Dhananjaya Pratap Singh · Ratna Prabha
Editors

Microbial Interventions in Agriculture and Environment

Volume 3: Soil and Crop Health Management

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Dhananjaya Pratap Singh is a Principal Scientist in Biotechnology at the ICAR-National Bureau of Agriculturally Important Microorganisms, Maunath Bhanjan, India. He completed his master's degree from G. B. Pant University of Agriculture and Technology, Pantnagar, and his Ph.D. in Biotechnology from Banaras Hindu University, Varanasi, India. His research interests include plant-microbe interactions, bioprospecting of microbial and plant metabolites, microbe-mediated stress mitigation in plants, metabolomics-driven search for small molecules, and bioinformatics in microbial research. He has also been working on the societal implications of microbial biotechnology for microbe-mediated crop production practices and rapid composting of residual agricultural wastes at the individual farm level to promote large-scale applications. He was involved in the development of the supercomputing infrastructure for agricultural bioinformatics in the microbial domain at the ICAR-NBAIM under the ICAR's National Agricultural Bioinformatics Grid (NABG) program in India. He is an Associate of the National Academy of Agricultural Sciences (NAAS), India, and has received several prestigious awards, including Dr. A. P. J. Abdul Kalam Award for Scientific Excellence. He has published more than 150 papers in respected national and international journals and has also edited 6 books on microbial research.

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Role of Microorganisms in Managing Climate Change Impacts

1

Muhammad Rehan Dastagir

Abstract

Microorganisms are vital constituents of any agroecosystem. In the prevailing environmental conditions, climate change is a real-time response to mark its harmful impacts on the soils, plants, and the whole Earth. The future of climate change seems to be more impactful in negative terms. Among various adaptation method on climate change, the mechanisms of microbial mitigation, and adaptation to environmental conditions make them suitable agents for combating against climatic aberrations. Various promising aspects of microbial adaptation to environmental challenges have been discovered and documented. These mechanisms help to generate understanding to cope with the changing environment. Some of these very prominent mechanisms have been discussed here. More result will come from the research on microbial culture, identification and physiology, and DNA sequencing. The future of Earth will vastly depend on the research of this microbial life in the changing environmental conditions.

Keywords

Microorganisms · Climate change · Abiotic stress · Mitigation strategies · Temperature

1.1 Introduction

Climate change is the buzzing word in the twenty-first century. The postindustrial shift of economic development of human civilization and overexploitation of fossil fuel energy lead to receive negative feedback from Earth. This is visible in the inevitable mark of climate change. The last century had experienced the mean

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0.74 ± 0.18 °C (IPCC, AR4 2007) temperate increment with changing pattern of seasonal cycle, intensity, and extremes of natural disasters such as drought, flooding, cyclone, etc. Anthropogenic activity induces the greenhouse effect by emitting greenhouse gases (GHGs), commonly known as CO_2, CH_4, N_2O, and chlorofluorocarbons (CFCs). The present atmospheric concentration of carbon dioxide is over 400 ppm crossing the standard limit of 350 ppm (Stocker et al. 2013). The increment of global mean sea level in the twentieth century has risen by 1.7 ± 0.2 mm $year^{-1}$ (Church and White 2011), and ice cover in the Arctic Sea has been observed nearly 49% below in 2000 as compared to 1979 due to ice melting.

Climate change has a significant effect on agriculture mostly due to change in temperature, rainfall, CO_2 level, altering crop growing season, pest infestation, soil loss, sea level rise, etc. This shift in natural conditions will alter the regular pattern of agricultural practices leading to declining food security in the world. Climate change could decrease maize production by 30% in Southern Africa, and rice production could decrease by 10% within 2030 in South Asia (Lobell et al. 2008). Worldwide climate-related disasters have increased alarmingly in the last three decades with substantial economic losses of agricultural products. There were 149 disasters from 1980 to 1990 in comparison to 332 from 2004 to 2014 (FAO 2016). Subsequent economic losses were 14 billion USD (1980–1990) and 100 billion USD (2004–2014). Between 2003 and 2013, agriculture in developing countries absorbed approximately 25% of the total impact of climate-related disasters (FAO 2016). The losses due to climate-related disasters affecting agricultural sectors differently: floods and storms responsible for crop damages; droughts for damage to livestock; storms and hurricanes for damage to fisheries; and floods for damage to forestry (FAO 2016). Varying quantities of climate risks and vulnerabilities are found at the regional level. The major type of natural disaster in sub-Saharan Africa and the Near East was drought and floods in Asia, Latin America, and Caribbean countries (FAO 2016).

There are a number of ways to tackle the challenges of climate change, e.g., bringing genetically improved varieties, salt and drought tolerant variety development, renewable energy and biofuels, afforestation, traditional agricultural practices, etc. Though many adaptation and mitigation strategies have been practiced for last few decades, however, little attention has been given to the microbial adaptation leading to climate change. Knowledge gap and industrial agriculture of chemicals and fertilizers deteriorating the health of soil organic matter of agroecosystem are the major concerns. The role of microorganisms in maintaining soil health has been realized in recent years. This chapter will look into the role of microorganism in managing climate change impacts for sustainable agriculture and environment.

1.2 Role of Microorganisms in Agriculture

A microorganism or microbe is a microscopic creature existing as single-celled form or in a colony of cells. They are common in almost every habitat from the poles to the equator, deserts, rocks, and the deep sea. Some of the microorganisms adapt to extreme temperatures like very hot or very cold conditions. They are a vital

component of fertile soils. Microorganism comprises a small volume of soil organic matters that are mostly active in the portion of soil life. This small portion is responsible for all nutrient cycling in soil for plant uptake, nutrient availability from mineral to plant root zone named rhizosphere. The role of microorganism in agriculture is stated below.

1.2.1 Microbes for Plant Nutrition

Plants uptake nutrients directly from the soils through their rhizosphere. Microbes present in the soil and atmosphere play an essential function in the nutrient management (Adhya et al. 2015). The role of bacteria and fungi is very crucial in decomposition of soil organic matter (Neill and Gignoux 2006). Microorganisms such as *Aspergillus niger*, *A. chroococcum*, *Azospirillum brasilense*, *Bacillus subtilis*, *Pseudomonas corrugata*, *Rhizobium* sp., and *Streptomyces nojiriensis* enhance plant growth and development (Bhattacharyya and Jha 2012; Phukan et al. 2012). Antagonistic actinomycetes native to the soil habitat have also been effective in decreasing the impact of plant pathogens during the plant growth (Sarmah et al. 2005).

Beneficial microbes in plant roots help in supplying nutrients, e.g., nitrogen, phosphorus, and potassium. Symbiotic associations with the higher plant roots have been found in arbuscular mycorrhizal fungi (AMF) (Salvioli et al. 2016). It helps in the absorption of nutrients such as P, water, and other important essential elements. Fixation of atmospheric nitrogen has been done by various algal genera such as *Anabaena*, *Aphanocapsa*, *Chroococcus*, *Oscillatoria*, and *Phormidium* from the rice fields (Hasan 2013; Shridhar 2012). A number of blue-green algae have been accounted for symbiotic associations with other microorganisms such as fungi, mosses, liverworts, and aquatic ferns (*Azolla*).

1.2.2 Microbes for Plant Growth Regulators

Rhizosphere-living microorganisms synthesize and release auxin, a plant growth regulator (Kapoor et al. 2012). Various plant growth regulators are produced from soil microorganisms, e.g., bacteria, fungi, and algae (Ahemad and Kibret 2014). Plant growth-promoting rhizobacteria (PGPR) is responsible for producing various phytohormones such as indole acetic acid (IAA), gibberellic acid, and cytokinins (Kloepper et al. 2007) and important metabolites such as siderophores, HCN, and antibiotics. Along with PGPRs, many pathogenic, symbiotic, and free-living rhizobacterial species took part in the rhizosphere (Han et al. 2005). Fungi also count in this process (Rahi et al. 2009; Murali et al. 2012) by bio-controlling parasitic spores, sclerotia, or hyphae of pathogenic fungi (Mejia et al. 2008). This biocontrol process produces a large quantity of enzymes including chitinases, proteases, and glucanases. *Trichoderma* strains are reported to inhabit with diverse plant roots (Saba et al. 2012). This advantageous association of fungi with plant growth is known as mycoparasitism (Jeffries 1995).

1.2.3 Microbes for Phosphorus Solubilization

Phosphate is a least mobile element among plant macronutrients. Phosphorus-solubilizing microorganisms (PSMs) play an important role in solubilization and mineralization (Walpola and Min-Ho 2012; Sharma et al. 2013). The mechanism of phosphate solubilization follows a reduction in soil pH due to the production of organic acids by the microbial communities followed by the discharge of organic phosphorus by acid phosphatase. The efficiency of phosphorus solubilizing is achieved when PSM is co-inoculated with other beneficial bacteria or mycorrhizal fungi (Mohammadi 2012). The efficiency of bacteria is higher than fungi in phosphorous solubilization (Sharma et al. 2013). Bacterial population in the soils, ectorhizospheric strains of *Pseudomonas* and *Bacillus*, *Rhizobium*, *Enterobacter*, and endosymbiotic rhizobia constitute efficient microbial communities of phosphate solubilizers to enrich soils with P (Khan et al. 2009). Phosphate-solubilizing bacteria (PSB) remain in the normal soil by 1–50% population, while phosphate-solubilizing fungi (PSF) have only 0.1–0.5% population (Panhwar et al. 2011). Potential strains of phosphate-solubilizing species are *Bacillus megaterium*, *Bacillus circulans*, *Bacillus subtilis*, *Bacillus polymyxa*, *Bacillus sircalmous*, and *Pseudomonas striata* (Rodriguez and Fraga 1999).

1.2.4 Microbes for Potash Mobilization

Potassium (K) is an important essential element for the plant. K is found abundant in soils. The proportion of K in the top soil ranges from 3000 to 1,00,000 kg/ha (Bertsch and Thomas 1985). There are four different types of K found in soil such as water-soluble (solution K), exchangeable, nonexchangeable, and structural or mineral (Sparks and Huang 1985). The amount of K release in soils depends on various factors. Changes in soil parameters like pH, moisture content, texture, level of oxygen, temperature, soil tilling, topography, and biogeochemical characters impact the release of K (Basak and Biswas 2009). The role of microbes in K mobilization is remarkable. In mobilization of insoluble K in the soil for plants, some effective microorganisms such as *Acidithiobacillus ferrooxidans*, *Arthrobacter* sp., *Azotobacter* sp., *Bacillus mucilaginosus*, *Bacillus edaphicus*, *Frateuria* sp., *Klebsiella* sp., *Paenibacillus* sp., *Pseudomonas* sp., and *Rhizobium* sp. (Sheng 2005; Lian et al. 2008; Liu et al. 2012) play a very crucial role.

1.2.5 Microorganisms as Biofertilizer and Biopesticide

Microbial biofertilizers and biopesticides are best for sustainable agriculture (Bhardwaj et al. 2014). Microbial biofertilizer is the application of living microorganisms on the seed, plant surface, or soil promoting rhizosphere microbial growth and supply of nutrients for plants (Bhattacharyya and Jha 2012; Vessey 2003). Microbial biopesticides promote plant growth by production of antibiotics,

siderophores, HCN, production of hydrolytic enzymes, and acquired and induced systemic resistance against pathogen (Somers et al. 2004; Chandler et al. 2008). An effective species of bacteria, named *Rhizobium*, displays symbiotic interactions (Shridhar 2012; Wang and Martinez-Romero 2000) with leguminous plants. This symbiosis occurred in root nodules where ammoniacal nitrogen fixation is done by bacteria for plant availability. This can be used as biofertilizer. *Rhizobium* biofertilizer could replace chemical nitrogen up to 30–35% (Mia et al. 2010). Other bacterial species, e.g., *Bacillus*, *Mesorhizobium*, *Acetobacter*, *Azospirillum*, *Aspergillus*, *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Azotobacter*, *Allorhizobium*, *Penicillium*, *Pseudomonas*, etc., also have potential plant growth-promoting capacity (Vessey 2003).

1.2.6 Microbes in Bioremediation

Bioremediation is a process where living organisms consume and break down the complex compounds, turning it into harmless, natural substances (Kumar et al. 2011). The prime bioremediators are known as bacteria, archaea, and fungi. In mycoremediation, fungi play the dominant role in the breakdown of aromatic pollutants such as toxic petroleum and chlorinated compounds (Rhodes 2014). Mycofiltration process is used to remediate/metabolize pollutants using fungal mycelia to filter toxic wastes and microorganisms of water bodies as well as soil.

Various microorganisms are useful in agriculture and denoted as agriculturally important microflora (AIM) for their applications in agriculture, horticulture, and forestry.

1.3 Impact of Climate Change on Microbes

The microbial existence is under threat, and sign of vivid response is shown due to changing climate and environmental factors (Kardol et al. 2010). Various dynamic reactions of soil microorganisms to environmental conditions have been observed (Joergensen 2010). The effect due to temporal and spatial scales on microorganisms also varies here (Savage et al. 2009). At the higher latitudes, the impact of global warming could be highest on microbial population (Davidson et al. 2006; The Core Writing Team 2007). Impact of climate change on microbes is stated below.

1.3.1 Effects of Temperature

The microbial population in soil determines the process of carbon sequestration along with other abiotic factors. Global warming alters the physiology of soil decomposers leading to CO_2 emission from soil (Schindlbacher et al. 2011). A high rate of carbon emission from soil is likely to be observed due to temperature increment leading to fungal decomposition. Higher temperatures help in elevating soil

nitrogen levels and negatively affect microbial activity and diversity (American Society for Microbiology 2008). On the contrary, biochemical reactions of bacteria under the warming stress work less efficiently. Hence, the release of carbon as carbon dioxide by microbes becomes higher than converting it into biomass (Zimmer 2010). The other factors include decomposers' temperature sensitivity, substrate availability, environmental variables like moisture of the soils and potential physiological adaptation conditions (Schindlbacher et al. 2011). Higher temperature induced release of carbon dioxide by microbial decomposition, which varies from soil to soil. Carbon use efficiency is crucial for long-term stability of soil and microbial biomass (Conant et al. 2011; Cotrufo et al. 2013).

1.3.2 Change in Precipitation Pattern

Change in precipitation pattern due to climate change results in extreme drought and flooding and timing of snowmelt. The available soil moisture content depends on a regular rainfall pattern (Aanderud et al. 2011). Significant effect on soil organic matter and microbial community has been observed with a 20% increase or decrease in precipitation. The carbon emission has been increased from dried peatlands with more oxygen availability to stimulate the aerobic decomposition. Moisture regimes of soils have profound effects on the growth and distribution of bacteria and fungi (Castro et al. 2010). Winter soil respiration and microbial community are greatly affected by snowfall (Aanderud et al. 2013). Climate change can result in a shift in snowfall in various ecosystems of the world (IPCC 2007; Henry 2008). In the coniferous forest, an increment of microbial activity under snow cover due to temperature fluctuation could induce heterotrophic respiration (Mariko et al. 1994; Brooks et al. 1997; Rey et al. 2002). In late winter, snow molds have been developed by extremely low-temperature snow pack. These snow molds supply about 10–30% of the total annual carbon dioxide in these areas. The rise in temperature is likely to shorten the late winter period resulting in the snow mold population to produce lesser amounts of carbon dioxide and overall decrease in carbon fixation (American Society for Microbiology 2008).

1.3.3 Effect of Elevated Carbon Dioxide Levels

An elevated carbon dioxide atmospheric concentration could result in more emission of potential GHGs, methane, and nitrous oxide (Pathak and Pathak 2012). Higher CO_2 levels also decrease methane uptake by soil microorganisms (up to 30%) (Phillips et al. 2001; Ineson et al. 1998). Moreover, higher levels of carbon dioxide also alter important microbial communities of tree leaves, having widespread consequences on the food chain. This is because microorganisms are the basis of nutrients for the small phytophagous animals (American Society for Microbiology 2008). In addition, accelerated plant productivity has been found in

an increase in microbial respiration due to elevated CO_2, and this supplies more carbon substrate to soil microorganisms (De Graaff et al. 2006).

1.3.4 Effects Mediated Through Plants

The belowground soil is not as highly influenced as the aboveground vegetation due to climatic changes (Duran et al. 2014). However, various indirect effects pass to soil microbial community through plants. Environmental change acting on aboveground vegetation has a significant effect on soil communities (Fierer and Jackson 2006). A change in rainfall pattern has severe effects on plant–microbial relationship in soils (Yepez et al. 2007) and dynamics of soil respiration (Aanderud et al. 2011). Climate change has indirect impacts on soil by modifying soil pathogenic activities (Morrien et al. 2011). Change in microbial diversity can also alter functional traits of plant (Lau and Lennon 2011). An elevated soil temperature also has consequences of improved net plant productivity to provide more substrates for heterotrophs such as discharge of labile sugars, amino acids, and organic acids from plant roots (Trumbore 1997). Global warming is likely to raise nutrient availability in soil by greater mineralization of soil organic matter (Ruess et al. 1999). The diversity and activities of microbes depend upon the availability of nutrients and changes in CO_2 flux (Diaz et al. 1993; De Graaff et al. 2006; Bardgett et al. 2009). Moreover, composition of plant community modifies with warming (Harte et al. 2006; Walker et al. 2006; Hoeppner and Dukes 2012) leading to changes in microorganisms (Havstrom et al. 1993; Hobbie 1996). Moreover, northward advancement of plants occurring in tundra region in warming condition has unknown influence on microbes (Zimmer 2010).

1.3.5 Impact on Aquatic Ecosystem

In the twenty-first century, ocean surface temperature could increase by 4–8°F (IPCC 2007). Hence, the change in aquatic temperature can potentially trigger change and disappearance of life forms (NASA 2015). Expansion of oxygen-depleted zones increases ocean stratification and thus has likely impacts on the microbial ecosystem (Walsh 2015). Warm polar oceans activate marine microbes for the decomposition of organic matter (Zimmer 2010). A higher ocean surface temperature decreases its density. It results in less upwelling of nutrient-rich cooler and deeper water to the surface and an inadequate supply of nutrients to phytoplanktons in the upper layer. The consequence is lesser pumping of carbon to the deeper water (Walsh 2015). In the Arctic, there will be smaller cell-sized phytoplankton species with the elimination of larger cell-sized due to climate change. The smaller cells, phytoplankton, have greater surface-to-area ratio, than larger cells gets sunk more quickly. This will lead to less carbon pumping into the ocean (Walsh 2015).

1.4 Microbial Role in Managing Climate Change

1.4.1 Microbial Genetics in Changing Environment

Climate change is the change of the frequency of weather of a given area for a long time. Climate change could shift in drastic change in temperature and precipitation leading to extreme heat and flooding, rising sea level, and natural disasters. Adaptation to this changing environment is the best way when change is inevitable. In the previous discussion, it was well discussed that microbes have significant role in crop production; however, climate change could jeopardize the survivability of microbes. Proper understanding of microbial function could give us lots of insight, and we could exploit it in managing climate change-related situations. A lot of microbes have short generation time to produce new variants that other eukaryotic and large organisms are unable to do (Bang et al. 2018). Phenotypic plasticity or change in organism's behavior develops on them in the changing environment with change in certain morphological and physiological traits (Price et al. 2003). Bacterial species are found to display extensive phenotypic variability/heterogeneity (Raj and van Oudenaarden 2008) building resilience (Justice et al. 2008) to environmental changes and adaptation. Phase variation or genetic changes can occur at the individual level of bacterial cells (e.g., Van der Woude 2011).

However, this beneficial mutation seems to be small, e.g., 2×10^{-9} per genome per replication for *E. coli* (Imhof and Schlötterer 2001). Horizontal gene transfer of bacteria is another kind of adaptation that took place through exchange of genetic material such as plasmids, transposons, and phages. This HGT event occurs between closely related species, allows rapid access to genetic innovations of nonparental lineages, and contributes to the dissemination of beneficial mutations (Aminov 2011). Overall, the adaptation to extreme environments requires an understanding of the diverse responses within the microbial system. The study of microbial genetics for adaptation gives us the solid foundation of utilizing the role of them in the changing environment.

1.4.2 Rhizosphere Microbes Improves Plant Stress Tolerance

Plant rhizosphere is occupied with various microbes such as plant growth-promoting bacteria (PGPB) and plant growth-promoting fungi (PGPF). Mycorrhizae supply phosphate and nitrate to plants, and rhizobacteria play a role in fixing atmospheric nitrogen (Corradi and Bonfante 2012; Geurts et al. 2012). Some beneficial microbes can provide resistance to environmental stress factors (Lugtenberg and Kamilova 2009).

Growth of crops under abiotic stress conditions can be improved by different bacterial families (Egamberdievaand Kucharova 2009). Co-inoculation of *Rhizobium/Pseudomonas* with *Zea mays* can increase its salt tolerance due to decreased electrolyte leakage and balance of leaf water contents (Bano and Fatima, 2009). Various microorganisms produce plant growth hormones such as indole acetic acid and gibberellic acid, which promote root growth (Egamberdieva and

Kucharova 2009). PGPBs can also promote plant's immune system to fight with many pathogens (Van Hulten et al. 2006).

Certain PGPF, such as mycorrhizal and endophytic fungi, significantly enhance stress tolerance of the plants against a variety of conditions, i.e., drought, heat, pathogens, herbivores, or limiting nutrients (Rodriguez et al. 2008). Some PGPF can have beneficial effect on certain host plants and exerts pathogenicity to nonhost plants, for example, *Colletotrichum acutatum*, which is a pathogenic ascomycete for strawberry but beneficial when colonizing with pepper, eggplant, bean, and tomato (Freeman et al. 2001).

Microbes help to improve plant stress responses to an abiotic environment by influencing plant physiologically (De Zelicourt et al. 2013).

1.4.3 Microorganisms in Controlling Carbon Emission

Carbon sequestration by microbial processes is yet to be explored. Two important sinks of carbon are soil and ocean can play major role to mitigate anthropogenic carbon emission (Menon et al. 2007). There is a huge potential of the carbon sequestration process which can be modified by microbial community engineering, i.e., a shift in land use from arable land to grassland entails an average 18% higher carbon sequestration, with a yearly carbon input of 0.75 tonnes C/ha/year (Kampf et al. 2016). Limited degree of soil manipulation could bring a higher degree of microbial homoeostasis for sequestration (Cleveland and Liptzin 2007; Fontaine and Barot 2005; Manzoni et al. 2012). Addition of charcoal or biochar to the soil as a long-term carbon source improves soil quality and adsorption of nutrients to increase their bioavailability to the plants (Lehmann et al. 2006; Laird 2008; Prost et al. 2013). The concept of carbon sequestration can also be approached by using concentrated CO_2 sources. Microbial electro-synthesis generates valuable products from electricity, using CO_2 or other organic feedstocks as carbon source (Nevin et al. 2010). In this process, acetate (Gildemyn et al. 2015), butyrate, and other commodity chemicals (Arends et al. 2017) have been produced. These chemicals can be converted to medium-chain fatty acids like caproate and caprylate that can serve as bio-based building blocks for the chemical industries (Agler et al. 2012; Spirito et al. 2014; Angenent et al. 2016). An energy-efficient harvesting of carbon source could lead to microbial carbon sequestration (Gildemyn et al. 2015; Andersen et al. 2016).

1.4.4 Improving Salinity Tolerance

Soil salinity could decrease national agricultural crop production in arid and coastal regions in climate change situations. *Azospirillum* inoculation can alter salt-stressed maize variety (Hamdia et al. 2004). Osmotic stress of pepper can be decreased by inoculation with *Bacillus* sp.TW4 (Sziderics et al. 2007). For the salt-stressed plants, secondary inoculation with *Azospirillum* can result in prolonged root

exudation of plant flavonoids following inoculation with *Rhizobium* (Dardanelli et al. 2008). Thus, co-inoculation of plants with various bacterial species can improve abiotic stress tolerance.

1.4.5 Drought Stress Tolerance

The drought stress on plant can result in stomatal closure to minimize water loss by increased abscisic acid (ABA) levels in leaves (Cho et al. 2008) with some other compounds such as ethylene, salicylic acid, etc. PGPR has beneficial effect on plant's drought tolerance caused by changes in hormonal contents, mainly of ABA, ethylene, and cytokinins (Cohen et al. 2008). *Azospirillum lipoferum* strains when inoculated with wheat seedlings can reduce the drought stress (Arzanesh et al. 2011).

Root morphology can be changed by beneficial bacteria and hormone-like matters produced to excite the endogenous plant hormones (Dobbelaere et al. 1999). It was also evident that significant amount of nitric oxide is produced as a diffusible gas by *A. brasilense* in aerobic conditions signaling IAA-induced pathway for root growth (Creus et al. 2005; Molina-Favero et al. 2008). Inoculation of plant species with certain bacterium species can increase its drought stress tolerance by isolating its drought-responsive gene, ERD15, from *A. thaliana* when inoculated with *Paenibacillus polymyxa* (Timmusk and Wagner 1999).

1.5 Conclusion

Climate change is a real thing, and it is already marking its harmful impact on Earth. The future of climate change will be more harmful, and we need to act immediately. Among various adaptation methods on climate change, microbial mitigation and adaptation are the latest additions here. The role of microbes is least known among the scientific community. Various promising aspects of microbes have been discovered to cope with changing environment due to climate change. Some of them have been discussed here. More results will come from the research on microbial culture, identification and physiology, and DNA sequencing. The future of Earth will vastly depend on the research of microbial life in changing environmental conditions.

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Microbial Interventions in Soil and Plant Health for Improving Crop Efficiency

2

Dhiman Mukherjee

Abstract

Realization of nutrient security and foodstuff demand as a whole is a significant aspect for the whole community of farming system. Microbes as unicellular organisms play a significant role in the whole soil–plant diverse agro-ecosystem. Bacteria, fungi and other microbial creatures have friendly symbiotic relationship with other well-developed organisms, some of which are equally helpful (mutualism), while others can harm the host life or develop relationships such as synergism and commensalism. Microbial intervention mainly encompasses the method of intervening natural process in soil or in crop rhizosphere by the microbial population there in the root, which is mostly helpful for the improvement of food materials accessibility as well as expansion and yield of plants. Effective soil inoculants attack and stay in the crop field with naturally occurring bacteria and confined stress situation in erratic state and to set up a well-matched interface by the host that includes biochemical association with the crop-resistant features. Various microbes in the soil system not only help in mineralization process but also help to make firm soil with good amount of organic substance akin to humus and other natural carbon-related complexes. This process is very much influenced by various climatic factors mainly temperature and wind, precipitation, etc. Under changing climate situation, nature of microbes is also being changed and develops very complex type of interactions, which become very difficult to understand. Genetically modified crops (mainly nonleguminous) form N_2-accumulating competent nodules by *Rhizobium*, ensuing nitrogen accumulation by nitrogen fixation. The induction of nodules harbouring nitrogen-fixing bacteria is a result of complex interface between BNF microorganism and plant. It involves several sets of genes and signals from both partners in a coordinated expression. In totality, it may be possible that NSP1/NSP2, NF-YA and

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ERN1 work in association and are helpful to the plant systems. Various biofertilizers are capable of accumulating nitrogen from the atmosphere, assisting the right use of nutrients such as potassium and phosphorus from organic or natural fertilizers and earth stock, progressing drought tolerance, getting better crop health or boosting alkalinity resistance. Crop root notices microbes with pattern-recognition sensor, which attach microbial-linked molecular pattern and trigger a basal protection enough for expansion of various pathogenic bacteria. 'Omics' techniques facilitate the recognition of gene transcripts, proteins or metabolites and have been developed to give a more detailed account into the genes and function expressed in the crop microbiome. Microbial population in soil agro-ecosystem are affected by an accumulation of biotic and abiotic factors that lead to numerical and qualitative variations. Bioremediation is a universal suitable option mainly to eradicate ecological pollutants in a contaminated place. This method includes mainly bacteria and flora to rot, impound or take away soil pollutants, mainly chemical insecticides and synthetic chemicals. This is possible by a succession of complex metabolic exchanges, repeatedly linking numerous diverse organisms, and unwanted contaminants can be ruined down or removed.

Keywords
Agro-ecosystem · Crop · Climate change · Disease · Microbes

2.1 Introduction

Fulfilment of food demand is an important and challenging task for different scientists, growers and policymakers worldwide. This involves a huge bionetwork of factors delivered by farming community and its allied sectors. More need for food leads to more intensive agriculture system with use of modern tools and heavy load of agro-chemicals to our environments (Lareen et al. 2016). Soil is factually swarming with different forms of life and important components under various agro-ecosystems. A handful of soil comprises trillions of microbes denoted by few thousands to millions of group or genera. Less than 1% earth gross facade vicinity is controlled by microbes which are beneficial for agricultural crop production (Mukherjee 2002; Young and Crawford 2004). Various microbes spreading out in the soil are so tightly associated with the site, extent and value of accessible soil carbon status, and nutrients present in the soil become very complicated. Discharge of various natural compounds into earth by crop rhizosphere not only provides significant substance for microbes but also exerts noteworthy control on the soil biophysiological system by changing its micro- and macro-ambience inside the root. This might be both helpful and negative for crop depending on which species of soil biota propagate. Decomposed crop or plant residues and natural content of soil turnover give the available carbon necessary to uphold earth's living action. Interactions of various microorganism communities with a range of soils and crops encompass an important critical fraction for soil plant system, which leads to more crop per unit

area of land (Mukherjee 2016a). Soil–plant system enlists their own microbiome that acts together with them and their abiotic surroundings via different mechanisms, which have remained core design for studying the microbial interventions in soil and plant for crop efficiency. This helps to know inherent molecular, biochemical and genetic mechanisms of crop microbial connections and decipher the final payback to soil–plants' health. Various molecular-level interventions in microbial inoculums help to manipulate crop and soil ecology surrounding. The spectacular boost in the use of synthetic chemicals for getting more economic products has become an important part of present-day farming practice (Mukherjee 2016b). The recurrent and excessive use of synthetic dose of chemical nutrients is costly, and this may deteriorate the ambiance at a quicker speed and makes land resource inappropriate for farming. There is deterioration of soil quality, turmoil in symphony and useful structure of earth's microbe community, and as a result, there is land loss of its productivity and it becomes barren. To trounce such environmentally unwanted actions, we require extending a feasible alternative that could tackle the present situation in an effective and sustainable way. Functionally varied groups of microbes are an imperative part of the earth's eco-system, which provides a low-cost option to synthetic chemicals. In the modern era, much attention has been paid in exploiting microbial strategies to make easy crop physiological development and growth, and in some cases, they have been commercialized for diverse plants such as collego, biomeal, etc. (Mukherjee 2016a). Plant linked microbes (rhizosphere environment and endophytic) are capable of supporting crop development by production of phytochemicals and secondary metabolites which help in soil bioremediation and sinking various stresses inside earth (sickness and pest, etc.) (Mendes et al. 2013a, b). Plant rhizosphere-linked endophytes are capable of making phytohormones, namely, gibberellins and auxins, for enhancing crop productivity (Hardoim et al. 2015). Under natural farming, diverse microbes, with suitable initiative and knowledge of them, are used as soil inoculates, for seed treatment, etc. to offer various important plant nutrients like N, P, and other phytohormones (Mukherjee 2013). Further, microbial association in crop root zone plays global consideration due to their role in various plant disease control systems and management of poor and barren soil via remediation technology. Therefore, the microbe community in broad is a viable tool for sustainable agriculture and better crop production expertise in the near future.

2.2 Microbes and Microbial Interaction

Tiny creatures that are microscopic are called microbes or microorganisms (very small to be realized or seen with the normal eye). Microbes are categorized as unicellular or single-celled organisms. Single-celled microbes play all the necessary roles of the living system. Microbes such as bacteria, fungi, etc. are microscopic; however, few eukaryotes are also tiny, including most protists and few fungi. Single-celled microbes are unicellular all through their existence process and play a crucial role in biotechnological intervention in the agriculture system. Single-celled

organisms generally hold merely a sole replica of their genome when not undergoing cell division; however, few organisms have manifold cell nuclei. Microbes reside in all territories of the environment, from the soil to the atmosphere. Few microbes have friendly symbiotic associations with other well-developed organisms, some of which are equally helpful (mutualism), while others can harm the host life. This phenomenon is called parasitism. Microbes in association with other microbes set up mutual benefit relationships and help higher plants or living beings (Chamoun et al. 2015). Generally, the bond is dietary, while other benefits might grow, and the union can turn necessary to the endurance of one or both partners. Numerous types of relationships such as synergism, commensalism, mutualism, amensalism, etc., are observed amongst the organisms (Lareen et al. 2016).

2.3 Microbial Intervention for Crop Health

Different approaches have shown that variation of microbes in the soil and rhizosphere microbiomes is very much unpredictable. Gans et al. (2005) assessed that 1 gram of dirt hold nearly one million different microbial genomes. Later on, Roesch et al. (2007) obtained 139,819 bacterial and 9340 crenarchaeotal rRNA gene sequences from 5 separate materials (soils) and counted an utmost of 52,000 operational taxonomic units (OTUs). Bacteroidetes, betaproteobacteria and alphaproteobacteria are a plentifully available microbial class in various cultivated lands under different findings (Roesch et al. 2007). Soil microbes play cumulatively for the benefit or harmful effect of soil. Basically, microbial intervention is the method of intervening natural process in soil or in crop rhizosphere by the microbial population present in the root which is mostly helpful for improvement of accessibility to food materials as well as expansion and yield of plants (Mukherjee 2017b). Microbe interference is very much supportive in attaining elevated output with sustainability in agriculture in numerous ways like more easy accessibility to crop foodstuff, fixation of atmospheric nitrogen, decay and recycling of raw wastes and residue, restraint of soil-associated pathogens, biodegradation of toxic chemicals mainly pesticides, synthesis of natural molecules for crop utilization, solubilization of food source, fabrication of cellulose to get better earth composition and numerous others.

Active microbe inoculants attack and stay in the crop field with naturally occurring bacteria and in a confined stress situation in an erratic state and set up a well-matched interface with the host that include biochemical association with crop-resistant features. During crop season, various soil available microbial communities undergo nonstop changes both over and under the ground, which influence crop vigour condition (Copeland et al. 2015). Microbes are seldom observed as sole type and are seen in a number of hosts or situations; thus, there is a great difference in microbe connections about the organisms concerned. Bacteria–fungi, fungi–crop or animal, microbes–plant or animal and microbes–fungi–crop or animal interactions lead to several kinds of exchange programme, which allow augmented host suitability. This may either be helpful for better yield of crop or improve crop

efficiency in many plants such as lettuce and spinach. According to Van et al. (2012), concern of a novel-type intruder in a milieu relies on the trait of limited microbe association which may be antagonistic or synergistic.

2.4 Microbial Interference for Disease Suppression

Several crop–microbe microbiome has a straight role in the soil microbial metacommunity, which, in turn, can be intensely influenced by farm practice (Fierer et al. 2013). Various classes of the root microbiome are helpful for crop development and its proper physiological function. Crop or root-colonized pathogenic microbes inhabit the rhizosphere striving to rupture from side-to-side microbial guard and conquer the inner crop defence system in order to start infection. To improve crop expansion and health, it is necessary to know important microbiome nearby roots of the crop and its role in crop production and management system, particularly what they are doing (Mendes et al. 2013a, b). Knowledge related to disease or various menace suppressive land has been correlated to change in micro biomass symphony as well as action. Microbial population in the soil can encourage erstwhile phenotypes in crops. Proper build-up and maintenance of the range and action of helpful soil microbial population give a protective system around the plant rhizosphere which compete with pathogen and give the right kind of protection to the vegetation. Some microbes under various soil media can inhibit different pathogens with the production of hydrogen cyanide (HCN) or fungal cell wall-degrading enzymes, such as chitinase and β-1,3-glucanase. Soil useful bacteria or microorganisms can help repress numerous plant rhizome- or root-consuming pests through their immature intensification phase with use as foodstuffs.

Under normal situation, plant and soil surrounding pathogens has a very critical role in farming process and whole ecosystems of land mass by enhancing decomposition of plant tissue and other waste of crop produces. Rigorous outbreaks of different infections are typical symptom of unevenness in a structure, whether it is a food surplus or requires need for genetic assortment, monocropping practices, etc. which gives a huge extent as host inhabitants. Natural check measures by utilizing soil-borne organisms work by diverse modes of action, which mainly comprise competitive exclusion, hyperparasitism, synthesis of normal antibiotics, systemic acquired resistance and induced systemic resistance. In competitive exclusion, one organism creates a milieu that is not desirable for a new organism, which efficiently excludes the second life form and is devoid of any kind of direct killing (Nebert et al. 2016). A lot of soil microbes produce antibiotics, which kill harmful pathogenic microorganisms, and this helps to bear crop expansion and maturity. One can recognize this by presence of *Penicillium* sp. and *Streptomyces* sp. in soil. They produce penicillin and streptomycin, respectively, which help to restrain the expansion of numerous pathogenic microbes in earth soil by distorting cell wall, production of various acids, modification of the metabolic system and protein synthesis. Natural antibiotic-producing organisms such as *Streptomyces* restrain unlike pathogens in earth system; as similar method its act in human and farm living beings; the

microbes generate the antibiotic that kill the bare pathogens. Explicit creatures are identified to defend seedlings and seeds from a range of diseases. For example, different *Bacillus, Pseudomonas* and *Trichoderma* species guard rhizosphere of crop from contagious plant diseases (Trabelsi and Mhamdi 2013). One can introduce such kinds of microbes or microorganisms by proper treatments of the earth's surface with specific culture. Effectiveness of soil culture varies broadly under different sorts of management practices.

2.5 Microbial Intervention in Soil Agro-ecosystem

Soil safety is a vital module for food sufficiency, as it is directly related to improving crop efficiency or economic yield under field conditions. The quality of soil has been distinct by the explicit nature of soil to carry out tasks within normal or systematic ecosystem, which limits to maintain natural efficiency, encourage ecological eminence and uphold crop and organism health. Use of a low amount of fertilizers or natural cultivation in few regions is significant and essential for monetary and community reasons. In this situation, the importance of microorganism in food materials accessibility for crop cultivation and restrain next to sickness and pests is of crucial value. Physicochemical properties of the soil and natural environment are the main parameter for primary output, and by exchange, these factors modulate whole farm structure efficiency. Deteriorating soil productivity and system production is a key global apprehension for attaining nutrition safety, mainly for escalating global inhabitants. As per different reports, soil health improvement with various bio-organic input can boost output only by 15–20% with use of proficient crop cultivars, and farm efficiency could be improved by 40–50% (Mukherjee 2016c). Food security is the global concern and foremost defy, and it is estimated that by 2050, food grain output needs to boost up to 45–65%. The major challenge for scientists, farmers and various policymakers is to enhance food grain/vegetable production under limited available land resources. Constraints mainly confined to limited soil productivity at global level due to intensive farming, soil erosion, depleting soil nutrient status, erroneous use of synthetic chemicals, and profound use of heavy machines.

Soil inhabitants, mainly bacteria, are a massive resource of hereditary wealth; however, many of these (>90%) are unproductive at present due to poor availability of local strains. Latest advances in omics technology bid an enormous potential to expose and exploit new genetic resources from earth microhabitat. Use of proteomics, metagenomics, metabolomics and transcriptomics becomes promising to recognize mechanisms with segregate genetic wealth for enhancing nutrient cycling and NUE with no taming earth microorganisms (Abhilash et al. 2012). For instance, metagenomics could give main information of novel hereditary assets for new characters in the soil. Various genes may be isolated or synthesized and used mainly for transgenic purposes. Moreover, metagenomics with conservative method of earth biophysical property may be utilized to resolve soil capability for offering various nutrients for plants, with minimal use of various agro-chemicals in farming. The

assortment of microorganisms connected with crop rhizosphere is massive and very complex. Current research in crop–bacterial exchange study revealed that crop is capable of shaping their root microenvironment as evidence from unlike crop variety host marked microbe community while cultivated on the identical soil (Berendsen et al. 2012). As per different research, it is quite obvious that different plant nutrients have an important role in various aspects of plant rhizome/root architecture, so this will effect profound development of rhizosphere. The parts surrounding crop roots have soaring plasticity to soil ecological change and can retort to availability of heterogeneous food particle in the soil profile in different patches (Jing et al. 2010). Almost all plant nutrients are absorbed by crops by their root rhizosphere, where exudates of roots mostly play a pivotal function in pouring exchanges amidst crop roots, soil and microbes. Exudates from root mainly contain different sugars, naturally occurring acid anions, phytosiderophores, acid phosphatases and phytase and amino acids that have a straight or tortuous effect on the gaining of plant food materials required for crop development. Split root trail of white lupin revealed that root exudation improved radically limited proton, citrate and acid phosphate when exposed to phosphate-lacking treatment (Shen et al. 2005). Acidification of root surroundings helps to enhance phosphorus uptake by crop and improve crop yield due to involvement of more microbes (Zhang et al. 2010). Different types of nitrogen are available to the plant in soil in large amounts controlled by the uptake ratio of anions and cations and so influence root and rhizosphere apoplastic pH (Marschner 2012).

Root acidification plays critical role in nutrient mobilization; this could strengthen by the use of appropriate doses of inorganic fertilizers and capable plant genotypes that can acidify root surroundings. This greatly helps to activate few microbes to some extent and help plant or crop growth. The role of microbes becomes more pronounced under acidic conditions to a great extent, and acid-loving microbes play a crucial role in crop development particularly in the case of rice and spinach. As per instance, use of single super phosphate and ammonium sulphate might lead to a lesser pH in the stimulant microsites in contrast to the use of diammonium phosphate (DAP), which is in support of nutrient movement and absorption by plant rhizosphere, particularly in calcium-rich soils. Use of proficient plant cultivars can secrete acid from the root and help to mobilize nonsoluble mineral in the soil as efficient method to boost phosphate accessibility in faba bean (*Vicia faba* L.), chickpea (*Cicer arietinum*), soybean (*Glycine max*), lupin and alfalfa (*Medicago sativa*). However, the root architectural process may also be triggered by hereditary alteration of plant and microbiological actions (Ryan et al. 2009). In exhaustive farming, various works point out that too much use of synthetic nitrogen (as chemical fertilizers) can endorse more acidification land mass for an extended period and become harmful for the microbial cycle (Guo et al. 2010). Significant use of microbial sources and decreased use of various synthetic chemicals with limited nitrogen and phosphate nutrients help in rhizosphere expansion of crop or plant for easy uptake of minerals, which assist in obtaining good vegetation yield and economic return to the growers.

2.6 Microbes and Nutrient Availability

Various microbes in the soil system not only help in mineralization process but also help to make firm soil with good amount of organic substance akin to humus (humic acid) and other natural carbon-related complexes. By this mechanism, soil available nutrients are recycled to mobilize the nutrients in a faster way and help in forming the soil structure to strengthen in a better static way (Pandit et al. 2017). The amount and value of microbial biomass with its decomposition are correlated with the available nutrient status of soil composition. Living organisms have a critical function in controlling the transformation of crop nutrients in earth. In the majority of soils, nutrients such as nitrogen, phosphorus and sulphur are mostly present as different organic compounds that are not utilized for crop uptake, showing various signs of nutrient unavailability (Mukherjee 2017e). Knowledge of the function of microbes in regulating the exchange of these organic pools into plant-accessible forms has acknowledged significant interest from microbiologists, soil chemistry researchers and crop management scientists. The microbial exchange of nutrients in a soluble form takes place through different procedures and mechanisms (Li et al. 2017). Proper knowledge of the important link between plant nutrient absorption and soil microbial interference will permit more well-versed management decision to be made for proper stewardship of soil wealth and for underneath suitable levels of plant economic output.

In normal ecosystem, crop–microbe relationships are key for principal crop nutrient availability. Crop or plants liberate carbon by rhizodeposition, which may be utilized by the microbial community for augmentation and action. Microorganisms, in return, provide different necessary nutrients (nitrogen and phosphorus) by atmospheric fixation of nitrogen or mobilization of soil organic carbon (SOC). A number of symbiotic and free-living microorganisms may be recognized to improve minerals and foodstuffs accessibility to crops (van der Heijden and Wagg 2013). There are plentiful free-living microbial habitats near the rhizosphere that can fix aerial nitrogen (Orr et al. 2012) and solubilize P (Richardson and Simpson 2011) for plant uptake. Under optimal conditions, the microbes are capable of fixing a noteworthy amount of nitrogen, which is cost-effective and environmentally significant. It is because the method of nitrogen fixation can notably reduce the amounts of synthetic form of fertilizer under both dry and humid conditions particularly in pulse-based cropping system. In moist conditions, nitrogen mobilization is high and therefore more leaching and denitrification, declining nitrogen accessibility to the crop (Miransari 2011). Few available soil microbes or bacteria can fix aerial nitrogen by nonsymbiotic association through its host plant. These are mainly *Achromobacter* spp., *Azotobacter* spp., *Azospirillum* spp. and *Pseudomonas* spp. (Saxena and Tilak 1998; Saharan and Nehra 2011; Mukherjee 2011). Crop uptake of varied nutrients by interference of AM fungi with host plant through their widespread hyphal growth becomes very effective mainly in root crops (Miransari 2011).

Various bacteria and fungi augment accessibility of phosphorus to crops from organic and unchanging phosphorus by mineralization and solubilization. This process helps to improve phosphorus gaining and accessibility by enhancing root growth in

a profuse manner. A wide array of contaminant microbes and fungi capable of solubilizing different forms of phosphorus are *Aspergillus* and *Pseudomonas* (Rodríguez and Fraga 1999; Whitelaw 2000). Symbiotic association between crop roots (particularly in pulses) and mycorrhizal hyphae plays a significant function by which crops utilize potash, phosphorus and many other minerals from earth substrates (Sanyal and Datta 1991; Mukherjee 2015b). The supply of phosphorus (organic or mineral) mainly depends on host plant interaction with microbial bacteria and mineralogical cycle existing in soil agro-ecosystem, which decide phosphorus mobility in the soil (Houser and Richardson 2010; Salimpour et al. 2010). Potassium is the third essential nutrient required by plants particularly in the early stage of crop growth. Potassium-solubilizing microorganisms play a vital role in making available insoluble forms of potassium by mineralization. They solubilize K from unavailable forms like mica, feldspar and others by producing organic acids, siderophores and also capsular polysaccharides (Ullman 1996). Another important plant nutrient in the present context is sulphur, and its availability becomes crucial particularly for oilseed production (Mukherjee 2014a). Sulphur availability to plant (mustard, toria) mainly depends on the microbial population availability and on its biological commotion. The genes concerning the mobilization of sulphonate and sulphate ester sulphur by various rhizosphere bacteria such as *Pseudomonas putida* (mutant S-313) have been pointed out (Kertsez and Mirleau 2004).

2.7 Microbial Signals for Crop Architecture

Microbe association with farming system plays a critical function in the performance of various crops with change in their physiological and growth process. This helps to change crop growth pattern and its architectural configuration. Plant roots and microbes in the rhizosphere zone have coexisted for millions of years, and they mutually benefit each other. Crops uphold a multifaceted interface with their root pattern, which would be vital for mineral absorption, growth and commencement of defence system. This may be useful due to crop and microbe signalling system. Root exudates play a critical role in signalling and provide benefit to crop-associated microbes in soil media. Its management involves manipulating root augmentation, rhizosphere alteration, restricted nutrient use, rhizosphere relationship in mixed cropping and the use of a well-organized plant variety with an endeavour to develop the organic latent for competent nutrient gaining by crop roots rather than excess utilization of synthetic nutrients such as urea, DAP, etc. Use of various fertilizers helps to offer mineral nutrition for crops and also more importantly to act as regulator of rhizosphere expansion with the help of signalling mechanism. The nutrient use under exhaustive agriculture practice would be maximized by optimizing rhizosphere architecture in an effective way and mineral solubilization and improving crop gaining. Crop development and growth depends on cell division, cell expansion and cell differentiation. Proper action of these mainly involves the transfer of signalling molecules amidst different parts of plant, which can be influenced by various stresses. The function of root exudates as signalling molecules showed that

roots produced malic acid, which allows the useful soil microorganisms *Bacillus subtilis* to reach the root, and this interface plays a significant role in crop defence against the foliar pathogen *Pseudomonas syringae* (Rudrappa et al. 2008). Likewise, alfalfa and tobacco crops are genetically modified to produce more citric or malic acid, which helps in the colonization of mycorrhizal fungi and rhizobacteria. This signifies the function of natural acids in crop–microbe exchange (Tesfaye et al. 2003). These kinds of research highlight the planning of organic acid biosynthesis and excretion from transgenic crop, which might symbolize striking techniques to adjust the roots' surrounding with latent use under different kinds of farming practices (Mackey and McFall 2006; Mukherjee 2014a). Exogenous use of defence signalling molecules, mainly methyl jasmonate, nitric oxide and salicylic acid, helps to build a broad array of secondary metabolites mainly indole glucosinolates, phytoalexins and alkamides, which might be useful in exchanging signals with microbe community (Zhao et al. 2005 ; Manuella et al. 2016).

Nitrogen nourishment remains the main limiting source in plant efficiency and the foremost cost to agriculture crop production system. Use of transgenic technology might reduce crop nitrogen requirement in two possible ways: first one is by genetically modified crops (nonleguminous mainly) to form N_2-accumulating competent nodules by *Rhizobium*, ensuing in nitrogen accumulation by nitrogen fixation (Jones et al. 2007). Moreover, nodule development in pulses engrosses a multifaceted signalling dialog amidst crop and rhizobia to begin colony formation, nodule arrangement and N_2 accumulation. Every phase is governed with numerous hereditary materials and as a result flourishing shift of nodule forming skill in cereals or other crops, which highlight the relationship amongst numerous crops with various microbial genes (NRC 2008). In the future, quickly rising omics technology would assist very much in understanding this composite sequence of relationships. Other possible approach to enhance N accessibility to crop include engineering crops with N-fixing (*nif*) genes. *Nif* genes encode nitrogenase enzyme, a key enzyme in the accumulation of N_2, and are distributed in numerous free-living and associated microbes (Wang et al. 2013). Numerous plants contain acid phosphatase enzymes for phosphate solubilization; however, this could predict that phytase genes and alkaline phosphatase can harness the use of transgenic techniques with various signalling methods, and as a result, the plant may sprightly use natural or unchanging phosphorus (Tian et al. 2012).

2.8 Microbes and Biological Nitrogen Fixation

Demand for cereals mainly wheat, maize, rice and small millets are gradually increasing so there is a need to improve the agronomic and molecular parameters to enhance the quality and productivity of cereals. Nitrogen is a vital essential nutrient intended for proper expansion and improvement of crop; however, cereals are unable to directly take up nitrogen from the environment. The nitrogen content of the soil is maintained through either fertilizer or organic farming. A surfeit use of nitrogen compounds in any form like water, air, and soil wreaks havoc on the

delicate rhizosphere of the plant root system. This crisis is overcome with the assist of atmospheric N_2 fixation process, which helps to reduce the undesirable effects of chemical nitrogen. The most effective and peculiar aspect of nitrogen fixation is symbiosis of the root nodule bacteria in legumes and nonlegumes. This occurs by different types of interface between the host plant and bacterium (Oldroyd and Downie 2008). It is assumed that about 20–25% of total nitrogen need is fulfilled by nitrogen fixation in cereal crops (Montanez et al. 2012). Another symbiosis process of nitrogen fixation takes place by cyanobacteria (e.g., *Nostoc* spp.), and they colonize different plant organs either intracellularly or extracellularly (Wagner 2012). These are novel methods by which nitrogen could be fixed directly in the soil with the help of soil or atmospheric microorganisms with the assist of other beneficial microbes in the ecosystem. Nowadays, researchers have a keen interest in introducing root nodule formation in cereals. But nodulation functioning in cereals is a tedious task, still if succeeded will be a novel achievement in the agricultural world. Nitrogen-fixing bacteria present in plant roots that can 'fix' atmospheric nitrogen as nitrate are known as *diazotrophs*. Similarly, cyanobacteria (blue–green algae) also fix the atmospheric nitrogen. However, these are generally endemic to the soil, and their efficiency towards nitrogen in rhizosphere is based on behaviour, concentrations of organic constituents of exudates secreted by plants as well as their corresponding ability to utilize organic compounds as carbon source (Florence et al. 2016). Genetic engineering with nitrogen-fixing symbiosis by following active signalling and developmental methodology to facilitate an appropriate setting for nitrogenase action in the crop nodule would be proved best solutions (Oldroyd and Dixon 2014; Rogers and Oldroyd 2014). The induction of nodules harbouring nitrogen-fixing bacteria is the result of complex interface between BNF microorganisms and plants. It involves several sets of genes and signals from both partners in a coordinated expression (Madsen et al. 2010). Collectively, it may be possible that NSP1/NSP2, NF-YA and ERN1 work in association to control the appearance of premature disease (Smit et al. 2005). One of the genes, *NAD1* (Nodules with Activated Defense 1), was very much useful in the maintenance of rhizobial endosymbiosis in nodules (Cerri et al. 2012). Moreover, the exact regulatory phase occupied in increasing nutrient uptake is yet to be deciphered.

2.9 Molecular Features in Biological Nitrogen Fixation

Nitrogen is one of the key components for crop growth and the whole physiological system. Its replacement becomes very difficult, and the only option is nitrogen accumulation in cereals equivalent to the pulse crop. However, this would be very tough under the present context. For nitrogen assessment, nitrogenase biosynthesis and N_2 fixation both are cumbersome processes. The use of *nif* genes using genetic markers is the preliminary approach of validation (Schmid and Hartmann 2007). Initially, in cyanobacterium, gene diversity was identified using nif gene probes and PCR fingerprinting using RFLP marker. Rai et al. (2014) demonstrated that 12 diverse terminal restriction fragments (TRF) were isolated using *nifH-RFLP* marker analysis

from the soil samples. Construction of library is an efficient way to reveal the gene diversity of uncharacterized diazotrophs in rhizosphere. Ueda et al. (1995) identified diazotrophs in rice using PCR-amplified *nif-H* sequences. The major problem with using RFLP is pattern of *nif-H* gene, as its behaviour differs within identical soil samples (Poly et al. 2001) which can be determined using cluster analysis of *nifH-RFLP* profile. This study could produce the data with a small difference in cluster analysis of *nifH-RFLP* profile in dirt area with various microbial communities (Burke et al. 2002). Two novel endophytic rhizobial strains having dual symbiosis property (*B. cepacia* and *R. leguminosarum*) were isolated from rice root using 16S rDNA sequences. They are competent to set up PGPR with rice plants and can stimulate nodules in common bean (*P. vulgaris*) roots. It is assumed that this *Rhizobium* strain isolated from rice transferred from the bean nodulated *Rhizobium* through horizontal gene transfer during the course of evolution (Singh et al. 2006). Besides this, the 16S rRNA is a good molecular marker due to its highly conserved function and ubiquitous distribution. The sequence of 16S rRNA varies from highly conserved to highly variable region. In a study of 16S rRNA series of cyanobionts, a single coralloid root of *Cycas revoluta* harbouring more than two cyanobacterial strains and in numerous roots from a single plant, diversity was also observed (Yamada et al. 2012). Important root architectural traits like root morphological features, nodulation traits and root hairs, which play a key role in BNF, are known to be genetically regulated by multiple genes or genomic regions referred to as quantitative trait loci (QTLs). Even though few QTLs have been reported to be playing a dominant effect on one trait, most have been found to have influence on many characters. The identification of major QTLs for these key BNF-influencing traits will be an important objective of genetic research and breeding programs aimed at enhancing BNF in cereals. RIL population (157 F2:7) and 105 SSR markers have been used to carry out a composite interval mapping and identified two QTLs for shoot dry weight, three QTLs for nodule number and one QTL for nodule dry weight, all QTLs having a small effect (Santos et al. 2013). In *Lotus japonicas*, using a RIL population, 34 QTLs controlling key BNF traits such as acetylene reduction activity (ARA) per plant, ARA for every nodule weight, ARA for each nodule number, nodule number for every crop and nodule weight for every plant were identified and mapped (Akiyoshi et al. 2012). A novel nitrogen-dependent gene *Ndhrl1* was isolated from wheat and mapped to the short arm of chromosome 2B which is associated with the lesion mimic trait (Li et al. 2016). Alike studies could be of great importance in cereals for identification of contrasting genotypes, which support BNF, is the first and foremost step in developing mapping populations and further mapping of QTLs. To introduce a symbiosis system in cereals, some essential genetic changes would be introduced such as detection of the Nod factor, organogenesis of the root nodule and relationship of an appropriate setting for nitrogenase action in the nodules (Curatti and Rubio 2014). One possible analysis to transfer the legume symbiosis into maize, wheat, etc. is linked to better claim of photosynthesis required to bear nitrogen accumulation. In this process, improved and well-advanced biotechnological approaches are presently explored, which may bring accumulated N to grain crop (Oldroyd and Dixon 2014; Beatty and Good

2011). Recently a key element that facilitates the movement of calcium in plants was identified which signals the nitrogen-accumulating microbes and stimulates the development of nodules on roots (John et al. 2007). As per different works, Nod factor is similar to Myc factors (fungal symbiosis), which may help for creation of a signalling (SYM) path (Maillet et al. 2011). Wheat crop inoculated with *nif-H* mutant of *Klebsiella pneumoniae* grown in nitrogen-deficient media showed unhealthy plant growth as compared to uncultivated *Klebsiella pneumoniae*-inoculated plants (Iniguez et al. 2004). Thus, *nif-H* gene plays a major role in biological nitrogen fixation, and this could be complemented if *nif-H* gene is possibly transformed in wheat.

2.10 Microbes and Weeds

Microorganisms play a critical role in various weed flora found in the farming system. Weeds are basically wild plants which have very low economic value. This grows spontaneously in cultivated and uncultivated soils and has several characters that allow their concern in different environments (Mukherjee and Karmakar 2015). The huge competitive aggressiveness mostly correlated to economic fatalities, because any unwanted plants take out earth nutrients, moisture, etc. from cultivated or non-cultivated areas (Mukherjee 2008). As like various invasive plants, weeds also have similar behaviour in different innate ecosystems including crop and pasture fields. Various works pointed out that unwanted plants are able to associate with AM fungi (Massenssini 2014) and that the possessions of this union vary depending on the ecological situation and soil factors (Mukherjee 2017c). Furthermore, the existence of a competing crop may change weed root colonization by AMF (Singh et al. 2004). Fialho (2014) found that *Bidens pilosa* and *Eleusine indica* show elevated fungal association when cultivated with maize. Such work revealed that weeds may have diverse competitive strategy and might form encouraging connections with unlike micro-biomass (Kundu et al. 2017). The microbes and its function differ with respect to plant cultivars and the company or lack of challenger crop etc. (Hedayetullaha et al. 2018). Furthermore, the configuration of the soil microbial biomass may alter with various plants and existing dirt setting. In broad, plant–soil microbes rivalry promote alternate soil microbe association, which vary when crop cultivate in single cropping system (Mukherjee 2017d). Sometime microbes are efficiently used to kill various weed and enhance crop productivity under different farming systems. This is one of the best methods for biological weed control through numerous bioherbicides (Mukherjee and Singh 2004). Most research is related to biological weed control measures confined to North America. This work is based on formulations of various fungal species and becomes successful in long-term experimental field only. Few notable results include use of BioMal (*Colletotrichum gloeosporioides*). This product mostly curbs problem of round leaf mallow (*Malva pusilla*) (PMRA 2006), and other species of product Cllego are used to control northern Jointvetch (*Aeschynomene virginica*) in the United States (Menaria 2007; Bailey 2014). Few important microorganisms, mainly *Pseudomonas fluorescens*

and *Xanthomonas campestris*, are involved in natural weed control measures. Weed control by bioloigical way, mainly using bacteria, have numerous compensation over the use of fungi because of more appropriateness for hereditary modification throughout either mutagenesis or gene exchange (Johnston-Monje and Raizada 2011). In few areas, viruses may influence different plant flora and also have bioherbicide potential. Such approach is usually measured for control of omnipresent class in large ecosystem rather than specially small manage localities. Viruses are mostly inappropriate for natural control due to their hereditary unpredictability and lack of host specificity (Diaz et al. 2014; Elliott et al. 2009).

2.11 Biofertilizer and Crop Efficiency

Various works to alleviate the deteriorating mineral nutrient pool sources concern mainly the worldwide biogeochemical and physicochemical cycles determined with the use of synthetic chemicals (Kahiluoto et al. 2014). Biofertilizers as different microbe cultures are a major method to decrease the employ of traditional synthetic chemical nutrients. Most of them can be used as biofertilizers, as they are capable of accumulating nitrogen from the atmosphere, assisting the right use of nutrients such as potassium and phosphorus from organic or natural fertilizers and earth stock, progressing drought tolerance, obtaining better crop health or boosting alkalinity resistance (Arora 2013; Augusto et al. 2013). This mainly includes latent microbes, which, when used to crop surfaces, seeds or soil, help to colonize the root surrounding or the core of the plant and enhance expansion by rising delivery or ease of use of basic nutrients to the host set. This is eco-friendly in nature and helps in minimal utilization of synthetic products (agro-chemicals, etc.). The microbes in biofertilizers refurbish the earth's usual nutrient cycle and build soil organic pool. By utilization of biofertilizers, healthy crop may be produced, with increasing food production and maintenance of the quality of the cultivated land. Because of numerous functions, an ideal word for the useful microbes is 'plant growth-promoting rhizobacteria' (PGPR). These are very much helpful in enhancing the productivity of the soil and pleasing crop nutrient needs by supply of natural foodstuffs by bacteria and its by-product. Thus, biofertilizers do not hold any chemicals which are injurious to the living earth matter and its use becomes very much friendly to growers. The use of bio-fertilizer is a capable expertise for future integrated crop management model in view of fast declining stocks of phosphorus and effective utilization of other nutrients such as N, K and S. Lukas et al. (2017) did an experiment on a meta-analysis to enumerate profit of biofertilizers in terms of economic output. Works revealed the supremacy of biofertilizers in dry climates over other climatic regions. Studies have pointed out yield increase in dry climate +20.0 ± 1.7%, tropical climate +14.9 ± 1.2%, oceanic climate +10.0 ± 3.7% and continental climate +8.5 ± 2.4% more compared to untreated ones. Demonstrated field trial revealed that better grain yield and oil content in rapeseed (*Brassica napus*) with use of culture comprised *Azospirillum* spp. and *Azotobacter* spp. (Namvar and Khandan 2015). These results have variously been accredited to indole acetic acid

production, gibberellins, a range of polyamines and amino acids and improved mineral accessibility to crop (Bashan and de Bashan 2010; Mukherjee 2012; Namvar and Khandan 2015). Inoculation of maize roots with the *Azospirillum brasiliense* (PGPR) had a positive result on microbial population and improvement in plant economic output (Herschkovitz et al. 2005). In totalling to useful microbes, the significance of mycorrhizal symbionts to numerous crop species is noticed by different scientific communities. Use of AMF is well acknowledged to augment host for absorption of plant nutrients mainly phosphate. Presence of AMF lowers down the bacterial foliar pathogens (Parniske 2008). Strains of *T. harzianum* supplement biofertilizer increase tomato yield by 20% with reduction in the use of inorganic fertilizer by 30% (Cai et al. 2014). Such work pointed out role of *T. harzianum* for increasing monetary benefits to growers while reducing the ecological damage of synthetic chemicals and other inorganic inputs. Cucumber soil with *T. harzianum*-enriched bioorganic fertilizer augmented microbial assortment. This was associated with reduction in rigorousness of *Fusarium* wilt sickness (Chen et al. 2012). Few researchers are very much interested in the *Sebacinales* fungus *Piriformospora indica*. This is an endophytic fungus capable of contaminating the rhizosphere of various crop genotypes (Weiß et al. 2011). Endophytic members of the *Sebacinales* are available everywhere in a series of ecology (Weiß et al. 2011), indicative of aggressive life strategies that potentially engross pressure over Microbial population dynamics in the rhizosphere. Infected crops create elevated yields and exhibit amplified patience of abiotic and biotic stresses in comparison with the untreated ones (Waller et al. 2005; Singh and Mukherjee 2009). Inoculation of *Cicer arietinum* (chickpea) with *P. indica* and the PGPR *Pseudomonas striata* leads for the time being increase in *P. striata* in the root zone and helpful for more crop output (Mukherjee 2006; Meena et al. 2010; Ghanem et al. 2014). Inoculation of the two bacteria has a synergistic effect on increase in *P. striata* inhabitants and crop biomass, which has become a very useful and practical tool for crop production (Meena et al. 2010). Possible synergism has also been reported for *chickpea* with *Meloidogyne incognita* (root-knot nematode) and *Macrophomina phaseolina* (root rot fungus) (Akhtar and Siddiqui 2008).

2.12 Plant Growth-Promoting Rhizobacteria

Microbial interactions in the rhizosphere of plant play critical function in solubilization, mobilization, and transformation of mineral from a restricted foodstuff source and then crop absorption of vital minerals to understand their complete hereditary latent (Mukherjee and Hedayetullaha 2018). Currently, the exploitation of natural approach is more accepted as a stabilizer to inorganic nutrients for getting better crop economic production in an integrated plant nutrient management system. With these facts, plant growth-promoting rhizobacteria (PGPR) have established a potent function in developing sustainable systems in plant output (Shoebitz et al. 2009). PGPRs have diverse relationships with dissimilar host flora. Broadly, the two main classes of associations are rhizospheric and endophytic. Rhizospheric associations

consist of the PGPRs that inhabit the exterior of the root or superficial intercellular places of the host plant, frequently forming root nodules. The leading class found in the rhizosphere is a microbe from the genus *Azospirillum* (Bloemberg and Lugtenberg 2001). Endophytic associations engross the PGPRs near and growing within the host plant in the apoplastic gap (Vessy 2003). They also assist in solubilization of fixed phosphates and erstwhile nutrients, improve resistance to stress, stabilize soil aggregates and pick up soil profile arrangement and organic matter substance. PGPR hold a high amount of available soil nitrogen in organic forms and various food nutrients in the crop–soil cycle; therefore, they assist in sinking need for phosphorus and nitrogen fertilizer and improve discharge of the nutrient source for vegetation.

2.13 Molecular Approaches for Microbial Interaction

The microbe–microbe or bacteria–host relations are the main approaches to inhabit and set up a range of diverse situations. Plant and soil microbe connections are critical for a successful growth and repair of microbe inhabitants in a system. These exchanges happen by the ecological identification of molecular and inherent massage which comprise several mechanism and modules of molecule. Which permit microbes to set up a society, which depends upon the multitrophic interface might outcome in high range. The effect of the numerous interfaces is often linked to pathogenic or beneficial effects to the host and soils of microbial communities. These exchanges occupy all environmental aspects, mainly biochemical change, metabolite swap, signalling, chemical secretion and inherent replace ensuing in a wide range. Microbe association transmits the molecular and hereditary information, and other various mechanisms might be concerning in this swap, such as secondary metabolites, siderophores, quorum-sensing scheme biofilm arrangement and cellular transduction signalling, amongst others. The final component of interface is the gene appearance of each organism in retort to an ecological related to its biotic or abiotic stimulus, which is accountable for various exchanges.

Crop root microbes have pattern-recognition sensors, which attach microbial-linked molecular pattern (MAMPs) and trigger a basal protection enough for expansion of various pathogenic bacteria (Jones and Dangl 2006; Bohm et al. 2014). The majority of nonpathogenic microbes and fungus linked to crops or cropping system are certain to make their own MAMPs, which prompt the problem of how useful microorganisms and flora handle to evade removal of the microorganisms via an immune retort. Crops probably classify pathogens from non-pathogens and react by any resisting microbial development, overlook it or strongly support it on or inside crop tissues (Vogel et al. 2016). This may symbolize a device of plant defence priming (Martinez-Medina et al. 2016) driven by crop microbiome. Using reliable approaches and concepts in human microbiome studies, Lundberg et al. (2012) and Bulgarelli et al. (2012) observed the spatial portion of microbial community in the root zone of diverse *Arabidopsis* accessions to determine the symphony of the nucleus microbiome. Lundberg et al. (2012) used pyrosequencing of the DNA from

bulk soil, rhizosphere and endophytic root compartments of more than 600 *Arabidopsis* plants for 16S rRNA gene segments of bacteria to show the impact of the soil type on microbial community structure. They concluded that endophytic root section was augmented with *Actinobacteria* and *Proteobacteria* and that the crop's growth phase and cultivar might steer differential enrolment and differential barring of Microbial population (Lundberg et al. 2012 ; Bulgarelli et al. 2012). Mark et al. (2005) used the entire genome transcriptome profile to assess the effects of rhizosphere exudates from two sugar beet varieties on gene expression in *Pseudomonas aeruginosa*. Genes are recognized in co-bacterial associations (mainly as chemotaxis, metabolism, type III secretion). Mark et al. (2005) showed that 104 genes are notably changed in response to both root exudates and that the common of these genes were regulated in response to only one of the two exudates. Further, a complete genome microarray was also used to determine endophytic colonization of rice by *Azoarcus* sp. BH72 (Shidore et al. 2012).

'Omics' techniques which facilitate the recognition of gene transcripts, proteins or metabolites have been developed to give a more detailed account about the genes and functions expressed in the crop microbiome. A metaproteogenomic technique was first observed for microbial population in the leaf area of *Arabidopsis*, soybean and clover plants (Delmotte et al. 2009). In root zone, metaproteomics works exposed multifaceted exchanges amongst crops and rhizosphere microbes in diverse crop sequences (Wang et al. 2011). Root microbiome of the therapeutic plant *Rehmannia glutinosa* and the phyllosphere and rhizosphere microbiomes of paddy (Knief et al. 2011) also showed various exchanges in a similar fashion. The importance of the root surrounded microbiome in the performance of crop ecology has been largely known, and the functions are restricted to its ability of rhizosphere surrounding microbes. A combination of conventional methods with new advanced sequencing technologies to measure organisms under new environment to know microbial existence in the root zone is very effective in the modern era of crop production. Proper recognition of the root secretion, signals and other main features in the root surrounding microbiome will be used as a chemical and microbial marker to clarify whether and how a crop engages with useful microbes. Unravelling the root micro environment also holds latent to get better plant defence and to expose various yet unidentified microbes present in soil, its functions and genes for various uses.

2.14 Climate Change and Microbes

Whole ecosystem changes with shifting of climate, and this will effect to various stresses, mainly the abiotic and biotic drivers of soil–aerial systems. Various changes from earth to the atmospheric surrounding could also be in harmony with soil microorganisms' features (Bardgett et al. 2008). Although Microbial population control a significant ecological unit, it is a lot indistinct how the profusion and symphony of microbe communities associate with perturbations of climate and interact to affect ecological behaviours (Mukherjee 2017a). Various changes in the context

of climate change are mainly addressed to more targeted whole parameters, such as microbial population, enzymatic action and microbial community profiles (Norby et al. 2004 ; Franklin et al. 2009). Key components of productive soils vary under a good management system and significantly rely on soil class, local weather situation, nature of plants cultivation and resource management techniques such as mulching and efficient genotype in use (Mukherjee 2018). The nature of microbes mainly depends on ecological phenomena such as heat, water-holding capacity of the soil, enzyme activity, temperature and nutrient ease of use, all of which are probable to be affected by a shift in climate (Solomon et al. 2007; NRC 2008; Mukherjee 2017b). Such modifications may have better impact on critical biological phenomena such as nutrient cycling, which depends on microbe movement. Weather forecast on each day plays a major role in crop health by allowing use of different culture media, which are beneficial for vegetation growth and physiological development (Mani and Mukherjee 2016). Soil temperature, moisture content and respiration of soil help boost microbial population or reduce as a result of a shift in rainfall and temperature pattern of atmospheres. Use of different beneficial microbes may be accurate with a proper forecasting mechanism. Change in soil respiration may have noteworthy reaction effects on the shift in climate and sternly modify aboveground population of *Actinobacteria* and underground microbial biomass (Austin et al. 2009). The behaviour of different soil microbes is accountable for the carbon cycling and soil nutrients' availability in the soil–crop system. Various climate modifications, mainly CO_2 concentration, rainfall ratio and variation in temperature pattern can possibly have effects on soil microbial biomass either in a direct or in an indirect way and enormity is doubtful (Austin et al. 2009). Precipitation and water availability of soil variation may change the ratio of fungi to bacteria availability and their population ratio (Williams 2007). Rising temperatures can augment microbial action, lead to a shift in Microbial population and transfer in favour of community which are suitable for elevated temperatures and quicker expansion patterns (Bradford et al. 2008). However, there are few possible outcomes for earth microbes in addition to carbon swap: (i) enhancement of microbial action with response to change in earth's atmospheric temperature may turn to augment land aeration and therefore effect nitrogen mineralization of newly and aged soil organic C (Schleppi et al. 2012) through 'priming' technology (Dijkstra and Cheng 2007), (ii) improvement of microbe accomplishment which might turn to arrest N and therefore limited availability of nitrogen to crops and create a harmful impact that constrains further enhancement in crop expansion and carbon movement in dirt (Friedlingstein et al. 2006), (iii) more crops–microbes struggle for nitrogen might turn to reduced bacteria putrefaction and so improved earth carbon accrual, as well as choice of useful microbial strain which assists its host crop assembly rising foodstuff need for crop expansion and carbon absorption and improved strength of earth's natural carbon through endorsement of dirt aggregation (Six et al. 2006; Strom et al. 2005). Population of useful microbes can be altered by use of various location-specific conservation agriculture practices mainly on residue retention and management aspects under various cropping systems (Mukherjee 2015c). Structural changes with various conservation practices, in turn,

may have significant effects on the performance of the soil ecosystem and microbial population, which interface crop cultivation. Various physiological stresses, mainly drought, lead to a decrease in microbe availability, favouring those microbes that are modified to mitigate under stressful situations (Jianbo et al. 2013) like water scarcity or alkalization (Solomon et al. 2007). The factors of severe changes of soil moisture affect the action of soil microbes and show their effect on soil hydrophobicity (Diamantis et al. 2013) and ultimately on crop efficiency. Climate-linked actions such as drought and freezing have more consequence on microbe behaviours than on temperature and precipitation (Schimel et al. 2007). Dry soil with low availability of water would have an effect on the action of lower microbes as reflected in the wild ecosystem by a noteworthy fall in litter phenol oxidase action and isoenzyme assortment, and soil Microbial population. On the other hand, more drought and dry situation in wetlands and peat lands would produce additional constructive situation for microbial action and, to some extent, beneficial for local vegetation (Albers et al. 2004). Peat lands and wetlands are the major stocks of earthly carbon and have key implication for the worldwide carbon cycle and ultimately to microbe community (Freeman et al. 2004). Worldwide land resources approximately hold two times more as much carbon as the ambience, making them one of the main sinks for atmospheric CO_2 and natural C (Williams 2007). This carbon is mainly stored in wetlands and peat lands, where microbial decomposition of carbon is restricted. Carbon stored in soil mainly relies on carbon access from leaf litter, decomposed earth matter and carbon availability from microbial respiration inside the soil (Davidson and Janssens 2006). Due to variation in temperature, few changes in decay rates could not merely influence carbon dioxide emission in the ambience but may well effect a larger change to the quantity of C store in the earth over decades (Davidson and Janssens 2006). Shifting of climate plays a vital function in biogeochemical cycles of C and N along with few biologically decomposing ecological contaminants. The earth biomass and microbial cycle have a significant function in mineral mobilization and are affected by long-term weather parameters (Mukherjee 2014b). Quan et al. (2016) studied the effects on soil microbial biomass carbon (MBC) and community composition in Moso bamboo plantations using high-throughput sequencing of the 16S rRNA gene. Intensive management and N addition, either alone or in mixture, notably improved earth's microbial biomass available C, with sinking bacteria availability. Intensive management practice improved the virtual availability of *Crenarchaeota and Actinobacteria;* however, this further reduced that of *Acidobacteria*. More use of nitrogen enhances the availability of *Acidobacteria* and decreases availability of *Proteobacteria*.

2.15 Plant–Microbe Interaction and Designer Plants

With advancement of various techniques, it is potential to influence microbial biomass and its function in the root zone to optimize the accessibility of mineral matter and other plant nutrient sources for crop utilization. Different types of microorganisms (actinomycetes, etc.) on the earth's surface assist decomposition of earth's

natural substance such as amorphous colloidal substance, which is recognized as humus. This complex has high CEC and water-holding capacity, which are very helpful for mounting plant growth and development. However, some crops are unable to utilize the humus due to some structural and physiological hindrance. Improvement of rhizosphere features in the plant helps to access various nutrients and water from different layers of the soil. Most appreciable root characters, which can draw valuable microbes, mainly PSB, have a significant consequence on crop efficiency. Moreover, limitations comprise feasibility of such approach (proper utilization), and achievement in field situation mainly cultured microorganisms has to fight with local availability of soil microbes for space and nutrient availability in the rhizosphere. Such kind of problem may, to a certain extent, be conquered with a new method known as 'designer plants' (Rayu et al. 2012). Although this technique is now formulated in many ways, it could be harnessed to boost farm output through increasingly available technology by optimum utilization of various resources, with respect to crop architecture and physiological system. Crop trait modification can be achieved by either conservative or transgenic breeding, which preferably draws more nitrogen-utilizing microbes and phosphate-solubilizing bacteria to plant roots for accessing organic nitrogen and phosphorus, which may vary with endosymbionts. There are numerous endophytic bacteria that can fix atmospheric nitrogen into the soil system, mainly as *Azosprillium, Beijerinkia, Pseudomonas,* etc. Treated entophytes (*Klebsiella pneumoniae*) showed up to 40–46% more nitrogen in treated wheat (Iniguez et al. 2004). If these approaches are collectively used in the designer plant, this might help to produce various seed materials required for farm utilization; this expertise can have noteworthy impact on crop and the whole system of farm production. There is a need for improved technologies to make sure that microorganisms applied to the seed stay ready for action in soils against native bacteria and form a tough union with mounting crops or plants under different situations ranging from wetland to forest trees. Crop roots have highly controlled morphological features to acclimatize to earth's ecosystem and notably modify the root surrounding environment by its physiological actions, mainly natural compounds such as organic acids, phosphatases, few signalling substances and redox exchanges (Marschner 2012). This can be easily accessible through designer plants. The root-induced rhizosphere process helps in solubilization and utilization of earth's nutrients simultaneously with microbial interaction behaviour, and it also helps in managing NUE by plants, therefore greatly affecting plant growth and its sustaining behaviours (Zhang et al. 2010). As a result, changing rhizosphere development or root expansion in a modified designer plant is a valuable approach to enhance optimum utilization of available food source and plant economic yield at the same time. The competence of roots for more nutrient movements, attainment, and utilize may be completely subjugated by (1) changing rhizosphere architecture (i.e. root expansion and mass, design, allocation); (2) modifiable rhizosphere development (i.e. restricted use of nutrients, rhizosphere exchanges and utilization of competent cultivars) and (3) maximizing root region management to coordinate rhizosphere expansion and earth nutrient supply with requirement of nutrients in cropping systems. Various works revealed that manipulation of root or its surroundings has

become an efficient move toward increase in both competence for nutrient utilization and plant efficiency for long-term plant economic yield from a single unit of land area (Shen et al. 2005).

2.16 Microbial Interactions and Molecular Perspectives

Microbes are hardly ever encountered as lone class communities in the milieu because study in dissimilar habitat has exposed massive prosperity, and plenty of differences are frequently observed in a miniature sample, signifying that microbe exchanges are intrinsic in the milieu. This includes soil deposit, plant residues, bacteria and other unicellular organisms. After a long time of evolution, a diverse class of associations has come out, which could ease mutual habitat; for example, endosymbiotic and mutualistic relationships or spirited, aggressive, pathogenic and parasitic relationships (Faust and Raes 2012). A lot of other metabolites have been observed to be linked with microbe–microbe exchanges. Such complexes are generally biologically very active and could act upon significant function in ecosystem relationships. Extensive work on microbe mechanisms and the interface with stimuli response helps to link cellular attentiveness towards microbe symbionts. Fabrication of signal molecule (auto-inducers) allows cells to exchange and react with the surrounding in synchronized pathway. Throughout the interface between host cells, microbial-linked molecular patterns are sealed for various microbial taxa, allowing relationship with soil and crop and regulating the microbial communications with dissimilar hosts. The microbiome linked with vegetation is measured by its genomic configuration. This helps to know crop health, development, strength and, as a result, yield, where every setting is linked with the crop root microbe association through explicit function (Lakshmanan et al. 2014). Metagenomics investigation by next-generation sequencing techniques shows that only 6% of bacterial population have been cultured by present techniques (Haldar and Sengupta 2015). The primary footstep in microbe–crop interface is microbial detection of crop exudates in the earth's ecosystem. There is an assumption that crops are capable of enlisting bacteria or microbes by crop root secretion, which are poised of various organic acids and sugars, which can differ according to the crop and its abiotic or biotic situations (Haldar and Sengupta 2015). Various crop selection explicit Microbial population as reported by Berg et al. (2016), when compared to the root surrounding and colony formation by microbes such as chamomile (*Matricaria chamomilla*) and nightshade (*Solanum distichum*) in spite of being cultivated in an alike situation; it gives dissimilar structural (analysing 16S rRNA genes) and functional (analysing nitrogen-fixing *nifH* genes) microbe community. However, crop exudates of alike crops differ mainly by the microbe community in the crop-growing phase. Scientists have now recognized a number of plant and/or crop exudate compounds accountable for specific exchanges, for example, flavonoids in pulse–*Rhizobium* (Peters et al. 1986) and strigolactone as an indicator for AMF (Akiyama et al. 2005).

Mutualistic microbes care for crops of different pathogens with induced crop resistance through antibiosis. The induced systemic resistance (ISR) in crops leads to more acceptance to pathogens. Mendes et al. (2011) worked on the microbiome of soil repressive to *Rhizoctonia solani* (fungal pathogen), which leads to damping off in some plants by 16S rDNA oligonucleotide microarray (PhyloChip). Ardanov et al. (2012) pointed out that treatment of *Methylobacterium* strains protects crops from pathogen attack and affects endophyte communities. As a result, with such ideas, scientists have worked on inoculating crops with different microbial cultures through balancing character (Mendes et al. 2013a, b). Microbes create a wide range of chemical complex substances called secondary metabolites which play an important function in enlargement, expansion and replica of produced compounds (Tata et al. 2015). However, such chemical substances are very much biologically active and can carry out a significant role in resistance, antagonism, signal system and environmental relationships (Bilyk and Luzhetskyy 2016).

2.17 Microorganism in Soil Agro-Ecosystem

Microbes are affected by an accumulation of biotic and abiotic factor, which leads to numerous and qualitative variations (Grayston et al. Grayston et al. 1998). Regarding the importance of microorganisms' association with roots, more scientific evidence shows the vital role they play in the degenerative processes of soil biogeochemical cycles (Taylor et al. 2009); plant protection against some pathogens; the synthesis of antibiotics, toxins, surfactants and organic compounds; and promotion of plant growth by producing specific nutrients, such as phytohormones and macro- and microelements (De Werra et al. 2009). Knowledge of the basics of bacteria–fungi, bacteria–bacteria and crop–bacteria relationships is useful to microbe community in cultivation aspects. The main constraint is whether such kind of microbial association is viable enough and firm in cultivated soil. As per various reports, organic soil with disease-reducing capacity gives more grain yield per unit area than inorganic fertilizer-treated soil, and this might be due to more survival of beneficial pathogens in natural soil (Shoebitz et al. 2009). Crops cultivated in organic-rich soils had lesser disease sternness and frequency than adjacent land of fertilizer-treated ones (Rogers and Oldroyd 2014). Such kind of observation becomes very critical for enhancement of food grain production with improved quality aspects. Recommendation from different microbiologist forums dictates that firm populations of useful microorganisms that are selectively recognized and maintained in the root surrounding restrain pathogens by release of secondary metabolites that are beneficial for crops and plants (Doornbos and van Loon 2012). Such phenomenon leads to infection repression, which may completely or partially keep out various pathogens from the earth. The two main categories of sickness-suppressive soils are specific and general restraint. Pathogen perseverance and virulence in the soil are sternly repressed in both cases (Janvier et al. 2007). In general, suppression of microbes' actions in the root surrounding restrain infection augmentation, which might be induced by the addition of organic matter in the soil that

enhances microbes' action and antagonism, so helping in infection control (Mukherjee 2015a). Specific repression happens when explicit microorganisms alienate the pathogens, which help soils to control disease (Berendsen et al. 2012). Presently, to combat the problem of soil toxicity or ambience problem near the root zone, bioremediation techniques have become very effective and helpful for microbial culture.

Biological remediation is essential in farming soils as conservative cultivation practice using synthetic chemicals and other agrochemical, contaminate our soil biological system. A number of pollutants could survive in soil and have unpleasant effect on the earth's system, plantation and living beings for a long time. In due course of time, for changing from exhaustive farming to natural farming, growers face the problem of remediating contaminated soil (mainly from heavy metal, etc.). Bioremediation is a worldwide acceptable option that mainly eradicates ecological pollutants in the infected place (Madsen 2003). This method includes bacteria, microbes and flora to rot, impound or take away soil pollutants, mainly chemical insecticides and synthetic chemicals. This is possible by a succession of complex metabolic exchanges, repeatedly linking numerous diverse organisms, and unnecessary contaminants can be wrecked down or removed. Under natural farming, growers may select the suitable bioremediation method for their ranch on the basis of contaminants needed to be separated, soil type and climate situation. Use of microbes at farm levels assists to decrease profound metal toxicity and give environment for proper vegetation augmentation and works as important bioremediation tools.

2.18 Conclusion

Nutrition security for the growing global population needs systematic breakthrough and skill developments at numerous levels from soil to aerial system. Improving land production per unit area on the basis of enhanced plant–soil and microbial continuum, resource availability and exploit effectiveness in cultivable land is a major confront. Techniques for seed dressing with beneficial microbial spores play an important role in crop production and productivity enhancement. The nutrient input in exhaustive crop husbandry practices ought to be maximized to attain more crop yield by changing root architecture through designer plants, which has become an effective approach under natural farming. Various planning approaches of modification of the underground portion of the plant with respect to known microbial physiological system have become a challenging task and ultimately affect nutrient use efficiency and plant yield per unit area of land. Microbe exchanges are extremely intricate and complex process, which involve many exchange process between plant–microbe continuum. Recent work in this direction has given novel insights into microbe association and their use in natural science and contiguous agroecosystem particularly with respect to shifting climate scenario with improvement in crop and soil health.

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Fusarium Wilts of Chickpea, Pigeon Pea and Lentil and Their Management

3

Suseelendra Desai, R. D. Prasad, and G. Praveen Kumar

Abstract

Fungal wilts caused by species of *Fusarium* on crop plants are threat to the food and nutritional security. Chickpea, pigeon pea and lentils are staple pulse crops of Indian diet and form principal protein source, especially for vegetarians. Wilt is a major disease of all these crops causing huge economic losses. The fungus *Fusarium oxysporum*, with several *formae specialis*, is ubiquitous and has been recognized as a threat to crop production among food, commercial and horticultural crops. Though most of the species are saprophytic, a few of them are highly pathogenic, and in some crops, physiologic races have also been reported.

Sometimes, the pathogen occurs in combination with other pathogens forming a complex. The wilt-causing fusaria in general show certain degree of host specificity. The fungus is soilborne, and the disease is monocyclic in nature. Major management strategies include breeding for host resistance, use of biological control agents and cultural and physical practices. Conventional breeding techniques and modern molecular tools have enabled to breed disease-resistant plants and thus reduce overall cost of disease management. Extensive research has been conducted to develop wilt-resistant cultivars due to which there is considerable reduction in losses due to this disease. Being a soilborne pathogen, cheap and sustainable methods such as development of formulations of potential biocontrol agents have helped in the reduction of crop losses. Strains of *Trichoderma* and *Pseudomonas* have been harnessed and commercialized, and these products are popular in farming community. However, no single method helps to minimize losses, and hence, integrated disease management packages need to be developed to reduce crop losses. In this paper, an effort is made to

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review the current status of the disease, pathogen, management strategies and way forward to manage the wilt pathogens efficiently.

Keywords
Fusarium wilt · *Trichoderma* · *Pseudomonas* · Resistant cultivars · Pulses

3.1 Introduction

India is among the highest producer as well as consumer of pulse commodities in the world. Among them, chickpea, pigeon pea, urdbean, mung bean, horse gram, lentil, pea, rajmash and lathyrus are important crops, chiefly grown in India, occupying about 23 million hectares in area. Pulses are grown in both kharif and rabi seasons. While chickpea and lentil are rabi crops, pigeon pea is grown mostly in kharif. Chickpea contributes to 40% of total pulse production in India, followed by pigeon pea (about 20%), and area under lentils is <10%. Due to increasing demand for pulses, annually 3–4 million tonnes of pulses are imported from countries like Australia, Canada and Myanmar. Pulse crops form an important vegetarian dietary for majority of Indian population due to their perfect protein component of high biological value when supplemented with cereals. Pulse crops fix atmospheric nitrogen, the predominant mechanism to meet their nitrogen requirement. Some of the pulses are also an excellent feed and fodder for livestock. The biomass after separation of the grains is fed to the animals as feed concentrate. Pulses contain about 20–25% of protein and 55–60% of carbohydrates, and they are rich in calcium and iron also. About 80% of the pulses are grown under rainfed conditions on marginal soils with poor soil health due to which their productivity levels are low. The net per capita per day availability of pulses for the population decreased by 61 g to 32 g from 1951 to 2010, while decreased production further created an imbalance in the demand and supply (Joshi and Saxena 2002). It is estimated that the deficit of pulses in coming time will be by 24.9 million tonnes till 2020. The major factors for this are the increase in population, rise in income of the people, geographical shift, climate change, emergence of complex diseases and pests and socio-economic considerations and input limitations (Ali and Gupta 2012).

3.2 Chickpea, Pigeon Pea and Lentil

In India, chickpea is the most important pulse crop that contributes up to 30% of total pulse acreage and about 40% of total pulse production in the country. This is the world's second most important legume crop after dry beans (*Phaseolus vulgaris*

L.). The crop is grown extensively throughout tropical, subtropical and temperate regions in South and West Asia, East and North Africa, Southern Europe, North and South America and Australia (FAOSTAT 2014). The major chickpea-growing countries are India, Pakistan, Turkey, Iran, Myanmar and Iraq in Asia, Ethiopia in Africa, Mexico, Canada and Australia. Chickpeas are rich in potassium, iron, zinc, phosphorus, magnesium, antioxidants, folate and vitamin B6. The area, production and productivity of chickpea in these countries are given in Fig. 3.1. Among major growing countries of chickpea, India tops in area and production. However, in terms of productivity, it is far below than countries like Canada, Australia, Ethiopia, Mexico and Myanmar.

Pigeon pea (*Cajanus cajan* L.) is a major pulse crop in the semi-arid tropics. India is the largest producer as well as consumer of pigeon pea in the world. India and Myanmar account for 83% of the pigeon peas produced in the world. Other major countries are Malawi, Tanzania, Kenya and Uganda. Apart from using the dry split pigeon pea as a protein source, fresh pods are also used as vegetable. Pigeon peas are rich source of calcium, manganese, magnesium, phenylalanine, aspartic acid, glutamic acid, leucine, lysine, folate and vitamin B6. The area, production and productivity of pigeon pea in major growing countries are presented in Fig. 3.2. Among the major pigeon pea-growing countries, India has the largest area followed by Malawi. However, production and productivity are the highest in Malawi.

Lentil (*Lens culinaris* Medik.) is among the main grain legume crop that plays important role in the supply of the protein to undernourished vegetarian population of the country. Lentils are grown mainly in Australia, Canada, Bangladesh, India, the United States, Turkey, Syria, Morocco and Pakistan. It is mainly grown in north-eastern plain zone as sole and intercrop under rain-fed conditions. It is one of the oldest crops that originated in near East and Mediterranean regions. Lentil is a staple pulse in Middle Eastern and Indian diets and one popular in the cuisines throughout the world (Anonymous, FAQ 2013).

Lentil is recognized as one of the most nutritious pulse crops ranking next to chickpea amongst rabi pulses. It is a rich source of calcium, phosphorus, iron, vitamin C, riboflavin, zeaxanthin, folate and carotenoids. Lentil is grown mainly in northern plains and central and eastern parts in India, especially in the states like Madhya Pradesh, Uttar Pradesh, Bihar, Uttarakhand and West Bengal. The area, production and productivity of lentils in major growing countries are presented in Fig. 3.3. Until 2015, India planted the largest area under lentils. However, in 2016, Canada has surpassed India in terms of area, production and productivity. Productivity of lentils is also high in Turkey and the United States.

3.3 Fusarium Wilt Pathogens

Fusarium wilt is a major yield-restricting and devastating factor in most of the pulse crops. The disease is caused by soilborne fungus belonging to the genus *Fusarium*. The genus belongs to *Nectriaceae* family, *Hypocreales* order, *Sordariomycetes* class and *Ascomycotina* division. Butler first reported chickpea wilt caused by *Fusarium*

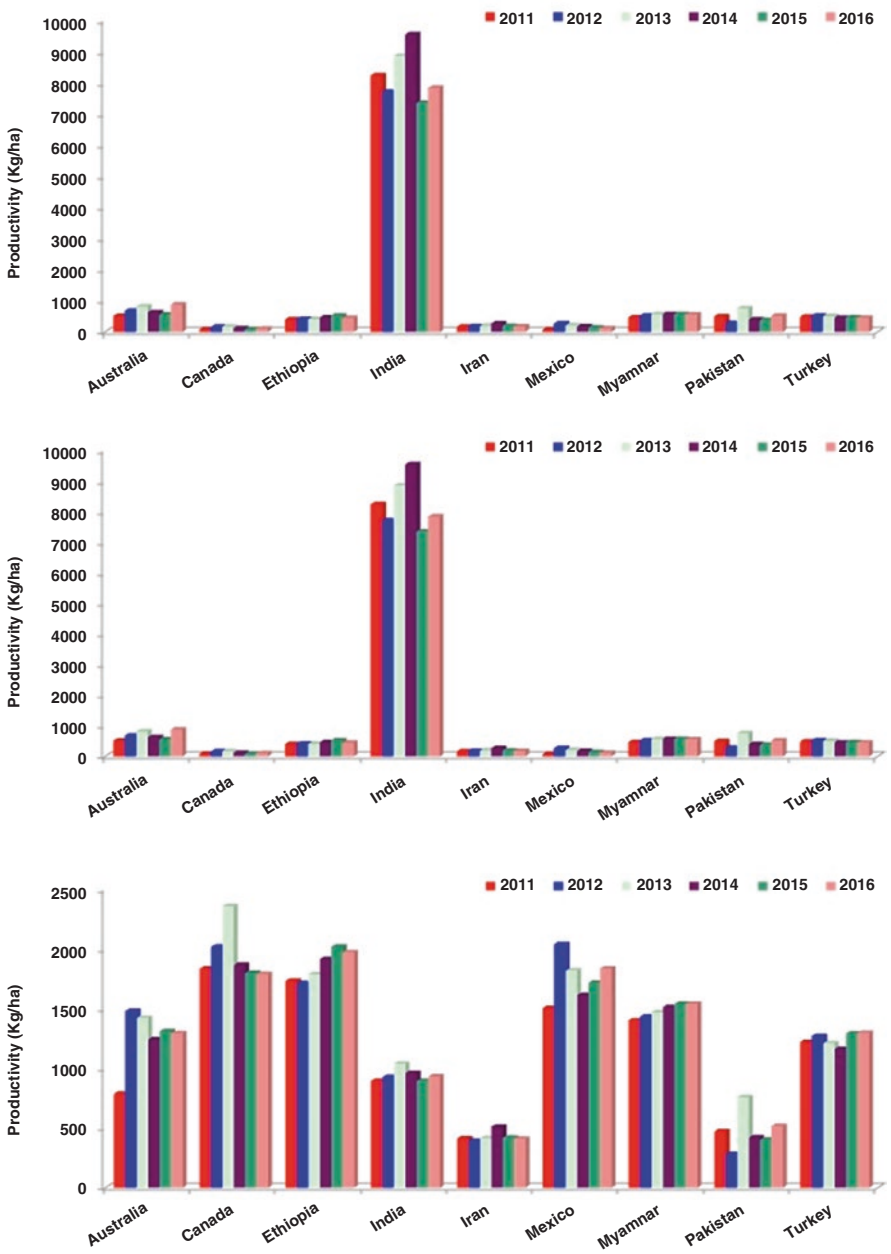

Fig. 3.1 Area, production and productivity of chickpea in recent years in major growing countries. (Source: http://www.fao.org/faostat/en/?#home)

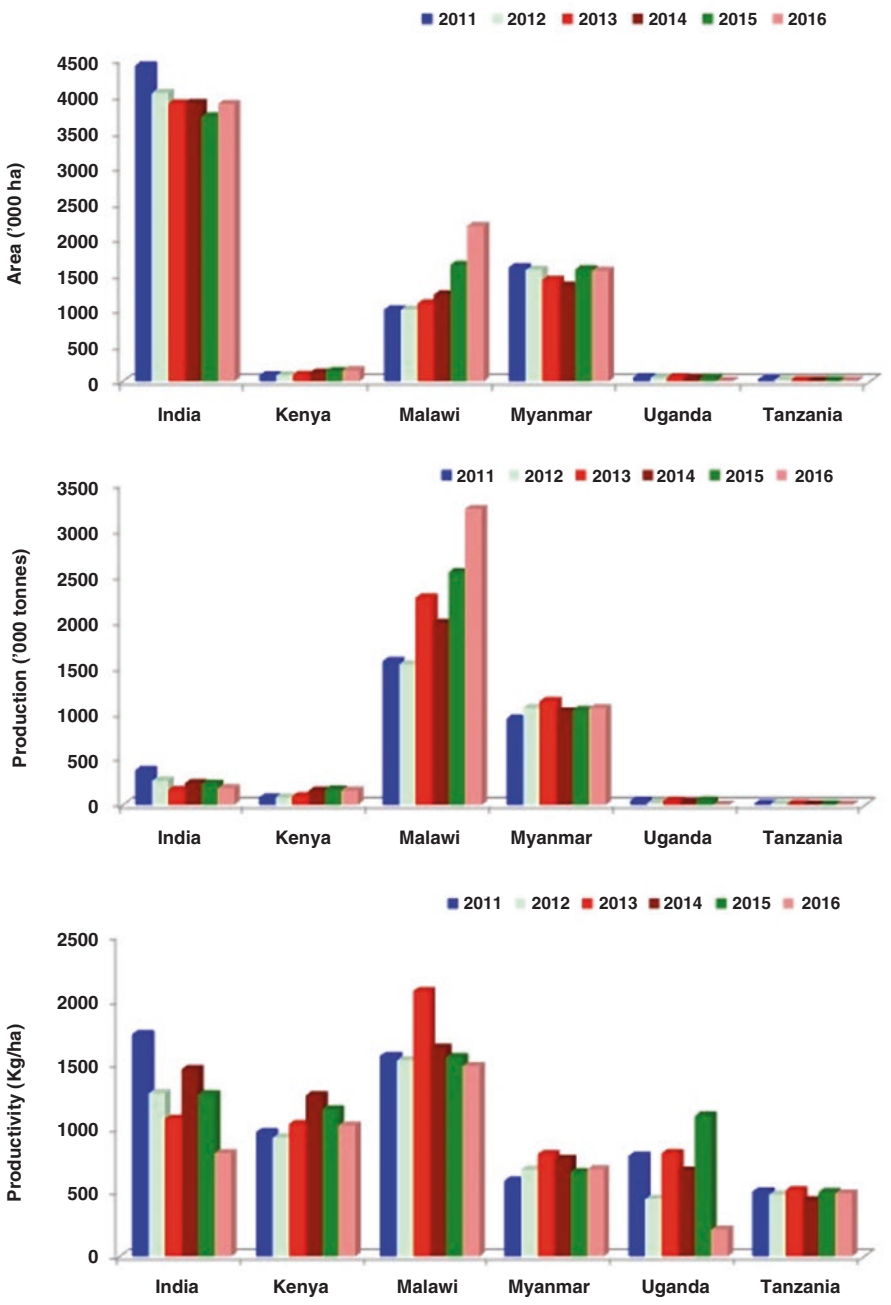

Fig. 3.2 Area, production and productivity of pigeon pea in recent years in major growing countries. (Source: http://www.fao.org/faostat/en/?#home)

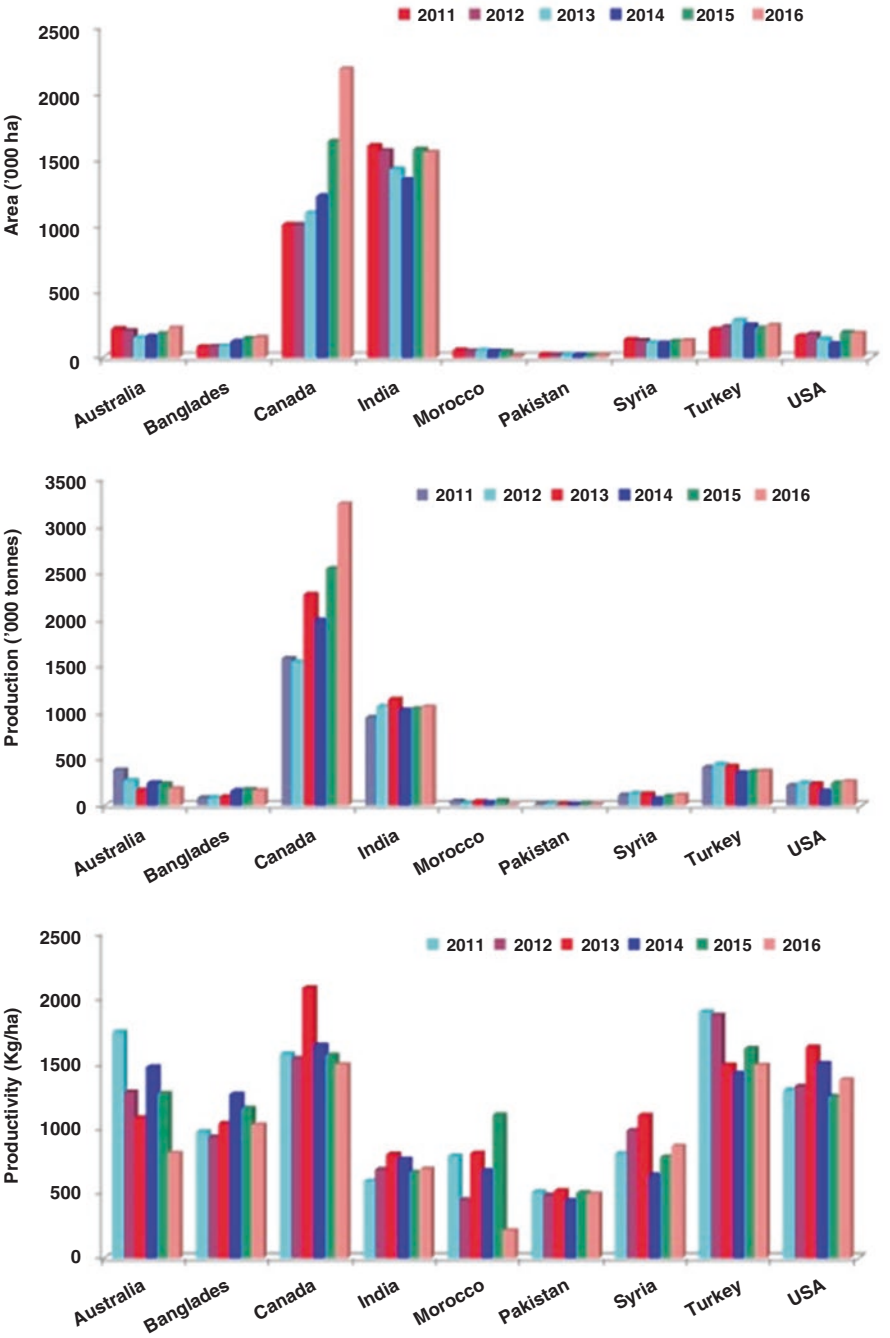

Fig. 3.3 Area, production and productivity of lentil in recent years in major growing countries. (Source: http://www.fao.org/faostat/en/?#home)

oxysporum f. sp. *ciceri* in India in 1918. Fusarium wilt of pigeon pea causes significant yield losses in susceptible cultivars throughout the pigeon pea-growing areas (Reddy et al. 1990). Butler reported pigeon pea wilt for the first time in India, and he identified the pathogen as *Fusarium udum* in 1910. The perfect stage of the pathogen, though reported as *Gibberella udum*, needs further confirmation. The pigeon pea wilt fungus is host specific being pathogenic only on pigeon pea and its wild relative, *Atylosia* spp. (Kannaiyan et al. 1985). The pathogen is specific in parasitism, being pathogenic to pigeon pea only (Upadhyay and Rai 1989; Kannaiyan et al. 1985). Wilt of lentil is a serious disease caused by *Fusarium oxysporum* f. sp. *lentis* and plays major role in reducing lentil yield in India and world over (Hamdi and Hassanein 1996). Severe wilt incidence was reported in 1949 resulting in more than 60% yield losses (Vasudeva and Srinivasan 1952).

3.4 Economic Importance of Wilts

Fusarium wilts affect not only pulses but also many other commercial crops. Fusarium wilt is one of the most devastating diseases affecting chickpea, pigeon pea and lentils worldwide. The disease can incite wilt at any time from the seedling stage to pod formation stage. The yield losses due to this disease alone in chickpea have been reported to be up to 60%. The yield losses due to pigeon pea wilt depend on the stage at which the plants wilt, and it can be up to 100% if the disease occurs at the pre-pod stage, about 67% when it occurs at maturity and 30% when it occurs at the pre-harvest stage (Kannaiyan and Nene 1981). Saxena et al. (2010) reported that *Fusarium* wilt disease in pigeon pea is so devastating that it can cause production loss up to 97,000 tonnes per year in India alone. The annual pigeon pea crop loss due to wilt in India alone has been estimated at US $ 36 million, while in eastern Africa the annual losses were estimated at US $ five million (Kannaiyan et al. 1984). In India, up to 50% yield losses due to lentil wilt have been reported (Anonymous 1999). The incidence of the wilt in recent years has been on the rise causing substantial lentil yield losses. This wilt pathogen survives in the soil as chlamydospores that can remain viable for several years (Erskine and Bayaa 1996) and is capable of colonizing residues and roots of most crops grown in rotation with lentil.

3.5 Symptomatology

Wilts in general can attack the plants from seedling to pod development stage. When the disease occurs at seedling stage, infected plants usually wither, wilt, collapse on the ground and die. Generally, wilt-infected plants do not show any damage to the roots, and thus root system appears healthy. However, when infected plants are split-open, they show a brown discolouration of the xylem vessels. Greyish-green chlorosis of the foliage is observed starting from the lower leaves and then extending to whole plant, and it eventually turns to dull yellow colour. In some cases, leaf

vein clearing also could be noticed before wilting appears. When mature plants get infected, the top portion of the plants droop and the foliage turns pale green to yellow. In highly susceptible genotypes, the plants get affected in 2–3 days. In some genotypes, partial wilting also could be observed affecting only one side of the plant. When infection occurs during the mid- to late-pod filling stages, seeds are often shriveled.

While in chickpea, the symptoms are generally noticed 2–3 weeks after sowing, symptoms can occur at both the seedling and adult stages of plant development. The root system will appear healthy, but with a reduced proliferation and nodulation rate. Leaves are retained on wilted plants. At later stages, the branches dry up from top to downwards, and finally the whole plant dries up. Lateral root infection results in partial wilting, whereas tap root infection results in complete wilting. In lentil, the seedling stage symptoms appear as sudden drooping followed by drying of leaves and death. In the field, the disease is seen in patches, and adult plant shows wilt symptoms usually from flowering to late-pod formation stages.

3.6 Pathogen Morphology

It is a common soil inhabitant and produces three types of asexual spores, macroconidia, microconidia and chlamydospores. The microconidia are ellipsoidal and have either no septum or a single one. The chlamydospores are globose and have thick walls. They are formed from the hyphae or alternatively by the modification enlargement and thickening of hyphal cells. They are important as endurance organs in soils where they act as inoculum in primary infection. The hyphae of *F. oxysporum* f. sp. *ciceri* are septate and branched. Macroconidia are straight to slightly curved, slender and thin walled usually with three or four septa. The microconidia are ellipsoidal with no or one septum. The conidia are formed on phialides. They are important in secondary infection. The chlamydospores are globose with thick walls. The teleomorph or sexual reproductive stage of *Fusarium oxysporum* is unknown.

F. oxysporum f. sp. *lentis* produces septate fluffy or submerged mycelium. Microconidia are usually produced on simple and short conidiophores arising laterally on hyphae. Microconidia measure 2.5–3.5 × 5–11 μm and are oval to cylindrical, straight or curved. Macroconidia measure 3.5–4.5 × 25–65 μm and are thin walled, with one to six septate, fusoid and pointed at both ends. Chlamydospores are smooth or rough walled and formed singly or in chains.

3.7 Physiological Specialization

Physiological specialization is occurrence of several forms within a species that are morphologically identical but differ in physiology. This variability is reflected in their selective pathogenicity towards varieties of host crop. The evolution of physiological specialization is often correlated with the strong selection pressure exerted when disease-resistant crop varieties are introduced over large areas. *Fusarium*

oxysporum is one of the most variable and highly dispersed species, and variability is reflected in the ecology and distribution. Though strains of *Fusarium oxysporum* are genetically distinguished on the basis of their vegetative compatibility, genetic uniformity is assured in some vegetative compatibility group (Leslie and Summerell 2006).

