone producers, and other types of beneficial association makes them very peculiar group of microbes interacting natural flora. Apart from higher plants, methylotrophic interaction was observed with bryophytes also as epiphytic as well as endophytic bacteria.

Keywords

Methylotrophs • PGPRs • Endophyte • Bacterial community • Phytohormone

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30.1 Introduction

Methylotrophic bacteria are ubiquitous, and a strong association and interaction with plants, within plants, and around the plant system is very common. They are abundantly present over plant leaf surfaces as pink-pigmented facultative methylotrophs. They participate in various plant-associated activities also. They can solubilize phosphate also and provide to the plants, promoting their growth. Plants in stress environment or extreme condition are influenced by this promising group of microbes. There are various novel potential methylotrophic bacteria reported to be in strong association with plants. They are also recognized as plant growth-promoting rhizobacteria, and several finding showed their colonization with plant roots. These specific reduced carbon-utilizing communities are present as both plant endophytes and epiphytes. Taking these considerations, methylotrophs associated with plants are here discussed with examples and recent developments.

30.2 Novel Methylotrophic Bacteria Associated with Plants

Like several novel bacterial strains, methylotrophic bacteria are also isolated from various ecosystems and reported novel after subsequent molecular characterization. Moreover, plant-associated epiphytic and endophytic methylotrophic strains were found to be novel after polyphasic approach. Most of the studies conclude *Methylobacterium* sp. as predominant plant-associated novel methylotrophic bacteria.

From *Populus delitoidesnigra* (poplar tree), a novel aerobic PPFM (pink-pigmented facultative methylotroph) was isolated and identified as *Methylobacterium* sp. strain BJ001T. The phylogenetic analysis revealed its relatedness with *Methylobacterium extorquens*, *Methylobacterium thiocynatum*, *Methylobacterium rhodesianum*, and *Methylobacterium zatmanii*. The carbon substrate utilization pattern of bacterium was differed from other phylogenetically closed methylotrophs, specifically methane carbon source (Aken et al. 2004).

Novel methylotrophic bacteria were isolated from leaf tissue of rice plant and were identified as *Methylobacterium oryzae* CBMB27T. Molecular chronometer 16S rRNA gene-based phylogenetic analysis revealed the taxonomic position of *Oryza sativa* L. CBMB27T in a clad of *Methylobacterium oryzae*, *Methylobacterium fujisawaense*, and *Methylobacterium mesophilicum* showed homology with 98.3%, 98.5%, and 97.3%, respectively. The experiment was validated by DNA-DNA hybridization together with polyphasic approaches to observe the relatedness of novel strain CBMB27T with its closest species (Madhaiyan et al. 2009b).

In an earlier finding, three novel restricted facultative methylotrophic bacterial strains 301 T, 30S, and SIP-4, utilizing C1 carbon compounds, were isolated from Lake Washington, Seattle, USA. Their phylogenetic position was determined by genotypic and phenotypic characterization using polyphasic approach. The above characterization method and results assigned SIP-3 strain as *Methylovorus glucosotrophus*, while strains 301 T and 30S were grouped in *Methylotenera mobilis*

JLW8T clad based on 16S rRNA gene sequence homology, but they were proposed as a novel strain *Methylotenera versatilis* sp. nov. based on their genomic and phenotypic characterization (Kalyuzhnaya et al. 2011).

Three different strains of methylotrophic yeast were isolated from leaf phylloplane of mango tree (*Mangifera indica*) and wine grapes (*Vitis vinifera*). Two strains KM13 and KM15 from grape leaf and one strain KM03 T from mango leaf were isolated. On the basis of various polyphasic characterization and nucleotide sequence-based phylogeny, these three strains were placed in the *Ogataea wickerhamii* clade. On the basis of various polyphasic approaches along with ITS region sequence analysis, strain KM03T was proposed as a novel species named *Ogataea kanchanaburiensis* sp. nov., while two other strains KM13T and KM15 were assigned as *Ogataea wangdongensis* sp. nov. (Limtong et al. 2013).