In chickpea, physiological specialization was reported in the early 1980s (Haware and Nene 1982). Pathotypes have distinct geographical distribution, and their races 2, 3 and 4 have only been described from India (Haware and Nene 1982), whereas races 0, 1B/C, 5 and 6 are found mainly in the Mediterranean region and the United States (Jiménez-Díaz et al. 1993; Halila and Strange 1996). Desai et al. (1992, 1994) reported alternative methods for distinction of races of *F. oxysporum* f. sp. *ciceri* based on morpho-physiologic characters and biochemical and molecular characters. Race 1A is reported in India (Haware and Nene 1982) and California and the Mediterranean region (Jiménez-Díaz et al. 1993). In pigeon pea, differential response of genotypes to wilt incidence across locations has been attributed to variability in pathogen. For instance, Sharma et al. (2016) reported that ICP 12749 (2) and ICP 14819 (3) expressed resistance in Akola, Badnapur, Patancheru and Sehore but susceptibility in Bangalore, Kanpur and Khargone. This variation may be attributed to the different climatic conditions, presence of different fungal variants and virulence of the pathogen at those locations. Similar observations were made by Mishra and Dhar (2003). So far, five variants (strains) of *F. udum* have been identified and documented (Reddy et al. 1996, Mishra 2004). In lentils, until recently no races were reported (Bayaa and Erskine 1998; Belabid et al. 2004; Mohammadi et al. 2012). However, Hiremani and Dubey (2018) based on the resistant and susceptible reactions on the differential cultivars grouped isolates of *F. oxysporum* f. sp. *lentis* into eight races/pathotypes and identified differential cultivar for each race/pathotype. Apart from standardizing a set of differential cultivars, they also reported the existence of races from India which will benefit in developing race-specific wilt-resistant lentil cultivars and help in identification of races/pathotypes prevalent in other lentil-growing countries around the world.

3.8 Disease Cycle and Epidemiology

Fusarium wilt of chickpea, pigeon pea and lentil are monocyclic in nature which are driven by the pathogen's primary inoculum. Since the pathogen is soilborne, it spreads within and between fields over seasons/years, thereby causing severe crop losses. The pathogen survives in resting spores called chlamydospores, which can withstand aberrant conditions for long periods. In general, *Fusarium oxysporum* spores including chlamydospores rest in the soil for several years. For instance, *Fusarium oxysporum* f. sp. *ciceri* is mainly soilborne and a facultative saprophyte. It can survive in the soil up to 6 years in the absence of susceptible host (Haware et al. 1978). The pathogen remains dormant and immobile in the soil as a saprophyte until it is stimulated by the root exudates of the host plant. The root exudates contain the nutrients required for germination and growth. Utilizing these exudates, the

pathogen produces mycelium, which invades the roots. Infection of the host involves a series of regulated steps starting from adhesion to the root surface. While adhesion could be nonspecific, site-specific adhesion appears to be important in placing positioning the propagule at the root surface for penetration and colonization. Penetration of the root cells is dependent on plant surface structures and activators. The pathogen enters root cells either directly or indirectly, and the most common site of penetration is at or near the tip of the roots. Postinfection, the mycelium moves intercellularly and enters xylem vessels through pits. Often pathogen proliferates in the vessels by producing conidia and thereby plugging the xylem vessels. In addition to plugging, the pathogen also produces gum, gels and tyloses, which clog the vessels. Infected vessels are damaged physically due to multiplication of the pathogen in the adjoining cells. This will lead to blockage of water supply to the upper parts, thus leading to drooping, yellowing, wilting and finally death of the plant. The most prominent symptom by which fusarium wilt could be distinguished from other diseases is vascular browning.

The primary inoculum survives in the soil and with the onset of favourable conditions; the resting structures germinate and produce mycelium, microconidia, macroconidia and chlamydospores. These propagules help the pathogen multiply in the rhizosphere. When the pathogen comes in contact with the host roots, it infects and advances intracellularly to infect the vascular tissues. The infected plants wilt and die and thereby add inoculum to the soil. The disease is monocyclic and is spread from field to field through runoff or irrigation water. Hence, if not managed, small patches of wilted fields could develop into endemic fields over years. A typical disease cycle is shown in Fig. 3.4.

3.9 Disease Management

The management of the wilt disease can be done through cultural, chemical and biological methods and use of resistant varieties. In the absence of resistant/tolerant variety, it is difficult to manage the disease caused by soilborne pathogens because of complex soil environment of physical, chemical and biological origin. Disease management strategies thus should aim at:

(i) Using pathogen-free seeds.
(ii) Avoiding endemic and high-risk wilt-infested areas.
(iii) Reducing or eliminating inoculum in soil.
(iv) Using resistant cultivars.
(v) Practicing clean cultivation to reduce spread of wilt within and between fields.
(vi) Using seed/soil treatment with chemical/biocontrol agents/organic residues to reduce soil inoculum load.
(vii) Practicing crop husbandry to avoid/minimize wilt infection.

Fusarium wilt management can best achieved if integrated strategy is applied (Haware et al. 1990; Jimenez-Díaz and Jimenez-Gasco 2011).

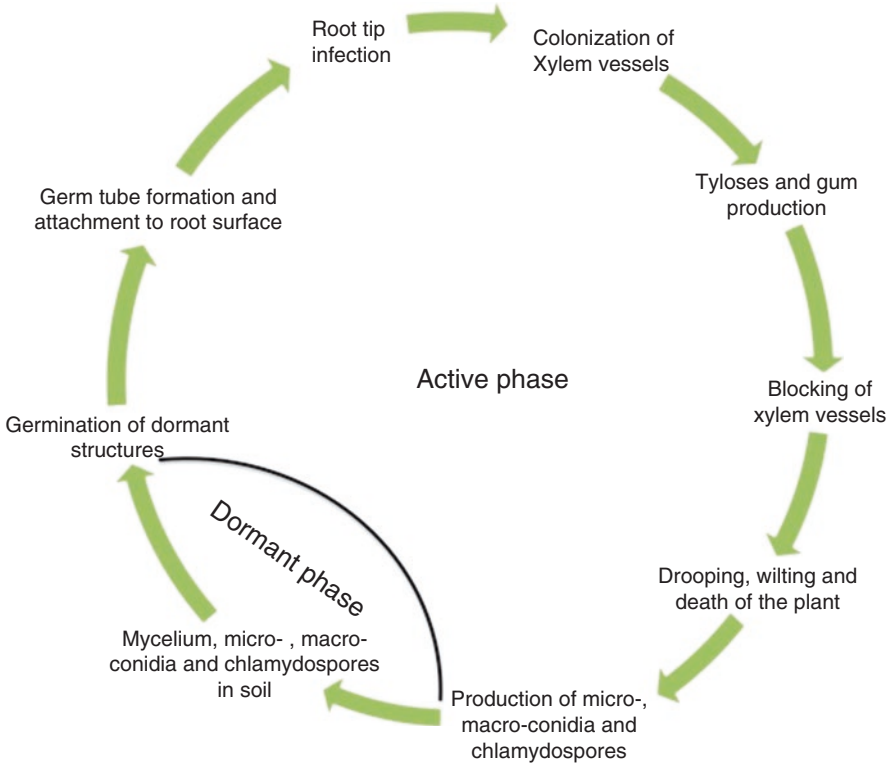

Fig. 3.4 Typical disease cycle of fusarium wilt of chickpea, pigeon pea and lentil

3.9.1 Host Plant Resistance

Host plant resistance has been successfully exploited for management of wilts in several crops. In chickpea, pigeon pea and lentils, several resistant cultivars have been released, and they are popular among farmers. Use of resistant varieties is the most important approach to control wilt disease. Both conventional breeding methods and modern molecular breeding methods such as quantitative trait loci-based methods and molecular-assisted breeding methods are being employed to develop promising resistant genotypes. Wilt-resistant varieties of chickpea, pigeon pea and lentils released by various agencies are presented in Tables 3.1, 3.2 and 3.3.

Several researchers have reported sources of resistance across germplasm accessions in chickpea, pigeon pea and lentil. A lot of efforts have gone into collection, characterization and cataloguing of the germplasm accessions of these crops, and the collections are available at CGIAR institutes like ICRISAT, and Indian national germplasm is being maintained at IIPR, Kanpur. Genetic resources are a valuable

Table 3.1 List of chickpea wilt-resistant varieties released since 2000 in India

S. no	Variety name	Released by	Year of release
1	Gujarat Gram-4	GAU	2000
2	SAKI-9516 (Jawahar gram 16)	JNKVV	2001
3	Kranti (ICCC-37)	ICRISAT	2001
4	Haryana Kabuli 1 (HK- 89-131)	CSSHAU	2002
5	Virat (Kabuli)	MPKV	2002
6	JG-130 (Jawahar gram)	JNKVV	2002
7	Vihar(Phule G-95311)	MPKV	2002
8	Pusa 1088	IARI	2003
9	Haryana Kabuli Chana 2 (HK 94134)	CCS HAU	2004
10	Haryana Chana-5 (H 96-99)	HAU, Hisar	2005
11	Himachal chana-2	CSKHPKVV	2006
12	JAKI -9218	PDKV, Akola	2006
13	Himachal chana-2 (HK-94-134)	CSK HP	2006
14	Digvijay	MPKV	2006
15	JG-63	JNKVV	2006
16	Akash (BDNG-797)	MPKV	2007
17	Rajas (Phule-G-9425-9)	MPKV	2007
18	Lam shanaya (LBeG 7)	ANGRAU	2007
19	JGK-3 (JGK 19)	JNKVV	2007
20	Jawahar Gram 226 (JG 226)	JNKVV	2007
21	GNG 421 (Gauri)	ARS, Sri Ganga Nagar	2007
22	JAKI 9218	PDKV	2008
23	JG6	JNKVV	2008
24	BGD 103	UAS	2009
25	Phule G 0517	MPKV	2010
26	Raj Vijay Kabuli gram 101 (JSC 42)	RVSKVV	2012
27	Raj Vijay gram 201 (JSC 40)	RVSKVV	2012
28	HK 4 (HK 05-169)	CCSHAU	2012
29	PKV Harita (AKG 9303-12)	PDKV	2012
30	GJG 0809	Junagadh	2013

pool of variability and thus could be exploited for targeted breeding programs. These accessions have been successfully deployed in breeding programs, thereby exploiting the heterosis. Singh and Mishra (1976) screened about 530 lines of pigeon pea, but none of them showed less than 5% incidence.

Defence response is based on the recognition phenomenon that operated between the host and the pathogen (Prasad et al. 2003). In case of resistant plants, it will lead to top triggering of a wide array of genetic responses leading to synthesis of defence enzymes and metabolites; ion fluxes across plant membranes; generation of reactive oxygen species; phosphorylation of specific proteins; production of cell wall-strengthening enzymes; induction of phytoalexins; HR response and induction of systemic acquired resistance in distal plant organs (Gupta et al. 2010); early and overexpression of lysyl oxidase genes in resistant cultivars upon inoculation by *F. oxysporum* f. sp. *ciceri* (Garcia-Limones et al. 2009); higher expression of CHS and

Table 3.2 List of pigeon pea wilt-resistant varieties released since 2000 in India

S. no	Variety name	Released by	Year of release
1	Vaishali (BSMR-853)	MAU	2002
2	Pusa 991	IARI	2003
3	Pusa 992	IARI	2004
4	GT-101	GAU	2004
5	ICPL-87119	ICRISAT, Patancheru	2004
6	VL Arhar-1	VPKAS, Almora	2006
7	CORG-9701	TNAU	2006
8	Vipula	MPKV	2007
9	Jawahar (JKM-189)	JNKVV	2007
10	TT-401	BARC	2007
11	Surya (MRG-1004)	ARS Madhira	2009
12	**TJT** – 501	RVSKVV	2009
13	IPA 204	IIPR	2010
14	TS-3R	ARS, Gulbarga	2011
15	ICPH 2740	ICRISAT	2015
16	ICPL 332 WR (TDRG 4)	ICRISAT	2015

Table 3.3 List of lentil wilt-resistant varieties released since 2000 in India

S. no	Variety name	Source	Year of release
1	Noori (IPL-81)	IIPR	2000
2	Malaviya Vishwanath (HUL 57)	BHU	2005
3	KLS 218	CSAUAT	2005
4	VL-Masoor-507	VPKAS, Almora	2006
5	VL Masoor 125	VPKAS, Almora	2006
6	IPL-406 (Angoori)	IIPR	2007
7	Moitree WBL 77	PORS, Berhampore	2009
8	Pant Lentil 7 (PL 024)	GBPUAT	2010
9	Pant Lentil-6 (PL-02)	GBPUAT	2010
10	VL Masoor – 129	VPKAS, Almora	2010
11	VL Masoor 133 (VL133)	VPKAS, Almora	2011

IFR gene-resistant cv. Digvijay as compared to cv. JG 62 (susceptible) and progressive reduction in expression with the progression of disease in the JG 62 (Gurjar et al. 2012); and severalfold upregulation of PR10 gene in resistant chickpea cultivar up to 48 h after inoculation but downregulation of the same in susceptible cultivar in 3, 4 and 5 days after inoculation (Saabale and Dubey 2012);

Recently Thudi et al. (2017) have re-sequenced 127 chickpea varieties to analyse genetic diversity and population structure and identified breeding signatures for targeted breeding programs. A review of status of marker-assisted selection approach for crop improvement suggested that a paradigm shift is required in breeding strategies for strengthening crop improvement programmes involving molecular marker technology (Kumar et al. 2011). Further, it is also highlighted that separation of specific molecular, physiological and biochemical characters that contribute to

abiotic and biotic stress tolerance could help to introgress these traits into otherwise agronomically accepted pulse cultivar.

3.9.2 Chemical Approach

The wilt caused by *Fusarium* spp. is primarily soilborne; hence, seed treatment with fungicide is a genuine method to control disease effectively (Vyas 1993). Systemic fungicides, viz., thiram, captan and vitavax, have been found effective against fusarism wilt and inhibited the infection by *F. oxysporum* f. sp. *lentis* by 100, 84.75 and 46.31%, respectively (Agarwal et al. 1974). Seed treatment with carbendazim, captan and thiram significantly increased the seed germination and seedling vigour of chickpea (Singh et al. 2004). Soil fumigation has also been tried especially in western countries. However, the broad-spectrum biocides used to fumigate soil such as methyl bromide are environmentally not safe as they pollute soil, water and air. Singh et al. (2010) found that carbendazim and carboxin completely inhibited the growth of *F. oxysporum* f. sp. *lentis*, whereas thiram and captafol could inhibit up to 87.5 and 83.1% of mycelial growth, respectively. Carbendazim and carboxin also improved seed germination and other plant growth parameters. Even though studies were conducted to manage wilts, as the pathogen is primarily soilborne and in small proportions seed-borne, application of fungicides has not given desirable results. Further, it is also not economically feasible to adopt these measures as they are very expensive.

3.9.3 Biocontrol Approach

Management of wilt diseases using biocontrol agents is successful mainly due to identification of potential strains, developing suitable formulation strategies, field demonstration of their efficacy and commercialization. Specific strains of *Trichoderma* colonize and penetrate plant root tissues and initiate morphological and biochemical changes in plants. It is considered to be part of the plant defence response that leads to induced systemic resistance (ISR) (Bailey and Lumsden 1998). Plant growth promotion by the *Trichoderma* is a well-established fact (Whipp and Lumsden 2001; Punja and Utkhede 2003). Root colonization by *Trichoderma* strains frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients (Arora et al. 1992). Biological control offers a potential alternative in agricultural production system for reducing the polluting chemical usage in the ecosystem (Kaur et al. 2010).

The manipulation of the crop rhizosphere with PGPR for the biocontrol of plant pathogens has shown considerable promise (Siddiqui 2006). Mixtures of biocontrol agents with taxonomically different organisms that require different optimum temperatures, pH and moisture conditions may colonize roots more aggressively, improving plant growth and the efficacy of biocontrol agents. An increase in suppression and enhanced consistency against multiple cucumber pathogens were

observed using strain mixtures of PGPR (Raupach and Kloepper 1998). Combined inoculation of *B. pumilus*, *P. alcaligenes* and *Rhizobium* sp. improved the growth of *F. oxysporum*-inoculated lentil plants (Akhtar et al. 2010). The effect could be due to direct antagonism, antibiotic production or competition with pathogens for essential nutrients (Gamliel and Katan 1993). *Bacillus* spp. are known to reduce the wilting index in *F. udum*-inoculated plants (Siddiqui and Mahmood 1995). Improvement in plant growth could be attributed to the inhibitory effects of *Bacillus* spp. on pathogens (Chan et al. 2003; Muhammad and Amusa 2003). Use of *Bacillus* spp. resulted in rapid colonization of all tissues in tomato, including the vascular stele, and induced resistance against *F. oxysporum* (Benhamou et al. 1996). Successful reduction in wilt index was reported when fluorescent pseudomonads and *Bacillus* spp. were applied in pigeon pea (Siddiqui et al. 2007). Similarly, inoculation with *Rhizobium* sp. alone resulted in better growth in both *F. oxysporum*-inoculated plants as it produced toxic metabolites that inhibit many plant pathogens (Haque and Ghaffar 1993). *P. fluorescens* produced phenazin, pyrolnintrin, phloroglucinol and siderophores, which may be involved in the suppression of the wilt fungus (Fridlender et al. 1993; Gamliel and Katan 1993). Leeman et al. (1995) reported satisfactory control of fusarium wilt of radish by treating the seed with *P. fluorescens*. In addition, *P. fluorescens* possesses other plant growth promoting traits. Among mycoparasites, *Trichoderma* includes the most widely used biocontrol agent of soilborne, seed-borne and other diseases (Chet et al. 1979; Chet and Baker 1981). *Trichoderma harzianum* and *T. virens* are active rhizosphere colonizers (Tronsmo and Harman 1992) that produce gliotoxin, viridin and some cell wall-degrading enzymes and also certain biologically active heat-stable metabolites such as ethyl acetate. Treatment of pigeon pea seeds with talc-based formulation of *Pseudomonas fluorescens* (Pf1) effectively helps to control fusarium wilt of pigeon pea (Vidhyasekaran et al. 1997).

3.9.4 Cultural and Physical Methods

Soil solarization is a non-chemical and environmentally friendly method of using solar energy for the management of soilborne plant pathogens including fungi, bacteria, nematodes, insect pests and mites in the soil. The soil is covered with a tarp, usually a transparent polyethylene cover, to trap solar energy. The trapped dry/moist solar energy causes physical, chemical and biological changes in the soil. The beneficial effects of soil solarization were first reported by Katan et al. (1976) after successfully demonstrating the management of soilborne pathogens under field conditions. The method has been reported to not only manage harmful pests but also help in mobilizing nutrients and manipulating the microenvironment in the rhizosphere to promote plant growth. Fusarism wilts have been successfully controlled by soil solarization (Stapleton and Vay 1986). The effects could be either direct kill of the pathogen or weakening of the organism, thus resulting in the reduction of aggressiveness and greater susceptibility to attack by other components of the soil microflora (Strange 2003). In addition, soilborne plant pathogen control could be

realized by flooding that destroys many soilborne pathogens (Strange 2003). Removing debris from fusarium wilt-affected chickpea crops and burning or flaming them to achieve thermal killing of *Fusarium oxysporum* f. sp. *ciceri* chlamydospores would reduce disease risk in the subsequent crop. Burning of wilt-affected crop residues greatly reduced the amount of soilborne inoculum (Jimenez-Diaz et al. 2015). Clean cultivation, intercropping and crop rotation have also been proved to reduce inoculum and thus help in the reduction of wilt incidence.

3.10 Conclusion and Way Forward

The concerted efforts of multidisciplinary teams of scientists so far have contributed to sustainable crop improvement and crop husbandry technologies to meet the growing pulse demand in India. The Indian Council of Agricultural Research through its network of research institutes and State Agricultural Universities has led the pulses improvement programs leading to a record production of more than 20 million tonnes of pulses. This initiative paved way to address burning issue of protein malnutrition by increasing access especially among the families below poverty line, as pulses apart from protein also supplement minerals and other nutritional factors. However, looking at the food and nutritional security issues of the future decades, the following issues need to be addressed.

It is pertinent to mention that among biotic stresses, wilts form important part as yield reducers and hence needs to be constantly addressed to find out viable options to manage them. Even after the development of wilt-resistant genotypes, still crop losses due to wilts are being experienced among farming communities.

1. Quick characterization of the germplasm accessions for desirable traits using modern phenotyping tools.
2. An assessment of response of the genotype x *Fusarium* interactions in the context of changing production system environments.
3. A revisit of the physiological specialization and if required suitable deployment of genetic variability across different agroecological regions.
4. Most often pulses are cultivated under resource-poor conditions which predispose the crop to biotic stresses like wilt. Hence, crop husbandry packages to overcome such scenario should be developed and popularized to bridge yield gaps.
5. There are some indications that future disease scenarios could be different in the light of anticipated climate change and climatic variability. The extreme weather events could be altering the host-pathogen interactions and hence need to be studied in detail under FATE and CTGC facilities.
6. Wilts, often in combination with other diseases like root rots, are posing more serious threats and thus need a thorough research for their mutualistic interactions.
7. Develop screening techniques for precise phenotyping of the genotypes.

8. Use of whole-genome sequencing tools in all these crops should now give an opportunity to unravel important information on genes and transcription factors associated with wilt resistance. Hence, novel bioinformatic tools should be employed to unearth this information quickly.
9. The recent advances in microbial research have given us many new tools to study host-pathogen interactions at molecular levels and thus characterize the recognition phenomena, effector genes, resistance induction factors, etc.

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Application of Arbuscular Mycorrhizae in Soil Management

4

Rajni Singh and Neha Sharma

Abstract

An arbuscular mycorrhizae (AM) fungus associates with plant by penetrating the root cells and enabling the plants to use various nutrients present in the soil. AM fungi help plants in phosphate absorption, and plants provide nutrition support to the fungus in the form of hexoses. Recently, in the presence of AM fungi, the degradation of organic pollutants and metals has been observed, and AM bioremediation is also a relevant technique for remediation of contamination sites. There are three types of bioremediation: microbial, mycoremediation, and phytoremediation. Among this, phytoremediation is most common. It involves degradation of the toxicants, and those toxicants are accumulated in the plants (which is called phytoextraction) from the soil or the toxicants can be converted into a nontoxic form and immobilized in the root surface (phytostabilization). AMF association with the plants can be explored in remediation of organic pollutants, sites which are polluted by heavy metals, radionuclides, PAH-polluted soils, and bioassay for soil pollution.

Keywords

Arbuscular mycorrhiza · Mycoremediation · Degradation · Bioremediation · Phytostabilization

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4.1 Introduction

An arbuscular mycorrhizal fungus gets attached with the roots as well as cortical cells of the plants. This fungus is categorized under phylum Glomeromycota and saprophytic in nature forming arbuscules. The arbuscular mycorrhiza (AM) in association with host plant root improves soil structure and enhances the plant resistance to environmental stress. The fungi absorb the carbon from plant through their arbuscules or intraradical hyphae. AM fungi take up hexoses via intraradical mycelium which is the product of the plant host's photosynthesis.

There are two distinct types of AM fungi, characterized by intraradical hyphal modifications:

- The Paris type where hyphal development is intracellular, forming coils in host plant cortical cells.
- The Arum type where intraradical hyphal development is mostly intercellular and forms arbuscules in root cortical cells.

4.1.1 The Development of Arbuscular Mycorrhizae Fungus

Arbuscular mycorrhizal fungal growth and development is rapid. The asymbiotic stage (the only stage in the phenology) has saprophytic ability (Azcón-Aguilar et al. 1999) and displays the lowest metabolic rate. The germ tube of a spore may grow up to 20–30 mm, but if a host root is not contacted within 15–20 days, it may cease growth and become septated due to limited metabolites availability. The spore may further produce another germ tube for growth.

At the pre-symbiotic stage, root exudate encourages germ tube growth toward the root (Giovannetti et al. 1993), stimulating multiple entry points into the root. The spore is not the principal infective unit in thriving habitats, mycorrhizal root fragments, and active hyphal networks being more effective (Smith and Read 2008). Appressoria are formed at predetermined intracellular points of contact with the root (Genre et al. 2005) through which penetration into the cortex occurs. Arbuscules are dichotomously highly branched hyphae which are in contact with the entire surface of plant cell plasma membrane and form the periarbuscular membrane (PAM). At this point of contact, the site of nutrient exchange is formed. Arbuscules develop within 1–6 days of penetration into cortex cells (Harley and Smith 1983). Arbuscules develop as intercellular hyphae spread through the root and continue to penetrate receptive cortical cells. The extent of root colonization also varies with soil biota interactions (Dauber et al. 2008) and with host plant species (Klironomos 2003) (Fig. 4.1).

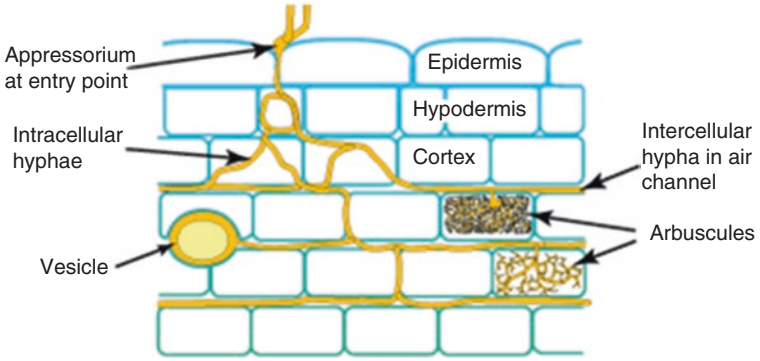

Fig. 4.1 Formation of arbuscules when fungi interact with plants

4.1.2 Bioremediation and Significance of Arbuscular Mycorrhizae

Bioremediation is a process that involves living microorganisms to cure and remediate polluted soils. This biological process involves conversion of polluting substances into less toxic forms. Among different bioremediation techniques, the most favorable is phytoremediation. Depending on the contaminants, there are different types of phytoremediation (phytoextraction, phytodegradation, phytofiltration, phytostabilization, phytovolatilization). Elemental pollutants (toxic heavy metals and radionuclides) are mostly removed by transformation, extraction, and sequestration, whereas organic pollutants which include hydrocarbons and chlorinated compounds are remediated by degradation, rhizoremediation, stabilization, and volatilization. AM associations are important in ecosystem because of the nutritional benefits to the symbiotic partner. The host root exudation pattern is changed by the AMF and which in turn changes the equilibrium associated with mycorrhizosphere. The process of bioremediation involves these types of interaction. The trace elements are localized in the external mycelium of the AMF.

Heavy metals are absorbed by the plants from the soil and either accumulated by roots or precipitated within the rhizosphere into nontoxic form or translocated to the shoots. AMF reduces the toxicity of metals to plants by decreasing translocation of metals from root to shoot (Leyval et al. 1997). The organic pollutant is degraded through microbial activity in root zone (rhizodegradation). AMF causes extension of roots outside the rhizosphere and affects the root exudation. There are certain enzymes which are being derived from enhanced root and rhizospheric microbial activity which causes removal of the pollutants by plant uptake. These are extracellular enzymes which break the complex macromolecules into smaller. These enzymes are hydrolases, lyases, oxidoreductases, and transferases which cause degradation of pollutants.

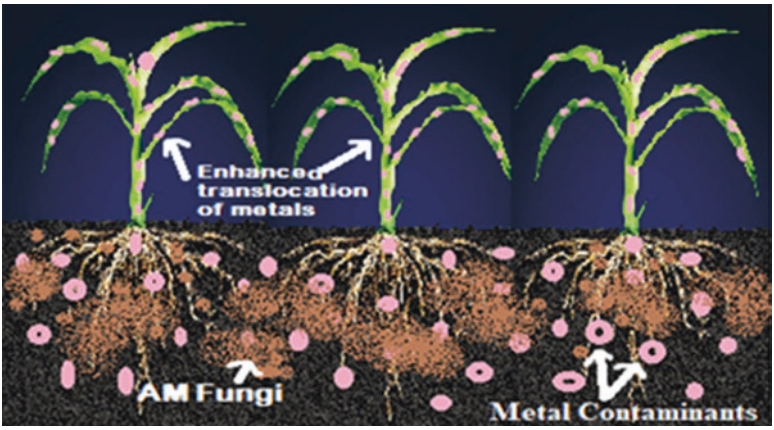

Fig. 4.2 AM-mediated phytoremediation of contaminants present in soil

There are many benefits of phytoremediation as the secondary waste is not generated which reduces the need for further treatment. It also enhances soil fertility and reduces the pollutant transfer through food chain to other ecosystem compartments (Fig. 4.2).

4.1.3 Harmful Effects of Pollutants

Heavy metals can be defined as inorganic contaminants which cause damage to the land. Heavy metals could be released from municipal compost, pesticides, or fertilizers. The residues from mines and smelting industries and emissions from municipal wastes could also lead to release of heavy metal. The accumulation of metals in the animal bodies can cause serious illness. Heavy metals cause various negative effects as they are toxic to soil as well as aquatic life. High concentration of heavy metal could also cause harm to human health, whereas its low concentration inhibits the physiological metabolism of plant. The heavy metals which are being uptaken by plants could be accumulated along the food chain and cause harmful effects to animal and plants. Plants consist of antioxidant enzymes, and it reduces the effect of various types of stresses. If the concentrations of heavy metals are high, then enzymes which are antioxidant in nature do not function. Reactive oxygen species are produced by heavy stress, and it decreases the activity of enzymes. The metal ions repress the activity of enzymes which are antioxidant. They also lead to the production of reactive oxygen species (ROS) that causes harm to aquatic life.

There are different types of organic pollutants commonly found in soils which include polychlorinated biphenyls, polycyclic aromatic hydrocarbons, organophosphorus and carbamate insecticides, herbicides, etc. Through various routes, poly aromatic hydrocarbons usually enter the environment and are present as a mixture containing two or more of these compounds, e.g., soot. These aromatic compounds

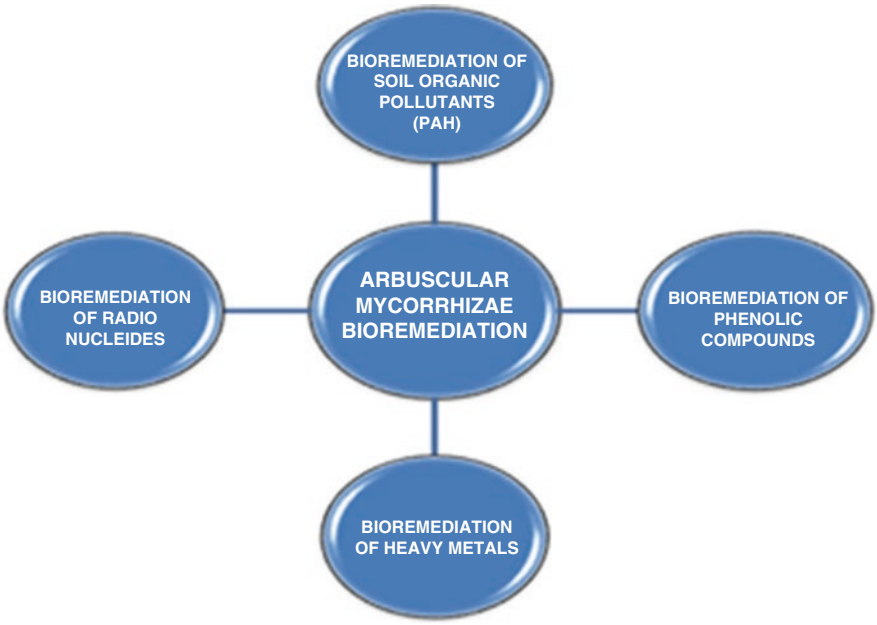

Fig. 4.3 Bioremediation of various substances by AM fungi

stick tightly to the particles and can move through soil to contaminate underground water. Thus, there are many harmful effects of pollutants, and this requires the need for bioremediation. Among many techniques, phytoremediation along with AM fungi is most favored (Fig. 4.3).

4.2 Bioremediation of Metals Present in the Soil

If the amount of metal present in soil is high, it would be harmful to bacteria, fungi, and various processes performed by them. By tolerating the metal concentrations, the soil microorganism adapts themselves to extreme environments. Similarly, mycorrhizal fungi act as a link between roots as well as soil and provide the heavy metal availability and toxicity to plants.

If the level of Cu and lead is high, they are being remediated with the help of AM fungi. There are certain species of AMF which can tolerate the concentration of metal, and thus, low concentration is present in shoots or in roots. The association of AMF with roots helps in increasing the surface area so that nutrients can be absorbed which are usually not absorbed by diffusion (P, Zn, Cu, etc.). Mycorrhizal hyphae of Glomalean family help in uptaking of the nutrients and transfer of metal to roots. Heavy metals are immobilized in the extraradical hyphal structures (Kaldorf et al. 1999). The retention of heavy metals is done by mycelium of fungus, and fixation is by polyphosphate granules. The cell wall of fungi is made up of chitin which

has metal binding capacity. AM fungi release glomalins (metal glycoproteins) which increase the immobilization of toxic metals. Certain protein called as metallothionein which is released by certain AM fungi alleviates the toxicity caused by heavy metal.

4.2.1 Bioremediation of Cu Present in the Soil by AM Fungi

The influence of AMF on soils which are polluted by Cu was seen. Copper (Cu) is present in environment and helps in growth of plant and also in the synthesis of enzymes and proteins required by the plants for various metabolic processes. It also regulates various biochemical and regulatory processes for metabolism of fungus and plant. If the concentration of Cu is high, it hampers the photosynthesis, is toxic to plant, and inhibits the process of respiration and synthesis of proteins, and the transfer of metals to the shoots is stopped.

AMF was used to inhibit Cu toxicity. The extraradical mycelium removes the metals by intracellular precipitation in the hyphal wall as chitin contains metal binding sites. The amount of glutathione reductase (GR), ascorbate peroxidase (APX), superoxide dismutase (SOD), and catalase (CAT) is decreased in the plants associated by AMF.

4.2.2 Bioremediation of Cadmium, Lead, Zinc, and Arsenic by AM Fungi

Heavy metals like cadmium and lead constrain various biochemical processes of plants. Hyperaccumulation of these heavy metals generates reactive oxygen species and methylglyoxal which cause inhibition of enzymes and DNA damage, peroxidation of lipids, and oxidation of proteins. These heavy metals hinder protein metabolism, respiration, photosynthesis, etc. Thus, bioremediation of these heavy metals is required. AM fungi form metallothionein proteins and enzymes because of stress caused by metals. These proteins support the plants against oxidative stress caused by excessive heavy metals in soil (Fabisiak et al. 1999). The association like ectomycorrhizal and ericoid is involved in immobilization which is toxic in nature and presents in soil. The effect of high concentration of metals was seen in the AM fungi (*Glomus intraradices*) which is observed as high spore formation and increase in the length of hyphae (Fig. 4.4, Table 4.1).

4.2.3 Bioremediation of Radionuclides

The level of radioactive elements in the environment has been increasing because of industrial activities, and it causes major problem to ecosystem. If accumulated in the food chain, it causes harm to human health. Radioactive elements occur naturally everywhere in the environment, and the major isotopic forms are uranium and radium

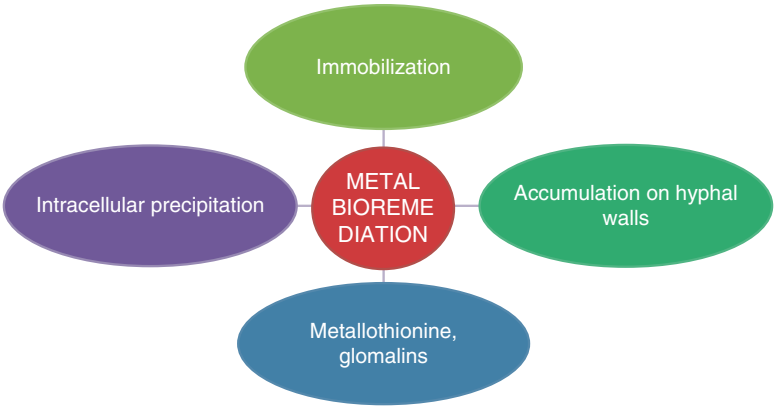

Fig. 4.4 Different processes for bioremediation of metals

which are present in the earth's crust. Free uranium dioxide is chemotoxic and leads to oxidative stress (Saenen et al. 2013). Thus, as the harmful effects are increasing, bioremediation of radionuclides is required. Majority of plant species show symbiotic association with AM fungi. Plants accumulate uranium in the roots. The isotope of uranium called as U^{238} is bioaccumulated into plant roots as uranium dioxide along with uranium dioxide phosphate and uranyl carbonate (Günther et al. 2003).

The important decay product of U^{238} is radium (Ra^{226}) and is present alongside with U^{238} in natural environments. The capacity for accumulation or tolerance of nonessential elements, such as Pb and Cd, and radionuclides, including ^{137}Cs (cesium), is increased with the help of AM fungi in the roots but is restricted or prevented in the shoots. If a plant is grown in high concentration of uranium, inoculation with AM fungi decreases the level of uranium present in the shoots. The immobilization of uranium in hyphal structures (Chen et al. 2008) shows that the fungus helps in the U^{238} accumulation and also its translocation above ground tissues. Macro fungi also translocate materials in the hyphal extension.

Mycorrhizal symbioses occur in most of the plants. In the soil, there is competition between K and Cs. When K fertilizers are added, the uptake of Cs is suppressed and vice versa. In some of the *Glomus* associations, the uptake of radioactive element depends upon hyphal length. The fungal hyphae have greater capacity as compared to roots, and thus, accumulation of metals takes place more in hyphal extension. Ericoid mycorrhizal plants accumulate less radio cesium than non-mycorrhizal plants (Dighton et al. 1991).

Mycorrhizal development in plant root leads to reduce Cs being taken up by the plants, and thus, it shows that Cs is immobilized in the extraradical hyphal structures of mycorrhizal fungus which reduce its translocation in the host plant. Thus, radionuclide uptake depends on:

- Competition between the metals
- Length of hyphae extension

Table 4.1 Bioremediation of metals by AM fungi

Metal name	Mechanism involved	Species of fungi	References
Cadmium (Cd)	Accumulation of heavy metals in vesicles	*Glomus intraradices*	Pawlowska et al. (1999) and Gonzalez-Chavez et al. (2004)
	Cell wall components such as free amino, hydroxyl, and carboxyl groups bind to heavy metals and act as bioabsorbants	*Glomus* and *Gigaspora*	
	Proteins in the cell wall of AM fungi also sequester toxic elements		
	AMF produces glomalin on hyphae that can enhance heavy metals sequestration		
	Metal dissolution by fungi takes place through ligand-promoted mechanism		
	Organic acids released by fungi can be used as source of protons for solubilization and metal-chelating anion complex and metal cations		Finlay (2008)
	Immobilization of metal in binding sites of hyphal extension		
Lead (Pb)	AM fungi bind heavy metals by releasing an insoluble glycoprotein called as glomalin	*Glomus intraradices*	Pawlowska et al. (1999)
	Chelation of metal by siderophores and metallothioneins by fungi		
	Sequestration of the metal by phytochelatins or phytates		
	Enhance uptake of phosphorus		Finlay (2008)
	Absorption by AM hyphae and then translocation from roots to shoots		
Aluminum (Al)	Mycelium of mycorrhizal fungus possesses strong metal binding capacity	*Gigaspora gigantic*	Bartolome-Esteban and Schenck (1994)
Zinc (Zn)	Immobilization of elements which are toxic in nature by polyphosphate granules in the upper of mycelium	*Pisolithus tinctorius*	Leyval et al. (1997)
Arsenic (As)	Retention of heavy metals present on the hyphal walls as chitin binds to the metal	*Glomus mosseae*	Chen et al. (2001) and Cornejo et al. (2017)
Copper (Cu)	Binding of metal to the glycoprotein glomalin	*Glomus etunicatum*	
		Glomus mosseae	

4.2.4 Bioremediation of Phenolic Compounds

AM fungi do not directly transform or degrade phenolic compounds. Previously, the fungi have not been reported to have abilities to degrade phenols, but known enzymes and their genes are being detected. It has been reported that the most rapid degradation of phenolics is caused by basidiomycetes fungi rather than bacteria.

4.2.5 Bioremediation of Soil Pollutants

The bioremediation with the help of arbuscular mycorrhiza causes the elimination of the pollutants present in the soil (organic as well as inorganic). It also improves the soil structure and helps in absorption of nutrients in a better way.

4.2.5.1 The Ability of the Bioremediation Is Affected by Following Factors

- The types of the mycorrhizal fungi.
- Fungi species origin.
- Different type of affected plants.
- Different type and amount of the pollutant.

Mycorrhiza helps in developing the ability of the plant to resist diseases (Harrier and Watson 2004). It also helps in the production of a substance called as glomalin, and it provides stability to the growth of plant in the soil. Polluted soils can be bioremediated with the help of two common types of mycorrhizae – ectomycorrhiza (ECM colonizes only woody species) and arbuscular mycorrhiza (AM). But the main function is performed by arbuscular mycorrhiza. The techniques of phytostabilization and phytoextraction are also used. AM hyphae influence the surrounding which is called as mycorrhizosphere which results in the formation of microbial communities as well. The efficiency of this process is improved when the communities associate with mycorrhizal fungi. AM fungi increase the phosphatase and dehydrogenase enzyme activity which causes oxidoreduction reaction of organic compounds.

There are many organic pollutants which are present in the soil: atrazine, DDT, DDE, fluorene, phenanthrene, pyrene-anthracene, chrysene, dibenz, and anthracene. The structure of organic pollutant influences the rate of removal of pollutant by fungus rate. The high molecular weight of the pollutant with low water solubility hampers the degradation rate. These compounds are degraded at a slower rate as compared to the compounds with low molecular weight. The fluorine translocation is greater than phenanthrene because of its lower molecular weight which facilitates the fluorene removal from the soil.

Polycystic aromatic hydrocarbons (PAH) are organic molecules which are hydrophobic in nature and consist of two or more fused benzene rings. The origin could be natural (organic residues) or anthropogenic (processing and incomplete

combustion of fossil fuels). Phytoremediation is allowed only when the levels of pollution and condition of the matrix which is polluted cause establishment of plants. Thus, arbuscular mycorrhizae fungi help in the plant cover establishment on polluted soil, modification of degradation rates of PAH, improvement of plant nutrient acquisition, improved water relations, tolerance level of pollutant, and sequestration.

4.3 Phytoremediation Mechanism

The soils which are polluted by PAH show low water-holding capacity and less inorganic nutrients. The AM fungi can help to improve the quality of soil in association with the plants.

Mechanisms that are involved in the phytoremediation are:

- Oxidation of contaminants with the help of activated oxygen species.
- The increased level of the oxidoreductases which protect the plant from oxidative stress.

The PAH can be degraded in the rhizosphere by both direct and indirect means. PAH are not directly absorbed by plants (Binet et al. 2000), and thus, they are intracellularly metabolized, and degradation of the pollutants takes place in soil or inside soil organism. The changes in the microbial community and the niche are being changed due to mineral nutrition competition, and the root exudation pressure is also changed.

In case of direct effects, there is increase in the production of extracellular peroxidases. The hydrogen peroxide causes the one-electron oxidation of chemicals to free radicals with the help of peroxidases enzymes. These enzymes biodegrade lignocelluloses and also participate in recalcitrant compounds bioconversion.

The treatment of the soil consisting of PAH by mycorrhiza as compared to non-mycorrhiza can be done in less time. If the exploitation of the soil occurs with the help of AMF hyphae, the microbial communities can be modified. The hyphae provide carbon outside the rhizosphere, and the microbial community can cause PAH degradation in enhanced way (Joner et al. 2000) (Fig. 4.5, Table 4.2).

4.4 Limitation of Arbuscular Mycorrhizae Bioremediation

- This process of bioremediation is relatively slow as compared to other methods of remediation.
- The process of soil remediation takes months to be accomplished by the pollutant-specific mycorrhizal fungi. The desired results may not be obtained if the wrong species is used for specific pollutant.
- The efficiency of the process depends on the type of plant used. There are some plants which do not form mycorrhizal association, and thus, remediation cannot be completed when these plants are used.

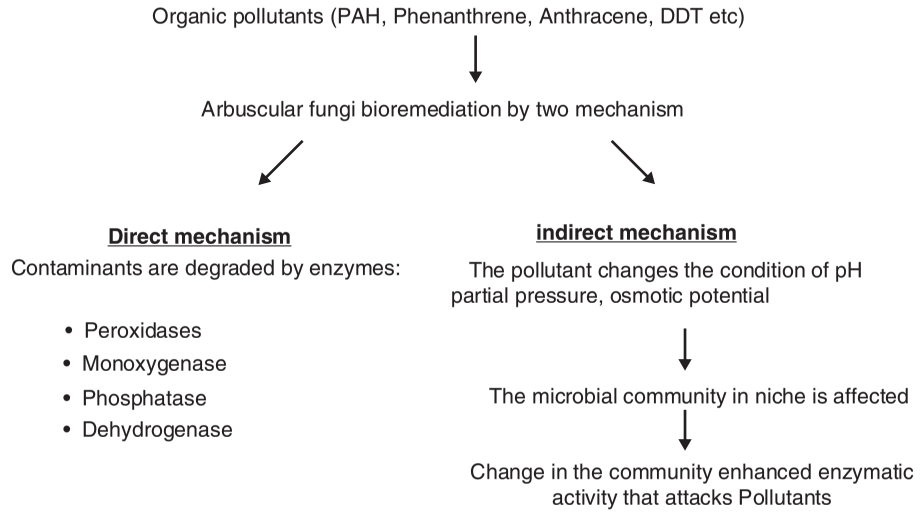

Fig. 4.5 Different mechanisms of phytoremediation

Table 4.2 Bioremediation of organic pollutants by AM fungi

Name of organic pollutant	Species of AM fungi and bacteria	Mechanism
Phenanthrene	*Glomus mosseae*	Microbiota in association with mycorrhiza causes PAH degradation
Pyrene	*Glomus etunicatum* and *Acinetobacter*	
Atrazine		
Phenanthrene	*Bacillus subtilis* and mycorrhizae	The production of root exudates by the mycorrhizal fungi is through extended root growth along with microbes
Polychlorinated biphenyls		
Petroleum (crude oil)	*Glomus intraradices* and *Sphingomonas paucimobilis*	Oxidation of contaminants by activated oxygen species (oxidoreductases)
		Oxidation of lignin by the extracellular enzymes released by fungi

- It can only degrade the pollutants which are present on the surface of the soil.
- The complete degradation of the pollutants is not caused.

4.5 Conclusion

AM fungi show the association between fungi and plants. This association shows mutualistic behavior. There are various techniques which are used to remediate the pollutants by natural means. AM fungi can also be used for the process of bioremediation. Various techniques of bioremediation can be used – phytoremediation, phytoextraction, rhizosphere degradation, etc. These techniques are used to reduce the level of pollutants in the environment which leads to toxicity. The pollutants could be metals, radioactive elements, phenolic compounds, and poly aromatic hydrocarbon compounds present in soil.

The AM fungi association performs the specific mechanism for the process of bioremediation. The pollutants like heavy metals are immobilized in the plants and thus are not released in the environment. The pollutants are also immobilized in the fungal hyphae or mycelium. AM fungi release specific compounds which provide signal to the plant to absorb the pollutant. Thus, AM fungi provide benefits to bioremediate the pollutant, whereas it has certain limitations as well as complete degradation of the pollutant does not take place. The research is in the direction to find the technique behind complete degradation by using mycorrhizal fungus.

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Plant Growth-Promoting Rhizobacteria (PGPRs): A Fruitful Resource

5

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Abstract

The rhizosphere is a unique zone because of its richness in comparison to the nearby soil areas and the accumulation of a variety of organic compounds secreted by the root through exudation and rhizodeposition. Rhizobacteria use rhizosphere as their niche. Rhizospheric microbial communities are members of a complex food web utilizing a huge amount of plant-released nutrients, affecting the carbon flow and transformation. The rhizospheric regions provide a congenial environment for the multiplication and metabolic activity of various microorganisms, through a variety of plant-released compounds like amino acids, sugars, and growth factors, that provide energy and nutrients to the microorganisms. Several rhizobacteria exhibits a commensal relationship with the host-plant, therefore does not effect its physiology and growth. Plant growth-promoting rhizobacteria (PGPRs) came into limelight after its sustainable agricultural and environment-friendly practices to serve the increased population. PGPRs are supposed to replace artificial growth regulators, chemical fertilizers, and pesticides which impose various adverse effects on sustainable agriculture. Innovative research and deep insight of the mechanism of PGPR-associated phytostimulation would enable us to find the way to isolate or develop a competent rhizobacterial strain which could sustain itself in varied agroecological conditions. With the advancements in technology and research, worldwide utilization of PGPRs will become a reality, which shall ensure the stability as well as productivity of agro-ecosystems for guiding us on the road to an ideal agricultural system.

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Rhizobacteria · Rhizosphere · Function · Benefits

5.1 Introduction

Rhizosphere is the term generally used to acknowledge the root zone of the plant system (Hartmann et al. 2008; Gouda et al. 2018). The zone is unique because of its richness in comparison to the nearby soil areas and presence of numerous organic compounds secreted by the roots via exudation, release, and rhizodeposition. The release of various organic compounds can be used as the energy source by the microbes and could initiate intense microbial activity within the rhizosphere. Therefore, it can be stated that rhizobacteria use rhizosphere as their niche. In the same way, those bacteria which induce growth of the plants are plant growth-promoting rhizobacteria (PGPRs). PGPRs attained limelight after knowing its sustainable agricultural and environment-friendly practices to serve the increased population. However, the abrupt exploitation of harmful fertilizers and pesticides causes severe adverse effects on the health of the environment. It is impossible to device a strategy which is eco-friendly to lessen the use of chemicals required for plant growth. In the late 1970s, the name PGPR was given by Kloepper and his colleagues, who described the PGPR (Kloepper and Schroth 1978). Numerous genera of soil bacteria come under PGPRs, promoting plant growth and development in association with the rhizosphere in most part of its life cycle (Saharan and Nehra 2011; Pandey et al. 2012). The PGPR-host relationship is confined to the rhizosphere (few of them colonize at the rhizosphere, rhizoplane, superficial intercellular spaces, or dead root cell layer) or is endophytic (some species exists in the apoplastic spaces present in the host plant inhabiting the structural and nonstructural nodules) (Vessey 2003).

The two major groups of PGPR are: (1) extracellular-plant growth promoting rhizobacteria (e-PGPRs): represents the microbial species that inhabit the rhizosphere over the rhizoplane, and (2) intracellular-plant growth promoting rhizobacteria (i-PGPRs): symbolizes the bacteria present in the intermediate spaces of the root cell cortex or within specialized structures called nodules (Gray and Smith 2005). The bacterial genera that are included as ePGPR are *Azospirillum*, *Azotobacter*, *Agrobacterium*, *Arthrobacter*, *Serratia*, *Bacillus*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcous*, *Pseudomonas*, and *Burkholderia*. The endophytic microbes representing the iPGPR are *Rhizobium*, *Bradyrhizobium*, *Allorhizobium*, *Mesorhizobium*, including Frankia species. PGPR induces plant growth by two broad mechanisms termed as: (I) direct and (II) indirect, although they do not show distinctive similarity (Lugtenberg and Kamilova 2009;

Ashraf et al. 2013). Nutrient availability is dependent upon direct mechanism which further depends upon the availability of a plant that fixes the available nitrogen, solubilizes insoluble phosphates, produces siderophores and mineralizes the organic matter (thus fulfilling the requirement for phosphorus, sulfur, and nitrogen nutrition of a plant). Apart from this, the mechanism includes plant growth hormone and stress hormone production like 1-aminocyclopropane-1-carboxylate (ACC) deaminase. On the other hand, indirect mechanism is related to those processes through which PGPR prevent or counteract the harmful effects of phytopathogens on host-plants by producing repressive substances that increase the natural resistance of the host-plants (Das et al. 2013). Thus, to sum up, the direct mechanisms include: nitrogen fixation, phosphate solubilization, potassium solubilization, phytohormone production, siderophore production, exopolysaccharide production, and rhizoremediation while the indirect mechanisms include: (i) stress management - (a) abiotic stress tolerance, and (b) biotic stress tolerance, (ii) disease resistance antibiosis, (iii) induced systemic resistance, (iv) production of protective enzymes, and production of VOCs. The PGPRs that are screened, well-studied and marketed includes *Agrobacterium, Azospirillum, Azotobacter, Bacillus, Burkholderia, Paenibacillus macerans, Pantoea agglomerans, Pseudomonas, Rhizobium, and Serratia* (Glick 2012). Although, several PGPR strains have been reported and studied but only few have been registered and commercialized (Bashan et al. 2014). Probably, this is because of the failure faced in field trials, due to the field conditions and the crop which was inoculated. The survival of any bacterial inoculant depends on its compatibility with the existing soil-microflora, along with the soil characteristics and environmental conditions (Martinez-Viveros et al. 2010). Glick (2012), coded some beneficial aspects which are to be prioritized before the commercialization of PGPRs. These include: (i) trait selection for effective functioning and selection of succeeding strains, (ii) coordination between regulatory bodies among different countries so as to work upon the environmental and agricultural aspects, (iii) improved understanding on the criteria of using rhizobacteria/endophytic bacteria, (iv) determining the particular strains for improved working in a specific environment which could be the strains which are well known to work efficiently in warm and sandy soil along with those which are compatible with cold and wet environment, (v) constructing an efficient site of application for setting up nurseries against the field, and (vi) improved understanding among the bacterial strains and PGPRs. It should be noticed that the suitable PGPR should possess rhizospheric competence, improved plant growth capabilities, easy multiplication properties, wide action spectrum, and consistent biological control activity (open applicability); should be non-harmful to the environment; must be friendly with the pre-existing microbiota; and should be flexible in tolerating dissection, high temperature, and oxidizing agents accompanied by UV radiations (Nakkeeran et al. 2005) (Tables 5.1–5.7).

Table 5.1 Growth-promoting substances released by PGPR

S. no.	PGPR	Plant growth-promoting traits																References
		ACCD	IAA	AR	AFA	S	NF	HCN	AP	PS	EPSs	NA	K	GA	CK	HMS/M	MR	
1	*Acinetobacter* sp. *Pseudomonas* sp.	√	√	–	√	–	√	–	–	√	–	–	–	–	–	–	–	Indiragandhi et al. (2008)
2	*Acinetobacter* spp.	–	√	–	–	√	–	√	√	√	√	–	–	–	–	–	–	Ahemad and Khan (2010f, g, 2011e, j) and Rokhbakhsh-Zamin et al. (2011)
3	*Azospirillum amazonense*	–	√	–	–	–	–	–	–	–	–	√	–	–	–	–	–	Rodrigues et al. (2008)
4	*Azospirillum brasilense*, *Azospirillum amazonense*	–	√	√	–	–	–	–	–	√	–	√	–	–	–	–	–	Thakuria et al. (2004)
5	*Azotobacter chroococcum*	–	√	–	–	–	–	–	–	–	–	–	√	√	–	–	–	Verma et al. (2001)
6	*Azotobacter chroococcum*	–	–	–	–	–	–	–	–	√	–	–	–	–	–	–	–	Kumar et al. (2001)
7	*Azotobacter* sp., *Mesorhizobium* sp., *Pseudomonas* sp., *Bacillus* sp.	–	√	–	√	√	–	√	√	–	–	–	–	–	–	–	–	Ahmad et al. (2008)
8	*Baciilus subtilis*	–	–	–	√	–	–	–	–	–	–	–	–	–	–	–	–	Cazorla et al. (2007)
9	*Bacillus* sp.	–	–	–	–	–	–	–	–	√	–	–	–	–	–	–	–	Canbolat et al. (2006)
10	*Bacillus* species PSB10	–	√	–	–	√	–	√	√	–	–	–	–	–	–	–	–	Wani and Khan (2010)

11	*Bacillus* spp., *Pseudomonas* spp., *Azotobacter* spp., *Rhizobium* spp.	–	√	–	–	–	–	–	√	–	–	–	–	–	–	–	–	Joseph et al. (2007)
12	*Bacillus subtilis*	–	√	–	–	–	–	–	–	√	–	–	–	–	–	–	–	Zaidi et al. (2006)
13	*Bacillus*, *Azospirillum* sp.	–	√	–	–	–	–	–	–	√	–	–	–	–	–	–	–	Yasmin et al. (2004)
14	*Bacillus*, *Pseudomons*, *Azotobacter*, *Azospirillum*, *Rhizobium*	–	√	–	–	–	–	–	–	√	–	–	–	–	–	–	–	Tank and Saraf (2003)
15	*Bradyrhizobium japonicum*	–	√	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Shaharoona et al. (2006)
16	*Bradyrhizobium japonicum*	–	–	–	–	√	–	–	–	–	–	–	–	–	–	–	–	Wittenberg et al. (1996)
17	*Bradyrhizobium* sp.	–	√	–	–	√	–	√	√	–	√	–	–	–	–	–	–	Ahemad and Khan (2011d, h, l, 2012f)
18	*Bradyrhizobium* sp.	–	√	–	–	√	–	√	√	–	–	–	–	–	–	–	–	Wani et al. (2007a)
19	*Bradyrhizobium* sp. 750, *Pseudomonas* sp., *Ochrobactrum cytisi*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	√	–	Dary et al. (2010)
20	*Bradyrhizobium*, *Rhizobium*	–	–	–	–	√	–	–	–	–	–	–	–	–	–	–	–	Duhan et al. (1998)
21	*Bradyrhizobium*, *Rhizobium*	–	√	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Antoun et al. (1998)

(continued)

Table 5.1 (continued)

S. no.	PGPR	Plant growth-promoting traits																References
		ACCD	IAA	AR	AFA	S	NF	HCN	AP	PS	EPSs	NA	K	GA	CK	HMS/M	MR	
22	*Bravibacterium* sp.	–	–	–	–	√	–	–	–	–	–	–	–	–	–	–	–	Noordman et al. (2006)
23	*Brevibacillus* spp.	–	√	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Vivas et al. (2006)
24	*Burkholderia*	√	√	–	–	√	–	–	–	√	–	–	–	–	–	√	–	Jiang et al. (2008)
25	*Enterobacter asburiae*	–	√	–	–	√	–	√	√	√	√	–	–	–	–	–	–	Ahemad and Khan (2010a, b)
26	*Enterobacter* sp.	√	√	–	–	√	–	–	–	√	–	–	–	–	–	–	–	Kumar et al. (2008)
27	*Gluconacetobacter diazotrophicus*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	√	–	Saravanan et al. (2007)
28	*Klebsiella oxytoca*	–	√	–	–	–	–	–	–	√	–	√	–	–	–	–	–	Jha and Kumar (2007)
29	*Klebsiella* sp.	–	√	–	–	√	–	√	√	√	√	–	–	–	–	–	–	Ahemad and Khan (2011b, f, g)
30	*Kluyvera ascorbata*	–	–	–	–	√	–	–	–	–	–	–	–	–	–	–	–	Burd et al. (2000)
31	*Kluyvera ascorbata*	√	–	–	–	√	–	–	–	–	–	–	–	–	–	–	√	Genrich et al. (1998)
32	*Mesorhizobium ciceri*, *Azotobacter chroococcum*	–	√	–	–	√	–	–	–	–	–	–	–	–	–	–	–	Wani et al. (2007c)
33	*Mesorhizobium* sp.	–	√	–	–	√	–	√	√	–	√	–	–	–	–	–	–	Ahemad and Khan (2009a, 2010e, g, 2012d)
34	*Mesorhizobium* sp.	–	√	–	–	√	–	√	√	–	–	–	–	–	–	–	–	Wani et al. (2008)
35	*Mesorhizobium*, *Bradyrhizobium* sp.	–	–	–	–	√	–	–	–	–	–	–	–	–	–	–	–	Khan et al. (2010)
36	*Paenibacillus polymyxa*	–	√	–	–	√	–	–	–	–	–	–	–	–	–	–	–	Phi et al. (2010)

37	*Proteus vulgaris*	–	–	–	–	√	–	–	–	–	–	–	–	–	–	–	–	Rani et al. (2009)
38	*Pseudomonas aeruginosa*	–	√	–	–	√	–	√	√	√	√	–	–	–	–	–	–	Ahemad and Khan (2010d, 2011a, k, 2012e)
39	*Pseudomonas aeruginosa*	–	–	–	–	√	–	–	–	–	–	–	–	–	–	–	–	Naik and Dubey (2011)
40	*Pseudomonas aeruginosa*	√	√	–	–	√	–	–	–	√	–	–	–	–	–	–	–	Ganesan (2008)
41	*Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Ralstonia metallidurans*	–	–	–	–	√	–	–	–	–	–	–	–	–	–	–	–	Braud et al. (2009)
42	*Pseudomonas chlororaphis*	–	–	–	√	–	–	–	–	–	–	–	–	–	–	–	–	Liu et al. (2007)
43	*Pseudomonas fluorescens*	√	–	–	–	–	–	–	–	√	–	–	–	–	–	–	–	Shaharoona et al. (2008)
44	*Pseudomonas fluorescens*	–	–	–	√	–	–	–	–	–	–	–	–	–	–	–	–	Saravanakumar et al. 2007
45	*Pseudomonas fluorescens*	–	√	–	√	√	–	–	–	–	–	–	–	–	–	–	–	Dey et al. (2004)
46	*Pseudomonas fluorescens*	–	√	–	–	–	–	–	–	√	–	–	–	–	–	–	–	Jeon et al. (2003)
47	*Pseudomonas fluorescens* PRS9, *Pseudomonas fluorescens* GRS1	–	√	–	–	√	–	–	–	√	–	–	–	–	–	–	–	Gupta et al. (2005)
48	*Pseudomonas jessenii*	√	√	–	–	√	–	–	–	√	–	–	–	–	–	√	–	Rajkumar and Freitas (2008)

(continued)

Table 5.1 (continued)

S. no.	PGPR	Plant growth-promoting traits																References
		ACCD	IAA	AR	AFA	S	NF	HCN	AP	PS	EPSs	NA	K	GA	CK	HMS/M	MR	
49	*Pseudomonas putida*	–	√	–	–	√	–	√	√	√	√	–	–	–	–	–	–	Ahemad and Khan (2011c, 2012a, c)
50	*Pseudomonas putida*	–	–	–	√	√	–	√	–	√	–	–	–	–	–	–	–	Pandey et al. (2006)
51	*Pseudomonas putida*	–	–	–	–	√	–	–	–	–	–	–	–	–	–	–	–	Tripathi et al. (2005)
52	*Pseudomonas* sp.	–	√	–	–	√	–	–	–	–	–	–	–	–	–	√	–	Ma et al. (2011b)
53	*Pseudomonas* sp.	–	√	–	–	√	–	√	–	√	–	–	–	–	–	–	–	Tank and Saraf (2009)
54	*Pseudomonas* sp.	√	√	–	–	√	–	–	–	–	–	–	–	–	–	–	–	Poonguzhali et al. (2008)
55	*Pseudomonas* sp.	√	√	–	–	√	–	–	–	√	–	–	–	–	–	√	–	Rajkumar and Freitas (2008)
56	*Pseudomonas* sp., *Bacillus* sp.	–	√	–	–	√	–	–	–	√	–	–	–	–	–	–	–	Rajkumar et al. (2006)
57	*Pseudomonas*, *Bacillus*	–	√	–	–	√	–	–	–	√	–	–	–	–	–	–	–	Wani et al. (2007c)
58	*Rahnella aquatilis*	√	√	–	–	–	–	–	–	√	–	–	–	–	–	–	–	Mehnaz et al. (2010)
59	*Rhizobium cicero*	–	–	–	–	√	–	–	–	–	–	–	–	–	–	–	–	Berraho et al. (1997)
60	*Rhizobium leguminosarum*	–	–	–	–	–	–	–	–	–	–	–	–	–	√	–	–	Noel et al. (1996)
61	*Rhizobium meliloti*	–	–	–	–	√	–	–	–	–	–	–	–	–	–	–	–	Arora et al. (2001)
62	*Rhizobium phaseoli*	–	√	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Zahir et al. (2010)
63	*Rhizobium* sp.	–	√	–	–	√	–	√	√	–	–	–	–	–	–	–	–	Wani et al. (2007b)
64	*Rhizobium* sp. (pea)	–	√	–	–	√	–	√	√	–	√	–	–	–	–	–	–	Ahemad and Khan (2009b, 2010c, 2011i, 2012b)

65	*Rhizobium*, *Bradyrhizobium*	–	√	–	–	√	–	√	–	√	–	–	–	–	–	–	–	Deshwal et al. (2003)
66	*Rhizobium*, *Bradyrhizobium*	–	–	–	–	–	–	–	–	√	–	–	–	–	–	–	–	Abd-Alla (1994)
67	*Serratia marcescens*	–	√	–	–	√	–	√	–	–	–	–	–	–	–	–	–	Selvakumar et al. (2008)
68	*Sphingomonas sp*, *Mycobacterium* sp, *Bacillus* sp, *Rhodococcus* sp, *Cellulomonas* sp., *Pseudomonas* sp	–	√	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Tsavkelova et al. (2005)
69	*Stenotrophomonas maltophilia*	√	√	–	–	–	–	–	–	√	–	√	–	–	–	–	–	Mehnaz et al. (2010)
70	*Variovorax paradoxus*, *Rhodococcus* sp., *Flavobacterium*	–	√	–	–	√	–	–	–	–	–	–	–	–	–	–	–	Belimov et al. (2005)
71	*Xanthomonas* sp. RJ3, *Azomonas* sp. RJ4, *Pseudomonas* sp. RJ10, *Bacillus* sp. RJ31	–	√	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Sheng and Xia (2006)

ACCD (1-aminocyclopropane-1-carboxylate) deaminase activity, *IAA* indole-3-acetic acid, *AR* antibiotic resistance, *AFA* antifungal activity, *S* siderophores, *NF* N_2 fixation, *HCN* hydrogen cyanide, *AP* ammonia production, *PS* phosphate solubilization, *EPSs* exopolysaccharides, *NA* nitrogenase activity, *K* kinetin, *GA* gibberellin, *CK* cytokinin, *HMS/M* heavy metal solubilization or mobilization, *MR* metal resistance

Table 5.2 Plant growth promoting rhizobacteria tested for various crop types

S. no.	PGPR	Plant	Results of the addition of bacteria to plants	References
1	*A. xylosoxidans* strain Ax10	*Brassica juncea*	Improved Cu uptake in plants and induced shoot length, dry weight, fresh weight, and root length of plants	Ma et al. (2009c)
2	*A. amazonense*	*Oryza sativa* L.	Increased panicle number, dry matter (7–11.6%), and nitrogen accumulation 3.5–18.5%) in grains	Rodrigues et al. (2008)
3	*A. brasilense* CW903, *B. pyrrocinia* CBPB-HOD, *M. oryzae* CBMB20	*Capsicum annuum* L.	Increase in root and shoot length by 0.4–17% and 4–35%, respectively. Production of IAA hormone and solubilization of phosphate were also observed	Madhaiyan et al. (2010)
4	*A. brasilense* CW903, *B. pyrrocinia* CBPB-HOD, *M. oryzae* CBMB20	*Oryza sativa* L.	Increase in root and shoot length by 20–31% and 1.5–8.55%, respectively	Madhaiyan et al. (2010)
5	*A. brasilense* CW903, *B. pyrrocinia* CBPB-HOD, *M. oryzae* CBMB20	*Lycopersicon esculentum* Mill.	Increase in root and shoot length by 1–13% and 8–13%, respectively	Madhaiyan et al. (2010)
6	*A. brasilense* Sp245	*Phaseolus vulgaris* L.	Increased root growth	Remans et al. (2008)
7	*Azotobacter* sp., *Azospirillum* sp., *Pseudomonas* sp.	*Avena sativa* L	Reduction in acetylene activity and IAA production. IAA production and acetylene-reducing activity. Increased root length (12–23%), root area (8–500%), dry weight of shoot (6–93%)	Yao et al. (2008)
7	*Azotobacter*	*Zea mays*	Production of IAA, increase in biomass, plant height, cob weight, cob length, etc.	Zahir et al. (2005)
8	*A. chroococcum*, *A. lipoferum*	*Gossypium hirsutum*	Increase in seed yield (21%), plant height (5%)	Anjum et al. (2007)
9	*B. cereus* (KBE7-8), *B. cereus, (*NAS4-3*)* and *S. maltophilia* (KBS9-B)	*Sorghum bicolour*	Increase in root and shoot length, respectively; production of IAA hormone and solubilization of phosphate were also observed	Idris et al. (2009)

(continued)

Table 5.2 (continued)

S. no.	PGPR	Plant	Results of the addition of bacteria to plants	References
10	*Bacillus edaphicus*	*Brassica juncea*	Pb mobilization, increase in root and shoot length; production of IAA hormone, and solubilization of phosphate were also observed	Sheng et al. (2008)
12	*Bacillus* M3	*Rubus* spp	Nitrogen fixation and production of IAA hormone and solubilization of phosphate were also observed	Orhan et al. (2006)
13	*Bacillus* M3, *Microbacterium* FS01, and *Bacillus* OSU-142	*Malus domestica*	Increased nitrogen (N) fixation and production of IAA hormone and solubilization of phosphate were also observed	Karlidag et al. (2007)
14	*Bacillus* sp., *Paenibacillus* sp.	*Oryza sativa*	Induced root and shoot growth	Beneduzi et al. (2008)
15	*Bacillus* species PSB10	*Cicer arietinum*	Significantly improved nodulation, grain protein; chlorophyll, leghemoglobin, seed yield, etc. Reduction in chromium uptake in grains, shoots, and roots	Wani and Khan (2010)
16	*B.subtilis* BEBlSbs (BS13)	*Lycopersicon esculentum*	Increase in the root, plant yield, and shoot length, respectively. Production of IAA hormone and solubilization of phosphate were also observed	Mena-Violante and Olalde-Portugal (2007)
17	*B. subtilis FZB 24®*	*Gossypium* sp.	Production of IAA hormone and solubilization of phosphate were also observed	Yao et al. (2006)
18	*B. subtilis*, *P. aeruginosa*	*Abelmoschus esculentus*, *Amaranthus* sp., *Solanum lycopersicum* L.	Increase in dry biomass, plant height, root length, etc. Production of IAA hormone and solubilization of phosphate were also observed	Adesemoye et al. (2008)
19	*B. weihenstephanensis* strain SM3	*Helianthus annuus*	Increased biomass of plant and the accretion of Zn and Cu in the shoot and root systems	Rajkumar et al. (2008)
20	*Bradyrhizobium* MRM6	*Vigna radiata*	Strain production of IAA hormone and solubilization of phosphate were also observed	Ahemad and Khan (2011h, l, 2012f)

(continued)

Table 5.2 (continued)

S. no.	PGPR	Plant	Results of the addition of bacteria to plants	References
21	*Bradyrhizobium* sp. (vigna) RM8	*Vigna radiata*	Increased biomass of the plant, nodule number, seed yield, leghemoglobin, shoot nitrogen, root nitrogen, grain protein	Wani et al. (2007a)
22	*Bradyrhizobium* sp. 750, *Pseudomonas* sp.	*Ochrobactrum cytisi*, *Lupinus luteus*	Increased biomass of the plant, nodule number, seed yield, leghemoglobin, shoot nitrogen, root nitrogen, grain protein	Dary et al. (2010)
23	*Brevundimonas*	Kro13	Cadmium sequestering	Robinson et al. (2001)
24	*Enterobacter cloacae*	*Brassica napus*	Both shoot and root lengths increased significantly	Saleh and Glick (2001)
25	*E. sakazakii* 8MR5, *Pseudomonas* sp. 4MKS8, *K.* oxytoca 10MKR7	*Zea mays*	Inoculation increases shoot and root length	Babalola et al. (2003)
26	*K. pneumonia*	*Triticum aestivum*	Significantly increased the root length and shoot length	Sachdev et al. (2009)
27	*K. ascorbata* SUD165	*Brassica juncea*, *Brassica napus*, *Solanum lycopersicum*	Increased resistance against heavy metals	Burd et al. (2000)
28	*Mesorhizobium* sp. RC3	*Cicer arietinum*	Increased biomass of the plant, nodule number, seed yield, protein content, shoot nitrogen, root nitrogen, grain protein, etc.	Wani et al. (2008)
29	*Mesorhizobium* strain MRC4	*Cicer arietinum*	Increased biomass of the plant, nodule number, seed yield, leghemoglobin, shoot nitrogen, root nitrogen, seed protein, etc.	Ahemad and Khan (2009a, 2010e, g)
30	*P. polymyxa*		Increased biomass of the plant, nodule number, seed yield, protein content, shoot nitrogen, root nitrogen, grain protein	Phi et al. (2010)

(continued)

Table 5.2 (continued)

S. no.	PGPR	Plant	Results of the addition of bacteria to plants	References
31	*A. lipoferum* DSM 1691, *A. brasilense* DSM 1690, *P. putida* strain R-168, *P. fluorescens* DSM 50090, *P. putida* DSM291, *P. fluorescens* strain R-93,	*Zea mays* L.	Increase in dry biomass, plant height, root length, leaf area	Gholami et al. (2009)
32	*P. aeruginosa*	*Brassica juncea*, *Cucurbita*	Reduction in Cu uptake and stimulated plant growth	Sinha and Mukherjee (2008)
33	*Pseudomonas aeruginosa* strain MKRh3	*Vigna mungo*	Reduction in Cd uptake and stimulated plant growth	Ganesan (2008)
34	*R. metallidurans, P. fluorescens*, *P. aeruginosa*	*Zea mays*	Enhanced Cr and Pb uptake and stimulated plant growth	Braud et al. (2009)
35	*Pseudomonas* BA-8 nd, *Bacillus* OSU- a	*Prunus avium*	Increased biomass of the plant, nodule number, seed yield, leghemoglobin, shoot nitrogen, root nitrogen, seed protein, total soluble solids, fruit weight	Esitken et al. (2006)
36	*Burkholderia* sp, *P. fluorescens* (MPp4)	*Zea mays*	Increase in dry biomass, plant height, root length, etc. Production of IAA hormone and solubilization of phosphate were also observed. Disease resistance was also observed	Hernández-Rodríguez et al. (2008)
37	*P. fluorescens* Avm.	*Medicago sativa*	Enhanced translocation of Fe and Cu from root to shoot	Carrillo-Castaneda et al. (2003)
38	*P. putida*, *Azospirilium*, *Azotobacter*	*Cynara scolymus*	Production of IAA hormone, solubilization of phosphate was also observed, vigor index, the velocity of germination decreased	Jahanian et al. (2012)
39	*Pseudomonas* sp.	*Triticum aestivum*	Production of IAA hormone, solubilization of phosphate, and soil enzyme activities were also observed	Sharma et al. (2011)
40	*Pseudomonas* sp.	*Cicer arietinum*	Enhanced dry and fresh weights of plants at a high concentration of Ni	Tank and Saraf (2009)

(continued)

Table 5.2 (continued)

S. no.	PGPR	Plant	Results of the addition of bacteria to plants	References
41	*Pseudomonas* sp.	*Triticum aestivum*	Enhanced plant growth	Gupta et al. (2002a, b)
42	*Pseudomonas* sp.	*Oryza sativa*, *Zea mays*	Antifungal and antibacterial properties.	Lawongsa et al. (2008)
43	*Pseudomonas* sp. A3R3	*Brassica juncea, Alyssum serpyllifolium*	Biomass increased under Ni stress conditions	Ma et al. (2011a)
44	*Pseudomonas* sp. PS1	*Vigna radiata*	Increased biomass of the plant, nodule number, seed yield, leghemoglobin, shoot nitrogen, root nitrogen, seed protein, total soluble solids, fruit weight	Ahemad and Khan (2010d, 2011k, 2012e)
45	*Pseudomonas* sp. SRI2, *Psychrobacter* sp. SRS8, *Bacillus* sp. SN9	*Brassica juncea*, *Brassica oxyrrhina*	Biomass increased under Ni stress conditions	Ma et al. (2009a)
46	*Alcaligenes* sp. ZN4, *P. fluorescens* ACC9, *P. tolaasii* ACC23, *Mycobacterium* sp. ACC14	*Brassica napus*	Resistance against cadmium	Dell'Amico et al. (2008)
47	*B.cereus* SRA10, *Psychrobacter* sp. SRA1	*Brassica oxyrrhina, Brassica juncea*	Resistance against metals (Ni)	Ma et al. (2009b)
48	*Psychrobacter* sp. SRS8	*Helianthus annuus, Ricinus communis*	Resistance against metals (Ni) increased biomass of the plant, nodule number, seed yield, leghemoglobin, shoot nitrogen, root nitrogen, seed protein, total soluble solids, fruit weight	Ma et al. (2011b)
49	*Rhizobium phaseoli*	*Vigna radiata* L.	Stress tolerance stimulates plant growth	Zahir et al. (2010)
50	*Rhizobium* strain MRL3	*Lens esculentus*	Increased biomass of the plant, nodule number, seed yield, leghemoglobin, shoot nitrogen, root nitrogen	Ahemad and Khan (2010f, g, 2011j)
51	*Rhizobium* strain MRP1	*Pisum sativum*	Significant increase in nodule number, seed yield, leghemoglobin, shoot nitrogen, root nitrogen, seed protein, total soluble solids, fruit weight	Ahemad and Khan (2009b, 2010c, 2011i)

Table 5.3 Efficient PGPR strains as phytohormone producer in numbers of plants

PGPR	Host	Hormone produced	References
Rhizobium leguminosarum	*Brassica napus* and *Lactuca sativa*	Cytokinin	Noel et al. (1996)
Rhizobium leguminosarum	*Raphanus sativus var. Longipinnatus*	IAA	Antoun et al. (1998)
Bradyrhizobium sp.	*Raphanus sativus var. Longipinnatus*	IAA	Antoun et al. (1998)
Agrobacterium sp.	*Lactuca sativa*	IAA	Barazani and Friedman (1999)
Alcaligenes piechaudii	*Lactuca sativa*	IAA	Barazani and Friedman (1999)
Comamonas acidovorans	*Lactuca sativa*	IAA	Barazani and Friedman (1999)
Paenibacillus polymyxa	*Triticum aestivum*	Cytokinin	Timmusk et al. (1999)
Azospirillum brasilense	*Triticum aestivum*	IAA	Kaushik et al. (2000)
Enterobacter cloacae	*Oryza sativa*	IAA	Mehnaz et al. (2001)
Pseudomonas fluorescens	*Glycine max*	Cytokinin	Garcia de Salamone et al. (2001)
Aeromonas veronii	*Oryza sativa*	IAA	Mehnaz et al. (2001)
Bacillus sp.	*Alnus glutinosa*	Gibberellin	Gutierrez-Manero et al. (2001)

Table 5.4 PGPR species and their ability to fix atmospheric N_2 in certain plants

Environment	PGPR	Crop	References
Rhizospheric	*Azospirillum* sp.	*Triticum aestivum*	Boddey et al. (1986)
	Azospirillum sp.	*Zea mays*	Garcia de Salamone et al. (1996)
	Azospirillum sp.	*Oryza sativa*	Malik et al. (1997)
	Azotobacter sp.	*Zea mays*	Pandey et al. (1998)
	Azotobacter sp.	*Triticum aestivum*	Mrkovacki and Milic (2001)
Endophytic	*Gluconacetobacter* sp.	*Sorghum bicolor*	Isopi et al. (1995)
	Azoarcus sp.	*Sorghum bicolor*	Stein et al. (1997)
	Herbaspirillum sp.	*Sorghum bicolor*	James et al. (1997)
	Burkholderia sp.	*Oryza sativa*	Baldani et al. (2000)
	Gluconacetobacter sp.	*Saccharum officinarum*	Boddey et al. (2001)
	Azoarcus sp.	*Leptochloa fusca*	Hurek et al. (2002)
	Herbaspirillum sp.	*Oryza sativa*	James et al. (2002)

Table 5.5 PGPR used as biocontrol agents against different diseases, pathogens, and insects affecting different crops

Disease/pathogen/insect	PGPR	Crop	References
Powdery mildew	*B. subtilis*	*Hordeum vulgare*	Schobeck et al. (1980)
Damping off	*P. fluorescens*	*Gossypium hirsutum*	Howell and Stipanovic (1980)
Take till disease	*Bacillus* sp.	*Triticum aestivum*	Renwick et al. (1991)
Take till disease	*Pseudomonas* sp.	*Triticum aestivum*	Renwick et al. (1991)
Take till disease	*Penicillium* sp.	*Triticum aestivum*	Renwick et al. (1991)
Take till disease	*Beauveria* sp.	*Triticum aestivum*	Renwick et al. (1991)
Take till disease	*Rhodococcus* sp.	*Triticum aestivum*	Renwick et al. (1991)
Fusarium wilt	*Pseudomonas* sp.	*Dianthus caryophyllus*	Van Peer et al. (1991)
Rhizoctonia solani	*P. cepacia*	*Gossypium hirsutum*	Fridlender et al. (1993)
Pythium ultimum	*P. cepacia*	*Cucumis sativus*	Fridlender et al. (1993)
Bacterial wilt	*P. putida*	*Cucumis sativus*	Kloepper et al. (1993)
Bacterial angular	*P. putida*	*Cucumis sativus*	Kloepper et al. (1993)
Bacterial angular	*F. oryzihabitans*	*Cucumis sativus*	Kloepper et al. (1993)
Cucumber antracnose	*P. putida*	*Cucumis sativus*	Wei et al. (1996)
Cucumber mosaic virus	*P. putida*	*Cucumis sativus*	Raupach et al. (1996)
Striped cucumber beetle	*P. putida*	*Cucumis sativus*	Zehnder et al. (1997)
Striped cucumber beetle	*F. oryzihabitans*	*Cucumis sativus*	Zehnder et al. (1997)
Rice sheath blight	*P. fluorescens*	*Oryza sativa*	Sung and Chung (1997)
Helocoverpa armigera	*P. gladioloi*	*Gossypium hirsutum*	Quingwen et al. (1998)
Rice sheath blight	*P. fluorescens*	*Oryza sativa*	Nandakumar (1998)
Rhizoctonia solani (sheath blight pathogen)	*P. fluorescens*	*Oryza sativa*	Vidhayasekaran and Muthamilan (1999)
Aspergillus sp.	*Pseudomonas* sp.	*Vigna radiata*	Sindhu et al. (1999)
Fusarium oxysporum	*Pseudomonas* sp.	*Vigna radiata*	Sindhu et al. (1999)
Rhizoctonia solani	*Pseudomonas* sp.	*Vigna radiata*	Sindhu et al. (1999)
Blue mold	*P. fluorescens*	*Oryza sativa*	Zhang et al. (2002)
Blue mold	*A. pasteurii*	*Oryza sativa*	Zhang et al. (2002)
Myzus persicae	*B. subtilis*	*Piper nigrum*	Kokalis-Burelle et al. (2002)
Rhizoctonia bataticola	*Pseudomonas* sp.	*Arachis hypogaea*	Gupta et al. (2002a, b)
Cotton aphids	*Bacillus* sp.	*Cucumis sativus*	Stout et al. (2002)
Acyrthosiphon kondoi	*Pseudomonas* sp.	*Trifolium repens*	Kempster et al. (2002)
Blue mold	*Bacillus pumilus*	*Nicotiana tabacum*	Zhang et al. (2003)
Blue mold	*S. marcescens*	*Nicotiana tabacum*	Zhang et al. (2003)
Myzus persicae	*B. licheniformis*	*Piper nigrum*	Lucas et al. (2004)
Fungal disease	*P. polymyxa*	*Sesamum indicum*	Ryu et al. (2006)
Fusarium avenaceum	*Enterobacter* sp.	*Cicer arietinum*	Hynes et al. (2008)
Rhizosphere fungi	*A. brasilense*	*Prunus cerasifera* L.	Russo et al. (2008)

Table 5.6 Commercial products developed using different PGPR strains

PGPR	Crop	Products
Agrobacterium radiobacter	Fruit, nut, ornamental nursery stock, and trees	Diegall, Galltrol-A, Nogall, Norbac 84 C
Azospirillum brasilense	Turf and forage crops	Azo-Green
Bacillus subtilis	Barley, beans, cotton, legumes peanut, pea, rice, and soybean	Epic, HiStick N/T, Kodiak, Rhizo-Plus, Serenade, Subtilex
B. amyloliquefaciens GB99	Broccoli, cabbage, cantaloupe, cauliflower, celery, cucumber, lettuce, ornamentals, peppers, tomato, and watermelon	Quantum 4000
Burlkholderia cepacia	Alfalfa, barley, beans, clover, cotton, maize, peas, sorghum, vegetables, and wheat	Blue Circle, Deny, Intercept
Pseudomonas fluorescens	Almond, apple, cherry, mushroom, peach, pear, potato, strawberry, and tomato	BlightBan A506, Conquer, Victus
P. syringae	Citrus and pome fruit	Bio-save10
Streptomyces griseovirdis K61	Field, ornamental, and vegetable crops	Mycostop

Table 5.7 PGPR species as biotic elicitors to elicit plant response

Induced metabolite	Plant	PGPR species	References
Ajmalicine	*Madagascar periwinkle*	*P. fluorescens*	Jaleel et al. (2007)
Picrocrocin	*Autumn crocus*	*B. subtilis*	Sharaf-Eldin et al. (2008)
Crocetin	*Autumn crocus*	*B. subtilis*	Sharaf-Eldin et al. (2008)
Safranal	*Autumn crocus*	*B. subtilis*	Sharaf-Eldin et al. (2008)
Serpentine	*Madagascar periwinkle*	*P. fluorescens*	Jaleel et al. (2009)
Hyoscyamine	*Black henbane*	*P. fluorescens* and *P. putida*	Ghorbanpour et al. (2010)
Scopolamine	*Black henbane*	*P. fluorescens* and *P. putida*	Ghorbanpour et al. (2010)
Tanshinone	*Red sage*	*B. cereus*	Zhao et al. (2010)

5.2 Rhizosphere: A Habitation for Typical Plant-Soil-Microbe Communications

Rhizosphere is defined as a confined area sandwiched between soil and roots functioning as an intricating ecosystem on Earth, comprising an integral plant root network, soil, and a wide range of microbial consortium containing bacteria, archaea, viruses, and microeukaryotes, for example, fungi, oomycetes, protozoa, nematodes, algae, and arthropods (Jones and Hinsinger 2008; Buee et al. 2009; Hinsinger et al.

2009). Based upon the complexity in networking of plants, soil, and microbes, the rhizosphere is differentiated into three zones: (i) endorhizosphere, location of the root cortex as well as endodermis over which microbes and mineral ions instigate into apoplastic space between cells; (ii) rhizoplane, inner zone present between the epidermal cells and mucilage; and (iii) ectorhizosphere, zone present on the outskirts extended from the rhizoplane to the bulk soil (McNear 2013).

The existing microflora in the rhizosphere completes their nutritional demand by feeding on plant metabolites/organic compounds released by roots (also known as rhizodeposition) (Hartmann et al. 2009; Dessaux et al. 2016) and plant debris. Rhizospheric microbial communities are members of complex food web utilizing a huge amount of plant-released nutrients, affecting the carbon flow and transformation (Raaijmakers et al. 2009). According to the reports, it has been reported that some part of photosynthetically fixed carbon (20–40%) is proportionate to the underground root system (Jones et al. 2009; Dessaux et al. 2016). Hence, the rhizospheric microbiota partially or completely affects the biomass productivity of natural plant communities. Although various microbial population lives in soil were having good plant growth promoting characterstics and are mutualistic to each other (Hooper et al. 2005; Van der Heijden et al. 2008; Lau and Lennon 2011; Wagg et al. 2011). Some other microorganisms of the rhizosphere are useful in plant growth, whereas some of them could be pathogenic (Mendes et al. 2013; Dessaux et al. 2016). Cook et al. (1995) stated that plants have the ability to manipulate the rhizospheric microbiota in a way to benefit by choosing precisely stimulating microorganisms exhibiting useful traits in plant physiology and growth. Similarly, Wagg et al. (2011) explained that belowground diversity participates in looking after plant productivity in adverse conditions. As they are sensitive to changes in abiotic conditions such as environmental stress and disquiets, rhizospheric microbes are utilized as bioindicators in soil quality. Thus, acquiring the need to safeguard the structural and functional practices of the rhizosphere will help in protecting plant-microbe interaction and similar rhizospheric activities as a method to improve and enhance plant ecosystem productivity and responses toward high-stress conditions which could include climatic changes due to mitigating effect formulated for lifelong soil carbon storage and environmental disruptions.

5.3 Rhizobacteria: Beneficial, Deleterious, or Neutral?

As per the above descriptions, the rhizospheric regions formulate a favorable habitat for the multiplication and metabolic activity of various microorganisms, because of a variety of plant discharges like amino acid, sugar, and growth factors, provident of energy and nutrient to the microorganism (Gray and Smith 2005). This has been a noticeable trait for a wide range of bacteria (named as rhizobacteria) colonizing the habitat (Schroth and Hancock 1982) for about 4–10% of the total root area, predominantly at the root tip and hair region. In the rhizospheric soil, the bacterial population ranges between 10^7 and 10^9 CFU/gram (Benizri et al. 2001; Compant et al. 2010), which is 100 times more than that in bulk soil (Weller and Thomashow

1994). Rhizobacteria usually belonging to the genera *Azotobacter*, *Agrobacterium*, *Arthrobacter*, *Alcaligenes*, *Bacillus, Cellulomonas*, *Mycobacterium*, *Flavobacter*, *Micrococcus,* and *Pseudomonas* are present, whereas very few aerobic bacteria are present because of less oxygen content due to root respiration.

On an average, positive, negative, and neutral types of interactions are observed between the plant and rhizobacteria (Whipps 2001; Dobbelaere et al. 2003; Beneduzi et al. 2012). The negative interaction states about the phytotoxic substances like C_2H_4 (ethylene) and HCN (hydrogen cyanide) secreted by rhizobacteria demolishing the growth and physiology of the plant. A large amount of rhizobacteria are in a commensal relationship with the plant, therefore building a neutral relationship with the plant host, thereby depicting no visible effect on plant physiology and growth. On the contrary, some of the microbial strains function in a way that they form a positive effect by establishing a direct or an indirect effect on the host plant by invading the root system. These are commonly termed PGPR (Kloepper et al. 1978, 1980a, b, 1989). Apart from vegetative growth elevation, PGPRs colonize the rhizosphere, root surface, and root tissues (Gray and Smith 2005; Beneduzi et al. 2012). It is evident in the literature that only 2% or less than 2% rhizobacteria enforce plant growth in the rhizosphere (Antoun and Kloepper 2001; Beneduzi et al. 2012). Gram-negative, rod-shaped rhizobacteria possess lower proportions and functions like Gram-positive cocci, rods, and pleomorphic. Different genera bacteria have been explored, and out of which *Pseudomonas* and *Bacillus* have turned out to be the most predominant ones (Podile and Kishore 2006). A brief discussion about PGPRs has been enlisted below.

5.4 Plant Growth-Promoting Rhizobacteria (PGPRs): Definition, Origin, and Introduction

They were first well-defined by Kloepper and Schroth (1978) to explain about the soil microbes that are intended to inhabit the plant root area succeeded by the seed inoculation to promote plant growth. Allochthonous or autochthonous PGPR initially colonizes onto the seed surface very quickly and later shows a quick response to chemically viable photosynthates produced by plant genotype in/around the root/soil surfaces (Frankenberger and Arshad 1995). To obtain successful colonization, a certain amount of major and minor soil supplements are provided such as NPK, BNF, PSM, and K fertilizers (Khan et al. 2013).

5.5 Rhizosphere and Rhizoplane Colonization

The reserach findings related to the colonization of beneficial bacteria in the rhizosphere were reported in the early 1990s. The detection of gfp- or gudA-labeled strains by fluorescence in situ hybridization or immunomarkers is secured using microscopic tools under gnotobiotic conditions. Furthermore, it has been found that bacteria colonize on soil inoculation (Gamalero et al. 2003). Later these bacteria are

observed as single cells which start adhering on root surfaces and multiply themselves, forming bacterial chains on the rhizodermis (Hansen et al. 1997). Colony formation could take place on the rhizodermal surface, and bacteria starts forming biofilms or microcolonies (Benizri et al. 2001). The in vitro rhizoplane study is not only conducted in matured plants but also on plants growing in normal soil, classified as high microbial diversity. It is important to note that both in gnotobiotic systems and natural soil, the root parts are not colonized in a systematic manner. Root zones offer diverse populace densities. *P. fluorescens* (A6RI strain) in association with tomato roots, constituting varied density and distribution according to root zone, has been well-defined by Gamalero et al. (2004). Various factors explain the nonuniform bacterial colonization, for example, bacterial quorum-sensing effects, root exudation pattern, and many more.

5.6 Chemotaxis Toward Root Exudates

Root exudation is dependent upon rhizoplane and rhizospheric colonization (Lugtenberg and Dekkers 1999). In photosynthesis, carbon fixation is translocated through the root zone system (Bais et al. 2006). Diverse types of amino acids, carbon source, and other constituents that are available to provide nutrients to bacteria adhere to the roots in the rhizospheric region (Walker et al. 2003). The microbes are attracted toward chemicals and move in the direction where exudate is present; this leads to microbe colonization, and they colonize both the rhizoplane and rhizosphere regions (Lugtenberg and Kamilova 2009). A mutant strain of *P. fluorescens* lacks the cheA gene which is responsible for chemotaxis hence lowers down the movement in the direction of root exudate (or toward specific exudate components) in the tomato rhizosphere and declines the colony formation in the root (de Weert et al. 2002). The colonization process is influenced by the difference in root exudate composition (Lugtenberg et al. 2001). Pleasant and repellent compounds show differences which hinder microbial colonization (reviewed in Bais et al. 2006) affecting microbial gene expression. The process of exudation is said to be heterogeneous in nature. Exudates accumulate in high concentration in some root spaces than others. Root exudation during massive exudation take place at the tips (Grayston et al. 1996). Just because of the varied exudation patterns, better colonization is observed at some specific sites (Kraffczyk et al. 1984; Paterson and Sim 2000; Gamalero et al. 2004). This suggests that in several root areas and at different development stages, unique rhizobacterial communities could maintain interaction with selected hosts (Rudrappa et al. 2008). Lately, it has been found that the plant may choose selective rhizosphere colonizers through root exudation when any of their organs gets infected by a plant pathogen. When *A. thaliana* was infected with *P. syringae* an elevated concentration of malic acid was observed in the rhizosphere . *B. subtilis* is attracted by malic acid which colonized at the rhizosphere of the same plant and resulted in the formation of biofilm which protected the roots via aggression from plant pathogen (Rudrappa et al. 2008). This investigation explains the role of plant and in particular the microbial community which get attracted to root mucilage (Knee et al. 2001).

5.7 Endophytic Colonization

Few microbes present on the rhizosphere prevents colonization of other microbes persisting in the rhizosphere and additionally the rhizoplane, yet they can permeate themselves in plants and colonize themselves inside tissues and show plant development advancing impacts (Hallmann 2001; Sessitsch et al. 2004; Compant et al. 2005, 2008; Hallmann and Berg 2007). Various recent studies approve that plants accommodate different endophytic populations (Idris et al. 2004; Krechel et al. 2004; Berg et al. 2005) and that endophytic microbes generally derive from the rhizosphere (Sessitsch et al. 2002; Compant et al. 2005; Hardoim et al. 2008). Endophytes express to a subgroup of the rhizobacterial systems, which can enter the endorhiza of their hosts after the rhizoplane is colonized (Gray and Smith 2005; Rosenblueth and Martínez-Romero 2006; Hallmann and Berg 2007). It has been reviewed that endophytes probably indicates plant development advancing impacts than microbes specifically colonizing only the rhizosphere (Conn et al. 1997; Chanway et al. 2000). The entrance procedure does not really include dynamic components, and accordingly, all rhizosphere microscopic organisms can be relied upon to be inside the roots at one phase of their life (Hardoim et al. 2008). Passive infiltration occurs at the ruptured area; for example, this happens at root rise destinations or made by harmful microbes and also at root tips (Reinhold-Hurek and Hurek 1998). For a particular microscopic organism, specific adjustments have been developed, for example, for nodulating microorganisms or organisms, which have particular instruments for dynamic infiltration of the root framework (inspected in Hardoim et al. 2008). In few plant-rhizobia interactions, for example, in the beneficial interaction between the semi-oceanic vegetable *Azorhizobium caulinodans* and *Sesbania rostrata* (Goormachtig et al. 2004), intrusion takes place through crevices in cortical intercellular disrupted passage and the horizontal root base. In other rhizobia-nodulating vegetables, colonization takes place inside shaggy roots as they enter root fleshy tissues, and henceforth concentrated organs are produced by the plant, known as knobs (Garg and Geetanjali 2007). As of now, it is known to be interceded by chemotaxis in the direction of flavonoid exudates and by bacterial flags; for example, gesture factors are required for the advantageous way of life of knob-shaping microorganisms. Flagella, jerking motility, lipopolysaccharides, and pili have been seen to influence bacterial versatility and endophytic colonization inside the host (Duijff et al. 1997; Dörr et al. 1998; Böhm et al. 2007). Even the emission of cell-divider debasing compounds (CWDEs) is engaged with microbial infiltration (Lodewyckx et al. 2002) and diffusing inside the plant. Dynamic or latent instruments have been used for translocation procedures of endophytic-microscopic organisms in the interior of the plant and have enabled them to advance in the direction of rhizoplane toward the root cortex. In spite of the fact that not being examined much of the time, it is notable that endophytes might disperse inside the plant and inhabit inside leaves or stems (Hardoim et al. 2008), where they can multiply and achieve populace densities of about 103–104 CFU g^{-1} of crisp mass under communal conditions (Hallmann 2001). A couple of concentrates revealed that some endophytic microorganisms colonize blossoms, products of the

soil (Hallmann 2001). In any case, under normal conditions, the larger part of blossoms does not contain endophytic microbes at all (Hallmann 2001). It is a perspective that just specific endophytes can colonize and make do in regenerative plant organs. A few strains having a place with *Pseudomonas* or potentially *Bacillus* and additionally to other genera, which likewise indicate plant development advancing capacities, were noted and detached from the inside of blossoms, and foods are grown from the grapevine ground (Compant et al. unpub. results). Hardly any species were disengaged from sanitized rice seeds (Okunishi et al. 2005). Strains having a place with *Rahnella* and *Pseudomonas* genera were also isolated from Norway spruce (Cankar et al. 2005) besides seeds of lupine (Barac et al. 2004) and also from different plants, giving rough data about the microbes-colonizing plant regenerative tissues.

5.8 Different Forms of PGPR

There are two principal types of PGPR, (I) ePGPR and (II) iPGPR (Viveros et al. 2010). ePGPR occupy the rhizosphere over the rhizoplane or in the voids present within the cells of the root cortex while iPGPR inhabit the nodular structures over the root cells. Microbial population belonging to ePGPR includes *Arthrobacter, Caulobacter, Azotobacter, Azospirillum, Bacillus, Burkholderia, Agrobacterium, Erwinia, Pseudomonas, Micrococcus,* etc., whereas endophytic microbes having a place with iPGPR incorporate *Allorhizobium, Mesorhizobium*, and *Rhizobium*, and also *Frankia* species, which can fix air N_2 particularly for vascular plants (Bhattacharyya and Jha 2012).

5.9 Mechanisms Employed by PGPR

5.9.1 Direct Mechanisms

PGPR utilization induces the development of plants by increasing the availability of nitrogen or natural minerals or by adding solubilizing minerals and phytohormones (Bhardwaj et al. 2014). By this approach, plant development can be directly influenced. Increase in the individual particles transition at the site of PGPR in root surface can coordinately improve the mineral uptake.

5.9.1.1 Nitrogen Fixation

Nitrogen (N) acts as a crucial supplement for the development and efficiency of plants. In spite of 78% atmospheric N_2, it is not accessible for the plant growth. Thus, atmospheric N_2 is initially converted to its functional form. Nitrogen-settling microorganisms utilize nitrogenase enzyme for the conversion of nitrogen into smelling salt (Kim and Rees 1994). These microorganisms include members of the *Rhizobiaceae* family which positively interact with rhizobia plants (such as leguminous), nonleguminous trees (e.g., *Frankia*), and independent endophytes, for example, cyanobacteria like *Azotobacter, Azocarus, Azospirillum,* and *G. diazotrophicus*

and more (Zahran 2001; Ahemad and Khan 2012d; Bhattacharyya and Jha 2012). Similarly, nonharmonious N_2-fixing microorganisms contributed less for nitrogen conversion as required by bacterially associated host plants (Glick 2012). Advantageous N_2-fixing microbes affect the member of the *Rhizobiaceae* family and build up cooperative association with the underlying foundations of leguminous plants. An unpredictable transaction among host and symbiont leads to advantageous interaction (Giordano and Hirsch 2004) which forms development knobs in which rhizobia inhabit itself as an intracellular symbiont.

5.9.1.2 Phosphate Solubilization

Phosphorus (P) is another critical plant supplement next to nitrogen, which is inexhaustibly available within soils (Khan et al. 2009). Irrespective of the massive availability of P, the measure of available P is very low in plants. Low availability of phosphorous is its insoluble nature, still plants acquire them in two dissolvable forms, i.e. H_2PO_4 and HPO_4^{2-} (Bhattacharyya and Jha 2012). The insoluble forms of P are available as an inorganic mineral like apatite, inositol phosphate, phosphomonoesters, and phosphotriesters (Glick 2012). Bacterial genera belonging to genus *Bacillus, Azotobacter, Erwinia, Burkholderia, Microbacterium, Enterobacter, Flavobacterium, Pseudomonas, Serratia, Rhizobium,* etc. are reliable phosphate solubilizers (Bhattacharyya and Jha 2012).

5.9.1.3 Potassium Solubilization

The third macronutrient important for plant growth is potassium (K). About 90% of potassium is present in an insoluble form or as silicate minerals, which contribute to low availability of dissolvable potassium within the soil (Parmar and Sindhu 2013). Further, potassium deficiency causes inadequate roots formation, low seed generation, moderate development rate, and a lower yield (Kumar and Dubey 2012). PGPRs like *Acidithiobacillus* sp., *B. edaphicus, B. mucilaginous, Ferrooxidans* sp., *Paenibacillus* sp., and *Pseudomonas* sp. have been found to be effective for potassium solubilization (by producing natural acids) from minerals containing potassium (Liu et al. 2012). The use of PGPRs, which is capable of potassium solubilization as a biofertilizer shall enhance the agribusiness (Setiawati and Mutmainnah 2016).

5.9.1.4 Phytohormone Production

The microbial activities with regard to the production of phytohormones like auxin (indole-3-acidic corrosive/indole acidic corrosive/IAA) has been very less explored. Eighty percent of microbes isolated from the rhizospheres have the ability to release and mix auxin as voluntary metabolite (Patten and Glick 1996). IAA triggers cell division in the plant, increases the rate of root formation and xylem, stimulates seed germination, regulates vegetative development, begins oblique and extrinsic root formation, and influences photosynthesis, color composition, biosynthesis of different metabolites, and protection from stress conditions. The release of IAA by rhizobacteria induces plant developmental procedures, which alter the plant IAA pool (Spaepen et al. 2007; Glick 2012). Bacterial-released IAA builds around root external surface, increases root length, and subsequently gives the plant more prominent

access to soil minerals. Similarly, rhizobacterial-liberated IAA also relaxes the division of plant cell and hence boosts a release of root exudation which gives extra minerals (Glick 2012). Subsequently, rhizobacterial-liberated IAA is considered to be an effective element in plant-microbe collaborations, for both phytostimulation and pathogenesis (Spaepen and Vanderleyden 2011).

5.9.1.5 Siderophore Production

Iron is an imperative supplement for all life forms. All microorganisms are known up to this point, except for specific lactobacilli, to basically require Fe (Neilands 1995). In natural conditions, iron is present as Fe^{3+} and probably forms insoluble hydroxides and oxyhydroxides, which cannot be uptaken in plants and microbial usage in insoluble form (Rajkumar et al. 2010). Usually, microorganisms obtain iron through the discharge of iron chelators described as siderophores, those who have a high affinity for iron complexes. Rhizobacteria forms a complex of Fe^{3+} and siderophore over the bacterial surface which is further converted into Fe^{2+} which gets permeable inside the cell (Neilands 1995; Rajkumar et al. 2010). Thus, siderophores play the role of the iron-solubilizing agent from minerals or natural blend under iron-stressed conditions (Indiragandhi et al. 2008). Siderophores additionally enclose stable edifices along with other metals, like Al, Cd, Ga, In, Pb, and Zn, plus radionuclides such as U and Np (Kiss and Farkas 1998; Neubauer et al. 2000; Rajkumar et al. 2010). Subsequently, microbial siderophores aids in relieving anxiety induced by plants due to the high concentration of metals in the soil. Plants acclimatize iron via bacterial siderophores through various unique mechanisms such as chelation and arrival of iron, or by the rapid uptake of Fe-siderophore complex (Schmidt 1999).

5.9.1.6 Exopolysaccharide Production

EPSs are assumed to maintain the water potential, fuse soil entities, and establish contact among rhizobacteria and plant roots, supporting the host during pathogenesis or stress condition induced due to saline soil, dry climate, and waterlogging (Pawar et al. 2016). *A. vinelandii, Agrobacterium* sp.*, B. drentensis, E. cloacae, R. leguminosarum, Rhizobium* sp., and *Xanthomonas* sp. are few EPS-producing PGPRs which play a role in soil ripeness-manageable horticulture (Mahmood et al. 2016).

5.9.1.7 Rhizoremediation

Removal of metals via phytoextract obtained from plants from debased soil and their remediation is stated as phytoremediation (Hamzah et al. 2016). Cooperative and nonharmonious interaction between plants and microbes, which are clarified by PGPRs, makes it an exceptional candidate for rhizoremediation. Presently, PGPR for rhizoremediation is confined to a couple of microbial, animal categories, for example, *P. aeruginosa*, *P. fluorescens*, and few *Bacillus* sp. (Kuiper et al. 2004). Further investigation on PGPR as bioremediators is needed for high removal of important metals or different pollutants from water and soil.

5.9.2 Indirect Mechanisms

Suppressive components in association with PGPR reduce the impression of plant pathogens by producing repressive substances that control the barrier induced on the host plant (Singh and Jha 2015). This can be considered as a procedure which helps plants to sustain itself under abiotic push or protects plants from contaminations that induce biotic pressure (Akhgar et al. 2014). The PGPR along with these suppressive components ensures the formation of hydrolytic chemicals (chitinases, cellulases, proteases, etc.), various antitoxins produced against plant pathogen or disease, utilization of deliberate opposition against different pathogens and irritations, and generation of siderophores, VOCs, EPSs, and so on (Gupta et al. 2014; Nivya 2015).

5.9.2.1 Stress Management

5.9.2.1.1 Abiotic Stress Tolerance

The vital abiotic stress that restricts plant efficiency and development is aridity push which is established by a dry spell, saltiness, and high temperature (Vejan et al. 2016). Since the bacterial strains, for example, *Pseudomonas putida* and *Pseudomonas fluorescens* have the ability to absorb cadmium from soil and can kill the hazardous effect of contamination of cadmium on grain plants, they can help to manage abiotic stress using PGPR (Baharlouei et al. 2011). Moreover, the effect of PGPR can be an increase in the water availability in leaf, especially under saline and abiotic pressure conditions (Ahmad et al. 2013; Naveed et al. 2014). The basis for the association between PGPR and dry season hindrance has been stated in a few yields, including chickpea, wheat, and soybean (Ngumbi and Kloepper 2016). Habib et al. (2016) reported that PGPR raises saltiness push resilience in okra via ROS-searching chemicals and improves the effectiveness of water usage.

5.9.2.1.2 Biotic Stress Tolerance

Biotic pressure results in a serious decline in agricultural yield and is triggered by various pathogens, for example, microorganisms, infections, organisms, nematodes, protists, creepy crawlies, and viroids (Haggag et al. 2015). PGPRs such as *B. amyloliquefaciens, B. licheniformis, B. subtilis, B. thuringiensis, P. favisporus*, and *P. polymyxa* can be utilized to understand such issues. Plants show great protection against different types of biotic pressure which are immunized by splashing their basic fundamentals or seeds medium term in cultures of PGPR (Ngumbi and Kloepper 2016).

5.9.2.2 Disease Resistance Antibiosis

The alternate for multiple pesticides can be the use of microbial antagonists against plant pathogens in rural yields. PGPR restricts virulent microorganisms through anti-infection agent delivery, similar to *Bacillus* spp. and *Pseudomonas* sp. Over the last two decades, the resistance of plant pathogens through the production of

anti-infection agents by PGPR has been the best and most considered biocontrol system (Ulloa-Ogaz et al. 2015). Largely, *Pseudomonas* sp. generates numerous antifungal, antibacterial, antitumor, and antiviral agents (Karalicine) (Ramadan et al. 2016).

5.9.2.3 Induced Systemic Resistance

Induced systematic resistance (ISR) is defined as a physical condition of enhanced protective limit induced because of a detailed regular advancement. PGPR instigates fundamental opposition in numerous plants against a few ecological constraints (Prathap and Ranjitha 2015). Guard components are enacted, and signs are directed by means of the vascular framework amid pathogenic attack which results in the actuation of a large number of safeguard compounds, for example, APX, CAT, chitinase, lipoxygenase, peroxidase, phenylalanine alkali lyase, polyphenol oxidase, and SOD along with certain proteinase repressor. ISR is not pathogen-definite; however it controls several plant infections (Kamal et al. 2014). ISR contains ethylene growth hormone monitoring in plants and induces protection responses against diverse phytopathogens. A number of bacterial spp. initiate ISR, like cyclic lipopeptides, siderophores, lipopolysaccharides, and volatiles similar to acetoin and 2,3-butanediol (Berendsen et al. 2015). In spite of the fact that most of the PGPR triggers ISR in plants, employing of PGPR could change agroindustry. Dynamic research using PGPR in current practices and systems will aid in the effective transfer of plants from in vitro conditions to the field, which is missing till date.

5.9.2.4 Production of Protective Enzymes

The plant development is enhanced by PGPR through the production of metabolites that control the machinery of plant pathogens (Meena et al. 2016). PGPR produces substances like ACC-deaminase, β-1,3-glucanase, and chitinase, which are mostly linked with lysing cell dividers as well as killing pathogens (Goswami et al. 2016). The parasitic cell divider parts are chitin and β-1,4-N-acetyl-glucoseamine; therefore, β-1,3-glucanase- and chitinase-delivering microscopic organisms regulate their growth. *P. fluorescens* and *S. fredii* deliver chitinase and beta-glucanases leads to *Fusarium* wither by *F. oxysporum* as well as *F. udum* (Ramadan et al. 2016). PGPR also represses the growth of *P. capsici* and *R. solani*, which are devastating phytopathogens (Islam et al. 2016).

5.9.2.5 Production of VOCs

The biocontrol strain generates VOCs that enhance plant development, restrict parasitic pathogens plus nematodes, and promote vital obstruction against phytopathogens (Raza et al. 2016a, b). The VOCs generated by specific microbial species belonging to different genera like *Arthrobacter*, *Bacillus*, *Pseudomonas*, *Serratia*, and *Stenotrophomonas* affect plant development. *Bacillus* spp. that deliver 2,3-butanediol and acetoin that restricts pathogen development as well as enhances plant development are considered as the best VOCs (Santoro et al. 2016).

5.10 Selection of PGPR

In light of the method of activity depicted above for PGPR, a few bacterial characteristics can be utilized to choose a competitor PGPR strain confined within the rhizosphere of a few plant-animal categories. The correct system by which PGPR advance plant development in various yields and under various natural conditions is not completely seen; however, it is ending up obvious that a few or all the plant development-advancing qualities do not work autonomously of one another yet additively (Ahemad and Kibret 2014). The most broad end that can be drawn from the above model is that to separate successfuly PGPRs, it is smarter to break down the dirt attributes where the plants will be developed and the particular prerequisites of the specific yield and afterward to recognize bacterial characteristics that may be gainful to these specific conditions (Ipek et al. 2014).

5.11 PGPR Inoculant Development and Production

Over-the-top utilization of composts has indicated a negative effect on yield profitability, soil and water defilement, edit powerlessness to illnesses, and eventually misfortune in the economy (Savci 2012; Cristina et al. 2013). To address such major issues, the approach of biofertilizer including both transporter-based and fluid biofertilizers (Pindi and Satyanarayana 2012) has given arrangements and have demonstrated promising outcomes (Bhardwaj et al. 2014). With respect to biofertilizer, India is one of the imperative nations in biofertilizer creation and utilization (Pindi and Satyanarayana 2012). The normal utilization in the nation is around 45,000 ton for each annum, while its creation is not exactly the half of utilization. The most extreme creation limit lies in Agro-Industries Corporation (AIC) pursued by State Agriculture Departments, National Biofertilizer Development Centers, State Agricultural colleges, and private divisions. The innovation used to deliver biofertilizer is in any case moderately new and advancing. In spite of ideal extension, there are sure issues in the creation of biofertilizers. These requirements incorporate (i) emergency of proficient PGPR strains: it has been discovered that the strains chosen for inoculants creation ought to be locale particular and sufficiently focused to build up in host soils and have the capacity to colonize plant roots adequately. Nonetheless, distinguishing reasonable PGPR strains for inoculant creation is truly troublesome because of their shifting capacities; (ii) nonaccessibility and shorter time span of usability of appropriate bearers (Ngampimol and Kunathigan 2008); (iii) variable resilience among PGPR toward the eccentric and indeterminate harvest fields temperature, odds of sullying, and poor security of the biofertilizer; and (iv) conceivable genotypic changes: amid biofertilizer generation, there are chances that particularly chosen life forms may connect with undesired living beings and subsequently may prompt changes in the essential character of creatures. Additionally, there is plausibility that amid maturation, the chosen PGPR strains may experience

changes prompting adjusted adequacy and practicality. This thus may result in sparing misfortune and expanded expense of generation. Regardless of communicating various critical attributes, the PGPR definitions have not been well known among agriculturists (Jangid et al. 2012). What's more, subsequently, is biofertilizers have not been embraced at a bigger scale. There are a few reasons why biofertilizers are not all that well known among ranchers. Major among them is the absence of mindfulness among the end-users (ranchers). Communication gap among agriculturists and producers and miscommunication about the quality and maintainability of biofertilizers are the other real obstacles in promoting the utilization of biofertilizers. A study by Srinivas and Bhalekar (2013) uncovered that about 85% respondents had no certainty towards biofertilizer practices, while half of the 85% respondents announced that lack of knowledge about biofertilizers was a reason for less use of this innovation. In this manner, with the end goal to make full-utilization of biofertilizers and to contend with manufactured manures, it is required to reliably create awareness among agriculturists by sorting out different network programs (Revellin et al. 2001). Low supply of biofertilizers to remote regions, moderate activity of biofertilizers, and accessibility of low-quality PGPR inocula in trade, are other significant issues in the promotion and selection of biofertilizers. Convincingly, the absence of comprehension and fears among ranchers about low yield and productivity are the real setback in the adoption of biofertilizer program.

5.12 Beneficial Aspects of PGPR

The microbes named as PGPR dwelling under dirt condition could initiate sensational transformation in plant development by generating development regulators and additionally enhancing nourishment to plant by providing and encouraging supplement take-up from soil (Zahir et al. 2004). What's more, a substantial quantity of these rhizobacterial strains can likewise enhance plant resilience against saltiness, dry spell, flooding, and substantial metal poisonous quality and, in this manner, empower plants to make due under negative ecological surroundings (Mayak et al. 2004; Nadeem et al. 2007; Zahir et al. 2008; Sandhya et al. 2009; Glick 2010; Ma et al. 2011c). Albeit different free-living soil microorganisms are believed as plant enhancers advancing rhizobacteria, every single bacterial strain of a specific variety does not have indistinguishable metabolic abilities for enhancing plant development to a similar degree (Gamalero et al. 2009). The two noteworthy routes through which PGPR can encourage plant development and improvement incorporate immediate and roundabout systems (Glick et al. 1995). Aberrant development advancement happens when PGPRs counteract or lessen a portion of the destructive impacts of plant pathogens by at least one of the few unique systems (Glick and Bashan 1997). These incorporated pathogens impart hindrance by generating unpleasant substances or by expanding the impediment of the host-plant in contradiction to pathogenic creatures (Nehl et al. 1996; Persello Cartieaux et al. 2003). For example, PGPR delivers secondary metabolites which decrease pathogen populace as well as create siderophores that diminish the iron accessibility for specific

pathogens in this way causing lessened plant development (Arora et al. 2001; Bhattacharyya and Jha 2012). Thus, PGPR can likewise build a plant barrier against maladies by evolving host-plant weakness, via an instrument termed instigated foundational opposition and along these lines give assurance against pathogen assault (Saravanakumar et al. 2007). Coordinate development advancement happens in various ways like giving valuable mixes to the host-plant associated with bacteria or potentially encouraging the uptake of supplements from the dirt condition (Kloepper et al. 1987). Further, it encourages the development of a plant by settling air nitrogen and siderophores discharge which solubilizes and sequester, subsequently escalating its availability for plant uptake, creating solubilizing minerals and phytohormones, for example, phosphorus, in order to build its accessibility (Kloepper et al. 1989; Glick et al. 1995; Patten and Glick 2002). Irrespective of these constituents, PGPR may likewise advance plant growth because of key compounds (ACC-deaminase, chitinase) and moreover by creating constituents like exopolysaccharides, rhizobitoxine, and so on that support plants to endure under stress conditions (Ashraf et al. 2004; Glick et al. 2007; Sandhya et al. 2009). Rhizobitoxine is an inhibitor of C_2H_4 amalgamation which promotes nodulation by weakening the negative effect of high C_2H_4 fixation (Vijayan et al. 2013). The adequacy of PGPR for advancing plant development additionally relies on the connection with host plant and soil condition other than their characteristic capacities.

5.13 Role of Plant Growth-Promoting Rhizobacteria for Plant Growth Enhancement

PGPR assumes a critical job in improving plant development via a wide range of components. The method that advances PGPR activity which further increases the plant growth includes:

(a) abiotic-stress resistance in plants;
(b) availability of supplements;
(c) plant growth regulators;
(d) siderophores production;
(e) development of unstable natural mixes; and
(f) insurance protein generation like ACC- deaminase, chitinase, and glucanase to avoid the plant diseases.

5.14 Conclusion

PGPR plays a critical role in enhancing plant growth, and it also maintains as well as remediates the degraded and contaminated wastelands, eutrophies water bodies, regulates the nitrogen and phosphorus runoff, and controls the pesticide pollution. The utilization of modern techniques and tools will enable us to enhance the ability of PGPR which can play a crucial role in sustainable agriculture by improving crop

productivity, soil fertility, and plant tolerance and maintaining a controlled nutrient cycle. Further studies are focusing on selecting suitable rhizosphere microbes and producing microbial communities plus searching for the opportunity of multidisciplinary research that combines multiple fields of science like agrobiotechnology, biotechnology, chemical engineering, material science, and nanotechnology. This interdisciplinary approach will help us to develop ecological and biological functional techniques which can provide new products of immense potential.

In the coming time, PGPR is supposed to replace the artificial-growth regulators, chemical fertilizers, and pesticides which impose various adverse effects on sustainable agriculture. Innovative research and deep insight of mechanism of PGPR-associated phytostimulation would enable us to find the way to isolate or develop a competent rhizobacterial strain which could sustain itself in varied agroecological conditions.

PGPR plays a functional role in context to biocontrol, biofertilizer, and bioremediation, all of which exhibit a positive effect on crop productivity and ecosystem functioning, and promotes its use in agriculture. With the advancing technology and research, utilization of PGPR will become a reality and will be of great help in the crucial process which ensures the stability as well as productivity of agroecosystems, hence guiding us on the road to an ideal agricultural system.

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Microbes for Bioremediation of Heavy Metals

6

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Abstract

Heavy metal pollution is expanding its arms to every nook and corner of this living world, thereby swamping our ecosystem with heavy metals that prove to be hazardous for plants, animals, and humans. One of the most common, eco-friendly strategies that can be employed to counter this problem effectively is bioremediation for alleviating the stress of heavy metal contamination. To implement this strategy, exploration and identification of heavy metal resistance microbes is need of the hour.

Keywords

Heavy metals · Bacteria · Bioremediation · Eco-friendly approach

6.1 Introduction

Heavy metals (e.g., As, Cd, Hg, Ni, Cr, Zn, etc.) are the group of metals whose atomic density is greater than 5 g/cm3. These are entered in the environment through various anthropogenic sources like activities of industries, mining, metal smelting, waste disposal, corrosion of metals in use, petroleum exploration, and agriculture

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activities (Ahemad 2012). The expulsion of effluents containing these heavy metals affects the environment and consequently causes several health hazards to animals, humans, and plants. Metal pollution of the environment by industrial and mining activities has resulted in worldwide pollution of soils, water, and air. Consumption of such contaminated water is extremely hazardous as these metals have carcinogenic and mutagenic effects. Further, these metals influence the growth and metabolic activities of microorganisms and thus eventually decrease their diversity (Roane and Pepper 1999; Qing et al. 2007). However, some microbes are able to survive and develop several resistance mechanisms against the toxic concentration of these heavy metals (Navarro et al. 2013; Neeta et al. 2016).

6.2 Sources of Heavy Metals

The increasing concentrations of metals in environment also include contributions from a wide range of industrial and domestic sources of contaminants. Their pollution has mainly occurred due to natural geological processes and anthropogenic activities. Some of the metals (e.g., As, Cd, Cu, Hg, Ni, Pb, and Zn) are deposited in the environment through geogenic processes or wastes discharged from industrial processes such as mining, smelting, metal forging, alkaline storage battery manufacturing, and burning of fossil fuels (Bradl 2005; Naja and Volesky 2009). Furthermore, the agricultural practices like the use of pesticides, phosphatic fertilizers, various agrochemicals, and sewage sludges used for irrigation purposes have also increased the concentration of these metals in soil and water. However, major sources of the heavy metals are coal-based thermal power plants and integrated iron and steel industries. Besides this, there are various other sources of metal contamination in environment like through soil erosion and natural weathering of the Earth's crust (Morais et al. 2012). Leachate pollution is also an emerging source of heavy and toxic metals in soil which percolate and lead to the leaching of pollutants into water and soil (Tiwari et al. 2015). The natural water sources are normally polluted by these discharges ultimately posing a great risk for aquatic ecosystems.

Among heavy metals, arsenic (As) is a metalloid with a complex chemistry, which naturally occurs in more than 245 different mineral forms including arsenates, sulfides, sulfosalts, arsenides, arsenites, oxides, silicates, and elemental arsenic (Mandal and Suzuki 2002). Arsenic trioxide (As_2O_3) is the most important commercial compound that is produced as a by-product of smelting industries of copper and lead ores. A low amount of arsenite is reduced to elemental arsenic during the manufacturing of semiconductors, components of lasers, colors of digital watches, alloys, microwave circuits, glasses, and light-emitting diodes (Ratnaike 2003). Weathering of rocks alters arsenic sulfides to arsenic trioxide, which get entry in the arsenic cycle as dust or by dissolution in rain, rivers, or groundwater (Mandal and Suzuki 2002). However, organic form of As enters in the environment as a result of biological activities.

Further, heavy metal cadmium occurs in the Earth's crust at a concentration of 0.1–0.5 ppm and is geologically associated with other heavy metals, viz., Zn, Pb,

and Cu ores (Morrow 2010). Its contamination in water, soil, and air has been occurring particularly in industrial and mining activity areas. Nonferrous metal mining and refining, coal and fossil fuel burning, use of phosphatic fertilizers, and waste incineration and disposal are usually the main anthropogenic reasons for Cd contamination in the environment. In mining areas, coal contains significant amounts of cadmium which are mostly deposited in the form of flue dust in environment. Surface soil contamination due to cadmium depends on various factors. Mobility, natural geochemistry, and magnitude of this heavy metal through the usage of fertilizers and atmospheric deposition majorly lead to its contamination. Natural emissions of cadmium to the environment can result from volcanic eruptions, forest fires, generation of salt aerosols, or other natural phenomena. However, Pan et al. (2010) estimated that more than 90% of cadmium in the surface environment is the consequences of industrial and agricultural process (Grant and Sheppard 2008; Roberts 2014). The use of municipal sewage sludge for agricultural purposes can enhance cadmium source. In the mining and industrial affected areas, house dust is also potentially a cause of cadmium exposure (Hogervorst et al. 2007). However various factors relating to soils, plants, and presence of other trace elements including Ca, Zn, Cu, Fe, Mn, Mo, and Se affect Cd availability (Lane et al. 2015). It is also used in silver–cadmium batteries, photography and television phosphors, and coating operations (Naja and Volesky 2009). Further, tobacco smoke is also one of the largest single sources of cadmium exposure in humans (Faroon et al. 2012).

Similarly, the metallic mercury is a naturally occurring metal which is a shiny silver-white, odorless liquid. It becomes colorless and odorless gas when heated. It belongs to heavy metals which are also toxic to living beings. Mercury exists in three forms, i.e., metallic elements, inorganic salts, and organic compounds. Each form has different toxicity level and bioavailability. Major sources of its pollution include anthropogenic activities such as agriculture, municipal wastewater discharges, mining, incineration, and discharges of industrial wastewater (Chen et al. 2012). Gold mining could produce waste, which contains mercury and causes mercury pollution.

Another most important heavy metal is lead (Pb). It is a highly toxic heavy metal whose widespread use has caused severe environmental contamination and health problems in numerous parts of the world. There are heavy deposits of coal and minerals such as pyrite, alumina, and dolomite in central parts of India. There are several thermal power plants and heavy industries such as steel, aluminum, and cement plants. These heavy metal industries increased the deposition of Pb and other metals in the environment. Further, chromium (Cr) is a naturally occurring metal present in the Earth's crust, with oxidation states ranging from Cr (II) to Cr (VI). Among various industries, tanneries are the main contributors of soil and water contamination with Cr and other toxic heavy metals (Tariq et al. 2008; Rajkumar et al. 2012; Reichman 2014). The Cr concentration in the soils may vary according to the natural composition of rocks and sediments). In soil, it may increase mainly through anthropogenic deposition, as, for example, atmospheric deposition (Rosas et al. 1989), also dumping of chromium-bearing liquids and solid wastes as chromium by-products, ferrochromium slag, or chromium plating baths (Kimbrough et al. 1999).

6.3 Effect of Heavy Metals on Environment and Health

In the world, millions of people from different countries are mostly dependent on groundwater for drinking purposes, but groundwaters are contaminated with elevated level of heavy metals. Heavy metal toxicity creates significant ecological, evolutionary, nutritional, and environmental problems (Benavides et al. 2005; Nagajyoti et al. 2010; Jaishankar et al. 2014). The toxicity depends upon the rapt dose and the route and duration of exposure. It can either be acute or chronic (Jaishankar et al. 2014). Further, short exposure of heavy metals can damage the functions of brain, lungs, kidney, liver, and other important organs, while long-term exposure causes variety of adverse health effects in humans such as dermal changes, respiratory, pulmonary, cardiovascular, hematological, neurological, developmental, reproductive, gastrointestinal, and carcinogenic effects (Mandal and Suzuki 2002; Ratnaike 2003). In plants, heavy metals affects shoot and root growth, while preventing homeostasis and nutrient uptake in it. (Asati et al. 2016). Both direct and indirect toxicity of heavy metals lead to a decline in plant growth which sometimes results in the death of plant (Chibuike and Obiora 2014). In plants, the use of arsenate-containing irrigation water reduces plant height, decreases yield, and affects development of root growth (Abedin et al. 2002).

Cadmium causes a wide range of organ toxicity due to its long half-life for elimination (Järup and Åkesson 2009). Various forms of cadmium like cadmium oxide, cadmium sulfate, and cadmium sulfide have high potential risk for carcinogenicity. The low concentration of cadmium from smoking is highly toxic to humans, as cadmium is absorbed more efficiently by the lungs than from the gastrointestinal tract (Eugenio 2008). Cadmium persuades changes at the biochemical, physical, and genetic levels in the plants and reduces plant growth. The effect of Cd toxicity can cause inhibition of growth processes and decrease of photosynthetic apparatus activity of plants (Gallego et al. 2012). It inhibits plant growth parameters including shoot and root lengths, number of leaves, and biomass and water and nutrient uptake (Alia et al. 2015). Furthermore, it also reduces the rate of new cells formations which ultimately results in plant death.

6.4 Bioremediation of Heavy Metals

Bioremediation is one of the most effective management tools for elimination of environmental hazards like toxic heavy metals. It is also an alternative that offers the possibility to destroy or render harmless by-products from various contaminants including heavy metals using natural biological activity. Bioremediation that involves the capabilities of microorganisms in the removal of pollutants is the most promising, relatively efficient, and cost-effective technology (Rajendran et al. 2003; Megharaj et al. 2011, Kulshreshtha et al. 2014; Ojuederie and Babalola 2017). It uses living organisms mainly including bacteria, fungi, or yeast to clean up polluted soil and water (Coelho et al. 2015; Gupta et al. 2016). Microbial approaches of bioremediation ensure more effective cleanup of polluted environment (Moghannem

et al. 2015). Bacteria being the most crucial microorganisms are frequently being used in the remediation of heavy metal-contaminated soils (Chen et al. 2015). The introduction of indigenous bacterium or bacterial consortium can provide a potential bioremediation process of heavy metal-affected soil and water without disturbing the target environment (Kang et al. 2016). The exceptional adaptation abilities and auspicious remediation efficiencies of endophytic bacteria could be useful for developing efficient heavy metal removal system (Guo et al. 2010). The application of bacterial mixtures could also be a greater resistance and efficiency for the bioremediation of heavy metals compared with single strain cultures (Kang et al. 2016). For this, many workers considered the best preference to ensure high treatment efficiency and performance under metal-affected area especially industrial effluents and mining areas (Bestawy et al. 2013). The highly toxic form of heavy metals can be altered to less toxic forms by heavy metal-resistant microorganisms through reactions of their metabolic processes like strategies such as bioaccumulation, bioextraction, biosorption, biotransformation, and rhizofiltration which are engaged for detoxifying the heavy metals by microorganisms (Verma and Sharma 2017). Some microbial cells secrete inorganic metabolic products in the form of sulfide, carbonate, or phosphate ions due to their respiratory metabolism. Thus, they help in precipitation of toxic metal ions in the form of nonenzymatic detoxification mechanisms.

The strategy for bioremediation of heavy metals mainly depends on the active metabolizing capabilities of microbial cells. Several bacteria require different amounts of heavy metals as primary and essential micronutrients for their growth and development. Interactions between microbial cells and metal ion can be active and passive based on the metabolism. The particularity of heavy metals lies with the lower metal concentration being promotional for microbial growth, however, high concentration being detrimental to the integrity of cell membrane, cell organelles, and its genetic material (Sengor et al. 2009). More importantly the intracellular metal accumulation causes interference with nutrient uptake processes, electron transport chain and/or the proton gradient force, and inhibition at DNA, RNA, and protein level (Maier et al. 2009) leading to altered protein stability and folding processes which resulted in protein aggregation (Jomova and Valko 2011; Lemire et al. 2013; Tamas et al. 2014). Further, the microbial systems get metals necessary for its metabolism and also counteract the ill effects of toxic metals to protect the cell by using a whole repertoire of mechanisms and to adapt themselves according to the immediate surrounding environmental conditions (Silver 1998; Sar et al. 2013; Girma 2015). Furthermore, in order to survive under metal toxicity condition, microbes have developed several mechanisms like metal exclusion through permeability barriers, active efflux pumps (Teitzel and Persek 2003), enzymatic conversion, volatilization, and bioprecipitation (Nies 1999; Zubair et al. 2016). In addition, bioremediation technologies have a potential to contribute in an eco-friendly manner by applying microorganisms as biosorbents for water, food, soil, and waste remediation (Bayat and Sari 2010; Monachese et al. 2012; García-García et al. 2016; Hansda et al. 2016). Current status of bioremediation process includes biostimulation and bioaugmentation approaches guided by specific microbes to

overcome the drawback lying with phytoremediation technique due to its slow and inadequate method of clearing the contaminated site (Ma et al. 2011).

In bioremediation, As metal-resistant bacteria, for example, are used to remove arsenic from the contaminated environments (Kumar et al. 2019). Several methods have been used to clean up and detoxify the As-polluted environment, but most of them are costly and difficult to get optimum results. Bacterial arsenic detoxification is an important event of interest in environmental bioremediation. This method is low in cost and environmentally friendly in comparison to other methods (Clausen 2000; Srinath et al. 2002; Tsuruta 2004). However, these bacteria are also capable in speedily oxidizing arsenite to arsenate or vice versa and are omnipresent in arsenic-contaminated groundwater and soil (Shakya and Pradhan 2009; Liao et al. 2011). For example, Dey et al. (2016) reported some gram-positive bacteria which were able to remove 51.99% and 53.29% of arsenite and arsenate from arsenic amended media, respectively. Similarly, Shakoori et al. (2010) reported that *K. oxytoca*, *C.* freundii, and *B. anthracis* showed high ability to reduce As(V) into As(III) 78%, 70%, and 84%, respectively. These bacterial strains can be exploited for bioremediation of arsenic from wastes (Bachate et al. 2009; Chang et al. 2012). Some arsenic-resistant bacteria having bioremediation potential are *Bacillus* spp., *Pseudomonas* spp., *Escherichia coli*, *Flavobacterium* spp., *Klebsiella* sp., *Enterobacter* spp., *Staphylococcus* spp., *Alcaligenes* spp., *Aeromonas* spp., *Microbacterium* sp. and *Acinetobacter* sp. (Anderson and Cook 2004; Abou-Shanab et al. 2007; Sultana et al. 2011; Anyanwu and Ugwu 2010) (Table 6.1).

Similarly cadmium is a toxic heavy metal that has a severe hazardous effect on living beings and their environment. Several processes have been used to remediate cadmium pollution from contaminated environment. Several bacteria use various mechanisms for survival in cadmium-contaminated sites that mainly include metal ion sequestration, efflux system, metal-binding proteins, and use of enzymatic conversion into nontoxic forms. Huang et al. (2014) investigated that accumulation of Cd by *Bacillus cereus* was due to extracellular biosorption. However, Sinha and Mukherjee (2009) revealed in their findings that membrane and periplasm can also help as a major accumulating site of cadmium in *Pseudomonas aeruginosa* (Pérez et al. 2015). In this connection, cadmium bioaccumulation ability are seen in *Alcaligenes*, *Pseudomonas* spp., *Enterobacter* sp., *Escherichia coli*, *Comamonas*, *Staphylococcus*, *Proteus* sp. *Gluconobacter* spp., *Bacillus* spp., *Lactobacillus. s*pp., *Ralstonia* spp., *Lactobacillus plantarum*, *Serratia* spp., *Klebsiella* spp., *Rhodotorula s*p., and *Stenotrophomonas* sp. (Sabdono 2011; Amoozegar et al. 2012).

Many metal-resistant genes like arsC, cadB, chrA, copAB, NiCoT, merA, czcA, and pbrA have been reported in bacterial systems for arsenic, cadmium, chromium, copper, nickel, mercury, and lead respectively. The phosphate efflux for Cu resistance was shown in the acidophilic bacterium *Acidithiobacillus ferrooxidans*, whose cells showed an increased exopolyphosphatase activity (Alvarez and Jerez 2004). Further, antioxidant system in response to heavy metal also acts as a good resistance mechanism against bacteria such as *Anabaena* (Singh et al. 2012; Panda et al. 2017). During heavy metal toxicity, bacterial cell evolve with unique sequence of genes which have been acquired, recombined, and rearranged from a wide range of

Table 6.1 List of some important metal-resistant bacteria isolated from different sources with their removal capacity (studies conducted after 2000)

S.N.	Bacterial strains	Metal	Metal removal capacity (%)	References
1.	*Micrococcus roseus*	**Arsenic**	85.61%	Shakya et al. (2012)
2.	*Pseudomonas stutzeri* ASP3		82.97% of As (V)	Shakya and Pradhan (2009)
3.	*Bacillus anthracis*		84%	Shakoori et al. (2010)
4.	*Exiguobacterium* sp.		99%	Pandey and Bhatt (2015)
5.	*Bacillus megaterium*		92%	Ghodsi et al. (2011)
6.	*Brevibacillus reuszeri Rhodococcus* sp.		96.67% 94.17%	Neeratanaphan et al. (2016)
7.	*Pseudomonas* spp.		78% of As(V)	Jebelli et al. (2017)
8.	*Bacillus megaterium*		93%	Miyatake and Hayashi (2009)
9.	*Exiguobacterium* sp.	**Cadmium**	99%	
10.	*Caulobacter crescentus*		99%	Patel et al. (2010)
11.	*Klebsiella pneumoniae*		82%	Shamim and Rehman (2012)
12.	*Pseudomonas aeruginosa*		94.7%	Jabbari et al. (2010)
13.	*Stenotrophomonas maltophilia*		80%	Chien et al. (2007)
14.	*Lysinibacillus fusiformis*		92.3%	Mathivanan and Rajaram (2014)
15.	*Pseudomonas aeruginosa*		89%	Sinha and Mukherjee (2009)
16.	*Pseudomonas* sp. W6	Lead	61.2%	Kalita and Joshi (2017)
17.	*B. longum* 46		55%	Halttunen et al. (2007)
18.	*Bacillus cereus*		85.4%	Murthy et al. (2012)
19.	*Vibrio fluvialis*	Hg	60%	Saranya et al. (2017)
20.	*P. putida*		90%	Okino et al. (2000)

sources. An example of such alterations is the genome of *Cupriavidus metallidurans CH34*, which inhabits a wide range of environments containing high concentrations of toxic metals (Janssen et al. 2010; Nies 2016). In *Ralstonia metallidurans* and *Cupriavidus metallidurans* CH34, the czc gene cluster is responsible for cadmium resistance.

Further, some more indigenous microbial genera has to be explored for their application in heavy metal bioremediation using molecular intervention. There are several complexities involved in the conventional methods for heavy metal remediation of soil and water, and the application of microbial species or consortium has arisen as a time-saver for bioremediation. Future research should focus on the issues involved in improving bioremediation approaches using genetically modified/engineered microorganisms (GEM) in all the stress conditions developed due to heavy metal pollutions.

6.5 Our Lead

Since more than two decades, our group is pursuing a lot of studies related to bioremediation of heavy metals (Goel et al. 2017; Dash et al. 2019). In case of microbial bioremediation of arsenic, it was observed that the presence of an almost similar mechanism of metal resistance in the two bacterial isolates from two different sources may be due to horizontal gene transfer from soil to water system and vice versa which is an alarming situation for global concern. Our group had also worked on heavy metal-resistant mutants of *Pseudomonas* sp. having PGPR properties. Similarly we isolated a lead- and cadmium-resistant *Pseudomonas putida KNP9* with PGPR activity. We had also reported some rhizobacteria for declination of copper and cadmium toxicity in soil and plant system.

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Plant Growth-Promoting Endophytic Bacteria and Their Potential to Improve Agricultural Crop Yields

7

Anurag Yadav and Kusum Yadav

Abstract

Plant-associated bacteria are known to inhabit rhizosphere (*Rhizobacteria*), phyllosphere (e*piphytes*) and endosphere (*endophytes*). The action of bacterial endophytes residing in plant tissues remained unexplored due to culturing difficulties and lack of advanced identification techniques. Endophytes shield the plant from root pathogen attack by producing biofilm around roots. Rhizobia are perhaps the best example of plant-associated endobacteria as they facilitate N uptake in plants through *Rhizobium*-legume symbiosis. With certain physiological differences, several species of *Rhizobium* remain present in legume plants like alfalfa, clover and pea. In this chapter, if not otherwise stated, the 'endophytes' are mentioned with reference to endophyte bacteria only.

Keywords

Bacterial endophytes · Plant growth promotion · Bioremediation

7.1 Introduction

Plant-associated bacteria are known to inhabit rhizosphere (*Rhizobacteria*), phyllosphere (e*piphytes*) and endosphere (*endophytes*). However, they only thrive abundantly in the rhizosphere due to nutrient-enriched plant root exudation in the region. The rhizosphere bacteria capable of entering plant tissues are called endophytes. Endophytes reside at least some part of their life cycle inside any plant part and do

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not produce disease symptoms (Azevedo et al. 2000). Although the abundance of microorganisms in the rhizosphere is known since the beginning of the twentieth century, the endosphere was considered sterile for a long time. The earlier discovered endophytes were only from the fungal groups. As a result, our initial knowledge of endophytes remained restricted to fungi (Tervet and Hollis 1948). The action of bacterial endophytes residing in plant tissues remained unexplored due to culturing difficulties and lack of advanced identification techniques. From the past two decades, the endophytes have received considerable attention when their potential of host protection against insects-pests and pathogens was recognized along with plant growth-promoting properties. Endophytes associate with most of the plant species and seem ubiquitous in plant tissues. They have been isolated from flowers, fruits, leaves, stems, roots and seeds of various plant species (Kobayashi and Palumbo 2000). The holistic impression of endosphere projects the view that the region teems with bacteria, fungi, actinomycetes and archaea (Hardoim et al. 2015). Endophytes live in the host microenvironment and remain protected from environmental stress, face lesser competition and gain higher access for nutrients (Dutta et al. 2014). The influence of endophytes on plants may be stronger than of rhizosphere microflora due to the direct nature of interaction. Endophytes multiply in plant apoplast, enriched with nutrients like calcium, carbohydrates, chlorine, phosphorous, potassium, sulphur (Canny and McCully 1988; Madore and Webb 1981), several amino acids and organic acids (Canny and Huang 1993). Endophytes benefit plants either directly by stimulating growth or indirectly by decreasing disease incidences. Endophytes improve plant growth and survival by conferring host resistance against pests and drought and by improving host N assimilation to yield higher seed set (Fescue 1990). Endophytes shield plants from phytopathogen attack by producing biofilm around roots (Rybakova et al. 2015). Rhizobia are perhaps the best example of plant-associated endobacteria as they facilitate N uptake in plants through *Rhizobium*-legume symbiosis. With certain physiological differences, several species of *Rhizobium* were isolated from legume plants like alfalfa (Stajković et al. 2009), clover (Sturz et al. 1998) and pea (Saini et al. 2015). In this chapter, if not otherwise stated, the 'endophytes' are mentioned with reference to endophyte bacteria only. The Table 7.1 lists bacterial endophytes isolated from various plants in several studies.

7.2 Beneficial Endophytic Bacteria

Endophytes benefit plant through direct or indirect mechanisms. However, the exact mechanisms of endophyte-mediated growth promotion are mostly unknown (Hardoim et al. 2008). Since most endophytes gain entry in plants as rhizobacteria, it is presumed that they may retain their traits inside the host. The endophyte-mediated benefit to plants seems similar to rhizobacterial functioning as most of the endobacteria survive in the rhizosphere and are easily culturable. Several taxa of endobacteria isolated from plants like sweet corn and cotton are in fact the common soil bacteria (McInroy and Kloepper 1994).

Table 7.1 List of bacterial endophytes isolated from plants

	Plant species	Plant part	Identified bacterial endophyte	References
1.	Walnut (*Juglans regia*)	Mature fruits	*Bacillus subtilis* HB1310	Zhang et al. (2014)
2.	Scotspine (*Pinus sylvestris*)	Ectomycorrhizal roots	Genera of *Pseudomonas*, *Burkholderia,* and *Bacillus*	Izumi et al. (2006)
3.	Rice (*Oryza sativa*)	Roots	*Stenotrophomonas maltophilia* RR-10	Zhu et al. (2012)
4.	Rice (*Oryza sativa*)	Leaves, stems, and roots	*B. aryabhattai, B. megaterium, B. subtilis, Klebsiella pneumoniae, Paenibacillus kribbensis, Microbacterium binotii, Microbacterium trichotecenolyticum*	Ji et al. (2014)
5.	Xaxim (*Dicksonia sellowiana*)	Fern pinnae and rachis	*Amphibacillus* sp., *B. megaterium*, *B. pumilus* *B. subtilis*, *B. thuringiensis*, *Gracilibacillus* sp., *Micrococcus* sp., *Paenibacillus* sp., *Stenotrophomonas maltophilia*, *S. nitroreducens*	Barros et al. (2010)
6.	Blue agave (*Agave tequilana*)	Leaves	*Acinetobacter* sp., *A. baumanii, A. bereziniae, Cronobacter sakazakii, Enterobacter hormaechei, Bacillus* sp., *Klebsiella oxytoca, Pseudomonas* sp., *Enterococcus casseliflavus, Leuconostoc mesenteroides* subsp. *mesenteroides, Gluconobacter oxydans*	Martínez-Rodríguez et al. (2014)
7.	Common bean (*Phaseolus vulgaris*)	Leaves	*Acinetobacter radioresistens, Acinetobacter* sp., *Agromyces mediolanus, Agromyces* sp., *B. amyloliquefaciens, B. bataviensis, B. muralis, B. subtilis, B. thuringiensis, B. niacini, Bacillus* sp., *Brevibacillus agri, Brevundimonas vesicularis, Delftia tsuruhatensis, Dietzia cinnamea, Enterobacter asburiae, E. hormaechei, Frigoribacterium faeni, Kocuria palustris, Lysinibacillus sphaericus, Microbacterium foliorum, M. phyllosphaerae, M. testaceum, Microbacterium* sp., *Methylobacterium populi, Micrococcus luteus, Paenibacillus cineris, P. lautus, Paenibacillus* sp., *Pseudomonas aeruginosa, Rhizobium larrymoorei, Rhodococcus erythropolis, Staphylococcus caprae, S. epidermidis, S. kloosii, S. sanguinis, S. warneri, S. saprophyticus, Staphylococcus* sp., *Sphingobacterium multivorum, Sphingomonas dokdonensis, Sporosarcina aquimarina, Sporosarcina* sp., *Stenotrophomonas maltophilia, Stenotrophomonas* sp.	Costa et al. (2012)

(continued)

Table 7.1 (continued)

	Plant species	Plant part	Identified bacterial endophyte	References
8.	Lebanon oak (*Quercus libani*)	Leaves, stems, and roots	*B. firmus, Pseudomonas protegens*, *Stenotrophomonas maltophilia*	Tashi-Oshnoei et al. (2017)
9.	Brant's oak (*Quercus brantii*)	Leaves, stems, and roots	*Pseudomonas protegens*, *S. maltophilia*	Tashi-Oshnoei et al. (2017)
10.	Greater celandine (*Chelidonium majus*)	Stems	*B. thuringiensis, B. amyloliquefaciens*	Goryluk et al. (2009)
11.	Cotton (*Gossypium hirsutum* L.)	Stems and roots	*Enterobacter* sp.	Tian et al. (2017)
12.	Tomato (*Solanum lycopersicum*)	N/A	*Bacillus* sp., *Burkholderia* sp. *Enterobacter* sp., *Pseudomonas* sp., *Rhizobium* sp., *Staphylococcus* sp., *Stenotrophomonas* sp.	Patel and Archana (2017)
13.	Poaceae family (maize, wheat, pearl millet, sorghum, and rice)	Roots	*Achromobacter* sp., *Acinetobacter* sp., *Ralstonia* sp., *Rhizobium* sp.	(Patel and Archana 2017)
14.	Wheat (*Triticum aestivum*)	Roots	*Azorhizobium* sp.	Webster et al. (1997)
15.	Grapevine (*Vitis vinifera*)	N\A[a]	*Enterobacter* sp., *Pseudomonas* sp., *Pantoea* sp., *Klebsiella* sp., *Staphylococcus* sp., *Clavibacter* sp., *Bacillus* sp., *Curtobacterium* sp., *Xanthomonas* sp., *Rhodococcus* sp.	Bell et al. (1995)
16.	Sugar beet (*Beta vulgaris*)	Roots	*Bacillus* sp., *Erwinia* sp., *Pseudomonas* sp., *Corynebacterium* sp., *Lactobacillus* sp., *Xanthomonas* sp.	Jacobs et al. (1985)
17.	*Aquilaria beccariana, A. crassna, A. hirta, A. malaccensis, A. microcarpa, A. sinensis, and A. subintegra*	Stem and roots	*Acinetobacter radioresistens, B. altitudinis, B. anthracis, B. arbutinivorans, B. arsenicus, B. aryabhattai, B. cereus, B. licheniformis, B. megaterium, B. methylotrophicus, B. pumilus B. stratosphericus, B. subtilis, B. tequilensis, Pantoea agglomerans* niv, *Rahnella aquatilis, Roseomonas mucosa, Vibrio cholera*	Bhore et al. (2013)

[a]Grapevine xylem sap was used

Several endophytes are quite beneficial to plants and hold certain metabolically useful traits. Many endophyte strains produce bioactive secondary metabolites like alkaloids, benzopyranones, chinones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones, xanthones, etc. Endophyte-based metabolites are used in developing agrochemicals, antibiotics, immunosuppressants, antiparasitics, antioxidants and anticancer drugs. Certain plants also produce useful secondary metabolites. The wide-scale use of such plants could manage long-term site protection of plants from phytopathogens and environmental contaminants. The involvement of plant secondary metabolites that could stimulate microbial degradation of pollutants may open an avenue for the development of suitable technologies. Such technologies could help in remediating contamination-exposed sites.

Obtaining bacteria with desirable traits need active screening from plant sources. Isolation of novel endophytes demand screening from plants growing under extreme environment. In addition, novel endophyte screening needs focus on multiple traits. A novel entophyte should hold the following traits for agricultural use. The endophyte (1) must not cause plant disease, (2) should multiply rapidly and easily spread inside the apoplast, (3) should be culturable and (4) must spontaneously and obligately colonize plant parts with host specificity (Bacon and Hinton 2006).

Some of the direct and indirect mechanisms of endophyte action on plants are discussed below.

7.2.1 Directly Beneficial Mechanisms of Endophytes

Endophytes aid plant growth by producing antimicrobial metabolites (Castillo et al. 2003; Ding et al. 2011; Pinheiro et al. 2013), insecticidal by-products (Azevedo et al. 2000) and iron chelators (Long et al. 2008). They also solubilize insoluble phosphates and possess N-fixing abilities (James 2000; Knoth et al. 2014; Krause et al. 2006; Lee et al. 2000; Meneses et al. 2011; Oliveira et al. 2000; Santi et al. 2013; Song et al. 1998). Additionally, sulphur-oxidizing endophytes are known to oxidize elemental sulphur to sulphate for plant use (Banerjee and Yesmin 2009). Also, endophytes are a prolific source of phytochemicals (Nisa et al. 2015) useful in reducing plant pathogen attack (Benhamou et al. 1998; Chen et al. 2011). Endophytes are a chief source of bioactive metabolites (Brader et al. 2014; Schulz et al. 2002) and contribute to plant metabolism (Brader et al. 2014).

Some of the recognized direct mechanisms of endophyte-mediated plant benefit are discussed below.

7.2.1.1 Phytohormone Production

Endophyte bacterial phytohormone-mediated plant growth promotion is a well-recognized method that changes the morphology and structure of plants. These traits render endophytes as the best option for agricultural applications (Hallmann et al. 1997; Sturz et al. 2000). Endophytes enhance legume crop yield by producing indole acetic acid (Khan et al. 2014; Patel and Patel 2014), gibberellic acid (Khan et al. 2014), ethylene (Kang et al. 2012; Long et al. 2010; Straub et al. 2013) and

auxins (Dutta et al. 2014). Like rhizobacteria, endophytes produce phytohormones through similar mechanisms. For example, root ethylene signalling by the endophyte *Herbaspirillum frisingense* GSF30 (T) causes *Miscanthus sinensis* growth promotion (Straub et al. 2013). Similarly, auxins induce rapid growth in plants by triggering cell elongation, division and differentiation (Taghavi et al. 2009). Endophytes could aid plant growth by producing phytohormones and siderophores. In addition, they induce systemic tolerance through 1-aminocyclopropane-1-carboxylate (ACC) deaminase production and antagonize phytopathogens.

7.2.1.2 Nitrogen Fixation

Nitrogen is one of the most vital macro elements of plant but limitedly present in soil. As plants are incapable of reducing atmospheric N, they require its supply as nutrition. In general agricultural practices, chemical N fertilizers provide nitrogenous nutrition to plants. Chemical fertilizers are often costly and associated with environmental hazards. Endophyte-mediated biological N fixation is a greener substitute of chemical fertilizer. Henceforth, several symbiotic prokaryotic endophytes with N-fixing ability have potential in agriculture. Diazotrophic endophytes present competitive advantage over their rhizosphere counterparts since they receive better environmental protection in the endosphere and reduced oxygen partial pressure in plant tissues, which favours efficient N fixation. It is known that endophytes can directly transport N to plants. Henceforth, the free-living diazotrophic endophyte bacteria are the focus of prime research from few decades (Boddey et al. 1991; Dobereiner and Pedrosa 1987; Reis et al. 2004). A classic and well-studied *Rhizobium* genus endophyte is still under study. Research is underway to improve plant N fixation efficiency by altering the rhizobial genome. Research is also ongoing to extend the specificity of *Rhizobium* to nonlegume crops (Fisher and Long 1992).

7.2.1.3 Phosphate Solubilization

Phosphorous is the third most essential macronutrient for plants. It is present in soil as mineral salts or lies incorporated in organic compounds. Due to the sparingly soluble nature, the major portion of soil P remain unavailable to plants (Miller et al. 2010). Certain bacteria that transform insoluble P into the soluble form to make it plant accessible are called phosphate solubilization bacteria (PSB). Rhizosphere bacteria are known to exude organic acids into soil that solubilize phosphate complexes that convert to ortho-phosphates. Phosphate solubilization is one of the common traits of endophytes. For example, the endophyte *Pantoea* sp. from the family Enterobacteriaceae shows P-solubilizing feature (Sulbaran et al. 2009). Literature also supports that bacteria from the genus *Pantoea* are efficient phosphate solubilizers (Rodríguez et al. 2006; Son et al. 2006). Apart from P solubilization, PSBs can facilitate plants in multiple other ways (Vassileva et al. 2010). PSBs help plant growth by improving their nutrient uptake, phytohormone production and by providing protection against phytopathogens (Singh et al. 2010). Obtaining multi-trait phosphate-solubilizing endophyte strains for experiments would require intensive

and rigorous screening from plant hosts. Application of multi-trait P-solubilizing endobacteria with a range of metabolic activities in varied environments could pave path for endosphere tailoring for imparting multiple benefits to plant.

7.2.1.4 Siderophore Production

Siderophores are iron-chelating agents produced by some microorganisms under iron deficiency. During the deficiency of this micronutrient, the siderophore complex provides Fe to plants and deprive the pathogen of it (Compant et al. 2005). Some endophytes produce siderophores like catacholate, hydroxymate and phenolate with biocontrol potential (Rajkumar et al. 2010). A siderophore-producing trait is commonly observed in endophytes because the bacteria face scarcity of free iron ions inside plant tissues (Sessitsch et al. 2004). Furthermore, siderophore after binding to heavy metals could lower their toxic effects. Siderophores indirectly help plants by presenting Fe and Mo factors to endophytic diazotrophs for nitrogenase synthesis and metabolic functioning (Kraepiel et al. 2009). Bacterial siderophores also enhance bioavailability of metals other than iron to induce better plant growth (Rajkumar et al. 2010). Although metal-resistant siderophore binding to heavy metals is common in the rhizosphere and is a crucial step in phytoremediation, nevertheless there is little evidence to support metal tolerance of endophyte bacteria inside plants (Rajkumar et al. 2010).

7.2.1.5 Insecticidal Properties

A plethora of literature supports the insecticidal (Azevedo et al. 2000; Banerjee et al. 2005; Chanway 2002; Liarzi and Ezra 2014; Verma and Gange 2013) and nematicidal (Hallmann et al. 1997) properties of endophytes. For example, the insecticidal activity of the endophytes *Streptomyces albus* and *Claviceps purpurea* has been reported against cotton aphid (*Aphis gossypii* Glover) (Shi et al. 2013). Similarly, several species of *Bacillus* and *Pseudomonas* genus were shown to reduce cotton bollworm incidence (Rajendran et al. 2007). The insecticidal property of endophyte finds applicability as a biocontrol agent. The potential of bacterial endophytes in biocontrol is vast as they colonize the same ecological niche like phytopathogens and therefore impart direct effect in the endosphere (Berg et al. 2005). Endophyte-derived metabolites correspond to varied structural groups like terpenoids, steroids, xanthones, chinones, phenols, isocumarines, benzopyranones, tetralones, cytochalasines and enniatines (Schulz et al. 2002). Biosynthesis of secondary metabolites is vital for endophytes to overcome competition (Schulz et al. 1999). Most of such compounds possess antimicrobial or insecticidal properties. Several endophytes reduce pathogen penetration by inducing the thickening of host endodermal cell wall (Gwinn and Bernard 1993). Few others inactivate insects by producing secondary metabolites. Some of the toxic metabolites secreted by entophytes are the pyrrolopyrazine alkaloid peramine (Ball et al. 2011), ergot alkaloid ergovaline (Siegel et al. 1990) and pyrrolizidine loline alkaloids (Wilkinson et al. 2000).

7.2.2 Indirect Beneficial Mechanisms of Endophytes

Throughout life, plants face several biotic and abiotic stresses that reduce their productivity. Endophytes mitigate plant stresses through several direct and indirect mechanisms. Several bacterial metabolites interact with plant to augment its resistance to pathogens and the process is called induced systemic resistance (ISR). The mechanism of ISR suggests that endophytes evolved from plant pathogens and thus can induce defence responses in plants like phytopathogens.

All such mechanisms are thoroughly discussed below.

7.2.2.1 Bioremediation

Bioremediation refers to the biological methods of removing or breaking down environmental pollutants. Plants secrete several non-neutralizable toxic metabolites. Such metabolites can harm the ecology of surroundings and therefore require bioremediation with the aid of some 'associative bacteria'. The exact mechanism of bioremediation in endobacteria is yet unexplored. However, it is considered that the bioremediation mechanism of endophyte must have similarity with rhizosphere bacterial systems. Endophytes help plant bioremediation through various mechanisms. Within plant tissues, endophytes alleviate heavy metal stress (Zhang et al. 2012) and degrade toxic compounds and metabolites (Han et al. 2011). Outside plant tissues, entophytes eliminate greenhouse gases from air (Stępniewska and Kuźniar 2013) and control pest growth (Azevedo et al. 2000). Microbe-induced bioremediation can be accomplished using several methods. Some newer and cost-effective methods are under development. Better insights into bioremediation are anticipated with improvement in our understanding for microbial metabolism. Advanced knowledge of underlying metabolic process would facilitate alteration of mechanisms through molecular tools to augment bioremediation efficiency.

7.2.2.2 Phytoremediation

Phytoremediation refers to plant-based remediation against environmental and soil pollutants. The concept of phytoremediation is newer in agriculture and seems cheaper than available engineering solutions. This 'greener' and pragmatic approach is receiving wide attention from the scientific community. A better understanding of the plant-endophyte association could aid in remediating barren lands and groundwater. Endophytes could equip plants with required degradation pathways for improved biodegradation and reduced phytotoxicity (Weyens et al. 2009). They can improve phytoremediation and benefit plant by fixing nitrogen, solubilizing minerals, producing phytohormones, producing siderophores, transforming nutrients and administering ACC as the N source (Germaine et al. 2009; Germaine et al. 2006; Rajkumar et al. 2009; Stępniewska and Kuźniar 2013). In addition, endophytes decrease metal toxicity and modify its translocation and accumulation in plants. In an experiment, the inoculation of endophytic bacterium *Serratia nematodiphila* LRE07 alleviated the Cd-induced changes by accumulating more biomass and higher photosynthetic pigment content in leaves of *Solanum nigrum* L. compared with non-symbiotic ones (Wan et al. 2012). Similar results were obtained for the

endophyte *Bacillus* sp. SLS18 on sweet sorghum (Luo et al. 2012). Some plants accumulate toxic end products in tissues, leading to stunted growth (Glick 2003). Some endophytes can neutralize toxic products of plant metabolism and help plant to grow faster.

Rhizosphere bacteria are recognized for their effectiveness in cleaning (Radwan 2009) and remediating polyaromatic hydrocarbons from soil (Olson et al. 2008). Similarly, novel endophytes could be applied to heavy metal-contaminated plants to harness their benefits. Several endobacteria are known to facilitate heavy metal phytoextraction (Rajkumar et al. 2009). Many trials on endobacteria-mediated heavy metal removal from plants have been successfully accomplished. Endophytes also degrade polyaromatic hydrocarbons (PAHs). PAHs are widespread soil contaminants that are often the combinations of low- and high-molecular-weight chemicals. Plants are able to degrade PAHs with the aid of microbes in the rhizosphere and endosphere. Plant-associated bacteria induce catalysis of atmospheric oxygen into aliphatic or aromatic hydrocarbons to produce corresponding alcohols (Radwan 2009). Useful phytoremediating microflora can be isolated from chronically contaminated sites. For example, hydrocarbon-degrading microflora was isolated from halophyte *Halocnemum strobilaceum* native to the coastal areas of the Arabian Gulf (Al-Mailem et al. 2010). However, the screening of potential endophytes from plant host is time consuming. Generally, pure culture isolation is not possible in certain hosts. Commonly, the isolated endobacteria show moderate bioremediation under field conditions. The bioremediation efficiency of underperforming organisms could be improved by genetic modifications. Due to the vast potentials in phytoremediation, the use of genetically engineered endophyte strains in scientific studies is increasing in scientific studies. For example, the bioengineered *P. putida* VM1441 (pNAH7) was found to protect host plant from the phytotoxic effects of naphthalene (Germaine et al. 2009). In another example, genetically modified *Burkholderia cepacia* L.S.2.4 was degrading toluene in plant tissues (Barac et al. 2004).

7.2.2.3 Biocontrol

Biocontrol is an eco-friendly way of protecting crops from phytopathogens using antagonistic microorganisms (Rybakova et al. 2015). Rhizosphere bacteria from genus *Bacillus* and *Pseudomonas* are known biocontrol agents, but most are unable to survive in varied agricultural conditions. Also, rhizosphere bacteria have limited survival abilities in non-native microclimates. Endophytes hold survival advantage over rhizosphere bacteria as they live in the host's protected microenvironment. The antagonistic endophytes are mostly Gram-negative and members of the Pseudomonadaceae family. An entire group of fluorescent pseudomonads is recognized for biocontrol potential. The genus *Pseudomonas* is a preferred biocontrol agent due to the qualities like (1) rapid growth to utilize root exudates, (2) ability to compete aggressively with other microorganisms (by suppressing the growth of other microorganisms through antibiosis, siderophore production and extracellular enzymes production) and (3) quick adaptation to environmental stresses. Members of the genus *Bacillus* are also useful biocontrol agents due to (1) production of varied antimicrobial compounds as secondary metabolite, (2) induction of plant growth

responses and (3) possession of endospore, which equip them to function better under adverse environmental conditions. Generally, native bacterial strains of a specific region are effective biocontrol agents for the local plant hosts than non-native ones. This is due to the survival advantage of native strains over non-native ones in the host microenvironment (Principe et al. 2007). For example, the native isolates of *Bacillus subtilis* were found effective in Egypt against root pathogens of groundnut, namely, *Aspergillus niger* Vantighn and *Fusarium oxysporum* Schlecht (Ziedan 2006).

Endophytes affect plant pathogens directly or indirectly by altering the internnal ecology (Gao et al. 2010). Similar to rhizosphere bacteria, endophytes curtail phytopathogen severity by competing for nutrition in the same ecological niche and by producing chemical agents adverse for plant pathogens. Some endophytes release antibiotics in the endosphere which restrict phytopathogens to the rhizosphere (Bara et al. 2013; Castillo et al. 2003; Franco et al. 2007). Several endophytes produce antibiotics such as coronamycin, ecomycins, kakadumycins, munumbicins, pseudomycins, xiamycins, etc. (Castillo et al. 2003; Christina et al. 2013; Ezra et al. 2004). Endophytic actinobacteria, like rhizosphere actinobacteria, are a notable source of antibiotics. For example, the antibiotic ansamycin is produced from *Streptomyces* sp., an endophyte of the mangrove tree (Xu et al. 2014) and kakadumycins from *Streptomyces* sp., an endophyte of Darwin silky oak (*Grevillea pteridifolia)* (Castillo et al. 2003). Endophytes constitute the chief component of endorhiza, presenting them as an ideal candidate for biological control. A variety of endophytes are antagonistic to fungal pathogens. Endophytes have been reported to reduce *Fusarium* wilt on the plants of banana (Chen et al. 2011), tomato (Benhamou et al. 1998) and capsicum (Sundaramoorthy et al. 2012). A root endophyte, *Pseudomonas fluorescens* PICF7, was reported to hinder pathogenic colonization of *Verticillium dahliae* in olive tissues (Prieto et al. 2009). Similarly, endophyte *Pseudomonas putida* P9 isolated from potato plant suppressed the disease of *Phytophthora infestans* (Andreote et al. 2009).

Endophytes have another mechanism to control phytopathogen entry in the host. During pathogen attack, endophytes direct phytoalexin production in plants to initiate antibiosis by chelating insoluble cations through siderophore production. The endophytes from *Pseudomonas* genus are well recognized for biocontrol against several bacterial and fungal phytopathogens (Andreote et al. 2009; Duijff et al. 1997). A pseudomonad-based siderophore pseudobactin is known to inhibit the growth of *Erwinia cartovora* which causes soft rot in potato (Kloepper et al. 1980). Pyoverdines are another group of pseudomonad-derived siderophore types that has biocontrol properties. Endophytes produce a range of other useful metabolites which are applicable in agrochemical and pharmaceutical sectors. Endophyte-generated flavonoids and flavones are plant-signalling molecules with known antimicrobial properties. These metabolites are produced as a signalling response of microbial adhesion to the root surface (phytoalexins). Endophyte response in the form of such metabolites (Christina et al. 2013) shapes the endosphere microflora. Actinobacteria are also important endophytes in regulating plant growth. Actinobacterial endophytes effectively promote plant growth through nutrient

translocation, phytohormone production, removing soil contaminants, controlling plant pathogens and by inducing plant defence responses (Franco et al. 2007).

Plants are also attacked by several viral phytopathogens. Only limited options are available for controlling plant-associated viral diseases. Due to the noncellular nature of viruses, they are difficult to check through direct measures. Instead, viral phytopathogens are indirectly regulated by targeting the 'pests' involved as vector in the disease (Perring et al. 1999). The concept of endophyte-mediated biological control of viral pathogens is relevant from the current perspective because increasingly indiscriminate use of pesticides for controlling pests and viral pathogens is causing several undesirable effects on plants, causing resistance of pathogens and generating environmental pollution, which is severly impacting human health (Harish et al. 2008b). Involvement of selective endophytes in agriculture can reduce the intensity of viral disease to aid in reducing dependency on chemical pesticides. Inside the host, endophytes reduce viral load on the infected plant by facilitating host response (Gouda et al. 2016).

Following are some known mechanisms of biocontrol:

7.2.2.3.1 Induction of Plant Resistance

Plant defends phytopathogens by secreting signalling molecules in the endosphere. Plants elicit ISR to tackle divergently with associative, pathogenic, neutralistic or symbiotic microorganisms by releasing signalling molecules (Hayat et al. 2010). Evoking of ISR induces increased density of plant cell wall which restricts phytopathogens to the outer layer of the root cortex (Benhamou et al. 1996) by controlling potential pathogen penetration (Benhamou et al. 1998). The plant ISR gets activated by expressing pathogenesis realated proteins (chitinase and β-1,3-glucanase) as well as by inducing defence related and other oxidative enzymes (peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase) (Harish et al. 2009). Association of endophyte elicits ISR in plants, thus reducing disease severity and improving plant stress tolerance (Mei and Flinn 2010). Most of nonpathogenic endophytes actually elicit plant defence like phytopathogens (Schulz and Boyle 2006). Endophytes may also augment plant defence against grazing animals and pests (Clay and Schardl 2002; Hartley and Gange 2009). The cellular response due to signal transduction cascade evokes ISR in plants. In a leading study, it was found that *B. pumilus* SE34 induces ISR in a stepwise fashion, starting from elaboration of structural barriers to producing toxic substances (e.g. phenolics and phytoalexin) and accumulation of molecules (e.g. chitinase) and hydrolytic enzymes (e.g. β-1,3-glucanases), which contribute in releasing oligosaccharides to stimulate other defence reactions (Benhamou et al. 1998). Endobacterial-inhabited *Arabidopsis* plant was studied to elicit ISR against two pathovars of *P. syringae*. The study found that ISR is evoked by salicylic acid/jasmonic acid/ethylene-dependent or ethylene-independent pathway (Harish et al. 2009; Kavino et al. 2007; Kumar et al. 2007; Ryu et al. 2003). Endophytes also evoke ISR in plants to combat viral pathogens. Evoking of ISR in tomato plants by endophytes like *Bacillus subtilis* IN937b, *B. pumilus* SE34 and *B. amyloliquefaciens* IN937a towards cucumber mosaic *Cucumovirus* (CMV) has been demonstrated (Zehnder et al. 2000). Certain endophytes elicit ISR in plants

towards banana bunchy top virus (BBTV) (Harish et al. 2009; Harish et al. 2008a; Kavino et al. 2007; Kumar et al. 2007).

7.2.2.3.1.1 Ecological Niche Occupation

Rhizosphere microflora interacts with one another to form a large community. New invaders or pathogens have to encounter negative effects of interaction from the already established microbial community. Similarly, endophytes protect plants by rapidly colonizing the endosphere, thus limiting the available substrates for pathogens (Pal and Gardener 2006). After penetration, endophytes colonize the inter- and intracellular spaces of host tissues. The multistep process of colonization involves host recognition, penetration and multiplication. The successful colonization ensures endophytic niche establishment for continuous and reliable nutrient supply from host parts and root exudates. However, the endophyte colonization is limited by plant lignin and other cell wall deposits that refrain it from becoming virulent in the endosphere.

7.2.2.3.1.2 Volatile Emissions

Certain endophytes secrete volatile compounds in the endosphere to counter phytopathogen attack. A commonly available and environmentally widespread bacterial genus, *Paenibacillus* is known to inhibit phytopathogens by secreting soluble and volatile metabolites (Rybakova et al. 2015). An experiment on tall fescue (*Festuca arundinacea* Schreb.) demonstrated the production of volatile compound monoterpene β-ocimene from endophyte infection. Moreover, endophyte infection boosts production of monoterpenes such as (E,Z)-allo-ocimene, limonene, linalool, myrcene and other compounds like methyl salicylate, indole and nonanal (Yue et al. 2001). An in situ experiment reported production of volatile organic compounds by endophyte *Nodulisporium* sp. GS4d2II1a that regulates pathogenesis of *Pythium aphanidermatum* (Sánchez-Fernández et al. 2016).

7.2.2.3.1.3 Other Mechanisms

Most endophytes do not antagonize plant invaders with a single mechanism and adopt several strategies. For example, *Trichoderma* hyper-parasitize phytopathogens and secrete chitinases and cellulases upon contact with pathogens (Russo et al. 2012). Subsequent coiling of mycoparasite hyphae around hyphae of pathogens enable the fungus to enzymatically digest the pathogen cell walls. Another example shows that *Pseudomonas pseudoalcaligenes* antagonize *Magnaporthe grisea* (Jha and Subramanian 2011) in the presence of Cu^{++} by activating fusaric acid biosynthetic genes in *Fusarium oxysporum* ZZF51 followed by chelation with Cu^{++} (Pan et al. 2010).

7.2.2.4 Plant Stress Mitigation

Water is the single most important constituent for plant growth and metabolism. In fact, distribution of plant species on land is regulated by water availability. However, over 35% of the world land surface has arid or semiarid environment. Plants living in arid conditions bear steady water stress and survive by developing tolerance.

Knowledge of stress tolerance mechanism of plant cells is a vital prerequisite for developing strategies of crop improvement and survival under adverse conditions. Developing crops that are more tolerant to water deficits and could maintaining crop productivity is a field of worldwide research. Research is also underway to harness the potential of endophytes for alleviating stress in crops.

Several types of abiotic and biotic plant stress, mitigated by endophytes, are discussed below.

7.2.2.4.1 Abiotic Stress Mitigation

Plants cope with a range of stresses by remodelling their metabolism to get tolerant. Plants respond to environmental stress by regulating expression of certain genes. Sometimes such metabolic changes require mediation from other organisms. Some useful endophytic bacteria facilitate plants to adapt towards environmental stress (Quadt-Hallmann et al. 1997). Plant-benefiting endobacteria mitigate host stress from temperature, drought, heavy metal accumulation and solar ultraviolet-B radiation (280–315 nm). Endophytes also alleviate plant cold tolerance by altering photosynthesis and metabolism of carbohydrates, causing accumulation of proline and phenol-based metabolites (Barka et al. 2006; Fernandez et al. 2012). Endophytes show similar effects towards drought stress (Naveed et al. 2014). An endophyte, *Azospirillum lipoferum*, has been reported to mitigate water stress of maize plants by secreting abscisic acid (ABA). It is proposed that ABA signals moderation of stomata closure to reduce water loss (Zhang and Outlaw 2001).

Soil salinity is one of the critical stress types faced by plants. Soil salinity reduces crop yield through root growth inhibition by signalling ethylene biosynthesis in plants (Feng and Barker 1992). Endophytes are known to alleviate plant stress by reducing ethylene level through the secretion of ACC deaminase (Nadeem et al. 2010). In addition, endophytes augment salinity stress in plants by accumulating glycine betaine-like compounds (Jha et al. 2011).

7.2.2.4.2 Biotic Stress Mitigation

Several kinds of organisms like microorganisms, insect pests and mammals impart biological stress to plants. Plants have evolved defence regulation against microbes, herbivores and other plants which is induced by expression of defence-related genes to translate secondary metabolites and specific proteins (Howe and Jander 2008; Mithöfer and Boland 2012).

Some of the biotic stresses of plants, mitigated by endophytes, are discussed below.

7.2.2.4.2.1 Interspecific Competition

Endophytes help host competitiveness towards pathogens through some unknown mechanisms, which is mostly growth independent (Aschehoug et al. 2012). These mechanisms may involve increased allelochemical production, plant vigour, seed yield (Kuldau and Bacon 2008), tiller numbers, leaf elongation rate and alteration of root architecture (Malinowski et al. 2000).

7.2.2.4.2.2 Invertebrate Pests

Endophytes reduce the effect of insect pests from order Orthoptera on plant (Crawford et al. 2010). The secondary metabolites such as peramine (Tanaka et al. 2005), ergovaline (Popay et al. 1990) and loliterm B (Prestidge and Gallagher 1985) produced by endophytes restrict the non-vertebrate pests. The process of screening, identification and reintroduction of beneficial endophytes in pathogen-affected plant host is tedious and time consuming. Sometimes reproducibility of results is unreliable due to variation in environment, genotype and other factors. As an option, genetically engineered endophytes could be deployed to deliver biopesticides within the host plant. Such an approach could ensure targeted, long-lasting and protected delivery of inhibitory compounds.

7.2.2.4.2.3 Herbivory by Mammals

The experimental results with native grass show that herbivores prefer to eat endophyte-free plants. The first reported case of endophyte effect on herbivory is from toxic pastures in the United States where health disorder in cattle was correlated with a high level of endophyte infestation in plants (Bacon et al. 1977). Grazing of endophyte-infested grasses is known to cause decreased productivity in mammals (Burke and Rorie 2002), increased systemic relaxin level (Ryan et al. 2001), altered hemograms and serum levels (Oliver et al. 2000), increased phagocytosis (Saker et al. 1998) and abdominal lipomatosis (Wolfe et al. 1998). Grasses provide the unique example of animal grazing tolerance through endophyte-induced defence by enhanced silicon uptake, hosting of toxin-producing endophytic fungi and inducting secondary metabolite production (Huitu et al. 2014). As the grazing-affected and damaged plant parts grow, the new shoots tend to deposit silicon in the cell walls. The deposited silicon in the damaged plant part enhances its abrasiveness causing tooth damage to grazers (Massey and Hartley 2006). Some grazing animals have well-adapted teeth to eat silica-enriched grass blades but carry lesser evolved system compared to insects to detoxify harmful chemicals secreted from plants. For example, Si intake by herbivores inhibit nitrogen absorption from digested plant materials (Massey and Hartley 2006) causing net output loss in the dairy industry.

7.3 Endophyte Colonization

Endophytes are transmitted to plant host either vertically through seeds and pollens or horizontally via soil atmosphere and insects (Frank et al. 2017). Endophytes mostly enter plants by horizontal transfer through roots, leaves and flowers, especially during mechanical damage. However, *Enterobacter asburiae* JM22 is known to penetrate cotton plants without external injury (Quadt-Hallmann et al. 1997). The majority of times the endophyte entry begins from roots through cracks and wounds caused by microbes, nematodes and arthropods. Some endophytes, however, can penetrate directly in a host cell. Endophytes enter plant tissues through type IV pili, lipopolysaccharides and exopolysaccharides (Hardoim et al. 2008; Jesus and Ben 2014; Reinhold-Hurek and Hurek 2011). After successful entry, endophytes

colonize the host tissues and grow in the host apoplastic washing fluid. During colonization, endophytes spread systemically from the entry site to intercellular cortex spaces and distant plant parts (Hardoim et al. 2008; Reinhold-Hurek and Hurek 2011). The successful colonization of endophytes depend on factors like plant tissue types, plant genotype and microbial type. The types of exoenzymes present in endophytes are especially crucial in deciding the colonizing potential on host. In addition, endophyte colonization significantly influences antioxidant potential of host plants (Hamilton et al. 2012).

Endophyte population in plants is considered dynamic and limited by biotic and abiotic factors (Wani et al. 2015); nevertheless they may receive better protection from environmental and biotic stresses than rhizosphere bacteria (Weilharter et al. 2011). External influences like anthropogenic activities and agricultural practices affect endophyte colonization. For example, several fertilizers (Seghers et al. 2004), especially with high N content, are reported to inhibit endophyte colonization (Fuentes-Ramírez et al. 1999). Application of chitin supplemented with nitrogen as an organic amendment, on the other hand, enhances endophytic species and population (Hallmann et al. 1999). Also amendments in soil nutrition (Hallmann 2003) and fertilizer treatments (Seghers et al. 2004) influence plant preference to certain endophytes. For example, high N-fertilization inhibits endophyte colonization on sugarcane (Fuentes-Ramírez et al. 1999).

Bacterial diversity and colonization are conventionally analyzed through culture-dependent methods. As most of the endophytes are not easily culturable, the culture-independent methods like metagenomics, metatranscriptomics, metaproteomics and single-cell genomics are gaining popularity. Metagenomics involves the study of complete bacterial genome combined with subsequent cloning and analysis. This high-throughput culture-independent method resolves the ecology and functions of nonculturable bacteria. Metagenomics is also useful in exploring the microbial community of some rare endophyte members. It allows identification of already identified novel genes independent of endophyte cultivability. Metagenomic analysis of rice roots has revealed an abundance of phylum *Proteobacteria*, *Gammaproteobacteria* and *Alphaproteobacteria* including many rhizobia (Sessitsch et al. 2011). In a continuing study on rice roots, the bacteria from genus *Enterobacter* and class Alphaproteobacteria were found to be associated with rhizobia and members of *Verrucomicrobia* (Sessitsch et al. 2012). Similar observations were obtained with *Populus* plant endophytes (Gottel et al. 2011).

The r-RNA sequencing is also one of the reliable methods for understanding endophyte phylogeny distribution. The16S r-RNA gene sequencing has confirmed that the endosphere region is predominantly colonized by the genus *Paenibacillus* (Ulrich et al. 2008). The new genus *Paenibacillus* was introduced to accommodate ‘group 3’ of the genus *Bacillus* (Ash et al. 1993). Moreover, the r-RNA-based endophytic phylogenetic distribution corresponds well with the taxonomic distribution of protein-coding genes, thus providing a nonbiased approach of endobacterial phylogeny, unlike DNA amplification and cloning methods (Sessitsch et al. 2011).

7.4 Endophytes and Their Interactions with Hosts

The presence of endophytic bacteria is considered ubiquitous in the plant system (Sturz et al. 2000). Henceforth, the absence of endophyte microflora in plants is correlated with its inability to grow in the culture medium. Plants provide diverse and extensive niche for endophytes residing in bark, buds, fruits, rachis, ovules, seeds, stems, tubers and xylem. Bacterial endophytes living in plant tissues belong to several genera and species. They thrive at lower population densities than rhizosphere bacteria or plant pathogenic microbial populations (Hallmann et al. 1997). However, it is yet to be established if endophytes impart more benefit to plant compared to rhizosphere microorganisms. Plant benefits pertaining to endophytes are well understood, but the knowledge of all endophyte population types that help plant is sparse.

Plant-microbe interaction is a complex relationship regulated by several biochemical and physiological mechanisms. Although the interaction between endophytes and host plants is not fully understood, such interaction may become associative, symbiotic, neutralistic or parasitic as per the host defence response and the types of microbes present in the endosphere (Long et al. 2008). Plants interact with endophytes by initiating defence responses through the jasmonate signal pathway (Dangl and Jones 2001) to reduce the invading microbial population (Miche et al. 2006). On the other hand, most of the endophyte remain unaffected from plant defence response with the help of several newly discovered genes (Minamisawa 2006). In fact, bacteria-mediated plant defence responses increase the spectrum and population density of root endophytes (Hallmann 2003), which could be tenfold lower than rhizosphere bacterial population (Gottel et al. 2011). Figure 7.1 describes the chemicals and enzymes produced by endophytes and their effect on plants.

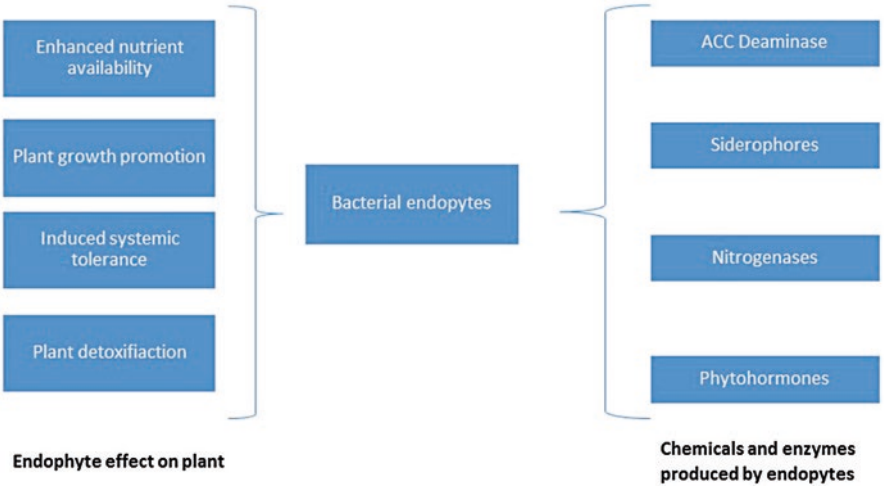

Fig. 7.1 Chemicals and enzymes produced by endophytes and their effect on plant

Cellular processes like metabolism, plant-microbe interaction and biofilm formation are induced by bacterial communications. In contrast, cell-cell signalling seems absent in the endophytes of grass *Azoarcus* sp. strain BH72. Nevertheless the pilA gene that encodes type IV structure proteins of pili is regulated with bacterial population density and has been reported essential for plant colonization (Hauberg-Lotte et al. 2012). Endophyte bacterial quorum sensing and their ability to surpass plant defence response are common traits that help them to signal the expression of targeted genes in high cell densities. Plant-associated bacteria and rhizobia share some common mechanisms that highlight their pathogenic or beneficial interaction with host plants. Like pathogenic bacteria, symbiotic rhizobia contain type III and IV secretion systems (Buttner and Bonas 2006; Thieme et al. 2005) and ethylene biosynthesis regulation pathway (Sugawara et al. 2006). Endophytes induce host-programmed cell death, stress responses, defence against pathogens and systemic stress signalling by producing reactive oxygen species and can be linked with host-microbe symbiosis. Endophytes secrete antioxidant compounds during biotic and abiotic stress. However, some endophytes appear neutral for their effect on plant and live on the cost of plant metabolites.

To find the relationship among members of the group, the genomes of several endophytes were sequenced. The whole-genome study of *Enterobacter cloacae* P101, an endophyte of switch grass (*Panicum virgatum*), was found related to other *E. cloacae* strains (Humann et al. 2014), which shows the nonspecific nature of association between endophytes and the host. The complete genome study needs meaningful analysis and methodology to develop highly adapted multi-trait endophyte strains for agriculture use. Such symbiotic strains would be applicable in varied environmental conditions and host ranges.

7.5 Conclusions and Future Outlook

Our current level of understanding about endophyte functioning is limited due to their unique microenvironment in endosphere. The relevant endophyte-specific research is scarce, and their concepts of metabolism are not fully understood (Ali et al. 2014). The proper endophyte study, which remained restricted due to non-cultivability, is now gaining momentum from culture-independent microbial identification methods. The methods are based on DNA extraction of sample followed by amplification of selected sequences through polymerase chain reaction (PCR) amplification. Most of the previous plant-bacterial research focused on interaction of single endophyte with plants under controlled conditions. Such approaches pose hurdle in obtaining the holistic view of the endophyte interaction with other organisms because in nature a bacterium interacts with several other beneficial and deleterious microorganisms under varied environmental conditions. Therefore, future research should study field-level interaction of endophyte consortium with plant host using evolved statistical methods and tools. This approach would ensure reliability of results with better reproducibility under varied land and environmental conditions. The future research should focus on understanding molecular-based

endophyte-host interaction as much of the current study is missing the involvement of host genotype in plant-microbe interaction. Thus it can be said that the exploration of host-endophyte interaction could pave path for low-input sustainable agriculture practices.

Crops productivity could also be improved by gene modification of plant or associated microflora. Adoption of gene modification methods could equip crops with pesticide resistance, phytoremediation, etc. to suitably regulate metabolism. However, newer bacterial exploration approaches like screening novel multi-facet endophytes or gene alteration are circumventing the requirement for plant genetic modifications. Nevertheless, endophytes can be much easily and cost-effectively engineered genetically and mass produced.

Endophyte bulk production for agricultural application requires an in-depth understanding of its growth kinetics outside the host in culture medium. Successful bulk production of multi-trait and genetically engineered endophytes demands thorough understanding of its physiology and metabolism. Such bacteria would need active formulations for survival during long-duration storage. Optimum plant variety-specific formulation of endophyte inoculants could maximize the beneficial effect of endophytes. To some extent, inoculant optimization can reduce bulk inoculant production cost. Such efforts could reduce our reliability on chemical fertilizers and pesticides. Moreover, the discovery of pesticidal synergistic effect on endophyte bioinoculants would control a wide range of pathogens. The research-based evolution of sprayable endophytes for co-application with chemical pesticides could impact commercial pesticide development for future integrated pest management (IPM). This newer microbial technology needs to prove its commercial viability to become successful. However, several hurdles impede the viability of endophytes in agriculture. For example, endophyte specificity to host restricts its wide-scale application to various crops. Another major hurdle in endophyte research and product commercialization is the consistency in retaining useful bacterial traits. Several endophytes have shown reduced action over the course of time. Long-term field trials are required to confirm the consistency of the introduced endophyte. Endophyte microbiology must overcome all such hurdles for active contribution towards sustainable agriculture.

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Importance and Utilization of Plant-Beneficial Rhizobacteria in Agriculture

8

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Abstract

Due to the use of a large amount of chemical fertilizers, continuous loss of soil fertility puts pressure on farmers toward more crop production in a sustainable manner. This problem creates a big challenge for farmers to fulfill the demand for the next generation. If an adequate amount of fertilizers is not supplied to crops, it raises major issue related to global food production and food security. Therefore, it requires adapting an eco-friendly, sustainable, and cost-effective approach for agricultural practices without arising environmental issues. Several natural rhizobacteria inhabiting the rhizospheric soil exist, which are used for plant growth promotion. They have tremendous capacity to provide directly or indirectly nutrient availability to the plants, stimulate plant hormones, and secrete certain compounds that help in the association of several other beneficial microbes with plant roots. In addition to restoring soil fertility, they have the capability to protect plants against soil-borne pathogens, thereby promoting plant growth. Further, application of plant growth-promoting rhizobacteria reduces the utilization of chemical fertilizers, pesticides, and other artificial growth regulators that cause severe health and environmental issues, soil infertility, water pollution, and biodiversity losses. In this context, sustainable use of rhizobacteria has been suggested to be an eco-friendly and cost-effective

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approach which increases crop yields and directly or indirectly protects plant from soil-borne pathogens for a long time.

Keywords
Food security · Plant growth · Rhizobacteria · Soil fertility

Abbreviations

ACC	1-Aminocyclopropane-1 carboxylic acid
BNF	Biological nitrogen fixation
ISR	Induced systemic resistance
PBR	Plant-beneficial rhizobacteria
PGR	Plant growth regulators
WHC	Water retention capacity

8.1 Introduction

Plant-beneficial rhizobacteria (PBR) has emerged as potential tools in creating sustainable agriculture owing to the issues of worldwide food security and environmental risk. PBR has a broad range of beneficial application in plant soil owing to its potential impact on soil health and plant growth development, protecting it from adverse conditions. The main impact of PBR includes increase in plant growth through enhanced nutrient availability for a longer period under adverse condition and increase in plant growth and quality of most commercial essential crops (Gray and Smith 2005; Silva et al. 2006; Figueiredo et al. 2011; Araujo 2008; Das et al. 2013). PBR constitute about 3–5% of the total population of bacteria that occur in rhizospheric soil (Antoun and Kloepper 2001). It refers to all the beneficial bacteria inhabiting at the surface of roots that participate in enhancing soil nutrient enrichment, promoting growth of plants, conferring resistance against stresses (includes both biotic and abiotic), and ultimately creating an improvement in agriculture (Gupta et al. 2015). In India, increased application of synthetic fertilizers in unsustainable manners deteriorates the soil health (Choudhary et al. 2018) and environment, leading to numerous ways of environmental pollution affecting other living beings which are reported in threshold value (Das et al. 2013). It is need of the hour to address these problems and promote the application of PBR instead of using synthetic fertilizers, pesticides, and other functional analogues to growth regulators of plants (Bahadur et al. 2014; Jat et al. 2015; Kumar et al. 2016).

PBR adapted two mechanisms for improving plant health and controlling plant diseases. In the direct pathway, PBR operates through biological fixation of atmospheric nitrogen and solubilization of mineral nutrients like phosphorus (P) and potassium (K) and acts as chelators by producing siderophores which binds with other metals resulting in increased Fe and Zn uptake from the soil,

exo-polysaccharides secretion, and production of plant hormones (e.g., indole acetic acid, gibberellins, ethylene, and cytokinin) (Bhardwaj et al. 2014; Singh et al. 2016; Kaur et al. 2016). Indirectly, PBR operates by secretion of antibiotic compounds, development of induced systemic resistance (ISR), and production of several hydrolytic enzymes, volatile compounds, hydrogen cyanide, and nutrient and space for competition, parasitism, and predation which ultimately lead to enhanced soil quality and increased plant health (Kaur et al. 2016). Sustainable agriculture implies the use of crops that possess disease management and tolerance toward salinity, drought, and heavy metal and balance the nutritional status in plants. Therefore, introduction of PBR may show potential to address the issues of environmental stresses.

Due to their ability to utilize root exudates and the high rate of the reproductive cycle, approximately 95% density of PBR resides in the rhizospheric soil (Glick 2012), which directly or indirectly affect growth and development of plants in many crops (Prashar et al. 2013). Likely, other groups of bacteria (e.g., *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Arthrobacter* etc.) are demonstrated as plant growth regulators and can be implemented in agricultural practices (Saharan and Nehra 2011).

8.2 Application of PBR as Bio-Fertilizers

Due to their abilities to induce positive responses in crop plants through their direct and indirect mechanisms, PBR continues to be a promising tool for various bio-fertilizer formulations. Application of bio-fertilizers and bio-enhancers can minimize chemical fertilizers utilization in the agricultural field and promote sustainable agriculture (Raghavendra et al. 2016; Zahedi 2016; Teotia et al. 2016). According to Mohapatra et al. (2013), bio-fertilizers application improves soil physical structure and maintenance of pH and improves water retention capacity (WHC), thereby increasing10–40% crop yield and nitrogen uptake/fixation up to 40–50 kg ha^{-1} (Mohapatra et al. 2013). Also, their parental inoculum is sufficient enough for a subsequent generation, which makes them a sustainably beneficial organism.

Bio-fertilizers includes different living strain of microorganisms which applied in soil to promote plant growth as well as enhance soil fertility in several ways such as increased availability, mobilization of nutrients, produce several metabolites, enhance decomposition of plant residues. It contains different sources such as K-, S-, and P-solubilizing microorganisms, N-fixing microorganisms, phytostimulation-promoting microorganisms, vesicular-arbuscular mycorrhiza, and siderophore-producing microorganisms (Prathap and Ranjitha Kumari 2015).

8.3 Application of PBR in Biological Nitrogen Fixation (BNF)

BNF is a step-determining biogeochemical process that shows the occurrence of certain microorganisms in the rhizosphere and is capable of converting atmospheric N into a reduced form of N. Nitrogenase enzyme and leghemoglobin are essential

components for operating this process. It has been well known that several symbiotic microorganisms such as *Frankia, Azospirillum,* and *Azotobacter* contain the nitrogenase enzyme that helps in N fixation (Franche et al. 2009; Suhag 2016).

8.4 Mechanism of Biological Nitrogen Fixation

8.4.1 Symbiotic N Fixation Between Interaction of *Rhizobium* and Legume/NonlegumePlant

Rhizobium which is found mostly in leguminous plants has the ability to colonize plant roots and encourage nodulation, enlarge cell elongation, and increase chances of bacterial association. Rhizobacteria residing in root nodules fix nitrogen into ammonia by the involvement of nitrogenase enzyme that helps in plant growth. Inoculation of these nitrogen-fixing microorganisms along with legume plants enhanced availability of N nutrient when plants are grown in soil that is scarce in the nutrient. Their symbiotic association sustains for a long time and increases various agronomic implications and facilitates N utilization. Indeed, a more understanding and management of N-fixing microorganisms could be initiated (Santi et al. 2013) to benefit the farmers through knowledge awareness. Sahgal and Johri (2003) reported that several genera of nitrogen-fixing microorganisms are able to fix N in a diverse range of plant species.

Like in leguminous plants, nitrogen fixation also occurs in certain species of nonleguminous plants. *Frankia* spp. is a nonleguminous N-fixing microbe associated with dicotyledonous species like *Alnus* and *Casuarina* (Dawson 2008). They are related to the genus of actinomycetes of the family *Frankiaceae* that possesses the capacity to fix atmospheric N under both symbiotic and free-living aerobic conditions.

8.4.2 Use of Symbiotic Association between *Anabaena and Azolla*

Azolla pinnata belongs to aquatic fern whereas *Anabaena* belongs to cyanobacterium or filamentous blue-green algae, and they seem to be considered as symbiotic partners. Their association enables plants to fix free atmospheric N, increase photosynthesis rate, and increase rice crop yield by 10–20%. Also, they can be used as a green manure, particularly in rice fields which have the capacity to increase soil porosity by about 3.7–4.2% and reduce soil bulk density. For example, *Aulosira fertilissima* are most active in a rice field as N fixer and they are demonstrated as a biofertilizer for cultivation of rice and supplement for animal nourishment in China (Mazid and Khan 2014; Kollah et al. 2016), whereas *Cylindrospermum* are found in sugarcane and maize fields. Application of these microorganisms in the agricultural field led to enhancement of about 20–30 kg N/hect/annum. Overall, *Azolla* exhibit improved soil health, level of nutrients status, as well as physicochemical status of the soil (Choudhary et al. 2017; Singh et al. 2016; Rawat et al. 2016; Masood and Bano 2016).

8.5 Restrictive N Fixation

Azospirillum spp. is also a root-colonizing, symbiotic bacteria and has no capability of nodulation and is mostly found on the surface of roots of dicot and monocot plants, e.g., sugarcane, wheat, sorghum, and corn (Glick 2012; Babalola and Glick 2012; Duca et al. 2014). They are considered an important PBR and utilized worldwide for their ability to increase growth, yield, and phytohormones in many bowls of cereal crops (Vurukonda et al. 2016). Some of the effective *Azospirillum* species such as *A. brasilense* and *A. lipoferum* have been identified for induction of plant growth, seed germination, plumule formation, and initiation for radical development. Similar to that, other free-living diazotrophs (e.g., *Azotobacter*, *Azoarcus* spp., *Herbaspirillum seropedicae*, *Acetobacter diazotrophicus)* are also considered as a plant growth enhancer and associates with plant roots, leading to benefit for plant development. *Azospirillum* association with plant promoted drought tolerance through production of indoleacetic acid (Dimkpa et al. (2009), whereas *A. lipoferum* that is able to produce the plant hormones abscisic acid and gibberellins in association with maize roots minimize the effect of drought stress (Cohen et al. 2009). Further, association of *Azospirillum* was able to increase yield up to 10–15% among cereal crops and fix N up to 20–40 kg ha^{-1}. Additionally, they secrete plant growth-promoting substances such as gibberellic acid, indole 3-acetic acid, and cytokinin which help in root development and N, P, and K nutrient acquisition from the soil system (Mohapatra et al. 2013; Mazid and Khan 2014).

8.6 N Fixation by Free-Living or Nonsymbiotic Rhizobacteria

Free-living or nonsymbiotic N-fixing microorganisms have huge application in sustainable agriculture practices. Several strains were identified, viz., *Azotobacter* sp., *Gluconacetobacter diazotrophicus, Azomonas, Achromobacter, Bacillus, Alcaligenes, Beijerinckia, Arthrobacter, Corynebacterium, Klebsiella, Derxia, Clostridium, Rhodopseudomonas, Xanthobacter, Enterobacter, Rhodospirillum,* and *Pseudomonas* (Vessey 2003; Barriuso and Solano 2008). Association of these bacterial strains with plants is able to fix ≤10–25 kg N/ha/annum. Application of *B. subtilis* Whlr-12 and *Bacillus* spp. Whlr-15 in wheat crop was able to enhance crop yield (Ahemad and Kibr et al. 2014; Baghaeeravari and Heidarzadeh 2014).

8.7 Role of Rhizobacteria in P Bioavailability

For plant growth and development, phosphorus is also an essential nutrient after nitrogen. It is involved in all metabolic processes like photosynthesis, respiration, energy, transduction, and biosynthesis of macromolecules (Khan et al. 2010). Abundance of phosphorus is generally found in both forms, namely, organic and inorganic. Approximately 70–90% phosphorus available in the soil is stable and

accumulated as insoluble compounds like calcium phosphate (occurs in most of the calcareous and alkaline soils), iron phosphate, and aluminum phosphate (generally occurs in acidic soils) (Chen et al. 2008). Out of the total applied P, only 0.1% is available to the plant, which might be due to low solubility and P fixation (Pereira and Castro 2014; Yasin et al. 2016). Several phosphate-solubilizing bacteria (PSB) are well documented which contribute to P bioavailability in the soil by converting insoluble or bounded P into the available form (Sundaram et al. 2016). These microbes enhance the availability of P through solubilization or mineralization of organic and inorganic P complexes which is found in soil (Kumar 2016). It has been demonstrated that seed treatment with PSB reduces 50% phosphatic fertilizers due to their ability to solubilize inorganic phosphate like di- and tri-calcium phosphate and hydroxyapatite (Singh et al. 2015; Yadav and Sidhu 2016).

8.8 Mechanism of P Solubilization by PSB

Mechanisms of PSB occurred in the following ways:

1. They secrete weak organic acids like malic acid, succinic acid, fumaric acid, 2-keto-gluconic acid, acetic acid, and gluconic acid. These acids facilitate decreased pH and increased chelation and compete with P for adsorption and convert it into soluble form through breakdown of various metal complexes (Ca, Fe, and Al).
2. Facilitate the removal of extracellular enzymes.
3. Biological phosphate mineralization.

8.9 Sulfur-Oxidizing Rhizobacteria

Sulfur (S) is another essential macronutrient among all nutrients, and about 90% is available in the inorganic form in the soil. Some microorganisms present in the soil facilitate sulfur availability for plant nutrition. Various amino acids containing sulfur, like cysteine, proteins, methionine, polypeptides, thiamine, biotin, etc., are metabolized through those microbes and increase the availability of $SO_4^{2-}$for plant nutrition. Various transformations of S in soil are accomplished through the activity of microbes (Vidyalakshmi et al. 2009; Mazid and Khan 2014). Transformation of S in the soil is operated under the following processes:

1. Mineralization: Breakdown of organic form of S improves the amount of inorganic compounds (sulfates) through microbial activities.
2. Immobilization: Transformation from inorganic complex of S into organic complex of S.
3. Oxidation: Elemental sulfur and inorganic complex of sulfur (H_2S, sulfite, and thiosulfate) are oxidized to sulfate by microbial activities mostly by chemoautotrophic and photosynthetic bacteria.

Proteins (amino acids) → breakdown → released sulfur → converted into sulfate through oxidation reaction (anaerobic condition) or form H_2S through reduction reaction under anaerobic condition (waterlogged soils).

The main microorganisms contributing to the conversion of elemental S to sulfates include the genus *Thiobacillus* such as *T. ferrooxidans*, *T. thiooxidans,* and *T. thioparus* which is an obligate chemolithotrophic and nonphotosynthetic organism. Other than these, some heterotrophic bacteria such as *Xanthobacter, Alcaligens, Bacillus, Arthrobacter, and Pseudomonas*, fungi which include *Penicillium* and Aspergillus, and few actinomycetes also exhibited their involvement in the oxidation of S compounds. Vidyalakshmi et al. (2009) also reported that some photolithotrophs belonging to the genera *Chlorobium*, *Rhodopseudomonas,* and *Chromatium*are are also involved in the oxidation of S in the aquatic environment.

8.10 Formation of S/Sulfuric Acid and Its Contribution to Agriculture Field

Many S-oxidizing microorganisms contribute to the total health and nutrient availability in the soil. For example, the formation of sulfuric acid which is a strong mineral anionic acid can render alkali soil and maintained the pH of the soil. It also solubilizes inorganic complexes of plant nutrients containing Na and enhances the amount of soluble K, P, Ca, Mg, etc. for plant uptake. Sulfate has been assimilated in soil through plants and microorganisms and incorporated in the form of proteins which are known as assimilatory reduction of sulfur. Sulfate can also be reduced to hydrogen sulfide by bacteria possessing reducing potential (e.g., *Desulfotomaculum* and *Desulfovibrio*) and may reduce the S availability for uptake by plants which is referred to as dissimilatory reduction of sulfur that is not considerable in the view of productivity and fertility of soil.

8.11 Production of Plant Hormones

Plant growth regulators (PGRs) generally made of organic compounds which are produced by plants and microorganisms (PBR) influence physiological and biochemical activities of plants at a low level (Jha and Saraf 2015) and contribute to defense responses under stressful conditions (Fahad et al. 2015) and fertility of soil (Verma et al. 2015). Significant PGR including IAA, GA, ABA, ethylene, and CK are listed in Table 8.1, and the schematic interaction and function of PBR is shown in Fig. 8.1.

Auxin contributes in the stimulation of cell division, elongation, differentiation of cells, and extension among plants (Kundan et al. 2015). It is synthesized in the young tips of stems, leaves (Kaur et al. 2016), and seeds through the transamination reaction and decarboxylation of tryptophan which is produced from root exudates, and it is also the essential precursor for IAA biosynthesis in bacteria (Etesami et al. 2009). *Azospirillum* secreted abundant auxin as compared to other phytohormones

Table 8.1 List of plant hormone-producing rhizobacteria in several crops

Plant hormones	Plant-beneficial rhizobacteria		Host plant	References
	Genus	Species		
Indole acetic acid	*Acinetobacter*	–	*Oryza sativa*	Gandhi and Muralidharan (2016)
	Azospirillum	–	*Triticum aestivum*, *Solanum tuberosum*	Prathap and Ranjitha Kumari (2015) and Ahemad and Kibret (2014)
		Brasilense	*Solanum lycopersicum*	Kumar et al. (2016) and Khan et al. (2016)
	Bacillus	–	*Triticum aestivum*, *Solanum tuberosum*	Prathap and Ranjitha Kumari (2015) and Ahemad and Kibret (2014)
		Thuringiensis	*Lavandula dentata*	Armada et al. (2014)
		–	*Asparagus racemosus*	Mitra et al. (2016)
		–	*Zea mays*	Zahid et al. (2015)
		Subtilis	*Solanum lycopersicum*	Kumar et al. (2016), Khan et al. (2016)
	Rhizobium	–	*Lactuca sativa*, *Daucus carota*	Flores-Felix et al. (2013)
		Leguminosarum	*Triticum aestivum*	Hussain et al. (2014)
		Phaseoli	*Triticum aestivum*	Hussain et al. (2014)
	Mesorhizobium	*Ciceri*	*Triticum aestivum*	Hussain et al. (2014)
	Pseudomonas	–	*Solanum lycopersicum*	Kumar et al. (2016) and Khan et al. (2016)
		Putida	*Brassica juncea*	Ahemad and Khan (2012)
		Aeruginosa	*Brassica juncea*	Ahemad and Khan (2012)
		–	*Zea mays*	Zahid et al. (2015)
Gibberellic acid	*Pseudomonas*	–	*Malus pumila* and pear	Kapoor et al. (2016)
		Putida	*Glycine max*	Sang-Mo et al. (2014)
	Azospirillum	*Lipoferum*	*Zea mays*	Cohen et al. (2009)
	Sphingomonas	–	*Solanum lycopersicum*	Khan et al. (2014)
	Bacillus	–	*Piper longum*	Joo et al. (2005)
Cytokinin	*Bacillus*	*Subtilis*	*Platycladus orientalis*	Liu et al. (2013)
		–	*Cucumis sativus*	Sokolova et al. (2011)
	Azobacter	–	*Cucumis sativus*	Sokolova et al. (2011)

(continued)

Table 8.1 (continued)

Plant hormones	Plant-beneficial rhizobacteria		Host plant	References
	Genus	Species		
Abscisic acid	*Phyllobacterium*	*Brassicacearum*	*Arabidopsis thaliana*	Bresson et al. (2013)
ACC deaminase	*Bacillus*	*Thuringiensis*	*Triticum aestivum*	Timmusk et al. (2014)
	Pseudomonas	*Fluorescens*	*Pisum sativum*	Zahir et al. (2008)
		Cepacia	*Glycine max*	Cattelan et al. (1999)
		Putida	*Vigna radiata*	Mayak et al. (1999)
	Alcaligens	–	*Brassica napus*	Belimov et al. (2001)

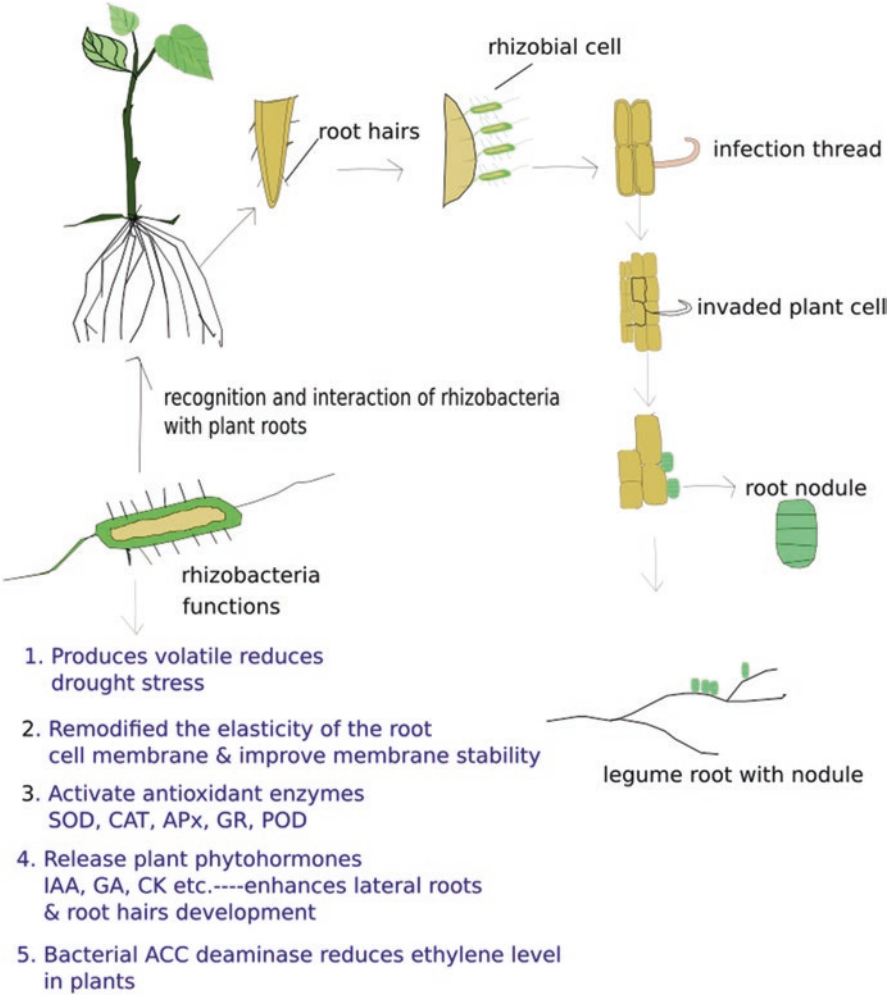

Fig. 8.1 Hypothetical mechanisms of plant growth promotion during plant-PBR interaction

(Kaur et al. 2016). Different species of PGPR, like *Acinetobacter* spp., *Rhizobium, Pseudomonas*, *Bacillus* spp., *Azospirillum*, and *Klebsiella*, contributes to indole-3-pyruvic acid biosynthesis and indole-3-acetic aldehyde biosynthesis (Shilev 2013) in rhizospheric soil of various crops. It was reported that the IAA production from *Pseudomonas* spp. plays a crucial role in increasing the growth and yield of tomato plant (*Lycopersicum esculentum*) (Sharma and Rai 2015). GA influence germination and emergence of seed; induction of flora, fruit, and flower development; and shoot induction (Spaepen and Vanderleyden 2011). Tomato plants treated with *Sphingomonas* sp. LK11 which is capable of producing GA results in a significant increment in different growth attributes (Khan et al. 2014).

CK enhance cell multiplication and control the development of roots by suppressing primary and lateral root elongation and promoting formation of root hair (Riefler et al. 2006). Some of the CK-producing bacteria include *B. subtilis* in *Platycladus orientalis* (Liu et al. 2013), *Azotobacter* spp. in *Cucumis sativus* (Sokolova et al. 2011) and *Triticum aestivum* (Timmusk et al. 1999); *Pseudomonas fluorescens* in *Glycine max* (de Salamone et al. 2001); and *R. leguminosarum* in *Pisum sativum* and *Lactuca sativa* (Noel et al. 1996).

Ethylene is an essential phytohormone which plays a crucial role in the initiation of root development, inhibition of root elongation, stimulation of seed germination, and leaf abscission promotion and activation of the synthesis of other plant-beneficial hormones. The enzyme 1-aminocyclopropane-1 carboxylic acid (ACC) is most essential for ethylene synthesis and is catalyzed through ACC oxidase. However, ethylene is also produced under stress conditions like heavy metals, phytopathogens, drought, flooding, and salinity. However, there is one way to minimize diseases developed through a wide range of phytopathogens by means of decreasing the response of plants for ethylene. To manage this risk in crops, PBR that produce ACC deaminase has been utilized like *Agrobacterium, Achromobacter, Acinetobacter, Azospirillum, Alcaligenes, Bacillus, Burkholderia, Enterobacter, Serratia, Ralstonia, Pseudomonas,* and *Rhizobium* which have potential to produce ethylene (Glick 2012; Das et al. 2013).

8.12 Siderophore-Producing Rhizobacteria

Siderophores are chelating molecules with high affinity for Fe secreted by certain fungi, bacteria, and grasses (Neilands 1995). They have a low molecular weight (approximately 400–1500 Da) and have a high affinity toward Fe^{+3}along with other micronutrients and membrane receptors. Membrane receptors have a tendency to bind with the complex of Fe/micronutrient-siderophore, thereby contribute in assessing the mobilization and facilitate the uptake of micronutrients in soil by microorganisms and ultimately stimulate plant growth and yield (Leong 1986). PBR may enhance translocation and abundance level of micronutrients through siderophore complex formation. Currently, there are around 500 well-characterized siderophores, and out of them, 270 siderophores and their chemical structures have been identified (Comelis 2010). Siderophores are normally grouped according to

the ligandsutilized for chelation of Fe^{3+}. Among siderophores, the major groups include the catecholate (phenolates), carboxylates, and hydroxamates (e.g., citric acid). Some of the important microorganisms include *Agrobacterium*, *Pseudomonas, Bacillus, E.coli*, *Rhizobium*, and many fungi which produce a wide spectrum of Fe-chelating substances (Zahir et al. 2004).

8.13 Effects of Siderophore on Plants

Research done on mungbean treated with *Pseudomonas* capable to produce siderophore that was subjected to Fe-deficient conditions showed reduced chlorotic symptoms and increase chlorophyll level in comparison to noninoculated plants. The Fe-pyoverdine complex synthesized through *P. fluorescens* C7 in *Arabidopsis thaliana* leads to increased Fe accumulation in plant tissues and improves growth and development (Noumavo et al. 2016).

8.14 Indirect Mechanism of Plant-Beneficial Rhizobacteria in Plants

Instead of the direct role of PBR in plant growth, it indirectly has effects on plant growth by killing many pathogenic fungi, caused by the secretion of many enzymes capable of degrading the cell wall like cellulase, 13-glucanases, protease, chitinase, and lipases. In addition, it also produces essential volatile substances and antibiotics that help in the inhibition of pathogens (Shrivastava et al. 2016; Velazquez et al. 2016).

8.15 PBR as Biocontrol Potential

In the current scenario, a continuous exposure to phytopathogenic microbe causes major problems for sustainable agricultural and ecosystem stability. Chemical pesticides application has led to continuous environmental issues and may respond to the development of super-resistant pathogen. In this regard, PBR plays a crucial role in the promotion of plant growth, increasing soil fertility and maintaining beneficial plant rhizospheric microbiomes by decreasing population density of pathogens in soil (Qi et al. 2016). Several PBRs are well known which are used in the agricultural sector for improving plant growth. *Pseudomonas fluorescence* is one of the important PBRs, considered as a biological control agent due to their abundant presence in the soil as well as plant roots (Panpatte et al. 2016). Another PBR like *Trichoderma* sp. is very efficiently used in the agricultural field for management of soil-borne pathogens and plant growth. According to Siddiqui (2006), PBR has the property of antagonism against several phytopathogenic fungi by using different mechanisms like competition, parasitism, and antibiosis. However, PBR is used against a broad spectrum of phytopathogens like viral, fungal, bacterial, and nematode diseases all over the world.

8.16 Role of PBR in Induction of Induced Systemic Resistance

PBR colonization with plant roots activated another kind of defense mechanism like ISR, and activation of it is to protect plants against several insects, herbivores, and phytopathogens. ISR sensitizes the plant immunity after elicitation with phytopathogen microorganisms that led to enhanced plant defense (Pieterse et al. 2014). PBR interaction with plant roots conveys ISR mechanism which strengthens the cell wall membrane stability, modulating the host biochemical reaction, thus leading to the synthesis of several chemical signals, viz. jasmonic acid, malic acid, salicylic acid, and phytoalexin synthesis and production of several hydrolytic enzymes. PBR interaction also induces modulation of cell wall stability by lignification (Benhamou et al. 1996) and its potential to build up a primary barrier for pathogens invasion. In addition callose formation and production of several phenolic compounds are generated by invasion of pathogens on the infected portion. Such type of formation or chemical changes at the infection site prolongs the fungal entry process, and the host develops a defense mechanism to suppress pathogen development at the outer surface. Therefore, priming of PBR is a cost-effective approach and sustains long-time use in the agricultural field (Pastor et al. 2013).

8.17 Importance of PBR in the Induction of Antibiosis Mechanism

Certain volatile and nonvolatile compounds are secreted between interactions of two or more organisms, in which one of them is eliminated. PBR are more powerful microbes that inhibit the pathogen proliferation and growth (Shiley 2013). These compounds include hydrogen cyanide, oligomycin tropolone, xanthobaccin, and tensin and are produced during interaction (Akhtar and Siddiqui 2010). Several pieces of evidence have been made, whether antibiotic compounds (2,4-diacetylphloroglucinol (Phl) and phenazine-1-carboxylic acid (PCA)) are isolated in the interaction of *Pseudomonas* with wheat rhizosphere (Raaijmakers et al. 1999). Further, PCA isolated from *Pseudomonas aureofaciens* was directly applied in the field of creeping bentgrass for controlling the *Sclerotinia homoeocarpa*. This evidence clearly suggested that PBRs are directly contributed in the suppression of pathogens via antibiosis mechanism.

8.18 Conclusion and Future Strategies

Nowadays, a huge amount of chemical fertilizers and pesticides have been directly used in agricultural practices that cause major issues in crops in the context of crop production, leading to food security and safety in the future (Pandey et al. 2018). These types of practices cause soil infertility which is directly affected to increase the cost of cultivation, farmers' income, and health. Therefore, there is need to adopt bio-fertilizer approaches which are nonhazardous for the environment, easy

to handle, nontoxic, and cheap and can improve crop production and minimize soil infertility (Mazid and Khan 2014). For this implementation, biocontrol application with organic manure makes a better technique to strengthen the soil nutrient status that has led to increasing soil fertility and minimizes the risk of environmental issues. These biocontrol agents add nutrient in soil by solubilizing K, mobilizing P, siderophore production, and biological nitrogen fixation. The application of biocontrol agents is an appropriate approach for efficient and coherent exercise in resources of agriculture with minimum production of negative effect on the surrounding environment that may cause water pollution. In addition, biocontrol agents have wide possibilities for agricultural practices in different geographical areas but have some limitations which require further research to identify and characterize such type of biocontrol agent which is an application in worldwide eco-friendly agricultural practices.