From wheat soil contaminated with tribenuron methyl, a novel bacterial strain was obtained. By the polyphasic approaches like phenotypic characterization, carbon substrate utilization, lipid estimation, G + C content analysis, DNA-DNA hybridization, and nucleotide-based phylogeny revealed this strain as *Methylopila henanense* sp. nov. This novel strain was placed in the clad of most closely related genus *Methylopila* after 16S rRNA gene sequence analysis (Wang et al. 2015). In an earlier finding, a novel bioinoculant for sustainable agriculture in the form of non-pathogenic phyllosphere methylotrophic bacteria was applied to agricultural fields to increase germination ability, storage ability, or seed vigor (Rajan et al. 2012).

30.3 Methylotrophs as PGPR (Plant Growth-Promoting Rhizobacteria)

Methylotrophs are well-known for their potential to minimize the biotic and abiotic stress factors affecting plants by their plant growth-promoting ability. Moreover, this subpopulation is indulged in the mechanism of plant growth promotion directly or indirecly.

Methylotrophic bacteria are also referred to as plant growth enhancer, and several species are isolated and reported earlier from rhizosphere region of plants (Meena et al. 2012; Anitha 2010; Madhaiyan et al. 2009a, b). In a study, methylotrophic bacteria having nitrogen-fixing ability were isolated from tropical legume plants *Sesbania aculeata* and *Crotalaria juncea*. Biochemical and molecular characterization identified the strain as *Methylobacterium nodulans* ORS2060. Under greenhouse condition, a significant increased nodulation in *C. juncea* and *M. atropurpureum* was observed along with higher nitrogenase activity (Madhaiyan et al. 2009a, b).

Looking at the potential of methylotrophic strains, a study was framed earlier to assess their plant growth-promoting ability together with the synergistic effect of methylotrophs, *Azospirillum*, and phosphobacteria. Investigation was done in a hybrid tomato plant CoTH1 under greenhouse condition. In pot experiment seeds were coated with the consortia of *Azospirillum* and phosphobacteria, while foliar spray of methylotrophs was also applied, and a significant growth and yield was recorded. Apart from plant growth-promoting rhizobacteria (PGPR), phyllosphere

bacterial inhabitants have also an ability to enhance and promote plant growth (Basile et al. 1969). Methanol is assimilated to CO_2 by methylotrophs similar to plants, and therefore toxicity produced because of formaldehyde accumulation is avoided and reduced in plants (McGiffen and Manthey 1996).

Methylotrophs associated with crop plants are reported as nitrogen fixers (Lee et al. 2006; Madhaiyan et al. 2004), as biofertilizers (Keerthi et al. 2015; Rekadwad 2014; Chauhan et al. 2010), and as IAA-producing bacteria (Anitha 2010) and used as inoculants for making agriculture sustainable (Kumar et al. 2016). In an earlier investigation, bacterial species of genus *Methylobacterium* were isolated from leaf phyllosphere of different crop plants and identified with the help of functional gene sequences of mxaF gene. The HPLC analysis of culture filtrate confirms the cytokinin production from numerous *Methylobacterium* strains that enhance the seed germination and seed vigor of wheat plant. Such kind of plant growth regulator (cytokinin) producing potent methylotrophic bacteria can be exploited in the development of bioinoculants (Meena et al. 2012).

Plant root growth is regulated by a very specific compound ethylene, a pathway component of auxin biosynthesis (Hardoim et al. 2008; Madhaiyan et al. 2007). High ethylene concentration works as stress condition in plants that inhibit the root growth which leads to plant aging. ACC (aminocyclopropane-1-carboxylic acid) is the precursor of ethylene and converted to ethylene by the action of enzymes ACC synthase and ACC oxidase during auxin biosynthesis pathway. This higher concentration of ethylene is restricted by the bacterium converting ACC to ammonia and alpha ketobutyrate with the help of enzyme ACC- deaminase rather than ethylene. The *Methylobacterium* species such as *Methylobacterium oryzae* (Madhaiyan et al. 2007), *M. radiotolerans*, and *M. nodulans* (Fedorov et al. 2013) interact with different plant roots in stress environment, diminishing the stress effect and making plant healthier (Dourado et al. 2015; Glick 1995).