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Potassium Solubilizing Bacteria (KSB)

9

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Abstract

Potassium (K) is reflected as a fundamental supplement and a noteworthy constituent inside every single living cell, which is required in vast sums by plants, animals, and people. In environment, soils normally contain K in bigger sums than some other supplements.As rocks gradually weathered, K is discharged, yet change of K from the basic portion to some other frame is as often as possible to ease back to give them a lot of this basic supplement required by crops. Utilization of chemical fertilizers has an extensive negative effect on ecological supportability. Potassium solubilizing bacteria (KSB) solubilize K-bearing minerals and change over the insoluble K to dissolvable types of K that plants can get to. Countless soil microscopic organisms, for example, *Acidithiobacillus ferrooxidans*, *Paenibacillus* spp., *Bacillus mucilaginosus*, *B. edaphicus*, and *B. circulans*, have ability to solubilize K minerals like biotite, muscovite, feldspar, mica, iolite, and orthoclase. KSB are normally present in every one of the soil, in spite of the fact that their number, assorted variety, and capacity for K solubilization differ which rely on the

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soil and climatic conditions. Despite that, KSB are the most essential microscopic organisms for solubilizing K minerals which demonstrate viable association amongst soil and plant frameworks. These microbes can be utilized productively as a wellspring of K-fertilizer for managing crop generation and keeping up soil K. Subsequently, generation and administration of organic manures containing KSB can scatter K inadequacy particularly in paddy field or zones where plants are normal for K and are likewise an approach to accomplish the objectives of the practical farming. This article shows a diagram of flow patterns and difficulties on the KSB, components, and their part in plant development advancement and in the end gives a few viewpoints for study on K in agriculture.

Keywords
Potassium solubilizing bacteria · Biofertilizer · Mineral bearing potassium · Potassium solubilization · Bacteria interaction with plant

9.1 Introduction

In the twenty-first century, agriculture faces different difficulties: to nourish a developing total populace, react to expanded worries about dealing with the natural resource base, receive more effective and maintainable production techniques, and adjust to environmental change and drought conditions in several developing regions (outstandingly in Europe, Central Asia, and the Horn of Africa). The total populace is anticipated to achieve 9 billion by 2050, and developing country of the Africa and Asia retain by far most of the expansion, while developed countries will encounter practically zero populace development in this century, and quite a bit of that development will be from migration from less developed countries (Haub et al. 2012).

The development in populace has expanded food production and the natural impacts which prompted expanded pressure on the land. This issue likewise concerned the fruitfulness of soil. In these circumstances, expanding the food production by and large can't be managed except if nutrients are applied to the soil to supplant those expelled through expanded yield generation. Accordingly, the food production challenge ahead is critical and requires expanding the efficiency of complex. With a specific end goal to expand world food production, farmers utilize the chemical fertilizers (Pacheco et al. 2001). While the chemical fertilizers assist a plant with growing, they don't enhance properties of the soil. Chemical fertilizers contain acids, as hydrochloric and sulfuric acids, which change the acidity (pH) of the soil. These progressions break down "soil fragments", the bonding material which canisters rock particles organized. At last, the outcome is a compacted surface that keeps rainwater from entering the soil. The acid fertilizers additionally change the sorts of microorganisms which can exist in the soil (Abbiramy and Ross 2013). Vast utilization of chemical fertilizers can cause the expansion in rate of lethal synthetic compounds, similar to cadmium, arsenic, and uranium in soil (Atafar et al. 2010). Chemical fertilizers may likewise influence osmatic pressure, conductivity, and water holding limit. These poisonous synthetic substances can

discover their way into the fruits and vegetable and at last human body (Tuli et al. 2010). Subsequently, the thought turn back to nature or utilizing sustainable materials is a requirement which prompts advance evergreen agriculture. After nitrogen (N) and phosphorus (P), potassium (K) is the most vital nutrient for plant. K has a key part in the growth of plant, development and metabolism, and furthermore expanded plant protection against diseases and pests (Maqsood et al. 2013). Biotite, muscovite, feldspar, mica, illite, and orthoclase are the major significant minerals with K, and most of the K have exist as a fixed form in soil and not directly taken up by plant (Meena et al. 2014). Also, one of the significant purposes behind the depilation of K in the soil is that these days agriculturists are not including crop residue in soil, which at last demonstrates the poor crop growth and yield (Meena et al. 2014). However, some valuable soil microorganisms including potassium solubilizing bacteria (KSB) could solubilize insoluble sources of K to soluble or available form of K by different mechanisms which include secretion of organic acids and inorganic acids and polysaccharides, acidolysis, complexolysis, chelation, and exchange responses (Meena et al. 2015; Keshavarz Zarjani et al. 2013). These analyses demonstrated that KSB can give an elective innovation to make K accessible for take-up by plants. Accordingly, detection of effective bacterial strains equipped for solubilizing K minerals rapidly can conserve our current resources and keep away from environmental pollution dangers caused by overwhelming use of K-composts. Consequently, in this chapter, we depict K status in soil and expand the investigations of KSB including separation and systems of solubilizing K-bearing minerals to develop productive bacterial inoculants for solubilization of K in soil.

9.2 Potassium Cycling

Potassium (K) is a fundamental supplement that assumes an essential part in development, metabolism, and plant growth. After N and P, K is the significant nutrient to confine productivity of crop. K is required to enact more than 80 distinct catalysts in charge of such nitrate reduction, starch synthesis, plant and animal's energy metabolism, photosynthesis, and sugar debasement. Without sufficient measures of K, water is lost from the cells, and the plant cells debilitate and begin to wither. K inadequate plants will have ineffectively developed cell walls and lower levels of store protein and starch, and they turn into a simple feast for sucking insects and an obvious objective for intrusion by parasitic spores (Meena et al. 2015). K is an essential macronutrient and the seventh most bounteous component in Earth's outside layer. Total K content in soils extend somewhere in the range of 0.04 and 3% K (0.4 to 30 g K kg^{-1} soil). In the upper 20 cm of the soil profile, a regular mineral soil can have 3000 and 100,000 kg ha^{-1} K. In spite of the fact that K present as abundant element in soil, just 1–2% of its total amount is accessible to plants (Sparks and Huang 1985). The rest are aggregated with different minerals and in this manner are inaccessible to plants. K is available in different forms in soil, which include mineral K, non-replaceable K, interchangeable K, and dissolved or solution K. Depending upon soil compose, from 90% to 98% soil K is mineral K and its

majority is inaccessible for plant take-up. Minerals comprising K are feldspar (orthoclase and microcline) and mica (biotite and muscovite). Different K-bearing minerals which have been utilized in various studies have been listed with their compositional analysis of elements (Table 9.1). At the point when these minerals climate, the K turns out to be more accessible as promptly replaceable, and dissolvable K can be adsorbed by plants' roots. The nonexchangeable type of K makes up around 1–10% of soil K and is caught between the layers or sheets of specific sorts of clay minerals (Sparks 1980). Dissolution of non-replaceable K to third interchangeable form happens when equilibrium of interchangeable and solution K is affected by runoff, crop removal, and disintegration and leaching. Both the clay particles and the organic matter have negatively charged locales that pull in and hold interchangeable K. Available K is the only form of K specifically and promptly utilized by plants and microorganisms in soil. Furthermore, this form is the frame that is subject to dissolve in soils. The concentration of available K in soil shifts from 2 to 5 mg $K\ L^{-1}$ for ordinary farming in humid region soils. The replaceable K is in fast balance with soil arrangement K. The discharge rate of replaceable K and non-interchangeable K to the soil arrangement is moderate. At the point when K particles build a surface complex by reacting with oxygen atoms in interlayers of certain silicate earth minerals, K fixation occurs. The limit of soils for K obsession relies upon the level of interlayering, the kind of earth mineral and the density of charge, the moisture level, the convergence of K particles and the centralization of contending cations, and the pH of the surrounding clay or soil (Shaimukhametov and Petrofanov 2008).

9.3 The Threats of Chemical Fertilizers

It has been well explained that the steady utilization of chemical fertilizers mostly ammonium, potassium, nitrate, and phosphate salts may have the unsafe impacts on the environments. Fertilizer industry is considered as a source of natural radionuclides (e.g. 238U, 232Th, and 210Po) and heavy metals (like Hg, Cd, As, Pb, Cu, Ni, and Cu) as a potential source (FAO 2009). The utilization of these fertilizers may influence the amassing of heavy metals in plant and soil system. As per the past investigations, because of the solid buffering power, the impacts of chemical fertilizers on soil are not rapidly under standable (Geisseler and Scow 2014). Through time, it expresses that rise up of the contamination, weakening of soil health, and soil degradation responses happening in the soil prompts crumbling of the adjust of the present components. Furthermore, toxic material like heavy metals collect inside the foods grown from the ground and cause issues in humans and animals fed on them. Fertilizers that mainly cause imbalance of sodium and K have negative effect on soil microbial populace, pH, and soil structure weakening. Constant utilization of these fertilizers causes a reduction in soil pH (Abbiramy and Ross 2013). The use of synthetic K-fertilizers in extensive amount disrupts equilibrium of nutrients and reduce the take-up of essential nutrients by the plants. The negative impacts of these fertilizers on soil microorganisms have been pulverizing and deadly. Notwithstanding the

Table 9.1 List of various K-bearing minerals and their compositional analysis of different elements

		Chemical composition (%)											
Insoluble K-minerals	K_2O	SiO_2	Al_2O_3	Fe_2O_3	Na_2O	CaO	MgO	TiO_2	MnO	P_2O_5	H_2O	Others	References
Biotite	9.12	39.99	18.98	14.75	0.28	0.07	13.69	–	–	–	–	–	Sheng et al. (2008)
Feldspar	13.54	63.56	18.35	1.76	0.20	0.04	0.10	–	–	–	–	–	Sheng et al. (2008)
	10.1	66.12	17.59	–	–	0.2	–	–	–	0.1	–	–	Girgis et al. (2008)
Illite	8.99	48.6	29.2	7.15	0.06	0.22	2.58	–	0.01	–	–	–	Zhou and Huang (2007)
Muscovite	9.28	51.04	28.63	5.09	0.58	0.06	0.72	–	–	–	–	–	Sheng et al. (2008)
K-rich shale	9.0	67.80	18.76	0.17	3.28	0.16	0.03	0.04	0.02	–	0.25	–	Lian et al. (2008)
Phosphorite	0.14	2.90	0.63	1.04	0.31	51.37	0.47	0.07	0.05	36.46	–	6.56	Congqiang et al. (2008)
Nanjing feldspar	13.6	62.73	15.98	1.73	0.25	0.05	0.08	–	–	–	–	–	Sheng and He (2006)
Suzhou illite	4.10	65.65	20.31	1.24	0.68	0.49	1.82	–	–	–	–	–	Sheng and He (2006)

unsafe impacts of the chemical fertilizers on the earth, cost of these fertilizers including K-fertilizers is additionally expanding each year (Meena et al. 2014).

9.4 Bacteria-Soil-Plant Interactions

Soils are complex blends of minerals, water, air, organic matter, and billions of organisms, and the progressions occurring in its organization are called biogeochemical changes. Soil fertility alludes to the limit of the soil to supply basic plant nutrients, for example, N, P, K, and iron (Fe), while the inorganic types of these minerals are made by microorganisms amid mineralization process (Zhao et al. 2016). In the soil, it is conceivable to discover different sorts of microorganisms, for example, bacteria, fungi, actinomycetes, protozoa, and algae, which microscopic organisms are by a wide margin the most well-known (i.e. ~ 95%). There are an several unique bacterial species, most of which presently can't seem to be even identified properly, and every species has its own specific importance and abilities. The number and variability of bacteria are affected by the soil structure, for example, organic carbon, temperature, moisture, and electrical conductivity, and different chemicals, and additionally by the number and kinds of plants found in those soils. Moreover, the majority of which underlying around plant roots in rhizosphere (Dessaux et al. 2009). This is a direct result of occurrence of nutritional substances including organic acids, sugars, amino acids, and other small molecules from exudates produced by roots (Walker et al. 2003). The bacteria may influence plant development in one of three different ways. The communication might be helpful (e.g. plant development advancing rhizobacteria and predatory enemies of herbivores), harmful (e.g. pathogens and herbivorous insects), or neutral for plant, and at times the effect of microbes may differ based on changes in soil conditions (Cheng et al. 2010). The bacteria that give a few advantages to plants are (I) those that form nodules on host plant roots (symbiotic relationship) and fix nitrogen; (ii) those that don't have any harmful effect on host plant while multiplying inside the plant tissues; (iii) those that have potential of competitiveness for their survivability in rhizosphere and surface of plant roots; and (iv) those that occur in soil in free living condition. In farming, useful microbes are generally characterized with their tendency of colonization in roots of plants following seed priming or seed treatment and improve plant development by expanding submergence of seeds, plant weight, and yield of crops. In spite of the constrained information of soil bacteria and plant connections, some of these bacteria are utilized economically as aides to farming practice. These bacteria comprise *Burkholderia cepacia*, *Delftia acidovorans*, *Paenibacillus macerans*, *Pantoea agglomerans*, *Pseudomonas* spp., *P. aureofaciens*, *P. chlororaphis*, *P. fluorescens*, *P. solanacearum*, *Bacillus* spp., *B.mucilaginous*, *B. pumilus*, *B. subtilis*, *B. amyloliquefaciens*, *B. fimus*, *B. licheniformis*, *B. megaterium*, *Agrobacterium radiobacter*, *Azospirillum brasilense*, *A. lipoferum*, *Azotobacter chroococcum*, *P. syringae*, *Serratia entomophila*, *Streptomyces* spp., *S. griseoviridis*, and *S. lydicus* (Amaral et al. 2016; Niu et al. 2015; Etesami et al.

2014a, b, 2015; Etesami and Alikhani 2016a, b). Generally, plant-advantageous bacteria help the plant development with two systems: (I) in direct mechanism by either aiding in acquisition of resources (N, P, Fe, and other essential nutrients) or directing levels of plant hormone or (II) in backhanded activity components by diminishing the pernicious impacts of different pathogens on the development and yield of plants as bio-control specialists. Till date, there are several studies that have been conducted in both pot and field experiments with significant contributions of plant growth-promoting bacteria that benefit to plant in various modes of aspects such as nutrient acquisition, growth, yield, and useful attributes related to crop productivity and soil health (Table 9.2).

9.5 Potassium Solubilizing Bacteria (KSB)

Microbial community impacts fertility of soil by means of various activities like dissolution, enhancing the availability of nutrients, and improving the nutrient acquisition (Parmar and Sindhu 2013). As of late, potassium solubilizing microbes have pulled in consideration of researchers as soil inoculant to improve the development of plant and yield. These microorganisms are powerful in discharging K from inorganic and insoluble pools of aggregate soil K by solubilization process (Sindhu et al. 2014). K solubilization is performed by an extensive range of saprophytic bacteria, fungal strains, and actinomycetes. There are solid confirmations that soil bacteria are equipped for changing soil K to the forms accessible to plant (Saiyad et al. 2015). The bacteria expanding the general execution of plants by giving for the most part dissolvable K to plants in various production systems are categorized as plant growth-promoting bacteria. There is an impressive population of KSB in soil and rhizosphere of plants. These incorporate both aerobic and anaerobic isolates in that the most frequent KSB in soil are aerobic. An extensively higher concentration of KSB is generally found in the rhizosphere in comparison with non-rhizosphere soil (Padma and Sukumar 2015). Solubilization of K by KSB from insoluble and settled forms is an important aspect as regards K accessibility in soils. Bacterial isolates having K-solubilizing potential can be screened by using modified Aleksandrov medium which is mainly based on halo zone formation surrounding the bacterial colonies as shown in Fig. 9.1 (Rajawat et al. 2016; Hu et al. 2006). The capacity to solubilize the silicate rocks by *B. mucilaginosus*, *B. circulanscan*, *B. edaphicus*, *Burkholderia*, *A. ferrooxidans*, *Arthrobacter* sp., *Enterobacter hormaechei*, *Paenibacillus mucilaginosus*, *P. frequentans*, *Cladosporium*, *Aminobacter*, *Sphingomonas*, *Burkholderia*, and *Paenibacillus glucanolyticus* has been described. Amongst the soil bacterial groups, *B. mucilaginosus*, *B. edaphicus*, and *B. circulans* have been explained as effective K solubilizers (Table 9.3). The microbial solubilization of K is strongly affected by pH, the bacterial strains utilized, oxygen, and sort of K-bearing minerals; in fact, moderate alkalinity supports the solubilization of silicate (Sheng and Huang 2001).

Table 9.2 Influence of different plant growth-promoting bacteria showed the beneficial effect on various parameters related to plant health and soil fertility amongst different crops

Crops	Bacteria	Pot/field trial	Results	References
Alfalfa	Unidentified	Pot	Shoot dry weight was significantly increased (16.2–59.0%)	Piccini and Azcon (1987)
Chickpea	*Bacillus polymyxa, Pseudomonas straita*	Pot	Increase in grain (14.3–21.4%) and straw (3.4–6.8%) yield. B.p. gave maximum grain yield, while P.s. improved straw yield	Alagawadi and Gaur (1988)
Mungbean	*Bacillus subtilis*	Field	Increased biomass, grain yield, and P and N uptake of mungbean grown in a P-deficient field on addition of rock phosphate and *B. subtilis*	Gaind and Gaur (1991)
Chilli	*Burkholderia tropica* KS04	Pot	Showed the greatest efficiency in promotion of chilli growth. It significantly increased the growth, flowering, and P-uptake, compared to uninoculated plants.	Surapat et al. (2013)
Wheat	*Pseudomonas striata*	Field	Significant increase in yield by inoculation of *P.s.* in presence of paddy straw	Varma and Mathur (1989)
Wheat	*Pseudomonas fluorescens* and *Serratia* sp.	Pot	Higher values around 64% in P uptake by wheat plants after 60 days of growth was observed with immobilized *P. fluorescens* + 3.25 mg P kg^{-1}	Schoebitz et al. (2013)
Sunflower	*Bacillus*	Field	Highest seed yield of sunflower possible with 100 kg P_2O_5 ha^{-1} fertilizer was achieved with about 50 kg P_2O_5 ha^{-1} when used in conjunction with PSB	Ekin (2010)
Alyssum serpyllifolium and *Brassica*	*Pseudomonas* sp.	Pot	Increased significantly the biomass (*B. juncea*) and Ni content (*A. serpyllifolium*) in plants grown in Ni-stressed soil	Ma et al. (2011)

(continued)

Table 9.2 (continued)

Crops	Bacteria	Pot/field trial	Results	References
Green gram (*Vigna radiata*)	*Bradyrhizobium*	Pot	When herbicide-tolerant *Rhizobium* strain MRP1 was used with herbicide, it increased the growth parameters at all tested concentrations of herbicides	Ahemad and Khan (2009)
Different genotypes of *Brachypodium distachyon*	*A. brasilense* and *Herbaspirillum Seropedicae*	Pot	Both bacterial and plant genotypes were critical to a successful interaction, and *H. seropedicae* showed strong epiphytic and endophytic colonization of roots	do Amaral et al. (2016)
Groundnut	Fluorescent *Pseudomonas*	Pot and field	PGPR1, PGPR2 and PGPR4 significantly enhanced pod yield (23–26%, 24–28% and 18–24%, respectively), haulm yield and nodule dry weight over the control in 3 years	Dey et al. (2004)
Cotton	*Bacillus* sp.	Field	Inoculation of *Bacillus* sp. significantly increased the seed cotton yield, number of boll/plant, boll weight, plant height, GOT (%) and staple length. Phosphorus in plant matter was also higher (0.39%) as compared with control (0.36%)	Akhtar et al. (2010)
Fababean	Unidentified PSB (JURB48+ JURMB69)	Pot	Plant height, root length, phosphorus content, P uptake and nodule number and weight were enhanced due to inoculation compared to uninoculated control in the presence or absence of phosphate sources	Demissie et al. (2013)
Black gram	*Pseudomonas aeruginosa*	Pot	Plants showed lessened cadmium accumulation, extensive response to improve plant growth	Ganesan (2008)

(continued)

Table 9.2 (continued)

Crops	Bacteria	Pot/field trial	Results	References
Maize	*P. aeruginosa*, *P. fluorescens*, and *Ralstonia metallidurans*	Pot	Promoted plant growth, facilitated soil metal mobilization, and enhanced Cr and Pb uptake	Braud et al. (2009)
Vigna radiata	*Rhizobium phaseoli*	Pot	In the presence of tryptophan, *Rhizobium* mitigated the adverse effects of salinity and increased the plant height, number of nodules per plant, plant biomass, grain yield, and grain N concentration significantly	Zahir et al. (2010)
Soybean and wheat	*Pseudomonas* sp.	Field	Significantly increased soil enzyme activities, total productivity, and nutrient uptake	Sharma et al. (2011)
Maize	*Klebsiella* sp. Br1, *Klebsiella pneumoniae* Fr1, *Bacillus pumilus* S1r1 and *Acinetobacter* sp. S3r2	Greenhouse	Showed the highest N_2-fixing capacity of 30.5% (262 mg N_2-fixed plant^{-1}) and 25.5% (304 mg N_2-fixed plant^{-1}) of the total N requirement of maize top at D_{50} and D_{65}, respectively. It also showed higher ear yield (up to 30.9%) with reduced fertilizer N input	Kuan et al. (2016)
Sedum plumbizincicola	*Bacillus* sp.	Pot	Significant enhancement in shoot & root biomass and leaf chlorophyll content. It also showed higher cd and Zn accumulation in root and shoot	Ma et al. (2015)
Calabrese	*B. oleracea* var. *italica*, *Bacillus amyloliquefaciens* subsp. *plantarum*, *B. subtilis* and *B. cereus*	Pot and field	Use of PGPR promoted size inequality within crop yield, but no significant change in yield	Gange and Gadhave (2018)

(continued)

Table 9.2 (continued)

Crops	Bacteria	Pot/field trial	Results	References
Myracrodruon urundeuva	*Azospirillum lipoferum*	Greenhouse	Increase of 30% root length, 50% root dry weight, 34% shoot dry weight and 10% soluble protein content with inoculation of *A. lipoferum* and inoculated plants showed 5% higher leaf water potential than control	de Oliveira et al. (2018)

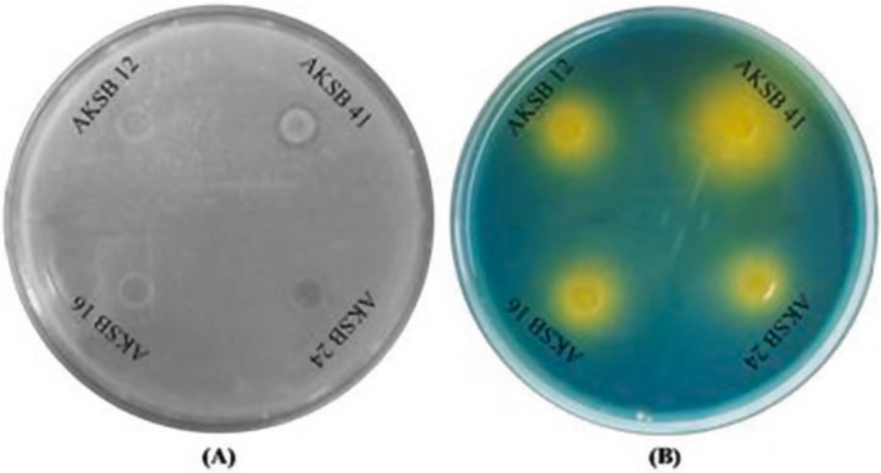

Fig. 9.1 Elucidation of halo zone on Aleksandrov and modified Aleksandrov medium. (Source: Rajawat et al. 2016)

9.6 Action Mechanisms of KSB in Solubilizing K

In present time there is small evidence accessible on K solubilization using KSB, which showed systems of silicate mineral dissolution to pass K to enhancing the growth and yield of various plants. Diminishing pH by means of produced organic acids and protons by KSB, expanding complex formation of cations by bounding to K, and acidolysis of encompassing region of KSB are some known activity components of KSB in process of K solubilization (Maurya et al. 2014). As happens on account of P solubilization, major system of K mineral solubilizations also have similar activity of organic and inorganic acids released by KSBs. Since organic acids are also supplemented by chelation, complex lysis, acidolysis, and exchange responses which are main means attributed to their translation in soluble form of K. The kinds of numerous organic acids that are generated by microbial strains

Table 9.3 List of bacteria which have potential to release K from different insoluble sources of K

Potash solubilizing bacteria		Insoluble potash minerals	References
Genus	Species		
Azotobacter	–	Feldspar	Yi et al. (2012)
Agrobacterium	*tumefaciens*	Waste mica (muscovite and biotite)	Meena et al. (2015)
Bacillus	–	Muscovite, potassium aluminosilicate, feldspar	Mikhailouskaya and Tcherhysh (2005), Rajawat et al. (2014), Yi et al. (2012), and Syed and Patel (2014)
	mucilaginosus	Illite powder, Montmorillonite, kaolinite, feldspar, Muscovite mica, and waste mica	Han and Lee (2005), Hu et al. (2006), Zhou et al. (2006), Sugumaran and Janarthanam (2007), Basak and Biswas (2009), Singh et al. (2010), and Basak and Biswas (2010)
	globisporus	Biotite	Sheng et al. (2008)
	pasteurii	Feldspar and bentonite	Youssef et al. (2010)
	megaterium	Kaolinite, muscovite and biotite mica	Diep and Hieu (2013) and Keshavarz Zarjani et al. (2013)
	coagulans	Kaolinite	Diep and Hieu (2013)
	metallica	Mica	Saiyad et al. (2015)
	firmus	Potassium aluminosilicate	Rajawat et al., (2014)
	cereus	Potassium aluminosilicate	Rajawat et al. (2014)
	mycoides	Potassium aluminosilicate	Rajawat et al. (2014)
	amyloliquefaciens	Mica powder	Gundala et al. (2013)
	licheniformis	Waste biotite	Saha et al. (2016)
Burkholderia		Mica	Mursyida et al. (2015)
Microbacterium		Feldspar	Yi et al. (2012)
Paenibacillus	*glucanolyticus*	Wood ash	Sangeeth et al. (2012)
Brevibacillus		Waste muscovite	Bahadur et al. (2017)
Enterobacter	*hormaechei*	Potassium aluminosilicate	Prajapati and Modi (2012) and Zhang and Kong (2014)
	cloacae		
		Mica	Bakhshandeh et al. (2017)
Pseudomonas	–	Potassium aluminosilicate	Syed and Patel (2014)
	putida	Mica	Mursyida et al. (2015)
	azotoformans	Waste biotite	Saha et al. (2016)
Klebsiella	*variicola*	Potassium aluminosilicate	Zhang and Kong (2014)
Alcaligenes	*piechaudii*	Aleksandrov medium	Verma et al. (2015)
Serratia	–	Mica	Mursyida et al. (2015)

(continued)

Table 9.3 (continued)

Potash solubilizing bacteria		Insoluble potash minerals	References
Genus	Species		
Rhizobium	*Pusense*	Waste mica (muscovite and biotite)	Meena et al. (2015)
	Potassium solubilizing bacteria	Feldspar, leucite, and trachyte	Setiawati and Mutmainnah (2016)
Pantoea	*ananatis*	Mica	Bakhshandeh et al. (2017)
Rahnella	*aquatilis*	Mica	Bakhshandeh et al. (2017)

Table 9.4 Various predominant organic acids produced by potassium solubilizing bacteria

KSB	Organic acids secreted	References
Bacillus mucilaginosus	Oxalic and citric	Sheng and He (2006)
Pseudomonas sp.	Tartaric and citric	Krishnamurthy (1989)
Pseudomonas aeruginosa	Acetic, citric, and oxalic	Badr et al. (2006) and Sheng et al. 2003
Paenibacillus mucilaginosus	Tartaric, citric, and oxalic	Liu et al. (2012) and Hu et al. (2006)
E.asburiae and *B. metallica*	Lactic and gluconic	Saiyad et al. (2015)
Bacillus megaterium, *Pseudomonas* sp. and *Bacillus subtilis*	Lactic, malic, and oxalic	Taha et al. (1969)
B. megaterium, *E. freundii*	Citric and gluconic	Taha et al. (1969)
Arthrobacter sp., *Bacillus* sp., *B. firmus*	Lactic and citric	Bajpai and Sundara (1971)

which differed in diverse organisms (Saiyad et al. 2015) are citric, gluconic, and oxalic acids released by KSB (Table 9.4). These acids convert insoluble K sources to soluble forms of K that are simply acquired by the plant. Binding of organic acids with metal ions like Fe^{2+}, Al^{3+} and Ca^{2+} results into solubilization of K (Fig. 9.2). Generation of capsular polysaccharides and oxidation causes release of K from K-bearing minerals for plant uptake (Shelobolina et al. 2012).

9.7 Effect of KSB on Crop Production

Availability of high-yielding varieties of crop and the raised intensification of agriculture, the soils are becoming depleted in K stock at a quicker rate. Microbial inoculants ready to release K from silicate have the impact on plant development parameters, yield, and K take-up through plants under both pot and field conditions as described in Table 9.5 (Meena et al. 2014). Earlier reports suggests inoculation with KSB showed advantageous impacts on growth of cotton and rape, eggplant, pepper and cucumber, peanut, maize, sorghum, wheat, sudan grass, sorghum, and

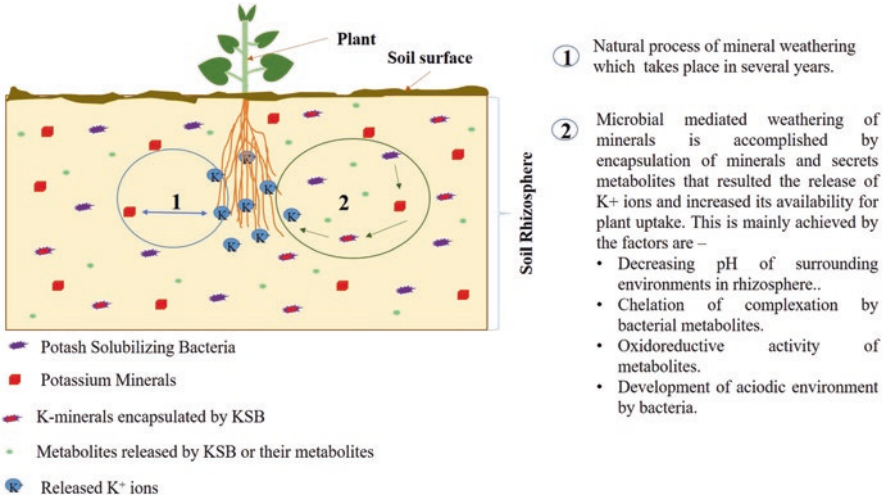

Fig. 9.2 Schematic diagram of interaction amongst plant, KSB and soil

tomato. Studies suggests that the application of KSB as biofertilizers for agriculture enhancement will result into decreased use of agrochemicals and help sustainable crop production (Archana et al. 2012).

9.8 Potentialities and Challenges of KSB in Industry

KSB increases weathering process of K minerals; particularly once in direct contact with mineral surfaces through various action means. Efforts have been made to use of K-mobilizing bacteria for solubilizing K from different K-bearing minerals (Saha et al. 2016) and therefore to increase plant nutrition. In spite of the fact that KSB could be a substitute and reasonable innovation to dissolve insoluble K sources into soluble forms, their application in farming practice is still avoided due to many factors. For instance, absence of information about biofertilizer amongst the farmers, moderate impact of the K biofertilizer on crop yield, low curiosity in scientific group on the advancement of K biofertilizer techniques, microbial deposition banks not yet established for KSB particularly because of this loss of proficient strains developed by scientists, and inadequacy in innovation in regard to carrier sustainability and product formulations are a portion of the real imperatives and constraints of the industry, which are expected to be improved soon.

9.9 Conclusions and Future Perspectives

Minerals bearing K showed leading place in the Earth's crust contributing K fertilization for crop plants. Plants acquired the K supply from soil solution that contains available K. Subsequent to this uptake, K is released into the soil from insoluble

Table 9.5 Effect of selected potassium solubilizing bacteria on various parameters related to plant health and soil fertility amongst different crops

KSB	Plant	Pot/field trials	Results	References
Mesorhizobium sp., *Paenibacillus* sp. and *Arthrobacter* sp.	Ryegrass	Pot	Inoculating the three strains into available K limit soil increased available K content significantly. The result of the pot experiment revealed that the three strains increased ryegrass growth vigour, biomass yield and K uptake to different degrees in available K-deficient soil. S-17 showed the most pronounced ryegrass growth promotion ability	Xiao et al. (2017)
Bacillus mucilaginosus	Sudan grass	Pot	Application of mica significantly enhanced biomass yield, uptake and per cent K recoveries by Sudan grass than control (no-K). Significant correlation between biomass yield, K uptake by Sudan grass and different pools of K in soils were observed	Basak and Biswas (2009)
Bacillus mucilaginosus, *Azotobacter chroococcum*, and *Rhizobium* spp.	Maize and wheat	Pot under phytotron growth chamber	Higher biomass accumulation, potassium content and uptake by plants as well as chlorophyll and crude protein content in plant tissue. Amongst the rhizobacteria, *Bacillus mucilaginosus* resulted in significantly higher mobilization of potassium than *Azotobacter chroococcum* and *Rhizobium* inoculation	Singh et al. (2010)
Bacillus mucilaginosus and *Azotobacter chroococcum* A-41	Sudan grass	Pot	Significantly higher biomass accumulation and nutrient acquisition were obtained in all the pots treated with mica and/or bacterial strain as compared to control. Co-inoculation of waste mica with *B. mucilaginosus* and *A. chroococcum* A-41 resulted in highest biomass production and nutrient acquisition	Basak and Biswas (2010)

(continued)

Table 9.5 (continued)

KSB	Plant	Pot/field trials	Results	References
Bacillus edaphicus	Cotton and rape	Pot	Found to increase root and shoot growth of cotton and rape. In cotton and rape growing in soils treated with insoluble potassium and inoculated with strain NBT, the potassium content was increased by 30% and 26%, respectively	Sheng (2005)
Bacillus edaphicus	Wheat	Pot	The root growth and shoot growth of wheat were significantly increased by *B. edaphicus* NBT and the mutants MPs^{++} and $MPs^{+}1$. Bacterial inoculation also resulted in significantly higher N, P, and K contents of plant components	Sheng and He (2006)
Bacillus megaterium var. *phosphaticum* and *Bacillus mucilaginosus*	Pepper and cucumber	Pot	Combined together, rock materials and both bacterial strains consistently increased further mineral availability, uptake and plant growth of pepper and cucumber, suggesting its potential use as fertilizer	Han et al. (2006)
Bacillus circulans	Khella	Field	Biofertilization with *B. circulans* F5 and their interactions. The highest values of all parameters were observed when the plants received calcium superphosphate and/or rock phosphate at the high rate. In regard to biofertilizer treatments, all of them led to a significantly increase in the growth criteria during the two successive seasons. The similar results were obtained in potassium treatment	Hassan et al. (2010)

minerals, but it is smaller as per the requirement of plants, because the amount of soluble K in the soil solution is very low and K is relatively immobile in the soil. Hence, to meet up requirements of plant, K-fertilizers should be used, which are a current exercise to provide accessible K in widespread agricultural systems (Zhang et al. 2013). Due to the higher price of these fertilizers, extended application cause enhanced cost of inputs. The farmers faces many direct or indirect problems like decline in the agricultural output, and multiple environmental constrains due to having heavy metal accumulation in soil and plant system. These toxic chemicals

accumulate into the fruits and vegetables and at last human body (Tuli et al. 2010). It has been notable that the utilization of KSB can be a promising strategy to solubilize K from soil and convert it into accessible form for plants, bringing about advancement of plant development and limiting the use of K-fertilizers. Solubilization of K is performed by numerous bacterial strains like *B. mucilaginosus*, *B. edaphicus*, *B. circulans*, *Pseudomonas*, *Burkholderia*, *Acidithiobacillus ferrooxidans*, and *Paenibacillus* spp. Earlier, researches well explained that by excreting organic acids KSB were capable to release K from various insoluble sources of K-minerals. Amongst achievement of KSB in making K accessible to plant, production of organic acids is major means, which can either directly increase K-releasing ability by either a proton- or ligand-mediated mechanism, or they can also indirectly increase release of K by the development of complexes in solution with insoluble sources of K. Hence, the use of KSB as biofertilizer not only enhances growth and yield of plant but also reduces the application of agrochemicals causing eco-friendly crop production.

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Seed Biopriming with Potential Microbial Inoculants as Sustainable Options for Stress Management in Crops

10

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Abstract

Biopriming of seeds represents standard approach for introduction of disease resistance via biocontrol agents. Priming of seeds with beneficial microorganisms and biocontrol agents has been reported more efficiently for the management of diseases and pests as compared to other available methodologies. The technique is also reported to stimulate cellular, molecular, and biochemical defense responses in plants toward resistance induction against abiotic stresses. Plants essentially live with microbial communities that colonize aerial parts as well as roots both externally (epiphytic) and internally (endophytic). By providing nutritional and defense-related support influencing distinct genetic cascades, biochemical pathways, and metabolite accumulation or excretion, microbes can fundamentally alter plant phenotypes and enable plants to tolerate stress conditions and at the same time enhance crop productivity. We discussed various techniques of seed biopriming as viable options for health management in crop plants and also presented case examples from rice fields.

Keywords

Microbial inoculants · Seed biopriming · Biocontrol · Disease management · Pest control · Crop health

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10.1 Introduction

Agriculture all over the country and that too, the crop production crops on which the livelihood security of a wider group of small and marginal farmers depends, is largely affected by biotic (caused due to pests and pathogens) and abiotic (salinity, alkalinity acidity of soils, moisture and drought stress, extremes of temperature conditions, etc.) stresses (Lopes and Foyer 2011). Although intrinsic capability of seed varieties is responsive to tolerate these stresses in various ways to protect plants and resist the losses in the productivity, it usually fails when the combined effects are more intensive. Although many chemical control options are available for the management of diseases and pests (biotic stresses) on crop plants, abiotic stresses are largely ignored due to no viable, sustainable, and long-term options exist. Plant pathogens cause many different kind of diseases in crop plants leading to severe loss in yield and productivity. At an estimate, 50–75% loss in yield is attributed to soilborne pathogens. Certain rapid and intense diseases like vascular wilts, root rot, and damping-off even causes more harsh effects and leads to ruining totally agriculture industries. Control of these soilborne pathogens imposes a problem as these pathogens are able to survive as sclerotia or mycelium for many years, even in varying environmental conditions. Initially, plant disease control depends on culture practices and chemical treatments, though they are not so effective in the current scenario and thus there is an urgent need of alternative approaches for sustainable agriculture. Organic approaches are emerging as an effective agent against soilborne pathogens in an environmentally friendly way (Aly et al. 2010; Mokhtar and El-Mougy 2014).

Diseases in plants can occurred due to structure or functional disorder of any system, mostly due to interference of any external factor like bacteria, viruses, fungi, or nematode. Most common plant diseases leading to loss in yield are wilt, blight, rust, root diseases, etc. A number of fungicides and bactericides are available in market for these pathogens, though they also have harmful effects over users of plant parts. To overcome their hazardous effects over human being, a number of alternative approaches are proposed. Seed priming is one of them; however, many different kinds of seed priming technologies are used including hydro-priming, biopriming, matrix priming, halo-priming, etc. Biopriming refers to biological seed treatment where seed hydration (physiological approach for disease suppression) and inoculation (biological approach for disease suppression) is carried out with beneficial organism. Biological seed treatment facilitates an improved and better substitute for chemical control, and preferentially, fungal antagonists are used against soil and seed-borne pathogens. It is reported that practice of *Trichoderma* for seed biopriming inhibits root rot pathogens in cowpea (Mondal and Bose 2014).

Agricultural production systems started using beneficial microbes approximately 60 years ago. Impact of microbes is evident in various crops like cereals, legumes, oilseeds, etc. Implication of seed biopriming through beneficial microbes is gaining recognition for management of biotic and abiotic stresses. In the present era of biological management of stress management, certain long-term and microbe-mediated viable options were developed and tested by the scientists to make plants defend

themselves from biotic and abiotic stresses in a better manner (Mondal and Bose 2014; Babalola 2010; Abuamsha et al. 2011a, b). Seed priming is extensively used over past decades for the purpose of physiological enhancement of germination. Seed priming is commercially accepted for seed germination over varying climatic conditions, especially for horticultural crops (e.g., carrot, lettuce, onion, pepper, etc.). Certain priming technologies also facilitate seed inoculation. In general, all the priming techniques involve incubation of hydrated seeds for limited period at specific temperature, followed by drying. Due to incubation, certain germination physiological processes are initiated in the seed, but the germination process did not lead to completion and thus seeds germinate faster.

10.2 Biopriming: A Potential Option

Crop productivity suffers from heavy loss due to diseases and pests under storage and field conditions. Majority of such diseases and pests are soilborne in nature (Ghanem et al. 2011). In usual practice, chemicals are being used for controlling seed and soilborne diseases. However, these methods, although viable, are less effective under field conditions due to various soil and environmental factors. Moreover, chemicals used for seed treatment mostly act as contact fungicides which are unable to protect the plants from foliar pathogens during the later stages of crop growth. Seed biopriming is a suitable alternative for chemicals as the microbes multiply continuously and occupy the growing root surfaces and form a biofilm around the roots to offer protection from soilborne pathogens in the growing plant stages (Mondal and Bose 2014). Further, the microbes can also elicit systemic resistance in the plants for protection from foliar pathogens during the later growth stages (Haas and Defago 2005). Due to these reasons, the concept of popularizing the seed biopriming technique among the farmers is gaining importance. This will not only ensure seed and crop health but at the same time also help to ensure long-term ecological sustainability at the field level (Verhagen et al. 2010; Nayata et al. 2010; Reddy 2013). In addition, seed biopriming can also enhance seed's nutritional and physiological characteristics for better germination and adaptation in various soil conditions. If entwined with other useful microorganisms, which are usually associated with the plant roots, it can further augment both plant productivity and immunity simultaneously (Moeinzaden et al. 2010; Dalling et al. 2011).

Biopriming represents standard approach for introduction of disease resistance via biocontrol agents. Priming of seeds with biocontrol agents is reported more efficient as compared to other available methodologies. It is also reported to stimulate other cellular defense responses which led to resistance induction. Plants essentially live with microbial communities that colonize aerial parts as well as roots both externally (epiphytic) and internally (endophytic). By providing nutritional and defense-related support influencing distinct genetic cascades, biochemical pathways, and metabolite accumulation or excretion, microbes can fundamentally alter plant phenotypes and enable plants to tolerate stress conditions and at the same time enhance crop productivity (Ghanem et al. 2011; Hardoim et al. 2012; Singh et al.

2013). The nature of microbe-mediated plant functional traits is widespread, effective, well proven in the literature, and quite diverse and can influence ecosystems through their effects on the functional values and population dynamics leading to defense against stress environment and plant growth promotion (Nelson 2004; Ma et al. 2011). Therefore, there is need to develop potential microbial inoculants for stress management in crops. The applicability of the same can be ensured at the level of facilitating low-cost commercial production of microbial inoculants and awareness generation among the farmers for adaptation of such methods and products. It can also be extended with the inoculation of efficient microbial strains with plants to deliver new avenues for enhanced crop productivity and soil fertility management (Mader et al. 2011; Tiwari et al. 2011).

Seed biopriming serves as crucial tool for coping with various stress conditions (biotic and abiotic). Owing to this, there is need for research activities for exploration of different novel biocontrol agents (fungi and bacteria) and their potential as biopriming agents. The most natural and intense microbial interactions not only help plants to adapt/tolerate environmental stresses that take place in the rhizosphere but can have an overall impact on the whole plant. Such interactions influence whole machinery of regulatory biosynthetic networks and their genes, proteome, and metabolic pathways not only in plant roots but at the distant parts of the plants also, leading to the activation of important responsive genes, protein, and enzymes and synthesis of a wide array of small-molecule metabolites that help plants withstand the challenges posed by the environmental stimuli and provide protection against instant damage (Babalda 2010). At the same time, signals and communicator molecules trigger long-term strategies in plant at genetic level to defend cells against oxidative stresses in distant parts also (Singh et al. 2013; Mariultto et al. 2014). Overall, the process of microbe-mediated Induced Systemic Stress Tolerance (ISST) in plants is integrated at the level of gene, protein, and metabolites and has proven capability of providing defense against abiotic stresses (Brotman et al. 2011; Adam et al. 2014). Understanding the impact of microbe-mediated biological, chemical, and physical complexities in the plants and the rhizosphere soil remains a great challenge which, if deciphered, can uncover the biological role of microbes for improved crop productivity in abiotic stress conditions, on the basis of which new microbial inoculants with stress-alleviating capacity in fields can be developed (Shoresh et al. 2010; Mader et al. 2011; Singh et al. 2013).

Biopriming is a simple farmer friendly and easily adaptable technique that can improve the vigor and seedling establishment and thereby plant efficiency in the field conditions especially in biotic or abiotic stresses (Jalilian et al. 2012; Negi et al. 2014). Sometimes, the early stages of germination are started but seedlings may not emerge, although there are reports which suggests that priming may allow the early DNA transcription and RNA and protein synthase to repair the physiological damage of seed cells and reduce the metabolic exudation (MacDonald 2000; Varier et al. 2010; Jabbarpour et al. 2014). These agents can improve seed germination characteristics and early emergence of seedlings to promote production of stranger plants. Being a viable and low-cost option with biologically sound

mechanism, this technique can be popularized among the farming communities as well as the extension workers to bring out mass penetration among wider rural sections for commercial gain (Moeinzadeh et al. 2010; Deryng et al. 2011).

10.3 Microbial Biopriming: Viable Technique

Microbial biopriming offers a viable technique of treating crop seeds using integrated physiochemical and biological methods. These options are safe, low-cost, and technically feasible in managing diseases, pests, and abiotic stress of crop plants as an alternative to control many seed and soilborne pathogens. Seed biopriming entrusts uniform emergence of the seeds sown even under adverse conditions of the environment. Various methods that have been used for priming are referred as hydro-priming, osmo-priming, drum priming, steeping priming, and solid matrix priming. Seed biopriming with bioagents (species of *Trichoderma*, *Pseudomonas*, *Bacillus*, *Beauveria*, etc. and actinomycetes) is one of the promising biological options for crop stress management being applied and tried in a successful manner. The methods are basically based on the natural management concept of plant-microbe mutual associations found throughout the biological kingdom and therefore are ecologically safer, naturally harmonic, economically cheaper, and biologically proven (Moeinzadeh et al. 2010; Mader et al. 2011; Piramyou et al. 2011; Siddikee et al. 2011; Singh et al. 2011; Kumar et al. 2013; Entesari et al. 2013; Monal and Bose 2014). Biopriming refers to the procedure of biological seed treatment which involves seed hydration followed by inoculation with useful microorganisms. It adds improvement to seeds in terms of viability, vigor indices, and germination. It also enhances plant growth and works as biocontrol agent against various diseases, ultimately leading to increase in crop yield. Mostly, bacteria or fungi are used for the seed biopriming. This approach represents an environmental friendly method in which specific microbes are used and they promote plant growth by different phenomenon, e.g., nutrient uptake enhancement, protection against plant pathogens, and production of plant growth-promoting substances. In current scenario, seed biopriming represents a better alternative over chemical treatment methods. It is an eco-friendly approach and safer for future agriculture and attaining recognition in the seed, plant, and soil health improvement projects.

Alternative options are considered; one among them is induction of plant resistance. As it is already known that plant defense mechanisms are induced and activated on simulation with proper agents leading to plant defense against pathogens, this process is called as induced systemic resistance (ISR) (van Loon et al. 1998). In crop sciences, Plant Growth Promoting Rhizobacteria (PGPR) are specifically reported as resistance inducers, though most of them are *Pseudomonas* spp. and are reported to be effective against numerous plant pathogens in a number of crops like cucumber, radish, tomato, sugarcane, and rice (Liu et al. 1995; Leeman et al. 1995; Raupach et al. 1996; Viswanathan and Samiyappan 1999; Burdman et al. 2000; Ongena et al. 2000; Ramamoorthy et al. 2001). ISR is emerging as a powerful alternative for chemical pesticides and is effective against a broad spectrum of

pathogens. Among possible sources for ISR, certain strains of nonpathogenic, root-colonizing PGPR are well characterized (Barka et al. 2000; Burdman et al. 2000; Ramamoorthy et al. 2001). A more specific term rhizobacteria-mediated induced systemic resistance (ISR) (van Loon et al. 1998) is applied for this phenomenon. *Pseudomonas fluorescens* strains are most widely used for this purpose, as they not only induce resistance toward pathogens but also enhance growth and development (Chen et al. 2000; Ongena et al. 2000; Ramamoorthy et al. 2001; Desai et al. 2002; Gnanamanickam et al. 2002).

Recent work by several research groups showed that microorganisms elicit "induced systemic resistance" (ISR) against biotic and abiotic stresses. Many of these organisms also increase nutrient uptake from soils, thus reducing the need for fertilizers and preventing the accumulation of nitrates and phosphates in agricultural soils (Dalling et al. 2011; Deryng et al. 2011). A reduction in fertilizer use would lessen the effects of water contamination from fertilizer runoff and lead to savings for farmers in addition to impart drought-tolerance capacity to plants. Several microorganisms capable of suppressing various soilborne diseases as well as foliar disease through induced systemic resistance mechanisms have been isolated.

Integration of chemicals, plant extracts, and biotic agents along with priming agents for managing plant diseases has been considered as a novel approach as it requires low amounts of chemicals, reducing the cost of control and pollution hazards while causing minimum interference with biological equilibrium (Reddy 2013). The use of fungicides, seed dressing chemicals, bioagents, microbial metabolites, or botanicals with priming agents has become an inevitable method of disease control, particularly in the absence of resistant cultivars (Deryng et al. 2011). Seed treatment with biocontrol agents along with priming agents may serve as an important means of managing many soil and seed-borne diseases, the process often known as "biopriming" (Singh et al. 2013; Yadav et al. 2013). Biopriming process had potential advantages over simple seed coating with bioagents and results in more rapid and uniform seedling emergence even under adverse soil conditions (Reddy 2013). Nano-biotechnology is being projected as one of the major relevant technologies for the effective and targeted delivery of bioformulation in the agricultural systems, and this technology also offers an economically viable option for minimizing ecological stresses and consumption of resources and leads to develop nano-carriers for the delivery of biocontrol agents within the bioprimed seed system (Hamza et al. 2013; Rangaraj et al. 2014).

10.4 Viable Methods

Seed biopriming involves soaking of seeds for 12 h in water, followed by addition of selected microbial bioformulation to presoaked seeds at the rate of 10 g/kg of seed. Treated seeds are then kept in polyethylene bags and covered with wet jute sack for preserving high humidity at 25–32 °C for 48 h. While in this duration, the bioagent over the seeds enhanced on overall surface as a protective layer on the seed

coat. These bioprimed seeds can be proceeded for sowing. Certain reports reflected storage of bioprimed seeds up to 2 months.

Currently, considerable interest is over-generation and incorporation of trait-specific microbial inoculants for seed biopriming to cope up with different abiotic stress conditions. Sufficient evidences are available for utilization of beneficial microbes for increasing plant's resistance toward different abiotic stresses, e.g., drought, salt, nutrient deficiency, heavy metal contamination, etc.). Seed biopriming exhibits competitive advantages over other delivery approaches and reduces physiological and pathological stresses in plants. Better plant promotion was observed for corn seeds after biopriming with *Pseudomonas fluorescens* AB254 in *Pythium ultimum*-infected soil. Further, biopriming of carrot seeds with *Clonostachys rosea* (IK726) provides resistance toward *Alternaria dauci* and *Alternaria radicina* (Jensen et al. 2004).

Vegetable crops are subjected to various pathogenic fungi during different stages, for instance, at sowing, seedling, flowering, etc., and lead to an extensive loss to farmers. Across the world, soilborne plant pathogens represent a major issue for farmers and cause significant loss in quantity and quality of yield. *Fusarium* spp., *Alternaria solani*, *Sclerotium rolfsii*, *Rhizoctonia solani*, *Macrophomina phaseolina*, and *Pythium* spp. are most prominent pathogen of vegetable crops (Abdel-Rehim et al. 1987; Celar 2000; Ramamoorthy et al. 2002; Hibar et al. 2006; Steinkellner et al. 2008). Currently, fungicides are widely used for the management of these pathogens, though due to malefic effects of these synthetic fungicides, non-synthetic safer alternatives are more preferred (Abdel-Kader et al. 2012).

In general, biopriming comprises seed coating with bacterial biocontrol agents (e.g., *Pseudomonas aureofaciens* Kluyver AB254) followed by hydration for 20 h at 23 °C in moist conditions; radicle growth is avoided. Seed priming leads to fast and homogenous germination of seedlings and is also effective in the unfavorable soil conditions (Rao et al. 2009). Seed biopriming with microbes involves seed coating with a microorganism suspension, followed by seed priming via different approaches, i.e., incubation in moist condition or solid matrix priming (Harman and Taylor 1988; Callan et al. 1991; Jensen et al. 2004; Pill et al. 2009).

Seed biopriming with biocontrol agents/microbes did not cause any modifications in the ecophysiological structure or physiological profiles of the microbial composition of rhizosphere contrary to the fungicides which modifies the metabolic profile of the rhizosphere bacteria (Correa et al. 2009). Selection of appropriate biocontrol agent for biopriming is also necessary as after the seed plantation, survival and growth of microorganism are essential for promoting plant growth and disease suppression. Different microbes owe different survival strategies in rhizosphere. For instance, *Pseudomonas chlororaphis* and *Pseudomonas fluorescens* are not able to proliferate well in rhizosphere, and their deficiency is reported, while different fungi (*C. rosea* and *T. harzianum*) are able to grow well (Bennett and Whipps 2008). Formulation ability also requires consideration before the biocontrol agent is selected for biopriming purposes. *Trichoderma* sp. is one such group of fungi which is extensively used as biopriming agents against a range of pathogens, e.g., *Pythium*, *Phytophthora*, *Rhizoctonia*, and *Fusarium* spp. (Ha 2010). Owing to

their plant growth-promoting traits and activity, next most important group for seed biopriming purpose is plant growth-promoting rhizobacteria (PGPR), which helps plant through colonization and synthesis of hormones (Lugtenberg et al. 2002; Somers et al. 2004), vitamins, and growth factors. They inhibit the growth of plant pathogens in rhizosphere via different mechanisms like induced systemic resistance, antibiosis, and competition for space and nutrients (Vessey 2003; Chandler et al. 2008; Kim et al. 2008; Lugtenberg and Kamilova 2009). They also possess good formulation ability due to which their large-scale use is also possible (Bhattacharyya and Jha 2012; Podile and Kishore 2006). Among PGPR, gram-negative *Pseudomonas* spp. (Weller 2007; Weller et al. 2002; Emmert and Handelsman 1999) and gram-positive *Bacillus* spp. (Richardson et al. 2009; Idris et al. 2007; Gutierrez-Manero et al. 2001; Whipps 2001; Kumar et al. 2011) are most widely used for biopriming (Mancini and Romanazzi 2014).

10.5 Cyanobacteria as Potential Priming Agent for Rice

Cyanobacteria are the potential candidates for biopriming of rice seeds. Many of the cyanobacterial strains have been used in the paddy fields as potential biofertilizers for fixing nitrogen and providing other benefits to the rice plants. However, there are several limitations such as uneven application in the field due to broadcasting of sand- or soil-mixed cultures, lack of point inoculation near the rice roots, need of high quantity of inoculum, and difficulties for the farmers to produce appropriate quantity of cultures for large field applications. Biopriming of rice seeds with potential cyanobacteria imparting the capabilities of high-nitrogen fixation, phytohormone production, and higher root association could be more potential and viable option as this will ensure point of inoculation at the site of rice roots, ease of delivery of inoculum, need of less inoculum size, and feasibility with the farmers to produce desired quantity of cultures with their own resources. Our rice seed biopriming and successive crop growth and developments in pots and fields for 3 successive years using various cultures of cyanobacteria, viz., *Nostoc commune*, *Anabaena doliolum*, and *Plectonema boryanum*, and a composite culture of all species prepared in equi-quantity composition yielded encouraging results (Fig. 10.1). Rice varieties (PR118, PR113, MTU1010, MTU7029, HUR105, PB1, PB115, and BPT5204) were coated with individual and composite cultures of cyanobacteria (5 g, moisture content 20 ± 2%; CFU 1.6×10^6), hydrated for 24 h, and then grown in pots and under field conditions. Both the rice seeds and cyanobacterial cultures remained viable and in good morphological and phenotypic appearance for more than 1 year. Bioprimed rice seeds showed enhanced germination percentage (10–16%), and primed plants showed increase in root length (5–9%), shoot length (12–17%), and seed vigor than non-primed plants. Increase in agronomic parameters was recorded in bioprimed plants, and the yield was enhanced by 5–9% in primed plants than non-primed plants in different varieties (Fig. 10.2).

Therefore, looking into the impact on rice seeds, the biopriming was proven to be an impactful technique for point inoculation of microbial species with definite

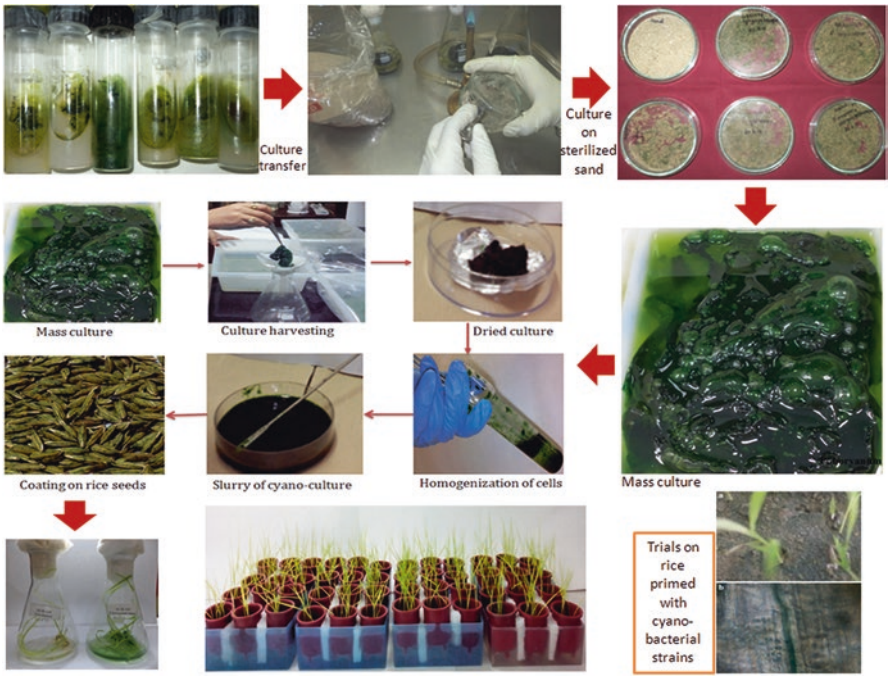

Fig. 10.1 From cyanobacterial isolates to mass culture: prospective ways of developing bulk cells for biopriming on rice seeds

traits and functions. This could not only boost crop health and development but support plant performance also under abiotic stressed conditions.

10.6 Conclusion

Nowadays, seed biopriming, development of efficient microbial biopriming agents, and their commercial circulation among the farmers are essentially needed. Identification of suitable microbial strains, formulation development, proper delivery mode, trials over fields at different locations, efficiency over different crops, and technology popularization among farmers and commercial production are extremely required. Apart from this, studies over the viability of the introduced microorganisms and its mode of work represent another area for instant attention. *Trichoderma* and *Pseudomonas* are broadly studied by different investigators, but there exist few reports over other beneficial microbes. Thus, research studies are required for identification and genetic manipulations of novel microbial agents with improved viability. Integrating bio-inoculants with proven advantages to seed through biopriming can effectively reduce biotic and abiotic stresses in agricultural system, thereby

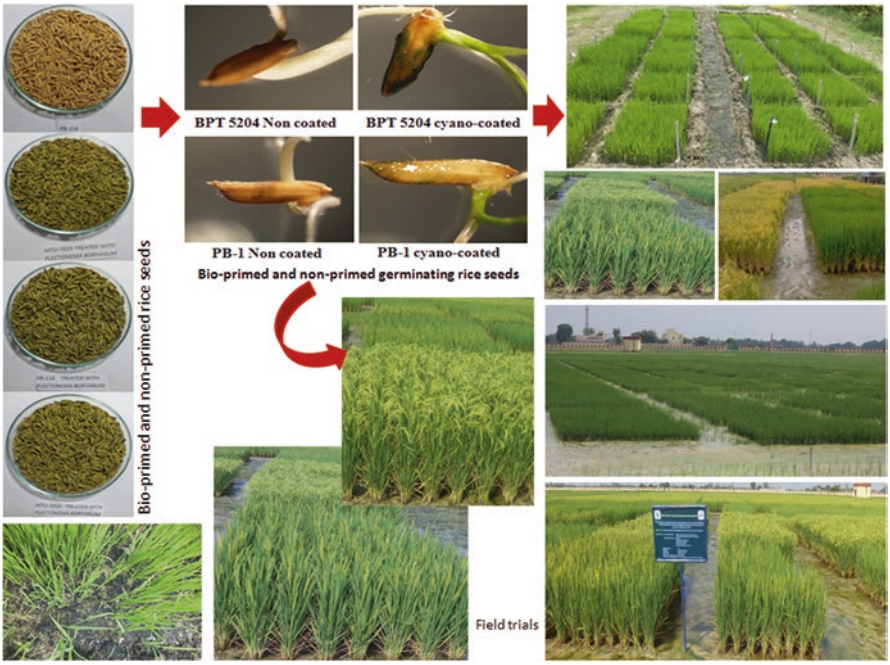

Fig. 10.2 Rice seed biopriming with cyanobacterial species: field-level impact assessment on different varieties at various agronomic parameters

enhancing the seed quality and crop yield in stressful environments with limited resources.

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Cropping Systems Effect on Soil Biological Health and Sustainability

11

Krishna Saharan, Ummed Singh, K. C. Kumawat, and C. S. Praharaj

Abstract

The influence on the chemical and physical soil composition, exerted from the applied cropping system, is dominated by the amount and kind of residual plant material. The cropping system, defined by the cropping sequence and type, as well as by plant residual management and natural and/or artificial fertilization, shapes the biological soil activities and environment for the soil micro-biotic habitat. Also climate and soil type exert an influence on the soil's biological activity in a significant amount. The effects, exerted from the farming practice on the soil microbial biomass, accumulate in a slow way and are often measureable only in the late stage, when changes in the microbial biomass already negatively affect fertility and stability of the soil ecosystem. Measuring the classical soil nutrition parameters does not always reveal these changes, and suitable soil health indicators are not established as a common standard. Soil microbial biomass turns out to be a good indicator for changes in the soil composition and shows potential for an early soil health indicator.

Keywords

Cropping systems · Pulses · Soil biology · Soil health · Soil microbial biomass · Soil enzymatic activity

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11.1 Introduction

Global agriculture is facing a changing scenario, an outcome from globalized agriculture production and worldwide trading of the products. With the industrialization of the food production, a trend to large-scale monoculture production systems has taken over the traditional crop rotation cultures, with their benefits for the soil health. Agriculture systems in many countries and regions are facing so-called second-generation problems characterized by degradation of the soil composition and texture, nutritional depletion (imbalance) of the soil, accumulation of herbicides and pesticides in the soil, resurgence of plant diseases and pest, depletion of groundwater, and increasing soil salinity (Fig. 11.1). These problems are on the short term alleviated by higher input of fertilizers, manpower (labor), and natural resources (e.g., artificial watering) which leads to decline in farm profits if the higher cost cannot be forwarded to the consumers. Crop rotation, as employed since long time ago in small-scale farming, shows a promising way to counteract these problems, enhances environmental safety, withstands weather aberrations, dampens price fluctuations, and regulates income from farming by maintaining or enhancing the soil health. Soil health can be seen as the overall soil capability to yield healthy plants in a sustainable long-term view, with a constant input of labor and external resources (e.g., fertilizer), and holds the key to sustainable food production in order to feed the increasing human population. Healthy soil can be defined by the ability to (a) provide physical support for the landscape itself (hills, mountains), vegetation, and external structures (e.g., buildings); (b) buffer natural rainfalls and filter/maintain the quality and level of groundwater; (c) produce plants, supply them with

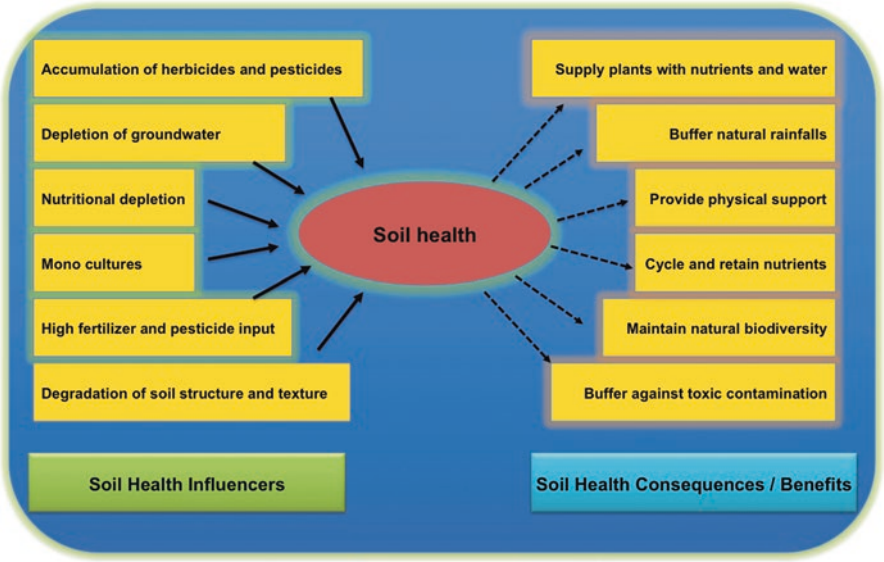

Fig. 11.1 Soil health influencers and benefits in the cropping system

sufficient water, and provide the habitat for soil organisms; (d) biochemically cycle and to retain nutrients that are essential for the growth and development of plants, such as nitrogen, phosphorus, potassium, or carbon; and (e) maintain the natural biodiversity and buffer against toxic contamination. These attributes are often influenced by agricultural management practices using excessive artificial inputs and the choice of the cropping system (Norris and Congreves 2018). In order to promote a successful and sustainable plant growth, the soil has to provide beneficial functions to the plants, which include (i) provide mineral nutrients for plant roots in proper form, within root-vicinity (space) and at the required time; (ii) supply water in the right quantity and with appropriate potential energy, available for ideally continuous uptake by plant roots; (iii) support the growth and spread of the macro- and micro-fauna as earthworms (*Lumbricidae*) and plant growth-promoting soil organisms as rhizobacteria and mycorrhiza fungi; (iv) facilitate sufficient root growth in providing low physical resistance by connected pores, supplying oxygen and removing carbon dioxide and toxic gases, and allowing sufficient rooting depth to generate the physical support needed.

Soil organic matter content is influencing most of these functions to a high degree. A high level of this soil organic matter is typically associated with higher soil aggregation and reduced erosion, improved nutrient cycling, as well as infiltration and also water retention and mobility (Meng et al. 2012). Recent research focus areas to elucidate the interactions and relationship between soil quality and the organic matter in soil are mainly (i) chelating agents (organic compounds) controlling the availability and toxicity of micronutrients for plants and related microorganisms, (ii) soluble or easy oxidable carbon as source of energy for microbial biomass, and (iii) conversion process of organic matter and its chemical energy in the nutrition chain (trophic levels) of the soil ecosystem which cycles nutrients and carbon. The productivity of the soil is primarily depending on its biological health, which includes the composition and amount of the microbial biomass with respect to organic carbon, soil nitrogen, and enzymatic activities. Microbes are the active agents for transforming organic matter and for recycling nutrients, affecting the sustainability in a large amount.

Another highly important biotic component of the soil ecosystem are microarthropods. They are involved in organic material decomposition, thereby increasing their availability to microorganisms and stimulating the overall nutrient turnover. Lacking general standards and minimum data sets turns objective assessment of soil health parameters into a challenge. Current available indicators for soil health include chemical properties (organic carbon, potentially mineralizable nitrogen), microbial biomass as well as soil enzymes, and respiration activities. As rhizospheric micro-organisms are contributing largely to the soil health condition, they shall be incorporated into any biological indicators for soil quality (Schloter et al. 2018). Recent studies have already emphasized the need to include soil organisms as an important parameter for soil health in order to reflect their importance in nutrient cycling, soil aggregation, and soil structure development. Linking proposed soil health indicators directly to soil functions is suggested by several authors; nevertheless, till to date there are no common standards or general guidelines of data

interpretation and value metrics describing the relation between soil biology composition/activity and soil health. This chapter's objective is to provide a summary of the soil health influencers and their indicators. Subsequently a brief description of commonly applied cropping systems and their exerted effects on soil fertility and productivity of succeeding crops is given.

11.2 Dominant Cropping Systems

The term "cropping system" describes the crops, the cropping sequences, and planting techniques used in a repeating sequence on a given agricultural area over a period of years. It represents the planting pattern employed by a farm, the allocation of farm resources, and deployment of available technology, determining their makeup. It comprises all time and physically related aspects in managing an agricultural production system. This includes also cropping a number of different crops grown simultaneously or in short succession on the same field. Using natural resources in an efficient and sustainable manner while generating a high yield and stable income for the farmer without negative side effects on the ecological soil environment characterizes ideal cropping systems. Cropping systems are either a result of improvements in agriculture technique, driven by changing market demand or available resources, defined by landowners or government decisions or simply environment- and climate-imposed facts as, e.g., nonproductive periods in winter times. Cropping systems can be mainly separated into sequential cropping systems with a planned and time-wise regular pattern of different crops, grown on a certain agricultural area, one after the other (crop rotation) and into intercropping systems where two or more different crops are grown together (at the same time) and in a spatial recurring sequence on a defined area of land. This means that different plant species are either grown simultaneously in short succession of each other or time-wise overlapping. Growing different plant species in a time sequential manner is referred to as crop rotation, and growing different plants simultaneously on a defined area is called intercropping (Malezieux et al. 2009). Cereal crops, legumes, oilseeds, and forage/fodder crops are the most important plants, and planting systems based on these crop types are worldwide dominating.

Climate change and resulting drought conditions are widely expected to exert higher challenges on food production systems in the future. Cropping yield is influenced by agronomic factors and several environmental parameters, with water availability and optimum temperature ranges among the most critical environment parameters (Awika 2011). Daryanto et al. (2016) have reported that agricultural yield correlates with both optimum environmental conditions (e.g., temperature, water, aridity) and agronomic parameters (i.e., crop species, phenological cycle, soil texture) at the same time. In this entry, we describe the major following cropping systems and soil enzymes, which affect the biological health of soil.

11.2.1 Cereal Systems

The cereals comprise a wide range of cultivated members of the grass family (monocotyledonous Poaceae, former Gramineae), often grown in an annual cycle. The plants feature a single growing cycle (monocarpic or semelparous species) and are having usually long, thin stalks with their fruits (grains) concentrated at the end. Examples of important cereals, where the starchy grains are used for food, are wheat, rye, maize, rice, oats, sorghum, millet, and barley. The terminus cereal is also used for secondary products that are processed out of the starchy grains of cereal plants like flours, breads, or pasta as further products. Cereals are a classical, worldwide-grown staple food with a higher (nutritional) energy contribution than any other type of crops. They are also a rich vitamin, mineral, and carbohydrate source and provide important fats, oils, and protein in their natural form as a whole grain (Sarwar et al. 2013).

Cereal cropping systems represent a vast range of agricultural production methods with the large-scale wheat and rice production areas worldwide, where both are often a classical monoculture cultivation system (Awika 2011). The specialization of large wheat farms in North America or the growing conditions in water-flooded fields for rice are resulting in these monoculture systems, but for rice, there are also crop rotation sequences, with, e.g., rice-legume employed. In contrast to legumes, cereals do not accumulate atmospheric nitrogen in nodules and require therefore artificial nitrogen supply for plant growth. The impact on the soil health of large-scale monoculture production areas is an ongoing discussion. Despite huge yield increase from this kind of cropping system, the needed artificial nutrients supply and the applied pesticides are affecting the soil health in a negative amount, which is not denied anymore. The dominance of cereals has a reported number of disadvantages for the farming systems: (a) depletion of soil nutrients over time, requiring replenishment by artificial sources of nitrogen and other nutrients; (b) declining factor productivity; (c) over reliance on high quantity of soil nutrients; (d) declining soil health; (e) in cereal cultures hard-to-control weed population development; (f) disease carryover between cereals, such as the root-borne crown root disease (*Fusarium pseudograminearum*) and the take-all disease (*Gaeumannomyces graminis* var. *tritici*); and (g) cyclic and simultaneous tendencies of market price movements of cereal crops and the resulting income dependency of the farmers (Brennan et al. 2004).

11.2.2 Pulse Systems

The second important group of crops, after cereals, are pulses. They provide a significant and balanced contribution for the nutrition of predominantly vegetarian populations. Their ability to biologically fix atmospheric nitrogen (BNF) and to release parts of unused nitrate into the soil makes them a highly valuable contributor to soil nutrition and soil health. They are also known to improve the soil microbial environment generally and to exudate organic compounds with low molecular

weight. These compounds serve as a nutritional substrate to soil microorganisms, resulting in the build-up of soil microbe populations (Lupwayi and Soon 2016). Having deeper-reaching and more abundant roots, they can reach and utilize higher amounts of water, stored in areas below the top-soil surface region, and are therefore more resistant to drought conditions, compared to shallow-rooted plants. The deep-reaching tap root system of pulse crops, like pigeon peas, makes them very suitable for intercropping with cereals and oilseeds, having shallow roots and which are often rain-fed. The table below is showing the various cropping systems for pulses used in India, depending on the regional cropping zone within the vast country (Singh et al. 2009). As indicated in the table, a sequential cropping system is employed in many regions with an alternating cereal-pulse sequence, especially in combination with rice as one seasonal cereal. Other cropping systems with the sole rice-wheat sequence, as found in the Indo-Gangetic plains, are under threat as a long-term decline in soil organic carbon (SOC) is observed, leading to a reduction of the overall productivity (Table 11.1).

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11.2.3 Oilseed Systems

Oilseeds are hardy crops and are reported as a suitable choice under rainfed conditions. They have potential for increasing overall return (profitability) by raising the cropping intensity with their stable return under harsh environment conditions. With their wide ability to adapt to environmental stress conditions, they benefit not only in terms of price. New introduced high-yield varieties are replacing lower yielding traditional crops because of higher returns gained by the better utilization of moisture and rainfall. The popular soybean delivers satisfactory yields in many countries when grown in the post-rainy season (rabi/summer). Sunflower can adapt to a wide range of soil types and is suitable for late planting in case of delayed or failed monsoon rain, or in case crops planted in the *Kharif* season have failed to grow. As a summer crop under limited irrigation, sesame shows a great potential in the highlands of Deccan (e.g., Andhra Pradesh/Telangana region). Safflower also shows economic advantage over other popular crops like coriander, chickpea, or rainfed wheat. Brennan et al. (2004) reported that intercropping of pulses with oilseeds turns out to be a profitable combination, as often the growth density of pulses can be kept and oilseed crops are grown additionally. Intercropping of winter pulses as chickpea and lentils with oilseeds is a common practice in rainfed areas of India. Studies conducted under AICPIP (during 1982–2006) showed that mustard-lentil, mustard-chickpea combinations in northern plains, chickpea-linseed in Central

Table 11.1 Important pulse-based cropping systems in different agro-climatic zones

Sl. no.	Agro climatic zones	States represented	Annual rainfall (mm)	Cropping systems
1	Western Himalayan Region	Jammu and Kashmir, Himachal Pradesh, Uttar Pradesh	1650–2000	Rice-chickpea/lentil/field pea, maize-chickpea/ field pea, ragi-chickpea/lentil/ field pea, maize/urdbean/mung bean-wheat, pigeon pea-wheat, mungbean/urdbean-mustard, common bean-potato
2	Eastern Himalayan Region	Assam, West Bengal, Manipur, Meghalaya, Nagaland, Arunachal Pradesh	1840–3530	Summer rice-urdbean/mungbean, rice-lathyrus, maize-maize-urdbean, maize-pigeon pea/horse gram, maize-chickpea/lentil/field pea, jute-urdbean-chickpea/lentil
3	Lower Gangetic Plains Region	West Bengal	1300–1600	Maize-chickpea/lentil/field pea, rice-chickpea/lentil/field pea, rice-chickpea+mustard/lentil
4	Middle Gangetic Plains Region	Uttar Pradesh and Bihar	1200–1470	Maize-wheat-summer mungbean/ urdbean, rice-potato-summer mungbean/ urdbean, rice-chickpea/lentil
5	Upper Gangetic Plains Region	Uttar Pradesh	720–980	Rice-wheat/potato-summer mungbean, maize-wheat/potato-summer mungbean, pigeon pea-wheat, mungbean/urdbean-wheat, sorghum (fodder)-chickpea
6	Trans Gangetic Plains Region	Punjab, Haryana	360–890	Maize-potato-summer mungbean/ urdbean, rice/maize-wheat-summer mungbean/ urdbean, maize-early potato-late potato-summer mungbean/ urdbean, rice- chickpea/lentil, maize-chickpea/ lentil/field pea
7	Eastern Plateau and Hills Region	Madhya Pradesh, Maharashtra, Odisha, West Bengal	1270–1430	Early rice-urdbean, rice-rice-cowpea, jute-maize-cowpea, jute-urdbean
8	Central Plateau and Hill Region	Madhya Pradesh, Rajasthan, Uttar Pradesh	490–1570	Sorghum (grain/fodder)-chickpea, fallow- chickpea, sorghum+pigeon pea-fallow, pearl millet+pigeon pea-fallow, rice/maize- chickpea/lentil/ field pea, moth bean/mungbean/ urdbean-wheat, pearl millet-chickpea
9	Western Plateau and Hill Region	Maharashtra, Madhya Pradesh, Rajasthan	600–1040	Urdbean-rabi sorghum, sorghum-potato-mungbean, cotton+urdbean/mungbean-fallow, sorghum-wheat-cowpea/ mungbean, cotton/sorghum-chickpea, mungbean/urdbean-safflower

(continued)

Table 11.1 (continued)

Sl. no.	Agro climatic zones	States represented	Annual rainfall (mm)	Cropping systems
10	Southern Plateau and Hill Region	Andhra Pradesh, Tamil Nadu, Karnataka	680–1000	Maize-sorghum+pigeon pea, sorghum-chickpea, pearl millet-horse gram, mungbean/urdbean-safflower, rice-mungbean/urdbean/cowpea, mungbean-sorghum/safflower, mungbean-pigeon pea, rice+rice mungbean/urdbean/cowpea
11	East coast Plains and Hills Region	Odisha, Andhra Pradesh, Tamil Nadu, Puducherry	780–1290	Rice-mungbean/urdbean, sorghum-mungbean/urdbean, tapoic+mungbean/urdbean, rice-rice mungbean/urdbean, rice-maize/cowpea, maize-horse gram/pigeon pea/chickpea
12	West Coast Plains and Hills Region	Tamil Nadu, Kerala, Goa, Karnataka, Maharashtra	2230–3640	Rice-urdbean/cowpea/chickpea, sugarcane+urdbean
13	Gujarat Plains and Hills Region	Gujarat	340–1790	Urdbean-safflower/niger, cowpea-safflower, mungbean-tobacco, pearl millet/sorghum+ pigeon pea-chickpea
14	Western Dry Region	Rajasthan	400	Pearl millet/ sorghum-chickpea+mustard, moth bean/mungbean-wheat, Cotton-chickpea

Adapted from Singh et al. (2009)

Plateau, and chickpea-safflower in the peninsular zone are the intercropping arrangements yielding highest return for the mentioned regions (Ali 1992; Singh and Rathi 2003).

11.2.4 Forage and Fodder Systems

Forage and fodder crops are a simple but also significant contributor in cropping systems. They are a simple answer to a common problem created by modern cultivation and fallowing practices, the decline in soil fertility, soil organic matter, and erosion. Forage is positively used on any type of land but particularly on marginal soils. It provides numerous benefits as improvement of soil quality, enhanced water management, reduction in weed population, increase in soil fertility (with legumes used), and subsequent yield and health increase for the following (cereal) crops. It also provides a more intense and deeper carbon sequestering and contributes therefore in reducing greenhouse gases. Forages can also aid to lower cost for nitrogen fertilizer and energy associated with applying nutrients (Singh et al. 2012). Farmers are using forage for positive results particularly on marginal cropland but are achieving them on any type of land.

Table 11.2 Different cropping sequences for fodder crop production

No.	Different cropping sequences	Expected yield
1	Maize + cowpea – maize + cowpea + seem + mustard	(300 q/ha) – (450 q/ha) – (1000 q/ha)
2	Sweet sudan + cowpea – berseem + oats	(1000 q/ha) – (1000 q/ha)
3	Hybrid Napier + Lucerne	(1250 q/ha) – (850 q/ha)
4	Maize + cowpea – jowar + cowpea – berseem + mustard	(300 q/ha) – (400 q/ha) – (1000 q/ha)
5	Teosinte + bajra + cowpea – berseem + oats	(1000 q/ha) – (1000 q/ha)
6	Sweet sudan + cowpea – mustard – oats + peas	(1000 q/ha) – (250 q/ha) – (500 q/ha)
7	Jowar – turnips – oats	(1800 q/ha)

Adapted from Geoffrey and James (2006)

The numerous benefits in both situations include higher soil fertility with leguminous crops, increased soil quality, improved water filtration and internal drainage, fewer disease in following cereal crops, reduced weed populations, higher yield and better economics in subsequent crops, and intensified and deeper carbon sequestering for greenhouse gas reduction. Research findings reported that the system of fodder production can vary regionally as well as locally or even from one farmer to the next (Singh et al. 2012). The individual fodder production system depends on available inputs as irrigation and fertilizers and also on insecticides/pesticides as well as on the landscape (topography) and is typically optimized for maximum livestock output per available production area. Maximum yield per production site, measured in either digestible nutrients or maximum livestock products, characterizes an ideal fodder system. Production shall also ensure sufficient succulent, palatable, and nutritive fodder to feed livestock on a daily basis throughout the year, and it shall be from high quality in terms of nutritional and flavor parameters. Growing high-yielding fodder crops, either as single or crop mixture, can increase overall yield. Also growing several (three or four) fodder crops in succession is helping to enhance production output on the given area. Even though forage requires specialized harvesting machinery, it needs less input in financial capital (cash). Compared to earlier times, harvesting equipment can be shared more easily with other farmers or rented from specialized organizations when needed. Some important fodder crops, crop rotating schemes, and expected yield under different regions in India are summarized in Table 11.2.

11.3 Soil Biological Health Indicators

11.3.1 Soil Microbial Biomass

The microbial biomass in the soil is considered as the living fraction/anchor of the soil organic matter (SOM), including bacteria, actinomycetes, fungi, algae, and microfauna in general, and represents typically 3–5% of the organic carbon within

the soil. It also serves as reservoir for the nutrients, even though, generally, the proportion of the biomass represents only 2–3% of the organic carbon (C) in soil. It is reported that declines in crop diversity tend to reduce soil microbial biomass, alter microbial functions, and threaten the provision of soil ecosystem services (McDaniel and Grandy 2016). Soil organic matter, created by decay of plant material and acting as an important source of plant nutrients, forms the variable (or labile) pool of the soil microbial biomass (SMB) and is perceived as one of the highly important contribution factors to soil fertility (Singh et al. 1989; Rai et al. 2018). Changes in microbial biomass affect the cycling of soil organic matter, stability, and fertility of the ecosystem in a negative way. Studies on soil microbial biomass carbon (SMBC), nitrogen (SMBN), and phosphorus (SMBP) in different natural and disturbed ecosystems showed an important influence on labile pool of carbon (C) and mineral nutrients (Smith and Paul 1990; Wardle 1992, 1999; Christos et al. 2014). The microbial biomass is an important factor in the transformation of soil nutrients and determines largely the biogeochemical cycle rate of C, N, and other nutrients. The applied cropping system affects the soil microbial biomass. It has been reported that crop rotations show to have large positive influence on soil carbon, nitrogen microbial biomass (McDaniel et al. 2014), plant pathogen suppression (Krupinsky et al. 2002), and yields (Smith et al. 2008; Riedell et al. 2009). This positive influence on the crop production has been generally referred to as the "rotation effect." Any change in the microbial biomass composition may influence the fertility and organic matter recycling in the soil and therewith the stability of that ecosystem. Many studies indicate a raise in soil microbial biomass with the addition of pulses in the cropping system. Including mungbean in a rice-wheat sequence shows increase of SMB. Similar results are found in the maize-based cropping systems, with maize-wheat-mungbean returning higher soil microbial biomass carbon (SMBC) as compared to maize-wheat only cropping (Singh et al. 2009). The effect of various cropping systems and their influence on the soil microbial biomass carbon and nitrogen are compared in Table 11.3. The type of vegetation, availability of substrate, and other abiotic factors in an ecosystem are influencing the microbial activity. Increased microbial activity has effect on the mineralization and reduction of mobilization of important plant nutrients as N, P, and S. As a biological indicator or index for soil, microbial activity can serve the dehydrogenase enzyme activity, which shows positive correlation to pulse cropping. As a dynamic and living organism, the SMB and its activity determine the organic matter transformation and regulation of the associated nutrient and energy cycling in soil. A turnover time of less than once per year and a quick response to conditions which leads eventually to an alteration of the soil quality turn the soil microbial biomass into a good pre-indicator for changes in soil health. Seasonal fluctuations induced from changes in climate conditions also affect microbial biomass, which tends to positive correlation (increase) with annual precipitation and shows negative correlation (decrease) with higher annual temperatures. Crop residues and root biomass as well as nutrient amendments, clay content, soil water content, and temperature influence the SMB, but also soil pH, C, N, and concentration of pesticides and heavy metals are affecting the quantity and quality of the soil microbial biomass. Measuring the SMB is

Table 11.3 Soil microbial biomass carbon (SMBC), SMBN, SMBP, and SMBK parameters by various cropping systems

Cropping system	Tillage practices		SMB (mg/kg soil)				References
			C	N	P	K	
Maize-wheat	BF + FYM		298	–	–	–	Singh et al. (2009)
Maize-wheat-mungbean	BF + FYM		*350*	–	–	–	
Maize-wheat-maize-chickpea	BF + FYM		*338*	–	–	–	
Pigeonpea-wheat	BF + FYM		*305*	–	–	–	
Rice-wheat			*305*				
Rice-wheat-mungbean			*376*				
Rice-chickpea-rice-wheat			*342*				
Rice-chickpea			*336*				
Maize + wheat			132				Venkatesh et al. (2013)
Maize + wheat + maize + chickpea			135				
Maize + wheat + mungbean			142				
Pigeonpea + wheat			150				
Rice-wheat	CT	-R	646	*201*	144	–	Choudhary et al. (2018)
		+Ri	1113	*343*	176	–	
	ZT	-R	890	*239*	153	–	
		+Rm	1181	*364*	163	–	
Maize-Wheat	CT	-R	895	*244*	157	–	
		+Ri	1500	*590*	208	–	
	ZT	-R	1278	*416*	188	–	
		+Rm	1990	*729*	213	–	
Chickpea	Sole	Rhizosphere	180	–	16		Tang et al. (2014)
Chickpea	Sole	Bulk soil	150	–	14		
Chickpea + durum wheat	Intercropped	Rhizosphere	380	–	35		

(continued)

Table 11.3 (continued)

Cropping system	Tillage practices		SMB (mg/kg soil)				References
			C	N	P	K	
Durum wheat + chickpea	Intercropped	Rhizosphere	170	–	11		
Chickpea + durum wheat	Intercrop	Bulk soil	250	–	20		
Durum wheat	Sole	Rhizosphere	230	–	18		
Durum wheat	Sole	Bulk soil	190	–	23		
Durum wheat + lentil	Intercropped	Rhizosphere	275	–	13		
Lentil + durum wheat	Intercropped	Rhizosphere	170	–	36		
Lentil + durum wheat	Intercrop	Bulk soil	430	–	28		
Lentil	Sole	Rhizosphere	155	–	24		
Lentil	Sole	Bulk soil	160	–	20		
Maize-Weat-Mungbean	WS	–	448.4	–	–	–	Parihar et al. (2018)
Maize-Chickpea-Sesbania	WS	–	470.0	–	–	–	
Maize-Mustard-Mungbean	WS	–	344.4	–	–	–	
Maize-Maize-Sesbania	WS	–	373.2	–	–	–	
Rice + Wheat	Field A	MC	119	21.9	–	27.0	Yamashita et al. (2014)
Rice + Wheat	–	MCC	88.7	*18.6*	–	*17.0*	
Rice + Wheat	–	CF	63.8	*8.78*	–	*7.0*	
Rice + Wheat	–	NF	47.8	*3.55*	–	*8.5*	
Rice + Wheat	Field B	RSC	119	*19.5*	–	*18.5*	
Rice + Wheat	–	NPK	52.7	*7.61*	–	*4.8*	
Rice + Wheat	–	NP	42.8	*3.71*	–	5.0	

[a]*MC* livestock manure compost plot, *MCCF* livestock manure compost plus chemical fertilizer plot, *CF* chemical fertilizer plot without application of livestock Manure compost, *NF* no fertilizer plot, *RSC* rice straw compost plus chemical fertilizer plot, *NPK* chemical fertilizer plot, *NP* no potassium fertilizer plot, *Field A* long-term application of livestock manure compost, *Field B* rice straw compost and chemical fertilizers, *£ WS* winter soil, *MWMb* maize-wheat-mung bean, *MCS* maize chickpea-sesbania, *MMuMb* maize-mustard-mung bean, *MMS* maize-maize-sesbania, *ΨCT* conventional till, *ZT* zero till, *R* residue, *i* incorporated, *m* mulched

therefore considered to be the most general and practical indicator, and an increase is generally seen as a desired and beneficial change of the soil health (Shukla et al. 2006).

11.3.1.1 Soil Microbial Biomass Carbon

A small portion of the biologically significant soil labile C comes from the SMBC. As a fertility and soil health indicator, it is a sensitive parameter for soil management practices and serves as reservoir of nutrients (as N, P, S), and content in soil correlates in a positive way with the available soil organic matter. It has been demonstrated that straw incorporation over 18 years increased the biomass by about 50%, while changes in total organic matter remained undetected (Powlson et al. 1987). Chander and Brookes (1991) showed that the ratio of SMBC to soil organic-C was a sensitive indication for heavy metal effects on the microbial biomass using soils from two different field experiments. Under tropical conditions, continuous applications of fertilizers and organic manures have shown an increase in soil microbial biomass-C and biomass-N with a balanced fertilization. The studies by Wang et al. (2011) on SMBC and SMBN content from mixed plant residues revealed that incorporating residues from more than two plant species into soils could increase both SMBC and SMBN which then can contribute to restore vegetation and soil fertility in the Loess Plateau. The sensitiveness of the soil microbial biomass to changes in soil management qualifies it as a good indicator for soil quality. Tropical conditions accelerate the decomposition of plant materials and enhance the transformation of SMB to SMBC. Supplemental applications of organic fertilizers further increase the creation of SMBC in comparison to sole application of inorganic fertilizers. For example, the applications of farmyard manure along with N-P-K fertilizer result in higher SMBC concentrations as compared to fertilization with N-P-K only.

11.3.1.2 Soil Microbial Biomass Nitrogen

Part of the nitrogen potentially available for mineralization and available for plants is out of the soil microbial biomass (Choudhary et al. 2018). This SMBN represents a significant sink or source for nitrogen to the plants. A substantial amount of soil-borne N originates from pulses after their harvesting. Their unique ability fixing atmospheric N_2 makes them a valuable SMBN donor, with a contribution to the soil N budget in the range of 4–20 kg/ha and with chickpea in the upper range of the contribution.

11.3.1.3 Soil Microbial Biomass Phosphorus

Phosphatic fertilizer continues to be a significant player in intensive agriculture, even though declining availability of phosphorus (P) and raising production cost from depletion of natural resources turn it into a future critical issue. Legume crops are a valuable source for soil N, but they also aid in the efficient utilization of native P. The secretions of certain organic acid (root exudates) facilitate the solubilization of various phosphorus forms and increase the available P as a result of P-acquisition from insoluble phosphates through roots. This capacity makes legumes efficient in

native utilization of P present in different forms. As an example, the ability of chickpea to access P, normally unavailable to other crops, in mobilizing hardly soluble Ca-P by rhizosphere acidification through its citric acid root exudates in Vertisols, whereas pigeon pea is known having the ability to dissolute Fe-P in Alfisol.

11.3.1.4 Soil Microbial Biomass Potassium

Potassium (K) in microbial cells inhabiting the soil is considered to be the major K pool for plant growth. The high potassium demand of plants for their proper growth turns it into one of the essential nutrients, with K uptake equivalent or greater than the nitrogen uptake by the crops (Yamashita et al. 2014, Owa 2006). K is available in four different forms in soil: water-soluble, exchangeable, non-exchangeable or fixed, and structural or mineral form. Most readily available for plants are the water-soluble and exchangeable forms (Sparks 2011). The concentration of K is generally regulated higher within inside the cells than in the outside environment (Uozumi 2011). Also bacteria and fungi accumulate K inside their cells to a concentration above 0.18–0.2 M (Slayman and Tatum 1964). This turns the soil microbial biomass into a rich K pool. Despite this, relatively less is known in dealing with this potential K source.

11.3.2 Soil Enzymatic Activities

Microbiota, a particular form of soil microorganisms, have an essential role in elements cycling and soil structure stabilization (Saha et al. 2008). They are also taking the dual role as a source and sink for carbon and labile nutrients. Enzyme activities are linked to the decomposition of organic matter and soil remediation processes and to indicators of biochemical activities. In combination with other chemical or physical parameters, they can determine the quality level of soil (Gelsomino et al. 2006), and enzyme activity estimates are often used as indicators for soil fertility and microbial activity (Skujins 1978). Soil enzymes are reported to be important in soil functions (Dick 1997; Alkorta et al. 2013), and their activity may serve as useful indicators for changes in soil biology and biochemistry due to external management and environmental factors (Dick 1994) as enzymes react on changes in soil management long before changes in any other soil quality parameter becomes detectable. Soil enzyme activities catalyze the principal biochemical reactions involved in nutrient cycling and are highly responsive to natural and anthropogenic-induced changes. They also serve a relevant role in organic matter decomposition and the cycling of plant nutrients.

Soil enzyme activity can be considered as the accumulated long-term effect of soil microbial activity and viable population at the sampling site. As a large amount of samples can be analyzed in a short time (within few days) requiring only a small amount of soil, they are suggested as sensitive indicators for soil fertility (Nannipieri et al. 2012; Doran and Parkin 1994). The major soil enzymes and their related functions are given in Table 11.4 (Srinivasa et al. 2011; Das and Varma 2011). The main groups of enzymes involved in nutrient cycles including dehydrogenases,

Table 11.4 The effect of different field practices/ecosystem on various soil enzymes activities

Field practice	C-cycling enzymes	N-cycling enzymes	Phosphatase	Aryl-sulfatase	Dehydrogenase	Urease	References
Horticulture land use system	–	–	High	–	High	High	Bhavya et al. (2018)
Continuous cropping system	First increase then decline (invertase)	–	High	–	–	Low	Sun et al. (2018)
Continuous cropping system	First increase then decline (invertase)		High			Low	Sun et al. (2018)
Conventional tillage vs. no tillage	–	–	High	–	High	–	Choudhary et al. (2018)
Degraded vs. native vegetation	Low (cellulose)	–	–	–	Low	–	Araújo et al. (2013)
Forest vs. pasture vs. agricultural	Highest (β-glucosidase) in forest, lowest in agricultural soil	Highest (urease) in pasture, lowest in agricultural soil	Highest (alkaline phosphatase) in forest, lowest in agricultural soil	–	–	–	Kizilkaya and Dengiz (2010)
Organic residue with RDF (maize residue in rice and wheat cultivation)	High (invertase)	High protease	High alkaline phosphatase		High	High	Tao et al. (2009)
Organic vs. unamended (in bell pepper)	High (β-glucosidase)	High acid phosphatase	–	–	High	High	Gopinath et al. (2009)
Continuous fertilization, no fertilizer	High	High	High		High	Nonsignificant	Saha et al. (2008)

(continued)

Table 11.4 (continued)

Field practice	C-cycling enzymes	N-cycling enzymes	Phosphatase	Aryl-sulfatase	Dehydrogenase	Urease	References
Conservational vs. conventional tillage	High (β-glucosidase)	High protease	High		High	High	Roldan et al. (2005)
Conventional tillage vs. no tillage	High (cellulose) under no tillage	–	High under no tillage	High under no tillage	–	–	Balota et al. (2004)
Continuous cropping system	First increase then decline (invertase)		High			Low	Sun et al. (2018)

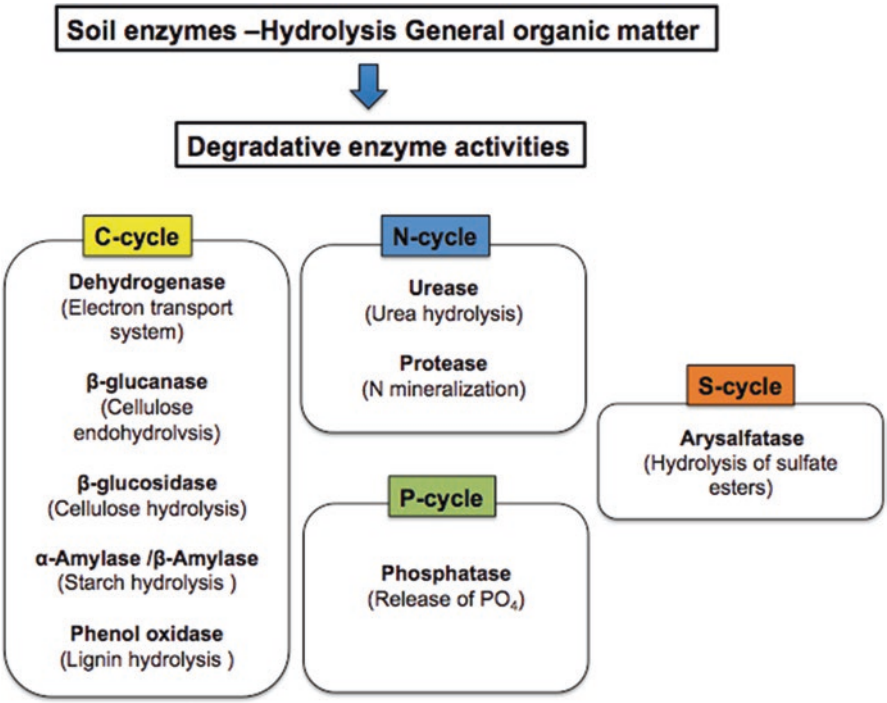

Fig. 11.2 Major soil enzymes as biological indicator of soil health

glucosidases, ureases, amidases, phosphatases, arylsulfatase, cellulases, and phenol oxidases are described (Fig. 11.2).

11.3.2.1 Carbon Cycling Enzymes

The carbon cycle process denotes the main constituent process of all living organisms, where primary producers fix atmospheric carbon dioxide and transform it to organic material. Microbes play a further important role in this cycle where autotrophic microbes are capable to fix carbon dioxide within the soil. Plants, as primary organic material producers in our terrestrial ecosystems contribute in significant amount to carbon fixation, although surface-dwelling algae and cyanobacteria, both free-living and symbiotic as lichens, may add to carbon fixation in some ecosystems in significant amount (Gougoulias et al. 2014). The organic material originating from the primary production is incorporated in living organisms and forms part of the nonliving organic materials, derived from decaying life. The ultimate recyclers of decaying organic material are heterotrophic bacteria and fungi. This kind of saprotrophic microorganisms closes the carbon cycle by converting the organic material, formed by the primary producers, back to carbon dioxide during respiration. This process of organic matter decomposition utilizes the degradation of nonliving organic material to derive energy for growth. Higher life forms, as

herbivore and carnivore beings, digest with gastrointestinal tract-inhabiting microbes organic material and support in this way the carbon dioxide cycle.

The mineralization of organic compounds occurs when they are entirely degraded to inorganic components, like carbon dioxide, ammonia, and water. The main activists for organic matter decomposition in soil ecosystems are fungi, representing the majority of the soil biomass. Nevertheless, bacteria as well as fungi are able to decompose and degrade complex organic molecules that cannot be broken up by higher organisms. A range of bacteria, especially out of *Actinobacteria* and *Proteobacteria*, are able to degrade soluble organic molecules such as organic acids, amino acids, and sugars (Eilers et al. 2010). Likewise, bacteria from phylum *Bacteroidetes* can aid in degrading more recalcitrant carbon compounds like cellulose, chitin, or lignin. Recalcitrant carbon compound-targeting bacteria may require quite large amounts of available N for supporting the creation of extracellular and transportation enzymes (Treseder et al. 2011), contrary to bacteria suited for low N environments, which are more proficient in metabolizing organic N compounds, such as amino acids. In soils with abundance of *Proteobacteria* and *Bacteroidetes*, a positive correlation of the net carbon mineralization rate was found, whereas it correlated negatively with *Acidobacteria* (Craine et al. 2013).

11.3.2.2 Nitrogen Cycling Enzymes

Nitrogen (N) is an essential element for protein and nucleic acids and is required by all organisms. Organic sources deliver the needed nitrogen for animals, whereas plants need nitrogen in inorganic forms, like ammonium and nitrate, or relatively depolymerized N sources such as single amino acids (e.g., glycine) (Schimel and Bennett 2004). Most microbes can utilize ammonium or nitrate for their growth, and they also take an important role in the nitrogen cycle. These microbes execute several processes not carried out by other organisms, like nitrogen fixation, dissimilatory nitrate reduction to ammonia (DNRA), ammonification, nitrification, and denitrification. The conversion rates of these microbial processes determine the availability of nitrogen where low rates can result in limiting the productivity of the underlying ecosystem. Only few microbial groups (e.g., nitrogen fixation or nitrification) mediate some of the process steps in the nitrogen cycle. These steps are known as narrow processes, whereas other steps are mediated by many groups (e.g., DRNA) and are considered as broad processes. Ammonification is known as the release of ammonium from soil organic matter during decomposition (Prosser 1989). Bacteria and archaea only carry out the biological reduction of atmospheric nitrogen to ammonium (biological nitrogen fixation – BNF). This BNF process is of crucial importance for the functioning of the entire ecosystem as it is the sole natural process through which atmospheric N enters the biosphere (Aislabie and Deslippe 2013). N-fixation is catalyzed by the enzyme nitrogenase, an extremely oxygen-sensitive enzyme, requiring an environment with low oxygen content for activity. The N-fixation is a process of high-energy expense; fixing 1 Mol of N_2 consumes the amount of 16 Mol of ATP. The produced ammonium becomes assimilated into amino acids and subsequently polymerized into proteins. Nitrogen-limiting conditions create an advantage for N-fixing microbes. Plant exudates may

supply some of the energy required for N-fixation which is carried out by free-living microbes (e.g., *Azotobacter, Burkholderia, Clostridium,* and some methanogens), some of them associated with the rhizosphere of plants, and by bacteria which form symbiotic relationships with plants (e.g., *Rhizobium, Mesorhizobium,* and *Frankia*). Rhizobia-forming root nodules in symbiotic relationships with human-introduced legumes such as clover, lucerne, or lotus became a significant nitrogen source for New Zealand's agricultural soils. In a similar way are native legumes (e.g., Sophora and Clianthus) forming symbiotic relationships with *Mesorhizobium* or *Rhizobium leguminosarum* (Weir et al. 2004). As reported, the nitrogen fixation rate generated by symbiotic rhizobia is often higher by a magnitude of two or three orders compared to free-living soil bacteria, indicating a mutual benefit for symbiotic life forms.

11.3.2.3 Phosphate Activity

The abundant organic phosphorus (P) in soil is able to provide nutrient P for plants and soil-borne microbes after hydrolysis and the release of free phosphates into the soil environment (Utobo and Tewari 2014; Condron et al. 2005). Plants and microbes secrete phosphatase enzymes into the soil, which are catalyzing this process. This secretion is actively driven by the demand for nutrient P or results from decaying cell, as a passive form of release. While microorganisms belonging to genera Actinomycetes produce rather negligible quantities of phosphatases are fungi, especially genera belonging to the *Aspergillus* and *Penicillium* type, as well as *Bacillus* and *Pseudomonas* bacteria mostly neutral phosphatase producer, as reported by Tarafdar and Chhonkar (1979). Phosphomonoesterase soil enzymes are showing activity under alkaline as well as under acid conditions and are therefore among the most studied enzymes. They can serve as biological soil quality indicators as they are acting on P-compounds with low molecular structure, including polyphosphates, sugar phosphates, and nucleotides (Makoi and Ndakidemi 2008). The evaluation of phosphatase activity in grassland in the temperate climate zone revealed a strong correlation between soil properties (P, N, pH, and clay content) and enzyme activity, as reported by Turner and Haygarth (2005). The amount of plant roots-exuded acid phosphatase differs between plant species, with legumes showing higher secretion as compared to cereals (Ndakidemi 2006; Yadav and Tarafdar 2001; Li et al. 2004). The higher P requirement of legume crops for the nitrogen fixation process in symbiosis with bacteria may attribute to this observation (Joachim and Patrick 2008). Crop management practice is also an active influencer of the phosphatase process, as the capability of soil mineral solubilization by phosphomonoesterases is considered to be on a higher level in the soil system with higher organic C content. Several studies confirmed a positive correlation between soil organic matter content and alkaline or acid phosphatase activity (Aon and Colaneri 2001; Aon et al. 2001), even though only few studies are available investigating the influence of crop management options on phosphatase activity in the soil ecosystem (Joachim and Patrick 2008). Understanding the phosphatase activity dynamics in the soil ecosystem is an important asset for anticipating the interactions as plant nutrient uptake and, in consequence, plant growth are governed by these interactions (Das and Varma 2011).

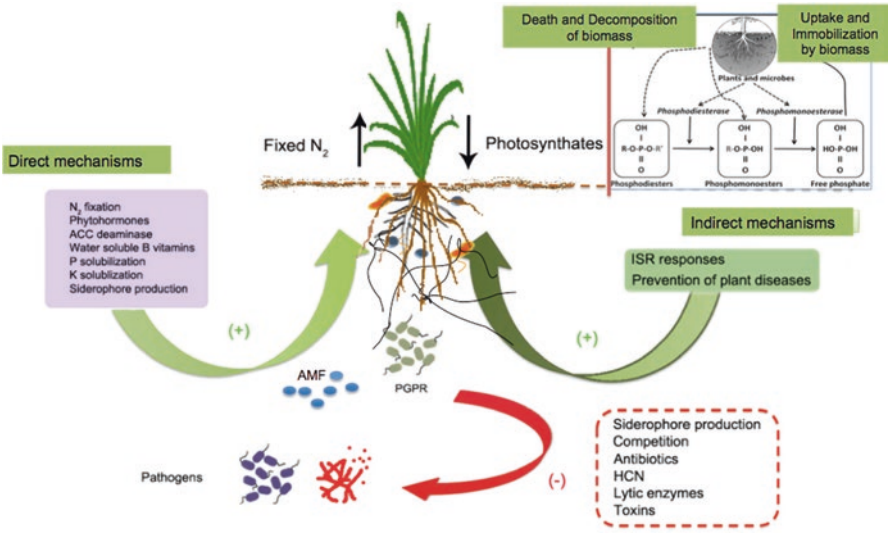

Fig. 11.3 A simplified conceptual model of plant nutrient uptake by microorganisms through direct and indirect mechanisms and turnover of organic phosphorus inputs from plants and microbes in soil

Phosphodiesterases in soil and related microorganisms are even less studied. Considering that the larger input of fresh organic P into the soil is out of the decomposition of phospholipids and nucleic acids, derived from the phosphodiesterase activity (Cosgrove 1967, 1980), the research on these topics is clearly underrepresented compared to its importance. For releasing free phosphate from a phosphate diester, both phosphodiesterase and phosphomonoesterase are required (Turner and Haygarth 2005). Phosphodiesterase releases by an initial hydrolysis a phosphate monoester which requires subsequent hydrolysis to release free phosphate. This second step is carried out by the phosphomonoesterase and creates P available for biological uptake (Fig. 11.3).

11.3.2.4 Arylsulfatase Activity

Arylsulfatase, a widely available soil enzyme, catalyzes the hydrolysis of organic sulfate ester to phenols and sulfate, or sulfate sulfur (Kertesz and Mirleau 2004; Utobo and Tewari 2014). The enzyme is found in bacteria strains of *Pseudomonas* sp., *Actinobacteria* sp., *Klebsiella* sp., and *Raoultella* sp., as well as in fungi like *Eupenicillium* sp. and *Trichoderma* sp. It is also found in plants and animals (Nicholls and Roy 1971) and was initially detected by Tabatabai and Bremner (1970) in soils. The secretion of arylsulfatases into the soil environment is mainly by bacteria as a response to sulfur limitation, as reported by Das and Varma (2011). According to the findings of McGill and Colle (1981) and Klose et al. (1999), the occurrence of arylsulfatase in various soils is many times correlated with the amount of microbial biomass and rate of sulfur (S) immobilization. Various soil environment

parameters influence the release of S from soluble and insoluble sulfate esters and depend on the type and content of organic matter (Sarathchandra and Perrott 1981), changes in the pH of the soil (Acosta-Martinez and Tabatabai 2000), heavy metal content (pollution) or organic sulfate esters concentration, and the extent of protection against enzymatic hydrolysis of organic sulfate esters, like sorption to particle surfaces in soils (Joachim and Patrick 2008). By now the knowledge about specific microbial genera or species having an important role in the soil organosulfur circle with arylsulfatase as the key enzyme is little (Kertesz and Mirleau 2004). Considering the importance of sulfate in plant nutrition, the role of arylsulfatase in S mobilization in agriculture soils is still a critical factor and requires more attention from the scientific institutions.