30.4 Methylotrophic Bacteria as Plant Colonizers

Methanol-utilizing pink-pigmented facultative methylotrophs, commonly found over the surface of plant leaves, are characterized by both biochemical and molecular approaches. The colonization of this PPFM population was observed in red clover and winter wheat leaves in a study. The more consistent colonization was observed in the isolates from red clover leaves. These microbial communities have the potential to colonize the plant rhizosphere also after inoculation of seeds (Omer et al. 2004).

The colonization of *Methylobacterium extorquens* was observed by gfp (green fluorescent protein) expression in an investigation (Figueira et al. 2000) in which transformation was reported with a gfp-containing plasmid under the control of methane monooxygenase and lacZ promoter. With the help of epifluorescence microscope, the colonization ability of *Methylobacterium* sp. was monitored in the study.

After seed bacterization the colonization of *Methylobacteria* in phyllosphere and rhizosphere was observed along with phyllosphere colonization after foliar spray was also studied. The experiment was validated and confirmed by site localization

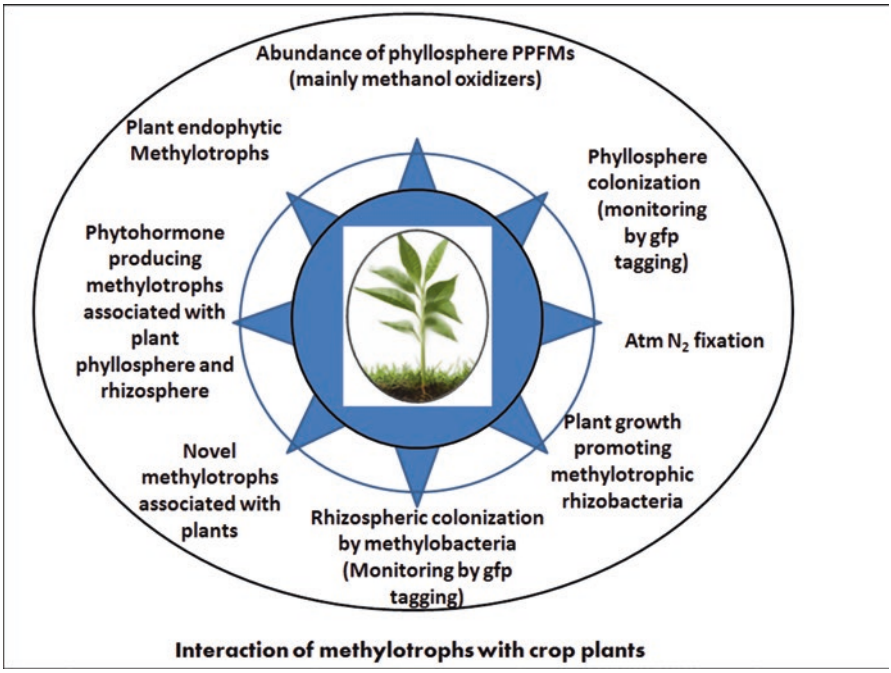

Fig. 30.1 Various interactions between plants and methylotrophic bacteria

of gfp-tagged mutants using confocal microscopy (Fig. 30.1). The abundance of *Methylobacterium* sp. was found in phyllosphere of wheat plant in an earlier investigation (Verma et al. 2014).

In another study on *Perilla* plant, the distribution and colonization of facultative *Methylobacterium* spp. was determined. *Perilla*, a herb, is widely used as food in Japan, and the interaction of this herb with methylotrophic bacteria was studied extensively. The distribution study of PPFM in different vegetable leaves results in the maximum abundance of methylotrophs over *Perilla* plant leaves and about 15% of the total bacterial inhabitants there. The isolates from the seeds of *Perilla* plant were identified as *M. fujisawaense* DSM5686T and *M. radiotolerans* JCM2831T, indicating a special type of interaction between PPFM and *Perilla* plant (Mizuno 2013).