11.3.2.5 Dehydrogenase Activity

Dehydrogenase enzyme is able to oxidize soil organic matter and is seen as an integral element of intact cells. During the oxidation process, a transfer of electrons and protons from substrates to acceptors takes place, but the enzyme does not extracellularly accumulate in the soil (Das and Varma 2011). Dehydrogenase activities as abundant metabolic processes in healthy microorganisms to decompose organic matter are a general bio-indicator of microbial respiration activities in soils (Bolton et al. 1985), and this activity can therefore be used to indicate biological soil activity (Utobo and Tewari 2014). This enzyme requires a bacterium as host and is found only within certain soil bacteria, e.g., genus *Pseudomonas*, with most abundant in *Pseudomonas entomophila*. The presence of dehydrogenase in soil is therefore a valid indicator for the presence of soil bacterial cultures (Walls-Thumma 2000).

Addition of triphenyltetrazolium chloride to the soil makes organic materials more available to microorganisms, and this chloride becomes converted to formazan, a chemical substance which can then be extracted for analysis from the soil. This test for dehydrogenase activity in soil indicates the presence of healthy bacteria with higher formazan levels and concludes for active metabolic processes enhancing the soil fertility (Alef and Nannipieri 1995; Walls-Thumma 2000). This determination of dehydrogenase levels leads to a more intense understanding of side effects from agricultural practices as application of artificial fertilizers, herbicides, or pesticides. As a direct indicator of the microbial activity in the soil, it can also serve as soil pollution indicator. McCarthy et al. (1994) reported higher levels of dehydrogenase enzyme activities in soils polluted with effluents from pulp and paper mills but low enzyme activities in fly-ash-polluted soils. Similar results are reported by Pitchel and Hayes (1990).

11.3.2.6 Urease Activity

Urease is the driving and required enzyme for the urea fertilizer hydrolysis into NH_3 and CO_2, accompanied with the pH rise of the soil and loss of N to the atmosphere through NH_3 volatilization (Frankenberger and Tabatabai 1982). Urease is widely found as intra- as well as extracellular enzyme in nature, being present mainly in plants and microorganisms (Burns 1982). Urease extracted from plants or microorganisms degrades rapidly in soil by proteolytic enzymes (Pettit et al. 1976; Zantua

and Bremner 1977). This leads to the conclusion that a relevant share of the soil ureolytic activity is carried out by extracellular urease, stabilized from the immobilization on organic and mineral soil colloids. Urease activity rises with organic fertilization and reduces with tillage of the soil (Saviozzi et al. 2001), so it is also widely used for evaluating changes in the soil management related to soil quality. Soil management-related parameters as soil depth, organic matter content, or cropping history, as well as environmental factors like pH, temperature, or heavy metal depositions, also influence the urease activity, which can therefore be used as a biological indicator of the soil constitution (Yang et al. 2006). The urease activity depends also on the physical and chemical soil properties and also on the microbial community (Corstanje et al. 2007). The enzyme stability is influenced by factors as humic substances or organo-mineral complexes, which makes it resistant against denaturation from heat and proteolytic effects (Makoi and Ndakidemi 2008). Urease activity generally increases with higher temperatures, and temperature dependency of the urea hydrolysis has drawn a significant attention in research. A better management of urea fertilizers requires the intense understanding of urease activity, especially in warm areas with a high amount of rainfall and irrigated or flooded soil conditions (Makoi and Ndakidemi 2008). Urease can be produced by bacteria, yeasts, algae, and fungi, as well as by plants. It may also become synthesized in some organisms, but mostly urease expression is under nitrogen regulation (Anna 2014). The synthesis of the enzyme is suppressed when growing cells have access to a preferred source of nitrogen (e.g., $NH4^{+}$) and activated under availability of urea or alternative sources of N. N supply regulating role for plants, after urea fertilization, created high attention for the soil urease activity.

11.4 Cellulose-Degrading Microorganisms

Soil microorganisms exert an important role in the degradation of cellulose. Cellulose-degrading microorganisms are abundant and ubiquitous in nature. Fungi or bacteria, including mesophilic or thermophilic anaerobic or aerobic bacteria, are able to perform the task of degrading cellulose (Wilson 2011). Even though present in high amounts, only a small fraction of microorganisms are able to degrade cellulose, likely due to its presence in recalcitrant cell walls. Cellulose degradation follows several mechanisms employed by different types of microorganisms, but all of them involve cellulases. The plant cell walls, the natural substrate of the cellulases and cellulolytic organisms, turn them to highly diversified organisms. Despite the great amount of information available, there is still not the full understanding about the cellulose degradation and microbial ecology in any given environment. The vast diversity of cellulose-degrading microorganisms in most of the active environments and lack of culture techniques to grow them artificially still limit our understanding of these topics. Cellulases are highly diverse enzymes, catalyzing a single chemical reaction which is the hydrolysis of β-1,4 linkage, joining two glucose molecules within a cellulose molecule. The fact that cellulases are able to degrade an insoluble substrate makes them a very unique enzyme (Wilson 2008).

The enzyme has to diffuse into the substrate and subsequently to move a segment from a cellulose molecule away from the insoluble particle to its active site. Soluble substrates are, in contrast, diffusing to the enzyme and bind themselves into the active site. Also cellulase activities may be used as a primary indicator of some chemical or physical soil properties and provide strategic support in agricultural soil management (Joachim and Patrick 2008). Any improved understanding of this enzyme is of high importance as the cellulose enzymes exert a very important role in natural cellulose recycling, a globally abundant polymer. With a better understanding, it may also be used as a sort of prediction tool in programs to enhance the soil fertility (Das and Varma 2011).

11.4.1 Cellulose-Degrading Bacteria

The bacteria involved in cellulase enzyme production are classified into aerobic, e.g., *Acinetobacter junii*, *Bacillus subtilis*, *Cellulomonas biazotea*, *Paenibacillus* sp., and *Pseudomonas*; cellulose; and anaerobic, e.g., *Acetivibrio cellulolyticus, Butyrivibrio fibrisolvens,* and *Clostridium thermocellum* (Islam and Roy 2018; Sukumaran et al. 2005; Sadhu et al. 2013).

11.4.2 Cellulose-Degrading Fungi

Fungi-synthesized cellulase enzymes occupy a critical role in recycling C and nutrients and in maintaining soil fertility in nature.

The fungi-based cellulolytic enzyme systems are usually separated into three groups: (i) soft-rot fungi with members *Aspergillus niger, A. oryzae, Fusarium solani, T. harzianum, Trichoderma reesei, Trichoderma atroviride, and Mucor circinelloides*; (ii) brown-rot fungi with *Poria placenta, Coniophora puteana, Lanzites trabeum, Tyromyces palustris, and Fomitopsis* sp.; and (iii) white-rot fungi with *Phanerochaete chrysosporium, Agaricus arvensis, Sporotrichum thermophile, Pleurotus ostreatus* as members (Kleman-Leyer et al. 1996; Nutt 2006; Sukumaran et al. 2005; Kuhad et al. 2011).

11.5 Phosphatase Activity

Phosphorous (P) represents the second major nutrient element after N in higher organisms. It is necessary for the growth of the plants and crop yield. However, a large quantity is immobilized due to the intrinsic characteristics of soils like pH, affecting the nutrient availability and activity of enzymes and altering the equilibrium of the soil solid phase (Martinez-Salgado et al. 2010; Dick and Tabatabai 1983).

Phosphatases are enzymes capable of hydrolyzing phosphoric esters with the liberation of inorganic phosphate. They can be found widely distributed in the nature and form two groups, “alkaline” and “acid” phosphatases. Their activity

depends largely on the moisture content in the soil and environmental temperature. They are usually classified according to their pH optimum as neutral (EC 3.1.3), alkaline (EC 3.1.3.1), and acid (EC 3.1.3.2). This classification is driven by the fact that some are optimally active at an alkaline and some others at an acid pH. Even though the pH value varies with a given substrate, using phenyl phosphate maximizes alkaline phosphatase activity at a pH of 9.8, whereas acid phosphatases show an optimum activity at pH of 4.9. The large spread in between these two optimum pH values allows determination of one of the phosphatase groups, even in the presence of the other one.

The phosphatase activity has an important role in the P-conversion, from soil organic matter into forms of P available for plant uptake, as organisms are only able to absorb phosphate in dissolved forms (Caldwell 2005). Plant roots, bacteria, and fungi produce phosphatase enzymes which serve to split off a phosphate group from its substrates and to convert a complex or an unavailable form of organic P into available phosphate for plants. The generation of phosphatase is therefore controlled by a combination of demand for P from the plants, microbes, availability of organic P substrates, and limitation of P the soil. Phosphatase secretions from roots and mycorrhiza and other enzymes directly influence the rhizosphere, a narrow soil region with a dense population of root-associated and free-living microorganisms (Margalef et al. 2017). Soil contains therefore a large quantity of phosphatase enzymes, either inside living microbial cells (intracellular enzyme) or as secretion of living cells or as decayed cellular material (as extracellular enzymes). Stabilization of phosphatases in soil can be achieved on surface-reactive particles as clay and on oxides of iron or aluminum. Because of their participation in the phosphorus cycle, phosphatase enzymes release inorganic phosphate that can be taken up by plants and microorganisms from organic moiety and complex inorganic materials.

Phosphorus has several important functions in the enumerable metabolic pathways and may be described as the maker of the energy currency of living systems (Ushasri et al. 2013).

11.6 Microbe-Mediated Mineral Solubilization

11.6.1 Nitrogen Solubilizers

Nitrogen forms an inherent component of proteins, nucleic acids, as well as other essential biomolecules and is therefore among the most important nutrients needed for the growth of plants and for the productivity in agriculture systems (Bockman 1996). The atmosphere on our Earth contains more than 80% nitrogen, but this is not directly accessible (is unavailable) for plants. To become available for plants and other eukaryotes, it must be converted into ammonia. For conversion into ammonia, three types of processes are possible: (a) atmospheric nitrogen is directly, in the atmosphere, converted into nitrogen oxides; (b) industrial nitrogen generation/fixation, which involves a high-energy input (due to high process temperatures of 300–500 °C) and catalyzation to ammonia; and (c) biological nitrogen fixation

(BNF) by microorganisms, using nitrogenase, a complex but natural enzyme system. The biological nitrogen fixation is environmentally sound and a very suitable alternative option to chemical fertilizers. This biological process represents also an important economic factor as about 60% of the available, and nitrogen is fixed by this kind of biological processes. Nitrogen fixation in nonleguminous plants is performed by PGPR (diazotrophs), engaging a nonobligate interaction with their host plant (Glick et al. 1999). A nitrogenase enzyme, coded by *nif g*enes, carries out this nitrogen fixation process (Masepohl and Klipp 1996). Dean and Jacobson (1992) elucidated the structural composition of the nitrogenase as a two-component metalloenzyme consisting of (i) dinitrogenase reductase, the iron protein, and (ii) dinitrogenase, with a metal cofactor. Masepohl and Klipp (1996) discovered three different nitrogen-fixing systems, based on the metal cofactor: (i) Mo-nitrogenase, (ii) V-nitrogenase, and (iii) Fe-only nitrogenase. The existence of these nitrogen-fixing systems differs among the bacteria, based on the growing conditions (Bishop and Jorerger 1990). There are free-living organisms, such as *Azospirillum*, *Azotobacter*, *Burkholderia*, *Herbaspirillum,* and *Bacillus* sp., inhabiting the rhizosphere and establishing a very close relationship with the plant, although they are not penetrating the plant tissues (Vessey 2003). They live in sufficient root proximity that the plants can take up excess nitrogen, fixed by the bacteria from the atmosphere but not used for its own. This unspecific and loose symbiosis is generating an additional nitrogen source for the plants. BNF is a high energy-consuming process, and bacterial strains which are able to perform this process fulfill first their physiological needs, creating little leftover nitrogen available for the plants. However, the growth promotion exerted by nitrogen-fixing PGPR was attributed for many years to the excess N, until additional effects were revealed with the use of nitrogen isotopes in research. Nitrogen-isotope tracing revealed that free nitrogen-fixing bacteria are enhancing the production of beneficial plant growth regulators and fixation of (excess) nitrogen is a secondary benefit for the plants (Nakkeeran et al. 2005). These findings led to inoculant development and applications, resulting in remarkable crop yield increases, especially for cereals, with *Azotobacter chroococcum* and *Azospirillum brasilense* as highly important PGPRs. These two species include strains that are capable to release vitamins and plant growth regulators, exerting direct influence on the growth of plants (Nakkeeran et al. 2005).

11.6.2 Phosphorus Solubilizers

The most limiting plant nutrient after nitrogen is phosphorus. Even though P reserves are abundant, they are not available in a suitable form for plants. Plants can only absorb soluble mono- and dibasicphosphate forms of P. Of considerable importance is also P present in organic matter, besides the inorganic forms of soil-stored phosphorous. Estimations of the deposited organic phosphorus range between 30% and 50% of the total available P in soil. This reservoir of soil-stored P can become mineralized by microorganisms and converted into soluble phosphates, suitable for uptake by plants (Gyaneshwar et al. 2002). Phosphate-solubilizing bacteria employ

two different mechanisms for this conversion: (i) release of organic acids, which produce ionic interactions with the phosphate salt cations and mobilize the phosphorous, and (ii) release of phosphatases which in turn are responsible for fracturing phosphate groups bound to organic matter (Gyaneshwar et al. 2002). Many microorganisms from different genera are capable of solubilizing phosphate and include the following genera: *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aerobacter*, *Flavobacterium*, *Chryseobacterium,* and *Erwinia*.

11.6.3 Potassium Solubilizers

The third major essential plant nutrient in crop production, after N and P, is K. It has an essential role in the activation of the enzyme and in the protein and photosynthesis and is important for the quality of products. Potassium is a dominant constituent of several soil minerals (Meena et al. 2015, 2016) as it ranks on seventh place among all the elements in the earth's crust. K-bearing minerals can become solubilized by potassium-solubilizing bacteria (KSB), which convert insoluble forms of K into soluble forms of K, accessible for uptake by plants. The number of microorganisms having the ability to solubilize K-bearing minerals as biotite, feldspar, illite, muscovite, orthoclase, and mica is large. Among these microorganisms are *Acidothiobacillus ferrooxidans*, *B. circulans*, *B. edaphicus*, *Bacillus mucilaginosus,* and *Paenibacillus* spp. type. KSB are typically found in all kinds of soils, but their number, diversity, and capability for K solubilization may vary depending upon the soil structure and climatic conditions. K release is through dissolving silicate minerals and production of organic and inorganic acids acidolysis, polysaccharides, complexolysis, chelation, and various exchange reactions. Biological fertilizers based on potassium solubilizers (KSBs) are therefore a viable alternative to chemical fertilizers (Etesami et al. 2017).

11.6.4 Sulfur Solubilizers

For recycling of sulfur compounds, a group of sulfate-reducing bacteria takes up the active role. They take up the sulfate as nutrient and reduce it to sulfide which is subsequently utilized in the amino acid synthesis (as cystine or methionine) and to synthesize sulfur-containing enzymes. In this sulfur transformation process, chemolithotrophic sulfur- and sulfate-reducing bacteria become important actors in the oxidation and reduction reactions. These reactions generate metabolic energy through sulfide oxidation and dissimilatory sulfate reduction (Muyzer and Stams 2008). Sulfur solubilizer bacteria use the highly oxidized form of sulfur (SO_4^{2-}), also known as sulfate, as the terminal electron acceptor to produce hydrogen sulfide (H_2S) during the catabolism of organic matter. The so formed sulfide can become oxidized from chemolithotrophic sulfur-oxidizing bacteria, either in an aerobic way (*Thiobacillus* or *Beggiatoa* spp.) or in an anaerobic process (*Chlorobium* spp.), to

elementary sulfur (S°) and SO_4^{2-}. Many different bacteria groups are also involved, e.g., *Desulfuromonas* spp. and *Desulfovibrio sulfodismutans*. Agostino and Rosenbaum (2018) reported that most cultured sulfur solubilizer microorganisms belong to four bacterial (*Deltaproteobacteria*, *Nitrospirae*, *Firmicutes*, and *Thermodesulfobacteria*) and two archaeal (*Euryarchaeota* and *Crenarchaeota*) phyla.

11.6.5 Zinc Solubilizers

Zinc, an important micronutrient for human beings, animals, as well as for crops, is a relevant component of different enzymes which catalyze many metabolic plant reactions. Zinc plays also a relevant role in the resistance of plants against diseases, in the photosynthesis, for the cell membrane integrity, in protein synthesis, or in pollen formation (Gurmani et al. 2012). It also enhances the antioxidant enzyme level and chlorophyll content within the plant tissues (Sbartai et al. 2011). Zinc also influences essential life processes in plants, such as (a) quality of N and protein uptake (nitrogen metabolism); (b) synthesis of chlorophyll (photosynthesis) and carbon anhydrase activity; (c) biotic and abiotic stress resistance, i.e., resistance against oxidative damage (Hussain et al. 2015; Alloway 2008).

Acidification is one of the various mechanisms through which zinc-solubilizing microorganisms solubilize zinc. Organic acids, produced by these microbes in soil, sequester the zinc cations and reduce the pH of the soil nearby. Additionally, the anions are able to chelate zinc and enhance therefore the zinc solubility. The production of siderophores and protons or oxido-reductive systems on cell membranes is another mechanism possibly involved in zinc solubilization (Saravanan et al. 2011); also production of chelated ligands is among them (Chang et al. 2005). Various biofertilizers as *Pseudomonas*, *Rhizobium* strains, *Bacillus aryabhattai*, *Bacillus* sp. and *Azospirillum*, *Oidiodendron maius,* etc. have shown enhanced plant growth and amplified zinc content in plant tissues. Zinc solubilization on lab-scale is reported from bacterial strains like *Pseudomonas aeruginosa, Gluconacetobacter diazotrophicus, Bacillus* sp., *Pseudomonas striata, Pseudomonas fluorescence, Burkholderia cenocepacia, Serratia liquefaciens, S. marcescens, and Bacillus thuringiensis* (Kamran et al. 2017).

11.6.6 Iron Solubilizers

Iron is another essential plant nutrient, and iron deficiency exhibits metabolic changes due to its role as a co-factor in numerous enzymes that are essential to important physiological processes in the plants, like respiration, photosynthesis, and nitrogen fixation. Iron is often unavailable for plants or soil microorganism's uptake, despite its abundance in soils. The predominant, in soil available, chemical form is Fe^{3+}, the oxidized form of iron that reacts to build oxides and hydroxides which are insoluble and hence inaccessible to plants and microorganisms (Brait

1992; Bultreys et al. 2001). For efficient iron absorption, plants are releasing iron-chelating organic compounds, thus rendering the insoluble oxides or hydroxides into soluble forms. The iron then diffuses toward the plant and becomes reduced and, with an enzymatic system present in the cell membrane, absorbed. Another strategy for iron uptake is in absorbing a complex, which is formed by Fe^{3+} and the organic compound, where the iron is then reduced within the plant and readily incorporated. There are also bacteria in the rhizosphere which are capable to exudate iron-chelating molecules (siderophores) into the rhizosphere, performing therefore a similar function as the plants (O'Sullivan and O'Gara 1992). Siderophores are compounds with low molecular weight (usually below 1 kDa), containing functional groups that are capable of iron-binding in a reversible way. Catechols and hydroxamates are the mostly found functional groups, with optimal distances to bind iron among the groups involved. Bacteria producing siderophore typically belong to the genus *Pseudomonas* with pyochelin- and pyoverdine-releasing *Pseudomonas fluorescens* as the most common type. As these substances show antibiotic activity and can improve the plant's iron nutrition, the rhizosphere bacteria increase their competitive potential in releasing these compounds (Glick 1995).

Siderophore-producing rhizobacteria also improve the health of plant at different levels. They can enhance the iron nutrition of the plant, can suppress the growth of other microorganisms in releasing antibiotic molecules, or suppress pathogen growth by diminishing the available iron for pathogens, usually fungi that are not capable to absorb the iron-siderophore complex (Cecile and Philippe 2004). Siderophores are chromo-peptides consisting of three structural parts, a quinoline chromophore, a peptide chain, and a side chain. Siderophores are assembled by nonribosomal, cytoplasmic peptide synthetases resembling the machinery described for antibiotic synthesis. Biosynthetic enzymes encoding genes are iron regulated and are often clustered with genes involved in the siderophore uptake (Glick 1995). Most of the bacterial genes that are involved in the iron assimilation are expressed only under iron-deficiency conditions (Hantke 2001). The mechanism of fluorescent pseudomonads for siderophore-mediated disease-suppression has been reviewed by Loper and Buyer (1991). The producing fluorescent pseudomonas strain can use the resulting ferric-siderophore complex via a specific receptor, located in its outer cell membrane, but the complex is not available to other organisms (Buyer and Leong 1986). The fluorescent pseudomonas strain may inhibit the growth of harmful bacteria and fungi at the plant root, as well as reduce or prevent the germination of fungal spores due to iron starvation conditions. A model for fluorescent pseudomonas siderophores-induced root pathogens suppression is shown in Fig. 11.4.

The unavailability of the ferric iron in the soil restricts the growth of deleterious or harmful organisms (Saharan et al. 2010; Daniel et al. 1992). Iron deficiency or deprivation leads to a kind of chlorosis in plants. Reports show (Moores et al. 1984) that the fluorescent siderophores from *Pseudomonas* spp. strain B10 inhibit the uptake of iron by maize plants and peas. In contrast, there are also numerous reports suggesting that plant species are able to obtain iron from certain microbial siderophores. Iron, derived from microbial hydroxamate siderophores, may become

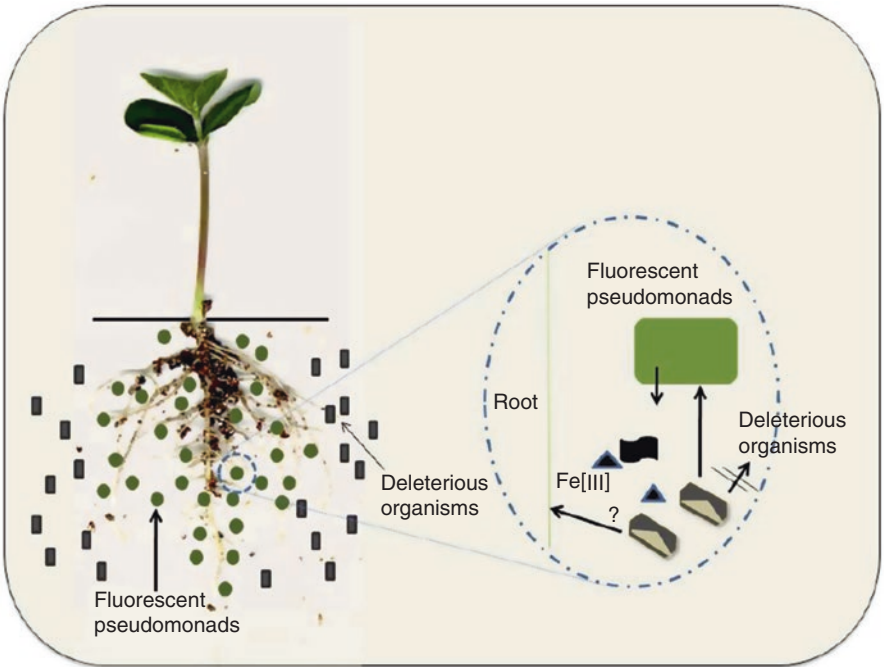

Fig. 11.4 Model for suppression of root pathogens by siderophores from fluorescent pseudomonads

accessible for plants, in nutrient solution as well as in soil. Furthermore, fluorescent pseudomonad siderophores have also been implicated in the remedy of lime-induced chlorosis by peanuts or in the iron uptake of tomato plants (Persello-Cartieaux et al. 2003; Lemenceau et al. 1993). Figure 11.5 shows the mechanisms of iron removal from siderophore complex by plants (Clarke et al. 2001), indicating that some plant species may acquire the needed iron via certain microbial siderophores. The siderophore concentration in soil is approximately in the range of 10–30 M.

11.7 Soil Respiration

Soil respiration is among the most important soil biological indicators that reflect the biological activity within the soil. The microbial activity is a fundamental process, providing energy and nutrients for recycling processes in an ecosystem. This is because soil microorganisms have some highly relevant roles in the biogeochemical cycling of organic C, N, P, K, S, etc. (Maharana and Patel 2013; Bandick and Dick 1999). High microbial respiration indicates loss of valuable organic carbon and low nutrient cycling activity in the soil (Alef 1995; Pankhurst et al. 1997), whereas low microbial respiration indicates immobilization and/or the presence of pollutants such as fungicides or pesticides (Pankhurst et al. 1997). Soil

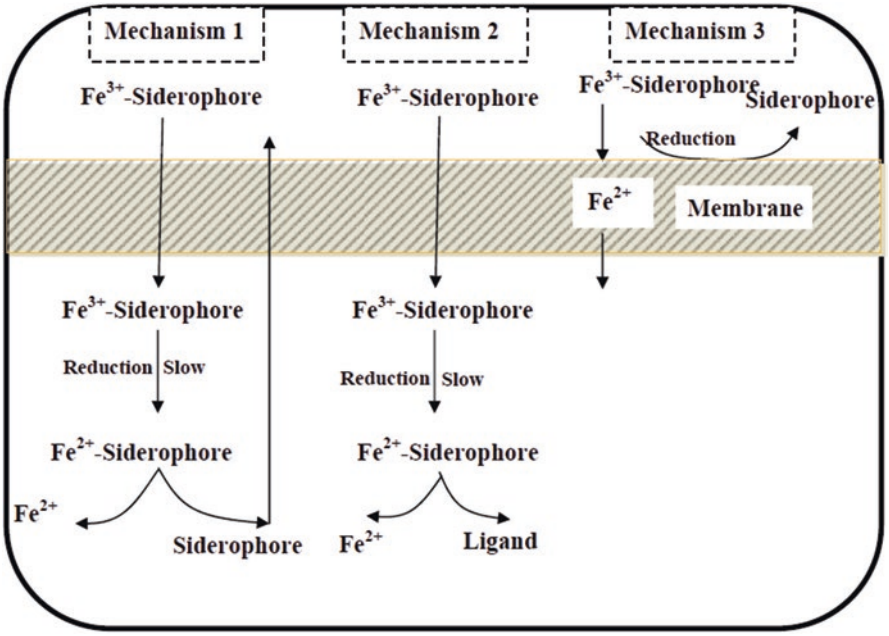

Fig. 11.5 Mechanisms of removal of iron from the siderophore complex by reduction of Fe^{3+}. Mechanisms 1 and 2 are used by plants. Microorganisms use any of the three stated mechanisms. (Saharan et al. 2010; Clarke et al. 2001)

microbial respiration has a linear relationship with mineralization of soil organic matter (SOM). Respiration is estimated as either CO_2 production or O_2 consumption, using basal respiration such as short-term laboratory assays (Parkin et al. 1996). In general, changes in precipitation, management practice, microbial community structure, aeration, soil structure, nutrient conditions, and pH affect the soil microbial respiration (Anderson and Domsch 1993; Singh et al. 2011). In addition, respiration is a temperature-sensitive process and has a close relationship with climate change and global C cycling. According to reports, soil provides a very large sink of carbon (C) in the terrestrial ecosystems and makes a major contribution to the global carbon equilibrium. The agricultural soil takes up an important role in the cycle of global carbon and accounts for around 11% of the global anthropogenic CO_2 emissions, as reported by Gao et al. (2013). To minimize the soil respiration and to retain more C sequestered in agricultural soils is therefore of high importance. Autotrophic respiration from plant roots and heterotrophic respiration of plant residues, root litter, and exudates as well as soil organic matter by soil microorganisms are the main contributor to soil respiration. Tillage practices in cropland and straw management is affecting the soil respiration in a large amount. The largest increase is observed directly after tillage operations; hence reducing the tillage-intensity can therefore lower the cumulative CO_2 emissions in a significant amount (Gao et al. 2013).

11.8 Conclusion

The balanced interaction between plants, plant nutrients, soil, and soil-borne microorganisms is an important factor for the performance of the agriculture system. Soil nutrients are consumed during plant growth and must be replenished for a sustainable agricultural growth cycle. This can be done by donation of artificially created nutrients (e.g., chemical fertilizer), by recycling plant material, by donating converted plant material (manure), and other forms of organic residues or any combination of these. Soil-borne microorganisms have a key role in preparing and converting available nutrients into a plant accessible form, as many nutrients are not in a for plants "ready-made" form present in the soil. One such group are the mineral solubilizers; they convert minerals into plant-accessible forms. Other microorganisms can, for example, fix atmospheric nitrogen, a major nutritional element for all plants. Atmospheric nitrogen can also be fixed by plants from the legume group. They form therefore an important factor within a sustainable agriculture system, with minimal external fertilizer input. Recycling organic material as fertilizer involves cellulose degradation. Again, we find microorganisms in the form of bacteria and fungi performing this task. The soil itself represents the host of all these activities. It provides the physical structure needed for the plants to grow, supplies the nutrients and water, and is home of the microorganisms. A healthy soil is therefore the key element for a sustainable agriculture system. The soil status (health) can be expressed in various ways, and there is still no common definition and metric for measuring and classifying the quality status of the soil health. Soil health indicators, such as soil microbial biomass or soil nutrient content (e.g., N, P, and K), are direct measurable parameters, giving a measure about the physical status of the soil. Another group of soil health indicators is an enzymatic activity parameter, revealing the status of the microbial activity, the "living part" in the soil. Many research studies indicate that not only proper physical soil parameters are sufficient for a solid agricultural base, but also the microbe system plays at least the same important role, and this must be considered in all aspects of research and farming. All these parameters are influenced by the agriculture system applied on the soil, the cropping system. There is no general optimum cropping system, as the climate zone, the soil structure, and many other parameters determine the growing sequence and cycle on a particular land area. Also the human factor must be considered as an influencer of the ideal cropping system for a given area, as the available input (labor, machinery, fertilizer, etc.) and the requested output (the return from the agricultural activities) are a key factor determining the soil state and the entire soil ecosystem in a holistic way.

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Influence of Endophytic Bacteria on Growth Promotion and Protection against Diseases in Associated Plants

12

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Abstract

Plants are colonized by different endophytic microbial communities. These endophytic microbiomes have been reportedly associated with improved growth, metabolism and defence against other physical factors. The endophytic population varies with plant species, genotypes and crop growth stages. They contribute plant growth promotion through nitrogen (N) fixation, phosphate solubilization and phytohormone production. Several phytohormones, such as indole-3-acetic acid (IAA), gibberellins (GA) and cytokinins (CK), synthesized by the plant endophytes can enhance different stages of plant growth, such as root formation, stimulation of cell division, extension, differentiation and regulation of fruit ripening. The low-molecular-weight siderophore molecules produced by these endophytes show high affinity for ferrous iron. Endophytes aid in the host's survival against biotic stress by the production of HCN and secondary metabolites that suppress the soilborne pathogens. They also enhance plant fitness by producing novel bioactive compounds. Different kinds of alkaloids produced by the endophytes also provide resistance to plants against environmental stresses. The amines and amides produced by the plant endophytes have shown toxic effects to insects. The endophytic bacteria can trigger strawberry flavour. Advanced techniques, such as metagenomics based on next-generation sequencing is useful to study the taxonomical diversity of microbial communities associated with the economically and agriculturally important crops. This chapter reviews the important role of plant-associated bacterial endophytes in agricultural crops.

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Endophytes · Microbiome · Plant growth promotion · Antibiosis · Bioactive molecules

12.1 Bacterial Endophytes

De Bary (1866) introduced the term 'bacterial endophytes' ('endon', within, and 'phyte', plant) for pathogenic fungi entering the leaves, and later, all microbes which enter into plant tissues were called as endophytes. These endophytes can complete their life cycle either partly or completely inside the plant. They may not show any disease symptoms in the host; however, they can cause imperceptible and asymptomatic infections (Wilson 1995). Various plant tissues can be colonized by endophytic bacteria and fungi (Bacon and White 2000). A large number of endophytic bacteria have been isolated from the surface-sterilized plant tissues (Reinhold-Hurek and Hurek 1998a). These endophytes are derived from the rhizospheric soil (Gao et al. 2004; Castro-Sowinski et al. 2007; Compant et al. 2010). The endophytes from the plant tissues are protected from environmental stresses or microbial competitions (Hallmann et al. 1997). A large number of genera (Gram-positive and Gram-negative), such as *Alcaligenes*, *Arthrobacter*, *Azoarcus*, *Azomonas*, *Azotobacter*, *Azospirillum*, *Beijerinckia*, *Burkholderia*, *Chromobacterium*, *Corynebacterium*, *Derxia*, *Devosia*, *Enterobacter*, *Flavimonas*, *Flavobacterium*, *Flexibacter*, *Herbaspirillum*, *Pantoea*, *Ralstonia*, *Rhizobium*, *Sphingomonas*, *Stenotrophomonas*, *Streptomyces*, *Vibrio*, *Xanthomonas* and *Zymomonas*, can colonize plants as endophyte. Even pink-pigmented facultative methylotrophic bacteria and characterized *Bacillus* and *Pseudomonas* species have been reported as endophytes (Kobayashi and Palumbo 2000). Bacteria which are from the root surfaces and leaves are termed as epiphytes (Andrews and Harris 2000), and these can have both epiphytic and endophytic populations (Hallmann et al. 1997).

The members of *Streptomyces*, *Azoarcus*, *Gluconobacter*, *Pseudomonas*, *Bacillus*, *Paenibacillus*, *Serratia*, *Stenotrophomonas* and *Enterobacter* belonging to major phyla, such as Actinobacteria, Proteobacteria and Firmicutes, belong to the endophytic population. A range of legume nodules can be colonized by other plant growth-promoting endophytes which are non-rhizobial forms. These belong to *Aerobacter*, *Aeromonas*, *Agrobacterium*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Chryseomonas*, *Curtobacterium*, *Devosia*, *Dyella*, *Ensifer*, *Enterobacter*, *Erwinia*, *Flavimonas*, *Herbaspirillum*, *Methylobacterium*, *Microbacterium*, *Mycobacterium*, *Paenibacillus*, *Pseudomonas*, *Phyllobacterium*, *Ochrobactrum*, *Staphylococcus*, *Streptomyces* and *Sphingomonas*, and these may occupy root, shoot and nodule tissues (Bai et al. 2002; Dudeja et al. 2012; Gagne et al. 1987; Tokala et al. 2002; Sturz et al. 1997). These endophytic bacterial populations have been isolated from different plant parts, particularly roots and nodule tissues of legumes (Muresu et al. 2008; Hoque et al. 2011; Dudeja et al. 2012) belonging to alfalfa (Gagne et al. 1987), clover (Sturz et al. 1997) and pea (Elvira-Recuenco and van Vuurde 2000).

12.2 Distribution of Endophytes

The endophytes have been reported to occur in different plant tissues depending on the colonization potential and resource allocation. Endophytes are reported to colonize different leaves, stems, roots, flowers, seeds and fruits, and no single plant is devoid of endophytes (Hallmann et al. 1997; Hallmann and Berg 2006). Endophytic population of bacteria varies with environmental conditions, species, plant genotypes, crop growth stages and microbial load (Pillay and Nowak 1997; Tan et al. 2003). In soybean, endophytic microbes can be affected by the cultivar, age of the plant, tissue used and the season (Kuklinsky-Sobral et al. 2004). About more than 3, 00,000 plant species are known to harbour endophytes (Strobel et al. 2004), but very few have been studied for plant endophytic interactions. According to plant-inhabiting strategies, the endophytes are categorized into three broad groups, namely, obligate, facultative and passive (Hardoim et al. 2008). Obligate endophytes are transmitted via seeds, whereas facultative endophytes are mostly found as free-living state in soil, and these can later enter into the plant (Hardoim et al. 2008). The passive endophytes enter through open wounds along the root hairs in which signalling mechanisms required for their colonization is absent (Verma et al. 2004; Rosenblueth and Martinez-romero 2006; Hardoim et al. 2008) and, hence, may have little significance as plant growth promoters. The endophytic community structure is shaped by survival and competency of endophytes in root, soil and plant factors, and legume nodules have more endophytic colonization compared with roots (Kumar et al. 2013). The endophytic diversity was lower than rhizoplane population; therefore, endophytes have probably been derived from the latter (Germida et al. 1998).

12.3 Colonization of Endophytes from the Rhizosphere to the Internal Plant Tissues

The plants harbour endophytic microbiome from soil (Mahaffee and Kloepper 1997; Rasche et al. 2006; van Overbeek and van Elsas 2008; Long et al. 2010). The structure or species diversity (richness and relative abundance) of endophytic microbial community is dynamic within the plant and can be influenced by soil type, geographical distribution, plant species, microbe– microbe interactions and plant–microbe interactions. In wheat, soil type, particularly the rhizospheric soil, determines the source and composition of the endophytic population (Conn and Franco 2004; Hallmann et al. 1997). Plant root exudates, which contain various organic compounds, can stimulate rhizospheric microbial community structure (Lemanceau et al. 1995; Miethling et al. 2000), which, in turn, may affect plant-associated microbial communities. Endophytic bacterial diversity is the subset of the rhizospheric microbial population (Germida et al. 1998; Marquez-Santacruz et al. 2010), and it is well known that plant-associated endophytes determine the plant fitness (Frommel et al. 1993; McInroy and Kloepper 1995; Sturz 1995). All plants harbour different microbial communities called plant microbiome. The plant–microbiome interaction

can determine the overall plant health and function. The influence of rhizospheric microbiome composition has been reported on the growth and health of plants (Berg and Smalla 2009; Mendes et al. 2011; Berendsen et al. 2012). The difference in the composition of plant and root microbiome probably suggests an influence of plants on root-associated microbiome (Germida et al. 1998; Gottel et al. 2011), and in the whole plant systems, the roots are most heavily colonized (Hallmann et al. 1997).

The population density of endophytes is less diverse than the root colonizers, and the endophytes seem to originate from the roots (Germida et al. 1998); however, the population density of endophytic bacteria is extremely variable, and these are less abundant compared with rhizospheric soil. In wheat, a higher population of *Bacillus polymyxa* in rhizospheric and non-rhizospheric soil over rhizoplane indicates plant-driven selection of particular endophytic bacteria (Mavingui et al. 1992). The higher population of endophytes within carrot crown than the metaxylem tissue was due to the availability of more photosynthate for proliferation of the larger community (Surette et al. 2003), and the potato stems showed the higher population of *Pseudomonas* sp. than roots (Garbeva et al. 2001). The endophytic bacterial population can vary with plant tissues (Johnston-Monje and Raizada 2011). Bacterial colony-forming units (CFU) recovered from xylem tissue of alfalfa varied from 6.0×10^3 to 4.3×10^4 per g (Gagne et al. 1987), and that of cotton ranged from 1×10^2 to 11×10^3 per g (Misaghi and Donndelinger 1990). The range of bacterial CFU was from 3.3×10^3 to 7.0×10^5 per g in sugarbeet (Jacobs et al. 1985), whereas in potato tubers, it varied from 0 to 1.6×10^4 per g (De Boer and Copeman 1974); however, Kobayashi and Palumbo (2000) reported viable endophytic bacterial population of 10^4 per gram of plant tissue. About 15 bacterial species were reported in red clover nodules with the population density of 10^4 viable bacteria per g of fresh nodule (Sturz et al. 1997).

12.4 Endophytes in Root Nodules of Legumes

Legumes form a tripartite symbiosis with N-fixing *Rhizobium* and plant-associated microorganisms. The first evidence of non-rhizobial bacteria (*Agrobacterium radiobacter*) in clover nodules was reported by Beijerinck and Van Delden (1902). Nodules of red clover showed the presence of *Rhizobium rhizogenes* and *Rhizobium leguminosarum* bv. *trifolii* (Sturz et al. 1997). Members of Proteobacteria can be co-occupants in the nodules of *Hedysarum* (Benhizia et al. 2004); however, these cannot form nodules in most of the cases. Ibanez et al. (2009) recovered nodule endophytic bacteria from peanut, and these were opportunistic during co-inoculation with *Bradyrhizobium* strain. The endophytic bacteria belonging to α, β and γ Proteobacteria were isolated from a wide range of legumes irrespective of their symbiotic specificity (Zakhia et al. 2006; Kan et al. 2007). There has been an enhanced nodulation and growth during cooperative interaction between PGPR (plant growth-promoting rhizobacteria) and *Rhizobia* (Tilak et al. 2006; Barea et al.

2005). Co-inoculation of *Mesorhizobium* sp. with nodule inhabiting *Pseudomonas chlororaphis* significantly enhanced root and shoot growth of *Sophora alopecuroides* (Zhao et al. 2011). The nodule-associated *Exiguobacterium* sp. from Fenugreek was characterized for its plant growth-promoting potential, and these microorganisms may have beneficial relation with the root nodules (Rajendran et al. 2012). Koli et al. (2015) characterized the plant growth-promoting potential of endophytes from chickpea nodules. Stajkovic et al. (2009) isolated and characterized non-rhizobial Gram-positive endophytes, namely, *Bacillus megaterium*, *Brevibacillus choshinensis* and *Microbacterium trichothecenolyticum*, from alfalfa root nodules. The positive influence on nodulation potential with comparable increase in plant growth was shown under co-inoculation of non-rhizobial strains with *Ensifer* (*Sinorhizobium*) *meliloti* in alfalfa plants. Similarly, in *Vigna radiata*, nodule endophytic bacteria showed a positive influence (Pandya et al. 2015), which could be due to IAA production resulting in phytostimulation and circumvention of plant defence mechanisms as part of colonization strategy (Spaepen and Vanderleyden 2011). Fungal symbionts, such as vesicular mycorrhiza, are also reported to colonize legumes, and these may improve nodulation, plant health and seed yield when co-inoculated with *Rhizobia* (Sturz et al. 1997; Bai et al. 2002; Rajendran et al. 2008). On the other hand, *Rhizobium etli*, a root nodule endophyte, can also colonize maize plants when grown with bean under mixed cropping (Zamora and Romero 2001).

12.5 Interaction Between Endophytes and Host Plants

The plant-associated endophytes form a range of different relationships, including communalistic, symbiotic, mutuality and trophobiotic. In addition, different types of nonpathogenic relationships, such as beneficial, neutral and detrimental, are formed by these bacteria with their hosts. The endophytes can influence plant growth promotion or inhibition, or there can be a neutral influence of endophytes on plant growth. The endophytic effect of plant growth promotion in one plant species may have no effect or can inhibit the growth of other plant species (Arsac et al. 1990; Chanway and Holl 1994; Lazarovits and Nowak 1997), and the overall benefits are well documented, and growth promotional activities of these bacteria can be cultivar specific as well (Pillay and Nowak 1997; Conn et al. 1997; Bensalim et al. 1998). The endophytic microorganisms showed plant growth-promoting potential (Hallmann 2001; Compant et al. Compant et al. 2003, 2005; Sessitsch et al. 2004) and may exhibit more pronounced plant growth-promoting effects than bacteria which colonize the rhizosphere (Conn et al. 1997; Chanway et al. 2000). The endophytic bacteria, after their entry, can translocate through active or passive mechanisms and can move from the rhizoplane to the root cortex, followed by aerial parts with a declining population density compared with rhizospheric population or root colonizers. The endophytes are able to pass through the endodermis by secreting cell wall-degrading enzymes and can colonize the endorhiza (James et al. 2002).

12.6 The Role of Endophytes in Plant Growth Promotion and Biocontrol

Analogous to PGPR, the endophytic bacteria can aid in growth promotion and phytoremediation, and these have an excellent potential with legumes and non-legumes (Antoun et al. 1998; Dudeja 2016). Endophytic bacteria are believed to elicit plant growth promotion indirectly by helping plants to acquire nutrients via N fixation, phosphate solubilization (Wakelin et al. 2004) and iron chelation (Costa and Loper 1994). According to Ali et al. (2012) and Coutinho et al. (2015), bacterial endophytes offer several benefits to the host plant, particularly growth promotion, which can be due to N fixation (Stoltzfus et al. 1997; Reinhold-Hurek and Hurek 1998a), and protection against soilborne pathogens (Table 12.1). Krishnamurthy and Gnanamanickam (1997) reported the role of endophytic microorganisms in controlling plant pathogens. These may prevent pathogenic infections via antimicrobial

Table 12.1 Plant growth-promoting potential of endophytic bacteria on various plants

Organism	Property	Host plant	References
Acetobacter diazotrophicus	N_2 fixation	Sugar cane (*Saccharum officinarum*)	Dobereiner et al. (1995a)
Klebsiella sp., *Paenibacillus odorifer*, *Sinorhizobium meliloti*	N_2 fixation	Sweet potato (*Ipomea batatas)*	Reiter et al. (2003)
Klebsiella sp.	N_2 fixation	Wheat (*Triticum aestivum*)	Iniguez et al. (2004)
Klebsiella sp., *Pseudomonas* sp.	N_2 fixation	Maize (*Zea mays*)	Riggs et al. (2001) and Yanni et al. (1997)
Microbacterium, Xanthomonas sp.	N_2 fixation Cellulase and pectinase activity	Rice (*Oryza sativa*)	Walitang et al. (2017)
Flavobacterium sp.	N_2 fixation Phosphate solubilization, IAA production	Rice (*Oryza sativa*)	Walitang et al. (2017)
Pseudomonas sp.	N_2 fixation and siderophore production	Rice (*Oryza sativa*)	Walitang et al. (2017)
Pseudomonas	IAA production	Soybean (*Glycine max)*	Sobral et al. (2004)
Sphingomonas sp.	Plant growth promotion Gibberellin and IAA production	Tomato (*Solanum lycopersicum)*	Khan et al. (2014)
Bacillus subtilis, *B. licheniformis*	Plant growth promotion	Chickpea (*Cicer arietinum*)	Saini et al. (2015)
Pseudomonas fluorescens, *Microbacterium* sp.	Lead resistance	Mustard (*Brassica nigra*)	Sheng et al. (2008)

metabolites or outcompete pathogens for nutrients through siderophore production or by manifesting the plant's systemic resistance. Direct influence can be through phytohormone, namely, auxins or cytokinins, production (Madhaiyan et al. 2006), or these may produce 1-aminocyclopropane-1-carboxylate deaminase, which lowers plant ethylene levels (Glick 1995). Minorsky (2008) reported a correlation between vigorous colonization of root endophyte (*Pseudomonas fluorescens* B16) and enhanced yield in tomato. The soybean root nodule endophytes, such as *Acinetobacter*, *Agrobacterium*, *Bacillus*, *Burkholderia*, *Pantoea* and *Serratia*, are reported to assist in phosphate solubilization, IAA production and N fixation (Li et al. 2008), and these may also suppress soilborne pathogens (Senthilkumar et al. 2009). Hydrolytic enzymes, such as pectinases and cellulases, produced by the endophytes facilitate penetration and persistence in the host plant (Hallmann et al. 1997; Reinhold-Hurek and Hurek 1998b). The endophytic fluorescent pseudomonads isolated from chickpea promote plant growth and symbiotic potential (Parmar and Dadarwal 1999).

(a) *Nitrogen Fixation*

N_2-fixing bacteria (diazotrophs) constitute a small proportion of total endophytic bacteria (Ladha et al. 1983; Barraquio et al. 1997; Martínez et al. 2003). Extensive evidence showed that symbiotic N fixers (*Rhizobia*) provide fixed N to plants in exchange for carbon; however, free-living diazotrophic bacteria contribute limited N, which may not be sufficient to support the requirements of host plants (Hong et al. 1991). Some endophytic diazotrophs, such as *Azospirillum* and *Azotobacter*, have an advantage over rhizospheric N fixer as these can colonize the interior of the plants and utilize the carbon substrates provided by the plants (Dobereiner et al. 1995b; McInroy and Kloepper 1995; Boddey et al. 1995; Sprent and James 1995; Triplett 1996). The significant contribution of endophytic diazotrophs in economically important graminaceous species, such as sugar cane (Urquiaga et al. 1992), rice (Shrestha and Ladha 1996; Jha et al. 2009) and kallar grass (Malik et al. 1997), has been reported. A diverse range of N-fixing endophytic bacteria were reported to colonize *Lasiurus sindicus*, a perennial drought-tolerant grass from the Thar Desert of Rajasthan (Chowdhury et al. 2009). The most likely candidates for biological N fixation in grasses are *Acetobacter diazotrophicus*, *Herbaspirillum* sp. and *Burkholderia* in sugar cane (Dobereiner et al. 1995a; Boddey et al. 1995, 2001; Baldani et al. 1997; Govindarajan et al. 2006), *Azoarcus* sp. in kallar grass (Reinhold-Hurek and Hurek 1998b) and *Alcaligenes* sp., *Azospirillum* sp., *Bacillus* sp., *Enterobacter* sp., *Herbaspirillum* sp., *Klebsiella* sp., *Pseudomonas* sp. and *Rhizobium* sp. in rice and maize (Patriquin et al. 1983; Boddey et al. 1995; Triplett 1996; Malik et al. 1997; Stoltzfus et al. 1997; Yanni et al. 1997; James et al. 2000). These studies have indicated the important role of endophytic diazotrophs in nonlegumes (Boddey et al. 1995, 2001; Dobereiner et al. 1995a, b; Ladha and Reddy 1995; Triplett 1996; Kennedy et al. 1997; Reinhold-Hurek and Hurek 1998a).

The N-fixing endophytic population of sweet potato was identified by the amplification of nitrogenase (*nif*H) genes under N-limited conditions. The *nif*H gene

sequences from endophytes resemble *Sinorhizobium meliloti*, *Sinorhizobium* sp. NGR234 and *Rhizobium etli*, *Klebsiella* sp. and *Paenibacillus odorifer* (Reiter et al. 2003). The application of endophytic *Acetobacter diazotrophicus* increased sugar cane production (Dobereiner et al. 1992) where plant acquired 20%–60% of its N requirements from the symbiont (Boddey et al. 2001). The *Gluconacetobacter diazotrophicus* that forms an endophytic association with sugar cane makes a significant contribution to N nutrition (Sevilla et al. 2000). Another diazotrophic endophyte, namely, *Herbaspirillum seropedicae* in sugar cane, is also shown to infect rice and increase $15N_2$ incorporation (James et al. 2002). *Burkholderia sp.* improved N uptake in grasses in nutrient-poor sand dunes (Dalton et al. 2004). Iniguez et al. (2004) reported that *Klebsiella* sp. strain Kp342 fixes N_2 in field-grown wheat and maize (Riggs et al. 2001).

(b) *Phosphate Solubilization*

Phosphorus (P) is an essential and the most limiting nutrient next to N for plant growth promotion (Gyaneshwar et al. 2002), and a significant portion of applied P is quickly fixed in soil; hence, it becomes unavailable (Nautiyal 1999; Rodríguez and Fraga 1999). The low availability of P is due to its presence as an insoluble form as plants can absorb P in either monobasic ($H_2PO_4^-$) or diabasic (HPO_4^{2-}) form (Glass 1989). A group of microorganisms which can solubilize P and make it available to plants are collectively called phosphate-solubilizing microorganisms (PSM). The P solubilization ability has been shown to be associated with root exudates (Nautiyal 1999; Rodriguez et al. 2000; Vazquez et al. 2000; Gyaneshwar et al. 2002; Vassilev and Vassileva 2003), and endophytic bacteria are capable of solubilizing insoluble phosphates (Rodríguez and Fraga 1999; Verma et al. 2001) during their initial colonization, which, in turn, may enhance P availability. These organisms may produce various organic acids, such as acetate, lactate, oxalate, tartrate, succinate, citrate, gluconate and glycolate, which, in turn, can solubilize insoluble phosphates in soil (Gyaneshwar et al. 1998).

(c) *Phytohormone Production*

Inoculation with Nif-mutants of *Azoarcus* BH72 significantly promoted rice growth (Hurek et al. 1994), indicating the other mechanisms involved for plant growth promotion by endophytic bacteria. Endophytic bacteria synthesize several phytohormones, such as indole-3-acetic acid (IAA), gibberellins (GA) and cytokinins (CK), which can enhance different stages of plant growth (Lee et al. 2004). IAA has been reported to have an important role in plant development and activation of the plant defence system (Navarro et al. 2006). Involvement of IAA in various growth-promoting functions, such as root formation, stimulation of cell division, extension, differentiation and regulation of fruit ripening, has been indicated (Glick 2012). This hormone is produced by root-associated bacteria, such as *Enterobacter* sp., *Pseudomonas* sp., *Azospirillum* sp. or *Streptomyces* sp. Zhao et al. (2011)

isolated endophytic bacteria from *Sophora alopecuroides* root nodules and found that 1 out of 28 produced a significant amount of IAA. The contribution of IAA for bacterial epiphytic fitness was reported by Brandl and Lindow (1998), and these observations were supported by other works as well (Glick 1995; Patten and Glick 1996; Bastian et al. 1998; Dobbelaere et al. 1999; Verma et al. 2001). Plant-associated bacteria produce IAA via indole-3-pyruvate (IPyA) pathway as the IAA production is positively correlated with plant growth stimulation. The expression of the gene *ipd*C (indole-3-pyruvate decarboxylase) was examined in wheat endophyte *Azospirillum brasilense Sp7* (Rothballer et al. 2005). Lowering the ethylene levels in plant roots relieves the auxin suppression response factor synthesis and thus indirectly increases plant growth (Gao et al. 2010). The abscisic acid (ABA) and gibberellic acid (GA) produced by the endophytic *Azospirillum lipoferum* impart water stress alleviation in maize (Cohen et al. 2009).

(d) *Siderophore Production*

Iron is an essential micro nutrient, with ferric (Fe^{3+}) ion being the most common form in well-aerated soil. However, plants absorb ferrous (Fe^{2+}) form of iron (Salisbury and Ross 1992). Endophytic bacteria produce siderophores, low-molecular-weight compounds with high Fe^{3+} chelating affinity. These bacterial siderophores can deliver the Fe^{3+} to the plant root surface where it is reduced to $Fe^{2+}$and absorbed (Bar-Ness et al. (1992). This is known as 'Strategy I' in plants. In 'Strategy II', siderophores excreted by grasses are absorbed with Fe^{3+} across the plasma lemma (Von Wiren et al. 2000). Siderophores can solubilize and transport ferric iron into bacterial cell via ABC-type transporter (TonB-dependent receptors) proteins (Neilands 1981; Hider and Kong 2010). Mitter et al. (2013) reported that genes encoding these membrane-bound TonB-dependent iron receptors are present in genomes *Burkholderia phytofirmans* PsJN and *Gluconacetobacter diazotrophicus* PAl5. A diazotrophic endophyte, *Herbaspirillum seropedicae* Z67, that colonizes the interior tissues of rice, wheat, corn and sorghum produces a lipopeptide siderophore, namely, serobactins A, B and C, via NRPS for iron acquisition (Rosconi et al. 2013). Endophytes that produce siderophore were reported in roots, leaves and grains of rice plant (Loaces et al. 2011). According to Lodewyckx et al. (2002) and Whipps (2001), endophytic bacteria can take up Fe^{3+} siderophore complexes of neighbouring microorganisms, thereby outcompeting those microorganisms. A comparative genomic analysis of endophytes revealed non- siderophore-producing endophytes comprise a larger number of genes encoding membrane receptors than the siderophore producers, hence potentially allowing them to sequester iron from heterologous siderophores produced by other endophytes (Mitter et al. 2013). The siderophores produced by the rhizospheric microorganisms are uncompetitive effects associated with plant pathogens (Hofte et al. 1994). Siderophores produced by endophytic *Methylobacterium* strains suppressed *Xylella fastidiosa*, the causative agent of citrus variegated chlorosis (Araujo et al. 2008).

(e) *Biocontrol Agents*

Endophytes can contribute to the host's successful survival against pathogens (Table 12.2). Their biocontrol potential may be through HCN production, a volatile, secondary metabolite that suppresses the multiplication of soilborne pathogens (Siddiqui et al. 2006). It is an active inhibitor of metal enzymes particularly copper containing cytochrome C oxidases. HCN is synthesized from glycine via HCN

Table 12.2 Biocontrol potential of endophytic bacteria against plant pathogens

Organism	Biocontrol organism	Host plant	References
Streptomyces sp.	Root rot *Phytopthora*	Faba bean (*Vicia faba*)	Misk and Franco (2011)
Paenibacillus sp., *Bacillus* sp.	Charcoal rot *Rhizoctonia bataticola*, *Macrophomina phaseolina* *Fusarium udum*, *Sclerotium rolfsii*	Soybean (*Glycine max*)	Senthilkumar et al. (2009)
Bacillus subtilis	White heads *Gaeumannomyces graminis* var. *tritici*	Wheat (*Triticum aestivum* L.)	Liu et al. (2009)
Pseudomonas, *Serratia*, *Bacillus* sp., *Arthrobacter* sp., *Micrococcus* sp., *Curtobacterium* sp.	Foot rot disease *Phytophthora capsici*	Black pepper (*Piper nigrum* L)	Aravind et al. (2009)
Bacillus cereus	Root rot *Rhizoctonia solani*	Cotton (*Gossypium sp.*)	Pleban et al. (1997)
Pseudomonas fluorescens	Damping off Pythium *aphanidermatum*	Chilli (*Capsicum annuum* L.)	Muthukumar et al. (2011)
Paenibacillus polymyxa AC-1	Blight *Phytophthora. Capsici* Die back *Ceratocystis fimbriata*, *Pseudomonas syringae* pv. Tomato DC3000	Chilli	Hong et al. (2016)
Bacillus cereus, *Bacillus thuringiensis*, *Bacillus pumilus*, *Pseudomonas putida*, *Clavibacter michiganensis*	Soft rot *Fusarium solani* Leaf spot *Alternaria pullulans*, *Alternaria alternata*, *Brachypsectra fulva*	Turmeric rhizomes (*Cucurma longa*)	Kumar et al. (2016)
Bacillus amyloliquefaciens	Anthracnose *Colletotrichum acutatum*	Chilli (*Capsicum annuum* L.)	Kim et al. (2015)
Bacillus amyloliquefaciens	Bacterial wilt *Ralstonia solanacearum*	Peanut	Wang and Liang (2014)

synthetase enzyme, which is present in the plasma membrane of particular bacteria (Blumer and Haas 2000). Different bacterial genera, *Alcaligenes*, *Aeromonas*, *Bacillus*, *Pseudomonas* and *Rhizobium*, are reported to produce HCN (Devi et al. 2007; Ahmad et al. 2008). Studies showed 50% of pseudomonads can produce HCN in vitro (Bakker and Schippers 1987; Schippers et al. 1991). The overproduction of HCN controls fungal pathogens of wheat (Flaishman et al. 1996). HCN production is necessary under field applications to improve plant resistance to pathogens under natural conditions if the host-associated bacteria produce this component. Dalal et al. (2014) showed an antagonistic activity of HCN-producing soybean endophytes against soilborne fungal pathogens, namely, *Rhizoctonia solani*, *Fusarium oxysporum*, *Sclerotium rolfsii*, *Colletotrichum truncatum*, *Macrophomina phaseolina* and *Alternaria alternata*, under in vitro conditions. Besides HCN, other volatile substances, such as 2,3-butanediol and acetoin, produced by endophytic bacteria are also responsible for pathogen suppression (Ryu et al. 2003). The genetically engineered endophytes, namely, *Herbaspirillum seropedicae* and *Clavibacter xyli*, produce δ-endotoxin of *Bacillus thuringiensis*, which can control insect pests (Turner et al. 1991; Downing et al. 2000). Studies revealed that endophytic colonization can trigger the genes for carbon metabolism, N assimilation and plant growth and genes for a limited plant defence (Elvira-Recuenco and Van Vuurde 2000). However, limited carbon sources in the apoplastic fluid can restrict endophytic growth (Rediers et al. 2005). Molecular studies using *Medicago truncatula* and *Arabidopsis thaliana* mutants showed plant defence-response pathway-mediated regulation via endophytes (Boller 1995; Iniguez et al. 2005). The endophytic actinobacteria that produce a broad spectrum of antibiotics have also proved their biotechnological significance (Coombs et al. 2004; Taechowisan et al. 2005; Swarnalakshmi et al. 2016).

12.7 Endophytes in Plants' Secondary Metabolite Production

The endophytes are a valuable source of new bioactive compounds (Tadych et al. 2009; Priti et al. 2013; Gouda et al. 2016), which are promising for medicine, agriculture and industry (Guo et al. 2008). Different kinds of alkaloids produced by the endophytes may provide resistance in plants against environmental stresses. The amines and amides produced have shown toxic effects to insects. Similarly, steroids, terpenoids and diterpenes are produced by endophytes (Tan and Zou 2001). Endophytes are reported to produce alkaloids and other fine chemicals, which, in turn, may induce resistance to nematodes, insect herbivores and livestock. The main advantage of endophyte infection to plants may be that it increases production of chemical toxins after damage to the plant has occurred (Bultman and Murphy 2000). Endophytic bacteria enhance plant fitness by producing novel bioactive compounds. Lipopeptides (non-ribosomal peptide synthetases (NRPS)) produced by the endophytic *Bacillus* and *Pseudomonas* play an important role in antibiosis and induce

plant defence mechanisms (Raaijmakers et al. 2010). The endophytic *Streptomyces* sp. HKI0595 (Ding et al. 2011) and *Streptosporangium oxazolinicum* K07-0450^T (Inahashi et al. 2011) produce multicyclic indolosesquiterpenes and antitrypanosomal alkaloids spoxazomicins A–C, respectively. Interaction between plant (*Echinacea purpurea*) and endophytes on alkamide production suggests their possible role on host's secondary metabolism, which, in turn, may influence the therapeutic properties of host plants (Maggini et al. 2017). The endophytic communities associated with medicinal plants may have antitumor and antimicrobial potential. The crude extracts of these endophytes showed cytotoxic activity against multiple myeloma RPMI-8226 cells and antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*.

These endophytes exhibit structurally diverse gene clusters of NRPS and PKS (polyketide synthases), which produce novel bioactive compounds and play a possible role in host plant bioactivity in medicinal plants (Miller et al. 2012). Indole-based derivatives, such as 6-isoprenylindole-3-carboxylic acid produced from *Artemisia annua*, show activity against Gram-positive and Gram-negative bacteria and plant pathogenic fungi, and some may behave as growth-promoting phytohormones (Lu et al. 2000). An endophytic *Phomopsis* sp. originated from *Salix gracilistyla* var. melanostachys produced phomopsichalasin, a novel cytochalasin. This metabolite inhibited the growth of *Bacillus subtilis*, *Staphylococcus aureus* and *Salmonella gallinarum* and human pathogenic yeast, *Candida albicans* (Tan and Zhou 2001). Various endophytic bacteria, including *Actinocorallia*, *Actinopolyspora*, *Dietzia*, *Isopterico*, *Kytococcus*, *Micromonospora*, *Microtetraspra*, *Nocardia*, *Promicromonospora*, *Rhodococcus*, *Streptomyces*, *Saccharopolyspora*, *Streptosporangium* and *Verrucosispora*, are isolated from medicinal plants, and out of these, *Streptomyces* is the predominant genus. Passari et al. (2017) isolated endophytic actinobacteria, such as *Streptomyces*, *Brevibacterium*, *Microbacterium* and *Leifsonia*, from *Rhynchotechum ellipticum*, a traditional medicinal plant from India. Antibiotic sensitivity assay in combination with the amplification polyketide synthase (PKS-I) and non-ribosomal peptide synthetase (NRPS) genes showed that these endophytes have broad-spectrum antimicrobial activity. The actinobacterial endophytic *Pseudonocardia* sp. strain YIM 63111 induces artemisinin (antimalarial compound) synthesis in the host plant (Li et al. 2012).

The endophytes can influence the host plant's secondary metabolism. The inoculation of endophytic *Methylobacterium extorquens* influenced the flavour-inducing furanone synthesis in the strawberry plants. The alcohol dehydrogenase (ADH) produced by the endophytic bacteria oxidize 1,2-propanediol to lactaldehyde, which is then converted by plants to 2,5-dimethyl-4-hydroxy-2H-furan-3-one (DMHF) and mesifurane, furanones (Zabetakis 1997). The presence of four endophytic ADH and plant DHMF transcripts in the vascular and achene tissues of strawberry fruits indicates the role of plant associated *Methylobacterium* with biosynthetic potential of strawberry flavour (Nasopoulou et al. 2014).

12.8 Methods Used in Endophytic Study

The endophytes, which are either culturable or non-culturable, reside mainly in intercellular space or inside vascular tissues. The techniques used in endophytic study are schematically depicted in Fig. 12.1. The culturable endophytes can be isolated from the surface-sterilized plant tissues. In surface sterilization, prewashed plant samples are rinsed in 70% ethanol for 30–40s and 2%–4% sodium hypochlorite for 5–10 min. The plant samples are then washed with sterilized distilled water several times along with Tween 20 before the final washing (Elbeltagy et al. 2000). The tissues can be placed on medium, and the aliquots of the sterile distilled water used in the final rinse can also be plated onto the same medium and incubated at room temperature to determine any bacterial growth. The surface-sterilized tissues can be cut into 1–2 cm pieces and homogenized with 0.85% sodium chloride or saline phosphate buffer solution. Samples (100 µl) of tissue extract with different dilutions are incubated on media plates and allowed to grow at 25 °C–28 °C. The plates are observed for colonies up to 15 days, and colony count after every 2 days

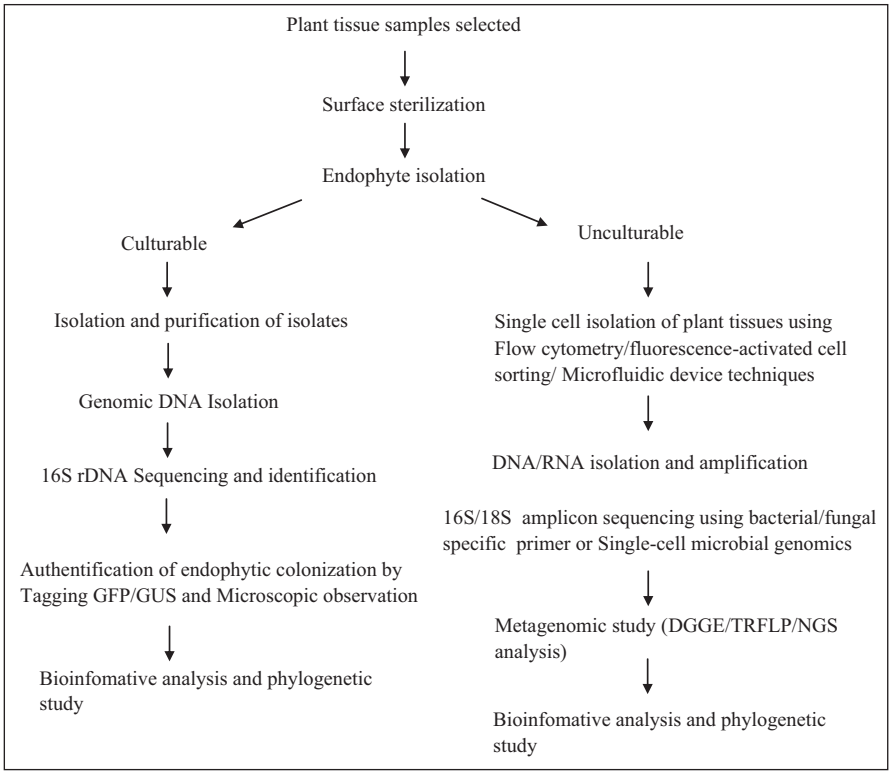

Fig. 12.1 Schematic diagram of endophytic bacterial study

are recorded and expressed as CFU per gram of fresh tissue. Depending on the colony morphotype, bacteria are selected, purified, identified and characterized using 16S PCR (polymerase chain reaction) analysis.

The authentication of endophytic bacteria is carried out by tagging marker genes, such as GFP, with that of the housekeeping genes of bacteria and inoculating into model plants. The presence or absence of the signal inside the plant tissue will help to determine if the bacteria are endophytic or not. Tanaka et al. (2006) incorporated GFP gene to endophytic *Enterobacter* sp. and *Klebsiella* sp. using conjugative plasmid, pTn5Kmgfpmut1, and the fluorescence microscopic observation showed the localization of these bacteria inside the root tissues. Annapurna et al. (2013) reported the endophytic colonization of *Paenibacillus polymyxa* strain HKA-15 in soybean nodules using GFP tagging. Similarly, transposons containing beta-glucuronidase (*gus*) can also be used as a marker gene. The *gus* markers in the test strains are tagged by conjugation with *Escherichia coli* strains harbouring plasmids carrying the respective transposons (Stoltzfus et al. 1997). Naveed et al. (2014) detected endophytic localization of *Enterobacter* sp. in maize using gus marker.

More than 99% of prokaryotes cannot be cultured; however, it is important to understand the physiology, genetics and ecology of unculturable microbial communities (Schloss and Handelsman 2005). Unculturable endophytic bacterial communities can be studied through metagenomic approach, which is based on either expression or whole-genome sequencing (Schloss and Handelsman 2005). DGGE (denaturing gradient gel electrophoresis) was used to study the metagenomic analysis of unculturable endophytic bacterial species of rice (Hardoim et al. 2012). The DGGE patterns of the 16S rDNA PCR products of rice seeds revealed relationship between soil type and bacterial endophytes. The active diazotrophic community associated with rice plants grown with and without nitrogenous fertilizer was studied using PCR-DGGE of *nifH* mRNA (Wartiainen et al. 2008). DGGE profile showed the distribution of rice-associated diazotrophic community in α, β and γ Proteobacteria, Firmicutes and Archaea. Recently, next-generation sequencing (NGS) is a widely used method for studying plant microbiome. Edwards et al. (2015) characterized the rice root-associated microbiome by amplification of hypervariable region (V4-V5) of 16S rRNA gene using NGS.

They observed higher bacterial diversity in rhizosphere than in endosphere and reported that microbiome diversity can vary with various soil types with the genotype depicting the greatest effect on the microbiome. Rice cultivation also accounts for methane gas emissions produced by methanogenic archaea, and the study also supported higher abundance of *Methanobacterium* in endosphere and rhizoplane than in rhizosphere. In another study, Rascovan et al. (2016) carried out a comprehensive analysis of root microbiomes associated with wheat and soybean collected from agricultural fields. Microbiome associated with rhizospheric soil and roots were analysed by amplifying V4 region of 16S rDNA followed by pyrosequencing, and the results revealed that *Pseudomonas*, *Achromobacter*, *Burkholderia*, *Chryseobacterium*, *Halothiobacillus*, *Klebsiella*, *Pantoea*, *Ralstonia* and *Zavarzinia* were the most abundant bacterial community in wheat and soybean. Unculturable organisms are identified from complex microbial communities through genome

amplification of single cells. Single-cell microbial genomics, including flow cytometry or fluorescence-activated cell sorting (FACS), can be used to study the genomic profile of unculturable single cells isolated from the natural environments (Jager and Siezen 2011; Yuan et al. 2018; Fouchet et al. 1993). The technique provides deeper insight into diversity and function of microbial communities (Muller and Nebe-von-Caron 2010). Single-cell micromanipulation method (Kvist et al. 2007) or microfluidic device technique (Marcy et al. 2007) are also used for the isolation of individual cells from uncultured bacterial communities.

12.9 Conclusions

The utilization of endophytic bacteria in agricultural production depends on our knowledge of the plant–microbe interactions and our ability to maintain, manipulate and modify beneficial bacterial populations under field conditions. The study of plant-associated endophytic bacteria is important for understanding their ecological role and plant growth-promoting potential. The gene expression profiles of bacteria in planta are more structured and variable than cultivation-dependent methods under laboratory conditions. The plant signalling networks determine endophytic symbionts in legumes. Different methodologies are used by researchers for studying the bacteria with associated microbes and their roles in plant growth development through secondary metabolite production or as biocontrol. Advanced techniques, such as next-generation sequencing, is applied for determining the taxonomical diversity of the bacterial endophytes associated with the economically and agriculturally important crops. Such studies can be advancement in the microbial research as different initiatives can be taken from these endophytes in the field of agriculture.

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Agricultural Perspectives of Mycorrhizal Glomalin as "Soil Fertility Determinants"

13

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Abstract

Agriculture is a multifunctional unit that involves microorganisms, plants, and animals. They interact together by carrying out various metabolic functions either symbiotically or parasitically or mutualistically. Such interactions help maintain the ecological balance. However, microorganisms play an essential role in maintaining the integrity of soil ecology. In particular, arbuscular mycorrhizal (AM) fungi are the most common microorganisms symbiotically associated with plants. The AM fungi are important in agriculture and have been explored because of their plant growth-improving properties. However, the present review illustrates how the protein (glomalin) produced by AM fungi is helpful in enriching the soil nutrient pool. As soil fertility is one of the factors that determine the output of agriculture, functional properties of AMF are also responsible for mitigation of heavy metal contamination caused by anthropological activities in addition to soil nutrient enrichment.

Keywords

Glomalin · Soil aggregation · Soil fertility proteins · Carbon sequestration · AM fungi

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13.1 Introduction

The glue produced by arbuscular mycorrhizal (AM) fungi was named glomalin after *Glomales*, the taxonomic order of this group of fungi. The discovery of glomalin was reported by Wright et al. (1996). Glomalin is an iron containing glycoproteinaceous substance in red-brown color. The concentration of iron varies in different soils ranging from 2% to 5%. In native state, glomalin is insoluble in water and is stable to heat (Wright and Upadhyaya 1996). Two fractions of glomalin were identified, namely, *total glomalin* and *easily extractable glomalin*. Glomalin may be primarily contained in the hyphal/spore walls and later gets sloughed off from the hyphae into the soil. In the present decade, agriculture largely depends on fertilizers to meet the needs of the agricultural crops. As a microbial biofertilizer, AM fungi are given a wide attention due to its enhanced biological activities. As AM fungi act as nutrient mobilizers, rhizoremediators, and biocontrol agent, some of the wide range of activities is contributed by glomalin (Selvaraj et al. 2004, 2005; Wright and Upadhyaya 1996).

The central role played by glomalin in agricultural and ecological aspects is represented in Fig. 13.1. Glomalin may be indirectly involved in plant growth by protecting the AMF hyphal strands from nutrient loss. Secondly, it is involved in carbon sequestration mechanism, by forming soil aggregation, in which the sequestered organic matter undergoes microbial attack resulting in the release of the essential nutrients required for microorganisms. The increase in beneficial microbial community in the rhizosphere would substantially benefit the plant growth. In addition, the formation of aggregates by glomalin reframes the soil structure that facilitates water infiltration, moisture retention, air permeability, etc. (Wright and Upadhyaya 1996). These properties, in particular, enunciate increased plant growth. Even though the above said criteria are fulfilled, the additional capability of glomalin is indeed essential in obtaining pure agricultural products through sequestration of

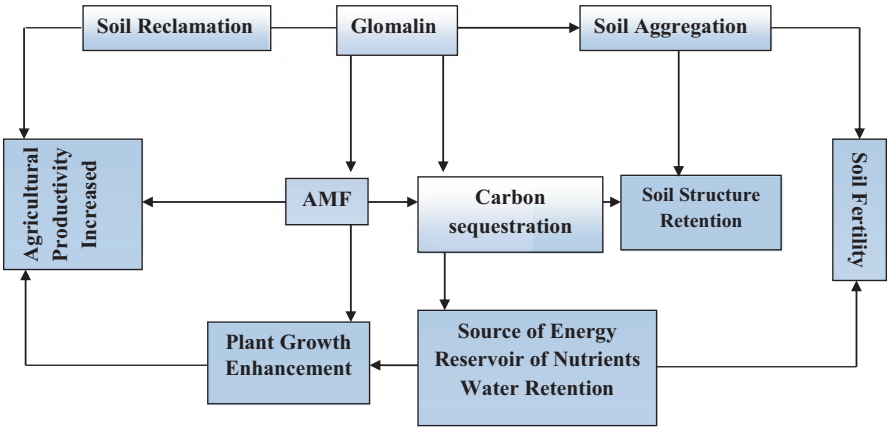

Fig. 13.1 Agriculturally Important Role Played by Glomalin

potentially toxic heavy metals (Gonzalez-Chavez et al. 2004). Hence, all these properties of glomalin likely increase the agricultural productivity. The detailed advantages of glomalin in ecological and agricultural prospects are as follows.

13.2 Origin of Glomalin Protein

The increasing accumulated evidence from decomposition studies suggested that this glomalin is of AMF origin. The importance of AMF for managing soil ecology is based on the presence of glycoproteinaceous substance called glomalin. Wright and Upadhyaya (1998) reported that the amount of glomalin protein in the soil is usually correlated with the aggregate water stability of the soil. Evidence that glomalin is produced by AM fungi, not plant roots, was obtained early in the investigation of the reaction of the monoclonal antibody against glomalin. In a blind experiment, immunofluorescence correctly identified glomalin only on roots that were later described as having AM colonization. Further, Steinberg and Rillig (2003) reported, when the AMF growth is eliminated (e.g., by incubating soil without host plants), concentration of glomalin in soil was reduced; also, they have observed the reduction in hyphal growth. Immunofluorescence assays show that glomalin coats AM fungal hyphae; sloughs from hyphae onto colonized roots, organic matter, soil particles, horticultural or nylon mesh, and glass beads; and is found on arbuscules within root cells (Wright et al. 1996; Wright and Upadhyaya 1999; Wright 2000). Glomalin is deposited in the soil, where it accumulates until it represents 5% of the soil C and N (Rillig et al. 2002; Lovelock et al. 2004). However, the ecophysiological function of glomalin protein is unknown, and it may be related to the glomalin protein (Gadkar and Rillig 2006). Glomalin was detected on AM fungal hyphae using an indirect immunofluorescence procedure that employs the antibody against glomalin and a second antibody tagged with fluorescein isothiocyanate (FITC) molecule (Wright 2000).

13.3 Glomalin Protein Production

Glomalin production is studied through short-term greenhouse studies. It is reported not to be exuded by the AMF hyphae but contained within the hyphal walls (Driver et al. 2005). Treseder and Allen (2000) reported that the AMF hyphae decay due to age and excrete glomalin as a residue mass in the soil. The hyphal glomalin content, standing stock, and turnover rate may determine the rate of the deposition of glomalin in the soils. Glomalin production rate is not always correlated with the abundance of AMF in the soils. Lovelock et al. (2004) used sand-filled ingrowth cores incubated in the tropical forest soils of Costa Rica and in the corn and sand cultures at the USDA in Maryland to estimate glomalin yields as a function of AMF hyphal length. Multiple mechanisms are responsible for the lack of a clear-cut correlation between AMF hyphal lengths and glomalin observed.

Standing stocks of glomalin in the soils are measured through the production, decomposition, and environmental conditions which may affect two fluxes independently. Rillig and Steinberg (2002) showed that the soil texture is linked to the yields of glomalin. In a global survey of soil glomalin amount, variation in biomes of glomalin stocks to net primary productivity (NPP) and AMF abundance was recorded. Both NPP and AMF influence glomalin production.

13.4 Role of Glomalin in Fertility

Glomalin may contribute to the long-term sustainability of agricultural ecosystems under subtropical conditions. Glomalin is the major and unique component of soil organic matter (SOM) (Pikul Jr et al. 2002). The weight of the glomalin is constituted by 30% of carbon. SOM has a greater significance in determining and influencing numerous aspects of soil quality, which include nutrient storage and water-holding capacities (Paul and Clark 1989). Organic C, organic N, and carbonate C are strongly correlated with glomalin (Bird et al. 2002). The glomalin was observed to increase with N availability in Harvard forests (Robinson 2002). The stability and resistant property of glomalin against proteolysis occur by binding to polymers like lignins, other carbohydrates, and phytates. This complex-forming property indeed increases the soil carbon, nitrogen, and phosphate pool. Thus, the increase in nutrient pool possibly occurs through the decomposition of soil organic matter, and it is the most significant mechanism in changing the nutrient C flux of the soil (Wright et al. 1998). Such decomposition is usually carried out by microorganisms, which makes the nutrients available to plant growth. Given the above information, the decomposition rate and time of glomalin under microbial influences would indicate the percentage of nutrient levels released from glomalin under controlled conditions. By using various native soil microbial isolates for efficient decomposition of glomalin, various percentage of nutrient release by microbial isolates can be identified.

13.5 Role of Glomalin in Soil Aggregation

Soil aggregates are dynamic. They form and reform over time, thereby making the organic material occluded within them accessible to degradative enzymes (De Gryze et al. 2005). Soil aggregation is a complex hierarchical process in which the concentration of glomalin is tightly correlated with aggregation stability (Wright and Upadhyaya 1998; Rillig 2004). It is an indicator of its quality directly relevant to carbon sequestration (Lal et al. 1998). In several ways, AM fungal colonization helps either directly or indirectly the growth of plants through production of glomalin. Glomalin is critically important in soil biological process because they carry out intense interactions with plant, with soil, as well as with soil microbes. Glomalin is released into the soil during the decomposition of hyphal strands, binds to the soil particles, and is capable of aggregating the soil together. The glomalin produced

acts as a "glue" by making the soil debris stick to the plant roots and AM fungal hyphae. Glomalin also forms a hydrophobic lattice around the aggregates and makes it water stable (Nichols and Wright 2004). The glomalin at higher levels is able to improve water infiltration rate, increase soil permeability to air, and promote greater root development, higher microbial activity, and greater resistance to surface sealing and erosion. This obviously leads to improved soil structure. If there is possibility of a situation without glomalin, the water would easily rush into intra-aggregate pore space causing the air molecules to condense.

The function of glomalin is to protect the fungal hyphae and maintain water and nutrients loss during the hyphal approach to the host plants. Thereby, it protects the hyphae from decomposition and microbial attack. When glomalin is present in the rhizosphere, the following combination of functions such as hydrophilic, acidic, complexing, and sorptive occur (Johnson et al. 2005; Rillig and Mummey 2006; Schubler et al. 2007). Glomalin is strongly influenced by the iron content in it, because the materials bound by polyvalent metal cations and polymers contribute to the persistence of aggregates (Wright and Upadhyaya 1998). The quantifiable amounts of glomalin are produced during the active colonization and ramification of AM fungal mycelium in the soil (Wright and Upadhyaya 1996). Soil aggregation aids in increased aeration, water infiltration, root development, and microbial activity. The minerals and organic matter present inside the aggregates are protected from the wind and water erosion. These aggregates undergo slow degradation through microbial attack, and the nutrients are released. Soil aggregates improve the structure, quality, and fertility and thereby obviously influence crop establishment and growth while also providing habitat for soil biota (Denef et al. 2002).

13.6 Role of Glomalin in Reclamation

AM fungi help in the sustainability of plant growth even in the disturbed or chemical-contaminated soils. AM fungi can alleviate the heavy metal stress caused to plants by binding to them into roots, thereby restricting their translocation into roots (Kaldorf et al. 1999). The additional tolerant mechanisms followed by AM fungi resist the metals including absorption onto fungal cell walls (Joner and Leyval 1997), siderophore-mediated chelation, and change in soil pH, microbial communities, and root exudation patterns. There occurs a high possibility of heavy metal accumulation in the fungal structures as they have high heavy-metal binding capacity, thereby representing them as a biological barrier (Dehn and Schuepp 1989). This may be due to the fact that the AM fungal hyphal structures are lined with glomalin (Joner and Leyval 1997). Glomalin is involved indirectly in reducing the levels of potentially toxic heavy metals such as Cd, Pb, Mn, and Fe in the plant host. The mechanism of heavy metal reduction by glomalin is through the molecular binding of these metals (Chern et al. 2007). It was found that AM fungi have the ability to absorb 3–14 mg Cu/g dry wt of AM fungal hyphae. The sequestration of Cu takes place by two means: electrostatic Cu sorption and strong complex formation. The complex of glomalin and Cu is highly stable (Gonzalez-Chavez et al.

2004). The complex formation of glomalin with other heavy metals like Zn, Al, U, etc. can be further analyzed. Even under the circumstances of survival in high heavy metal contaminated sites, AM fungi improve the plant growth and P nutrition. In addition, the carbon sequestration on the lands applied for the agriculture and forestry purposes can be reclaimed, and this could be a potential option to mitigate global climate change (Lal 2003). Such carbon sequestration activity is greatly carried out by glomalin, which thus indirectly helps soil reclamation. AM fungi along with glomalin protein has a wide range of functional abilities in improving the soil fertility, plant growth, and crop yield as well as in cleaning up of heavy metal-contaminated sites. From this, we could infer that sustainable agriculture is feasible through increasing the production of glomalin.

13.7 Role of Glomalin in Stress Tolerance

Glomalin has been closely related with heat shock protein 60 (hsp60). These proteins are produced by eukaryotic and prokaryotic cells when under stressed environmental conditions (increased temperatures, pH change, and nutrient starvation) (Gadkar and Rillig 2006; Purin and Rillig 2007). Gadkar and Rillig (2006) have reported that the amino acid sequences of glomalin are linked to hsp60 using liquid chromatography mass spectrometry. Further, they have reported that these glomalin protein may be serving as a protective function for AMF as a stress-induced protein (Rillig and Steinberg 2002; Driver et al. 2005). Cornejo et al. (2008) relating the glomalin protein with heat shock protein clarify how stress imposed by heavy metals may rapidly increase glomalin production by AMF and GRSP concentration in polluted soils. Rillig and Steinberg (2002) demonstrated that the increased space of AMF has influence on the reduction of glomalin. The study shows that unfavorable growth conditions may enhance glomalin production by AMF. Glomalin performs a protective function in a living fungus, and AMF allocates many of its resource to glomalin production (Rillig and Steinberg 2002).

13.8 Role of Glomalin in Carbon Storage

Glomalin is reported to account for 4–5% of total carbon (C) and nitrogen (N) in the Hawaiian soils. It contributed to the production of glycoprotein comprising of high total C than that of the microbial biomass carbon (Zhu and Miller 2003; Rillig 2004). It facilitates soil carbon storage (Rillig et al. 2001). Wilson et al. (2009) observed low level of C and N in soil due to the suppression of AMF and its relation to significant decreases in AMF hyphae and GRSP concentrations. Further, they have speculated that the reduction in AMF hyphae and GRSP concentration leads to the loss of C and N and in macroaggregates by reducing aggregation and stabilization. Fenney et al. (2004) reported that not much is known about the direct influence of glomalin on organic storage, since most of its relation to C storage is by virtue of stabilizing aggregates.

13.9 Factors Influencing Glomalin Production

The higher glomalin production is, generally, related to the type of AM fungal species, their diversity, nature of extra-radical hyphae, and its activity (Helgason et al. 1998; Ryan and Graham 2002; Auge 2004). In addition, the concentrations of glomalin are highly dependent on the levels of soil C and N. However, the controls on the production of glomalin are still unknown (Rillig et al. 2001). If the control mechanisms for the glomalin production are identified, the creation of mutation in the particular gene would express the defects caused when glomalin production is stopped/mutationally changed. Nutrient composition, iron concentrations in the soils (Wright and Upadhyaya 1996), climate aberrations (growing season length, temperature, moisture), the fungi involved (AM fungal species identity and possibly diversity), host plant(s), and their productivity could become important contributors in the production of glomalin, which is present in soils into the magnitude of >60 mg cm^{-3} or over 100 mg g^{-1} (Rillig et al. 2001).

AM fungi community composition may be an important regulator of GRSP (glomalin-related soil protein) production in soils. Certain agricultural practices and management can influence the production of glomalin in higher or lower levels. The physiology of AM fungi controls the production of glomalin. The other factors that influence the production of glomalin include rhizosphere microbial population, physicochemical characteristics of the soil, and fungus host-species combinations (Rillig 2004). There is a strong correlation of glomalin with AM fungal hyphal length and stability of soil aggregates indirectly involved in soil carbon storage by forming soil aggregates. Sumathi et al. (2008) studied the climatological influence on glomalin and revealed that the maximum total glomalin concentrations were observed during the months of October and November. The biotic and abiotic influences on the concentrations of individual glomalin were studied; the glomalin was statistically significant and positively correlated with plant yield and quality. The variation in the concentration of glomalin is also based on the soil type, cultivation practice, water, etc. (Rillig et al. 1999).

13.10 Future Prospects

The management of several following agricultural practices would be helpful in increasing the agricultural productivity, by reducing the CO_2 and methane release, proper nutrient management in soil, replacing the use of chemical fertilizers with biofertilizers especially AM fungi, and mulching and tillage practices, as glomalin is susceptible to these factors. While discussing the agricultural perspectives, focus on biocontrol mechanisms against plant pathogens to protect the agricultural crops from damage is critically important. The agricultural crops are in general highly prone to microbial/nematode diseases resulting in a great loss in agricultural productivity. After the isolation of pure glomalin, toxicity patterns of glomalin as individual nematicidal/insecticidal/antagonists compound can be analyzed. This analysis would be a new approach in the field of biocontrol.

Even though the merits offered by glomalin are wide, during the soil and roots extraction, glomalin extracted along with phenolic compounds would show erroneous results. The presence of tannic acid substances in the glomalin extracts may produce spurious results of colorimetric Bradford assay, and there occurs a problem of overestimation of glomalin when tannins are present (Rillig et al. 2001). Additional problem is that once the glomalin is extracted from the soil or plant roots, it loses its native form. Further effort to study the characteristics of glomalin is becoming a difficult task. An alternative method, which eliminates the interfering compounds at the time of extraction, is necessary because the concentration of glomalin varies according to vegetation and soil type. From the above discussion, it can be ascertained that glomalin production is greatly influenced by several biotic and abiotic factors. A slight change in the biotic/abiotic factors would create a stress that directly reflects the glomalin production. We have little idea of the relationships between glomalin, biotic, and abiotic factors. The impact of every single factor on glomalin is necessary, and there is a need to design special experiments to identify the role of that particular factor in glomalin production. Such studies would be immensely helpful in knowing the biochemical mechanisms influenced by the individual factor. In fact, there is increasing knowledge about the glomalin concentrations and its variations according to soil type and cultivation practices. The interest in biochemical nature of glomalin is increasing as evidenced by various structural analyses being performed. However, there is a need to understand the molecular mechanisms, which are involved in increasing the production of glomalin. Further extensive research is warranted at a molecular level to confirm this.

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Perspectives of Plant Growth-Promoting Rhizobacteria in Conferring Salinity Tolerance in Crops

14

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Abstract

Soil salinity is imposing serious threats for crop production particularly in arid and semi-arid regions. Various causes for increasing soil salinity in agricultural lands around the globe include weathering of rocks, excessive irrigation, deforestation and poor drainage. Scraping, flushing and leaching are physical means by which soil salinity can be managed, but to a limited extent. Salt-tolerant crop plant varieties are developed by plant biotechnologists to overcome the salinity issues. Bacteria that exist in the rhizoplane and rhizosphere and that are endophytic have shown positive effects on the crop with respect to nutrient availability and therefore are of great importance. The current chapter encompasses the adverse effects of salinity on crop plants and direct and indirect effects of plant growth-promoting rhizobacteria (PGPR) in amelioration of salinity stress and the mechanisms involved thereby. Nitrogen fixation, phosphate solubilisation, phytohormones and the siderophores produced by PGPRs directly make the nutrients available to the plants and allow the crops to grow vigorously. The indirect mechanisms involve production of lytic enzymes, antibiotics that inhibit the pathogen. PGPRs produce osmotolerant chemicals, reactive oxygen species scavenging enzymes and the enzymes that reduce the oxidative stress on the plant system and thereby induce systemic resistance to saline conditions in the plants. In conclusion, the PGPRs can be used as alternate strategy for not just flourishing of the crop plants but also allowing them to withstand a stress condition and thus can be used so that the barren saline lands can be brought under cultivation.

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Keywords
Soil salinity · NaCl · Plant growth-promoting rhizobacteria (PGPR) · Phytohormones · Siderophores

Abbreviations

ACC)-deaminase	1-aminocyclopropane-1-carboxylate deaminase
ePGPR	Exo-PGPR
EPS	Exopolysaccharides
IAA	Indole-3-acetic acid
IAM	Indole-3-acetamide
iPGPR	Internal PGPR
IPyA	Indole-3-pyruvic acid
PGPB	Plant growth-promoting bacteria
PGPR	Plant growth-promoting rhizobacteria
PSB	Phosphate-solubilising bacteria
ROS	Reactive oxygen species
VOC	Volatile organic compounds

14.1 Introduction

Under unfavourable conditions, plants face challenges and deviate from optimal growth and reproduction phase. Under these unfavourable conditions, the plants are said to be 'stressed'. A wide range of environmental stresses such as temperature, drought, high and low light, sodicity (alkalinity), acidity and salinity show adverse effects ranging from growth retardation to even the death of the plants. Temperate and tropical agriculture is severely affected by salinity, an abiotic stress that accounts for 20% of agriculture worldwide (Pessarakli 1999; Mayak et al. 2004; Glick et al. 2007). Agriculture is facing a lot of challenges in producing healthy seed sets and accelerating assimilates from source to sink due to environmental challenges including salinity (Ahmad et al. 2012; Mantri et al. 2012). Salinity is a major abiotic stress which hinders the productivity of various crops in agriculture (Shanker and Venkateswarlu 2011; Khare et al. 2015; Kumar et al. 2017; Kumar and Khare 2016; Khare et al. 2018). Saline soils contain high concentrations of one or more soluble salts particularly chlorides, sulphates and carbonates of sodium, calcium and/or magnesium, leaving substantial negative impacts on plant productivity (Kumar and Khare 2015, 2016).

The soil salinity is a serious problem in dry and arid/semi-arid climates (Shrivastava and Kumar 2015). It results in the formation of salt marshes and salt lakes which is caused mainly by weathering of rocks and minerals, precipitation and

washing off of salts and their deposits (Rengasamy 2002). Salts occur in these soils in the form of charged ions that are released from weathering process. The weathering of rocks releases soluble salts such as sulphates, carbonates and chlorides of calcium, magnesium and sodium. Of these, sodium chloride (NaCl) is the most soluble and abundant salt in saline lands which is also carried from the oceans by rain and wind (Pitman and Läuchli 2002; Parihar et al. 2015; Kumar and Khare 2015). On the other hand, secondary salinity is caused by human activities like aggressive irrigation, deforestation and poor drainage. In most of the areas where the wild flora is replaced by annual crops, the water table gradually rises. Dissolved salts get accumulated in the topsoil as water evaporates resulting in salinity and forms a salt scald. Hence, irrigated lands become more saline as compared to drylands as water leaves behind salt deposits year after year. Salinity impairs plant growth by causing osmotic imbalance, ion imbalance and toxicity, and oxidative bursts (Srivastav et al. 2018; Kumar and Khare 2019).

On the other hand, salinity stress is also caused by over-irrigated areas leading to waterlogging or occasionally water-deficit conditions causing salt accumulation hampering nutrient supply to plants. Saline conditions are also exhibited in groundwater due to irrigation with salt-rich water. The amount of salinity stress experienced by the crops also depends upon the type of soil in a particular region like clayey soils have high capacity to accumulate Na+ ions as compared to more sandy soils.

Salinity stress is detrimental to crop growth, yield and quality of produce and is termed as a serious problem for agriculture (Munns and Tester 2008). Hyper soil salinity affects the plants (particularly glycophytes or salt-sensitive crops) at different levels ranging from physiological, biochemical and molecular. Owing to the severity of salinity problem and its implications on crop yields, several attempts have been made to understand various mechanisms underlying salt stress responses and tolerance in plants (Kumar et al. 2018). Considering the limited success with conventional breeding programs for developing salt-tolerant high-yielding crops, several approaches including genetic engineering and molecular breeding approaches have been explored by the researchers around the globe for conferring salinity stress tolerance in important crop species (Kumar et al. 2017). Different signal transduction pathways and gene regulatory networks are worked upon to enhance tolerance to salinity stress experienced by plants at biochemical and molecular levels (Hasegawa et al. 2000).

However, owing to the severity of the problem and urgent necessity of the effective solutions, scientific community advocates for other potent, novel and easier approaches to overcome soil salinity problems. One of the potent approaches is the use of beneficial microbial inoculants to improve salt tolerance in plants in a viable, economic and feasible option. This may help to reclaim salinity-prone areas being used for the cultivation of different crops (Berg 2009). Plants, in association with their inhabitant microbial communities, the phytomicrobiome, function as a halobiont. The biology of the host plant is affected by the phytomicrobiome which facilitates them by modulating the regulatory path for adaptations in the existing habitats. This may be helpful in altering biochemical and molecular levels of the plants in favour of resistance or tolerance of stresses. Members of the phytomicrobiome, which include plant growth-promoting rhizobacteria (PGPR), are inoculated as

microbial consortia, and this strategy has gained interest to improve salinity-tolerant crops (Smith et al. 2015). Through this chapter, we are presenting herein the potential use of PGPR for enhanced plant growth and in conferring salinity tolerance in the crops treated with the PGPR. The current knowledge, successful events and challenges are also discussed.

14.2 The Plant Growth-Promoting Rhizobacteria (PGPR)

Rhizosphere is a dynamic zone around the plant roots influenced by root secretions and the microorganisms residing there. The plant root system confers a great influence on this narrow zone around them as the root exudates such as sugars and amino acids accumulate in this region. This provides a good source of nutrients and energy to the soil bacteria. This is directly reflected by 10- to 100-fold increase in the bacterial counts in the rhizosphere as compared to the bulk soil. These microorganisms show significant influence on the plant growth and yield (Singh 2013; Singh et al. 2015).

The effects of plant-associated bacteria are known to be both adverse and beneficial (Dobbelaere et al. 2003). The bacteria that prove to be beneficial are referred to as plant growth-promoting bacteria/rhizobacteria (PGPB/PGPR). PGPR colonise the rhizosphere, rhizoplane (root surface) or the root itself and promote the plant vegetative growth (Gray and Smith 2005). The PGPR are also referred to as exo-PGPR (ePGPR) which resides in the rhizosphere or rhizoplane and internal PGPR (iPGPR) that are found within the root cells especially in the nodular structures. Both ePGPR and iPGPR are involved in plant growth promotion in different ways (Gray and Smith 2005).

Bacteria belonging to family Rhizobiaceae (includes genera *Rhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Allorhizobium* and *Mesorhizobium*, collectively termed rhizobia) invade plant root system and form root nodules. These gram-negative rods and some gram-positive cocci and rod-shaped bacteria other than rhizobia promote plant growth by increasing nitrogen availability as they fix atmospheric nitrogen. However, the ePGPR do not form nodules but enhance plant growth by several mechanisms (mentioned in Table 14.1) along with free-living N_2 fixation, which will be discussed in the subsequent sections of this chapter. Some of the ePGPR are free-living nitrogen-fixing bacteria and bacteria from genera *Bacillus*, *Pseudomonas*, *Erwinia*, *Aeromonas*, *Actinobacter*, *Serratia*, *Micrococcus*, *Arthrobacter*, *Flavobacterium*, *Chromobacterium*, *Agrobacterium*, *Hyphomicrobium*, *Caulobacter* and *Enterobacter.*

14.3 Mechanism of Action: Direct

The PGPR promote plant growth by various mechanisms that include both direct and indirect mechanisms. The direct mechanisms involved facilitate nutrient uptake or make them available to the plants by nitrogen fixation, mineralisation of organic compounds, solubilisation of mineral nutrients and phytohormone production, as discussed below.

Table 14.1 Activity and effects of plant growth-promoting bacteria on crop plants under salinity stress

Sr. No.	Organism	Activity/mechanism (effect)	Crop	References
1	*Achromobacter piechaudii*	ACC deaminase (tolerance up to 172 mM NaCl)	Tomato (*Lycopersicon esculentum*)	Mayak et al. (2004)
2	*Pseudomonas* spp.	2,4-diacetylphloroglucinol - 2,4-DAPG (antibiosis/ suppression of pathogenesis)	Wheat (*Triticum aestivum*)	de Souza et al. (2003)
3	*Bacillus* sp., *Pseudomonas* sp. and *Serratia marcescens*	Ammonia production and hydrogen cyanide	Maize (*Zea mays* L.)	Agbodjato et al. (2015)
4	*Bacillus subtilis BBG100*	Mycosubtilin: antagonistic activities against several yeasts and pathogenic fungi	Tomato (*Lycopersicon esculentum*)	Leclère et al. (2005)
5	*Pseudomonas* spp.	Catecholate siderophores, hydroxamate siderophores	Chickpea (*Cicer arietinum* L.)	Sujatha (2013)
6	*Bacillus pumilus* and *Bacillus licheniformis*	Gibberellin production	Alder (*Alnus glutinosa* [L.] Gaertn.)	Gutierrez-Manero et al. (2001)
7	*Pseudomonas putida* GR12-2	IAA production	Mung bean (*Vigna radiata*)	Patten and Glick (2002)
8	Phosphate-solubilising bacteria (PSB) from solid waste-composting samples	Organic acid production, P solubilisation	-	Wei et al. (2018)
9	*Frankia*	Symbiotic N2 fixation	Dicotyledonous plants	Pawlowski and Sirrenberg (2003)
10	*Chitinophaga*, *Nitrospira*, *Flavobacterium*	Produce antibiotics, nitrogen fixation, phosphate solubilisation	Maize (*Zea mays* L.)	(Yang et al. 2017)
11	*Azotobacter salinestris*	N2 fixation, IAA and GA production and phosphate solubilisation	Maize, sorghum and wheat	Chennappa et al. (2018)
12	PGPR	IAA production, phosphate solubilisation, degrade cellulose	Chickpea (*Cicer arietinum* L.)	Hossain et al. (2016)
13	*Burkholderia cepacia SE4*, *Promicromonospora* sp. *SE188* and *Acinetobacter calcoaceticus SE370*	Low ABA, higher GA production, anti-oxidative enzymes produced (abiotic stress management)	*Cucumis sativus*	Kang et al. (2014)

(continued)

Table 14.1 (continued)

Sr. No.	Organism	Activity/mechanism (effect)	Crop	References
14	*Ochrobactrum intermedium*	Indole acetic acid and siderophores and present ACC deaminase activity, biofilm production (high temperature and salt stress up to 300mM tolerated)	*Arachis hypogaea*	Paulucci et al. (2015)
15	*Burkholderia phytofirmans* (PsJN) and *Enterobacter* sp. (FD17)	ACC deaminase, exopolysaccharide production (salinity stress)	Maize (*Zea mays* L.)	Akhtar et al. (2015)
16	*Pseudomonas pseudoalcaligenes* and *Bacillus pumilus*	Caspase-like protease activity and programmed cell death and hence tolerance to salinity	*Oryza sativa*	Jha and Subramanian (2014)
17	*P. fluorescens* NT1, *P. stutzeri* C4, *P. aeruginosa* T15	ACC deaminase, siderophore production, exopolysaccharide production (salinity stress)	Tomato (*Lycopersicon esculentum*)	Tank and Saraf (2010)
18	*Pseudomonas strains* PF1 and TDK1	Tolerance to salinity	*Oryza sativa*	Sen and Chandrashekhar (2014)
19	*Enterobacter* sp. UPMR18	Antioxidant enzyme activities (SOD, APX, and CAT), ACC deaminase (tolerance to salinity)	Okra (*Abelmoschus esculentus* L.)	Habib et al. (2016)
20	*Rhizobium tropici*, *P. polymyxa*	ACC deaminase	Pepper (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum*)	Yang et al. (2009)

14.3.1 Biological Nitrogen Fixation (BNF)

The plant species are not able to convert the 78% of nitrogen present in the atmosphere to a usable form and greatly depend on the process of BNF carried out by soil bacteria. The soil bacteria convert dinitrogen to ammonia which can be used by the plants. Bacteria fix atmospheric nitrogen both symbiotically and non-symbiotically.

In symbiotic nitrogen fixation, the microbes enter the plant roots, form nodules and fix the atmospheric nitrogen. This successful mutualistic relationship is well established in leguminous plants and rhizobia and between nonlegume plants and an actinomycete *Frankia* (Santi et al. 2013). Plants belonging to families Casuarinaceae, Coriariaceae, Elaeagnaceae, Datiscaceae and Myricaceae, and occasionally in Betulaceae, Rhamnaceae and Rosaceae, are found to be involved in this

actinorhizal relationship (Pawlowski and Sirrenberg 2003). Also, bryophytes, pteridophytes, gymnosperms and angiosperms along with some fungi and marine eukaryotes form symbiotic relationship with heterocystous cyanobacteria *Nostoc* and *Anabaena* which fix atmospheric nitrogen (Franche et al. 2009).

Non-symbiotic nitrogen-fixing bacteria are free-living bacteria in the rhizosphere and belonging to genera that include *Azoarcus*, *Azotobacter*, *Acetobacter*, *Azospirillum*, *Burkholderia*, *Diazotrophicus*, *Enterobacter*, *Gluconacetobacter*, *Pseudomonas* and cyanobacteria (*Anabaena*, *Nostoc*).

14.3.2 Phosphate Solubilisation

Phosphorous (P) is the second most essential nutrient for plants after nitrogen. Soil has abundant P (400–1200 mg/kg) in the organic and inorganic form. In spite of this large presence, only ≥1mg/kg of P is available in soluble form and hence has to be supplied in the form of fertilizer. Most of this applied form of fertilizer is precipitated and very less is available for the plants. Hence, utilisation of the phosphate-solubilising property of phosphate-solubilising bacteria (PSB) proves to be an economical and eco-friendly alternative. Apart from phosphate solubilisation, these bacteria elicit other indirect effects that promote plant growth. They include production of indole-3-acetic acid (IAA), hydrogen cyanide (HCN), siderophores, 1-amin ocyclopropane-1-carboxylate (ACC)-deaminase and N_2 fixation and production of biocontrol agents.

14.3.3 PGPR-Induced Phytohormones Production

PGPR have potential to produce phytohormones that include auxins, gibberellins, cytokinins, ethylene and abscisic acid which can mediate processes including plant cell enlargement, division and extension in symbiotic as well as non-symbiotic roots.

14.3.3.1 Auxins

IAA is the most common natural auxin synthesised by plants. The rhizosphere bacteria can synthesise IAA and along with the constituent IAA to stimulate plant growth (Glick 2012). The pathways for synthesis of IAA are either tryptophan-dependent or tryptophan-independent pathways. Pathogenic bacteria such as *Pseudomonas syringae*, *Agrobacterium tumefaciens* and *Erwinia herbicola* synthesise IAA predominantly via the indole-3-acetamide (IAM) pathway (constitutive route). The PGPR predominantly use the trp-dependant pathway where they utilise the L-tryptophan from the plant root exudate and the pathway where indole-3-pyruvic acid (IPyA) is the intermediate (Patten and Glick 1996; Dobbelaere et al. 2003).

Auxins have effect on the whole plant; however, the IAA released in the rhizosphere by PGPR shows significant effect on the plant root system with remarkable increase in plant size, branching number and thereby surface area in soil contact

(Goswami et al. 2016). This increase in surface area leads to more efficient nutrient uptake and directly affects the growth of the plant.

14.3.3.2 Cytokinins

Cell division, seed germination, root elongation, chlorophyll accumulation, leaf expansion and delay senescence are the plant functions affected by cytokinins. These N6-substituted aminopurines (30 growth-promoting structures) are produced by almost 90% rhizobacteria. They influence plant development such as emergence of the seedling and increase root length of several crop species (Gray and Smith 2005).

14.3.3.3 Gibberellins

PGPR belonging to genus *Rhizobium*, *Azospirillum*, *Acetobacter*, *Herbaspirillum* and few species of *Bacillus* are reported to produce gibberellins. Gibberellins are molecules made from a skeleton of 19–20 carbon atoms. 136 different molecules constitute this class of phytohormones, of that four (GA_1, GA_2, GA_3 and GA_{20}) are reported to be produced by bacteria (Gutierrez-Manero et al. 2001). Gibberellins influence developmental processes in higher plants such as seed germination, stem elongation, flowering and fruit setting. Along with auxins (facilitate root development thereby more nutrient uptake), these hormones can be translocated from the roots to the aerial parts of the plant and show pronounced effect on stem and shoot elongation.

14.3.4 Siderophore Production

Iron is abundantly present in the soil as ferric ions (Fe^{3+}), but the Fe^{3+} are sparingly soluble, hence available in very low concentration to plants and microbes. For assimilation of iron, soil microorganisms produce low molecular weight, iron-chelating compounds. These are called siderophores which can transport iron into the cells. Around 500 known siderophores are classified chemically as hydroxamates, catecholates and carboxylates. Plants could uptake labelled iron in large quantities when inoculated with PGPR such as *Aeromonas*, *Azadirachta*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Pseudomonas*, *Rhizobium*, *Serratia* and *Streptomyces* sp. as compared to the uninoculated controls (Sujatha 2013). Availability of iron directly influenced plant growth and chlorophyll content.

Siderophores produced by PGPR also benefit the plants indirectly. They act as biocontrol agents. Under iron limitation, the secreted siderophores show a very high affinity for ferric iron and form a ferric-siderophore complex. This complex is available only to the siderophore-producing organisms and unavailable to other organisms. The producing strain can utilise this complex via a specific receptor in its outer cell membrane. Thus, siderophore-producing PGPR may restrict the growth of phytopathogens.

14.4 Mechanism of Action: Indirect

14.4.1 Production of Lytic Enzymes

Both gram-positive and gram-negative rhizobacteria have shown potential to degrade cell wall of plant pathogens by producing certain enzymes such as chitinases, phosphatases, β-glucanase, proteases, lipases, dehydrogenase, etc. (Hayat et al. 2010; Singh et al. 2015; Goswami et al. 2016). Chitinases degrades chitin, an insoluble linear polymer of β-1,4-N-acetylglucoseamine, which is the major component of fungal cell wall and affects the structural integrity of the pathogen. Various cell wall-degrading enzymes produced by the rhizobacteria impact structural integrity of plant walls against the targeted pathogen and thus act as effective biocontrol agents.

14.4.2 Hydrogen Cyanide and Antibiotic Production

Many rhizobacteria colonise on/around specific plant roots and have ability to produce cyanide. The toxic cyanides are considered as effective means of weed control (Bhawsar 2014; Kamei et al. 2014). Glycine secreted by plant roots acts as a precursor for production of HCN. Cyanides are also considered as part of geochemical cycles and making phosphorous available to the plants (Rijavec and Lapanje 2016). Besides this, the antibiotics produced by PGPR (Reetha et al. 2014; Goswami et al. 2016) inhibit the phytopathogens, thereby improving on plant health.

14.5 Exploration of PGPR in Conferring Salinity Stress Tolerance in Crops

It is an established fact that soil salinity hampers the water uptake by the plants. This causes ionic imbalance and ionic toxicity besides exerting osmotic stress (Munns and Tester 2008). Amongst potent approaches for conferring salinity tolerance in glycophytic crops, the use of PGPR holds significance and is emerging as a sound approach for developing salt-tolerant crops. These bacteria are tremendously beneficial for plant growth under stressful conditions. Figure 14.1 illustrates the beneficial effects of PGPRs on plants and their mechanism of action. Traditionally, bacteria were identified and known to be the symbionts that affect the growth and vigour of crop plants. In mutualistic associations such as classic legume – *Rhizobium* symbiosis – the bacteria are endophytic wherein they invade the plant tissue to form root nodules and fix atmospheric nitrogen. Many other exist in the rhizosphere or on the rhizoplane and are free-living bacteria having ability to fix nitrogen, solubilise phosphate or sequester iron. The root exudates direct the signalling pathways for the activity of these free-living bacteria (Jin et al. 2014; Ilangumaran and Smith 2017; Bharti and Barnawal 2019). Along with nutrient assimilation activity, other beneficial activities such as production of biocontrol agents and degrading pollutants lead

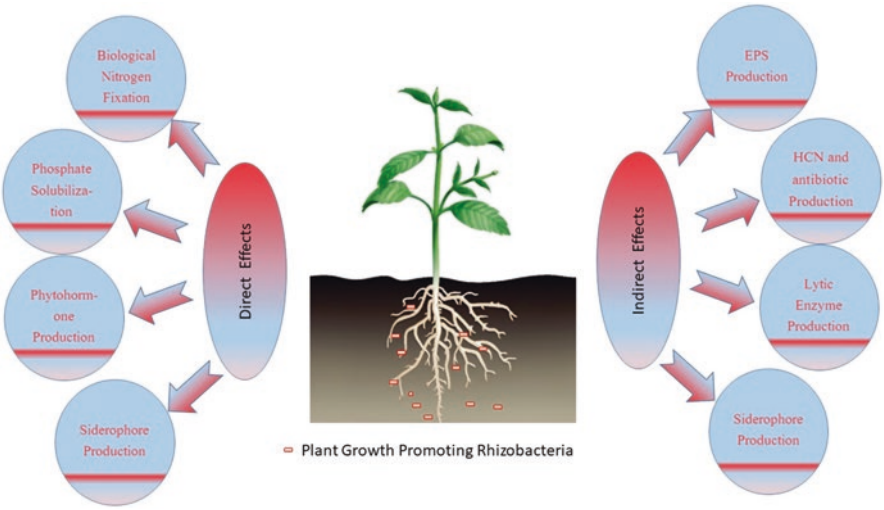

Fig. 14.1 Beneficial effects of plant growth-promoting rhizobacteria on plants and their mechanism of action

to phytoremediation (Beneduzi et al. 2012; Chennappa et al. 2018). The PGPR are also involved directly or indirectly under abiotic stress conditions which are regulated by induction of systemic resistance in plants.

To withstand salt stress, plants tend to accumulate compatible solutes such as proline (decreases the cytoplasmic osmotic potential, facilitating water absorption) and scavenge reactive oxygen species (ROS) molecules (Pottosin et al. 2014; Khare et al. 2015). PGPR are known to induce the biosynthesis and accumulation of compatible solutes in plant tissues, thereby helping the plants to cope up with salinity stress. The salt-stressed pepper plants when inoculated with strains of *Microbacterium* sp., *Brevibacterium* sp. and *Rhizobium* sp. exhibited greater accumulation of proline and rise in the catalase activity as compared to the uninoculated plants, thus alleviating the harmful effects of salt stress on plant growth (Hahm et al. 2017). These plants also showed significant increase in total chlorophyll, plant height, fresh weight, dry weight and content than non-inoculated plants. Sen and Chandrasekhar (2014) observed similar effect on a rice variety inoculated with *Pseudomonas* sp. under salt stress. Improved soil water-holding capacity and reduced soil water evaporation were found for PGPR-treated soil samples. *Arabidopsis* inoculated with *Paenibacillus* (Zheng et al. 2018) increased water availability. The exopolysaccharides (EPS) produced by the PGPR were responsible for this change in water-holding capacity.

Wild-type *Pseudomonas* and an IAA-deficient mutant were used for treatment of canola seeds. The primary roots developed from the seedlings treated with the wild type were 35–50% longer than the untreated seeds and those treated with the mutant (Patten and Glick 2002). These results suggest that bacterial IAA plays a major role in the development of the host plant root system. Habib et al. (2016) reported

treating salt-sensitive okra seeds with ACC deaminase containing *Enterobacter* sp. Enhanced seed germination and growth of okra seedlings under salinity were observed in treated seeds as compared to the uninoculated seeds. Enhanced activity of antioxidant enzymes such as superoxide dismutase, peroxidase, glutathione reductase, mono-hydroascorbate reductase, ascorbate peroxidase and catalase and expression of ROS pathway genes induced by PRPR was helpful in amelioration of salinity. Similar findings were reported in potato plants when treated with PGPR *Bacillus* (Gururani et al. 2013) and enhanced tolerance to salinity stress and in tomato when inoculated with *Achromobacter* (Mayak et al. 2004).

Bacillus amyloliquefaciens SQR9 conferred salt tolerance in maize plants (Chen et al. 2016). The study was conducted in a hydroponic system, and the researchers proposed that the mechanism involved could be decrease in cell destruction due to increased soluble sugar levels, scavenging of ROS due to enhanced peroxidase/catalase activity and glutathione content and Na+ toxicity reduction due to reduced Na^+ levels. The reduction in sodium levels was due to inhibition of uptake or expelling it from roots. *Bacillus amyloliquefaciens* SQR9 also shows upregulation of the expression of genes related to salt tolerance and down-regulates the expression of genes related to abscisic acid in plants. In a similar study, Ashraf et al. (2004) showed that the PGPR produced EPS that restricted uptake of Na^+, thereby conferring salinity tolerance in wheat plants. Chen et al. (2016) also found upregulation of *NHX1* and *NHX7* gene expression (encoding Na^+/K^+ antiporter) in *Arabidopsis* when inoculated with *Bacillus amyloliquefaciens* SQR9 and correlated it to reduced sodium toxicity. In a hydroponic study, Dong et al. (2017) inoculated *Stylosanthes guianensis* with *Bradyrhizobium* strain RJS9-2. In the PGPR-inoculated plant, accumulation of osmoprotectants proline, betaine, ectoine and trehalose and increase in IAA production were suggested as mechanism of salt tolerance. This possible mechanism was further confirmed with the proteomic analysis that showed regulation of 14 salt stress-regulated proteins.

Sinorhizobium meliloti 1021 enabled soya bean plants to adapt to saline conditions (Qu et al. 2016). This adaptation was due to reduced ionic stress by exclusion of sodium; reduction in osmotic stress due to production of osmoprotectants (soluble sugar compounds); and regulating transcription of enzymes involved in ROS scavenging (catalases, ascorbate peroxidase, glutathione S-transferase and superoxide dismutase), salt-responsive genes (stress-induced protein SAM22, PR10-like protein and phosphatidyl inositol-specific phospholipase C) and flavonoids metabolism (cytochrome P450 monooxygenase, chalcone synthase and chalcone isomerase) in soya bean seedlings.

Volatile organic compounds (VOC) such as aldehydes, ketones, alcohols, aliphatic hydrocarbons and sulphur compounds produced by PGPR are involved in antibiosis against phytopathogenic fungi, bacteria and nematodes, whereas methyl jasmonate and ethylene are implicated in development of induced systemic resistance in plants including Arabidopsis, tobacco, tomato, pepper and cucumber (Ali et al. 2015). HCN produced by PGPR is considered as a common biocontrol agent. It shows significant antibiotic activity by regulating availability of key nutrients

such as phosphorous (Rijavec and Lapanje, 2016) and also active as a weedicide (Kamei et al. 2014).

In conclusion, the benefits of organic farming are far more than just as a biofertilizer. The formulations of the beneficial bacteria can be effectively applied to improve the crop affected due to abiotic stress conditions. Plants themselves have mechanisms to tolerate abiotic stress, and development of a stress-tolerant plant variety is an alternative to deal with the abiotic stress conditions. However, both the ways are complex and not so cost-effective. Application of PGPR formulation may effectively enable the plants to sustain in the salinity-stressed conditions. The signalling molecules secreted extracellularly by the PGPRs may also improve the soil quality. Thus, application of microbiota in stress-adapted crops in saline regions has future prospects which are yet not completely explored.

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Microbe-Mediated Biotic and Abiotic Stress Tolerance in Crop Plants

15

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Abstract

Fluctuating global climate has increasing influence on the occurrence of biotic and abiotic stresses in agriculture resulting in reduced productivity. The scenario has been estimated to be intensified owing to the increased drought, soil and water salinity, and shortage of water resources. Biotic stress was also encountered in terms of outbreaks of various pathogens. Diseases caused by pathogens are the foremost factor affecting agricultural produce. Copious mechanisms are implemented by plant to tolerate the stressor(s). Key strategies were designed for developing biotic and abiotic stress-tolerant crop varieties, cultivation techniques, and microbial inoculant and products to enhance the tolerance of plants toward biotic and abiotic stresses. In this literature, we focus on the response of plants toward biotic-abiotic stress, plant-beneficial microbes, and microbe-mediated tolerance in crop plants.

Keywords

Biotic stress · Abiotic stress · PGPR · Phytohormones · Microbial mitigation

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15.1 Introduction

Global environmental changes have adversely affected crop production and have posed a major challenge in maintaining food security, sustainability, and reproducibility to scientific managers during the past few decades. Numerous forecasts have been made regarding increasing temperature, fluctuating levels of atmospheric CO_2, and erratic precipitation. In the early twenty-first century itself, the global agriculture is facing serious problems including severity of water stress, pollution, and salinization of water and soil. Agricultural sustainability is currently facing two major challenges—rising human population and limited availability of land for cultivation (Shahbaz and Ashraf 2013). Remediation of problems such as soil pollution, salinization, degradation, and desertification and other stress-imposing problems is the key to ensure sustainability and food security for the ever-growing population. Multi-disciplinary techniques are essential to improve crop productivity and maintain soil health through enhanced plant-microbe interactions (Lugtenberg et al. 2002; Meena et al. 2017). Drought stress lowers soil water potential, which decreases the availability of moisture in cells, eventually restricting their development and cellular division. The condition ultimately leads to generation of reactive oxygen species (ROS) that ultimately make the plant suffer oxidative stress (Vurukonda et al. 2016). Major consequences happen in plants due to salinity condition, particularly due to restricted water uptake, altered soil quality, and decreased porosity (Munns and Tester 2008). Additionally, high levels of salinity also damage the membrane transport mechanism which ultimately affects nutrient uptake (Tiwari et al. 2011; Sorty et al. 2016; Meena et al. 2017).

Globally, around 20% of the total cultivated and 33% of the irrigated agricultural land are affected by salinity. In addition, around 10% annual increase has been estimated in the saline area because of multiple reasons including low rainfall, increasing surface evaporation, use of saline water for irrigation, and use of poor cultural practices. According to an estimate, more than 50% of the arable land may be salinized by 2050 (Jamil et al. 2011). Under water stress conditions, root length and root structure play a major role in water and nutrient uptake from the soil. Plant root systems have been shown to elongate under drought conditions for efficient fetching of soil water and nutrients (Lopes et al. 2011). Also it has been demonstrated that higher number of primary and secondary roots are developed during moisture stress to increase the root surface area for increased water absorption capacity (Miyahar et al. 2011).

15.2 Plants' responses to biotic stress

Biotic stresses can affect the crop at both the pre-harvest and post-harvest stages. Unlike vertebrates, plants lack adaptive immune system, thus lacking the ability to recognize past infections and counter response ability. On the other hand, plants have evolved with multiple defense mechanisms to counteract disease infections, majority of which are based on secondary metabolites. Biotic (living organisms

such as pathogen, bacteria, herbivores, etc.) and abiotic (drought, salinity, heavy metal, cold, etc.,) stresses represent a form of environmental stress which affects the survival, productivity, and reproducibility (Atkinson et al. 2013; Pandey et al. 2017). Bacterial infections like those triggered by *Ralstonia solanacearum*, the causal agent of wilt in tomato, *Acidovorax avenae* causing seedling blight and bacterial fruit blotch of cucurbits, and *Burkholderia glumae* causing bacterial panicle blight in rice (Kudela 2009) and rise in temperature have been correlated with the improved growth and reproduction of these pathogens (Ladanyi and Horvath 2010). Many studies highlight the defense system of plants suffering from biotic stress such as coffee rust in Brazil, maize leaf blight in the USA, and potato blight in Ireland-Irish potato famine in 1845–1849 (Hussain 2015). The molecular mechanisms behind the nonspecific pathogen resistance are yet to be understood. However, these responses probably depend on both the integral obstacles and the inducible responses that involve proteins and other organic molecules synthesized prior to infection or during pathogen attack (Kiraly et al. 2007; Jones and Dangl 2006). Integral defenses consist of morphological and structural barriers, including cell walls, epidermis layer, trichomes, thorns, etc., and chemical compounds including metabolites such as phenolics, nitrogenous compounds, saponins, terpenoids, steroids and glucosinolates, proteins/peptides, and enzymes (Ferreira et al. 2007; Freeman and Beattie 2008; Dahal et al. 2009). These compounds confer tolerance or resistance to biotic stresses by not only defending the plant from the infectious pathogen but also giving the plant strength and rigidity. Inducible responses involved in biotic stress tolerance are mainly categorized into two forms: systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Bitla et al. 2017; Kannojia et al. 2017). Salicylic acid (SA) and its derivatives (aspirin: acetyl SA) play a major role in biotic stress tolerance in crops. Treatment of aspirin to tobacco plants induced resistance against tobacco mosaic virus (White 1979; Antoniw et al. 1980). SA also has been shown to induce the expression of pathogenesis-related PR genes in plants.

Plant cell wall is the most important physical barrier responsible for restricting microbial infection. The plant cell wall is composed of cellulose, hemicelluloses, pectins, and glycoproteins (Carpita and Gibeaut 1993). When the pathogen manages to pass the cell wall barrier, the pathogen is recognized, and cascades inducing an array of chemical and structural changes happen in the cell, aiming to restrict the infection and protect further pathogen development (Eggert et al. 2014; Voigt 2014; Vorwerk et al. 2004). Some of the defined changes include induction of lignification (Vance et al. 1980; Zhao and Dixon 2014), deposition of cellulose (Luna et al. 2011), cell wall-protein cross-linking (Bradley et al. 1992), accumulation of reactive oxygen species, and synthesis of antimicrobial compounds (phytoalexins) (Franke et al. 2005; Lamb et al. 1997; O'Brien et al. 2012).

Necrotrophic pathogen infection leads to degradation of cell wall, the pathogens are sensed, and defense mechanism signaling cascades are activated through plasma membrane receptors, and ultimately, inducible defense response is raised (Fry et al. 1993; Monaghan and Zipfel 2012) (Table 15.1).

Table 15.1 Biotic stress-responsive genes in plants

Crop	Gene	Function	References
Potato	*NAC* genes	Expression induced of wounding and bacterial infection	Collinge and Boller (2001), Hegedus et al. (2003), and Mysore et al. (2002)
Rice	Xa21	Bacterial blight resistance	Song et al. (1995)
Rice	Xa1	Bacterial blight resistance	Yoshimura et al. (1998)
Rice	Pib	Rice blast resistance gene	Zi-Xuan Wang et al. (1999)
Arabidopsis	WALLS ARE THIN 1 (wat1)	Enhanced resistance to *Ralstonia solanacearum*	Denance et al. (2012) and Ranocha et al. (2010)

15.3 Plants' Responses to Abiotic Stress

Plants have evolved indigenous stress-response mechanisms; however, they exhibit inherent physical, morphological, and molecular restrictions that limit their capability of responding to diverse abiotic stresses (Meena et al. 2017; Atkinson et al. 2013). Abiotic conditions like drought, salt, temperature, and metal contamination can induce production of ROS by limiting the ability of a plant to utilize light energy through photosynthesis (Shinozaki and Yamaguchi-Shinozaki 2000). Stress-sensing ability of plants varies physically, morphologically, and molecularly among wild-type and modern cultivars. However, the underlying mechanisms still remain poorly understood. Plants can sense and respond to stresses in various ways (Ahmad et al. 2015; Jiang et al. 2016). Many of the underlying molecular mechanisms are predominantly unknown. The most noticeable effect of unfavorable conditions initially appears at the cellular levels; afterward, physiological indicators are apparent.

After stress sensing, plants show an immediate and effective response to initiate complex stress-specific signaling cascade (Andreasson and Ellis 2010). Jasmonic acid (JA), ethylene (ET), abscisic acid (ABA), auxin, gibberellins (GA), brassinosteroids, and cytokinins (CKs) are the growth regulators known to play a major role in plant signaling pathways (Pieterse et al. 2012). ABA plays a significant role as a stress-hardener in plants during abiotic stress and also has emerged as an important element of plant's immune-signaling pathway (Cao et al. 2011; Qin et al. 2004; Todaka et al. 2012). Under normal and HT conditions, phytochrome interacting factor 4 (PIF4), a basic helix-loop-helix transcription factor, forms part of the central regulatory pivot facilitating the diurnal growth of plants (Li et al. 2018). The significant role of phyB-PIF4 signaling module in balancing plant growth and defenses during the response to HT stress was demonstrated by Gangappa et al. (2017). Elaboration of antioxidants and osmolytes and activation of transcription factors (TFs) are initiated through the expression of stress-responsive genes for mounting appropriate defense action (Atkinson et al. 2013; Prasch and Sonnewald 2013). In rice, salt tolerance activation-2 (OsSta2) was studied. Plants with overexpression of OsSta2-Ox were more tolerant to osmotic stress and maintain healthier growth pattern than wild-type (WT) seedlings against mannitol application, indicating that OsSta2 may respond to both salt and drought stresses (Kumar et al. 2017). Xie et al. (2017) observed the RNA-seq and sRNA-seq and found that there were 2574

mRNAs and 76 miRNAs individually that were differentially expressed in citrus root under salt and drought conditions. Likewise, eight novel miRNAs and their targets against salinity stress have been identified in maize. A total of 37 potential new miRNAs were screened in response to the salt stress responses (Fu et al. 2017).

15.4 Microbial Mitigation of Biotic Stress

Occurrence of diseases is a major threat to crop production worldwide from sowing to harvest and even during storage of the produce (Amusa 2006). In the rhizosphere region, microbial activity plays a key role in inhibiting the soil-borne plant diseases (Hariprasad and Umesha 2007; Rani et al. 2007). Soils have their own level of plant disease restriction ability (Baker and Cook 1975; Cook 2000). A number of microbes have been shown to play a major role as a biocontrol agent against plant pathogens. Representative examples of these microbes include plant-beneficial microbes such as *Azotobacter* spp., *Bacillus subtilis*, fluorescent *Pseudomonas*, *Rhizobium* spp., etc. (Tuzun, 2001). Biocontrol ability of the microbes is related with to the efficient root colonizing ability, catabolic versatility, and their capacity to produce a wide range of enzymes and metabolites that are responsible to antagonize the pathogen (Anith et al. 1999; Ramamurthy et al. 2001; Mayak et al. 2004a, b; Vivekananthan et al. 2004; Singh et al. 2012). Siderophore-producing *Pseudomonas* have been shown to colonize the roots of a variety of crop plants including cereals, pulses, oilseed, and vegetables (Elad and Baker 1985; Neilands and Leong 1986; Loper and Buyer 1991). Plant pathogenic bacteria cause several dangerous diseases to plants across the world (Vidhyasekaran 2002). Management of insect-pests and diseases by biological control method or with the help of microorganisms that restrict the growth of phytopathogens is the most prominent substitute for ecologically detrimental chemical products in agriculture (Azevedo et al. 2000). Extensive utilization of biological control agents over the existing chemical agents for soil-borne diseases could significantly contribute to sustainable, green crop production under biotic stress conditions. Literature exists to endorse the implementation of biological control strategies in modern agriculture (Table 15.2).

15.5 Microbe-Based Mitigation of Abiotic Stress

Abiotic stresses such as heat, drought, salinity, alkalinity, acidity, flood, wind, intense/low light, heat, etc. affect plant productivity and yield, leading to low income (Meena et al. 2017). Implementation of management practices such as culture practices, irrigation, and utilization of crop residue for mulching purposes, soil management, and selection of more appropriate crop varieties can potentially alleviate the effects of abiotic stress. Application of beneficial microbial communities in integral agricultural practices is being considered as a promising technology to be endorsed to enhance crop productivity in a sustainable and environment-friendly manner under stressed environmental conditions (Gill et al. 2016; Sorty et al. 2016). Focused

Table 15.2 Microbial agents for disease control

Crop	Disease	Causal organism	Biological control	References
Rice	Bacterial panicle blight of rice	*Burkholderia glumae* and *B. gladioli*	*Bacillus* (RAB) sp.	Shrestha et al. (2016)
Onion	Onion bacterial disease		*P. agglomerans* 2066-7 strain	Sadik et al. (2013)
Tomato	Bacterial wilt disease	*Ralstonia solanacearum* race 1 biovar 3	*Bacillus subtilis*	Sinha et al. (2012)
Red pine	Root rot	*Fusarium* species	*Paxillus involutus*	Pal and Gardener (2006)
Common sage	Wilt and root rot diseases	*Fusarium oxysporum* and *F. solani*	*Brevibacillus formosus*, *Brevibacillus brevis*, and *Stenotrophomonas maltophilia*	Omar and Ahmed (2014)
Chili	Anthracnose (fruit rot) and damping off	*Colletotrichum gloeosporioides* and *Rhizoctonia solani*	*Pseudomonas aeruginosa* FP6	Bakthavatchalu Sasirekha and Srividya (2016)

utilization of plant growth regulators such as ABA, cytokinins, auxins, salicylic acid, etc. can play an important role in increasing the water potential in plants under drought stress condition (Zhang et al. 2004). 1-Aminocyclopropane-1-carboxylate (ACC) deaminase enzyme found in many plant growth-promoting bacteria restricts the rising levels of plant ethylene precursor, thus lowering the level of ethylene under stress conditions (Glick, 2004). Plant growth-promoting rhizobacteria (PGPR) can potentially contribute a significant role toward alleviation of abiotic stresses in crop plants under present prospective of varying agro-climatic scenario; simultaneously, the microbes can also help to reduce the excessive dependence on chemical fertilizers, thus maintaining soil health (Tiwari et al. 2011; Yandigiri et al. 2012; Nautiyal et al. 2013; Sorty et al. 2016, 2018; Bitla et al. 2017; Meena et al. 2012, 2017). Under saline conditions, PGPR help the plant in root and shoot development, increase nutrient availability and chlorophyll content, and develop salt tolerance (Qurashi and Sabri 2012).

Many PGPR impart good effects under abiotic stress conditions by direct and indirect mechanisms such as biofilm formation; chemotaxis; siderophore, EPS, and indole acetic acid (IAA) production; and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Srivastava et al. 2012; Nautiyal et al. 2013). PGPR *Pseudomonas mendocina* strains were demonstrated for their favorable effects on soil by stabilizing soil aggregates (Kohler et al. 2006). PGPR *Pseudomonas mendocina*-inoculated plants exhibited increased shoot biomass (Kohler et al. 2009). PGPR *Pseudomonas mendocina* strain co-inoculated with AMF (*Glomus intraradices* or *G. mosseae*) in lettuce improved the activity of the antioxidant enzyme catalase and reduced oxidative damage in lettuce (Kohler et al. (2008) (Table 15.3).

Table 15.3 Microbial agents to enhance abiotic stress tolerance in plants

Sr. no.	Microorganisms	Mechanisms	Stress	Plant	References
1	*Azospirillum*	Produces IAA	Drought		Dimkpa et al. (2009)
2	*A. brasilense*	Nitric oxide helps in IAA-inducing pathway	Drought	Tomato	Creus et al. (2005) and Molina-Favero et al. (2008)
3	*A. brasilense Cd*	Increases root length and root area	Drought	Common bean	German et al. (2000)
4	*Phyllobacterium brassicacearum* strain STM196	Increases the ABA content, leading to decreased leaf transpiration	Osmotic stress tolerance	*Arabidopsis*	Bresson et al. (2013)
5	*P. putida* H-2-3	Improves plant growth	Drought	Soybean	Sang-Mo et al. (2014)
6	*A. brasilense*	Increases root growth, proline accumulation plant, and water potential	Drought	Maize	Casanovas et al. (2002)
7	*Azospirillum lipoferum*	Produces of ABA and gibberellins	Drought	Maize	Cohen et al. (2009)
8	*Azospirillum*	Induces decrease in leaf water potential and increase in leaf water content, enhanced root growth, and production of IAA	Drought	Wheat	Arzanesh et al. (2011)
9	*Achromobacter piechaudii* ARV8	Produces ACC	Drought and salt	Pepper and tomato	Mayak et al. (2004a, b)
10	*Bacillus subtilis*	proBA genes for the production of free proline	Osmotic stress	*Arabidopsis*	Chen et al. (2007)
11	Co-inoculation of *Rhizobium* and *Pseudomonas*	Increases production of proline; maintains relative water content in leaves	Salt	Maize	Bano and Fatima et al. (2009)
12	Co-inoculation of *Rhizobium tropici* and *P. polymyxa*	Increases nodulation, N content, and plant growth	Drought	Green bean	Figueiredo et al. (2008)
13	*T. asperelloides T203*	Improves seed germination	Salt	*Arabidopsis* and cucumber	Brotman et al. (2013)
14	*Pseudomonas* AKM-P6	Improves thermo-tolerant capacity	Heat stress	*Sorghum*	Ali et al. (2009)

(continued)

Table 15.3 (continued)

Sr. no.	Microorganisms	Mechanisms	Stress	Plant	References
15	*P. fluorescens* Pf1	Increases the activity of catalase and peroxidase	Water stress	Green gram	Saravanakumar et al. (2010)
16	*Pseudomonas putida* GAP-P45	Improves plant biomass, relative water content, leaf water potential, proline, and sugar	Drought	Maize	Sandhya et al. (2010)

Phytohormones are crucial for the regulation of plant growth and development and also spontaneously involved in the survival of plants under abiotic stress conditions (Skirycz and Inze 2010; Fahad et al. 2015; Sorty et al. 2016; Meena et al. 2012, 2017). Wheat crops inoculated with *A. brasilense* Sp245 increased grain yield and mineral quality (Mg, K, and Ca), along with improved relative water status, and water potential was recorded under water stress condition (Creus et al. 2004). In pot trials involving green gram inoculated with plant growth-promoting *Pseudomonas* sp. PS1, a significant enhancement of plant growth, dry matter, nodule number, total chlorophyll content, root and shoot development, seed yield, and seed protein content was noted by Ahemad and Khan (2010, 2011, 2012). These evidences therefore encourage ignition of keen efforts to develop new strategies for microbial mitigation of abiotic stresses.

Under water stress conditions, exopolysaccharides play a major role in developing biofilms, increasing soil aggregation, and improving water-holding capacity around the plant root and also improving the water stress tolerance ability of the plant (Bensalim et al. 1998; Sandhya et al. 2009; Meena et al. 2017). EPS-producing bacteria provide a promising environment for maintaining moisture around the root and rhizospheric area and protect the plant and bacteria against shear (Hepper 1975). In vitro inoculation of grape (*Vitis vinifera* cv. chardonnay) explants with a PGPR, *Burkholderia phytofirmans* strain PsJN, under low-temperature conditions increased grapevine root growth, plantlet biomass, and physiological activity (Barka et al. 2006). PGPR also synthesize indole-3-acetic acid (IAA) which facilitate shoot and root growth along with improved water uptake, thus ensuring sustainable growth and survival under abiotic stress conditions (Marulanda et al. 2009; Sorty et al. 2016; Meena et al. 2017). Increased root growth was observed in wheat seedlings, tomato, and cucumber plants following inoculation with IAA-producing *P. chlororaphis* TSAU13. The strain increased phytohormonal content in plants, consequently enhancing water conductance under saline conditions (Egamberdieva and Kucharova 2009; Egamberdieva 2012). An increase in lateral root density and length as well as root hair density and length (59% and 200%), respectively, was observed in drought-stressed wheat plants when inoculated with 1-aminocycloprop ane-1-carboxylate (ACC) deaminase and IAA-producing *Bacillus thuringiensis* (Timmusk et al. 2014). GA-producing *Azospirillum lipoferum* inoculated in maize

plants conferred drought tolerance (Cohen et al., 2009). Elevated endogenous GAs in PGPR (*Burkholderia cepacia* SE4, *Promicromonospora* spp. SE188, and *Acinetobacter calcoaceticus* SE370)-treated cucumber plants exhibited augmented plant growth under drought and salinity stress conditions (Kang et al. 2014a). Gibberellin-secreting *Rhizobacterium* and *Pseudomonas putida* H-2-3 inoculation in soybean improved tolerance to drought and salinity stress (Kang et al. 2014b). Implementing similar strains in routine agriculture either singly or in the form of consortium could be a promising strategy for mitigating drought stress in plants.

15.6 Future prospectives

Stresses both biotic and abiotic are the major constraints and challenges for the crop quality and productivity and a threat to the global food security. The answer to these problems of plants is to develop microbial products and practices of plant-microbe-soil interaction. Efforts are needed to increase the awareness regarding the use of stress-tolerant microbial strains and mycorrhizal fungi in agriculture for enhancing plant growth under biotic and abiotic stress conditions. These microbes might stimulate plant growth by regulating plant hormones, increase nutrition uptake and siderophore production, and enhance the antioxidant system. Microbes can also enhance disease tolerance through ASR and ISR. AM enhanced the availability of nutrients and water throughout the stress condition and increase tolerance to stress. The complication of strain-specific communications within a species suggests the survival of extremely specific and multifarious association mechanisms, and our empathy of what aspects manage the optimal specificity of plant-microbial associations and how microbes enhance stress tolerance to plants is still in its beginning. However, enlarging research in this field and applying the knowledge gains to crop plants could promise additional avenues to develop agriculture in a sustainable way. Considering a present consequence, imminent research is necessary to identify potential stress-tolerant PGPM. Certainly, diversity of microbial strains should be tested to formulate effective microbial consortia to overcome the negative impact of changing the environment.

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Application of Microbial Products for Enhancing the Nutritional Quality of Agricultural Produce

16

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Abstract

Frequently, altering environmental conditions threaten the agricultural productivity and nutritional quality of the produce. Nutritional requirements of human beings are totally dependent on agriculture. Pressure of increasing population on limited agricultural land to produce nutritionally improved agricultural produce is major concern. Copious strategies were suggested to enhance the nutrient quality of agriculture after the harvesting, but very few strategies were developed and applied in situ. The part of PGPR, AMF, and other endophytic microorganisms in enhancing agricultural productivity is well known. Our current knowledge regarding mechanism of microorganisms in enhancing nutrient quality is still in infancy. This chapter characteristically highlights the involvement of microbes in nutritional enhancement of crops produced and focuses on the probable strategies for nutritional improvement in agricultural produce.

Keywords

Nutritional quality · PGPR · Agricultural produce · Microbial products · Biofertilizers

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16.1 Introduction

Plants are the major human diet covering bioactive constituents that employ nutrition promoting human health and well-being. Agricultural food harvests with high nutritional value should constantly be ideal over food products with low nutritional value. The same is more hopeful if the nutritional value of food is improved under natural environmental circumstances particularly in agricultural farms. Consumption of low-quality contaminated fruits and vegetables enhances the risk of chronic diseases like cancer, cardiovascular disease, stroke, Alzheimer's disease, cataract, and age-related functional decline. During the last few decades, increasing population demands more food, challenging the agriculture, more in emerging countries where croplands and resources barely contribute to an efficient crop production required to meet such a crucial demand for food. Worldwide food security issue will foster dependence on innovation, expansion, and transfer of technologies regarding green revolution that lead to improved food production while ensuring sustainable intensification of agriculture. However, the process had caused harmful impacts on the environment and also represented a covert problem for human health (Baez-Rogelio et al. 2017). The widespread usage of synthetic fertilizers in farms is currently under dispute due to environmental concerns and safety for consumer health.

Plants continuously interact with various kinds of microbes from soil microbial communities of the extreme pool of biological diversity in the nature (Berendsen 2012; Sahu et al. 2018). The seeds and roots exterior provide ideal habitat for microbial growth and development. Beneficial plant–microbe and microbiome interactions might characterize a promising sustainable solution to improve agricultural production both qualitatively and quantitatively. Plants establish association with a vast diversity of beneficial microorganisms such as arbuscular mycorrhizal fungi (AMF) and plant growth-promoting bacteria (PGPB), which can enhance both the plant health and productivity (Timmusk et al. 2017). The benefits of PGPR interactions for plants generally enhance seed germination rate, root growth, yield, leaf area, chlorophyll content, nutrient uptake, protein content, hydraulic activity, tolerance to abiotic stress, shoot and root weights, biocontrol, and delayed senescence (Adesemoye and Kloepper et al. 2009; Compant 2010; Tiwari et al. 2011; Srinivasan et al. 2012; Yandigiri et al. 2012). Advanced understanding of genomic, postgenomic, and biochemistry and ecological understanding on the symbiotic association of beneficial microbial interactions have led to the development and commercialization of efficacious microbial products like biofertilizers, biostimulants, and biopesticides with proven success in improving crop production and adaptation to the environmental challenges (Lindemann et al. 2016; Mishra et al. 2016; Sorty et al. 2018; Umesha et al. 2018).

Formulations of microbial inoculants composed of beneficial microbial inoculants that perform a significant role in soil health are widely available. Microbial inoculants are the probable substitute to chemical fertilizers and pesticides (Babalola and Glick 2012). Microbial-based bio-products are those bioactive compounds necessary to stimulate and advance biological processes of the intricate plant–microbe–soil band (Singh et al. 2016). Microbial inoculants pose promise for integrated

solutions to the agro-environmental concerns due to their capacity to promote plant growth, enhance nutrient availability and uptake, and support soil health. Microbial inoculants include three major groups: arbuscular mycorrhizal fungi (AMF), PGPR, and the nitrogen-fixing rhizobia. PGPRs also improve nutritional quality of fruits and vegetables. Several studies have proved that they can increase the sweetness, moisture content, secondary metabolites content (anthocyanins, flavonoids, and carotenoids) with antioxidant potential, and minerals quantity in the fruits in the human diet (Ruzzi and Aroca 2015; Bona et al. 2016). In this chapter, we focus on the use of beneficial rhizosphere microorganisms for improving not only growth and yield but also the nutrient quality of crops that make them a promising tool capable of responding to the challenges for today's agriculture and horticulture.

16.2 Microbes for Agricultural Quality Improvement

Microbial inoculants could exist in different forms such as solid or liquid, constituting of bacteria, fungi, actinomycetes, algae, etc. It could also consist of either a pure culture or a mixed culture (Reddy and Saravanan 2013). The group of microorganisms promoting plant growth is better known as PGPR (plant growth-promoting rhizobacteria) and includes species of *Pseudomonas*, *Burkholderia*, *Bacillus*, *Azotobacter*, *Azospirillum*, *Gluconacetobacter*, *Rhizobium*, *Achromobacter*, *Arthobacter*, *Azoarcus*, *Clostridium*, *Enterobacter*, *Flavobacterium*, *Frankia*, *Hydrogenophaga*, *Kluyvera*, *Microcoleus*, *Phyllobacterium*, *Serratia*, *Staphylococcus*, *Streptomyces*, *Vibrio*, etc. (Bashan and de-bashan 2005; Ahmad et al. 2008; Saravana-Kumar et al. 2008; Supanekar et al. 2013; Sorty et al. 2016; Meena et al. 2017). Leguminous crops can fix nitrogen through symbiotic bacteria *Rhizobia* in their root nodules. The use of plant growth-promoting (PGP) organisms-based biofertilizers (Rhizobium with phosphobacteria) increases crop yield by fixing the atmospheric nitrogen and improving the availability of phosphorus in leguminous crops (Selvakumar et al. 2012). Biofertilizer such as *Rhizobium* improves the formation of root nodules and helps in biological nitrogen fixation. These organisms belong to the bacterial communities, the classical example being the symbiotic nitrogen fixers. The symbiotic association can be formed by either single species or more than one species, for instance, *Bradyrhizobium* and *Bacillus polymyxa* alone or in combination markedly increased the number of root nodules due to synergistic interaction among phosphate-solubilizing microorganisms and *Bradyrhizobium* which lead to increased nodulation and enhanced nitrogen fixation in soybean crop (Jain and Trivedi, 2005). The symbiotic bacteria infect the legume root and form root nodules within which they reduce molecular nitrogen to ammonia which is radically utilized by the plant to produce valuable proteins, vitamins, and other nitrogen-containing compounds. The production of horticultural crops with high contents of carotenoids, flavonoids, and polyphenols is a primary goal that encounters the demands of consumers and investigators due to their health benefit effects (Rouphael et al. 2010). Such improvements can be achieved with the help of AMF symbiosis which has been shown to induce modifications in the plant

secondary metabolism for enhancing the content of phytochemicals with health-promoting impacts (Sbrana et al. 2014). Several studies demonstrate the microbes-based improvement of crop quality by enhancing growth, nutrient uptake, protein content, vitamins, oil content, etc. Inoculation of *Azospirillum lipoferum* in maize improved plant growth through accumulation of free amino acids and soluble sugars (Bano et al. 2013). Pea seeds when inoculated with *Variovorax paradoxus 5C-2* exhibited enhanced nodulation, seed yield, seed number, and seed nitrogen content (Dodd et al. 2005). The most important plant growth-stimulating bacteria are *Azotobacter*, *Azospirillum*, and *Pseudomonas*, which, in addition to biologically stabilizing nitrogen and solubilizing soil phosphate, affect the yield performance of the plants through production of a significant amount of growth-stimulating hormones especially auxins, gibberellins, and cytokinins (Sumana and Bagyaraj 2002).

16.3 Microbes-Based Enhancement of Quantitative and Qualitative Traits in Plants

Different mechanisms are incorporated by the associative microbes to induce qualitative and quantitative improvements in plant. It was demonstrated that the fluorescent *Pseudomonas*, *Trichoderma*, and *Mesorhizobium* species inoculated in chickpea (Singh et al. 2014) improved content of phenolic compounds; similarly, mixture of microbial strains inoculation in pea seeds enhanced the antioxidant phenolics to severalfolds (Jain et al. 2014). PGPR can also improve the nutritional quality of fruits and vegetables and increase sweetness and mineral content in the plant produce for additional human diet (grapes, apples, strawberries, blackberries, sweet cherries, tomatoes) (Ruzzi and Aroca 2015; Bona et al. 2016; Bitla et al. 2017). Enhanced contents of minerals and chlorophylls were reported in cabbages supplied with PGPR (Bona et al. 2016).

16.4 Vitamins, Flavonoids, and Sugars

Vitamins are among the nutrients essential for many biological functions crucial to life. Despite being presented in minute amounts in the diet, vitamins prevent specific deficiency syndromes which can affect people when there is an absence or a reduction of their contents (Combs and McClung, 2016). Moreover, vitamin deficiency in humans can lead to several diseases such as ocular surface abnormalities (Simkin et al. 2016) or neurodegenerative problems (Sechi et al. 2016). Due to the importance of vitamins, one of the proposals presented by the World Health Organization (WHO) deals with the improvement of the content of essential vitamins in food in order to decrease worldwide malnutrition (Garcia et al. 2016).

Increased levels of vitamin C after bacterial treatment have been described in vegetables. For instance, Bona et al. (2017) showed that inoculation with *Pseudomonas* sp. 19Fv1T not only enhances yield but also positively affects the concentration of vitamin C in tomato fruits. Additionally, Shen et al. (2016) showed

that vermicompost combined with plant probiotic *Bacillus megaterium* and *Bacillus amyloliquefaciens* also increases tomato yield and vitamin C content. The maximum levels of vitamin C content in tomato fruits were achieved after the inoculation of two bacterial strains *Bacillus amyloliquefaciens* (FZB2 and FZB42) (Gul et al. 2008). Berry crops are regarded as a good source of vitamins in accumulation to their anticarcinogenic and antimutagenic properties (Seeram 2006, Zeljic et al. 2017). Enhancement of vitamin B9 and vitamin C content in strawberry fruits following the inoculation with arbuscular mycorrhizal fungi (AMF) and different strains of plant growth-promoting bacteria (PGPB) (Bona et al. 2015) has been reported; substantial alterations in ascorbic acid levels after inoculation with *Pseudomonas* sp. 5Vm1K, a mixture of AMF, and co-inoculation formed by AMF and *Pseudomonas* sp. 5Vm1K were also reported. Strawberries acquired from plants inoculated with the strain *Phyllobacterium* sp. PEPV15 contained significantly higher quantities of vitamin C (Flores-Félix et al. 2015). Similarly, high levels of vitamin C content in strawberry fruits after inoculation with *Paenibacillus polymyxa* RC05 was also reported (Erturk et al. 2012). AM *Glomus intraradices* colonization toward strawberry roots stimulated plant growth and also increased the sugars and anthocyanin content in fruit (Castellanos-Morales et al. 2010). AMF also increases glucose and malate content in tomato (Copetta et al. 2011) and enhance nutritionally significant elements like copper (Cu) and iron (Fe) in lettuce (Baslam et al. 2011).

Flavonoids are beneficial to human health when consumed in large quantities; thus, they are important not only for the food industry but also for pharmaceutical companies. García-Seco et al. (2015) studied the inoculation of blackberry plants with *Pseudomonas fluorescens* N21.4 which significantly improved flavonoid content. Buckwheat inoculated with *Azospirillum* spp. and *Azotobacter* spp. showed increased concentrations of flavonoid and phenolic contents (Singh et al. 2015).

16.5 Oil Content

Presence of important fatty acids like oleic, linolenic, palmitic, and stearic acid and the pumpkin seed oil has high nutritional value. Many oilseeds are cultivated as rainfed crop with poor input resources, which exert greater impact on plant health particularly plant nutrition. The oilseed forms an essential part of human diet; thus, the nutritional quality of oil is critically significant to the human health. Literature is available to signify the microbes influence on oil content and oil quality in seed oil crops. Inoculation of the pumpkin seeds with phosphate-solubilizing bacteria, *P. putida* and *B. lentus*, and nitrogen-fixing bacteria *Azotobacter sp.* and *Azospirillum sp.* induced significant enhancement of the content of oil, seed, and fruit yield, particularly the fatty acid (Afsaneh et al. 2013). Shoghi-Kalkhoran et al. (2013) studied the pooled impact of organic fertilizers, urea, and inoculation with various PGPRs including *Azotobacter* and *Azospirillum* on grain yield, protein, fatty acids, and oil contents in sunflower crop. The integrated fertilization process enhanced the crop productivity, seed oil content, and quality of sunflower crop. *Jatropha curcas* seeds

inoculated with *Trichoderma viride*, *Azospirillum*, and *Phosphobacterium* showed improved plant height, seed yield, and oil content (Sathianachiyar and Devaraj 2013). Combined inoculation of PSB, VAM, and *Azotobacter* significantly improved crop yield and oil content in sunflower (*Helianthus annuus* L.) (Patra et al. 2013). Oil contents were significantly higher under the treatment of *Rhizobium*. PSB at the 75% dose of fertilizer due to the phosphorus is structural element of certain coenzymes involved in biosynthesis of groundnut oil (Vala et al. 2017).

16.6 Essential Oils

Essential oils (EOs) are lipophilic mixture of volatile secondary compounds in the plants. The composition usually contains monoterpenes, sesquiterpenes, and phenylpropanoids. These oils have versatile ecological functions in the plants (Harborne and Tomas-Barberan 1991; Harrewijn et al. 2001) and are used as flavors and fragrances, antimicrobials and antioxidants, and medicines (Deans and Waterman 1993). It is known that soil microorganisms can amend the secondary metabolic ways of plants, inducing the synthesis of mixture of essential oils that are of great importance for the food and pharmaceutical industries (Lingua et al. 2013). The most volatile compounds contained in *Origanum majorana* L. are essential oils and have an important economic interest because of their use as flavoring, fragrances, fungicides, and insecticides. Some researchers have demonstrated the effects of root colonization by PGPRs on the composition and amount of essential oils in different crops, and the inoculation of *Origanum majorana* with *P. fluorescens* and *Bradyrhizobium sp*. (Banchio et al. 2008) and the inoculation of peppermint (*Mentha piperita*) with *P. fluorescens* (Santoro et al. 2011) yielded an escalation in the total essential oil content without altering its composition. The highest oil yield in fenugreek was obtained by a mixture of biofertilizers *Azospirillum lipoferum*, *Azotobacter chroococcum*, and *Bacillus megaterium* (Mahfouz and Sharaf Eldin 2007). Marjoram (*Majorana hortensis* L.) is used worldwide as a spice and a medicinal source in the form of the essential oil in aromatherapy due to its stimulant and antispasmodic properties. Increased level of essential oil component terpinen-4-ol, γ- and α-terpinene, trans-sabinene hydrate, phellandrene, p-menth-1-en-8-ol is accompanied by a decrease in the proportions of *cis* sabinene hydrate, pcymene, α-terpinolene, linalyl acetate, β-caryophyllene, and spathulene when *Majorana hortensis* plant inoculated with compost extract and biofertilizer mixture *Azospirillum brasiliensis*, *Azotobacter chroococcum*, *Bacillus polymyxa*, and *B. circulans* (Gharib et al. 2008). *Azotobacter chroococcum* and *Azospirillum lipoferum* could cause increased yield and essential oil content in some spices and medicinal plant like coriander (Kumar et al. 2002), fennel (Mahfouz and Sharaf Eldin 2007; Abdou et al. 2004; Azzaz et al. 2009), davana (Swaminathan et al. 2008; Kumar et al. 2009), dill (Darzi et al. 2012), black cumin (Valadabadi and Farahani 2011), and turmeric (Velmurugan et al. 2008).

16.7 Proteins

Proteins are major part of human regular diet. Microbial inoculation in agricultural crops has been shown to improve protein content in agricultural produce, particularly in cereals and other grain crops. Nitrogen is an important constituent of protein and amino acids; protein content in crop shoot, leaf, fruit, and seed depends on availability of nitrogen in soil and capability of plant to uptake the nitrogen. Zalate and Padmani (2009) demonstrated that seed inoculation with biofertilizers such as *Rhizobium* strains and phosphate-solubilizing bacteria significantly amplified the protein content of groundnut due to improved nitrogen content in grain, as nitrogen is an integral part of protein. Saharan and Nehra (2011) demonstrated that *Azospirillum*, *Azotobacter*, and *Pseudomonas* enhanced plant growth and yield through various mechanisms including the production of phytohormones. Phytohormones are the principal constituent of protein fluctuations and can increase the yield and quality of oilseed crops (Lone et al. 2005). Amino acids help in the synthesis of proteins and are an important feature of PGPRs. The amino acids produced by the PGPRs include methionine, glutamine, glutamic acid, isoleucine, leucine, and aspartic acid (Babalola, 2010). *Azotobacter chroccoccum* and *Azospirillum lipoferum* inoculation promotes the higher protein concentration in the achene of sunflowers (Mohsennia and Jalilian, 2012). Mycorrhizae *Glomus* spp. inoculated in wheat significantly increased the content of proline, free amino acids, total soluble, and crude protein and also improved activities of antioxidant enzymes under water stress (Khalafallah and Abo-Ghalia, 2008); similarly, Habibzadeh et al. (2008) studied that *Glomus mosseae* and *G. intraradices* enhanced seed yield, leaf P, leaf N, proteins, and water use efficiency in mung bean. Wani et al. (2008a, b) demonstrated that protein content in chickpea was improved by 16% and in pea by 8% through inoculation with *Mesorhizobium* sp. RC3 and *Rhizobium* sp. RP5, respectively. *Azospirillum* treatment in fenugreek also enhanced the protein and lipid content in seeds (Kumutha 2005) (Table 16.1).

16.8 Microbial Products for Sustainable Farming Under Abiotic Stress

Abiotic stresses drastically affect the agriculture yield, productivity, and nutritional value of the crops. There are evidences of productivity decline in agriculture crops in the world due to increasing water stress, reduction in number of rainy days and high temperature, hailstones, salt, cold, heavy metal, etc. Recent studies indicate that microorganisms can help crops to cope up with the abiotic stresses. They alleviate the impact of abiotic stresses in crop plants, mainly by synthesizing the phytohormones including indole-3-acetic (IAA) acid (auxin), cytokinins, gibberellins, and abscisic acid. These compounds consequently result in increased root length, root surface area, and number of root tips, leading to enhanced uptake of nutrients (Egamberdieva and Kucharova 2009; Meena et al. 2012). Phytohormones contribute significant role in plants suffering abiotic stress to escape or survive under the

Table 16.1 Nutritional value of agricultural produce enhancing microbes

Microorganism	Crop	Nutritional value improved	References
Azotobacter chroococcum, Azospirillum lipoferum	Ajowan (Carum copticum)	Essential oil	Ghilavizadeh et al. (2013)
B. japonicum	Soybean	Oil, protein	Blazinkov et al. (2015)
Trichoderma harzianum	Tomato	Protein content, sugar, ascorbic acid, b-carotene, lycopene	Molla et al. (2012)
Paenibacillus polymyxa RC14	Brassica oleracea var. capitata cv Yalova 1	N, P, K, S, Fe, and Cu	Yildirim et al. (2015)
R. Intraradices	Cucurbita pepo	P, K, Fe, Zn, and Mn	Rouphael et al. (2010)
Pseudomonas fluorescens Ap14	Berries	Flavonoids	Ramos-Solano et al. (2015)
Bacillus licheniformis	Tomato	Flavonoids	Ochoa-Velasco et al. (2016)
Pseudomonas putida, *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Glomus intraradices*, *Glomus mosseae*, *Glomus etunicatum*	Tomato	Lycopene	Ordookhani et al. (2010)
Rhizobium strain TVP08	Capsicum annuum	Flavonoids	Silva et al. (2014)
Pseudomonas sp., Bacillus lentus, and *Azospirillum brasilense.*	Ocimum basilicum	Antioxidant activity and chlorophyll	Heidari and Golpayegani (2012)
Azotobacter, Azospirillum (nitroxin), Bacillus and pseudomonas (phosphate-solubilizing bacteria)	Capsicum annum L.	Vitamin C	Tayeb Rezvani et al. (2013)
Providencia sp. 2 strains of Anabaena sp. Calothrix sp.	Wheat	Enhancement 18.6% protein content	Rana et al. (2012)
Pseudomonas spp.	Safflower	Oil	Sharifi (2012)
Bacillus pumilus, *Bacillus mycoides*	Runner bean	Protein	Stefan et al. (2013)
Azotobacter, *Azospirillum*, *Pseudomonas*, *Rhizobium,* and *Bacillus*	Dill (Anethum graveolens L., Apiaceae)	Flavonoids	Hussein et al. (2015)
Azospirillum and *Azotobacter*	Safflower	Protein	Nosheen et al. (2016)

(continued)

Table 16.1 (continued)

Microorganism	Crop	Nutritional value improved	References
Bacillus subtilis and *Pseudomonas fluorescence*	Sorghum	Protein	Prathibha and Siddalingeshwara (2013)
Azospirillum, *Azotobacter,* and *Rhizobium*	Black gram (Vigna mungo L. Hepper)	Protein	Selvakumar et al. (2012)

stressful conditions (Fahad et al. 2015). Moreover, the PGPRs (synthesized phytohormones) also elicit plant cell growth and division and help them tolerate against environmental stresses (Glick and Pasternak 2003). Biofertilizers like *Azospirillum* may release phytohormones like auxin which develop root branching and induce root elongation. This would be a clear benefit for plants in dry regions, where a highly developed root system is needful for efficient water uptake (Dobbelaere et al. 1999; Steenhoudt and Vandereyden 2000). Additionally, biofertilizers like *Azotobacter* are able to produce other plant hormones like gibberellins and cytokinins which attenuate the stress symptoms in plants and help stabilizing the yield (Bhardwaj et al. 2014).

Inoculation of *Pisum sativum* with ACC deaminase producing *Pseudomonas fluorescens* biotype G (ACC-5) induced longer roots, which led to an amplified uptake of water from soil under water scarcity (Zahir et al. 2008). Rhizobacteria having the ability to produce exopolysaccharides can be used effectively for enhancing drought resistance in sunflower plants (Sandhya et al. 2009). The exopolysaccharides are mainly responsible for water holding, and aggregation of soil, which promotes better growth and development by ensuring sustained moisture supply and improved soil health. Arbuscular mycorrhizae improve the nutritional eminence of plants, enable plant adaptation to different ecosystems, and increase plant tolerance to abiotic stress factors, and they are also considered to be biocontrol agents (Singh et al. 2012). *Azotobacter chroococcum* and *Streptomyces niveus* inoculated in maize plants growing under diverse salinity levels were found to stimulate total soluble sugars, total free amino acids, proline, and total soluble proteins which lead to greater salt tolerance of the plants (Magda et al. 2003). Enhanced oil content in salt stress-affected *Brassica juncea* was observed after *Trichoderma harzianum* application which improved the uptake of essential nutrients, enhanced accumulation of antioxidants and osmolytes, and decreased Na^+ uptake (Ahmad et al. 2015).

16.9 Strategic Enhancement of Nutritional Quality in Agricultural Products Using Microbes

Convalesce nutritional quality of food is necessary during the yield attempts targeting yield improvement. Development of new microbe-based strategies and approaches can provide a powerful, sustainable option that could maintain the

quality along with increased yield. The manipulation of the crop microbiome in situ can be considered as prominent strategies for enhancing the nutritional quality of food crops (Singh and Trivedi 2017) and the external application of commercial inocula containing beneficial microorganisms in soils (Vosatka et al. 2012; Rouphael et al. 2015). Another green revolution is needed where crops are to be developed particularly for improved quality and yield under environmental extremes with low input of chemical pesticides and fertilizers while simultaneously promoting the increased use of organic fertilizers (organic manures, compost, and microbial biofertilizers). Consortium of native bacterial strains is more advantageous over the individual strains originating from another niche. AMF and combined application of P solubilizers and N fixers are the best inoculants. The yield enhancement is more by the combinations of the two functional traits N fixation and P solubilization than their distinct application as there is absence of competition and presence of positive interactions between the two traits (Schütz et al. 2018). A plethora of research appears directed toward development of good biological control agents for controlling of agricultural pests and pathogens, as well as yield improvement, and tolerance to abiotic stress; however, minor efforts were led to identify microbes and their mechanisms to enhancing nutritional quality of agricultural produce (Meena et al. 2010). It is therefore important to ensure nutritional security through advanced strategies involving the use of improved biofertilizers in the modern agriculture.

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Microbial Products: Protein, Enzyme, Secondary Metabolites and Chemicals

17

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Abstract

Microbial products are described as products derived from microbes. Microbial products have been contributing in almost every sphere of human life. These products have proved their importance and value in field of food and feed sector, agriculture, healthcare, and many other industries. Microbes have the ability to grow in wide variety of substrate on large scale to produce many valuable primary metabolites such as amino acids, enzymes, vitamins, organic acids, alcohol and bioactive metabolites such as antibiotics, alkaloids, peptides, growth factor, etc. This chapter describes the importance of microorganism for production of protein, enzymes, secondary metabolites and chemicals.

Keywords

Microbial products · Protein · Enzyme · Secondary metabolites and chemicals

17.1 Introduction

Microorganisms have been used from centuries for production of valuable products (Du et al. 2011). The first industrial process, the production of alcohol by yeast from malt or fruit extracts, is still being carried out for many years. Since then, microorganisms are used in mass production of various range of products such as food additivities, whole enzymes and cells, protein, agrochemicals, biofuels, antibiotics, solvents and many more (Cipriano 2006). Microorganism to be useful for industrial

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production of any product should possess certain characteristics such as ability to grow fast in relatively inexpensive medium, should be easily inoculated, should be non-pathogenic and should be able to produce desired product quickly and easily amenable to genetic manipulation (Zhang et al. 2016).

17.2 Microbial Proteins

The dried cells of microorganisms (algae, bacteria, fungi and yeast) used as protein-rich food and feed additives are collectively known as 'microbial protein' (MP) (Matassa et al. 2016; Uphadhya et al. 2016). A number of microbes have been used as a part of diet all over the world, since ancient times. Microbial protein has nowadays replaced animal or vegetable protein as an alternative source of protein. It can also be used for human consumption directly as food. The term 'microbial protein' was substituted with the single-cell protein (SCP) during 60s. Single-cell proteins are usually the microbial biomass or protein extract to be used as food or feed sources or additives (Gour et al. 2015).

Owing to the population pressure in near future, especially in several developing countries, there may not be enough animal or vegetable proteins to fulfil the requirements of humans. Therefore, in the protein deficiency, microbes provide viable alternative of various protein supplements (Goldberg 1985; Nasseri et al. 2011a, b). One of the nutritional advantages of MP for human and animal consumption is rich in essential amino acids (lysine, methionine) which are usually limiting in most plant and animal food. About 25% of the world's population presently suffer from hunger and malnutrition. Therefore, MP deserves a serious consideration for its use as food or feed supplement (Matassa et al. 2016). Apart from being protein rich, MP also contains carbohydrates, fats, nucleic acids, vitamins and minerals (Gour et al. 2015). When MP is used as feed for animals, but not suitable for human consumption, it is said to be of feed grade. However, the food grade MP is suitable for human consumption.

The MP has several advantages over conventional proteins. They are healthy source of vitamins, carotenes and carbohydrates and may be produced under normal conditions throughout the year (Upadhyay et al. 2016). Unlike protein production from conventional crop, shortage of land and environmental disasters (such as drought or flood) are not problems in MP production. The significance of MP as protein supplements is very high, and thus, sustainable technology for its production on mass scale with economy and sustainability is in demand for global requirements (Ali et al. 2017).

The first MP to be produced in large scale and commercialised was 'Pruteen' by Imperial Chemical Industries (ICI) in 1983. It is produced from oxidation of methanol using *Methylophilus methylotrophus* (Westlake 1986). Natural and artificial organic substrates which are by-products from sugar industry, food processing industries and food waste are also used for MP production. The breakthrough in the MP production was hampered mainly due to low prices of protein sources like fish-meal and soybean including underdeveloped fermentation technology products (Matassa et al. 2016).

17.2.1 Substrates for MP Production

MP can be produced by a number of different substrates (Nasseri et al. 2011a, b) the microorganisms can grow over huge substrate ranging from fruit juices to hydrocarbon as well as waste materials and able to recycle different polluting agents. Therefore, microorganisms may not only be cultivable properly on different cost-effective substrates to fulfil the requirements of our daily diet but also serve to be the mediators of environmental renovation (Adebule et al. 2018). MP production using low-value materials as substrate for protein proves economically feasible for use in animal feed (Spalvins et al. 2018). The design and strategy for MP production depend on availability of substrates and its closeness to production plant (Gour et al. 2015). The major classes of substrates that can be used for MP production can be grouped into renewable carbon sources (CO_2, starch hydrolysate, cellulose hydrolysate, whey, molasses, industrial effluent and cellulosic waste) and nonrenewable carbon source (methanol, ethanol, liquid hydrocarbon and gaseous hydrocarbon). Low-value materials can be converted to nutritive microbial products using microbes and become asset to the environment (Matassa et al. 2016).

17.2.2 Microorganisms for Single-Cell Protein Production

Algae, bacteria, fungi and yeasts produce microbial biomass. The parameters for the selection of microorganisms depend on various factors including the fast growth of microorganism on broader range of substrate materials (Ghimire et al. 2014). The other criteria may be nutritional requirement (energy value, protein content, amino acid balance) and technical requirement (type of culture, type of separation, nutritional requirements). The desired microbial species should be cultured on the medium under sterile condition. Table 17.1 summarises the comparison of characteristics of different group of microorganisms for MP production. The microorganism used for microbial protein production should possess the following characteristics:

I. Specific growth rate (m) and biomass yield should be high.
II. Affinity for the substrate should be high.
III. Nutritional requirements should be low, i.e., few indispensable growth factors requirements.
IV. Able to utilise complex substrates.
V. Able to develop high cell density.
VI. Stable during multiplication.
VII. Capacity for genetic modification.
VIII. Good tolerance of temperature and pH.
IX. Balanced protein and lipid composition.
X. Should be nontoxic and have low nucleic acid content and good digestibility.

Table 17.1 Comparison of characteristics of different groups of microorganism for MP production

S. No.	Parameters	Algae	Bacteria	Fungi (yeast)	Fungi (filamentous)
1.	Growth rate	Low	Highest	Quite high	Lower than bacteria and yeast
2.	Substrate	Light, CO_2 and inorganic matter	Wide range	Wide range except CO_2	Mostly lignocellulosic
3.	pH range	Up to 11	5–7	5–7	3–8
4.	Cultivation method	Open pond, bioreactors	Bioreactors	Bioreactors	Bioreactors
5.	Risk of contamination	Serious	High, precaution necessary	Low	Low if grown below pH 5
6.	Recovery of biomass	Difficult and expensive using unicellular algae	Problematic	Easy	Easy
7.	Amino acid	Generally good	Good	Good	Low in Sulphur-containing amino acid
8.	Nucleic acid content	–	Very high (20% RNA)	High (15% RNA)	High (15% RNA)
9.	Protein content	Up to 60%	80% more	55–60%	50–55%
10.	Toxin	–	Gram-negative bacteria may produce endotoxins	–	Many species produce mycotoxins

17.2.3 Microorganism Involved in Production of Microbial Protein

Microorganisms like algae, fungi, yeast and bacteria have been utilised for microbial protein production (Goldberg 1985). Among all microorganism, yeast is most suitable as MP due to its high nutritive value (Nasseri et al. 2011a, b). But nowadays, other groups of microorganism are also widely explored for MP production due to several characteristics and advantages of these group of microorganism. Table 17.2 shows some important microorganism and the substrate used by them for microbial protein production.

17.2.3.1 Algae

The algae are used in human diets since very early time, and they are good source of proteins for the people in many countries of East Asia and Central Africa. Members of the genera *Chlorella*, *Scenedesmus* and *Spirulina* are generally

Table 17.2 Some important microorganism and substrates used for MP production

Microorganism	Substrate
Algae	
Chlorella sp.	CO_2+ sunlight
Scenedesmus acutus	CO_2+ sunlight
Spirulina maxima	CO_2+ sunlight
Yeast	
Candida utilis	Confectionary effluents
Candida utilis	Ethanol
Paecilomyces variotii	Sulphite liquor (from wood pulp mills)
Candida intermedia	Whey
Candida krusei	Whey
Candida lipolytica	N alkanes + ammonia
Kluyveromyces fragilis	Whey
Saccharomyces cerevisiae (baker's yeast)	Molasses
Fungi	
Aspergillus fumigatus	Maltose, glucose
Fusarium graminearum	Starch hydrolysate, glucose
Aspergillus Niger	Starch, cellulose, hemicellulose
Aspergillus oryzae, *Cephalosporium eichhorniae*	Cellulose, hemicellulose
Calvatia gigantea	Brewery waste
Penicillium cyclopium	Glucose, lactose, galactose
Rhizopus chinensis	Glucose, maltose
Agaricus campestris	Malt molasses
Agaricus blazei, *A. campestris*	Glucose
Chaetomium cellulolyticum	Cellulosic waste (straw, bagasse, sawdust)
Mushroom	
Paecilomyces variotii	Sulphite liquor
Bacteria	
Brevibacterium sp.	C_1–C_4 hydrocarbons
Methylophilus methylotrophus, streptomyces, *Flavobacterium* sp., *Pseudomonas fluorescens*, *P. utilis*	Methanol
Acinetobacter	–

cultivated in mass in ponds and tanks. They use no-cost CO_2 and sunlight as primary substrates (Pulz and Gross 2004). Generally, the limiting factor in their large-scale production is illumination. Algal MP has almost 60% crude protein including good-quality amino acid composition except for low quantity of sulphur-containing amino acids (Ugboguand and Ugbogu 2016). However, there are some disadvantages of using algae as MP which are:

I. Rich chlorophyll content which is not suitable for human use
II. Serious problems when *Chlorella* and *Scenedesmus* are used in human diet (*Spirulina* is more suited for human use)

III. Low cell density, e.g., 1–2 g dry weight/l
IV. Serious risk of contamination
V. Costly recovery methods for unicellular algae (*Spirulina* harvested by filtration or simply by skimming)

17.2.3.2 Filamentous Fungi

Filamentous fungi with polysaccharide hydrolysates, e.g., starch hydrolysates and sulphate liquor from wood pulp industries, have been used to produce MP (Asadollahzadeh et al. 2018). These are usually grown as submerged cultures in which they grow as yeastlike cells, in filamentous form or in pellets. They have crude protein content of 50–55%; the protein is low in S-containing amino acids but otherwise is excellent in amino acid composition. The recovery of filamentous and pellet forms is rather easy by filtration. The most successful mycoprotein which is commercialised and sold in many countries is the Quorn™ (Wiebe 2004). Since mycoproteins taste like meat, they are successfully used as alternative to the conventional animal proteins. However, there are also some problems associated with fungi which are listed below:

I. Slower growth rates than bacteria and yeast.
II. Contamination by yeast may be frequent if sterility is not maintained, while that by bacteria can be minimised by keeping the pH of broth below 5.
III. They have high nucleic acid content (up to 15% RNA).
IV. The strains have to be thoroughly evaluated for mycotoxin production.

17.2.3.3 Yeasts

Yeast has been used for long time as an additional source of MP. During World War I, Germany produced torula yeast (*Candida utilis*) and consumed it in making sausages and soups (Srividya et al. 2013). Members of *Saccharomyces*, *Candida* and *Torulopsis* have been widely studied for MP production, and those of the first two genera are used for some commercial processes using various substrates (Ali et al. 2017). The feed supplementation of pet animals such as dog, cat and fish is obtained from yeasts, which make the supplement more edible for the animals (Ali et al. 2017). Commonly, it is rich in vitamin B. The difficulties in use of yeasts as MP are:

1. Slower growth rates than fastest-growing bacteria.
2. High nucleic acid content (up to 15%) which needs to be reduced.
3. Methionine supplementation may be done to overcome S-containing amino acid deficiency of its proteins.

17.2.3.4 Bacteria

A number of bacterial species have been evaluated for MP production by using a wide variety of substrates (Rudravaram et al. 2009). Bacteria owing to their fast growth, short generation time and doubling time were found to be more effective in the production of MP (Knight and Leitsberger 2016). They also have the ability to grow on wide range of carbohydrates from simple carbohydrates (sugars and starch)

to hydrocarbons (methane) and fractions of petrochemicals (methanol and ethanol) (Bamberg 2000). Methanol is among preferred substrate for carbon source for the bacterial growth due to its solubility in water, nonexplosive and free from hydrocarbon impurities.

Bacteria can utilise both inorganic nitrogen in form of ammonium salt, ammonia, nitrates, urea and organic nitrogen present in the waste. Mineral nutrient is added in the bacterial culture in concentration sufficient to support microbial growth to fulfil nutrients deficiency in natural waters. Large quantities of microbial proteins for animal feed can be obtained from microbial species like bacteria *Brevibacterium*, *Methylophilus methylotrophus*, *Achromobacter delvaevate*, *Acinetobacter calcoaceticus*, *Aeromonas hydrophila*, *Bacillus megaterium*, *Bacillus subtilis*, *Cellulomonas* species, *Flavobacterium* species, *Lactobacillus* species, *Methylomonas methylotrophus*, *Pseudomonas fluorescens*, *Rhodopseudomonas capsulata*, *Streptomyces* spp. and *Thermomonospora fusca* (Adedayo et al. 2011; Gomashe et al. 2014; Dhanasekaran et al. 2011). However, bacteria also have few limitations as a producer of microbial protein such as:

I. High nucleic acid, especially RNA content.
II. Maintenance of sterility and pH between 5 and 7.
III. Risk of pathogenic bacterial contamination and recovery of microbial cell.
IV. Also, careful evaluation for endotoxin production is essential particularly when gram-negative bacteria are used.

17.2.4 Biotechnological Method for Cultivation of MP

Microorganisms are endowed with metabolic capabilities of using a wide range of various substrates both from renewable and nonrenewable source, but all of them require carbon, nitrogen and phosphorus sources as well as other minerals and vitamins. The main stages of single-cell protein production are medium preparation, fermentation and downstream processing. Few processes used for MP production by different commercial plants are described below:

A. *The Symba process*: In this method, two yeasts, the amylase-producing *Endomycopsis fibuligera* with fast-growing *Candida utilis*, are used in sequential mixed culture using starchy waste as substrate (Oura 1983). It is a two-stage process; in the first stage, *Endomycopsis fibuligera* is grown in a small reactor containing sterilised potato waste, which is supplemented with phosphorus and nitrogen sources. Starch is hydrolysed at this stage. In the second stage, the broth is pumped into next reactor where both the organisms are present. After few days of fermentation, biomass is recovered by centrifugation and dried by spray or drum drying. This process can be operated continuously, and after 10 days, up to 90% reduction in pollution load of waste is recorded. In this process, *C. utilis* dominates the final product and constitutes 90% of the MP. Protein-rich biomasses are concentrated by centrifugation, filtered and finally spray-dried

or drum-dried before entering the market as nutritional supplement. The final product called as 'Symba yeast' contains about 45% protein besides vitamins (Jarl 1969).

B. *The Bel process*: This is the most popular process for the production of MP from dairy industry waste using *Kluyveromyces lactis* or *Kluyveromyces marxianus* by Bel Industry (France). Whey invariably contains about 5% lactose, 0.8% protein and 0.2–0.6% lactic acid and is used as a substrate. The MP produced by this process used for animal and human consumption is marketed as Proteibel. In this process, initially, pasteurisation of whey is done during which almost 75% of whey protein got precipitated. The amount of lactose is adjusted to 34 g/l with the addition of mineral salts. Supplemented whey is added at 22 m^3 of continuous fermenter and maintained at 38 °C, pH 3.5 and aeration rate of 1700 m^3/h (Moulin et al. 1983). The yeast utilises the lactose and attains a biomass concentration of 25 g/l, with a biomass yield of 0.45–0.55 g/g lactose. Then centrifugation is done to recover yeast cells which are finally roller-dried to 95% solids. Less than 1 g/l of residual sugar are left in spent medium (Waites et al. 2002).

C. *The Bioprotein process*: MP were produced using alkanes (methane) and straight chain hydrocarbon by several oil companies during the late 1970s when the prices of conventional feed protein were high and oil prices were low. However, due to their immiscibility in water and explosiveness when mixed with oxygen especially methane, use of these compounds posed some problems. In 1990, a company named Norferm produced MP by growing *Methylococcus capsulatus* in a medium fed with methane-rich natural gas as the sole source of carbon and energy, and the process is known as Bioprotein process. Fermentation is carried out in continuous loop fermenters containing medium enrich with ammonia, minerals and methane. Downstream processing comprises centrifugation, ultrafiltration and spray-drying to harvest the biomass. The final product obtained is marketed as Pronin (Waites et al. 2002). However, microorganisms can only tolerate a low concentration (0.1–1.0% v/v) of methanol. The advantages of using methanol over methane and many other carbon sources are the complete miscibility with water and its availability in a pure form.

17.2.5 Advantages of Microbial Protein

There are number of advantages of using microbial biomass as a source of protein as compared to protein from conventional crops as source of feed and food such as:

I. A high protein, vitamin (especially B complex) and amino acid contents and low-fat content.
II. Possibility of genetic modification for production of amino acid of specific interest.
III. Continuous yearly production which is independent of climatic and seasonal changes.

IV. Utilisation of even waste material as their substrate, thereby helping in reduction of pollution by recycling waste materials.
V. Owing to high rate of microbial multiplication, large amount of microbial proteins are produced in small portions of land within short time.

17.2.6 Problems

MP can be considered as potential source of protein; however, it also contains other biomolecules such as carbohydrates, lipids, nucleic acid, mineral and vitamin. The major problem in the use of microbial protein as a human food is the presence of high concentration of nucleic acids (Anupama 2000). In fungi and yeast, 10–15% of total nitrogen is in the form of nucleic acid, which follows different route of metabolism and not metabolised in the same way as protein. The consumption of proteins with high concentration of nucleic acids (8–25 g/100 g of protein dry weight) causes increase in uric acid level in blood resulting in kidney stones and gout. The problems associated with the MP from hydrocarbons are the presence of residual alkanes, polycyclic aromatic hydrocarbons and fatty acids. The accumulation of these compounds in the adipose tissues of animal causes serious health hazards. Consumption of MP may also cause skin reactions or allergies and gastrointestinal reactions resulting in nausea and vomiting (Adedayo et al. 2011). The assimilation of some heavy metals, microbial toxins and chemical residues from nutrient media can also cause serious health hazards. MP has an unpleasant colour, odour and taste which make them unpalatable even for animal consumption. It is therefore necessary to reduce nucleic acid content in MP to an acceptable low level.

17.2.7 Application of Microbial Protein

MP has potential of application in various sectors. Table 17.3 enlists various application of MP.

Table 17.3 Applications of microbial protein

S. No.	Industrial sectors	Applications
1.	In animal diet	In calves, poultry and pigs for fattening ability
		Breeding of fish
		As a feed for laying hens
		As a feed for household animal
2.	As a part of foodstuffs	As vitamin carrier
		Emulsifying agent
		As a carrier of scent
		Improving the nutritional quality of baked items
		In readymade meals
3.	In technological field	Foam-stabilising agent
		Processing of leather and paper

17.3 Microbial Enzymes

Enzymes are biological catalysts that play a vital role in metabolic and biochemical reactions by lowering the activation energy (Nigam 2013). They are highly specific in nature, catalysing only one particular type of reaction. They are proteinaceous in nature, except catalytic RNA molecule, known as ribozymes. The cellular processes are mainly regulated by a coordinated reaction sequence with greater specificity using set of enzymes. Therefore, the enzymes are vital for support of life (Cech and Bass 1986).

With the increasing use of enzymes in many industrial and commercial applications, the demands for production of enzymes have risen (Pandey et al. 1999). Various chemicals and pharmaceuticals produced by industrial processes have several disadvantages such as they show low catalytic efficiency, lack of specificity, need high temperature and pressure for their synthesis and also use of organic solvent leads to generation of waste and pollution. However, productions of such compounds via enzymes have several advantages: firstly, enzymes can work under mild reaction condition, stereo- and regioselective chemicals are produced and have long half-life, and enzymes can work on wide range of substrate. Enzymes do suffer from limitation such as use of certain enzymes requires cofactors. However, approaches such as cofactor recycling and use of whole cell could resolve such problems (Adrio and Demain 2014). Enzymes can be produced from animals, plants and microorganism. However, microbes as source of enzymes produced from different groups of microorganism like bacteria, fungi and yeast are more preferred over plant and animal sources (Anbu et al. 2013) because microbial enzymes:

I. Are more active and stable.
II. Can be produced in large scale.
III. Extraction and purification of microbial enzyme are much easier.
IV. Require limited space and time period for production.
V. Microbes can work under different sets of environmental conditions.
VI. Fermentative production is independent of seasonal variation.
VII. Microbes exhibit convenient and safe production methods.

17.3.1 Production of Microbial Enzymes

The development of fermentation method for production of microbial enzymes has provided unlimited supply of enzymes (Vittaladevaram 2017). Earlier surface culture methods were used for commercial production of enzymes; however, within last few years, submerged culture methods have been extensively used. Both these methods have their own advantages and disadvantages. Solid-state fermentation in which microorganism cultivation and production of enzyme are done on a solid substrate is successfully employed in enzyme production (Pandey et al. 1999; Wang and Yang 2007).

After fermentative production of enzymes, precipitation method is widely used for its recovery from broth. The enzymes are with the help of solvents like acetone and alcohol. The precipitated enzyme is then filtered and dried at low temperature or vacuum dried. Microbial enzyme may be sold out in dry powder form or in concentrates (Underkofler et al. 1958). Most of the commercial enzymes are stable dry forms. However, some may need presence of stabilisers and activators for maximum showing stability and efficiency.

Due to problems like loss of enzyme activity and low recovery of enzymes associated with conventional fermentation and downstream processing method, membrane-augmented downstream method is the most suitable way equipped with microfiltration and ultrafiltration membranes (Verma et al. 2012). Membrane-augmented downstream processing has many advantages over conventional recovery processes such as purity, yield, quality and percentage recovery of enzyme is good; fewer steps is required for recovery, thereby reducing overall cost; design of recovery system is flexible and easy to operate; and also, this method is environmentally friendly (Binod et al. 2013).

The low concentration of enzymes which are normally produced by wild strain is considerable hindrance for enzyme production. But with advent in technology for improvement of strain, this problem can be solved. For the process of strain improvement, a wild-type strain is isolated to increase its productivity (Tiwari et al. 2015). The isolated strain should exhibit features like rapid growth, genetic stability, requiring less fermentation time, nontoxic to humans and exhibit tolerance to high concentration of carbon and nitrogen source. For faster growth rate, downstream processing and behaviour of bioreactor are increased through cellular genetics. For example, in case of yeast fermentation, more emphases are given to processes involving gene regulation and ploidy in which carbon source has a predominant role in protein production. In case of fungal source, the emphasis is given on the cell wall, differentiation, secretion and branching. Site-directed mutagenesis (Zhang et al. 2017) recombination, protoplast fusion (Agyei et al. 2016) and RDT technology (Aguilar-Toalá et al. 2016) are being used for strain improvement.

17.3.2 Application of Microbial Enzymes

The demand for microbial enzymes is on a continuous rise driven by application of enzymes in various industries such as paper and pulp, leather, pharmaceutical and analytical industries, food and feed industries and many more (Singh et al. 2016). With advancement in field of protein biochemistry, bioinformatics, molecular biology and bioanalytical techniques, the horizon of enzyme utilisation in various fields is expanding day by day. The extensive usage of microorganisms in various bioprocesses can be applied in industries. Table 17.4 summarises several applications of microorganisms for delivering different valuable products.

Table 17.4 Biotechnological applications of microbial enzymes

Industries	Enzymes	Application	Microorganism source
Food and beverages	α-Amylase	Process of baking, brewing, liquefaction of starch, improvement of bread quality, clarification of fruit juice	*Aspergillus* sp., *Rhizopus* sp. and *Endomyces*
	Glucoamylase	In production of beer, improvement of bread quality, high glucose and fructose syrups	*Bacillus* sp., *Clostridium* sp., *Rhizopus* sp., *Aspergillus* sp.
	Protease	Brewing industry	*Aspergillus niger*, *A. oryzae*, *Bacillus amyloliquefaciens*, *B. stearothermophilus*, *Mucor miehei*, *M. pusillus*
		Tenderisation of meat	
		Milk coagulation	
		Improvement of bread quality	
	Lactase (β-galactosidase)	Reduction of lactose intolerance in people, as prebiotic food ingredients	*Lactobacillus acidophilus*, *Bifidobacterium longum*, *Enterococcus faecalis*
	Lipase	Development of cheese flavour, cheddar cheese production	*Aspergillus niger*, *Burkholderia cepacia*, *Candida antarctica*, *C. rugosa*, *Pseudomonas mendocina*, *P alcaligenes*
	Phospholipase	Development of cheese flavour, lipolysed milk fat production	*Aspergillus oryzae*, *A. fumigatus*, *Serratia* sp., *S. liquefaciens*, *Fusarium oxysporum*
	Esterase	Flavour and fragrance enhancement in fruit juice, de-esterification of dietary fibre, short-chain flavour esters production	*Trichoderma reesei*, *Aspergillus niger*, *Schizophyllum commune* and *Aureobasidium pullulans*
	Cellulase	Feed for animal, clarification of fruit juice	*Trichoderma*, *Chaetomium*, *Penicillium*, *Aspergillus sp*, *Fusarium*
	Xylanase	Clarification of fruit juice, improvement of beer quality	*Bacillus*, *Cellulomonas*, *Micrococcus*, *Streptomyces*, *Actinomadura*, *Nonomuraea*
	Pectinase	Clarification of fruit juice	*Aspergillus* sp., *Bacillus* sp., *Erwinia* sp., *Fusarium* sp., *Kluyveromyces* sp., *Pseudomonas* sp., *Penicillium*, *Rhizopus* sp., *Trichoderma* sp.
	Glucose oxidase	Shelf life improvement of food	*Aspergillus Niger and Penicillium amagasakiense*
		Improvement of food flavour	

(continued)

Table 17.4 (continued)

Industries	Enzymes	Application	Microorganism source
	Laccase	Polyphenol removal from wine, in baking	*Trichoderma* species, *Pycnoporus cinnabarinus*
	Catalase	Food preservation (with glucose oxidase), removal of hydrogen peroxide from milk prior to cheese production	*Aspergillus niger*, *Micrococcus luteus*
	Peroxidase	Flavour and colour development in food, improvement of nutritional quality of food	*Bacillus* spp., *Pseudomonas* sp., *Citrobacter* sp., *Candida krusei*, *Coprinopsis cinerea*, *Phanerochaete chrysosporium*
	Asparaginase	Reduction of formation of acrylamide during baking	*Escherichia coli*, *Erwinia chrysanthemi*
	Debittering enzymes: Naringinase	Removal of bitter taste in fruit juice, enhancement of wine aroma	*Aspergillus*, *Bacillus*
Detergent	Amylase	Removal of starch-based stain	*Aspergillus* sp., *Bacillus* sp.
	Cellulase	Softening, colour brightening	*Aspergillus niger*, *Bacillus* sp.
	Cutinase	Removes triglyceride stains	*Fusarium solani*, *F. pisi*
	Lipase	Fat decomposition, removal of any fatty stain	*Aspergillus oryzae*, *Bacillus* sp., *Candida* sp.
	Protease	Removes protein stains	*Aspergillus* sp., *Bacillus* sp.
Leather and textile	Alkaline protease	During soaking process removal of non-fibrillar protein, making leather soft and other uses	*Alcaligenes faecalis*, *Bacillus* sp.
	Amylase	Dehairing, fibre splitting and desizing in textile	Aspergillus sp., *Bacillus subtilis*
	Neutral protease	Waste water reduction, dehairing	*Aspergillus niger*, *A. flavus*, *Bacillus subtilis*
	Lipase	Degreasing	*Rhizopus*, *A. Niger*
	Transglutaminases	Waste processing	Streptoverticillium
	Cellulase and pectinase	Bioscouring	

(continued)

Table 17.4 (continued)

Industries	Enzymes	Application	Microorganism source
Cosmetic	Endoglycosidase	Teeth whitening, removal of plaque and odour-causing deposits in teeth and gum	*Mucor hiemalis*
	Laccase, peroxidase	As a hair dye	*Bacillus subtilis*, *Trametes versicolor*
	Papain	Teeth and gum care, skin and hair care	
	Lipases	Preparation of hair waving, used in skin care creams and ointments	*Aspergillus oryzae*, *A. flavus*
	Protease	In smoothening and cleaning of skin by removing dead skin cells	*Aspergillus niger*, *A. flavus*, *Bacillus subtilis*
	Superoxide dismutase	Scavenging of free radical skin care	*Lactobacillus plantarum*, *Corynebacterium glutamicum*
Paper and pulp	Amylase, cellulase	Improvement of drainage and deinking	*Aspergillus niger*, *Bacillus* spp.
	Lipase	Pitch control	*Candida antarctica*
	Protease	Removal of biofilm	*Bacillus subtilis*
	Xylanase	Enhance delignification	*Aureobasidium pullulans*
		Bleaching	*Trichoderma reesei*
			Thermomyces lanuginosus
			Streptomyces lividans
	Ligninolytic enzymes: Laccase, peroxidase	Non-chlorine bleaching, delignification	*Bacillus subtilis*
Therapeutic	Asparaginase, glutaminase	Treatment of leukaemia	*E. coli*
	Collagenase	Skin ulcers	*C. perfringens*
	Ribonuclease	Antiviral	Yeast and bacteriophages
	Streptokinase	Blood clots	*Streptococci* sp.
	Uricase	Gout	*A. flavus*
	Urokinase	Blood clots	*Bacillus subtilis*
	β-Lactamase	Antibiotic resistance	*Citrobacter freundii*, *Serratia marcescens*, *Klebsiella pneumonia*
	Penicillin acylase	Penicillin production/ broad spectrum, antibiotic production	*Penicillium* sp.
Polymer	Lipase	Polycondensation, polymerisation and polyaddition reactions	*Candida antarctica*
	Laccase, transglutaminase	Crosslinking in polymers	*Trametes hirsute*, *Trichoderma reesei*

17.3.2.1 Application in Food and Beverages Industries

Enzymes such as amylase, cellulase, pectinase, lactase and others are widely used in food industries (Raveendran et al. 2018). These enzymes are mainly used in fruit juice industries for clarification of fruit juice (Kumar 2015), in baking industries for improvement of bread quality and cake making (James et al. 1996) and in wine making and brewing industries to improve flavour, texture and aroma of wine and beer (Galante et al. 1993). Enzymes such as α-amylases and glucoamylases dominate the food enzyme market followed by protease and lipase. Protease and lipase find their application mainly in dairy sector where they are used in the production of bakery products, dough conditioning, as sweeteners, chocolate syrups and meat tenderising, in egg products, seafood, flavour extracts, flavour development and many others (Aravindan et al. 2007). Some application of various other food enzymes such as pectinases, glucose isomerases, cellulases and hemicellulases is presented in Table 17.3 above.

17.3.2.2 Application in Detergent Industry

In detergent industries, enzymes find application mainly in removal of protein, oil, fat and other stains from clothes. Enzymes such as lipases, proteases and amylases which break down lipids, protein and carbohydrates, respectively, are incorporated in detergents to remove these hard stains which occur due to spillage of blood, grease, oil, chocolate, curries, etc. in clothes (Raveendran et al. 2018).

17.3.2.3 Application in Leather and Textile Industry

Enzymes like proteases and lipases help in making leather smooth and soft by removing the hair on the skin and also proteins and fats in between the leather. Enzymes like cellulase are used to give smooth and glossy appearance to natural cotton, wool and synthetic fabrics. Amylase enzymes are used to control the fabric size and thickness of the thread (de Souza and Magalhães 2010). A hydrogen peroxide residue after bleaching is removed by catalases.

17.3.2.4 Application in Cosmetics

With the rapid development of cosmetics industry, the use of enzymes will also be more and more widespread. Enzyme can be used as an antioxidant in the cosmetics industry as well as moisturising agents, whitening and other functional additives (Smythe 1951). Among them, superoxide dismutase is the biologic enzyme most widely studied and widely used in the cosmetics industry. SOD is the abbreviation of superoxide dismutase, which is the first line of defence against free radicals in the body. Superoxide anion free radicals are produced when the human body absorbs oxygen to carry on metabolism. If free radicals are not eliminated, the body will produce a chain reaction, destroying human cells. Modern medicine proved that free radical is an important factor that causes a variety of diseases and ageing. SOD is a natural killer of free radicals. Cosmetics containing SOD have some functions of sunscreen, anti-radiation, whitening, antiwrinkle, anti-inflammation and anti-ageing (Babizhayev 2006). SOD makes skin more delicate, especially suiting for those who work in front of computers and under hot sun to effectively prevent the damage of

ultraviolet rays and to inhibit the formation of melanin, senile plaques and facial acne.

17.3.2.5 Application in Paper and Pulp Industry

In paper and pulp industries, enzymes are mainly used for bleaching, pitch removal and deinking of paper wastes (Kirk et al. 2002). With use of xylanase for bio bleaching of pulp which is eco-friendly bleaching techniques, the technology was widespread used by several mills worldwide. After xylanases, potential application of laccase in paper and pulp industry was realised where it is used for delignification and brightening of pulp, removal of lipophilic extractives and improving physiochemical as well as mechanical properties of pulp by either forming reactive radicals with lignin or by functionalising lignocellulosic fibres (de Souza and Magalhães 2010). Laccases exhibit detoxification of the coloured and toxic compounds released as effluents from pulp and paper industries and also render them nontoxic through polymerisation and depolymerisation reactions (Upadhyaya et al. 2016).

17.3.2.6 Application of Enzymes in Therapeutics

Enzymes are being used in treatment of various diseases mainly because they are highly specific and fast. Therapeutically useful enzymes are required in low concentration but with a very high degree of purity; therefore, sources of such enzymes are selected with great care, avoiding any possibility of contamination and incompatibility (Gurung et al. 2013). Enzymes in therapeutics mainly find applications as thrombolytic agents which are capable of rapidly lysing the clots that can cause many allied conditions such as myocardial infarction, phlebitis, pulmonary embolisms and occluded catheters, for the control of the growth of selected neoplasms or leukaemias and as antidotes to poisons or as counteragents capable of mitigating the delirious effects of toxins, etc. (Mane and Tale 2015). Another major application of enzyme is in treatment of cancers such as leukemia. Enzyme such as asparaginase has promised if worked upon for the treatment of acute lymphocytic leukaemia.

17.4 Secondary Metabolites

Secondary metabolites were first recognised by Sashs in 1873, which are natural small organic product/molecules which do not have primary function in growth, development and reproduction of organisms but are very important for human health (Cragg and Newman 2013). Microorganisms also produce primary metabolites; the key difference between them is that primary metabolites are essential for growth of cell and are produced during growth phase, whereas secondary metabolites don't play physiological role in growth and development and produced mainly during idiophase or stationary phase. Other differences between primary and secondary metabolites are illustrated in Table 17.5.

Secondary metabolites are usually produced when growth is limited by exhaustion of growth-limiting substrates such as carbon, nitrogen, phosphate, etc. Their synthesis is greatly influenced by manipulating the media composition for culturing these organisms (Ruiz et al. 2010). One of the example is biosynthesis of antibiotic

Table 17.5 Primary and secondary metabolites

S. No.	Primary metabolites	Secondary metabolites
1.	Essential for growth and development	Not essential for growth and development
2.	Not important for ecological adaptation	Important for ecological adaptation
3.	Uniform	Variable
4.	Conservative	Diverse
5.	Constant	Adaptive
6.	Relatively simpler structures	Highly complex structure and a large number of specific enzymatic reaction for synthesis
7.	Les genetic variation	Highly genetic variation
8.	Constitutive	Constitutive as well as inducible production

penicillin which starts when glucose is completely depleted from the medium and fungus (*Penicillium chrysogenum)* starts consuming lactose, a less readily utilised sugar. Microbial metabolites play important role for the development of various sectors such as agriculture, pharmaceutical and food (Sharma et al. 2016). They can further be exploited for the production of novel products and method development.

Most secondary metabolites produced by actinomycetes commonly of genus *Streptomyces* and fungi are of economical importance. Structural diversity of secondary metabolites exhibits a variety of bioactivities such as antimicrobial, antioxidant, antitumour, immunosuppressive, antiparasitic agents and inhibitors of enzymes. Mostly secondary metabolites are produced in stationary phase after active growth in log phase and usually have an unusual chemical structure. They have a major effect on the health, nutrition and economics of our society.

Secondary metabolites after growing in selective media have been subjected to combinatorial chemistry. Secondary metabolites also exhibit a vast diversity in their chemical structures (Ncube and Staden 2015). The biosynthetic pathway of secondary metabolites is however linked to network of primary metabolism using the same intermediates and regulatory mechanisms (such as feedback inhibition, induction, catabolite) and is formed by pathways branching off from primary metabolic pathways at a relatively small number of points. In addition, genes responsible for synthesis of secondary metabolites are clustered together, and expression of these genes is induced by one or few regulators (Osbourn 2010). The following are the biosynthetic categories which are usually involved in synthesis of secondary metabolites:

1. Metabolites derived from shikimic acid: This family includes production of aromatic amino acids, ergot alkaloids and the antibiotics candicidin and chloramphenicol.
2. Metabolites derived from amino acids: This family includes antibiotics such as penicillin, cephalosporin, cephamycins, cyclic peptide antibiotics (gramicidin) and immunosuppressive agent cyclosporine.
3. Metabolites derived from acetyl-CoA and related compounds, as well as Krebs cycle intermediates: This family is further divided into polyketides producing antibiotics such as erythromycin, the insecticidal-antiparasitic compound aver-

mectin and the antitumour agent doxorubicin and terpenes producing, for example, non-cytotoxic antitumour agent Taxol.
4. Metabolites derived from sugars.

17.4.1 Microorganisms as Source of Secondary Metabolites

Microbes are an important and novel resources for producing natural secondary metabolites with potent biotechnological application. Many secondary metabolites such as alkaloids, terpenoids, steroids, quinones, isocoumarins, lignans, phenylpropanoids, phenols and lactones which are beneficial for plant as well as human health are well known to be produced from many microbes (Sharma et al. 2016).

The discovery of penicillin from a fungal sp. *Penicillium notatum* in the 1940s and its subsequent use in clinic soon lead to the discovery of number of antibiotics from microorganisms especially actinomycetes and fungi (Demain and Fang 2000). Bacterial resistance against antibiotics is a challenge as a long term. *Staphylococcus aureus* is the first bacterium in which penicillin resistance was observed in 1947, just 4 years after the drug started being mass-produced.

Many infectous diseases which were earlier treated only by synthetic drugs, nowadays being treated by microbial metabolites (Singh et al. 2017) showing antimicrobial, anti-inflammatory, antidiabetic, antitumour, anticholesterolemic, antioxidant, immunosuppressive and enzyme inhibitors activities (Table 17.6). Moreover, new microbial metabolites are also being employed as plant growth regulators; as antiparasitic, pesticide and herbicide agents; and in other agricultural applications.

17.4.2 Endophytic Microbes as a Source of Secondary Metabolites

The complex relationship among endophytic microorganisms and plants remains unique. The symbiotic relationship gives endophytes powerful ability to produce novel bioactive substances beneficial for plant health as well as human health (Strobel and Daisy 2003). Endophytic microorganisms comprise unicellular bacteria, actinomycetes and fungi, spending all and sometimes part of its life cycle colonising in healthy plant tissues inter- or intracellular (Bhardwaj and Agrawal 2014). Endophytic microbes are explored and exploited for their ability to produce various phytochemicals of their host plant which can additionally possess medicinal properties (Stierle et al. 1993). Endophytes are capable of producing different classes of secondary metabolites having bioactive compounds belonging to structural classes such as alkaloids, steroids, terpenoids, phenols, quinines, flavonoids, phenylpropanoids, aliphatic compounds, polyketides and peptides. These compounds have shown different activities from interdisciplinary perspectives of biochemistry, genetics, fungal biology and host plant biology.

Table 17.6 Biological activities of secondary metabolites of industrial importance

S. No.	Microbes	Bioactive compound	Biological activities
1.	*Acremonium chrysogenum*	Cephalosporin	Antibacterial
2.	*Alternaria arborescens*	Alternariols	Mycotoxin
3.	*Amycolatopsis mediterranei*	Rifamycin	Antibacterial
4.	*Ashbya gossypii*	Riboflavin	Nutrient
5.	*Aspergillus flavus*	Aspergillic acid	Antifungal
6.	*Aspergillus fumigates*	Asperfumin	Antifungal
7.	*Aspergillus parasiticus*	Aflatoxins	Mycotoxin
8.	*Aspergillus terreus*	Lovastatin	Anticholesterolemics
9.	*Beauveria nivea*	Cyclosporines	Immunosuppressive
10.	*Candidatus entotheonella*	Calyculin	Phosphatase inhibitor
11.	*Claviceps purpurea*	Ergotamines	Mycotoxin
12.	*Cryptosporiopsis quercina*	Cryptocandin	Antioxidant
13.	*Emericella* sp.	Emerimidine A and B, emeriphenolicins A and D	Antiviral
14.	*Endoecteinacidia frumentenis*	Ecteinascidin 743	Antitumour activity
15.	*Entrophospora infrequens*	Camptothecin	Anticancer
16.	*Exiguobacterium indicum*	Alkaloids	Antidiabetic activity
17.	*Fusarium* spp.	Zearalenone	Mycotoxin
18.	*Fusarium solani*	Camptothecin	Anticancer
19.	*Fusarium subglutinans*	Subglutinol A and B	Immunosupressive
20.	*Gibberella fujikuroi*	Gibberellin	Plant growth regulator
21.	*Gliocladium* sp.	10-DAB III	Anticancer
22.	*Micromonospora*	Gentamicin	Antibacterial
23.	*Monascus purpureus*	Monascin	Pigment
24.	*Monascus ruber*	Monacolin	Anticholestrolemics
25.	*Penicillium chrysogenum*	Penicillin	Antibacterial
26.	*Penicillium citrinum*	Pravastatin	Anticholestrolemics
27.	*Penicillium griseofulvin*	Griseofulvin	Antifungal
28.	*Periconia* sp.	Piperine	Antibiotic
29.	*Pestalotiopsis microspora*	Isopestacin and pestacin	Antioxidant
30.	*Mucor fragilis*	Podophyllotoxin and kaempferol	Biocontrol assay
31.	*Streptomyces antibioticus*	Actinomycin-D	Antitumour

(continued)

Table 17.6 (continued)

S. No.	Microbes	Bioactive compound	Biological activities
32.	*Streptomyces aureofaciens*	Aureofacin	Antifungal
33.	*Streptomyces aureofaciens*	Tetracycline	Antibacterial
34.	*Streptomyces avermitilis*	Avermectin	Insecticidal
35.	*Streptomyces cinnamonensis*	Monensin	Growth promoter
36.	*Streptomyces aureofaciens*	4-Arylcoumarins	Antitumour
37.	*Streptomyces* sp.	Dinactin, cyclononactic acid	Antineoplastic
38.	*Streptomyces* sp.	Ansamycins, naphthomycin K	Cytotoxic activity
39.	*Streptomyces venezuelae*	Chloramphenicol	Antibacterial
40.	*Streptomyces verticillus*	Bleomycin	Antitumour
41.	*Streptomyces clavuligerus*	Clavulanic acid	Plant enzyme inhibitor
42.	*Taxomyces andreanae*	Taxol	Antitumour
43.	*Tolypocladium inflatum*	Cyclosporin-A	Immunosuppressive
44.	*Trichoderma flavofuscum*	L-DOPA	Parkinson's disease
45.	*Zygosporium masonii*	Zygosporin-A	Antibacterial
46.	*Xylaria* sp.	Cytochalasin D	Cytotoxic, antifungal and antibacterial

17.4.3 Biological Activity of Secondary Metabolites

Microbial metabolites possess various biological activities like antimicrobial, antioxidant, antitumour, immunosuppressant, anti-inflammatory, insecticidal, antihypercholesterolemic and antidiabetic, which show potent applications in field of agriculture, pharmaceuticals and food industry.

17.4.3.1 Antimicrobial Activity

One of the major concerns faced by health services these days is the rate at which the existing pathogenic microbes are getting resistant to the available commercial drugs (Bhardwaj et al. 2015). Because of which, intensive search for new and effective antimicrobial agents is the need of the time, and that is encouraged by investigating novel corners and natural surroundings. Many common microbial diseases that previously caused suffering of human beings because of unavailability of drug for the treatment have now been eradicated or can be routinely treated, mostly due to the availability of secondary metabolite as antibiotics (Gouda et al. 2016).

Secondary metabolites are produced by organism to combat other organism. So far, many microbes have been used for isolation of large number of metabolites

showing antimicrobial activity. These secondary metabolites show their activity against other microorganism at low concentration. These metabolites show antibacterial, antifungal and antiviral activities (Berdy 2012). Examples of antifungal activity include cryptocandin, cryptocin, ecomycins, pseudomycins, pestaloside and pestalopyrone, and antibacterial activity includes periconicins A and B, phomopsichalasin and javanicin, whereas antiviral activity includes cytonic acid A and B.

Some of the antimicrobial agents from microbes especially from endophytic fungi are active not only against human pathogens but also against plant pathogens, leading to their application in agriculture fields (Dutta et al. 2014). Secondary antimicrobial metabolites ergosta-5,7, 22-trien-3-ol, 4-hydroxymellein and 2,3-dihydro-5-hydroxy-α,α-dimethyl-2-benzofuranmethanol were obtained from the endophytic fungus *Gliomastix* of medicinal plant *Parispolyphylla* var. *yunnanensis* (Zhao et al. 2012).

17.4.3.2 Antioxidant Activity

Antioxidant compounds play a significant role in improving human health and prevention of disease (Gouda et al. 2016). Free radicals catalyse oxidative reactions that develop toxic lipid peroxides which play a major role in the origin of numerous diseases like high blood pressure, diabetes, cancer, cardiovascular, neurodegenerative, etc. (Lobo et al. 2010). Microbial metabolites also inhibit the enzymes of mitochondria respiratory chain and damage its DNA and proteins which cause lethal effect for the cell (Murphy 2009). Nowadays, alternatives of natural antioxidant compounds are sought and developed to obtain compound which is specific and has better biological activity without any side effects. These bioactive compounds with biomedical potential play a significant role in the prevention or treatment of human diseases, associated with oxidative damage that has a high impact in world society.

Several medicinal plants, fruits and vegetable have been reported to possess natural antioxidant compound along with their free radical scavenging activity. However, secondary metabolites producing microbes can be a possible cause of novel naturally produced antioxidants. Various groups of microorganisms have been identified as the source of antioxidants. It was observed that antioxidant activity assumed to be associated with lipid component of cell protected an oil-soluble fraction of *Mycobacterium phlei* added to cottonseed oil against oxidation (Viswanathan et al. 2014; Liu et al. 2017). *Shewanella* sp., epiphytic bacteria associated with marine brown alga, *Bifurcaria bifurcata*, revealed to be excellent sources of natural antioxidant and antimicrobial compounds.

Besides bacteria, some fungi, few actinomycetes, yeasts and algae were also found to produce the compounds with antioxidant activity. Family Actinomycetaceae are the group of microorganisms as the source of secondary metabolites, pivotal compounds, for drug-based recovery due to biological activities of those compounds. *Streptomyces* spp. SRDP-H03 and BI244 exhibit antioxidant activity (Rakesh et al. 2013). The antioxidant used for flavours, 2-(hydroxy-2-metho xy-3,4-methylene dioxyphenyl)-benzofuran, recovered from baker's and brewer's yeast, was effective in protecting food quality of *Aspergillus oryzae* preventing oxidative rancidity. Antioxidant compounds such as pestacin and isopestacin were obtained from *Pestalotiopsis microspora* (Strobel et al. 2002). The antioxidant activity of

pestacin and isopestacin was attributed to the scavenging ability of both superoxide and hydroxyl free radicals.

17.4.3.3 Anticancer Activity

The most known examples of usage of bacteria and their metabolites for the cancer treatment are investigations made by William Coley (1891), who utilised *Streptococcus pyogenes* and *Serratia marcescens* supernatants in the treatment of patients with tumours. This mixture, called today as 'Coley's toxins', was used in approximately 1200 patients with malignancy (McCarthy 2006). Chemotherapeutic agents for cancer treatment are secondary metabolites of microbial origin and are produced by the genus *Streptomyces* (Manivasagana et al. 2014). Actinomycetes, *Streptomyces antibioticus*, reported as a source for actinomycin-D, one of the first natural metabolites used for treatment of tumour (Ginell et al. 1988). The anthracycline class of antitumour agents isolated from *Streptomyces peucetius* is the most clinically efficacious agents, whereas paclitaxel (Taxol) the most famous and fascinating compound in the history of secondary metabolites from endophytic fungi. Taxol is the world's first billion dollar anticancer drug from endophytic fungi *Taxomyces andreanae*, isolated from bark of yew tree *Taxus brevifolia* (Stierle et al. 1993). *Pestalotiopsis microspora* produce high amount of Taxol (Li et al. 1996). Other fungi like *Nodulisporium sylviforme* Zhao et al. (2011) and *Botryodiplodia theobromae* Venkatachalam et al. (2008) also produce Taxol. An alkaloid, 22-oxa-12 cytochalasins, which displayed antitumour activity, was isolated from *Rhinocladiella* sp., an endophyte on *Tripterygium wilfordii.*

The endophytic fungus *Mucor fragilis* from *Cercospora* sp. is able to produce antitumour compound, i.e., podophyllotoxin and kaempferol and guanacastane diterpenoids (Huang et al. 2014). *Fusarium griseum* is reported for production of fusidienol which acts as inhibitor of farnesyl transferase enzyme which is responsible for tumour (Singh et al. 1997). Some of the commercialised antitumour agents isolated from fungi are pentostatin, peplomycin and epirubicin. *Verticillium balanoides* produces balanol, which is a potent protein kinase-C inhibitor (Kulanthaivel et al. 1993). Remarkable improvement have also occured in the sepration based chromatographic and spectroscopic techniques over the last two decades facilitates the identification and characterization, of known microbial metabolites which has increased rapidly, making it necessary to get rapid fingerprinting of the metabolites present in an extract before isolating the compounds.

17.4.3.3.1 Immunosuppressive Activity

Although most of the antimicrobial drugs are safe and effective, many of them may lead to immunosuppression, causing immune dysfunction (Leekha et al. 2011). It is induced by immunosuppressant drugs and had a profound effect on lymphocytes function. Immunosuppressive agents prevent the activity of the immune system and employed in the transplantation of organs or tissues to prevent allograft rejection, in

the treatment of autoimmune disorders (Rainsford 2007) or diseases such as rheumatoid arthritis and insulin-dependent diabetes and also in the treatment of nonautoimmune inflammatory conditions (Li et al. 2017). Common drugs used for this purpose include cyclosporin A, tacrolimus and rapamycin.

Cyclosporine isolated from soil fungus *Tolypocladium inflatum* is being used as immunopharmacological active metabolites (Borel et al. 1976). Cyclosporine is widely used in organ and tissue transplantation surgery that selectively regulates T-cell proliferations without exhibiting excessive toxicity, whereas tacrolimus (FK-506) is a macrolide class of natural product isolated from *Streptomyces* and is used in allogeneic organ transplantation surgery. Rapamycin was first isolated from *Streptomyces hygroscopicus* used to prevent organ transplant rejection (Vezina et al. 1975; Borel et al. 1976).

Recently, endophytic microorganisms have also been used as an under explored resource for the discovery of new bioactive molecules with immunosuppressive property. The endophytic fungus *Fusarium subglutinans*, isolated from *T. wilfordii*, produces the immunosuppressive but non-cytotoxic diterpene pyrones subglutinol A and B (Lee et al. 1995). Compared to immunosuppressant drug cyclosporine, subglutinols A and B are found to be more potent in the thymocyte proliferation assay. Since subglutinols A and B do not show toxicity associated with them, they can be explored for wider application such as treatment of autoimmune diseases like rheumatoid arthritis and insulin-dependent diabetes in future.

17.4.3.4 Anti-Inflammatory Activity

The formation of inflammation mostly involves both innate and adaptive immune response. Nonsteroidal anti-inflammatory drugs (NSAIDs), steroidal drugs and immunosuppressant drugs, which have been usually used for the relief of inflammation worldwide, are often associated with severe side effects such as gastrointestinal bleeding and peptic ulcer (Steinmeyer 2000). Secondary metabolites such as penicillinolide isolated from organic extract of marine fungus *Penicillium* sp. SF-5292 (Lee et al. 2013), terpenoides from fruiting body of the fungus *Fomitopsis pinicola* (Dresch et al. 2015) and *Ganoderma colossum* and cyathane diterpenes from the fruiting body of the fungus *Sarcodon glocaupus* and *Sarcodon scabrosus* are reported as anti-inflammatory agents (Kamo et al. 2004).

17.4.3.5 Other Activities

Nodulisporic acids are novel indole diterpenes, exhibiting potential insecticidal properties against larvae of the blowfly (Bills et al. 2012). L-783,281, a nonpeptidal fungal metabolite, is used as an antidiabetic agent as an insulin mimetic (Qureshi et al. 2000). Chlorinated, epimeric 1,3-oxazinane derivatives isolated from the endophytic fungal strain *Geotrichum* sp. AL4 showed clear bioactivities against the nematodes *Bursaphelenchus xylophilus* and *Panagrellus redivivus* (Li et al. 2007).