The abundant methylotrophic bacteria over the plant surface actively participate in carbon dioxide fixation and affect the plant growth. The culture-independent assessment of three different plant phyllosphere bacteria using metaproteogenomic approach revealed predominant alpha proteobacterial genus *Methylobacterium* along with *Sphingomonas*. Soybean, clover, and *Arabidopsis* phyllosphere-associated *Methylobacteria* have the ability to utilize methanol as sole carbon and energy. The results show the identification of unique methylotrophic traits and therefore give a good example of plant-microbe interaction (Delmotte et al. 2009).

In a model legume plant, *Medicago truncatula*, colonization of methylotrophic bacteria especially *Methylobacterium* sp. was observed by an inoculation experiment.

The ability to utilize methanol as a source of carbon and energy provides a selective advantage at the time of colonization of model legume plant. The competitive colonization ability between mutant and wild type *Methylobacterium extorquens* AM1 was determined. The fluorescent protein-labeled *Methylobacterium extorquens* AM1 were observed under fluorescence microscopy which revealed the major bacterial communities on leaf surface while abundance on the lower leaf side (Sy et al. 2005).

Interaction of Methylotrophs with Water Plants Possibly, metabolite exchange between water plants and methylotrophs displays a beneficial interaction. Macrophyte-associated methanotrophs were found in the water column in a study where methanotrophs oxidize methane by utilizing oxygen released by plants, while CO_2 and ammonia are provided to plants by oxidation. Methane oxidation carried out by methanotrophs therefore reduces the greenhouse effect in the environment by this mutual relationship (Iguchi et al. 2015). Ultimately methylotrophic bacteria maintain the biogeochemical cycle of carbon in the ecosystem.

The community structure of methanotrophs associated with both dryland and flooded rice ecosystem was illustrated in earlier investigation. Molecular characterization-based community composition showed the abundance of Type I and Type II methanotrophic community in flooded rice field soils (Dubey and Singh 2001).

The PPFM (pink-pigmented facultative methylotroph) abundance apart from crop association was also observed with coastal region plants. Root region soil of different plant species of Southern California coastal region showed the PPFM abundance, that is, a best example of water plant-PPFM interaction. The PPFM abundance was in the range of 10^2–10^5 CFU/g dry soil of plant roots with variations across different plant species. In this natural ecosystem, this type of interaction suggests PPFM to be a better target for future work related to plant-microbe feedback. In the rhizosphere region of coastal sage scrub plants, PPFM abundance depends on both immediate and surrounding plant species (Irvine et al. 2012). Mangrove forest is another natural ecosystem of importance to mankind, and a diversified soil methylotrophic population was recorded from this fertile and dynamic ecosystem earlier. These potent methylotrophic strains have the ability to resist pathogenic fungus *Macrophomina phaseolina* (Kumar et al. 2015).

Wetlands are rich source of microbial assemblages, and this ecosystem is engaged in ecological control process. A number of methylotrophs are associated with wetland plants and are actively participating in nitrogen fixation process (Prasad et al. 2002; Barraquio and watanabe 1981). Fast accumulation of nitrogen in the roots of wetland plants indicates the methanotrophy during the development of peatland that ultimately induces the nitrogen fixation process. Additional CO_2 derived from methane is also fixed by methanotrophy in this ecosystem (Larmola et al. 2014). A novel methanotrophic bacterium *Methylocystis rosea* sp. from an arctic wetland soil of Norway was reported in a study having nitrogenase reductase structural gene (nifH) (Wartiainen et al. 2006).