17.5 Chemicals

17.5.1 Organic Solvents

17.5.1.1 Ethanol

Ethanol is a biofuel primary metabolite used as a chemical feedstock for many chemical industries (Singh et al. 2017). Microbes play a pivotal role in fermentation of sugars for bioethanol production. Some microorganisms have the ability to produce ethanol and CO_2 by utilising glucose under anaerobic conditions. Some microorganisms such as *Saccharomyces cerevisiae* (dried yeast), *Pichia kudriavzevii*, *S. diastaticus*, *Kluyveromyces marxianus*, *Escherichia coli* strain KO11 and *Zymomonas mobilis* are capable of producing ethanol from sugar juices (Zabed et al. 2014). *S. cerevisiae* is widely used in bioethanol fermentation due to its greater efficiency of converting sugars into ethanol, production of flocs during growth making it easier to settle/suspend and higher tolerance to ethanol.

Bioethanol production mainly involves three categories of substrates: sugar (sugarcane, sweet sorghum, sugar beet), starch (corn, potato, sweet potato, etc.) and cellulose (wood, grass, agriculture residue) using batch, fed-batch or continuous fermentation process. Batch fermentation is a type of closed system wherein feedstock, microorganisms, nutrients and other ingredients are added to the fermentation vessel, and when the process is complete, ethanol is recovered. In fed-batch mode, there is only intermittent or sometimes continuous feeding of one or more ingredients during the fermentation process, whereas continuous fermentation is an open system in which there is constant input and output of ingredients from the fermentation vessel.

Sugars are directly fermented into ethanol using yeast. Starchy materials are first liquefied with help of alpha amylase isolated from microorganism such as *Aspergillus niger* and *Bacillus subtilis* into oligosaccharide and dextrin, further saccharification is done where dextrin is converted into glucose with help of glucoamylase, and finally glucose is fermented to ethanol (de Souza 2010). In case when cellulosic substrate is used for bioethanol production prior to saccharification and fermentation step, substrates are pretreated by physical, chemical or biological means for removal of lignin and hemicellulose, increase porosity of material and reduce crystallinity of cellulose (Maurya et al. 2015).

17.5.1.2 Acetone and Butanol

The commonly used organism in the industrial acetone-butanol fermentation process is *Clostridium acetobutylicum*. *Clostridium acetobutylicum* is a spore-forming bacterium and can ferment a large number of carbohydrates such as glucose, lactose, fructose, maltose, sucrose, starch and lignocellulosic materials. Acetone and butanol are widely used as solvents and used in chemical industries.

17.5.1.3 Citric Acid

Citric acid is a common metabolite and the natural constituent to be widely used as organic acid in food and pharmaceutical industries. It derived its name from a tree

called *citrus* in Latin, producing lemon-like fruit. In 1917, Currie reported that *Aspergillus niger* is capable of accumulating significant amounts of citric acid in sugar and salt containing medium at an initial pH of 2.5–3.5. He also exhibited that under growth-limiting condition, high concentrations of sugar favoured the production of citric acid. A large amount of citric acid is produced throughout the growth phase of such strains, which established the basis for industrial production.

Various strains of genera fungi, yeast and bacteria were reported for production of citric acid such as *Mucor piriformis*, *Penicillium citrinum*, *P. janthinellum*, *Penicillium luteum*, *P. purpurogenum*, *P. restrictum*, *Paecilomyces divaricatum*, *Botrytis* sp., *Trichoderma viride*, *Saccharomycopsis lipolytica*, *Arthrobacter paraffineus*, *Corynebacterium* sp., *Trichoderma viride*, *Ustulina vulgaris* and others. Among these organisms, fungus *A. niger* is used commercially for its production due to various reasons such as it is able to ferment variety of cheap raw materials, it is easy to handle and it has high yield of citric acid production (Show et al. 2015). For selecting citric acid-producing microorganism, two methods are widely used: 'the single-spore technique' and the 'passage method'. A variety of starch-, sucrose- and hydrocarbon-based media in liquid fermentation are most widely used for citric acid production. The properties of citric acid such as safe, pleasant acid taste, high water solubility, chelating and buffering properties account for its extensive usage in food and pharmaceutical industries. In cosmetic industries and toiletries, citric acid is used as buffer and chelating agent.

17.5.2 Antibiotics

Antibiotics are product of secondary metabolism that can inhibit their growth process of other organism even when used at low concentration and are therefore used to fight infections in humans or animals. Variety of bacteria, fungi and actinomycetes are producing antibiotics on a large scale. Over 8000 antibiotics were isolated from bacterial cultures (both gram positive and negative) and of fungi (mostly filamentous) although only about 100 of these have been commercially used to treat human, animal and plant diseases. In addition, around 2500 antibiotic active substances have been reported in lichen, algae, higher animals and plants.

The *Streptomyces* are responsible for the production of more than 60% of the known antibiotics, while 15% of the rest are produced by the members of the related actinomycetes. *Micromonospora*, *Actinomadura*, *Actinoplanes*, *Nocardia*, *Streptosporangium*, *Streptoverticillium* and *Thermoactinomyces* are some of the well-known genera. Various medically useful peptide antibiotics are produced by members of the genus *Bacillus*. The classical lactam antibiotics, penicillin and cephalosporin, are usually produced by the filamentous fungi *Penicillium* and *Cephalosporium.* Table 17.7 summarises some antibiotics produced by microorganism.

Table 17.7 List of some antibiotics producing microorganism

S. No.	Name of antibiotics	Producer microorganisms	Activity	Mechanism of action
1.	Penicillin	*Penicillium chrysogenum*	Gram-positive bacteria	Disrupt cell wall synthesis
2.	Griseofulvin	*Penicillium griseofulvum*	Dermatophytic fungi	Microtubules
3.	Cephalosporin	*Cephalosporium acremonium*	Broad spectrum	Disrupt cell wall synthesis
4.	Bacitracin	*Bacillus subtilis*	Gram-positive bacteria	Disrupt cell wall synthesis
5.	Polymyxin B	*Bacillus polymyxa*	Gram-negative bacteria	Attack on cell membrane
6.	Amphotericin B	*Streptomyces nodosus*	Fungi	Disrupt cell membrane
7.	Erythromycin	*Streptomyces erythreus*	Gram-positive bacteria	Disrupt protein synthesis
8.	Neomycin	*Streptomyces fradiae*	Broad spectrum	Disrupt protein synthesis
9.	Streptomycin	*Streptomyces rimosus*	Gram-negative bacteria	Disrupt protein synthesis
10.	Tetracycline	*Streptomyces griseus*	Broad spectrum	Disrupt protein synthesis
11.	Vancomycin	*Streptomyces orientalis*	Gram-positive bacteria	Disrupt protein synthesis
12.	Rifamycin	*Streptomyces mediterranei*	Tuberculosis	Disrupt protein synthesis
13.	Gentamicin	*Micromonospora purpurea*	Broad spectrum	Disrupt protein synthesis

17.5.2.1 Penicillin Production

Penicillin from *Penicillium notatum* showed its efficacy in laboratory cultures against bacterial pathogens (Tan and Tatsumura 2015). The fungus *Penicillium chrysogenum* to grow well in the medium for penicillin production requires sugars mainly lactose and a nitrogen source (in this case, a yeast extract). Penicillin being a secondary metabolite is produced in stationary phase like many of other antibiotics. The industrial production of penicillin involves following steps: (1) preparation of inoculum; (2) preparations of medium and its sterilisation; (3) medium inoculation in the fermenter; (4) forced aeration with sterile air during incubation; (5) when fermentation is complete, removal of mould/mycelium; and (6) extraction and then purification of the penicillin.

Cultivation of *Penicillium chrysogenum* for penicillin production occurs in three phases. In the first phase, growth of mycelium occurs, lactic acid present in media corn steep liquor is utilised by the microorganism, and liberation of ammonia results in increase in pH. Production of antibiotic is low. During second phase, antibiotic production (penicillin) is maximum, owing to fast utilisation of ammonia and lactose. The mycelial mass increases, but pH remains unchanged. During third, the

last, phase, the antibiotic concentration decreases in the media. There is slight increase in pH due to liberation of ammonia and autolysis of mycelium starts. After the production/fermentation process is completed, mycelium of the fungus is removed by filtration from the broth and further processed by processes like adsorption, precipitation and crystallisation to yield final product. Solvent extraction at an acidic pH at temperature below 100 °C is usually preferred method for penicillin recovery from broth. Mycelium which is recovered after filtration can be used as soil conditioner after being treated and dried. To remove pigments and other impurities from the penicillin-rich solvent, it is treated with activated carbon. Penicillin is recovered as salt of the potassium and sodium by adding potassium or sodium acetate to the solvent.

17.5.3 Amino Acids

Amino acids are monomeric units of proteins that contain a high percentage of nitrogen (~16%). It can be categorised as essential amino acids which body cannot synthesise and has to be supplemented in diet and non-essential amino acid which body can synthesise. Amino acid production is gaining increasing demand in view of their importance/applications in industries like food as nutrient, in feed as additives, in personal care as rejuvenators and in pharmaceutical as drugs (Mahmood 2015). The increasing demand for some essential amino acid such as lysine and methionine and non-essential amino acid like glutamic acid in last two decades in feed, food and pharma industries has led to their global production worldwide (D'Este et al. 2018). The primary foodstuff of many underdeveloped and overpopulated countries across the globe has deficiency of these essential amino acids. There are basically three methods available for production of amino acids:

1. Extraction from protein hydrolysates
2. Chemical synthesis
3. Microbial processes involving fermentation and enzymatic synthesis

Since microbial processes have certain advantages over other methods, it is widely used method for industrial production of amino acids. Microorganisms have the ability to convert sugars present in the substrate to amino acid under aerobic and anaerobic conditions. Many amino acid-producing microorganisms are being developed by mutagenesis and screening programmes. Researchers are developing new amino acid overproducing strains via genetic recombination, RDT technology and use of auxotrophic mutant. Currently, *Corynebacterium glutamicum* and *E. coli* are the most common microorganism used for fermentative production of amino acids worldwide (Nakayama 1985; D'Este et al. 2018). Amino acids like lysine and glutamic acid have been successfully produced with the help of genetically modified *C. glutamicum*, while *Escherichia coli* has been modified for production of aromatic acids (Kinoshita 1985).

17.5.3.1 Corynebacterium Glutamicum

Corynebacterium glutamicum is an aerobic non-pathogenic gram-positive soil bacterium which is widely used in the amino acid production. L forms of several amino acids such as glutamate, lysine, phenylalanine, threonine, tryptophan, serine, proline, glutamine, arginine and isoleucine are being produced by *C. glutamicum* (Schneider et al. 2011). It can use sugars such as glucose (mostly preferred), sucrose, fructose, ribose, mannose and maltose as carbon source with pH of 7 and temperature of 30 °C for its optimal growth (Liebl 2005; Zahoor et al. 2012). Many inhibition studies have shown that substrate can be growth limiting for some amino acid production. Glycolysis, hexose monophosphate pathway and the Krebs cycle are the three main central metabolic pathways which are linked to the biosynthesis of the amino acids. Different enzymes are involved in the conversion of carbon between TCA cycle and glycolysis such as 6-phosphogluconate dehydrogenase and isocitrate dehydrogenase.

17.5.3.2 Escherichia Coli

E. coli is an aerobic gram-negative bacterium commonly used to produce several amino acids such as L-methionine, L-lysine and L-threonine and the aromatic amino acids (Leuchtenberger et al. 2005). Mutant strain of *E. coli* is able to produce L form of branched chain amino acids like valine, leucine and isoleucine (Park and Lee 2010). *E. coli* is able to ferment glucose, sucrose, mannose, xylose, arabinose, galactose and fructose as its carbon source. The optimum temperature required for growth is 37 °C and pH of 7 (Noor et al. 2013). Glycolysis, the hexose monophosphate pathway and the Krebs cycle are the central carbon metabolism pathways used by *E. coli* that is responsible for the breakdown of the carbon sources.

17.5.4 Applications of Amino Acid

17.5.4.1 Application in Food Industry

Amino acids are used either alone or in combination as flavour enhancers. Monosodium glutamate is the most frequently used in food industry. Glycine and alanine also enhance taste and flavour (Gunlu and Gunlu 2014). Tryptophan, in association with histidine, acts as an antioxidant to preserve milk powder. For the preservation of fruit juices, cysteine serves as an antioxidant. Aspartame, a dipeptide (aspartyl-phenylalanine methyl ester) produced by a combination of aspartic acid and phenylalanine, is about 200 times sweeter than sucrose. It is used as a low-calorie artificial sweetener in soft drink industry. There are certain essential amino acids that are deficient or limiting in plant proteins. These include lysine, methionine, threonine and tryptophan. Addition of the deficient amino acid(s) improves the nutritional quality of human foods as well as animal feeds. Thus, bread enriched with lysine and soy products supplemented with methionine are of better nutritional value. Methionine-added soybean meal is a better feed for pigs and other animals.

17.5.4.2 Application in Pharmaceutical Industry

The amino acids can be used as medicines. Essential amino acids are useful as ingredients of infusion fluids for administration to patients in post-operative treatment (Bozzetti and Bozzetti 2012).

17.5.4.3 Application in Chemical Industry

Amino acids serve as starting materials for producing several compounds. Glycine is used as a precursor for the synthesis of glyphosate (a herbicide) (Amrhein et al. 1980), while threonine is the starting material for the production of aztreonam (another herbicide). Poly-methyl glutamate is utilised for manufacturing synthetic leather. Some amino acids in the form of N-acyl derivatives are useful for the preparation of cosmetics.

17.5.4.4 Application in Vitamins-Related Industry

Vitamins are essential micronutrients required in trace quantities that cannot be synthesised by mammals. Vitamins are synthesised by plants and microorganism and are essential for the metabolism of all living organism. They have many nutritional and physiological roles in vivo such as they are required as growth factor for men, animal, plants and microorganism. They are now increasingly being used as additives in food/feed, as agents in medical therapeutics and also as health and technical aids. Vitamins are mainly categorised in two groups, water soluble and fat soluble, for which chemical synthesis and microbial/enzymatic conversion process are reported. Production of vitamins through chemical synthesis process is energy-intensive and cost-intensive; therefore, nowadays, microbial fermentation processes are currently being used in the production of vitamins (Ledesma-Amaro et al. 2013; Wang et al. 2016). Table 17.8 summarises various microorganism exhibiting production of vitamins and their function.

17.5.4.5 Application of Microbial Pigments

The natural pigments extracted from microorganism are termed as ‘microbial pigments’. Pigments produced from biological sources like microorganisms are natural, safe for health and environment friendly. Due to which, there is growing interest in production of such type of pigments. Different species of bacteria, yeast, mould and algae producing such pigments exhibit wide applications in food, cosmetics, textiles (dyes) and fish industry (such as enhancing the pink colour of farmed salmon) and can also be used as potent antioxidant agents (Tuli et al. 2015). Various pigments like quinones, carotenoids, melanins, flavins, astaxanthin, anthraquinone, prodigiosins, monascins, violacein, indigo, etc. are produced by microorganism. Some of the examples of microorganisms producing natural pigments and their colour and uses are given in Table 17.9.

17.5.4.6 Application of Microbial Flavour and Perfumes

Flavours mostly extracted from plant/animal sources or produced through chemical synthesis are putting stress in their uses due to health awareness among people. Flavours find wide range of applications in field of food, feed, beverages,

Table 17.8 A list of vitamins produced through microbial fermentation and their function is given below

S. No.	Name of vitamin	Microorganism	Method	Function of vitamin
1	Vitamin E	Freshwater microalgae *Euglena gracilis*, *Spirulina platensis*, *Dunaliella tertiolecta*, *Synechocystis*, *Chlorella*, *Chlamydomonas* and *Ochromonas*	Fermentative production from glucose	Antioxidant; protects cell walls
2	Vitamin K	*Flavobacterium* sp., *B. subtilis* and *Propionibacterium freudenreichii*	Fermentation using soybean extract	Needed for proper blood clotting
3	Vitamin B2 (riboflavin)	*Clostridium butylicum*, *Eremothecium gossypii*, *Ashbya gossypii*	Fermentative production from glucose	Part of an enzyme needed for metabolism of energy; important for healthy skin and vision
4	Vitamin B12 (cobalamin)	*Pseudomonas denitrificans*, *Propionibacterium shermanii*, *Propionibacterium* or *Salmonella typhimurium*	Fermentative production from glucose	Part of an enzyme used for generation of new cells; important for proper functioning of nerve
5	Vitamin B7 (biotin)	*Serratia marcescens*	Fermentative production from glucose by using genetically engineered microbe.	Part of enzyme needed for metabolism of energy
		Multiple enzyme system (*Bacillus sphaericus*)	Using the biotin biosynthetic enzyme system of mutant (*Bacillus sphaericus)* while conversion from diaminopimelic acid	
6	Vitamin C (2-keto-L-gulonic acid)	2,5-Diketo-D-gulonic acid reductase (*Corynebacterium* sp.)	2,5-diketo-D-gluconate obtained through fermentative process is enzymatically converted to 2-keto-L-gulonic and then chemically to L-ascorbic acid	Part of an enzyme used for metabolism of protein; helps in absorption of iron; acts as antioxidant; important for healthy immune system

Table 17.9 Some natural pigment and colour-producing microorganism

S. No.	Microorganism	Pigment	Colour	Uses
1.	*Blakeslea trispora*, *Mucor circinelloides*, *Phycomyces blakesleeanus*, *Dunaliella Salina*	β-Carotene	Yellowish	Antioxidant, potential positive properties against certain diseases
2.	*Penicillium oxalicum*	Arpink red	Red	As colourant in various food product
3.	*Ashbya gossypii*	Riboflavin	Yellow	As colourant in various food product
4.	*Fusarium sporotrichioide*	Lycopene	Red	Antioxidant activity
5.	*Fusarium graminearum*	Anthocyanin	Red, purple, blue	Food colourant and additives
6.	*Chromobacterium violaceum*	Violacein	Violet	In textile industries as dye agent for pure silk, cotton, rayon and other fabrics.
				In pharmaceutical as antitumoural, antiparasitary, antiprotozoan, anticancer, antiviral, antibacterial and antioxidant activities
7.	*Vibrio psychroerythrus*, *Serratia marcescens*, *Pseudomonas magnesiorubra*	Prodigiosin	Red, yellowish orange	Cytotoxic activity, as dye agent for wool, nylon, acrylics and silk fibre
8.	*Phaffia rhodozyma*, *Haematococcus pluvialis*	Astaxanthin	Red	Fish feed
9.	*Monascus* sp.	Monascorubramin, rubropunctatin	Yellow, orange, red	Flavour agent in food products
10.	*Dermocybe sanguinea*, *Aspergillus oryzae*	Anthraquinone	Pink, red or violet	Dye agent for wool fibres
11.	*Haematococcus*, *Chlorella*, *Chlamydomonas*	Canthaxanthin	Reddish-orange colour	Poultry feed and fish feed

detergents, cosmetics and pharmaceutical formulations. Microbes are well known for production of aromas and fragrances (Bomgardner 2012). Man is using various groups of microbes to impart new fragrances and aromas to products like beer, wine, cheese, etc. from ages which are produced through fermentation (Gupta et al. 2015). Many volatile and non-volatile components are responsible for imparting the characteristic flavour to any compound with diverse physicochemical attributes.

Table 17.10 Some microorganism and enzymes producing flavours and fragrances

S. No.	Flavour type	Bioactive chemical component	Microorganism or enzyme involved in production
1	Vanilla	Vanillin	*Pycnoporus cinnabarinus*
2	Butter flavour	Diacetyl	*Lactococcus lactis*, Lactobacillus sp., *Streptococcus thermophilus*, *Leuconostoc mesenteroides*
3	Flavour components in dairy products	Lactones	*Trichoderma viride*, *Candida tropicalis*, *Tyromyces sambuceus*, *Cladosporium suaveolens*, *Yarrowia lipolytica*
4	Fruity aromas	Esters	*Hanseniaspora guilliermondii*, *Pichia anomala*, *Lactococcus lactis*
5	Nutty and roasted flavour	Pyrazines	*Corynebacterium glutamicum*
6	Essential oils	Terpenes	*Ceratocystis moniliformis*
7	Aroma-related alcohols, rose smell	2-Phenylethanol	*Hansenula anomala*, *Kluyveromyces marxianus*, *Saccharomyces cerevisiae*
8	Cherry and other natural fruit flavour	Benzaldehyde	*Bjerkandera adusta*, *Phanerochaete chrysosporium*, *Pseudomonas putida*, *Polyporus tuberaster*, *Trametes suaveolens*
9	Blue cheese and fruit flavours	Methyl ketone	*Aspergillus niger*, *Penicillium roqueforti*, *Penicillium glaucum*, *Agaricus bisporus*
10	Mint	(−)-menthol	Lipase (*Candida rugosa*)
11	Citrus-type fragrance	Isopulegol	Lipase (*Pseudomonas* sp.)
12	Apple and pineapple	Butyric acid	*Clostridium butyricum*

These flavoured compounds can be naturally produced during fermentation of microbial cultures or their enzyme preparations. Also, microorganism or enzymes derived from them can transform natural precursor into valuable single-flavour molecule called impact substances or top notes or useful flavouring mixture, known as flavour building block. Some of the microorganism or enzymes producing flavour and fragrances are provided in Table 17.10.

17.6 Conclusions

In the view of ever-increasing demand of commercially important compounds which were earlier synthesised by chemical methods, microbes have revolutionised the production methods through the fermentative route. The advancement in fermentative and biotechnological production processes of the microbial products is not only eco-friendly and cost-effective but is also capable of meeting the growing global demands of such products. The development of microbial-based processes gives emphasis mainly to reduce the harmful effects of chemical/synthetic processes to environment and ultimately to society. The global development in microbial

technology since more than five decades has resulted in patented production of many microbial products and enzymes showing their widespread applications in industries such as food, feed, pharmaceutical, paper, pulp, textile, detergents, personal care products and many more. Microbial production of many fine and commodity chemical and also enzymes clearly indicates the shift in the paradigm. As we all are aware that microbes are present in all environment on the earth, and only a fraction of such microorganism have been utilised for the production of industrially important products, a huge diversity of microbes is still to be explored for the production of new products and processes.

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18 Microbial Products and Biotechnological Applications Thereof: Proteins, Enzymes, Secondary Metabolites, and Valuable Chemicals

Fatemeh Dabbagh, Zahra Moradpour, and Abdollah Ghasemian

Abstract

Microbial species are among prominent producers of useful natural products, which are a very diverse collection of molecules. These natural products or better defined as specialized metabolites occur in various structural and functional classes and have been used by humans historically for different purposes: pharmaceuticals, chemical industry, agriculture, food and feed sector, etc. To the best of our knowledge, only a small fraction of microbial products is exploited and yet remains a larger chest to be reached. The most advantageous microbial products not only are restricted to useful proteins and enzymes, antibiotics, antitumor agents, immunosuppressants but also include antivirals, anthelmintics, nutraceuticals, polymers, enzyme inhibitors, surfactants, bioherbicides, biopesticides, and many more agricultural and industrial products.

In this regard, the objective of this chapter is to focus attention on the world of microbial natural products and their application from a biotechnological point of view. Microbial sources, biological activities, structures, biodiscovery, and, to some extent, biosynthesis and genetic engineering of natural products obtained from microorganisms are reviewed.

Keywords

Natural products · Microbial diversity · Proteins · Enzymes · Metabolites · Bioactivity

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18.1 Introduction

Microbial species are among prominent producers of useful natural products. Soon, it was realized by researches that microbes are crucially integrated in human, animal, and plant health by producing a variety of chemicals, in addition to promoting processes such as fermentation and transformation and decomposition of organic substances. For more than 70 years, microbes have provided us with valuable compounds to treat or even alleviate disorders and improve human life quality. Many therapeutics, ranging from antibiotics to antitumor agents, are themselves microbial products or alternatively targets for further drug discovery. To this end, an assessment of FDA approvals granted to new molecular entities (NMEs) reveals that natural products and their derivatives constitute over one-third of all NMEs, among which, one-quarter are from the microbial origin (Patridge et al. 2016). In addition to the therapeutic importance of microbial natural products, they have numerous usage in the food, feed, agriculture, chemical, pharmaceutical, and biofuel sectors (Du et al. 2011). The aforementioned microbial metabolic products are classified as the primary and secondary metabolites. A wide range of small molecules consisting of vitamins, amino acids, alcohols, nucleosides, and organic acids, which are mediators of processes such as microbe–microbe signaling, immune activation and inflammation, host–microbe crosstalk, and microbial metabolism, are microbial products of primary metabolism (Ióca et al. 2014). These primary metabolites are made during the exponential phase of growth and are intrinsically essential for growth. On the other hand, secondary metabolites, which are comprehensively discussed in the following sections, are compounds produced usually late in the growth cycle of the cell and come in various structural and functional classes.

The objective of the present chapter is to describe the world of microbial natural products and their applications from a biotechnological point of view. Microbial sources, biological activities, structures, biodiscovery, and, to some extent, biosynthesis and genetic engineering of natural products obtained from microorganisms are reviewed.

18.2 Secondary Metabolites

Secondary metabolites, which are also referred to as “natural products” from either plants or microorganisms, are fertile sources of drug discovery and chemical biology tools (Kato et al. 2012). Secondary metabolites or natural products are organic compounds of relatively small molecular weight (<3000 Da) with considerable structural diversity that are not essential for primary (housekeeping) metabolism or growth of the organism under laboratory conditions and, with this regard, take the name secondary metabolites. Davies (2013) employs the wise phrase of “specialized metabolites” as an alternative to “secondary metabolites” which is believed to be a more adequate term. Based on an estimation, up to 15% of some microorganisms’ genome content is assigned to the production of secondary metabolites.

In addition to prime evolutionary fitness to the producing organism, secondary metabolites are of major noteworthiness to humankind owing to their beneficial effects as bioactive and pharmaceutical agents (Wiemann and Keller 2014). In terms of their origin and their application, natural products are constituents of many pharmacological categories. For intance, they can be categorized into various pharmacological groups including, but not limited to, analgesic, anti-Alzheimer's, anti-Parkinsonism, antiallergic, antiarrhythmic, antiarthritic, antiasthmatic, antibacterial, anticancer, anticoagulant, antidiabetic, antifungal, antiglaucoma, antihyperprolactinemia, antihypertensive, anti-inflammatory, antiobesity, antiparasitic, antipsoriatic, antithrombotic, antiulcer, antiviral, benign prostatic hypertrophy, bronchodilator, calcium metabolism, cardiotonic, contraception, hematopoiesis, hemophilia, hormone replacement therapy, hypocholesterolemic, hypolipidemic, immunomodulator, immunostimulant, immunosuppressant, muscle relaxant, nootropic, vasodilator, and vulnerary (Ghasemian and Moradpour 2017; Newman and Cragg 2007). In addition, secondary metabolites possess leading ecological roles in microorganisms, in terms of the nutrient acquisition, chemical communication, and defense (Giordano et al. 2015).

18.2.1 Screening and Discovering Secondary Metabolites

Among the approaches currently employed to discover natural molecules, high-throughput screening approaches including high-throughput DNA sequencing and novel genomic-type techniques (Charlop-Powers et al. 2014; Kang and Brady 2013; Owen et al. 2015; Rutledge and Challis 2015), bioinformatics, cheminformatics, and structure-determination strategies have boosted the discovery of microbial natural products (Pereira et al. 2014; Scanlon et al. 2014).

18.2.2 Alkaloids

As amino acid-derived nitrogen-containing compounds of low molecular weight, a variety of organisms such as bacteria, fungi, plants, and animals produce a wide array of alkaloids. These are classified into various structural groups according to the amino acid of origin in their biosynthesis: tropane-, pyrrolidine-, and pyrrolizidine-alkaloids (derived from ornithine), benzylisoquinoline (derived from tyrosine), quinolizidine- and piperidine-alkaloids (derived from lysine), and indole-alkaloids (derived from tryptophan). Alkaloids are important natural products with significant therapeutic values meant for the treatment of cancer and neurodegenerative diseases. Benzylisoquinoline-type alkaloids derived from tyrosine, found a unique class of pharmaceutical molecules, compromising, for instance, the narcotic analgesic morphine and antibacterial agents berberine, magnoflorine, palmatine, and scoulerine. Indolocarbazole alkaloids are another main class of alkaloids, containing staurosporine (product of both *Streptomyces staurosporeus* and *Streptomyces actuosis*) and rebeccamycin as members, which inhibit protein kinases and mammalian DNA topoisomerase I, respectively (Song et al. 2014).

18.2.3 Terpenes (Terpenoids)

Also called isoprenoids, terpenoids (sesquiterpenes, diterpenoids, and triterpenoids) are derivatives of five-carbon atom isoprene units. They are known as the largest and most diverse group of natural products and are synthesized from the condensation of two C5 units as starting blocks, accordingly, isopentenyl-pyrophosphate (IPP) and its isomer dimethylallyl-pyrophosphate (DMAPP). Condensation of the two aforementioned C5 units and larger IPP- and DMAPP-derived starting building blocks such as the C10 unit, geranyl pyrophosphate (GPP), the C15 unit, farnesyl pyrophosphate (FPP) and the C20 unit, geranylgeranyl pyrophosphate (GGPP), results in the formation of monoterpenes, sesquiterpenes (for instance, artemisinin), diterpenes such as taxol, triterpenes (steroids), and tetraterpenes (carotenoids) (Song et al. 2014). In addition to having role in respiration, electron transport, photosynthesis, and hormone signaling, terpenoids and derivatives thereof may serve as antiparasitic agents. Refer to Table 18.1 for a summary of microbial terpenoids, application thereof, and their chemical structures (Bhosale and Bernstein 2005; Ghimire et al. 2016; Mousa and Raizada 2013).

Table 18.1 Examples of microbial terpenoids

Compound	Application(s)	Producing microorganism(s)
Sesquiterpenes		
Trichodermin	Used as template for chemical synthesis of pharmaceutical compounds and plant growth regulators	*Trichoderma harzianum*
Phomenone	Antifungal against plant pathogens	*Xylaria* sp.
Triterpenes		
Squalene	Potential pharmaceutical application, antioxidant	Various microorganism, such as *Aurantiochytrium* sp., *Kluyveromyces lactis*
Tetraterpenes (carotenoids and derivatives)		
Astaxanthin	Antioxidant used in nutricosmetics, feed additive	*Xanthophyllomyces dendrorhous, Haematococcus pluvialis*
β-Carotene	Food colorant, feed additive, nutraceutical	*Blakeslea trispora, Dunaliella salina, Streptomyces chrestomcyeticus* subsp. *Rubescens, Rhodotorula glutinis*
β-Cryptoxanthin	Antioxidant, vitamin A precursor	*Brevibacterium linens, Flavobacterium lutescens*
Fucoxanthin	Antioxidant, nutraceutical	*Undaria pinnatifida, Sargassum fusiforme, Laminaria japonica*
Canthaxanthin	Food colorant, feed additive	*Micrococcus roseus, Gordonia jacobaea, Brevibacterium* sp.
Lutein	Nutraceutical, nutritional supplement	*Chlorella zofingiensis*, *Chlorella protothecoides*, *Muriellopsis* sp., *Scenedesmus almeriensis*
Zeaxanthin	Nutraceutical	*Dunaliella salina*, *Phormidium laminosum*, *Flavobacterium multivorum*, *Microcystis aeruginosa*

18.2.4 Polyketides and Nonribosomal Peptides

Polyketides (PKs) are produced by a multi-enzyme assembly line referred to as polyketide synthase (PKS). PKSs are grouped as three different classes: type I is assigned to large and multifunctional enzymes, type II is assigned to dissociable complexes formed from monofunctional enzymes present in bacteria, and type III is assigned to homodimeric enzymes of relatively small size found in plants, bacteria, and fungi (Abe and Morita 2010; Funa et al. 1999; Seshime et al. 2005; Shen 2003). The structural and functional versatility present in the polyketide family is ensued from the combinatorial usage of few plain building blocks (namely, acyl-CoA thioesters including acetyl-CoA, malonyl-CoA, and methymalonyl-CoA) during chain elongation. So, many polyketides and nonribosomal peptides and combinations thereof represent clinically significant biological activities including anticancer (such as calicheamicin and bleomycin), immunosuppressant (such as rapamycin), and antibacterial (such as erythromycin and vancomycin) activities. Accordingly, the detailed description of each compound is discussed in its relevant following sections.

18.2.5 Flavonoids and Stilbenoids

Flavonoids and stilbenoids are important groups of plant-specific secondary metabolites with significant antioxidant and radical scavenging bioactivities. Being true for many natural products, the isolation of flavonoids and stilbenoids from plants is limited owing to low productivity from the natural plant sources and complexity of the recovered mixtures. In addition, total synthesis of these compounds is too costly and inefficient. Accordingly, the semi-synthesis of these plant-derived natural products and their heterologous production in microbial species are currently applicable (refer to the following sections for further details) (Kumar and Pandey 2013).

18.2.6 Antibiotics and Other Bioactive Substances

Natural products are invariably best recognized for their crucial importance in the identification and advancement of antimicrobial agents or "antibiotic-ome" (determined as natural products possessing antibiotic activity). A well-known background (the exploration of penicillin from the fungal genus *Penicillium* in the 1940s and, subsequently, the discovery of numerous other antibiotics from microbes) indicates that most of antibiotics currently available on the market are natural products or their derivatives isolated from microorganisms (Peláez 2006). Selman Waksman was the only one who systematically explored the microbial sources for novel natural products for the first time in 1943, along with isolation of streptomycin from the Gram-positive soil-dwelling actinomycete *Streptomyces griseus* (Milshteyn et al. 2014; Sakula 1988). From 1945 up to now, hundreds of thousands of secondary metabolites have been isolated and explored for the ability to treat bacterial, fungal,

parasitic, and viral infections (Davies 2013). Of all antibiotics (including β-lactams, aminoglycosides, macrolides, glycopeptides, etc.), more than a half are semisynthetic derivatives produced by actinomycetes and 10–15% by nonfilamentous bacteria (Demain 2014). In fact, it is assumed that antibiotics and natural products are closely related terms. Although the expression antibiotic no longer refers merely to natural products, a huge range of recently developed and marketed antibiotics are based on natural chemotypes (Peláez 2006). Table 18.2 summarizes different

Table 18.2 Examples of marketed antibiotics originated from microbial natural products

Original metabolite or antibiotic category	Commercial preparation(s)	Producing microorganism(s)
Penicillins (β-Lactams)	Penicillin G	*Penicillium* spp.
	Penicillin V	*Aspergillus* spp.
	Ampicillin	
	Amoxicillin	
	Methicillin	
Cephalosporins (β-Lactams)	Cefoxitin	*Acremonium* spp.
	Cefaclor	*Emericellopsis* spp.
	Cefotaxime	*Amycolatopsis lactamdurans*
	Ceftriaxone	*Streptomyces clavuligerus*
	Cefuroxime	
Carbapenem (β-Lactams)	Imipenem, meropenem, doripenem, ertapenem	*Streptomyces clavuligerus, Streptomyces* spp. (currently produced by chemical synthesis)
Monobactam (β-Lactams)	Aztreonam	*Chromobacterium violaceum* (produced completely via chemical synthesis)
Aminoglycosides	Streptomycin	*Streptomyces griseus*
	Neomycin	*Streptomyces fradiae*
	Kanamycin	*Streptomyces kanamyceticus*
	Gentamicin	*Micromonospora purpurea*
	Tobramycin	*Streptomyces tenebrarius*
Chloramphenicols	Chloramphenicol	*Streptomyces venezuelae*
Macrolides	Erythromycin	*Saccharopolyspora erythraea*
	Azithromycin	
	Clarithromycin	
Glycopeptides	Vancomycin	*Streptomyces orientalis*
	Teicoplanin	
Fosfomycin	Fosfomycin	*Streptomyces fradiae*
Mupirocin	Mupirocin	*Pseudomonas fluorescens*
Streptogramins	Streptogramin B	*Streptomyces roseosporus*
Cyclopeptides	Polymyxin B	*Bacillus polymyxa*
Tetracyclines	Tetracycline	*Streptomyces aureofaciens*
	Chlortetracycline	
Ansamycins	Rifampicin, rifamycin	*Streptomyces mediterranei*
Lincosamide	Lincomycin	*Streptomyces lincolnensis*
Lipopeptide	Daptomycin	*Streptomyces roseosporus*

generations of antibiotics and their origin as a natural microbial product (Begg and Barclay 1995; Papp-Wallace et al. 2011; Spízek and Rezanka 2004).

18.2.7 Cytotoxic and Immunosuppressive Compounds

Microbial natural products and derivatives thereof have historically been a rich source of cytotoxic and antitumor pharmaceuticals. Table 18.3 summarizes some small-molecule antitumor drugs of microbial origin, either direct microbial product or derived from microbial secondary metabolites. In the table, different sources of N and ND, respectively, refer to natural product (N) and derived from a natural product, usually a semisynthetic modification (ND) (Giddings and Newman 2013).

18.2.7.1 Actinomycins, Anthracyclines, and Bleomycins

Actinomycin C, obtained from various species of soil *Streptomyces*, was the first antibiotic with *in vitro* antitumor activity (Waksman and Woodruff 1940). Thereupon, actinomycin D was able to receive the FDA approval for the treatment of highly malignant tumors. Actinomycin D, as a DNA-intercalating agent, competes for transcription factor DNA-binding sequences and thus inhibits RNA and protein synthesis (Gniazdowski et al. 2003). Subsequently, a wide variety of antibiotics, namely, bleomycin, mitomycins, mithramycins, and anthracyclines, were isolated from microbial sources and investigated for antitumor activity in addition to being evaluated for clinical use. Daunorubicin and doxorubicin (also known as adriamycin) are two most profitable anthracyclines which are isolated from *Streptomyces peucetius* and other related strains. Both daunorubicin and doxorubicin are FDA approved for

Table 18.3 Antitumor drugs of microbial source

Generic name	Source[a]	Generic name	Source[a]
Aclarubicin	N	Leucovorin	N
Actinomycin D	N	Mifamurtide	ND
Amrubicin hydrochloride	ND	Mitomycin C	N
Asparaginase	N	Mithramycin	N
Bleomycin	N	Neocarzinostatin	N
Carfilzomib	ND	Pentostatin	N
Carzinophilin	N	Peplomycin	N
Chromomycin A3	N	Pirarubicin	ND
Cytarabine ocfosfate	ND	Romidepsin	N
Daunomycin	N	Sarkomycin	N
Doxorubicin	N	Streptozocin	N
Epirubicin hydrochloride	ND	Temsirolimus	ND
Gemtuzumab ozogamicin	ND	Trabectedin	N
Idarubicin hydrochloride	ND	Valrubicin	ND
Ixabepilone	ND		

[a]N, natural product; ND, derived from a natural product, usually a semisynthetic modification

cancer chemotherapy. Epirubicin, pirarubicin, idarubicin, valrubicin, amrubicin, aclarubicin, sabarubicin, annamycin (a liposomal variant of doxorubicin), berubicin, and a combination of anthracycline and anthracene dione structural classes, mitoxantrone hydrochloride, and pixantrone dimaleate are all anthracycline analogs that have been structurally modified or synthesized through semisynthesis or total synthesis (Giddings and Newman 2013).

18.2.7.2 Bleomycins

Bleomycins are another group of extremely important glycopeptide antibiotics mainly isolated from Actinomycetales. Bleomycins have a shared core structure but are different based on the presence of diverse positively charged functional groups and disaccharides. The aforementioned molecules were originally isolated and developed as antitumor agents from *Streptomyces verticillus*. As the mechanism of action, bleomycins require a metal ion (Cu^{2+} or Fe^{2+}) in order to activate the sequence-specific oxidative cleavage of the DNA and RNA (Hecht 1986; Hecht 1994; Stubbe and Kozarich 1987).

18.2.7.3 Enediynes

The enediynes are a structurally unprecedented class of antitumor antibiotics, encompassing important and useful microbial compounds, calicheamicin γ1I. In 1987, it was for the first time that the isolation of ten-membered calicheamicins from *Micromonospora echinospora* spp. *calichensis* was reported. Calicheamicin γ1I along with its close relative, dynemicin A, became the progenitor of a new chemical class of natural products, the enediynes. Currently, this class covers 13 enediynes with its core being composed of two acetylenic groups conjugated by a double bond within either a nine- or ten-membered ring. As the mechanism of action, enediynes undergo a unique rearrangement upon activation and subsequently interact with DNA which results in cleaved double-stranded DNA and following cell death. The enediyne natural product being exploited to date is summarized in Table 18.4 (Van Lanen and Shen 2008).

18.2.7.4 Epothilones

The soil-dwelling Gram-negative myxobacterium *Sorangium cellulosum* is the producer of 16-membered macrolides epothilones A and B. Epothilones are tubulin stabilizers that enhance the polymerization of microtubules (Forli 2014).

18.2.7.5 Geldanamycin Derivatives and HSP90 Inhibitors

Geldanamycin is a benzoquinone ansamycin antibiotic produced by *Streptomyces hygroscopicus* var. *geldanus* with antitumor properties. Initially, the mechanism of action of geldanamycin was thought to be the inhibition of the tyrosine-specific kinase (v-Src) associated with growth regulation and cell proliferation. However, it was subsequently unraveled that this compound binds to heat shock protein (HSP) 90 and acts as an HSP 90 inhibitor (DeBoer et al. 1970; Uehara et al. 1986; Uehara et al. 1988).

Table 18.4 Examples of enediyne natural products and their sources

Compound	Producing microorganism
Nine-membered category	
Auromomycin	*Streptomyces macromomyceticus*
Largomycin	*Streptomyces pluricolorescens*
Actinoxanthin	*Actinomyces globisporus*
Sporamycin	*Streptosporangium pseudovulgare*
Neocarzinostatin	*Streptomyces carzinostaticus*
C-1027	*Streptomyces globisporus*
Maduropeptin	*Actinomadura madurea*
Kedarcidin	*Actinomycete L585-6*
N1999A2	*Streptomyces* sp. AJ9493
Sporolides A and B	*Salinispora tropica*
Cyanosporasides A and B	*Salinispora pacifica*
Ten-membered category	
Esperamicin	*Actinomadura verrucosospora*
Calicheamicin	*Micromonospora echinospora* sp. *calichensis*
Dynemicin	*Micromonospora chersina*
Namenamicin	*Polysyncraton lithostrotum*
Shishijimicin	*Didemnum proliferum*
Uncialamycin	*Streptomyces cyanogenus*

18.2.7.6 Histone Deacetylase Inhibitors

Romidepsin (FK228) is the solely approved histone deacetylase inhibitor, produced as a fermentation product from the Gram-negative bacterium *Chromobacterium violaceum*. Romidepsin is a bicyclic depsipeptide possessing an unusual disulfide bond connection between a thiol and D-cysteine (Bertino and Otterson 2011). Santacruzamate A, derived from the marine cyanobacterium *Symploca* sp.; trichostatin A (TSA) isolated from the actinomycete *Streptomyces hygroscopicus*; apicidin isolated from the endophytic fungus *Fusarium pallidoroseum*; chlamydocin isolated from the fungus *Diheterospora chlamydosporia*; FR235222 isolated from the fermentation broth of *Acremonium* sp.; largazole isolated from the cyanobacterium *Symploca* sp.; and spiruchostatins isolated from *Pseudomonas* sp. and *Burkholderia thailandensis* are all examples of microbial natural products possessing histone deacetylase inhibitory action (Tan and Liu 2015).

18.2.7.7 Cyclosporins and Other Microbial Immunosuppressants

Cyclosporin A, as a principal immunosuppressive drug, is among the several tightly related cyclic undecapeptides which are produced by filamentous fungi. These closely related cyclic undecapeptides are secondary metabolites produced by *Cylindrocarpum lucidum* and *Tolypocladium inflatum*. The advent of cyclosporin A made a great advance in the immunotherapy of bone marrow and solid organ transplantations (Survase et al. 2011). There are reports of cyclosporin A production by

other microorganisms including *Fusarium solon* (Sawai et al. 1981), *Neocosmospora varinfecta* (Nakajima et al. 1988), and *Aspergillus terreus* (Sallam et al. 2003).

Rapamycin, which was originally isolated from *Streptomyces hygroscopicus* in 1975, is a 31-membered macrocyclic antibiotic. At this moment, although the initial antitumor activity of rapamycin is not further developed, discrete molecules with different pharmacological activities are produced based upon the rapamycin's core structure. Modifications made on the rapamycin led to the development of four clinically approved drugs as immunosuppressive and/or chemotherapeutic agents, namely, sirolimus (rapamycin), everolimus, temsirolimus, and zotarolimus (Law 2005; Li et al. 2014a).

Other significant compounds with potent immunosuppressive activity are a series of macrolides, to be specific, polyketide-nonribosomal peptide hybrid (refer to the Sect. 18.2.4) (for instance, FK506, also known as tacrolimus or fujimycin) produced by many *Streptomyces* species, such as *Streptomyces tsukubaensis* and *Streptomyces hygroscopicus* subsp. *yakushimaensis* (Kino et al. 1987). Table 18.5 summarizes the mechanism of action, target, and source organisms of some natural products with immunosuppressive activity.

18.2.8 Antivirals

Almost all of the available approved antivirals are chemical synthesis products. However, natural products are of immense significance in gaining insights for the synthesis of antiviral compounds (Takizawa and Yamasaki 2017). Nucleoside

Table 18.5 Microbial immunosuppressing agents

Compound(s)	Mechanism of action and target	Source microorganism(s)
Cyclosporin	Binds with high affinity to cyclophilins, and this complex specifically and competitively binds to and inhibits calcineurin, a calcium- and calmodulin-dependent phosphatase	*Cylindrocarpum lucidum, Tolypocladium inflatum, Fusarium solon, Neocosmospora varinfecta, Aspergillus terreus*
Gliotoxin	An inhibitor of NF-κB activation	*Aspergillus fumigatus*
FK-506 (tacrolimus or fujimycin)	Acts by inhibiting T cell activation and binds to a cytosolic protein (although not cyclophilin but has peptidyl-prolyl isomerase activity)	*Streptomyces tsukubaensis*
Immunomycin (ascomicin)	Inhibition of calcineurin	*Streptomyces hygroscopicus* var. *ascomyceticus*
Rapamycin (sirolimus)	Binds to the FK-binding protein and presumably modulates the activity of the mTOR. The mTOR inhibits interleukin (IL)-2-mediated signal transduction, resulting in cell cycle arrest in the G1-S phase	*Streptomyces hygroscopicus*
Microcolin	To be elucidated	*Microcoleus* sp.

Table 18.6 Microbial natural products with antiviral activity

Compound(s)	Mechanism of action and target	Source microorganism
Leupeptin	An inhibitor of serine and cysteine proteases, prevention of glycoprotein-mediated entry of Marburg virus	*Streptomyces roseus*
Antipain and elastatinal	Inhibitors of serine and cysteine proteases, inhibition of poliovirus 2A protease	*Actinomycetes*
Pepstatin	Aspartic proteinase inhibitor, contribution to the development of a key class of anti-HIV drugs (proteinase inhibitors) in highly active antiretroviral therapy	*Streptomyces* spp.
Siastatin B	Sialidase inhibitor	*Streptomyces verticillus* var. *quantum*
Stachyflin	Anti-influenza virus, inhibits conformational changes of hemagglutinin	*Stachybotrys* sp.
Statins	Hydroxymethylglutaryl coenzyme A reductase inhibitors, with antiviral effects for HBV, HIV, influenza virus, dengue virus, human cytomegalovirus and HCV	*Penicillium citrinum*
Myriocin	Serine palmitoyltransferase inhibitors, active against HCV, HBV, and influenza virus	*Myriococcum albomyces*

analogs from actinobacteria, including formycin, coformycin, and oxanosine, exhibit antiviral activity (Shimada et al. 1981; Takeuchi et al. 1996). Benanomicins A and B, kijimicin, and bellenamine and homologs thereof are isolated microbial products exerting anti-HIV activity (Kondo et al. 1996; Nakamura et al. 1991). A summarized overview of some natural products exhibiting antiviral activity and their mechanism of action and producing microorganisms is presented in Table 18.6 (Aoyagi et al. 1969; Gnirss et al. 2012; Martínez-Gutierrez et al. 2011; Minagawa et al. 2002; Molla et al. 1993; Nishimura et al. 1993; Sadanari et al. 2013; Suda et al. 1972; Umezawa et al. 1974; Umezawa et al. 1970; Umezawa et al. 1973).

18.2.9 Anthelmintics

Avermectins (the most important of them, ivermectin), produced by *Streptomyces avermitilis*, are a group of macrolide compounds discovered as anthelmintics. Possessing potent activity against arthropods and helminths, avermectins act by means of the GABA (γ-aminobutyric acid) receptor system, blocking neuromuscular transmission and therefore paralyzing the susceptible organisms and leading to death (Hotson 1982). In addition, paraherquamide, synthesized by both *Penicillium paraherquie* and *Penicillium charlesii*, and its dehydro-derivative exerts anthelmintic activity (Lee et al. 2002).

18.2.10 Enzyme Inhibitors

Many pharmaceutical/active biological agents are specific enzyme inhibitors. Enzyme inhibitors are precious means for the study of enzyme structures and elucidation of their mechanisms and with many applications in medicine, agriculture, and biotechnology. The outlined description of these compounds is summarized in Table 18.7 (Endo et al. 1983; Gani and Engh 2010; Hasumi et al. 1987; Ishimaru et al. 1988; Kido et al. 1983; Manivasagan et al. 2015; Matsuura et al. 1993; Miyazaki et al. 1980; Nishida et al. 1991; Omura et al. 1986; Umezawa et al. 1985; Vesselinova et al. 1991).

Phthoxazolin, a metabolite of *Streptomyces* sp., specifically inhibits cellulose synthetase, the key enzyme in cellulose biosynthesis in bacteria, fungi, algae, and plants (Omura et al. 1990). Bestatin also known as Ubenimex is produced by *Streptomyces olivoreticuli* and has a dipeptide-like structure that specifically inhibits aminopeptidase B and leucine aminopeptidase. Moreover, bestatin is reported to restore impaired immune function, activate cytotoxic phagocytes, stimulate cell-mediated immunity, and enhance IL-1 and IL-2 release from macrophage and spleen cells (Monaghan and Tkacz 1990).

Table 18.7 Microbial enzyme inhibitors of medical/pharmacological/biotechnological interest

Compound(s)	Target enzyme/disease	Source microorganism(s)
Aldostatin	Aldose reductase (diabetes)	*Pseudorotium zonatum*
Acarbose	α-Glucosidase and sucrase	*Streptomyces* sp., *Actinoplanes* sp.
Trestatin	α-Amylase	*Streptomyces dimorphogenes*
Lipstatin	Pancreatic lipase (obesity and diabetes)	*Streptomyces toxytricini*
Nojirimycin	α-Amylase	*Streptomyces nojirensis*
Erbstatin	Tyrosine kinase	*Streptomyces* sp.
Adecypenol	Adenosine deaminase inhibitor	*Streptomyces* sp.
Bestatin	Aminopeptidase B	*Streptoverticillium olivoreticuli*
Clavulanic acid	β-Lactamase (suppressor of penicillin resistance)	*Streptomyces clavuligerus*
Fibrostatin	Proline hydroxylase (pathological fibrosis)	*Streptomyces catenulae*
Asperlicin	Cholecystokinin-antagonist (antiulcer)	*Aspergillus alliaceus*
Ancovenin	Angiotensin-converting enzyme (ACE) (hypertension)	*Streptomyces* sp.
Muracein	Angiotensin-converting enzyme (ACE) (hypertension)	*Nocardia orientalis*
Phenacein	Angiotensin-converting enzyme (ACE) (hypertension)	*Streptomyces tanashiensis*
Foroxymithine	Angiotensin-converting enzyme (ACE) (hypertension)	*Streptomyces nitrosporeus*

(continued)

Table 18.7 (continued)

Compound(s)	Target enzyme/disease	Source microorganism(s)
Aspergillomarasmine	Endothelin converting enzyme inhibitor	*Aspergillus oryzae*
Streptovaricins, Streptonigrin	Reverse transcriptase (retroviral infections)	*Streptomyces spectabilis*
Lovastatin (mevinolin)	Hydroxymethyl glutaryl CoA reductase (hypercholesteremia)	*Aspergillus terreus, Monascus ruber*
Compactin (mevastatin)	Hydroxymethyl glutaryl CoA reductase (hypercholesteremia)	*Penicillium citrinum*
Phenicin	Hydroxymethyl glutaryl CoA reductase (hypercholesteremia)	*Penicillium phoeniceum, Penicillium rubrum*
Triacsin	Acyl CoA synthetase	*Streptomyces* sp.
Purpactin	Acyl CoA cholesterol acyltransferase	*Penicillium purpurogenum*
Squalestatin	Squalene synthetase	*Streptomyces* sp.
Leupeptin	Serine protease (inflammation, pancreatitis)	*Streptomyces roseus, Streptomyces albireticuli*
Stauroporine	Protein kinases	*Streptomyces staurosporeus*
E-64 (loxistatin)	Thiol proteases, such as papain and cathepsin B (muscular dystrophy)	*Aspergillus japonicus*
K-76	Complement cascade (anaphylactic shock)	*Stachybotrys complementi*
Complestatin	Complement cascade (anaphylactic shock)	*Streptomyces lavendulae*
Mutastein	Inhibition of insoluble glucan synthesis by *Streptococcus mutans* (prophylactic agent for tooth decay)	*Aspergillus terreus*

18.2.11 Receptor Antagonists

Among the diverse source of microbial natural products, there are compounds that bind and antagonize biological receptors. A variety of compounds are reported to be chemokine receptor antagonists (Yuan 2014). Oxytocin receptor antagonist, L156373, is a cyclic hexapeptide produced by *Streptomyces silvensis*. Oxytocin is a pituitary hormone, which regulates uterine contraction and lactation, and antagonism of its receptor might find a therapeutic activity for delaying premature labor (Pettibone et al. 1989). On the other hand, migrastatin isolated from a cultured broth of *Streptomyces* sp. acts as a muscarinic acetylcholine receptor antagonist (Nakae et al. 2006).

18.2.12 Nutraceuticals

Nutraceuticals (a hybrid of nutrition and pharmaceutical) are either substances that are food or part of food providing medical/health benefits or products isolated or purified from foods that are generally supplied in medicinal forms. However,

nowadays, nutraceuticals are functionally diverse bioactive compounds, which in part are obtained from microorganisms, including amino acids, prebiotics, and polysaccharides (Wang et al. 2016).

18.2.12.1 Prebiotics

A prebiotic is a nonviable food constituent that can confer health benefit to the host through modulation of the microbiota (The Food and Agriculture Organization of the United States (FAO)). Prebiotics are often nondigestible saccharide polymers of 3–10 monomeric sugar units, including inulin, fructo-oligosaccharides, and galacto-oligosaccharides. Strains of *Lactobacillus gasseri* produce inulin and inulin-type fructans, also known as soluble dietary fibers. In addition, galacto-oligosaccharides can be produced by *Kluyveromyces lactis* (Wang et al. 2016).

18.2.12.2 Polysaccharides

Microbial polysaccharides (sugar polymers of versatile structures) can be regarded as a source for nutraceuticals due to their health-beneficial properties, among are xanthan, gellan, dextrans, and alginate (refer to Sect. 18.2.13 for further details). Polysaccharide scleroglucan excreted by the fungus *Sclerotium rolfsii* is considered a nutraceutical with potential antitumor and antiviral properties (Giavasis 2014). Several animal polysaccharides such as hyaluronic acid (HA), chondroitin, and heparosan are also produced by microbial host species (Sheng et al. 2015; Yoshimura et al. 2015; Yu and Stephanopoulos 2008).

18.2.12.3 Polyamino Acids

Polyamino acids are produced in microorganisms from a couple of amino acids by means of ribosome-independent enzymatic processes. Three sorts of polyamino acids, to be specific, poly-γ-glutamic acid (γ-PGA), poly-ε-L-lysine (ε-PL), and multi-L-arginyl-poly (L-aspartic acid), are found in nature and are regarded as a nutraceutical (Wang et al. 2016).

18.2.13 Polymers

Microbial species synthesize biopolymers or natural polymers as intracellular, structural, and extracellular polymers of diverse and specific functions. Currently, the number of microbial polymers and their applications are increasing rapidly. Among microbial exopolysaccharides, gellan (produced by *Sphingomonas paucimobilis*) and curdlan are utilized in the preparation of gels. Pullulan (mainly produced by *Aureobasidium pullulans*), dextran, and xanthan are other polymers used as viscosifying agents (Vijayendra and Shamala 2014). The main biopolymeric polysaccharides are alginate, bacterial cellulose, curdlan, dextran, gellan, hyaluronic acid, levan, pullulan, scleroglucan, succinoglycan, and xanthan gum.

Pullulan is an extracellular, linear, unbranched, and water-soluble bacterial exopolysaccharide of maltotriose repeating units linked by α-1,6-glucosidic bonds. Pullulan, with the molecular formula $(C_6H_{10}O_5)_n$, is produced mainly by dimorphic

fungi *Aureobasidium pullulans*, *Eurotium chevalieri*, *Tremella mesenterica*, *Cytaria* sp., *Cryphonectria parasitica*, and *Rhodototula bacarum* (Gaur et al. 2010).

Bacterial cellulose is an unbranched polymer of β-1,4-linked glucopyranose units. This bacterial exopolysaccharide is mainly produced by bacterial species of *Gluconobacter*, *Azotobacter*, *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Escherichia*, *Salmonella*, *Sacrina*, and *Rhizobium*. Food additive, oil recovery, paper industry, and wound dressing are among the reported applications of bacterial cellulose (Römling and Galperin 2015; Ross et al. 1991; Valera et al. 2015).

Curdlan, a polysaccharide consisting of β-1,3-linked glucose residues, is produced by species of *Agrobacterium*, *Rhizobium*, *Pseudomonas*, and *Cellulomonas*. This polysaccharide is used as a food additive, concrete additive, and immune stimulator and in heavy metal removal processes (Liu et al. 2015; Siriwardana et al. 2011; Yang et al. 2016).

Dextran: *Leuconostoc*, *Streptococcus*, *Gluconobacter* sp., *Pediococcus pentosaceus*, and lactic acid bacteria are reported to be the main producers of this polysaccharide. This bacterial exopolysaccharide is made of D-glucopyranose units with predominantly α-(1,6) linkages in the main chain and a variable amount of α-(1,2), α-(1,3), and α-(1,4) branched linkages. Dextran has many applications as blood plasma substitute, as molecular sieves (Sephadex), in heavy metal removal, in cosmetics, and as an emulsifying and thickening agent (Nácher-Vázquez et al. 2015; Sarwat et al. 2008; Ul-Qader et al. 2001).

Gellan extracellular polysaccharide is composed of tetrasaccharide repeating units of two β-D-glucose residues, one β-D-glucuronic residue, and one of α-L-rhamnose. Gellan is mainly produced by *Pseudomonas elodea* and *Sphingomonas* spp. with applications as agar substitute, coating material, and food additive, and in food thickening, cell immobilization, gel electrophoresis, tissue engineering, cosmetics, and medicine (Prajapati et al. 2013; Raghunandan et al. 2018; Zhang et al. 2015a).

Hyaluronic acid, as a glycosaminoglycan, is composed of monosaccharide units, glucuronic acid, and N-acetylglucosamine. This polymer is mainly produced by *Streptococcus zooepidemicus*, *Streptococcus equi*, and *Pasteurella multocida* and has many uses in cosmetics, viscosupplementation, and wound dressing (Liu et al. 2011; Pan et al. 2017).

Levan: Levan, a β-(2,6)-linked fructose polymer, is mainly produced by *Zymomonas mobilis*, *Bacillus* spp., *Streptococcus* spp., and *Alcaligenes viscosus*. This biopolymer is utilized as a blood plasma substitute, in the cosmetics industry, as an emulsifying agent, and as a food additive (Gu et al. 2017; Öner et al. 2016; Silbir et al. 2014).

Scleroglucan, a β-1,3- and β-1,6-glucan, is mainly isolated from *Sclerotium rolfsii*, *Sclerotium glucanicum*, *Schizophyllum commune*, *Botrytis cinerea*, and *Epicoccum nigrum*. This biopolymer is useful for different biotechnological applications, such as cosmetics and pharmaceutical products, drug delivery, immune stimulator, oil recovery, and food additive (Castillo et al. 2015; Schmid et al. 2011).

The exopolysaccharide succinoglycan is produced mainly by *Sinorhizobium meliloti*, *Agrobacterium* spp., *Alcaligenes faecalis*, *Pseudomonas* spp., *Rhizobium*

spp., and a large number of soil microbes with applications as a food additive and in the oil recovery sector (Halder et al. 2017).

Xanthan heteropolysaccharide consists of β-D-glucose units linked at positions 1 and 4. In this cellulose-like backbone, every other glucose unit is attached to a trisaccharide side chain, composed of glucuronic acid and two mannose residues. *Xanthomonas campestris* is the main producer of this biopolymer. Owing to high viscosity even at low concentrations and nontoxic characteristics, xanthan has various applications in the food and oil industry. Other important fields considering xanthan applications are agricultural products, coatings, cosmetics, food additive, and paper industry and as a thickening agent (Kreyenschulte et al. 2014).

The microbial biopolymer of γ-polyglutamic acid is an anionic extracellular polyamide consisting of glutamic acid repeat units linked between the α-amino and γ-carboxylic acid functional moieties, produced by a variety of microorganisms including *Bacillus* spp., *Staphylococcus epidermidis*, *Natrialba aegyptiaca*, *Natronococcus occultus*, and *Fusobacterium nucleatum*. However, *Bacillus subtilis* and *Bacillus licheniformis* are the most important strains for γ-polyglutamic acid production. The polymer, which can be classified as pseudo-poly(amino acid), is regarded as a biodegradable plastic and has a wide variety of other applications such as fertilizer, as food thickener, in medical adhesives, in skin care, in tissue scaffolds, as drug delivery system, and in wastewater treatment (Ogunleye et al. 2015).

Poly-ε-lysine: The polymer is produced by *Streptomyces albulus* with many applications as a coating material, dietary agent, emulsifying agent, and food preservative (Xu et al. 2017; Zhou et al. 2015).

The polysaccharide alginate is a biopolymer mainly produced by the species of *Pseudomonas* and *Azotobacter*. Application of alginate is widely reported in the following sectors: cell immobilization, drug delivery, food additive, textile/paper industry, wound dressing, and water treatment (Maleki et al. 2015; Urtuvia et al. 2017).

Cyclodextrins (CD): Cyclodextrins as water-soluble nonreducing cyclic oligosaccharides are mainly produced by the direct act of microbial cyclodextrin glycotransferases on starch substrates. In fact, cyclodextrins consist of α-1,4-linked D-glucopyranosyl units, and based on the number of glucose units, they are classified as alpha-, beta-, and gamma-cyclodextrins. *Bacillus* species are the main producers of this pharmaceutically important polymer (Ahmed and El-Refai 2010; Goo et al. 2014). Polyhydroxyalkanoates (PHAs) are polyesters of microbial origin, or better defined as biopolyesters, which are regarded as biodegradable green plastics. As polymers of R-3-hydroxyalkanoic acids, PHAs are produced by a variety of bacteria (Gholami et al. 2016).

18.2.14 Surfactants

Microbial surfactants or biosurfactants are a wide variety of structurally diverse microbial products exhibiting surface activity at the interfaces. These amphipathic compounds can be classified according to their mode of action, molecular weight,

Table 18.8 Microbial surfactants

Compound(s)	Source microorganism(s)
Glycolipid class	
Rhamnolipids	*Pseudomonas aeruginosa*
Trehalolipids	*Rhodococcus erythropolis*
	Arthrobacter sp.
	Mycobacterium spp.
	Nocardia spp.
Sophorolipids	*Candida bombicola*
	Candida apicola
Mannosylerythritol lipids	*Candida antarctica*
	Pseudozyma spp.
Lipopeptide class	
Surfactin	*Bacillus subtilis*
Iturin	*Bacillus subtilis*
Fengycin/plipastatin	*Bacillus subtilis*
Lichenysin	*Bacillus licheniformis*
Liposan	*Candida lipolytica*

and physicochemical characteristics. To this end, microbial surface-active compounds are generally categorized as low (such as glycolipids and lipopeptides) and high molecular weight (e.g., polysaccharides, proteins, and lipoproteins) surfactants. Glycolipids, consisting of mono- or disaccharides, combined with long-chain aliphatic acids or hydroxyaliphatic acids, mainly include rhamnolipids produced by *Pseudomonas aeruginosa*, trehalolipids produced by *Rhodococcus erythropolis*, sophorolipids produced by *Candida bombicola*, and mannosylerythritol lipids (MEL) isolated from *Pseudozyma* yeasts. Included in lipopeptides, examples comprise surfactin, iturin, and fengicyn cyclic lipopeptides produced by *Bacillus* species (Rodrigues 2015). Refer to Table 18.8 for a summarized review of microbial surfactants.

Owing to diversity, biodegradability, and low toxicity, microbial surfactants are considered superior to their chemical counterparts and hence have many applications in the food and cosmetics industries, enhanced oil recovery, emulsification, detergency, lubrication, moisture retention, solubilization, and bioremediation (Campos et al. 2013).

18.2.15 Biopesticides

Biological pesticides or biopesticides with microbial origin (from bacteria and fungi) include agents which are active against a wide range of invertebrate pests (for instance, arthropods and nematodes) and also weeds, plant diseases, and some vertebrates. These biopesticides comprise bioinsecticides, acaricides, nematicides, fungicides, bactericides, and herbicides.

Table 18.9 Microbial or microbial-derived products as bioinsecticides

Active ingredient	Target(s)
Bacteria	
Bacillus thuringiensis subsp. *aizawai*	Caterpillars
Bacillus thuringiensis subsp. *kurstaki*	Caterpillars
Bacillus thuringiensis subsp. *galleriae*	Certain beetles
Bacillus thuringiensis subsp. *tenebrionis*	Colorado potato *(L. decemlineata)* and elm leaf *(P. luteola)*
Bacillus thuringiensis subsp. *israelensis*	Mosquitoes, black flies, fungus gnats, and other nuisance flies
Bacillus firmus	Plant parasitic nematodes
Bacillus subtilis	Soil-borne and plant pathogenic fungi, Psyllid,
Bacillus sphaericus	Mosquito larvae
Burkholderia rinojensis	Broad-spectrum insecticide/acaracide Bionematicide
Chromobacterium subtsugae	Broad-spectrum insecticide/acaracide
Paenibacillus popilliae	Japanese beetle, *Popillia japonica*
Pasteuria spp.	Plant parasitic (cyst) nematodes
Pseudomonas fluorescens	Zebra and quagga dreissenid mussels
Fungi	
Beauveria bassiana	Thrips, aphids, whiteflies, plant bugs, mites, and other arthropods
Myrothecium verrucaria	Plant parasitic nematodes
Metarhizium brunneum (*M. anisopliae*)	Thrips, whiteflies, mites, weevils, and ticks
Isaria fumosorosea (formerly *Paecilomyces fumosoroseus*)	Whiteflies, aphids, thrips, leafminers, plant bugs, mites, some soil pests
Paranosema locustae	Grasshoppers and mormon crickets (rangeland)
Purpureocillium lilacinum (formerly *Paecilomyces lilacinus*)	Plant parasitic nematodes
Trichoderma harzianum	Soil-borne and plant pathogenic fungi

Microbial bioherbicides obtained from bacteria and fungi for the control of both pre- and post-emergent grass and broad-leaf weeds have drawn much attention over the past decades. Microbial bioinsecticides, with applications worldwide, are mainly products of *Bacillus thuringiensis* species. Spores, as well as insecticidal Cry and Cyt toxins obtained from the soil bacterium *Bacillus thuringiensis*, are among the most widely produced biopesticides. Microbial pesticides based on species/strains of microbes or their pesticidal metabolite, comprising bacteria, fungi, and baculoviruses are summarized in Table 18.9 (Arthurs and Dara 2018; Barka et al. 2015; Brun et al. 2016; Harding and Raizada 2015; Mupondwa et al. 2015).

18.2.16 Plant Growth Regulators

Phytohormones or plant hormones are roughly plant growth-promoting agents that can be classified into five classes, namely, abscisic acid, auxins, ethylene, cytokinin, and gibberellins. Gibberellins (GAs) are a family of tetracyclic diterpenes first

discovered from the ascomycetous fungus *Gibberella fujikuroi*. In spite of the ubiquitous presence of gibberellins in plants, algae, fungi, and bacteria, currently, the filamentous fungus *Gibberella fujikuroi* is mainly utilized as the sole strain for industrial production (Shi et al. 2017).

Abscisic acid is another important phytohormone playing a significant role in regulating plant growth. The earliest discovery of abscisic acid dates back to 1969 from the *Penicillium italicum*. However, there are reports of microbial production of this compound in *Cercospora rosicola*, *Botrytis cinerea*, and other filamentous fungi including the genus of *Aspergillus* and *Rhizopus* (Shi et al. 2017).

Eco-friendly alternatives in sustainable agriculture during the last decades have been studied extensively. In this context, volatile compounds from microorganisms emerged as low-cost, effective, efficient, and eco-friendly alternatives. As lipophilic compounds derived from microbial metabolic pathways with low molecular weight, low boiling point, and high vapor pressure, volatile organic compounds released from diverse microorganisms (such as *Bacillus*, *Pseudomonas*, *Arthrobacter*, *Fusarium*, and *Alternaria*) enhance plant growth by direct or indirect mechanisms. These compounds mainly belong to alkanes, alkenes, alcohols, esters, ketones, terpenoids, and sulfur families (Fincheira and Quiroz 2018).

18.2.17 Bioflavors

Microorganisms are highly engaged in the production of flavors and fruit, flower, and essential oil scents which have many applications in cosmetics, perfumes, soaps, cleaning products, candles, food, and beverage industries. These bioflavor compounds belong to many chemical categories of esters, terpenoids, aldehydes, and ketones, which naturally act as insecticides, pheromones, or precursor molecules for various other natural products (Carroll et al. 2016). The main biotechnological processes contributing to flavor formation are limited to the de novo biosynthesis of flavor compounds, bioconversion of added precursors, and in situ microbial flavoring. As an important example, yeast microbial species of *Candida*, *Rhodotorula*, and *Sporobolomyces* are producers of γ-decalacton (the key component of peach and apricot flavors). Acetoin and diacetyl, as intermediates of natural bacterial fermentation of 2,3-butanediol, are compounds with a buttery flavor. Successful metabolic engineering of *Escherichia coli* and *Bacillus subtilis* or alternative pathways in yeasts such as *Candida glabrata* are reported for acetoin production (Chen and Jordan 1984; Li et al. 2014b; Nielsen et al. 2010; Zhang et al. 2014). Methyl ketones are found as a flavoring agent in dairy products and essential oil scents as well. Methyl ketones with various aliphatic carbon chain lengths can be produced from the fatty acid β-oxidation pathway in bacteria (Goh et al. 2014).

There are many compounds belonging to aldehydes and alcohols with flavoring characteristics. Isobutyraldehyde is a branched chain aldehyde having a malt-like odor accompanying wine and beer fermentation. Vanillin (vanilla flavorant) and benzaldehyde (a flavor with strong cherry and almond-like aroma) are other microbial product flavors belonging to this category. Linear aldehyde and alcohols, in addition to aromatic counterparts, are desired flavors with aromas including freshly

cut grass, cucumbers, foliage, apples, and leather (Krings and Berger 1998; Kunjapur et al. 2014). Natural flavoring compounds belonging to the ester chemical group represent bioflavors with the fruity or flowery aroma. As such, isobutylene acetate and 3-methyl-butyl acetate, which are flavors of raspberries/pears/pineapple and banana/pear, respectively, are produced by means of metabolic engineering in *Escherichia coli* (Park et al. 2009; Rodriguez et al. 2014). As terpenes, limonene (citrus scent), and germanic acid (sweet, woody, or leafy flavor with hints of citrus) are the main bioflavors obtained from microbial commercial sources.

18.2.18 Biopigments and Dyes

Compared to their chemically synthesized counterparts, biopigments are gaining attention in the food, feed, beverage, pharmaceutical, and cosmetic sector and recently in industries like textile, plastic, paint, paper, and printing. A variety of biopigments, namely, carotenoids, melanins, flavins, quinines, monascins, and violancein, have been produced by microorganisms (Duffose 2006). Among the carotenoids and xantophylls, there exists especial attention toward β-carotene and lycopene, lutein, zeaxanthin, canthaxanthin, rhodoxanthin, and astaxanthin. Table 18.10 presents a list of pigment-producing microorganisms and their proposed bioactivities (Agarwal et al. 2000; Andersen et al. 1991; Andrighetti-Fröhner et al. 2003; Antonisamy and Ignacimuthu 2010; Araújo et al. 2010; Clauditz et al. 2006; Cooney et al. 1966; Cude et al. 2012; Feng et al. 2012; Liu et al. 2005; Tuli et al. 2015; Vasanthabharathi et al. 2011; Venil et al. 2013).

18.2.19 Proteins and Enzymes

Enzymes, as biological macromolecules, act by catalyzing a specific biochemical reaction and are responsible for all those vital chemical interconversions that are essential to sustain life. In 1877, Wilhelm Friedrich Kühne, a professor at the University of Heidelberg, mentioned the term "enzyme" which is adopted from the Greek word "ενζμον" meaning "*in leaven*" and so was the first person to give a scientific terminology to this biomolecule (Kühne 1976). Owing to their vast range of activities, and based upon the nature of reactions, enzymes are being classified according to a numerical classification scheme, namely, Enzyme Commission number (EC number). As a system of enzyme nomenclature, each EC number in line with a recommended name is linked to a specific enzyme. Very most enzyme names end in "ase," with some of the originally studied enzymes being the exception, such as pepsin, trypsin, and rennin.

Enzymes, also known as "biocatalysts," are immensely isolated and purified from microorganisms, as the most fertile source due to the broad biochemical diversity, feasibility of mass culture, and the simplicity of genetic manipulation. Therewith, microbial enzymes are relatively more stable than their plant or animal counterparts (Zhang and Kim 2012). Since the ancient time, naturally occurring

Table 18.10 Microbial pigments and their proposed bioactivities

Compound(s)	Color	Source microorganism(s)	Bioactivities
β-Carotene	Yellow-orange	*Blakeslea trispora, Fusarium sporotrichioides, Mucor circinelloides, Neurospora crassa, Phycomyces blakesleeanus, Dunaliella salina*	Anti-cancer, antioxidant, suppression of cholesterol synthesis
Ankaflavin	Yellow	*Monascus* spp.	Antitumor, anti-inflammatory
Anthraquinone	Red	*Penicillium oxalicum*	Antifungal, virucidal
Astaxanthin	Pink-red	*Haematococcus pluvialis*, *Phaffia rhodozyma*, *Agrobacterium aurantiacum*	Antioxidant, photoprotectant, anticancer, anti-inflammatory
Canthaxanthin	Orange, pink	*Bradyrhizobium* spp., *Monascus roseus*	Antioxidant, anticancer
Cycloprodigiosin	Red	*Pseudoalteromonas denitrificans*	Antiplasmodial, anticancer
Granadaene	Orange-red	*Streptococcus agalactiae*	Antioxidant, detoxify ROS
Indigoidine	Blue	*Corynebacterium insidiosum*	Antimicrobial
Lycopene	Red	*Fusarium sporotrichioides, Blakeslea trispora*	Antioxidant, Anticancer
Melanin	Black	*Saccharomyces neoformans*	-
Monascin	Yellow	*Monascus* sp.	Immunomodulative effect, anticholesterolemic effect
Naphtoquinone	Deep blood red	*Cordyceps unilateralis*	Anticancer, antibacterial, trypanocidal
Prodigiosin	Red	*Serratia marcescens*, *Pseudoalteromonas rubra*	Anticancer, DNA cleavage, immunosuppressant
Pyocyanin	Blue, green	*Pseudomonas* spp.	Cytotoxicity, neutrophil apoptosis, ciliary dysmotility, pro-inflammatory
Riboflavin	Yellow	*Ashbya gossypi*	Anticancer, antioxidant, protection against cardiovascular diseases, in vision
Rubropunctatin	Orange	*Monascus* spp.	Anticancer
Staphyloxanthin	Golden	*Staphylococcus aureus*	Antioxidant, detoxify ROS
Violacein	Purple	*Janthinobacterium lividum, Pseudoalteromonas tunicate, Pseudoalteromonas* spp., *Chromobacterium violaceum*	Antioxidant, detoxify ROS
Xanthomonadin	Yellow	*Xanthomonas oryzae*	Protection against photodamage
Zeaxanthin	Yellow	*Flavobacterium* spp., *Staphylococcus aureus, Paracoccus zeaxanthinifaciens, Sphingobacterium Multivorum*	Antioxidant

enzymes have been used extensively, and nowadays, enzymes play key roles in numerous biotechnology processes that are quite often encountered in the production of food and beverages, clothing, paper products, pharmaceuticals, detergents and cleaning supplies, or any other (Gurung et al. 2013). Table 18.11 and the following sections summarize different applications of distinct categories of microbial enzymes.

18.2.19.1 Lipases

Lipases are ubiquitous enzymes in the esterase subclass (EC 3.1.1.3) that catalyze hydrolysis and synthesis of long-chain acylglycerols. Lipases are crucial enzymes in the digestion, transport, and processing of dietary lipids in most, if not all, living organisms. Owing to their broad range of applications and facile mass production, lipases are considered as biotechnologically valuable enzymes (Ghasemian and Moradpour 2019). *Bacillus*, *Pseudomonas*, and *Burkholderia* are among the main lipase-producing bacterial genera. Fungal lipases are also well isolated and produced by different species of filamentous fungi and yeasts. Due to four unique characteristics (their exquisite chemoselectivity, regioselectivity, and stereoselectivity; availability of large quantities and high yield production from microbial sources; availability of their crystal structure; and avoiding the need for any cofactor), lipases are the most widely utilized group of biocatalysts in organic chemistry (Jaeger and Eggert 2002). Lipases are receiving increasing attention as catalysts for polymeric synthesis and as catalysts for the production of biodiesel fuels and many fine chemicals. They also find applications in food modification (in the selective hydrolysis of fat triglycerides to release free fatty acids in dairy products, which are used to develop flavored products such as cheese, butter, margarine, milk chocolate, and sweets), detergent formation, cosmetics, and lipid-rich waste water treatments. The most commercially important biotechnological application of lipases is their incorporation into detergents to remove fat-containing stains. These commercially significant detergent lipases are mainly the ones from *Thermomyces* sp., expressed in the recombinant strains of *Aspergillus oryzae*, as well as from *Pseudomonas* species (Anobom et al. 2014; Jaeger and Reetz 1998).

18.2.19.2 Proteases

Proteases remain the dominant enzyme types, which are currently used as industrial enzymes. Proteases, either intracellular or extracellular, are produced by many microorganisms. Based on whether they are active under acidic, neutral, or alkaline conditions and based on the nature of the active site group of the enzyme, i.e., metallo- (EC 3.4.24), aspartic- (EC 3.4.23), cysteine- or sulphydryl- (EC 3.4.22), or serine-type (EC 3.4.21), microbial proteases are classified (Gupta et al. 2002). Although a wide cluster of bacteria and fungi produce extracellular proteases, protease is majorly produced by *Bacillus* species, namely, *Bacillus subtilis*, *Bacillus sphaericus*, *Bacillus licheniformis*, *Bacillus cohnii*, *Bacillus stearothermophilus,* and *Bacillus firmus* (Banerjee and Ray 2017). However, bacterial species belonging to the genera *Pseudoalteromonas*, *Psychrobacter*, *Photobacterium*, *Vibrio*,

Table 18.11 Biotechnological applications of microbial enzymes in different areas

Type of industries	Enzyme(s)	Application(s)
Alcohol/ beverages	Amylases, glucanases, proteases, arabinoxylans, amyloglucosidase, pullulanases, and acetolactate decarboxylase	Degradation of starch into simple sugars. Also for degradation of complex proteins into sugars resulting in increase of fermentation efficiency. Production of low calorie beer
Fruit drinks	Cellulase, pectinase	Clarification of fruit juices
Food processing	Amylase, protease, papain, and trypsin	Degradation of starch and complex proteins, softening of meat, predigest of baby foods
Dairy	Rennin, lipases, and lactases	Hydrolyzing protein, cheese production, and glucose production from lactose
Detergent	Protease, amylase, lipase, cellulases, and mannanase	To remove protein after staining, remove insoluble starch in dish washing, removing oils and fats, and to increase effectiveness of detergents
Textile	Amylase, pectinase, cellulases, catalase, and protease	To remove starch size, glue between the fiber core and the waxes, fabric finishing in denims, degrading residual hydrogen peroxide after the bleaching of cotton, wool treatment, and the degumming of raw silk also known as biopolishing
Paper and pulp	Amylases, xylanases, cellulases, hemicellulase, ligninases, and esterase	Degrade starch to lower viscosity, aiding sizing, deinking, and coating paper. Xylanases reduce bleach required for decolorizing; cellulases and hemicellulase smooth fibers, enhance water drainage, and promote ink removal; lipases reduce pitch, and lignin-degrading enzymes remove lignin to soften paper, for esterification
Animal feed stock	Phytase	Increases total phosphorous content for growth, increases in phytic acid need
Rubber	Catalase	Generates oxygen from peroxide to convert latex into foam rubber
Oil and petroleum	Cellulases, ligninases, and mannanase	Formation of ethanol, forming gel breaker in oil drilling
Biopolymer/ plastic	Laccases, peroxidases, lipases, and transglutaminases	Forming cross-links in biopolymers to produce materials in situ by means of polymerization processes
Pharmaceutical	Nitrile dehydratase, D-amino acid oxidase, glutaric acid acylase, penicillin amylase, penicillin G amylase, ammonia lyase	Producing water soluble intermediates, semisynthetic antibiotics, intermediate for aspartame
Molecular biology	Restriction enzymes, DNA ligase, and polymerases	Used to manipulate DNA in genetic engineering, crucial for restriction digestion and the polymerase chain reaction, also important in forensic science

Halobacillus, *Bacillus*, *Microbulbifer*, and *Shewanella* are reported to be dominant producers of serine proteases (Zhang et al. 2015b). Alternatively, cysteine proteases are mostly produced by fungal species like *Aspergillus oryzae* and *Sporotrichum pulverulentum* (de Souza et al. 2015).

Proteases have multiple applications in the market including the sectors of detergent, food/feed (in the preparation of protein hydrolysates of high nutritional value), pharmaceuticals, diagnostics, leather, waste management, silk degumming, silver recovery in photographic industry, and so forth (da Silva 2017; Theron and Divol 2014).

18.2.19.3 Polysaccharide-Degenerating Enzymes

Amylases: Amylases are enzymes responsible for the breakdown of starch into sugars by catalyzing the hydrolysis of internal glycosidic linkages in low molecular weight sugars (as a glycoside hydrolase). Being present in an abundant amount in human saliva, amylase starts the mechanical process of digestion. Starch-degrading amylolytic enzymes are of particular value in the biotechnological sector, ranging from food, fermentation, and textile to paper industries (Lin et al. 1997; Sidhu et al. 1997). Amylases are obtainable from various sources, like plants and animals; however, the microbial counterparts are generally industrially sustainable and had in the last three decades made a significant contribution to the food and beverage industry. Based on the bonding type, amylases are being subdivided into three categories of α-, β-, and γ-amylases. α-Amylases (EC 3.2.1.1) manage the hydrolysis of internal α-1,4-glycosidic linkages in starch in low molecular weight products, namely, glucose, maltose, and maltotriose units. As its application, α-amylase is used in ethanol production to break down the grain starch into fermentable sugars. Termamyl, an α-amylase originated from *Bacillus licheniformis*, is widely used in some detergents, principally for starch-removing or dishwashing. β-Amylase (EC 3.2.1.2) (also known as 1,4-α-D-glucan maltohydrolase, glycogenase, or saccharogen amylase) is also produced by bacteria, fungi, and plants. Acting from the nonreducing end, β-amylase catalyzes the hydrolysis of the second α-1,4 glycosidic linkages, breaking apart two glucose units (maltose) at once. γ-Amylase (EC 3.2.1.3) (alternative names: glucan 1,4-α-glucosidase; amyloglucosidase; exo-1,4-α-glucosidase; glucoamylase; lysosomal α-glucosidase; 1,4-α-D- glucan glucohydrolase) does break the α(1-6) glycosidic linkages, as well as the ultimate α(1-4) glycosidic linkages at the nonreducing end of amylose and amylopectin, resulting in glucose. The use of γ-amylase in food, pharmaceutical, drug delivery, and chemical industries as well as in agriculture and environmental sectors is well recognized (Gurung et al. 2013). As regards thermostability is an eligible characteristic of many industrial enzymes, thermostable amylolytic enzymes are popular and currently in research to improve industrial method of starch degradation and also in the production of valuable products, namely, crystalline dextrose, glucose, maltose, dextrose syrup, and maltodextrin (Asgher et al. 2007; Konsoula and Liakopoulou-Kyriakides 2007; Pandey et al. 2000). A great number of mesophilic fungi, including *Aspergillus* and *Penicillium* species, are also producers of α-amylase (Ray 2004; Santerre Henriksen et al. 1999).

Chitinases: Chitin is a linear polymer of β-1,4-N-acetylglucosamine (GlcNAC) that can be degraded by chitinase (EC 3.2.1.14) as a glycosyl hydrolase. Chitinases possess the ability to degrade chitin directly to low molecular weight chitooligomers of various applications and various industrial, agricultural, and medical functions (Hamid et al. 2013). To be specific in their biotechnological applications, chitinases are also gathering attention as biocontrol agents of fungal phytopathogens and harmful insects (Mathivanan et al. 1998; Mendonsa et al. 1996). In addition to being a target for biopesticides, chitinases have few medical applications and are used for the estimation of fungal biomass (Dahiya et al. 2006). There are many reports of chitinase production by bacterial species of *Streptomyces*, *Alteromonas*, *Escherichia*, *Serratia*, and *Aeromonas* (Blaak and Schrempf 1995; Frankowski et al. 2001; Kamensky et al. 2003; Tsujibo et al. 1993) and fungal species of *Trichoderma*, *Coccidioides*, and *Aspergillus* (Alcazar-Fuoli et al. 2011; Pishko et al. 1995; Seidl et al. 2005).

Alginate lyases: Alginate lyases, also known as alginases or alginate depolymerases (either EC 4.2.2.3 or EC 4.2.2.11), are enzymes responsible for the degradation of polysaccharide alginate, which consists of β-D-mannuronate and α-L-guluronate as monomeric units to alginate oligosaccharides (Zhu and Yin 2015). A number of alginate lyases have been identified and isolated from various sources, including bacteria, fungi, and algae (such as *Pseudomonas* spp., *Stenotrophomonas maltophilia*, *Flavobacterium* spp., *Pseudoalteromonas* spp., *Saccharophagus degradans*, *Vibrio* spp., and *Azotobacter* spp.) (Li et al. 2011; Wong et al. 2000). Currently, these enzymes are being used for producing alginate oligosaccharides, elucidation of alginate structure, preparation of red/brown algae protoplast, and a promising potential application in the treatment of cystic fibrosis in patients infected with alginate-producing *Pseudomonas aeruginosa* by degrading the polysaccharide biofilm of bacterium (Islan et al. 2014; Xiaoke et al. 2003).

Agarases: Agarases are the enzymes known for complete hydrolysis of agar polysaccharides to agaro-oligosaccharides. α-Agarase (EC 3.2.1.158), β-agarase (EC 3.2.1.81), and β-porphyranase are constituents of this enzyme group (Chi et al. 2012). Bacterial agarases are produced by species of *Streptomyces*, *Flammeovirga*, *Pseudoalteromonas*, *Agarivorans*, *Vibrio*, *Alteromonas*, and so forth (Dong et al. 2007; Long et al. 2010; Lu et al. 2009; Oh et al. 2010; Temuujin et al. 2011; Yang et al. 2011). Agarases might have possible future applications in generating oligosaccharides with various nutraceutical activities; in sustainable production of stock chemicals for biorefinement and bioenergy; and in the health food, pharmaceutical, and cosmetic industries (Chi et al. 2012; Fu and Kim 2010).

Carrageenases: Carrageenans are hydrophilic sulfated linear galactans (they are hydrocolloid polysaccharides like agar and alginate) with both technological and economic significance and various biotechnological applications. Based on the number of sulfate substituent present in the structure, carrageenans are divided into κ-carrageenan, ι-carrageenan, and λ-carrageenan. Therefore, the enzymes which degrade carrageenans are called κ-carrageenases (EC 3.2.1.83), ι-carrageenases (EC 3.2.1.157), and λ-carrageenases (EC 3.2.2.162). Oligo-carrageenans produced by the action of microbial enzymes provide many biotechnological applications and

can be more advantageous than acid hydrolysis products due to higher uniformity in molecular weight (Chauhan and Saxena 2016). There are reports of carrageenase isolation from bacterial sources of *Pseudoalteromonas*, *Alteromonas*, *Cellulophaga*, *Pseudomonas*, *Cytophaga*, *Tamlana*, *Vibrio*, *Catenovulum*, *Microbulbifer*, *Zobellia*, *Bacillus,* and *Cellulosimicrobium* (Dyrset et al. 1997; Li et al. 2013; Michel et al. 2001; Sarwar et al. 1987; Yao et al. 2013; Youssef et al. 2012; Zhu and Ning 2016; Ziayoddin et al. 2014).

Cellulose and hemicellulose hydrolase: Cellulases are the products of many microbial species including actinomycetes, bacteria, and fungi. Cellulose-degrading bacteria mostly comprise *Cellulomonas*, *Thermobifida*, *Cytophaga*, *Sporocytophaga*, *Caldicellulosiruptor*, *Clostridium*, *Ruminococcus*, *Acetivibrio*, *Butyrivibrio*, and *Fibrobacter*. Cellulases from microorganisms are either cell-bound or extracellular and mostly differ by their mode of action. Endoglucanase or glucanohydrolase (EC 3.2.1.4), exoglucanase or cellobiohydrolase (EC 3.2.1.91), exoglucanase or cellodextrinase (EC 3.2.1.74), β-glucosidase (EC 3.2.1.21), cellobiose phosphorylase or cellobiase (EC 2.4.1.20), cellodextrin phosphorylase (EC 2.4.1.49), and cellobiose epimerase (EC 5.1.3.11) are examples thereof (Lynd et al. 2002; Sharma et al. 2016).

18.2.19.4 Laccase

Laccases or benzenediol/oxygen oxidoreductases (EC 1.10.3.2) are multi-copper enzymes that act in the catalytic oxidation of phenolic and nonphenolic aromatic compounds. Laccases are mainly produced by fungi; however, bacterial laccases are also gaining attention due to their remarkable features compared to their fungal counterparts. In addition to their vast applications as multi-purpose biocatalysts, laccases are of biotechnological applications in pulp and paper biobleaching, decoloration and degradation of textile dyes/effluents, biosensor development, enzymatic removal of phenolic compounds in beverages, fruit juice processing, bioremediation, and detoxification of aromatic pollutants (Chauhan et al. 2017; Mate and Alcalde 2017; Upadhyay et al. 2016). Laccases produced by bacteria mainly belong to Gram-positive species such as *Bacillus*, *Geobacillus*, *Streptomyces*, *Rhodococcus*, *Staphylococcus*, *Azospirillum*, *Lysinibacillus*, and *Aquisalibacillus* and some Gram-negative bacteria like *Pseudomonas*, *Enterobacter*, *Delfia*, *Proteobacterium,* and *Alteromonas* (Forootanfar and Faramarzi 2015; Ghasemi et al. 2014). There are many reports of laccase isolation from the following fungal species: *Aspergillus flavus*, *Phanerochaete chrysosporium*, *Schizophyllum commune*, *Pycnoporus cinnabarinus*, *Coriolopsis gallica*, *Pichia pastoris*, *Pleurotus ostreatus*, *Pleurotus eryngii*, *Trametes pubescens*, *Marasmius quercophilus*, *Trametes versicolor*, *Myceliophthora thermophila*, *Coriolopsis gallica*, *Pycnoporus cinnabarinus*, *Botrytis cinerea*, *Phanerochaete chrysosporium,* and *Trametes versicolor* (Upadhyay et al. 2016; Yang et al. 2017).

18.2.19.5 Medicinally Important Enzymes (Therapeutic Proteins)

Enzymes have been exploited as therapeutic agents for several decades in three broad areas, namely, (a) to replace enzymes that are absent or are defective as a result of inherited disease; (b) to replace enzymes that are deficient as a result of

acquired disease in the producing organ(s); and (c) to perform a desired bioefficacy based on the catalytic activity of the enzyme (Goldberg 1992). In a more general respect, with regard to the category (c) and the catalytic activity of the enzyme, it should be noted that there are few important microbial enzymes in clinical use with notable therapeutic uses as oncolytics, thrombolytics, and anticoagulants or as replacements for metabolic deficiencies.

Asparaginase: L-asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1) is the enzyme, which hydrolyzes the amino acid L-asparagine into aspartic acid and ammonia. L-asparaginase is considered the most medicinally important microbial enzyme with a primary role in the treatment of acute lymphoblastic leukemia (ALL) (Krishnapura et al. 2016). The specific action mechanism of the compound employs lymphocytic leukemic cells that are deficient in L-asparagine synthase. In these cases, normal cells are being able to synthesize L-asparagine. Several microorganisms are endowed with the ability to produce L-asparaginase. However, *Escherichia coli*, *Erwinia aroideae*, *Erwinia carotovora*, and *Erwinia chrysanthemi* are the main commercial enzyme producers (Cachumba et al. 2016; Ghoshoon et al. 2015).

Arginine deiminase: Arginine deiminase (EC 3.5.3.6) is the enzyme that catalyzes the hydroxylation of arginine to citrulline and ammonium. Arginine, one of the nonessential amino acids in humans, is synthesized from citrulline. However, many tumors, such as hepatocellular carcinomas and melanomas, are auxotrophic for arginine and strictly depend on exogenous arginine. So, it is confirmed that arginine depletion by arginine deiminase is effective as one potential cancer therapy agent for the treatment of arginine-auxotrophic tumors (Han et al. 2016). Although arginine deiminase was primarily discovered from *Bacillus pyocyaneus*, there are many reports of its isolation from *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Pseudomonas plecoglossicida*, *Halobacterium salinarum*, *Mycoplasma arginini*, *Mycoplasma hominis*, *Streptococcus pyogenes*, *Enterococcus faecium,* and *Lactococcus lactis* (Fiedler et al. 2015; Kaur and Kaur 2016; Ni et al. 2008; Su et al. 2015; Xiong et al. 2016).

Collagenase: Microbial collagenases (EC 3.4.24.3) are the enzymes that cleave helical regions of fibrillar collagen molecules under physiological conditions (Duarte et al. 2016). Although commercial collagenases are mainly isolated from *Clostridium histolyticum*, species such as *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus licheniformis*, *Bacillus cereus*, *Microbacterium liquefaciens*, *Alicyclobacillus sendaiensis*, *Thermoactinomyces* sp., *Streptomyces parvulus,* and *Aeromonas* sp. have been described as good sources of collagenolytic enzymes (Baehaki et al. 2012; Kanayama and Sakai 2005; Makinen and Makinen 1987; Nagano and To 2000; Petrova et al. 2006; Sakurai et al. 2009; Tsuruoka et al. 2003; Wu et al. 2010). Collagenase is used in the treatment of several human diseases (including Dupuytren's disease), in debridement of wounds and burns, in cancer therapy, for treating lumbar disc herniation, and in treatment of chronic total occlusions (Cemazar et al. 2012; Chu 1987; Jordan 2008; Patry and Blanchette 2017; Ramundo and Gray 2008; Strauss et al. 2003; Thomas and Bayat 2010; Wu et al. 2009).

Glutaminase: Like L-asparaginase, L-glutaminase is a microbial anticancer enzyme proven effective against acute lymphocytic leukemia. L-glutaminase (EC

3.5.1.2) is the enzyme that catalyzes L-glutamine to L-glutamate and ammonia. Based on this catalytic action, depletion of L-glutamine occurs in cancerous cells which are auxotrophic for this amino acid and consume more of it for their energy need and have a higher rate of proliferation (Wise and Thompson 2010). A vast variety of reports exists on L-glutaminase isolation and characterization from microbial species including but not limited to *Bacillus* and *Pseudomonas* spp. and few reports with fungal, actinomycete, and yeast systems (Binod et al. 2017; Jesuraj et al. 2017; Shirazian et al. 2016; Sinsuwan et al. 2012).

Urate oxidase (uricase): Uricase or urate oxidase (urate: oxygen oxidoreductase, EC 1.7. 3.3) catalyzes the oxidative opening of the purine ring of urate to yield allantoin, carbon dioxide, and hydrogen peroxide. The enzyme is currently regarded as an important therapeutic one for the treatment of tumor lysis syndrome and gout (Dabbagh et al. 2016). Many bacterial (Dabbagh et al. 2012) and fungal species, mainly, *Aspergillus flavus,* are producers of this enzyme.

Fibrinolytic enzymes: Fibrinolytic enzymes are used to lyse blood clots, composed of fibrin, to avoid thrombosis in blood vessels. Due to side effects and expensive prices linked to common thrombolytic agents, microbial fibrinolytic enzymes have gained much more attention during the last decades. Streptokinase from *Streptococcus hemolyticus* and *Streptococcus pyogenes*, staphylokinase from *Staphylococcus aureus*, nattokinase from *Bacillus subtilis natto*, and subtilisin from *Bacillus subtilis* are principal examples (Dabbagh et al. 2014; Ebrahimi et al. 2011; Ghasemi et al. 2012b; Raee et al. 2017).

Cholesterol oxidase: Cholesterol oxidase (EC 1.1.3.6) is an enzyme of great commercial value, especially in laboratories for the determination of cholesterol concentration in serum and other clinical samples. Cholesterol oxidase has been reported from a variety of microorganisms, mostly from actinomycetes and other species such as *Arthrobacter*, *Brevibacterium*, *Corynebacterium*, *Mycobacterium*, *Nocardia*, *Burkholderia*, *Chromobacterium*, *Pseudomonas,* and *Rhodococcus* (Doukyu 2009; Ghasemian et al. 2009; Moradpour and Ghasemian 2016; Yazdi et al. 2008).

18.2.19.6 Enzymes Used in Bioremediation

Bioremediation is the exploitation of biological agents such as bacteria, fungi, and enzymes thereof as an attractive and effective method for cleaning the environment from toxic pollutants and solve the problem of industrial/environmental waste materials. Different bioactive natural products and enzymes have applications in the bioremediation of environments (Ruggaber and Talley 2006). Most of the enzymes applied in bioremedial techniques belong to the bacterial monooxygenases, dioxygenases, hydrolases, azoreductases, nitroreductases, aldo-keto reductases, dehalogenases, cytochrome P450 monooxygenases, and phosphotriesterases (Tanokura et al. 2015).

18.2.19.7 Enzymes Used in Molecular Biology

The discovery of the polymerase chain reaction (PCR) enzyme from the bacterium *Thermus aquaticus* (Taq polymerase) is an outstanding example of microbial importance in the production of valuable enzymes, which has revolutionized the world of molecular biology and genetic engineering since the late 1980s. Other critical enzymes with microbial origin in this area are DNA polymerases (EC 2.7.7.7), RNA polymerases (SP6 RNA polymerase purified from SP6 bacteriophage-infected *Salmonella typhimurium* LT2 and T7 RNA polymerase produced by the T7 bacteriophage), ligases, nucleases and restriction enzymes, phosphatases (EC 3.1.3.1, purified from *Escherichia coli*), methylases, and topoisomerases (Rittié and Perbal 2008).

18.2.20 Chemicals

Microbial biosynthesis or production of chemicals from microbial cell factories is an alternative route with several advantages for synthetic chemistry methodologies. In spite of being extensively environmentally friendly, only a few chemicals can be produced by microorganisms, and in some cases, the producing capabilities, including titer, yield, and productivity, are not satisfactory enough.

18.2.20.1 Organic Acids

Microbial production of organic acids through fermentation is a fast-growing area, which yields a variety of carboxylic acids, namely, acetic, lactic, citric, glyceric, glucaric, succinic, butyric, xylonic, fumaric, malic, itaconic, lactobionic, propionic, pyruvic, and adipic acids. Microbial platforms of production through microbial fermentation, in addition to low cost, renewable, or even waste feedstocks, are used efficiently for the production of value-added organic acids (Alonso et al. 2015). Organic acids, in addition to vast commercial and industrial applications, are emerging as novel building blocks for the synthesis of fine materials including pharmaceuticals, polymers, food additives, and different chemicals (Sauer et al. 2008). Table 18.12 summarizes major organic acids of great biotechnological applications that are produced in microbial species.

18.2.20.2 Alcohols and Polyols

Short-chain diols, such as 1,3-propanediol, 2,3-butanediol, and 1,4-butanediol, are building blocks for polyesters and other industrial chemicals. These compounds are naturally produced by a variety number of microorganisms, among them are *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae,* and *Serratia marcescens* (Celińska and Grajek 2009; Cho et al. 2014; Nakamura and Whited 2003; Zhang et al. 2010).

Table 18.12 Organic acids produced via microbial metabolism

Organic acid	Number of carbon atoms	Molecular formula	Main applications
Glycolic acid	C_2	$C_2H_4O_3$	Cosmetics and biopolymer precursor
Acetic acid	C_2	$C_2H_4O_2$	As green solvent, polymer precursor
Acrylic acid	C_2	$C_3H_4O_2$	Coating, adhesives, and detergents
Lactic acid	C_3	$C_3H_6O_3$	Food and pharmaceutical industry
Propionic acid	C_3	$C_3H_6O_2$	Chemical precursor, food and feed preservatives
3-Hydroxypropionic acid	C_3	$C_3H_6O_3$	Plastics, coatings, adhesives, and chemical precursor
Glyceric acid	C_3	$C_3H_6O_4$	Precursor of drugs, surfactants, and polymers
Butyric acid	C_4	$C_4H_8O_2$	Food additive and feed supplement
Fumaric acid	C_4	$C_4H_4O_4$	Polymer building block, food and feed additive
Succinic acid	C_4	$C_4H_6O_4$	Polymer building block and chemical precursor
Malic acid	C_4	$C_4H_6O_5$	Polymer intermediate and food additive
Itaconic acid	C_5	$C_5H_6O_4$	Coatings, detergents and polymer building blocks
α-Ketoglutaric acid	C_5	$C_5H_6O_5$	Chemical precursor for fine chemicals
Xylonic acid	C_5	$C_5H_{10}O_6$	Polymer precursor
Adipic acid	C_6	$C_6H_{10}O_4$	Nylon and polymer precursor
Galactonic acid	C_6	$C_6H_{12}O_7$	Detergents, solvents, and paints
Gluconic acid	C_6	$C_6H_{12}O_7$	Food additive and pharmaceutical ingredient
Glucaric acid	C_6	$C_6H_{10}O_8$	Detergent builder and polymer building block
Lactobionic acid	C_{12}	$C_{12}H_{22}O_{12}$	Cosmetics, personal care, and pharmaceutical products

Polyols or polyhydric alcohols are carbohydrates for which their carbonyl functional group is reduced to a hydroxyl group. Polyols are valuable compounds in the biotechnology area with a variety of applications in functional foods and nutraceutical production industry. Common commercially important polyols include sorbitol, arabitol, mannitol, xylitol, lactitol, maltitol, and erythritol. Xylitol (a naturally occurring five-carbon sugar alcohol) is produced from the microbial conversion of D-xylose by reported microorganisms, including yeast *Debaryomyces hansenii*. This sugar alcohol is a second-generation sweetener with many applications in the food industry (Dominguez et al. 1997). Erythritol (a four-carbon sugar alcohol) is also produced by microbial fermentation processes using various yeasts (such as *Torula corallina*, *Candida magnoliae*, and *Pseudozyma tsukubaensis*) and bacteria (Moon et al. 2010a).

18.2.20.3 Aromatic Chemicals

Aromatic natural products are a wide group of compounds possessing various applications and serving as building blocks for the synthesis of a vast range of chemicals. Traditionally, aromatic natural products are produced via chemical synthesis from petroleum-derived feed stocks; however, biological synthetic pathways in microorganisms are promising green alternatives. Phenolic acid derivatives (hydroxybenzoic acids, phenol, gallic acid, salicylic acid, muonic acid, caffeic acid, p-coumaric acid, rosmarinic acid, ferric acid), flavonoids (pinocembrin, naringenin, and eriodictyol), stilbenoids (pinosylvin, resveratrol, and piceatannol), coumarins (umbelliferone, esculetin, and scopoletin), and aromatic amino acids are among important aromatic chemicals obtained from microbial sources (Wang et al. 2018). In terms of valuable aromatic chemicals production, host organisms of *Escherichia coli* and *Corynebacterium glutamicum* and yeast platforms are mainly exploited (Noda and Kondo 2017).

18.2.20.4 Diamines

Diamines are another prominent chemicals originating from microorganisms. The compounds are utilized as monomers to synthesize co-polymerized polyamides. These important diamines are named 1,4-diaminobutane (putrescine) and 1,5-diaminopentane (cadaverine) (Benner et al. 2004; Ma et al. 2017; Nguyen et al. 2015).

18.2.21 Vitamins, Biofactors, and Co-enzymes

Several vitamins and biofactors are solely produced by organic chemical synthesis, however, several of these compounds are considered as microbial natural products. For example, β-carotene; vitamins E, K2, B1, B2 (riboflavin), B3 (niacin), B5, B6, B8, B12 (cyanocobalamin), B13 (orotic acid), and C (L-ascorbic acid); ATP; nucleoside and coenzymes (NAD, NADP, FAD, coenzyme A and Q, pyrroloquinoline quinone or PQQ); and S-adenosyl-L-methionine and S-adenosyl-L-homocysteine are produced by microbial biosynthesis (Vandamme 1994). Table 18.13 summarizes the microbial synthesis of water-soluble and fat-soluble vitamins and biofactors.

18.2.22 Biofuels

Microbial biofuels are highly biodegradable and a renewable source of energy including biodiesel, bioethanol, biobutanol, biomethane, biohydrogen, or bioelectricity obtained from either bacteria, yeasts, or microalgae (Ghasemi et al. 2012a; da Silva et al. 2014). Biofuels can be produced by many oleaginous microorganisms, including algae, yeasts, fungi, and bacteria. Metabolic engineering of mentioned organisms has made a great impact on the microbial production of biofuels.

Table 18.13 Microbial synthesis of vitamins and biofactors

Compound(s)	Source microorganism(s)
Vitamin B1 (Thiamine)	*Saccharomyces cervisiae* (bioconversion)
Vitamin B2 (Riboflavin)	*Ashbya gossypii*
Vitamin B3 (Niacin)	*Nocardia rhodochrous* (bioconversion of 3-cyanopyridine)
Vitamin B5 (Pantothenic acid)	*Rhodotorula minuta*, *Candida parapilosis*, *Rhodococcus erythropolis* (bioconversion of ketopantoylactone)
Coenzyme A	*Brevibacterium ammoniagenes*
Vitamin B6 (pyridoxine)	*Flavobacterium* spp., *Pichia guillermondii*
Vitamin B8 (H, biotin)	*Bacillus sphaericus*
Vitamin B12	*Propionibacterium shermanii, Pseudomonas denitrificans*
Vitamin B13 (orotic acid)	*Corynebacterium glutamicum*, *Brevibacterium ammoniagenes*, *Bacillus* spp.
Vitamin C	*Gluconobacter oxydans*
ATP	Yeasts, *Brevibacterium ammoniagenes*
NADP	*Achromobacter aceris*
Coenzyme Q	Bacteria, yeasts
S-adenosyl-L-methionine	Yeasts
S-adenosyl-L-homocysteine	*Psedomonas putida*, *Alcaligenes faecalis*
Vitamin D2	*Saccharomyces cervisiae*
Vitamin E	*Euglena gracilis*
Vitamin K2	*Flavobacterium meningosepticum*

18.3 Engineering Microbial Factories for the Production of Natural Products

There are many reports of successful genetic engineering of microorganisms, which is applied for the development of strains devoted to overproduction of natural products. In this context, engineering microbial cells for the biosynthesis of natural compounds of pharmaceutical significance is of great importance. Rapid growth and biomass accumulation, ease of characterizing, and isolating final products are the main advantages that lead microbial species to be used as producers of numerous valuable molecules including antitumors, antivirals, antibiotics, and many others (Jeandet et al. 2013). Superiorities of the production process in an engineered microbial system compared to the conventional chemical synthesis are summarized in environmentally benign route by avoiding the use of strong acids and bases, organic solvents, and heavy metal catalyzers.

Currently, among the different heterologous systems, *Escherichia coli* and *Saccharomyces cerevisiae* are the main production hosts for the biosynthesis of

almost all natural products of interest. In addition, other novel heterologous platforms, consisting of *Bacillus subtilis*, *Lactococcus lactis*, *Pichia pastoris,* and Chinese hamster ovary (CHO) cells, are emerging (Overton 2014).

In the field of organic acid production, engineered or so-called "tailored" microbial species with novel product-specific enzymes or metabolic pathways which are capable of producing unnatural bioproducts including glucaric acid and adipic acid, are reported (Moon et al. 2009; Moon et al. 2010b). In the field of medicinally important plant polypeptide biosynthesis, engineered microbial species also offer great opportunities. Accordingly, there are reports of genetically engineered microbes for the biosynthesis of antibiotics (rifamycin, erythromycin, and tetracyclines), anticancer drugs (anthracyclines and epothilones), antiparasitic agents (avermectin, artemisinic acid as the precursor of the antimalarial agent artemisinin) (Dietrich et al. 2009; Ro et al. 2006), therapeutic enzymes (urate oxidase and asparaginase) (Ghoshoon et al. 2015), cholesterol-lowering agents (lovastatin), hormones and immunological agents (immunoglobulin G antibodies, human interleukins, human interferons, and gonadotropin-releasing hormone) (Mazor et al. 2007; Medina-Rivero et al. 2007; Westers et al. 2006; Xu et al. 2006), and immunosuppressants (rapamycin) (Horinouchi 2009).