30.5 Methylotrophs as Plant Endophytic Bacteria

This unique group of bacteria has properties to inhabit the plant internal compartments where they utilize methanol and other reduced carbon substrates to grow. They are actively participating in the metabolism of different plant metabolites. They are not pathogenic and are beneficial for plants in various aspects (Podolich et al. 2008; Pirttila et al. 2005; Madmony et al. 2005). Several endophytic bacterial communities are found within plant reproductive organs also (Madmony et al. 2005). The presence of endophytic methylotrophic bacteria induces the root formation along with enhanced biomass of soybean seedlings in a study (Holland and Polacco 1992). The endophytic bacteria reported in scots pine shoot tips were studied for their metabolic activity by in situ hybridization technique. These methylotrophic and other bacterial communities were found more abundant in summer season to the shoot tip and undetectable during winter season. This finding shows the temporal variation of the endophytic community, and highest endophytic numbers were also detected during spring season. The identified endophytic population in the shoot tip comprises *Methylobacterium* spp., *Pseudomonas fluorescens*, *Mycobacterium* sp., and *R. minuta*. Among all endophytic bacteria, *Methylobacterium* spp. was found most common and observed throughout the year (Pirttila et al. 2005) (Table 30.1).

Some another endophytic methylotrophs were observed and identified from poplar tree as *Methylobacterium populi* (Tanaka et al. 2008), and the location of the *Methylobacteria* inside the plant was examined through fluorescent in situ

Table 30.1 A list of endophytic and epiphytic methylotrophic bacteria associated with various plant or plant parts

S.No.		Plant/plant parts	References
Endophytic methylotrophs			
1.	*Methylobacterium* sp.	Seed seedlings	Ferreira et al. (2008)
2.	*Methylovorus mays*	Plant shoot	Ivanova et al. (2000, 2008)
	Methylobacterium mesophilicum		Ulrich et al. (2008)
3.	*Methylobacterium extorquens* str. F and *Pseudomonas synxantha* str. G	Bud endophyte of scots pine	Laukkanen et al. (2000)
			Pirttila et al. (2000, 2003)
4.	*Methylobacterium* sp. and *Pseudomonas fluorescens*	Shoot tip	Pirttila et al. (2002, 2005)
5.	*Methylobacterium radiotolerans*, *M. oryzae*, *M. fujisawaense*	Mangrove plants	Dourado et al. (2012)
6.	Methanotrophs	Stem leaves of *Sphagnum* mosses	Raghoebarsing et al. (2005)
7.	*Methylobacterium* sp.	Potato tissues	Podolich et al. (2008)
Epiphytic methylotrophs			
8.	*Methylobacterium extorquens* (trans-zeatin producing)	*Arabidopsis*, maize, barley, and soybean surface	Koenig et al. (2002)
9.	*Methylobacterium* sp.	Crop plants phyllosphere	Meena et al. (2012)
10.	*Methylobacterium* sp.	*Funaria* protonema	Hornschuh et al. (2002)

hybridization technique. Mangrove plants like *Rizophora*, *Laguncularia*, and *Avicennia* spp. were also reported to be associated with the methylotrophic communities such as *Methylobacterium radiotolerans*, *M. oryzae*, and *M. fujisawaense*. These strains were also reported with their heavy metal tolerance ability (Dourado et al. 2012).

30.6 Conclusion

Methylotroph-plant interaction is a best example of plant-microbe interaction that reflects the impact of this beneficial group of bacteria to environmental flora. The positive influence and interaction of methylotrophs with different parts of the plant make them healthier and strong enough in adverse conditions. Greenhouse effect also is inhibiting the growth of wider plant species in the environment, and methylotrophic communities (methanotrophs) are minimizing this plant growth-inhibiting effect. The abundant pink-pigmented facultative methylotrophic bacteria over plant leaf surface are also involved in plant growth promotion apart from rhizospheric methylotrophs. Plant-colonizing methylotrophic communities are also responsible for creating induced systemic resistance in plants. The present compilation enlightens the facts that endophytic or epiphytic methylotrophs interacting with various plant species and plant parts enhance our antiquity to know the physiological mechanism, activity, and abundance of methylotrophic communities.

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