Novel techniques of metabolic engineering, procedures of generating high-quality libraries of enzyme variants, and high-throughput screening (HTS) technologies will pave the way for the engineering of enzymes and proteins in favor of the biosynthesis of various compounds with potent biological activities (Shivange et al. 2009; Yang and Withers 2009). Specifically, HTS techniques can swiftly lead to the identification of genes involved in the modulation of a particular biosynthesis pathway. Convening all genes encoding for a biomolecular pathway will make the assembly of genetic constructs for the synthesis of a given product possible. New methods for the facile and prompt cloning of single genes together with the availability of synthetic operons such as bacterial operons (generally used in the biosynthesis of many medically and pharmaceutically valuable compounds) have accelerated the construction of synthetic multigene pathways (Blanusa et al. 2010; Shao et al. 2009).

As a final point, systems biology, metabolic engineering, and "omics" technologies (genomics, functional genomics, and metagenomics) have shed new light on the protein and biomolecular pathway engineering. These new methodologies will thus pave the way for very important progress in the metabolic engineering of microbial cell factories (Jeandet et al. 2013).

18.4 Concluding Remarks

It is worth mentioning that obviously there remain many potential natural product producers to be screened and various natural products to be isolated and characterized. And in this context, new genome-guided discovery efforts are promising means to unravel valuable natural products from different sources. Recent efforts based on novel genomic technologies, bioinformatics tools, and comparative

metabolomics have also demonstrated that hard-to-culture or uncultivable microorganisms can be regarded as precious resources of new molecular targets that were missed to be discovered in previous surveys of underexplored resources. Despite this fact that a significant number of natural product substances are actually produced by microbes, it is considered that this area of natural product research should be expanded significantly.

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Systems and Synthetic Biology Approach to Understand the Importance of Host-Pathogen Interaction

19

Ashish A. Prabhu and V. Venkatadasu

Abstract

In this chapter, we have discussed the basic factors required to understand the systems biology of host-pathology interaction, which can be applied for modeling and simulating the interaction between plant and pathogens and to get an idea about drug discovery and metabolic engineering. Further, we highlight the high-throughput technologies, such as omics technologies (genomics, transcriptomics, proteomics, and metabolomics), which can be used as a tool for identifying molecular mechanisms of the cell and biochemical pathway of the host-pathogen system. Several mathematical models, such as genome-scale metabolic modeling (constrain-based modeling) and interaction-based modeling (e.g., gene regulatory networks and protein-protein-based interactions) have been demonstrated which help in understanding the genotypic-phenotypic relationship of the host-pathogen interactions.

Keywords

Systems biology · Host-pathogen interactions · Omics technology · Metabolic modeling

19.1 Introduction

In the present scenario, the major question is how to address the cause of crop yield and field stock infection, which is impacting the economy worldwide. Recent studies have shown that the amount of these infections may increase even more due to

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global warming. Several new variants of microorganisms, including viral, bacterial, and fungal pathogens, can find novel hosts and ecologic niches. Also by systems perspective, lack of understanding of the complex mechanism by which these pathogens evade the host defense machinery and adapt according to their lifestyle needs is evident. Hence, there is an absolute necessity to study the relationship between the host and pathogen in order develop suitable chemicals to reduce pathogenicity (Aderem et al. 2011). Over the past few decades, the advancement in technology has developed strategies for investigating the host-pathogen interaction on the scale of molecular levels by adapting various computational and analytical tools. With the outbreak of genome sequencing, various databases are present to show strains and variants of pathogens sequenced to date. At the same time, availability of vast data on population-level genetic variation for plant hosts offers a huge potential for the study of host-pathogen interaction.

Further to gain the insight into the pathogen virulence and how these pathogens rewire the cellular transcription and dynamics of protein networking of host systems (McDermott et al. 2011), several molecular tools, such as deep sequencing, high-throughput proteomics, and sophisticated interactome analysis, have been used (Peng et al. 2010; Niemann et al. 2011; de Chassey et al. 2008; Shapira et al. 2009; Mukhtar et al. 2011; Das and Kalpana 2009). During the course of evolution, the pathogens have developed a strong selection for the defense mechanism exerted by the host system and consequently adapt to their environment. It is very difficult to extract data through experimental observation of the host-pathogen relationship (Shi et al. 2006; Eriksson et al. 2003). In order to develop improved therapeutic agents, knowledge related to these interactions is essential. Previously most of the treatments, such as vaccines, antibiotics, and antivirals, were designed by exploiting the structural and molecular differences between the host and pathogen. However, most of the pathogens have developed resistance to antibiotics, which is again a major issue. Hence, periodic development of novel methodology based on the study of these pathogens to develop novel therapies is of utmost importance. The schematic of the PHI modeling system is depicted in Fig. 19.1.

19.2 Systems Biology as a Tool

The deeper understanding of the complex biological systems is very crucial in predicting the pathogen-host interactions (PHIs) (Durmuş et al. 2016). Systems biology helps to assemble a framework for models of biological systems for systematic measurements. It is an interdisciplinary field in life sciences integrating engineering, mathematical, bioengineering, medical, and computational disciplines to understand the nonlinear behavior in biological systems (Kitano 2002; Durmuş et al. 2015). Previously, reductionist approaches were used to understand the biological systems which consider only fewer molecules of interaction, whereas systems biology uses holistic approaches based on omics data, which gives the overall view of the interactions between protein, nucleotide sequences, ligands, and metabolites in PHIs. Further, noncoding RNAs and small molecules play a crucial role in

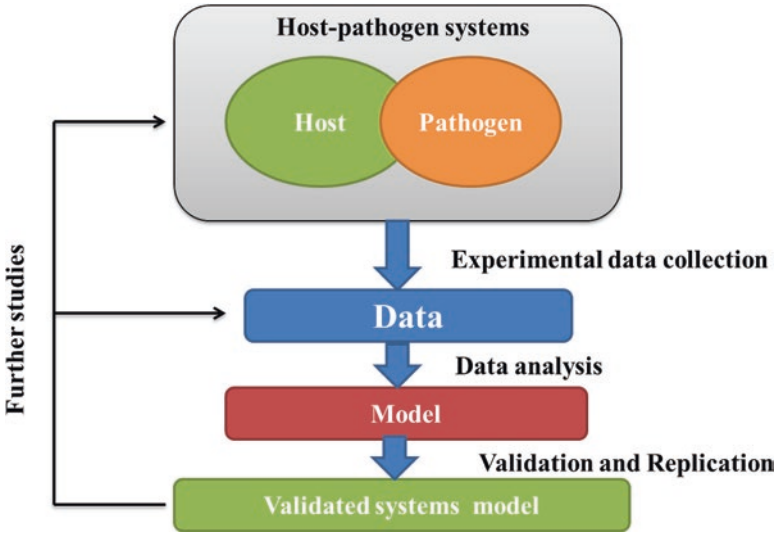

Fig. 19.1 Schematic modeling system for pathogen-host interaction (PHI)

understanding virus-host interactions and bacterial-host interactions (Durmuş et al. 2015; Raja et al. 2017; Likić et al. 2010).

It is very important to understand the biochemical networks of the system (viz., gene regulatory network, protein-protein interaction network, and metabolic network), which helps in deciphering the systems studies on biochemical subnetworks or cross-networks. Integrating the information from various biological levels provides complex and unanticipated global behavior of PHIs (Durmuş et al. 2015, 2016). The biochemical networks give the idea of how each component in the system behaves in the spatial and temporal ways and also how precisely the controls are excreted on them. The metabolomics approach makes it possible to precisely measure the metabolite concentration, whereas the transcriptomics and proteomics approaches provide the quantitative data of mRNA and protein levels, respectively (Karahalil 2016). Experimental approaches to assess in vivo reaction rates (fluxes) are again important parameters and are well developed to ascertain metabolic networks. The metabolic flux helps in determining the genotype-phenotype relationship (Antoniewicz 2015; Chen and Shachar-Hill 2012; Deidda et al. 2015). The omics data collected from infected cells and pathogens will be subjected to bioinformatics analysis to construct an infection-specific gene regulatory, metabolic, and protein-protein networks. The analysis of PHI omics data using computational systems biology tool unravels the infection mechanism, dynamics, and potential drug targets for the prevention of infections. Recently, web-based databases are available to accommodate the increasing data generated in PHI experiments, and also, they provide pathogen-host interactome data, which helps in focusing on specific pathogen or host system. Also novel text mining methods, which help in PHI data retrieval, are required (Durmuş et al. 2015).

19.3 Omics Technology: To Understand the Relationship between Host-Protein Interaction

During the 1920s, a botanist named Hans Winkler introduced a word genome by merging the words "GENe" and "chromosOME." It is known that omics involves a mass or a large number of measurements per end point. Today, more than 1000 omics fields are available for describing the properties of lipids, nutrients, etc. (Karahalil 2016; Antoniewicz 2015; Chen and Shachar-Hill 2012; Deidda et al. 2015). The generation of omics data through the application of high-throughput techniques and the data management and analysis via computational biology and mathematical modeling has brought the major revolution in the field of infection biology. A deeper insight of host immune response during infectious conditions gives an idea for the development of diagnostics, therapeutics, and vaccines. Also the systems biology of the infection led to the development of personalized medicines and novel therapeutic targets. The integrative personal omics profile (iPOP) combines genomics, transcriptomics, proteomics, metabolomics, and autoantibody profiles from a single individual over a 14-month period (Sarker et al. 2013; Chen et al. 2012).

19.4 Genomics and Transcriptomics Data for PHI

In genomics, the analysis of the nucleotide sequences, genome structure, and nucleotide composition will be carried out. Further this analysis helps in understanding the genetic variation among the individual and thereby providing the structure and functional relationship, their variants and diseases or response to therapy. Understanding the genetic variations helps in elucidating the genetic basis of diseases using genome-wide association study (GWAS) associated with genome linkage analysis and case-control studies with individual gene. To obtain the insight of this genetic information known as central dogma (DNA-mRNA-proteins), high-throughput techniques, such as microarray and next-generation sequencing (NGS), are being used. Further whole-genome sequencing helps to identify the type of pathogen and its nature of virulence, antibiotic resistance, and diagnosis and the development of new vaccines. A plethora of the literatures published show the relationship between gene polymorphism and disease susceptibility. Single-nucleotide polymorphism (SNP) can be used as an important tool for the identification and characterization of pathogen variants and disease susceptibility in plants and humans (McCourt et al. 2013; Yağar et al. 2011; Karahalil et al. 2011; Mardan-Nik et al. 2016). Over the past few decades, with the development of NGS, a large amount of genomic sequencing data are available in public databases. These sequencing technologies are capable of handling huge genome dataset in a timely and cost-effective manner. The phylogenetic studies based on whole-genome sequencing have helped in understanding the evolution of the PHIs and the possible prevention of infectious diseases. Metagenomics and metatranscriptomics of pathogens revealed how pathogenic microorganisms adapt to hosts, e.g., plants (Guttman et al. 2014).

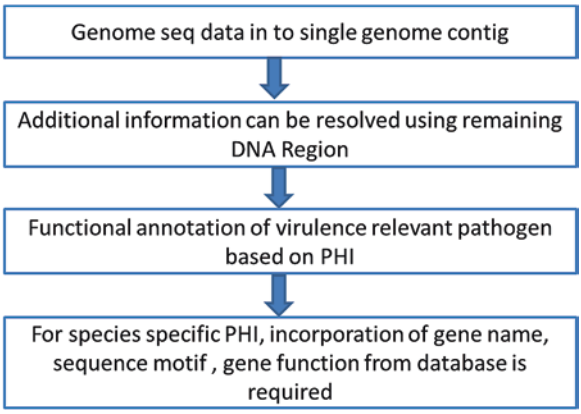

Fig. 19.2 Systematic whole-genome sequences procedure of PHI

The systematic whole-genome sequencing procedure of PHI is shown in Fig. 19.2. Whereas on the other hand, to get more insights into the evolution of pathogen, molecular pathogenesis and host specificity by using comparative genomics. Further NGS gives the molecular insight for diverse pathogens on genomic and transcriptomic levels (Fig. 19.3). Usually genomics is based on static data, whereas transcriptomics gives a dynamic profile of gene expressions with time. The genotype and expression phenotype can be linked through the through mRNAs match with particular genes in the genome (Karahalil 2016). The functionality differences between tissues and cells, interaction between genes, gene regulation and regulatory sequences, and identification of diseased states can be provided using RNA profiling (Durmuş et al. 2015). Some of the genomics and transcriptomics tools are provided in Table 19.1.

19.5 Proteomics and Metabolomics

The actual information related to metabolic and enzymatic processes can be obtained through a comprehensive study of the proteins. The characteristics of proteins and protein-protein interaction rapidly change cell proliferation and migration. Further characters, such as posttranslational modification, help to understand the dynamic proteome analysis (Wright et al. 2012; Larance and Lamond 2015). The protein structures and functional studies play a crucial role in PHIs as they can elucidate the role of the pathogens in eliciting the innate and adaptive immune responses. Pathogen-associated molecular patterns (PAMPs) are molecules or small molecular motifs within a group of pathogens (e.g., the protein flagellin, lipopeptides, lipopolysaccharide (LPS)) that are recognized by proteins, the so-called pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs (Qian and Cao 2013)). In many cases, the signal transduction is stimulated by PRRs via different pathways,

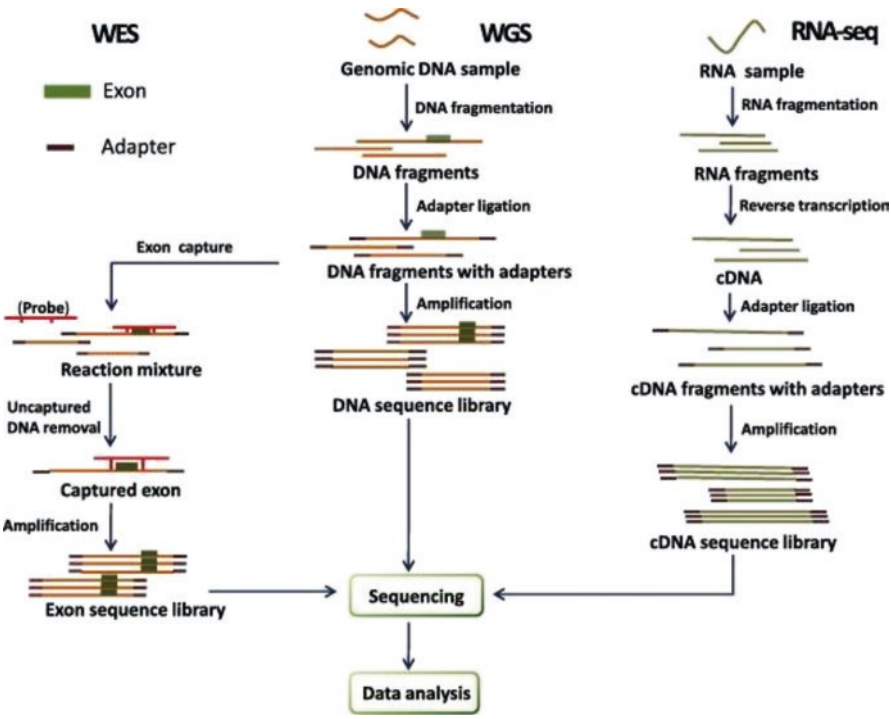

Fig. 19.3 Overview of next-generation sequencing technology used for sequencing PHI data

for example, JAK-STAT pathway, interferon gamma (IFNγ)-receptor pathway, and tumor necrosis factor-alpha (TNFα) signaling. During viral and microbial infections, the type II cytokines (IFN-γ) play a key role in innate and adaptive immunity (Prabhu et al. 2016, 2017, 2018). Transcription factor NF-κB also activated by various intra- and extracellular stimuli, such as bacterial or viral products, e.g., the TLRs signaling, and induces the expression of pro-inflammatory cytokines (interleukins, TNFα, Type I interferons) (Chen et al. 2012).

Utilizing bioinformatics as a tool for understanding the descriptive proteome analysis of the pathogen and its interaction with the host will give a better idea for designing the diagnostics and medicines. Several proteomics methods, such as mass spectrometry (MS), for protein and peptide analyses via, for instance, the matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI) techniques resulted in powerful MS instrumentations (Del Chierico et al. 2014). The detail of the techniques is mentioned in Table (19.1). Further the alteration to the environmental variations can be determined by estimation of metabolites, which are the end products of the cellular regulatory process. Because endogenous metabolites are fewer than genes, transcripts, and proteins, only fewer data can be interpreted. Hence, metabolomics has a great advantage over genomics and proteomics. The change in the metabolites reflects the biological states of organism. An in silico study, such as genome-scale metabolic models, utilizes metabolites to identify the

Table 19.1 Techniques used for genomics, transcriptomics, proteomics, and metabolomics and their applications

Omics technologies	Applications
Genomics	
RFLP ASO	Identification of single-nucleotide polymorphism (SNP) by Affymetrix SNP GeneChip and IIIuminaGoldenGateBeadChips assays, TaqMan assay
AFLP	Study on gene polymorphism
PCR RAPD DNA microarrays	Help in early diagnosis, treatment of similar disease, susceptibility to drugs, and variation among the individual
Transcriptomics	
Microarray, hybridization-based, sequence-based, Taq-based methods	Identification of metabolic pathway and drug response
Sequence-based, Taq-based methods (SAGE, CAGE, MPSS, etc.)	High-throughput techniques which provide gene expression profiles of organism
RNA-seq, whole transcriptome shotgun sequencing; WTSS EST SAGE	Predict absolute mRNA data and transcript profiles for better drug discovery
Proteomics and metabolomics	
Gel-based proteomics: 2DGE	High throughput (detection of hundreds of individual species within a single sample)
Gel-free proteomics: 2D-DIGE	Finding biomarkers for chronic diseases
TOF	Enable the analysis of proteins with low abundance in complex samples
MS, NMR spectroscopy MS-based proteomics: LC-MS,GC-MS, CE	Provide quantitative and comparative analysis of different samples

effective target of the drugs. One important PHI is the production of toxins by the pathogen that affects the host immune system. The fungus *Aspergillus fumigatus* which secretes gliotoxin induces apoptosis in host system. Systems biology-based models, including genetic regulatory networks (GRNs), help in understanding the uptake of important nutrients, such as nitrogen, carbon, and iron, by pathogens from the host system and how they regulate the biochemical network (Scharf et al. 2012; Gardiner and Howlett 2005).

19.6 Mathematical Modeling Assisting PHI Interaction

In the past few decades, the synthetic and systems biology field has witnessed a major paradigm shift with the availability of whole-genome sequencing for various organisms, which gave the whole picture of metabolic network, signaling and regulatory pathways in cells. For altering the metabolism of an organism, understanding

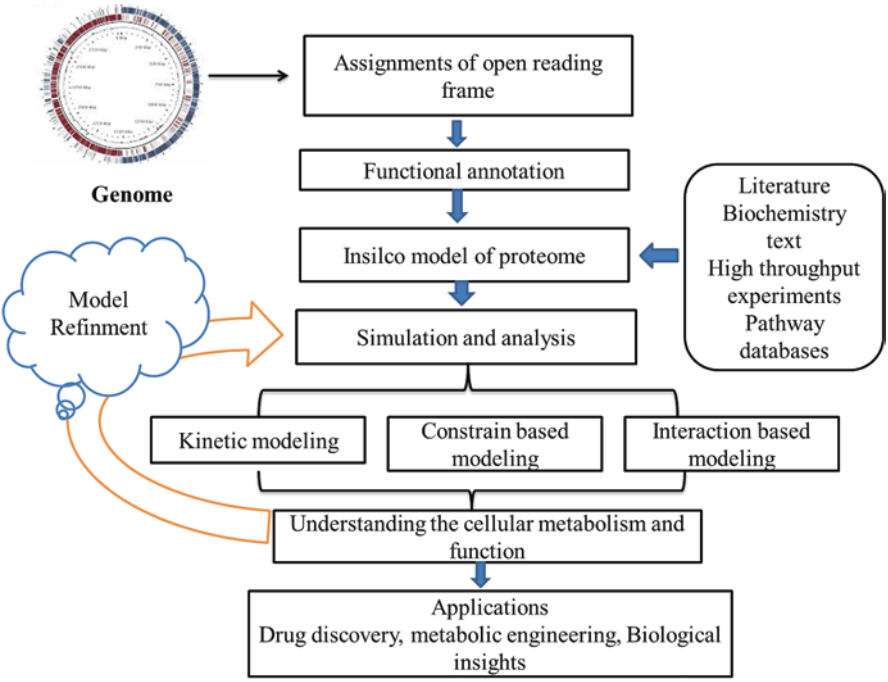

Fig. 19.4 Overview of the steps involved in designing metabolic modeling of organism

the cellular biochemical network is very much essential (Bose 2013; Chuang et al. 2010; Chae et al. 2017). With the evolution of systems-based approaches, a wide range of techniques were applied for the simulation and analysis of biochemical systems. The entire biochemical modeling can be classified into (i) constrain-based modeling, which relies on the reaction stoichiometry; (ii) kinetic modeling, which is based on comprehensive mechanistic modeling; (iii) interaction-based network (Raman and Chandra 2009). The steps involved in reconstruction of metabolic pathways are shown in Fig. 19.4.

Compared with kinetic modeling, which requires a detailed study for evaluating its parameters, constrain-based model offers a more precise quantification of genotype-phenotype relationship and hence is widely used in metabolic engineering (Antoniewicz 2015; Çalık and Özdamar 2011; Dai and Locasale 2016). In constrain-based analysis, the organism fine-tunes itself with the change in the environment satisfying the given constrain and achieves better survival capabilities. For in silico metabolic engineering, metabolic networks are simulated using constrain-based method and ultimately represent all biochemical networks in the organism. The metabolic network reconstruction may be focused on specific pathways/central metabolic pathways to encompass the entire genome leading to a genome-scale metabolic model. The reconstruction of genome-scale metabolic models involves various steps that includes (a) draft model creation, (b) detailed model reconstruction, (c) mathematical format conversion, (d) gap identification

and filling, and (e) simulation and visualization (Faust et al. 2011; Geng and Nielsen 2017; Kim et al. 2012).

In the PHI context, the pathogens are solely dependent on the host for getting the substrate, thereby maintaining the active metabolic state; hence, there is a continuous exchange of metabolites between hosts and plant pathogen (Orth et al. 2010; Kauffman et al. 2003). Also for the pathogenesis of an organism it depends on the availability of the nutrients in the host system there is a direct link between the metabolism and the virulence. Recently advanced version of bioinformatics tools for the reconstruction of metabolic network based on genomics data and constrain-based modeling, there in silico metabolic networks are very essential in understanding the physiology of pathogen for e.g. substrate availability in the host that decides the pathogenicity or the secretion of the toxins based on the host environmental conditions (Chavali et al. 2012; Eisenreich et al. 2013; Gouzy et al. 2014; Brown et al. 2008; Milenbachs et al. 1997). A constrain-based modeling of the Gram-negative bacterial pathogen, *Salmonella typhimurium*, showed a systematic metabolic modeling between the pathogens and the hosts (Raghunathan et al. 2009). The simulation of flux balance models for the reconstruction of genome-scale metabolic models answered the question of survival capabilities of pathogen. It has been shown that when the author used the media similar to the host cell, the model-predicting ability was superior. The author also showed that integration of transcriptome data with this flux analysis data led to a better understanding of transport mechanism. Recently, a dynamic flux balance analysis (FBA) model of a barley plant was constructed, which is capable of predicting the steady-state flux distribution of the metabolism of different organs throughout the entire plant development (Grafahrend-Belau et al. 2013).

19.7 Gene Regulatory Network Modeling in PHI

The phenotype of an organism is solely dependent on the gene expression, the gene regulation is an interconnection of regulatory circuits at molecular levels. The molecular mechanism includes controlling of transcription by transcriptional factors; RNA transporting, which is responsible for the posttranscriptional control of RNA; chromosomal remodeling; controlling of protein translation through signal transduction network; and posttranslational modifications, such as phosphorylation and acetylation (Thompson et al. 2015). Measuring the interactions between these molecular components is very difficult, but the advances made in the past two decades to precisely measure these components have enabled large-scale measurements of gene expression at steadily decreasing costs. With this data, the reconstruction of the molecular systems can be done using computational techniques, and the interaction underpinning patterns of gene expression can be easily interpreted (Vijesh et al. 2013). Interactions among the molecular components of the living systems are collectively known as gene regulatory network (GRN) models. Most of the biological models help in understanding the pathogenicity of the organisms,

ODE-based modeling are based on kinetic parameters describes PHI phenomenologically and does not consider the molecular mechanism (Hecker et al. 2009).

GRNs describe the logic of mode of infection by pathogens, adaption of pathogens to their hosts, and defense mechanism of hosts against pathogens. It is very difficult to reconstruct GRNs based solely on gene expression data. Proposed reverse engineering methods include those based on Boolean networks, Bayesian networks, differential or difference equations, and graphical Gaussian models that integrate gene expression data to better curate models (Hecker et al. 2009; Chai et al. 2014). In plant system, only few literatures based on GRN are available. Varala et al. (Varala et al. 2018) applied GRN to understand the temporal transcriptional logic underlying dynamic nitrogen (N) signaling in plant. The time series transcriptome analysis showed the dynamics of nitrogen signaling by a temporal cascade of *cis* elements. Recently, Ikeuchi et al. (Ikeuchi et al. 2018) used enhanced yeast one-hybrid (eY1H) screen to build GRN models, systematically showing the regulations between transcription factors and promoters. Also they showed that wound/hormone secretion invokes cross talks between genes and thereby regulates the common reprogramming-associated genes via multilayered regulatory cascades.

19.8 Protein-Protein Interaction Network Modeling in PHI

In recent years, the molecular structure and function of gene and proteins and their relationships are studied thoroughly, leading to a better identification of intra- and interspecies protein-protein interaction networks. Several characteristic features of PHIs, such as adhesion, colonization, and even invasion, can be interpreted through protein interaction map/protein-protein interaction (PPI) (Zhou et al. 2014). It has been observed that the PPI data used to predict the intra-species may not be applicable for interspecies host-pathogen PPIs. Several approaches of PPIs for understanding the PHI have been proposed among species. PPIs are broadly categorized into homology-based approach, structure-based approach, domain-motif interaction-based approach, and machine learning-based approach (Shao et al. 2012). Generally the protein-protein interaction network (PIN) is mathematically represented in the form of graphs where nodes symbolize proteins and edges connect the interacting protein pairs (Colizza et al. 2005). Interestingly it was observed that the datasets available for interaction show a similar nontrivial topological structure of the networks, defining a broad connectivity distribution P(k); i.e., the probability that any given protein interacts with k other proteins. This kind of pattern gives large hubs defining the nodes which have large number of connectivity leading complex architecture supporting nontrivial correlation and hierarchical features in network topology (Yook et al. 2004; Ravasz and Barabasi 2003; Maslov and Sneppen 2002). These features are shared among many biological networks that appear to have recurrent architectural principles that might point to common organizational mechanisms (Ravasz and Barabasi 2003; Dorogovtsev and Mendes 2002). A detailed review by Zhang et al. (Zhang et al. 2010) describes the importance of protein-protein interaction in the regulation of plant developmental, physiological, and

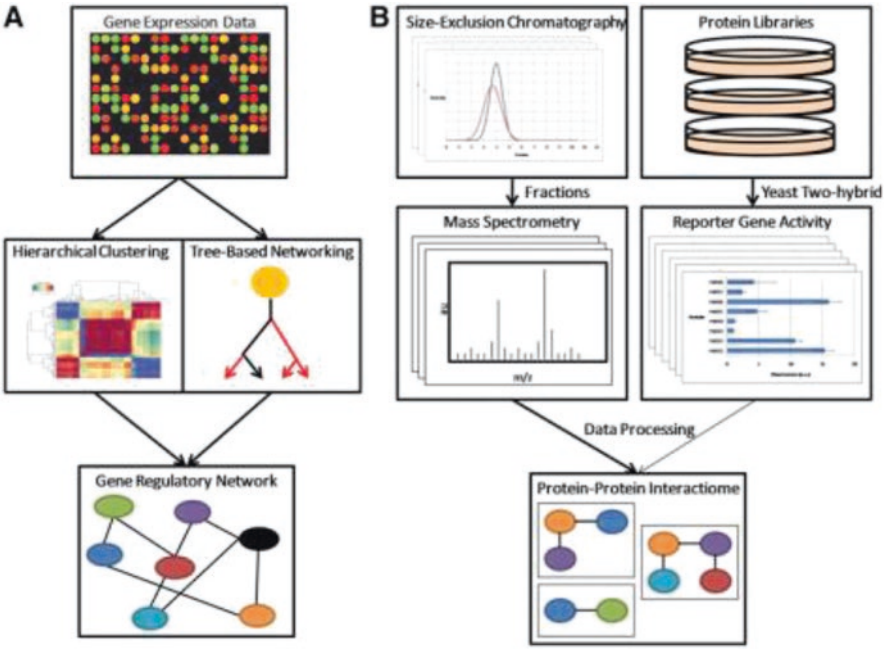

Fig. 19.5 (**a**) Gene regulatory network using gene expression data. (**b**) Protein-protein interaction modeling using proteomic data

pathological processes. Zhu et al. (Zhu et al. 2016) developed a protein-protein interaction database of maize plant. The architecture of gene regulatory networks and protein-protein interactions is shown in Figs. 19.5a and b, respectively.

19.9 Conclusion

With the advancement in omics technology, a huge amount of data is generated on genomics, transcriptomics, proteomics, and metabolomics. These data can be easily interpreted with computational biology techniques, which help in understanding the regulations between the gene and perturbation in the external environment. Further these tools are very useful in predicting the interactions between the pathogens and the hosts. With the application of flux balance analysis, it is possible to understand the genotype-phenotype relationship between the organisms. GRN modeling and protein-protein interaction-based modeling show the regulations of molecular mechanisms between the hosts and the pathogens. Systems biology has provided a better way to understand pathogenicity and drug discovery.

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Microbes-Mediated Nutrient Use Efficiency in Pulse Crops

20

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Abstract

Legumes are the major crops used in crop rotation practices to maintain soil fertility. Soil fertility is maintained mainly by microorganisms associated with roots either symbiotically or asymbiotically. Microbes have capability to fix atmospheric nitrogen (N_2) and enhance nutrient use efficiency by using a number of strategies like phosphate solubilization, potassium solubilization, mineral absorption, etc. Currently, use of microbial consortium (symbiotic as well as free-living) to increase nutrition use efficiency and activation of defense systems of plants is gaining importance. Microorganisms are eco-friendly, and their use is one of the best alternates of chemical fertilizers and pesticides. Additionally, efforts are also being made to develop transgenic plants for increasing nutrient use efficiency. These transgenes are mostly of microbial origin. The present review focuses on enhancement of nutrient use efficiency of plants by using either individual microbe or microbes in consortium mode. The review also discusses the strategies adopted by microbes to enhance use of nutrients from soil.

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Nutrient use efficiency · Microorganisms · Biofertilizers · Consortia · Pulse crops

20.1 Introduction

There is an urgent need of increase in food production to fulfil the need of ever-growing human population, without disturbing the environment and quality of food. The population of the world is continuously growing and is expected to be doubled by 2050 (Rubiales and Mikic 2015). The value of agricultural farm products increases when their nutritional content is embellished under natural environmental conditions in which they grow (Patel et al. 2015). However, poor farming practices and scarcity of land are the major causes of reduced nutrient sources in soil (Mmbaga et al. 2014). Modern agriculture is changing the concept of conventional agricultural practices for sustainability in agriculture, i.e., from "high input, high output" to "using less produces more." In the coming decades, one of the major problems is to meet the needs of the population without harming the environment and making a balance with natural resources (Shen et al. 2012). By sensing the scenario of present agricultural practices, there is a need for cropping systems which not only improves the production but also conserves soil fertility. Cropping legumes is one of the best agricultural practices because they fix atmospheric nitrogen, reduce energy, and improve soil physical conditions and biodiversity (Courty et al. 2015; Peix et al. 2015). Developing countries cover about 74% of the global pulses production, and the remaining 26% is covered by developed countries. If we consider country-wise production of the pulses, countries like India, China, Brazil, Canada, Myanmar, and Australia share 25, 10, 5, 5, and 4%, respectively. India has contributed 25–28% of total production of pulses and has the highest consuming population of pulse crops. India covers alone about 75% of the global chickpea (*Cicer arietinum* L.)-producing area (FAO STAT 2010). Some of the developing countries are still struggling to improve production, balancing application of nutrients and replacement of traditional varieties and therefore the main reason for poor production.

Use of effective microbial inoculants will be cost-effective and eco-friendly and is a renewable source of plant nutrients (Khan et al. 2007). Phosphate-solubilizing bacteria (PSB) and *Rhizobium* impart major role in N fixation and P solubilization (Tagore et al. 2013). Rhizosphere, the key zone of interaction between plants and soils, plays an important role in the uptake of nutrients from the soil. About 40% of the plant photosynthates are released in soil that provide a stable and strong base for the rhizosphere microbiome (Patel et al. 2015). The root exudates are good source of various organic nutrients, namely, organic acids, vitamins, mucilage, sugar, amino acids, nucleosides, phenolic compounds, and chemo-attractants. All these compounds play a significant role in attracting microbes and initiating the recycling process. Understanding the mechanism which governs the recruitment of microbes and their activity would be a great opportunity to enhance crop production (Sarma et al. 2015). Interaction in the rhizosphere between microbes and plant roots not

only influences the growth of roots but also influences the soil nutrient transformation, mobilization, and their efficient use by plants (Shen et al. 2012). Colonization of microorganisms around the roots may have neutralistic, symbiotic, associative, or parasitic relations within the plant. The relationship of microbes depends upon the status of nutrients in the soil, defense system of the plant, soil environment, and type of microorganisms residing in the rhizosphere (Verma et al. 2010).

A large number of studies reported the role of plant growth-promoting rhizobacteria (PGPR) inoculants for improving agricultural productivity and provided sufficient pieces of evidence to understand the basic mechanisms of interaction. According to their mode of interaction, PGPR can be classified as phytostimulators, biofertilizers, and biopesticides. However, some of them possessed both the characteristics, i.e., act as biofertilizers as well as biopesticides. Several mechanisms including nutrient solubilization, production of phytohormones, improvement in plant nutrition, and suppression of disease-causing organisms were reported for the PGPR over plant growth promotion. Microorganisms having the ability to improve nutrient uptake, increase nutrient availability, or stimulate plant growth are known as biofertilizers. Biofertilizers are the only alternative to complement to chemical fertilization for increasing production without harming soil fertility and environment (Mia and Shamsuddin 2013). A number of PGPRs have been reported to fix the atmospheric nitrogen, solubilize mineral nutrients, and mineralize organic compounds. Few of the PGPRs have better ability to be considered as biofertilizers in the sense of fixing atmospheric nitrogen as well as to solubilize phosphorus (Martinez-Viveros et al. 2010). Nitrogen, phosphorus, and potassium (NPK) are limiting nutrient factors for plant growth and play a crucial role in the physiological processes of the plant. Macronutrients are the main components for building a plant cell including genes and chromosomes (Mmbaga et al. 2014). Although concentrations of these elements are high in the atmosphere (nitrogen 78%), and soil (P 0.05%, K 0.03%), plants are not able to utilize them directly as nutrients and they remain in bind form or in complexes (Acharya et al. 2012). Beneficial soil microbes play a significant role in circulation of plant nutrients, which ultimately minimizes the use of chemical fertilizers. Supplementation with phosphorus, potassium, and rhizobial inoculants has prominent effect in improving nutrient uptake, growth, yield, photosynthesis, and economic benefits in legumes. *Rhizobium* inoculation improves soil health by fixing atmospheric nitrogen (Mmbaga et al. 2014). The use of biological and organic fertilizers minimizes the use of chemical fertilizers and forms the basis of sustainability in farms (Mohammadi and Sohrabi 2012). One of the robust biocontrol agents and biofertilizers, *Trichoderma* spp. have the ability to solubilize a number of plant nutrients like Fe^{3+}, Cu^{2+}, Mn^{4+}, and Zn^{2+} which are found in the unavailable form in certain soils. An isolate of *Trichoderma* T-22 has been reported to produce siderophores that chelate iron by lowering the oxidation of metallic ions and increase the solubility (Altomare et al. 1999).

The proportion of potential yield achieved under mineral deficiency or availability is known as nutrient use efficiency (NUE). NUE is the product of nutrient utilization efficiency (NUtE) and nutrient uptake efficiency (NUpE), which is the combined result of nutrient assimilation efficiency (NAE) and nutrient

Table 20.1 Microbes reported in better nutrient uptake in different leguminous plants

Sl. No.	Microorganism	Uptake of nutrients	Crop name	References
1	*Rhizobium*, *Bacillus megaterium* subsp. *Phospaticum*, *T. harzianum*	N and P	Chickpea	Rudresh et al. (2005)
2	*Glomus mosseae* and *Acaulospora laevis*, *Pseudomonas fluorescens*	P	Soybean	Yadav and Aggarwal (2014)
3	PSM	P	Soybean	Sandeep et al. (2008)
4	*Trichoderma* species	K, Mg, Ca, and Na	Bean	Abd-El-Khair et al. (2010)
5	*Funneliformis mosseae* + *T. viride*	N and P	Mung bean	Sharma et al. (2016)
6	*Azospirillum*	P	Chickpea	Rokhzadi et al. (2008)
7	*Pseudomonas* and *Rhizobium*	N, P, and K	Mung bean	Kumar et al. (2015)
8	*Pseudomonas, Azotobacter, Bacillus, M. ciceri*	P and N	Chickpea	Wani et al. (2007)
9	*Glomus aggregatum*	Zn, Mn, Cu, Fe, and B	Soybean	Fattah (2013)
10	*Trichoderma hamatum*	N and P	Urd bean	Badar and Qureshi (2012)
11	*Glomus* sp.	Ca, K, Mg, P, Fe, and Si	Cowpea	Yaseen et al. (2011)
12	*Bradyrhizobium japonicum, Pseudomonas* sp.	N and P	Soybean	Argaw (2012)

remobilization efficiency (NRE) (Masclaux-Daubresse et al. 2010). NUE can be defined as the capacity of the plant to acquire or utilize nutrients and can be chosen to emphasize productivity or internal nutrient requirement of the cells (Gourley et al. 1994). NUE can be divided into two components. Component 1 describes the ability of the plants to extract the nutrient from the soil and their utilization efficiency, whereas component 2 tells us about the ability of the plant to convert the absorbed nutrient into yield (Mehetre and Mukherjee 2015). Generally, plant nutrient uptake occurs in the ionic form, and microbes can use both organic and mineral forms of the nutrients. Thus, microorganisms are predominantly required to complete any nutrient cycle (Kumar et al. 2015). Mehetre and Mukherjee (2015) reported the use of *Trichoderma* spp. in nutrient recycling and nutrient availability to the plants. Use of beneficial microbial inoculants can improve the NUE in soil where nutrients are present in unavailable form (Table 20.1) and help in sustainable development of the agricultural systems (Qureshi et al. 2009).

20.2 NUE and Free-Living Microorganism

The growth of a plant is affected through both direct and indirect means by the range of activities which is associated with PGPR (Fig. 20.1) (Sarma et al. 2012). Some PGPRs elicit chemical and physical changes in the plant defense system by ISR which in turn leads to suppression of plant diseases caused by various phytopathogens (Sarma et al. 2002). There are also reports of a phenomenon called "induced systemic tolerance" which is linked to tolerance from the abiotic stresses that include salt, temperature, and drought (Yang et al. 2009). The genus *Pseudomonas* is found most abundantly in the rhizospheric soil among the gram-negative soil bacteria (Bardas et al. 2009). A number of studies have been conducted to see the use of root-associated *Pseudomonas* spp. for plant growth promotion effect (PGPE) or their use as potential biological control agents. Endophytes may also stimulate plant growth directly by increasing nutrient uptake, enhancing plant biomass, producing siderophores and phytohormones (IAA), solubilizing phosphorus

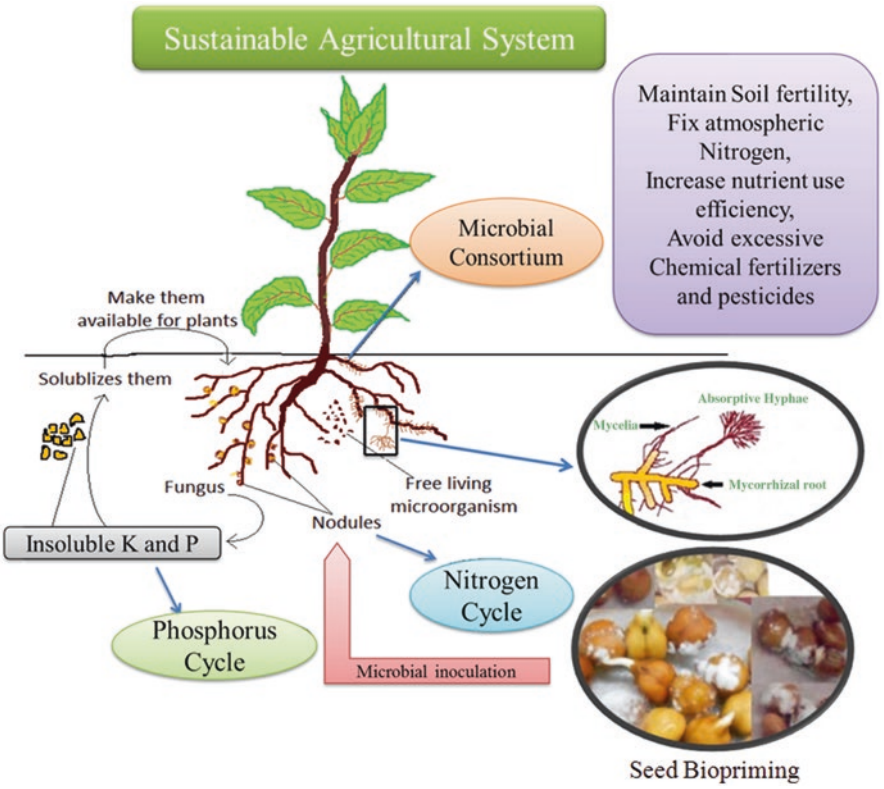

Fig. 20.1 Role of microbe in nutrient solubilization and make them available to plant: a sustainable approach in agriculture

(Lugtenberg and Kamilova 2009), decreasing heavy metal toxicity (Suranjana and Manas 2009), and fixing nitrogen (Yan et al. 2010).

Egamberdieva et al. (2013) reported use of *Pseudomonas* strains for enlargement of the root system and further enhancement of nutrient uptake, nodulation, and shoot growth of leguminous plants. Production of indole-3-acetic acid (IAA) from bacterial inoculants is mainly responsible for root enlargement (Tanimoto 2005; Tilak et al. 2006). Exogenous application of phytohormones on alfalfa (Gruodien and Zvironaite 1971) and groundnut (Srinivasan and Gopal 1977) also suggests their role in plant growth promotion and nodulation. A clear halo is formed around the colonies of both bacterial and fungal strains having P-solubilizing activity.

The second important key element as mineral nutrient after nitrogen is phosphorous in terms of quantitative plant requirement. Apart from *Pseudomonas* and *Bacillus*, *Serratia*, *Azotobacter*, *Xanthobacter agilis*, *Chryseobacterium*, and *Klebsiella* are some other bacterial P solubilizers reported (Vazquez et al. 2000). The P-solubilizing activity of bacterial strains is lost upon repeated subculturing, but that is not the case in the context of P-solubilizing fungal strains. In addition, soil fungi peregrinate longer distances more efficiently in comparison to bacteria which prove that they are worth as P solubilizers in soil. Greater P-solubilizing activity is shown by such P-solubilizing fungi compared to bacteria as they produce more acids compared to bacteria (Venkateswarlu et al. 1984). Some of the most potent P solubilizers among the filamentous fungi are *Aspergillus, Trichoderma, Rhizoctonia,* and *Penicillium* (Sharma et al. 2013). Due to N fixation and P solubilization, *Rhizobium* and PSB have greater importance in this stream. There are reports suggesting increased phosphorous availability in soil when efficient P-solubilizing strains such as *Bacillus megaterium* biovar *phosphaticum*, *Bacillus polymyxa*, *Pseudomonas striata*, *Aspergillus awamori*, and *Penicillium digitatum* were applied to crop's rhizosphere and soil (Tagore et al. 2013).

Many *Trichoderma* strains (*T. harzianum*, *T. asperellum*, *T. viride*, *T. virens*) produce volatile and nonvolatile antimicrobial compounds which help in colonization of *Trichoderma* on other pathogenic organisms. *Trichoderma* acquires a number of mechanisms for its biocontrol strategy like antibiosis, myco-parasitism, competition, and modification of the environmental conditions while promoting plant growth (Shakeri and Foster 2007; Reino et al. 2008). Development of plants is increased when seeds of pea are bioprimed with *T. asperellum* BHUT8 that increased germination of seeds in the initial step and protection of seedling emergence against the soilborne phytopathogens (Singh et al. 2016a). Pathogen requires efficient nutrient utilization ability for the nutrients available around the host for their successful colonization (Snoeijers et al. 2000). Abd-El-Khair et al. (2010) have reported that the effect of *Trichoderma* treatments is more on leaves of *Phaseolus vulgaris* than pods. However, an increase in macroelements like potassium, magnesium, and calcium and microelement like iron has been observed. These elements play a significant role in the plant defense system after pathogen attack. *T. hamatum* has been reported to increase the percent nitrogen in experimental crop *Vigna mungo* at 30th day (Badar and Qureshi 2012). By improving lignifications in the secondary cell walls, certain *Trichoderma* strains inhibit the invasion of *Fusarium oxysporum* f.sp.

ciceris in chickpea. Induced lignifications in chickpea plants by *Trichoderma* were also observed through histochemical staining, and upregulated expression of genes involved in lignin biosynthesis pathway was also observed (Meshram et al. 2019).

20.3 Role of Symbiotic and Endophytic Microorganisms in NUE

Soil microorganisms like *Rhizobium* and many other plant growth-promoting soil bacteria have been reported to enhance nutrient uptake constitutively and also influence the chemistry of soil (Dobbelaere et al. 2003; Bais et al. 2006; Lugtenberg and Kamilova 2009). Dorosinsky and Kadyrov (1975) reported not only increase in nodulation after seed priming with *Rhizobium* but also showed an increase in nitrogen uptake, growth, and yield response of pulse crops. Bambara and Ndakidemi (2010) have also reported an increase in the availability of nutrients to the plant by biological N_2 fixation after inoculation with *Rhizobium.* Nitrogen is one of the major nutrients needed by plant cells for maintenance of physical structure and genetic constituent of the plant and is involved in a number of growth and developmental processes for better grain yield (Graham and Ranalli 1997).

Tairo and Ndakidemi (2013) reported that *B. japonicum* and the combined use of phosphorus in cowpea (*Vigna unguiculata* L. Walp) have resulted in a significant uptake of NPK, Na, and other nutrients in roots, pods, shoots, and whole plant. After entry of the bacteria in plant roots, they get transformed into bacteriods which carry out the process of nitrogen fixation mediated through the enzyme nitrogenase as their primary function (Rees et al. 2005). A pink-colored nodule resembles efficient nitrogen fixation as the color is imparted by enzyme leghaemoglobin (Peix et al. 2015). The advantage which endophytic diazotrophs have over the root-surface-associated organisms is that they possess colonizing capacity in the interior parts of plant roots where they get established to a niche which is more conducive for effective fixation of N and its transfer to the host plant subsequently. Additionally, the free-living diazotrophs also promote growth and nutrition of the plants through various other mechanisms (Richardson et al. 2009).

There is an increase in the intracellular calcium (Ca^{2+}) levels at a very early stage of interaction of pathogenic, mycorrhizal, or endophytic microbes when the two partners are recognized by each other (Singh et al. 2011). The level of cellular Ca^{2+} is regulated tightly, and even a slight deviation in its concentration contributes to the information for activation of protein and signaling (Vadassery and Oelmüller 2009). The existence of arbuscular mycorrhizal (AM) fungi in most of the terrestrial ecosystems is well known (Smith and Read 2008). AM fungi have the ability to improve soil ingredients required for development of low-cost sustainable agricultural systems. By making micro- and macroaggregates, AM fungi can also check soil erosion (Miller and Jastrow 1994). AM fungi are basically obligate biological symbionts. They enhance the uptake of various elements, namely, P, N, K, Ca, S, Cu, and Zn, and produce glomalin too (Guo et al. 2012). AM fungi can increase the host resistance against soilborne diseases and enhance salt tolerance (Evelin et al.

2009) and sequestration of heavy metals (Tonin et al. 2001). AM fungi provide a lot of opportunities for sustainable development of the agricultural system. These are important for the area where nutrients availability is low because of their binding ability with soil particles and organic matter.

Recently, it has been shown that the inoculation of AMF as biofertilizers can be considered as an alternative of chemical fertilizer to meet nutrient deficiency (Halder et al. 2015). Farzaneh et al. (2009) have also reported the use of AM fungi to increase the growth by 43% in chickpea and nutrient uptake (Akhtar and Siddiqui 2007). Improved nutrient uptake, mainly phosphorus, has also been reported due to the colonization of roots by AM fungi (Farzaneh et al. 2011). The augmented nutrient content ultimately enhances the vigor and defense mechanisms of plants. When *Pseudomonas aeruginosa* PW09 (a wheat endophytic bacterium) was applied to the cucumber plants, it conferred increased protection against *Sclerotium rolfsii* and NaCl stresses through provocation of various defense responses in the plant that included augmented activation of antioxidant and phenylpropanoid activities (Pandey et al. 2012). The results were similar in chickpea plants when they were treated with two *Pseudomonas* strains (Cgr and S1) singly against *Sclerotinia sclerotiorum* infection and NaCl salt stress (Sarkar et al. 2014). Both of them were very efficient in reduction of the stresses through improvement in the proline content and activity of phenylalanine ammonia lyase.

20.4 Role of Microbial Consortia on NUE

A number of microorganisms such as *Pseudomonas*, *Agrobacterium*, *Bacillus*, *Burkholderia,* and *Enterobacteria* have been isolated from the root nodules of various leguminous plants including clover, alfalfa, soybean, and pigeon pea (Geetha et al. 2008; Zakhia et al. 2006; Kan et al. 2007; Li et al. 2008). Co-inoculation of nodule entophytes improved the plant yield and health under greenhouse conditions in the form of increased root weight and nodulation compared to inoculation with rhizobia alone (Bai et al. 2003). Co-inoculation of plant growth-promoting bacteria (PGPB) *Pseudomonas* with rhizobia has been reported to promote plant growth better compared to individual treatments (Chandra et al. 2010; Chanway et al. 1989). Similar results were also reported with *Bacillus* (Geetha et al. 2008), *Azospirillum* (Yahalom et al. 1988), and *Azotobacter* (Burns et al. 1981) with *Pseudomonas*. Parmar and Dadarwal (1999) have reported the use of *Rhizobium* spp. along with *Bacillus* strains for the stimulation of chickpea growth, nodulation, and N_2 fixation. Co-inoculation of *P. chlororaphis* Zong1 with *Mesorhizobium* sp. SQ1 has also been reported to promote plant growth (Zhao et al. 2013).

Combined inoculation of microorganisms also improves the nitrogen and P content in grain to that of their single inoculation. *Bacillus*, having highest solubilization efficiency (SE) and solubilization index (SI) capability, in combination with *Mesorhizobium* exhibited higher N and P content in the rhizospheric region. The proliferation of plant roots also occurred after the co-inoculation of microorganisms (Qureshi et al. 2009). Yuming et al. (2003) have concluded that IAA-producing

microbes enhances the N and P contents in *Glycine max* which ultimately enhanced the length and biomass of root. Co-inoculation of *Mesorhizobium* with P-solubilizing *Pseudomonas* and *Bacillus* spp. showed a significant increase in uptake of nitrogen and phosphorus. The combined use of *M. ciceri* RC4, *A. chroococuum* A10, and *Bacillus* PSB9 enhanced the grain yield after 145 days of sowing (Wani et al. 2007). The microbial consortium application and count (inoculum) are also very important phenomenon (Singh et al. 2016b). Application of a consortium consisting of three microbes, namely, *Pseudomonas fluorescens* OKC, *Trichoderma asperellum* T42, and *Rhizobium* sp. RH4, as seed bioprimers is very effective in enhancing crop yield and growth of chickpea and pea (Yadav et al. 2013).

Increase in various parameters, namely, seed germination, plant height, nutrient uptake, number of branches, nodulation, total biomass, and yield of chickpea, after combined inoculation of *Rhizobium*, a phosphate-solubilizing *B. megaterium* subsp. *phospaticum* strain PB and a biocontrol fungus *Trichoderma* spp. in chickpea plants has been reported. Co-inoculation of *T. harzianum* PDBCTH 10 with PSB and *Rhizobium* showed an increase in growth and yield parameters (Rudresh et al. 2005). A combined use of *Azospirillum brasilense, R. meliloti*, the obligate nitrogen fixers of alfalfa (*Medicago sativa* L.), with vesicular-arbuscular mycorrhizal fungus (*Glomus fasciculatum*) was found to be effective in plant growth improvement, increase in nutrient uptake, and abundance of the microsymbionts in the rhizosphere of alfalfa (Biró et al. 2000). As per another study, the microbial consortia consisting of two microbes, *Pseudomonas fluorescens* OKC and *Trichoderma asperellum* T42, leads to an added nutritional quality in edible parts of chickpea plants that include seed, pericarp, and foliage (Yadav et al. 2017). The consortium-treated plants exhibited increased accumulation of nutrients, namely, N, P, K, Na, and Ca, and an enhanced quality of nutrition, namely, total phenols, proteins, flavonoids, and carbohydrates, in all its edible parts. The partitioning of nutrition among the various edible parts of the chickpea plant was also very much evident in the microbial treatments in comparison to the uninoculated ones. So in this way, the consortium of microbes is capable of enhancing the dietary value which will ultimately be helpful in overcoming the problem of malnutrition as the seeds are consumed by humans and the pericarp and foliage (straw) are alternatives to the forage and roughage for the ruminants.

20.5 Conclusion

Improvement in NUE of plant systems is a major immerging concept for enhancing crop production to meet out the demands of ever-increasing population. However, maintenance of soil health is also important along with NUE. Chemical fertilizers have the ability to increase the available nutrients in soil, but their longer use can make soil less productive. Use of microorganisms either indigenous or isolated from anywhere else seems to be one of the promising alternatives. The present review mainly emphasized the use of microorganisms which may be free-living, symbionts, or consortia of microorganisms for enhancing agricultural productivity. The

importance of microorganisms is more in the soil where nutrients are present but in unavailable form to the plants. Biological N_2 fixation also improves soil fertility without harming the environment and ecological balance of elements in soil and atmosphere. Microbial inoculation along with phosphorus and potassium has constructive effect in improving the nutrient uptake, photosynthesis, growth, nodulation, economic benefits, and yield in legumes. Synergistic effect of microbial consortium has opened newer area to improve nutrient use efficiency of plants along with protection from a number of deleterious plant pathogens. Increase in the availability of N and P, the most limiting nutrients in legumes for plant growth, can be increased in soil by using microbial inoculants with legume crops. Thus, the use of microorganisms in various ways to improve and maintain soil fertility would be an effective alternate to chemicals and improve NUE in crops.

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Omics Data Integration in Microbial Research for Agricultural and Environmental Applications

21

Dhananjaya Pratap Singh and Ratna Prabha

Abstract

Essentiality of omics research clubbed with the bioinformatics data analysis has been perceived in a long time for the advancement of science and innovation. Bioinformatics finds a direct application in the crop improvement programs. The availability of complete genomes of microbial species, economically important crops, animals, and the whole environment (metagenomes) facilitated high-throughput studies for the opening of new avenues to improve crop programs. Different approaches, such as microbial and plant genome comparisons, genetic mapping strategies, and evolutionary analyses, involved in crop development programs are possible through bioinformatics data analysis. New genes, novel proteins and their functions, unique metabolites and their quantitative profile, and metabolic pathways generated from microbes, plants, and animals seemed to have yielded much expected values in terms of new targets or strategies for the development of crop plants in agriculture. Recent work on this subject helped us in dealing with such issues realistically and optimistically in a socially responsible way. Omics-aided research in microbial and plant sciences genuinely help us to consider that people are exploring novel scientific and technological systems to improve human health, human food and animal feed production, overall agricultural productivity, and environmental protection.

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Omics data · Agriculture · Bioinformatics · Microorganisms · Metagenomes · Genomics · Transcriptomics · Proteomics · Systems biology

21.1 Introduction

Agriculture is among the noblest acts on earth performed by the human civilization with the mission "to live and to let live." Ever since the beginning of the human evolution, access to food has remained central to every civilization, and only agriculture has fulfilled the need for food and feed. Agriculture encompasses multifaceted areas of life, including biological, physicochemical, ecological, social, commercial, economic, and livelihood-related activities to feed human population and, at the same time, sustain the environment (Green et al. 2005). Visibly, all human and animal life is directly dependent on agriculture being carried out by the farmers in the fields, but indirectly, agriculture hosts almost all kinds of biodiversity of plants, animals, microflora and fauna, and microorganisms (Emma-Okafor et al. 2010).

Biological diversity has three principal components: (i) genetic diversity comprising variation among different species; (ii) species diversity addressing variety of species; and (iii) Ecosystem diversity reflecting a variety of ecosystems (Turbé et al. 2010). All these components work in integration and balanced way to perform ecosystem function, and disturbance in any of the three components leads to a great loss to the overall ecology. Besides plant and animal species, which have the widest inhabitation on earth, soils constitute the basic hub for one-fourth of all living species on earth, including insects, worms and earthworm, ants, mites, termites, ground beetles and small invertebrates, nematodes, mites, pot worms, springtails, microflora and fauna, bacteria, fungi, protozoa, and other microorganisms (Phalan et al. 2011). These diverse organisms act as chemical engineers, biological regulators, and ecological managers to engineer biological dynamics of the soils for sustainable functioning of the whole ecosystem (Turbé et al. 2010). Agriculture all over the world is largely dependent on these basic factors acting on distinct spatiotemporal parameters, which may be influenced by the environmental and edaphic conditions, but, nonetheless, they can present a framework for the sustainable management options for agricultural problems.

In the last century, the global population grew fast to become quadrupled. Whereas in 1915 the world population was 1.8 billion, by 2015, it reached almost 7.3 billion and may even be almost 9.7 billion by 2025 (United Nations 2015). Following the rising pace of global population, the demand for food may increase from 59% to 98% by 2050 (Elferink and Schierhorn 2016). Therefore, to feed the people, farmers worldwide will be challenged to either increase productivity of food crops on the existing agricultural lands with the use of efficient farm inputs, including improved seed varieties, chemicals, fertilizers, potential irrigation system, and alternative agricultural practices such as precision farming, or bring more and more lands into agricultural practices to grow more crops (Foley et al. 2011). Although the ecological and social aspects of finding out more land for crop production have limitations, increasing crop yields per unit of cultivated land will satisfy the excessive demand for food worldwide (Ray et al. 2013). Statistical tracking of the global productivity trend of four major crops (maize, rice, wheat, and soybean), from

which almost two-third of the calories in the world come, resulted in interesting results. Data mining of a dataset comprising almost 2.5 million agricultural statistics collected across the world from over 13,500 geopolitical units indicated that the annual yield of these four top crops grew by 1.6%, 1.0%, 0.9%, and 1.3% per year, whereas the required rate to double global production by 2050 is 2.4% per year (Tilman et al. 2011; Ray et al. 2013). Different factors, like urbanization, industrialization, chemical and inorganic soil contaminants, climate change, lack of investments in agriculture and farmer's literacy towards improved agricultural practices and socioeconomic conditions of the rural communities are also imposing challenges over production of enough crops for food (Challinor et al. 2014). Other factors, such as water scarcity, rising global temperature, extreme and abrupt weather conditions, land areas under salinity and drought, and excess of water are posing problems for sustainable agricultural productivity.

Then where are the solutions for improvements? What are the potential options that can lead to improved productivity? We do not play any major role in controlling the changing global climate, except for adopting long-term programs to mitigate climate change. Also, we have only limited options to protect agricultural lands from industrialization and urbanization because of the great pressure of the fast-evolving developed society that needs better roads, houses, and other infrastructures. Further, agriculture in most parts of the world is dependent on natural rain which affects severely when rain is abrupt, and efforts to link every corner of the land with irrigation system is a money-intensive task that could be developed only with a slow pace. Therefore, looking into the present scenario, the search for the solutions of enhancing crop productivity by the management of genetic resources (crop plants, seeds, animals etc.), chemical and biological options associated with the agricultural fields, farm inputs, and exploitation of biodiversity would be key potentially viable and sustainable ways for the future agriculture (Kesavan and Swaminathan 2008).

Management and utilization of genetic resources of plants, animals, fishes, poultry, insects, microbes, and other organisms that are well adapted to climate change, tolerant to abiotic and biotic stresses, and fit to perform under adverse environmental conditions can strengthen agriculture (FAO 2010; Fujisaka et al. 2011). Natural habitats, e.g. soils, water represents the best custodians of the genetic resources that live, adapt, evolve and service there with their inherent genetic potential. Exploitation of intrinsic genetic potential of plants may lead to the production of high-yielding crops, disease-resistant and stress-tolerant varieties, high-value nutrient-rich products, functional foods, and bioactive metabolites that could be served to the society (Takeda and Matsuoka 2008; Spalding 2010). Similar qualitative and quantitative productivity enhancement options can also be followed for producing other agricultural produce, whether it is of animal origin or microbial origin. The genetic resources associated with the biodiversity of the agriculturally important and entwined organisms, e.g., microbes, microflora, and fauna, also pose a great influence on the crop productivity by strengthening crop plants, upper-layer soils and below-ground soils, and edaphic environment and its interaction with the soil.

Over the past few decades, molecular biology and omics studies covering many spheres and aspects of genomes, transcriptomes, proteomes, metabolome, and

phenome have paved new ways to inspect the holistic biology and functional characteristics of the organisms (Thottathil et al. 2016). *Omics* studies usually refer to the global utilization of high-throughput techniques in molecular biology and their applications for deciphering the holistic view of biology. Genomics addresses sequencing technologies that are dedicated for decoding genetic codes within the DNA of living organisms. Likewise, transcriptomics, proteomics, and metabolomics present deeper insights on the functional behavior of organisms by improving our understanding of key biosynthetic processes and molecules (genes, proteins, and metabolites) through which organisms respond to their environment and communicate with other organisms. Researchers across the world are deciphering the interface of theoretical principals and crop biology in the areas as diverse as genome sequencing and analysis; population genetics; evolutionary diversification among the organisms; adaptation mechanisms; studies in protein dynamics, interactions, identity, modeling, simulation, and networks; characterization of functional role in systems biology; analysis of communities and interactions in the habitats; and identification of metabolites having prominent functions in the biology and ecosystem (Paterson et al. 2010; Proost et al. 2011; Smith et al. 2011; Wendel et al. 2016).

In a more collaborative manner, these techniques including bio-imaging and visualization studies, molecular systems biology and network analysis, functional and comparative genomics, epigenomics, transcriptomics, and metabolomics can facilitate a deeper understanding about the crops and their associated environment, which possess transformational values. In the past decades, such complex studies have generated a huge data, the decipherization of which is yielding meaningful information that is beneficial for increasing crop productivity in a sustainable manner (Pichersky and Gerats 2011; Singh et al. 2011). However, there exists a major challenge to combine the high-throughput omics data from omics studies and apply in meaningful way. Deciphering crop biology at experimental and theoretical levels using omics strategies needs high-end computational support and cyberinfrastructure (Spalding 2009; Goff et al. 2011; Zivy et al. 2015; Thao and Tran 2016). Fortunately, for the analysis of the big data obtained from omics studies on crops, microbes, and crop-associated organisms, we are strengthened enough with the bioinformatics methods and computational tools to analyze, interpret, model, store, archive, and meaningfully use the data. Further, mining of this big data on crops and associated organisms may lead to a better understanding of environmental and biotic impacts on crops and development of improved varieties to support Second Green Revolution.

21.2 Agriculture Is a Living System

Among various biotic entities that majorly encompass agriculture, seeds, plants, animals, soil-inhabitant flora and fauna, and microorganisms are the major living drivers of the whole agricultural ecosystem. Their biodiversity and interactions within themselves and with the outside environment impact growth, development, and productivity of the crop plants and, at the same time, influence soil health and

fertility status (Kibblewhite et al. 2008). Such interactions may have both a negative (e.g., plant-pathogenic or pest interactions) or positive (e.g., plant-beneficial microbe interactions) impact on crop plants and may lead to reduced or enhanced productivity (Atkinson and Urwin 2012; De-la-Peña and Loyola-Vargas 2014). Besides, the microorganisms and microflora and fauna inhabiting the soils or associated with the plant system also constitute the most basic component of any agricultural ecosystem (Glick 2012). While working altogether in an integrated and balanced way, these components may substantially help to generate more food for the ever-increasing population.

Soil, the backbone of agriculture, is a multicomponent, multifunctional, living, and complex system. The expansion of the knowledge about soil biodiversity, especially the complexity of the underground life and its importance to the above-ground life-forms, including plants, microbes, and small flora and fauna, has tremendous applicability (Carey 2016). Findings indicate that more diverse soils with rich microbial communities of bacteria, fungi, actinomycetes, cyanobacteria, and microfauna, such as worms, mites, and nematodes, are healthier and improve the capacity of soils to hold more water and more nutrients and provide more minerals to plant roots (Wagg et al. 2014). A decline in the ecosystem functions, such as mineralization, nutrient fixation, retention and recycling, soil structure, organic richness, and plant diversity at the surface, is found directly linked with the soil biodiversity; it can directly impact the quality and quantity of crops that ultimately influence human health (Wall et al. 2015). Because biotic interactions, communications, exchange, and multifunctional associations of microbial communities in the soils are ever-going phenomena till the whole life of plants makes them more robust towards climate change (Crowther et al. 2015), such soils are climate-smart soils (Paustian et al. 2016). Soils with their huge biodiversity component persuade multifarious omics studies to explore microbial communities, their molecular networks, functions, interactions with plants, and abiotic factors. Molecular identification and characterization of traits of various microorganisms linked with improving the plant and soil health and development of microbial inoculants based on their functional characters to enhance agricultural productivity have benefitted agriculture (Adesemoye et al. 2009; Hayat et al. 2010).

In agricultural soils, individual beneficial microorganisms, e.g. PGPRs, symbionts, endophytes, and pathogens, and their functions are majorly identified (Barret et al. 2011). Still, much research is not carried out over the entire microbiome, despite of the fact that larger community of soil microbes are still unidentified but possess huge impact over soil fertility and crop productivity (Babalola 2010; Chaparro et al. 2012). In soils, the functions of microorganisms are coordinated to improve plant health, and the secretion of the plant roots shapes crop health and their community composition. In other words, plants recruit microbiome of their own choice as per the developmental stages, management practices, edaphic conditions, and neighboring communities in the rhizosphere (Chaparro et al. 2012).

Multi-omics studies, especially transcriptomics, proteomics, and metabolomics, have also helped greatly in deciphering genes, proteins, and metabolites that act as signals for chemical communicators and receptors in the plant roots and

microorganisms (Meena et al. 2017). Studies comprising metagenomics, metatranscriptomics, and metaproteomics improved our understanding on microbial partnerships and their functional roles and created deeper views on the characterization of microbial networks and their involvement in various biosynthetic pathways (DeLong 2013; Segata et al. 2013). Recently, metagenomics approaches have largely been used to decipher the unculturable complex microbial communities in the habitats. This has posed great challenges due to their complex interaction networks in different soils (Daniel 2005; Zhou et al. 2015).

Work on metabolomics for the unbiased characterization of biomolecules led to the characterization of biomolecules involved in the chemical biological aspects, biological networks, and interaction and helped in identifying signature small-molecule metabolites through which microbial communities communicate among them, with the plant roots and with their environment (Kuhlisch and Pohnert 2015). Based on such studies, biological and chemical activators (stressors) were identified which provide defense to plants against various kinds of abiotic stresses (Iriti and Faoro 2009; Perez and Brown 2014). Understanding how plants perceive chemical signals, such as volatile metabolites and small-molecule phenolics and flavonoids produced by the rhizosphere bacteria and fungi, and elucidating the molecular mechanisms that justify the ecological significance of such communications could potentially help scientists to enable farmers to grow hardier crops (Cossins 2014).

21.3 Omics in Agricultural Research Is Data-Intensive

Agriculture comprising microbes, plants, animals, and functionally live soils that inhabit macro- and microflora, fauna, and microbial communities is a live system. These living beings have their own genetic constitution: the genomes that make them structurally and functionally active to perform various ecosystem functions. This is how the data is integrated in the living agricultural system (Fig. 21.1). The interest in the genetic composition of plants, animals, and soil microbial communities led to the deciphering of the structural composition of different genomes and the analysis of the functional characteristics associated with their genes, proteins, and metabolites. Therefore, the root of the agricultural improvement is directly linked with the genetic alphabets of the living entities and their interactions that comprise the agricultural ecosystem (Bellard et al. 2012). The nucleotide alphabets of the complex genomes of plants, eukaryotic organisms, and even smaller genomes of prokaryotic microorganisms possess the key to document them phylogenetically, classify their evolutionary diversification, underpin the genetic mechanism behind their adaptation to environmental stresses, and uncover their interactions with other organisms (Koonin 2012).

However, crop genomes besides having economic and ecological significance are large, repetitive, and polyploidy in nature and, therefore, pose challenge for sequencing and comparative analysis (Paterson 2006). However, the advent of high-end sophistication in sequencing technologies through instrumentation and

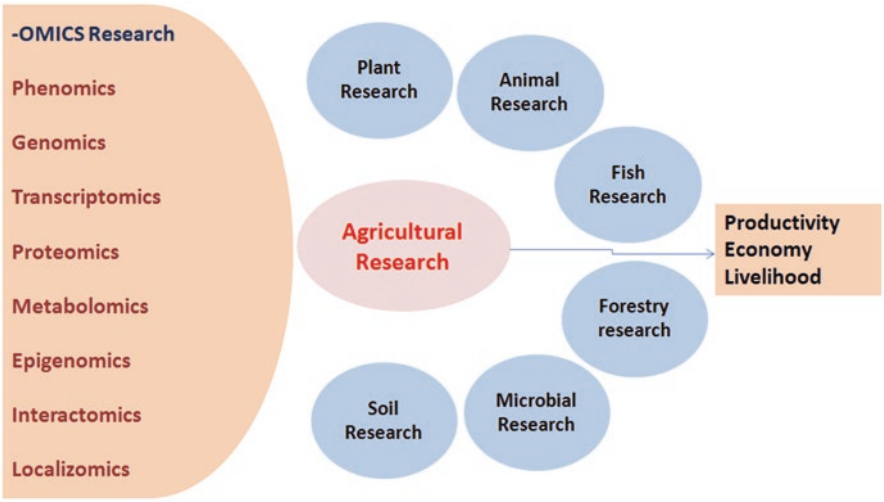

Fig. 21.1 Data-driven agricultural research

computational support has opened new scope and opportunities for omics-based studies of crop plants and associated agricultural organisms (Ehrhardt and Frommer 2012; Agarwal and Narayan 2015; Mba et al. 2012). However, scientists agree that besides ample opportunities, there are also challenges regarding sequencing technologies, computational biology, bioinformatics, data integration, storage and application, and big data analytics in supporting agricultural research (Emon 2016). Rapid and cost-effective sequencing technologies have changed the experimental way in plant research and made it possible to reveal genomic architecture of plant species, differential makeup of population genetics and targeted functional genes for specifically desired traits in crop plants to improve crop production, and better management of the associated environment (Pareek et al. 2011; Pingali 2012).

Technological revolution in the past few years has opened an unprecedented gateway of biological information covering sequence-based identity of genes, proteins, and their functions, phylogenetics, multidimensional distribution and localization of macromolecules, structure and function of small-molecule metabolites in cellular system, and mapping of specific genotypic and phenotypic traits, which has generated a huge amount of data for analysis and interpretation (Galbraith 2011; Barga et al. 2011). The perspectives that integrate applications of technological advancements in experimental biology with bioinformatics across all disciplines of biological sciences are considered as a "Fourth Paradigm" in science called "Data-Intensive Scientific Discovery" (Gray 2009). Genomes, transcriptomes, proteomes, and metabolomes of organisms comprise magnificent and voluminous biological information that constitute big data for biological studies.

21.4 Benefits of Omics-Driven Data Analysis in Agriculture

Bioinformatics is a data management science for restructuring biological information to obtain logical interpretations out of the data generated through omics efforts (Lai et al. 2012; Edwards 2013; Mehmood et al. 2014). Methods in bioinformatics basically include databases access and comparative information creation, confirmation, storage, analysis, and interpretation to yield meaning for the biological data (Vassilev et al. 2005; Singh et al. 2012). Bioinformatics analysis practically involves computational alignment techniques for the identification and annotation of genes in sequenced genomes, creation of mathematical modeling techniques (data mining, statistical analysis, neural networks, genetic algorithm, etc.) for functional analysis, method integration through tools and algorithms for information on gene hunting, detection of epigenetic variants, genome assembly and annotation, proteome analysis, gene expression analysis, and comparative genomics (Bansal 2005; Hu et al. 2011; Mehboob-ur-Rahman et al. 2016). Databases are becoming useful tools for searching specific biological information, research data analysis, downloading of large datasets for computational biology applications, data management, designing of biological experiments and tools, generation of *in silico* data, publication, data-sharing education and training, and resource integration (Robinson et al. 2010; Raza 2010; Marx 2013; Greene et al. 2015). This science standardizes the mutuality relationship of computational principles in biological systems (genes and gene products) to take information on biological system and processes (Narayanan 2005; Greene et al. 2015).

21.5 Omics Approaches for Crop Improvement

Agricultural production complementary to food security is a challenging task to address global climate change (Brown and Funk 2008). This further invites genetic advances for increasing crop productivity from the farms (Leegood et al. 2010). Breeding technologies underpin future enhancements in crop production and, coupled with the recent sophistication and advancements in the "omics" research, offer great opportunities to create massive datasets on crop species. Integration of genetic and phenotypic information using omics approaches clubbed with the bioinformatics leads to the identification of genes and pathways directly responsible for important agronomic phenotypes (Langridge and Fleury 2011). Here comes the role of genotyping technologies that help in the wide-scale high-throughput screening of germplasms to identify novel alleles from diverse from genetic resources (Fig. 21.2). This further expands our understanding on the genetic and trait variability available among the crop plants for incorporation into future breeding programs. Plant traits that are important for sustainable crop production are complex and multigenic, and high genetic variability in these traits makes them more difficult for breeding (Leegood et al. 2010). However, molecular breeding approaches simplified crop improvement programs by identifying the genes supporting these traits to incorporate them into new cultivars. A better understanding of the relationship between

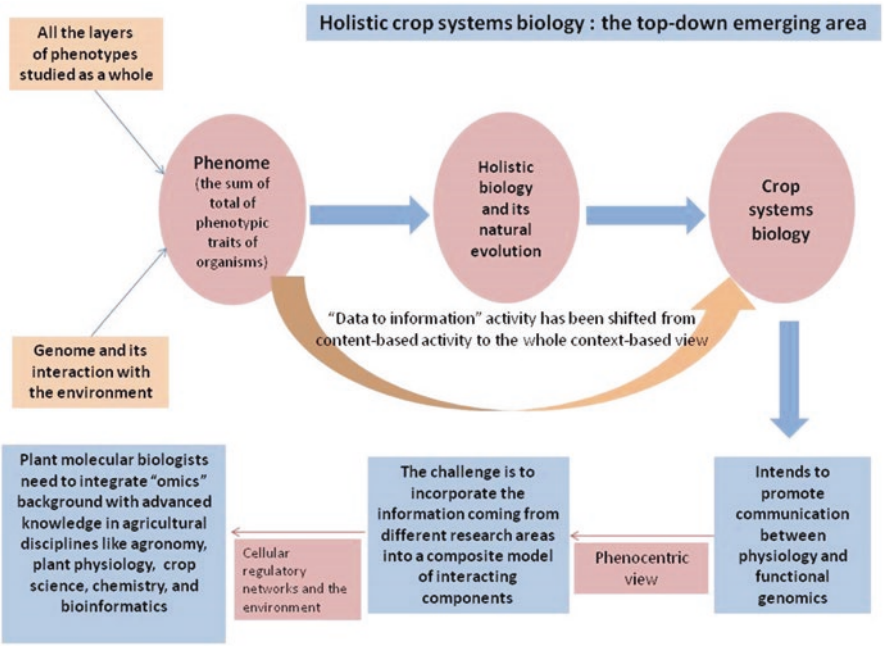

Fig. 21.2 Overview of bioinformatics and systems biology for crops improvement

genotype, component traits, and environment can be generated through a multidisciplinary way leading to the identification of candidate genes, QTLs, and traits underlying the processes that may lead to crop improvement (Parry and Hawkesford 2012).

Sequencing of many important food crop genomes opened a gateway to understand genetic diversity and genomic variability to improve crop varieties. Identification of the key genes regulating and monitoring important traits and comparison of genetic variations among the cultivars are now being facilitated with the access of the crop genome sequences. The knowledge gained and information obtained from the genomics, transcriptomics, epigenomics, and gene expression studies can help to develop new and improved crop varieties with more potential to produce and at the same time fight against stresses (Thottathil et al. 2016). Access to new genetic diversity pool is becoming a demand in agriculture to produce more food with high quality and more nutritional and health benefits for the growing population. Crop wild relatives (CWRs), close relatives of domesticated crop plants, are the rich gene pool that can be exploited as genetic resources for crop quality management and nutritional food sources. Genomics helps to characterize wild population, germplasm collection, and its conservation (Brozynska et al. 2016). Genome-wide analysis of wild plant species could yield novel gene pool for further exploitation as food resources (Brozynska et al. 2014). Genome sequencing of CWRs has revealed genetic diversity in their genomic constitution and will assist in

different, crop improvement strategies (Edwards and Henry 2011; Henry 2014a, b; Krishnan et al. 2012).

Expressed sequence tags (ESTs) are of great significance in crop genomics research because regardless of the complexity in plant genomes due to polyploidy or the presence of repetitive sequences, it can be applied to model crop plants (Mochida et al. 2008). ESTs represents robust sequence resources for the exploitation in gene discovery, genome annotation and comparative genomic analysis. Enormous ESTs and deep coverage genomic libraries were produced for barley, rice, sugarcane, maize, sorghum, and wheat in which genetic linkage conservation (collinearity) is widely recognized (Martin et al. 2005). Extensively expressed sequence databases and complete genome sequence can help in the identification of candidate genes, genetic analysis, and genetic improvements of crops. To assess the genomic differences between the members of Asparagales (asparagus, garlic, and onion) and Poales, including rice, Kuhl et al. (2004) generated 11,008 unique ESTs from a normalized cDNA library of onion. Sequence analysis revealed microsatellite markers, single-nucleotide polymorphisms (SNPs), and homologs of transposable elements, and analysis of ESTs and genomic feature showed strong differences between Asparagales and Poales. Physiological characteristics and genetics of melon fruits are important aspects to be covered under genomics programs for crop improvement (Nunez-Palenius et al. 2008). Construction of 11 full-length enriched and four cDNA libraries of different melon genotypes (fruits, flowers, leaves, roots, cotyledons, and calluses) revealed 71,577 and 22,179 ESTs, respectively (Clepet et al. 2011). Such studies provide a valuable resource for functional and comparative analysis that can be used for breeding improvements of melon and closely related species (Huang et al. 2009).

Peanut (*Arachis hypogaea*) is an important global crop for proteins and vegetable oil. The crop has lots of potential for genetic improvement. The peanut research community deposited 252,832 ESTs in NCBI EST database in 2011 (Feng et al. 2012). Further, Ranjan et al. (2015) identified certain stress-responsive candidate genes using peanut expressed sequence tags (ESTs). This resource is now facilitating as a valuable tool for genome-wide experiments on peanuts.

An analysis of 170,746 wheat ESTs resulted in a valuable data resource of non-anonymous molecular markers (Nicot et al. 2004). Among 492,832 ESTs available in the wheat database (http://wheat.pw.usda.gov/cgi-bin/ace/search/wEST), 36,520 (7.41%) had 43,598 eSSRs (Peng and Lapitan 2005). The eSSR markers developed by such studies were transferable among related Triticeae species, such as *Triticum aestivum*, *T. durum*, *T. dicoccoides*, *Hordeum spontaneum*, *H. vulgare*, and *Secale cereale*, *and are therefore useful for comparative genomic profiling, gene tagging, and gene cloning*. EST-SSRs markers provide important implications for the genetic analysis in wheat and related species (Peng and Lapitan 2005).

Rice is an important staple food crop for feeding more than half of the global population. Because of the worldwide importance of the crop, genomes of rice cultivars, including *japonica*, *indica*, and *aus*, were sequenced and annotated. *Sequencing of indica* rice enriched the global rice genomic data resources. This

helped in characterizing *indica* rice germplasm to identify genes of agronomic importance associated with yield, diseases, and pests (Mahesh et al. 2016).

Simple sequence repeats (SSRs) or microsatellite markers are short (1–6 bp long) repeat motifs with high levels of polymorphism and can be developed conventionally or from sequence databases (Thiel et al. 2003; Chen et al. 2006). SSR primers are already available for different crops including, barley, almond (*Prunus communis* Fritsch.), and peach (*P. persica* (L.) Batsch.), *T. aestivum*, and *O. Sativa.* These SSRs are useful molecular markers for developing an inexpensive way of representing transcribed genes and their putative functions by a homology search.

Comparative genomics on plants revealed that gene organization is highly conserved throughout the evolution. Omics-led bioinformatics studies for searching genes and their functions in the plant genomes helped in the gene discovery and incorporation of the desired traits for crop improvement (Mahalakshmi and Ortiz 2001; Mochida and Shinozaki 2010; Mehmood et al. 2014). Plant breeders are interested in the dissimilarities in plant varieties for developing improved crops with multiple benefits over the wild-type plants (Zamir 2001). Designing plants based on gene functions and regulatory networks to enhance tolerance to environmental stresses, growth, and development is important (Takeda and Matsuoka 2008). Molecular basis for particular traits is related with candidate genes (Flint and Mott 2001; Mackay et al. 2009). These information are available in different databases, such as Gramene (Liang et al. 2008), Gramene QTL database (Ni et al. 2009), ORGO (Yamamoto et al. 2012); LAILAPS (Esch et al. 2014), and Sol Genomics Network (SGN) (Fernandez-Pozo et al. 2015), which facilitate researchers in analyzing particular plant genomes with respect to gene sequence, putative function, or genetic map position (Tecle et al. 2010; Hassani-Pak et al. 2016).

Currently, a huge amount of data on DNA sequences and polymorphism of many crop plant varieties and cultivars is available in various databases (Pérez-de-Castro et al. 2012), which is useful for the detection of diverse cultivars along with their distances and similarities that are calculated by the polymorphism on a part of the chromosome with unidentified function (Govindaraj et al. 2015). Biological knowledge networks (BKNs) represent nodes that comprise genes, transcripts, proteins and proteins domains, biomolecules, biosynthetic pathways, ontology terms, networks, phenotypic traits, and literature resources (Liekens et al. 2011). A genome-scale knowledge network (GSKN) considers all genes of a genome of organism as nodes. Building crop-specific knowledge networks (CropNet) for barley, wheat, and other crops involves various steps, such as data integration, reference knowledge network from model species (e.g., *Arabidopsis* datasets) (RefNet), integrating crop-specific information (CropNet), and updating knowledge (Hassani-Pak et al. 2016). The GSKN for wheat (WheatNet) comprises almost 450 k concepts and 1.7 million relations, whereas that of barley (BarleyNet) contains 420 k concepts and 1.3 million relations, but the type and amount of data vary from species to species of crops (Hassani-Pak et al. 2016). The potential benefits of such data integration help to establish associations between distant characters (QTLs/traits) and link biological processes with the genes (Blake et al. 2016). The GSKN and text mining help scientists to link effective genes, such as barley gene MLOC_10687.2, with the biological knowledge discovery (e.g., seed size phenotype) (Hassani-Pak et al. 2016).

Therefore such user-friendly tools can improve our understanding on gene discovery on key phenotypic traits, such as yield, stress tolerance, and disease resistance.

21.6 Omics Studies Uncover Stress Tolerance Mechanisms in Crops

In the fields, crop plants continuously face different abiotic stresses (heat, cold, salinity, drought, radiation, and soil contaminants), which severely affect homeostasis and result in yield loss by as high as 50%. Plant's intrinsic biochemical, physiological, and molecular mechanisms evolved as a complex abiotic stress-tolerance trait, and its associated genes could be identified and functionally deciphered with emerging plant genome information. Being of multigenic character, deciphering crop molecular mechanisms to respond and adapt the stresses requires multidisciplinary and integrated approaches based on genetic, molecular, cellular, physiological, and developmental knowledge and information that influence tolerance to stress, including drought, which is among the most prime abiotic stresses in the world (Tuberosa and Salvi 2006).

Fast advancing knowledge on omics strategies are practically beneficial to fine-tune the molecular breeding and transformational programs for achieving crop improvement through knowledge on gene regulation (Ashraf 2010) and better understanding on the specific role of different metabolites and transduction of signals in plants (Valliyodan and Nguyen 2006). Defense responses to abiotic factors are regulated by the regulatory changes being activated due to several genes and pathways simultaneously or over time. Omics approaches, especially genomics, transcriptomics, proteomics, and metabolomics, have remained instrumental in addressing and dissecting multigenecity of stress response mechanisms in plants through genes and genome sequences, tissue-specific transcript pools, proteins, profiles of metabolites and intermediate products, dynamic changes associated with the biosynthetic routes, protein interactions, and observations on mutants (Bohnert et al. 2006). Experimentally, omics-led genome-wide expression profiling followed by the validation of the gene functions through mutants or transgenic analysis is extensively being used to identify genes associated with stress responses (Vij and Tyagi 2007).

Transcript analysis in rice (*Oryza sativa* L.) in response to high salinity condition was studied (Kawasaki et al. 2001). Induction of stress-responsive transcripts, followed by the transcripts related to defense functions and upregulation of transcripts (e.g., aquaporins) over a week, was observed. Upregulation and downregulation of thousands of DEGs in two rice genotypes, salinity-sensitive Nipponbare and salinity-tolerant Pokkali under high salinity stress conditions, were recorded (Jiang et al. 2013). Microarray analysis is used for study of expression of gene families which are involved in stress-responsive biological processes. Some gene families were preferentially regulated by high salinity stress. Comparative transcriptomic and metabolomic profile of rice seedlings under salt stress conditions led to the understanding of molecular mechanisms underlying salt tolerance in seedlings as

revealed by the phenotypic, metabolic, and transcriptomic analysis of two contrasting rice genotypes, IR64 and PL177 (Wang et al. 2016). The upregulation of several salt-specific genes related to important biological pathways provided combined genetic, metabolic, and transcriptomic evidence for improved salt tolerance in PL177 genotype. Further, different -omics studies had also assisted in identification, characterizationa and analysis of different genes, transcripts, proteins and metabolites of rice, which are involved either in stress-response or stress-tolerance mechanisms. Studies on heat shock protein genes (Zou et al. 2009; Ye et al. 2012), HAK potassium transporter gene family (Yang et al. 2009), genetic overlap of salt-tolerant QTLs (Zang et al. 2008), metabolites under chilling stress (Zhao et al. 2013), genes for acute dehydration (Minh-Thu et al. 2013), and low-phosphorus stress (Li et al. 2010) in rice revealed magnificent information that could be incorporated into the crop improvement programs leading to more stress-responsive and adaptive crops.

Similarly, tool kits like agriGO for the analysis of gene ontology in agricultural crops could also ease difficulties in finding out the genes and their pathways for comparative genomic and transcriptomic analysis (Du et al. 2010). Genetic and genomic tools for developing improved stress-tolerant wheat and maize are described in detail by Fleury et al. (2010). Analysis of global gene expression profile helps to identify differentially expressed genes critical for the heat stress response (HSR) in *Brassica rapa* (Dong et al. 2015). Gene expression in two Chinese cabbage inbred lines in response to heat stress revealed changes in 2142 genes in Chiifu and 1535 in Kensin, showing a distinct HSR in these species. Such data could help in developing molecular markers for heat stress in plants and engineered heat-tolerant crops. Genome-wide transcription profile of physic nut (*Jatropha curcas* L.) showed 1533 and 2900 differentially expressed genes in roots and leaves, respectively (Zhang et al. 2015). Also, the genes expressed in the droughted plants were found associated with the biosynthesis, transport, nucleobase-containing compounds, and cellular protein modifications. The upregulated genes in the roots were found to be associated with the synthesis of abscisic acid (ABA) and raffinose and signal transduction of ABA, whereas those in the leaves were related to ABA signal transduction and trehalose and raffinose synthesis. The genes and pathways identified in stress conditions can be useful for germplasm improvement and breeding for drought tolerance in *Jatropha* (Zhang et al. 2015). In chickpea, Garg et al. (2016) reported genotypic and development-stage associated molecular responses to drought and salinity through transcriptome analysis (Garg et al. 2016).

Overall, 4954 and 5545 genes, of which almost 47% were transcription factor encoding genes, were identified to be regulated in drought- and salinity-tolerant genotype of chickpea. Critical insights regarding key enzymes affected by drought/salinity stress, associated regulatory network, and transcriptome dynamics in chickpea in response to stress were obtained by such findings that may help in the generation of stress-tolerant chickpea varieties (Garg et al. 2016). Global gene expression analysis using high-throughput RNA-seq led to the identification of almost 3000 SR1-regulated genes that bind to promoters of several salt-responsive genes and act as negative regulator of salt stress (Prasad et al. 2016). Molecular mechanisms of tobacco root development under drought stress are not well understood. However,

genome-wide gene expression profile of tobacco roots generated over five million differentially expressed tags that resulted in 1476 upregulated and 1574 downregulated differentially expressed genes (DEGs) associated with 43 functional categories of 7 significant pathways (Yin et al. 2015). The study explores valuable molecular mechanisms that regulate root development of tobacco or other crop plants under drought stress.

Omics-driven bioinformatics facilitates better understanding of gene functions and helps to identify pathways which are involved in stress tolerance, development, and growth (Takeda and Matsuoka 2008; Mochida and Shinozaki 2010; Ma et al. 2012; Hu et al. 2015). It also helps to understand the complete prospective of post-genomic revolution in plant sciences and crop systems biology (Faccioli et al. 2009; Emon 2016; Thao and Tran 2016). It is now finding enormous applications in different areas of agriculture, specifically in the studies related to improvements in crop and plant resistance against pathogens, pests and stresses, nutritional quality of plants, plant growth and development in nutrient-deprived soils, and usage of remote sensing and GIS in agriculture sector (Atkinson and Urwin 2012; Birthal 2013). Mining valuable information from existing databases on crops using bioinformatics tools and translating such information in developing crop varieties with enhanced tolerance towards soil alkalinity, heavy metal toxicity, and other stresses can lead to an increase in yield and will be a milestone in the agriculture sector (Jewell et al. 2010; Fita et al. 2015). Such efforts led to the enhancement of the quality of crops or their molecular capabilities to perform better in severe environmental conditions (Bita and Gerats 2013; Silva 2015). This has further enabled researchers to develop and use pipelines for the prediction of genes linked with disease resistance, drought tolerance, and other specific traits (Xu et al. 2014; Sircar and Parekh 2015; Esposito et al. 2016).

21.7 Omics Research Support Soil Health Management Strategies

A rich diversity of microorganisms make soils live due to their critical and dynamic role in nutrient cycling, carbon recycling, soil structure make-up, ecosystem restoration, biodiversity functions, and plant interactions (Harris 2009). Therefore, the structural identification and functional characterization of microbial communities that regulate soil functions are among the important issues. Molecular taxonomy that evolved with the comparative analysis of ribosomal RNA (rRNA) sequences discriminated three primary domains: bacteria, archaea, and eukarya (Woese et al. 1990). The sequencing of small subunit ribosomal RNA (16S rRNA) gene of organisms isolated and cultured from different soil habitats, assists in their identification and classification (Rahendran and Gunasekaran 2011).

21.8 Bioinformatics-Driven Crop Research

Increasing the yield of agricultural crops has been the main concern all over the world in the last several decades (Pingali 2012). Advancements in the management of agronomic, edaphic, and soil parameters and strategies for the crop improvement have contributed significantly to the achievement of this goal (Edgerton 2009; Weekley et al. 2012). Both the agronomic practices and crop improvement based on genetic traits for particular intrinsic characters are presently entwined to deliver better yield, particularly when disease/pest control and tolerance to abiotic stresses are concerned (Dennis et al. 2008). Analysis of plant genomics has opened new doors to generate deep understanding on genetic structure, functional operations, and developmental patterns of crops under changing environment (Ehrhardt and Frommer 2012). By deciphering plant genomes, we now know the key gene functions throughout the various stages of the plants, regulation of repressing or stimulating gene networks facilitating morphogenesis of vegetative and reproductive tissues, and gene expression mechanisms when plants face biotic or abiotic stresses (Jackson 2006). Analysis of sequenced plant genomes enables us to identify the functional gene activities during plant interactions with pathogens and abiotic stresses (Atkinson and Urwin 2012; Meena et al. 2017), key genes enabling plants for their growth, development and productivity (Yuriko et al. 2014), gene networks for prominent biosynthetic pathways (Kim et al. 2012), and evolutionary diversification in crop plant species and their genetic cascades (Fucile et al. 2008; Meyer and Purugganan 2013; Andolfo et al. 2015).

In the past few decades, major challenges of growth and development and stress tolerance/resistance in plants are being addressed at the level of phenomics and systems biology that incorporated a holistic approach to resolve the problems associated with crop yield (Arvidsson et al. 2011; Rahaman et al. 2015; Chen et al. 2014b). Bioinformatics is now becoming a bridge between the data-driven omics science in plants and its translational manifestation for the field applications (Chen et al. 2014b). It not only offers an analytical platform on which problems of plant biology and phenology can be addressed but also acts to establish functional links between plant genome and phenome to create a complete genotypic-phenotypic map (Dalziel et al. 2009; Großkinsky et al. 2015). The newer tools and techniques in omics-driven science coupled with the bioinformatics are now becoming closer to connecting with agriculture to address the problems of crop responses to the environmental challenges and its mitigation strategies at genotypic and phenotypic levels (Chen et al. 2014a).

21.9 Bioinformatics to Decipher Microbial Role in Agriculture

Microbial communities are vital and integral components of plant and soil ecosystem. They successfully colonize roots, inhabit plant parts as epiphytes and endophytes, and architect soil health and fertility for mutual benefits (Farrar et al. 2014). On the other hand, their interactions with plants seriously impose threatening

diseases causing huge crop loses (Fletcher et al. 2006). Many essential ecosystem services, e.g., degradation and decomposition of wastes, soil sanitation, nitrogen and mineral fixation/solubilization, carbon sequestration, and water and air containment, are directly linked to microbial functions that support terrestrial biology, plant protection, and crop production (Aislabie and Deslippe 2013; Wommack and Ravel 2013). Similarly, the physical, chemical, and biological (organic) status of the soils is influenced by the microbiome and its structure and function (Lareen et al. 2016). Therefore, the role microbes play to maintain dynamic equilibrium and integrity of the agroecosystem is crucial for sustaining soils and maintaining plant health. The advent of next-generation sequencing technologies and improvements in the tools, techniques, databases, and software for bioinformatics data analysis have made it possible to decipher and annotate genome, transcriptome, proteome, and metabolome of prokaryotic (bacteria, actinobacteria, methylotrophs, cyanobacteria) and eukaryotic (fungi) organisms (Baldrian and López-Mondéjar 2014). Annotation of bacterial and fungal genomes has shown that many organisms possess potential for plant growth promotion, biological control of diseases, quorum sensing (QS), bioremediation, agrowaste decomposition, organic matter sequestration, degradation of soil contaminants, and production of small-molecule secondary metabolites (Chen et al. 2007; Milshteyn et al. 2014; Schmidt-Dannert 2015; Chan et al. 2015; Wang et al. 2016; Mukherjee and Roy 2016).

Determination of genome sequences and connecting their functions to decipher biological and ecological implications of whole genome of the organism is a challenging task (Zhou and Miller 2002). Additionally, genes in the genomes may encode for a number of proteins that interact and function in specific cellular processes. Annotation of genomes, identification of genes, characterization of gene functions, protein machinery, and regulatory networks are the tasks that may not be identified or defined without the applications of high-end computational data analysis (Singh et al. 2012). The applications of bioinformatics hugely support microbial genomics studies aiming at sequencing and comparatively analyzing genes, gene functions, and whole genomes of microorganisms (Chen and Pachter 2005; Zhulin 2015), proteomics studies that aim to identify proteins, establish role in metabolic and regulatory networks and characterize interactions and localization (Jensen 2006; Miteva et al. 2013), microscopy, cell visualization and simulation studies to understand cell behavior (Zengler 2009; Delile et al. 2016), combinatorial chemistry for microbial metabolites (Jung 2007; Kim et al. 2015) and development of antimicrobial/agrochemical agents and drugs from leads of microbial origin (Baker 2005; Brown and Wright 2005; Cragg and Newman 2014). Advances in area of bioinformatics tools and techniques along with availability of big datasets and information microbial genomics and transcriptomics projects are enhancing our understanding on the mechanisms of stress tolerance, growth promotion, disease control, bioremediation, environmental interaction, and adaptation in microorganisms. This is eventually useful for various applications in the fields.

Microbial functions in the ecosystem are conducted in complex, integrated, and intricate environment where communities interact and communicate among each other to perform ecosystem function. In communities, microorganisms are the key

players for the environmental sustainability, and therefore, it becomes challenging to decipher community structure and functions of the microbes (Tyson et al. 2004). Advancements in our knowledge expanded with the outcome of the isolation of community DNA from the environmental samples, sequencing the same and surveying the structural and functional genes that enable us to know about the microbial communities that can represent various ecosystem functions (Handelsman 2004). Metagenomics is a powerful technique which helps in integrating the genomic information obtained from the microbial communities about their structure and function and links it with the functional behaviors of the environmental samples (Thomas et al. 2012). The area involves a lot of computational exercise and tools to decipher the knowledge about the functional microbial communities in the soils and other environmental samples. This will help to develop genome-based microbial ecosystem models for stressed (saline and/or drought-affected), organic, polluted, and contaminated soils, microbe-based energy solutions and bioremediation practices, microbe-mediated management of diseases/pests in crop plants, and microbial community-based soil indicators for healthy soils (The New Science of Metagenomics 2007).

21.10 Application of Omics Approaches in Microbial Research

Microorganisms are the most primitive life-forms on earth. They are the key managers of the present-day agricultural ecosystem. They equally benefit the environment, natural resources, soil fertility, crop productivity, and public health by playing key role in facilitating valued ecological services and strengthening rural economy of the countries. Microbes are vital living components constituting a huge biodiversity that actually contributes to many of the functions of any live and sustainable agroecosystem that substantially performs well even under unfavorable circumstances. Diversification within the microbial communities and their strength in terms of their overall population can be witnessed by the fact that, even today, we are only aware about almost 1% of the total communities being a culturable population, whereas the rest of the microbial life-forms are non-culturable. Microbial cells evolved as complete cell factories performing thousands of chemical and metabolic reactions at a time to make themselves suitable for environmental pressure. With the possession of such kind of diverse metabolic diversity, microbial communities are fundamentally important for the functioning of the ecosystem, breaking down of complex animal and plant residues, detoxifying soil contaminants and chemical wastes, balancing soil nutrients, managing pest and diseases, and releasing essential minerals and natural products for plant growth promotion. Microbes always live in beneficial mutualistic/associative relationships with plants where their interactions benefit plants at several levels. They can be harnessed for producing valuable natural product molecules used as drugs for humans and animals, biocontrol chemicals against pests and pathogens, and bioremediator of environmental contaminants.

Technological revolution spurred by the most needed and timely required advancements in the biology of molecules, chemistry, biophysical sciences, and

agriculture has emerged in the past few decades. Since the advanced research techniques in the present day are mostly aided with computational tools to assist data generation, collection, mining, and analysis, the assemblage and interpretation of such data need bioinformatics and computational biological approaches to make interpretations of generated data forms. As we come across deeper insights into the basic biological mechanisms, their interaction patterns, and complex network behavior, we know processes that drive physiology and biochemistry of the organisms. This data-driven science can reflect manifestations of the impact of agroecological disturbance on agricultural productivity and climate change on crop pathogen and pest behavior. This can further widely address adaptation mechanisms in the organisms challenged to the stresses, microbial patterns of evolution, pathogen interaction with hosts (plants/animals), global carbon economy, and bioremediation of polluted soils.

The area of omics science encompasses all the segmented science into a holistic manner to address systems biology. Work carried out at global scale in genomics, proteomics, and other areas is creating lots of biological data. The number of whole-genome sequencing projects of prokaryotes and fungi along with proteomics studies is increasing constantly. Different research groups are involved in diverse omics programs like evolutionary diversification, environmental stresses adaptation, interactive biology of plant and microbes, plant growth promoting traits, biological control, root colonization, bioremediation, biofortification, rhizosphere community analysis, metagenomics, protein analysis and structure prediction, metabolic libraries preparation etc. Thus, very diverse nature of data is expected from these experiments. This will make it challenging for the people involved in handling, managing, annotating, analyzing, and storing such data for future reference and work.

With the large-scale developments in the faster, cheaper, and easier genome sequencing technologies, scientists are now becoming interested in opting for whole-genome sequencing projects at a very fast pace, and microbial genomes, being very small in size and easy to handle, are attracting the attention of biologists. However, all these genome and transcriptome projects and metagenomic- and metatranscriptomic-scale studies increasingly emphasize complementary pre-sequencing, functional genomics, and data analysis capabilities that will be required for taking up wide-scale system biology studies to trace out patterns in adaptation, evolutionary diversification, and benefits for the agricultural productivity.

Constantly fast emerging areas in this field, specifically for crop sciences and microbial research, can foster new developments in the future. Some of the examples are described here in brief.

21.10.1 The Data Connects Genotype to Phenotype

The structural and functional microbial communities derive from the interwoven matrix of biodiversity. This has evolved due to physical and chemical variations of habitats over time (Little et al. 2008). Although microbes occupy a central position in driving biosphere processes, our knowledge about ecological processes that

principally guide microbial community structure and function is limited. Predictive modeling encompassing a framework on complex interactions within and between species, evolutionary and ecological mechanisms in the habitats, and similarities and differences in microbial community ecology could be viable solutions to study and link functional attributes of organisms (or communities) with their structural behavior (Little et al. 2008; Larsen et al. 2015). For understanding the molecular basis of tough life style, climatic fitness, functional efficiency in diverse habitats, and environmental selection, the basic capability of inter-linked species to specific phenotypes needs to be addressed (Poisot et al. 2011). Comparative strategies for genes, proteins, or metabolites were needed to link particular gene, protein, or metabolite or their clusters for complex traits, such as plant growth promotion, biocontrol agent, colonization, associations, adaptive behavior, or climatic functions (Cadotte et al. 2011).

Wide-scale genome comparisons were performed to decipher plant interaction determinants, genetic variations in the rhizospheric or epiphytic microbial communities, and functional differences among species, e.g., *Rhizobia*, *Sinorhizobium*, and *Rhizobium leguminosarum* isolated from complex systems, such as soil samples, rumen, and plant root rhizosphere (Tian et al. 2012; Sugawara et al. 2013; Kumar et al. 2015; Seshadri et al. 2015). Phenotypic characterization was needed to take on organizational structure that regulates interactions among rhizospheric microbial communities (Huang et al. 2014) of metalliferous soils (Epelde et al. 2010), marine oil spills (Röling and van Bodegom 2014), and biomass-degrading and composting environment (Kong et al. 2011). Such studies on microbial interactions will pave the way for identifying and assigning functional characteristics to specific microbes or their communities. In the future, we need to evolve with advanced computational tools to determine or predict genes for specific traits, their functional network, and metabolic connections in microbial genomes and metagenomes to decipher function-linked microbial communities, their evolutionary pattern, and habitat-wise distribution in diverse environments.

21.10.2 Large-Volume Sequencing of Microbial Genomes and Metagenomes

Although microorganisms are critical to plant and soil health either due to their pertinent role as growth promoters, immunity developers, biocontrol agents, or plant pathogens, we know little about microbes at their genome level (Microbe Project 2001, National Science & Technology Council, Washington DC, 29 pp.). The microbial genome sequencing projects for individual prokaryotic and eukaryotic organisms are by and large prioritized, and many laboratories are expanding their work on sequencing novel microbes with good capabilities offering plant growth promotion, biological control, bioremediation, and agri-food processes (Microbial Genome Sequencing: Perspectives of the American Phytopathological Soc., https://www.apsnet.org/members/outreach/ppb/Documents/MicrobialGenomicsSeq08revisionfinal.pdf). However, since almost

99% of the microbial communities remained unrepresented till now due to the limitations in their culturing from the environment, environmental genomics or metagenomics came into existence to identify such communities and correlated the properties rendered by these communities in the ecosystem. Therefore, in the near future, massive-scale sequencing of not only individual microbes but also metagenomes from various habitats, such as soil, water, rhizosphere, and plants, would offer a great source of big data to be annotated and analyzed in the future.

21.10.3 Exploring Prospects of Single-Cell Genomics

Only a small fraction of the microbes are being made culturable in vitro. They represent a substantial bottleneck for exploring and exploiting the functional biology of a huge majority of microbial life. Most importantly, this includes a large number of microbes that are relevant to and needed for the benefit of agriculture, energy, and environmental applications. The inability to culture majority of microorganisms, especially in highly critical environments, can be resolved by the current single-cell technology, which allows recovery of genome and transcriptomes for uncultured individual prokaryotic and eukaryotic organisms. This can provide a link between phylogeny, metabolic networks, and expression activity. Single-cell technologies provide simplified datasets that will allow unprecedented insights into the biology of life. A major strategic target for the next few decades is increasing automation and streamlining of all the steps in single-cell pipelines with the goal of being able to handle huge number of single cells a day to generate simplified datasets and expand that to complementary systems-level approaches. The coupling of single-cell technologies with transcriptomics, proteomics, and metabolomics is expected to yield substantial improvements in the insights at comprehensive systems-level views.

21.10.4 Multidimensional Genome Annotation and Data Integration

Accumulation of huge data from the massive whole-genome and metagenome sequencing projects of prokaryotes and fungi enables the realization of the development of large-scale data processing, integration, pattern analysis, and functional annotation. In the coming years, many bacterial and fungal genomes of agricultural importance will be sequenced by the domain centers through in-house existing facilities or may be outsourced for the NGS data generation and also by many more collaborating institutions. All these scientific communities may seek help in genome or metagenome annotation and data integration for in-house whole-genome sequencing projects, for outside projects, and for all those available for agriculturally important microorganisms in public domain. It will also include data fusion strategies that involve employing integration and reduction of multidimensional data to improve analytical accuracy. These capabilities allow refinement of both structural annotation (the location of functional elements within sequences) and

functional annotation. Infrastructural facilities, machine upgrade, and human capabilities in such programs need to be enhanced to cater the needs for the future high-end genomics and metagenomics programs.

The generation of large amount of sequence data from genome projects is not a unique capability possessed by any organization. At the upstream, we need massive-scale and innovative sample procedures, whereas at the downstream, there is a need for more integrated informatics and strong linkage to functional annotation studies. Many of the most important scientific challenges in microbial biology, bioenergy, and environmental microbiology in the future will only be adequately tackled at large-scale facilities with multiple genomic capabilities and resources together with the support of multidisciplinary experts. Along with this, capable tool and algorithm developers, bioinformaticians, database designers, and data curators will also be required to join in the team of biologists to cater the needs for the next-generation sequencing.

21.10.5 Large-Scale Microbial Proteomics and Systems Biology Studies

Upregulation and downregulation of microbial and plant proteins during microbe-mediated interactions in biotic or abiotic stress conditions are among the major challenges in the near future. Such investigations will enable scientists to identify signature proteins responsible for many of the biological processes in interactive biology. Since proteins are the major biological products that drive organisms in the environment through the regulation of all the metabolic networks, their expression patterns can largely define phenotypic characters of the organisms, and thus, they are vital links in governing systems biology besides genes and metabolites. Therefore, such capabilities will be required in the future to enable us to develop, process, and interpret MALDI-TOF, MS-MS, NMR, or X-Ray crystallographic data and support biologists generating proteomic data for specialized purposes. Also, since this big data will require a huge space for its storage, infrastructural capabilities will again be required for proper storage and retrieval.

21.11 Conclusion

Omics-driven research entwined with the bioinformatics data analysis is now becoming key to resolving many biological questions pertaining to plants, animals, and microbial life. This data-intensive science finds a direct application in the crop improvement programs. The high growth in sequencing data generation mirrors expansive needs of large-scale systems-based science. However, to be useful to the researchers, such research must be accompanied by parallel improvements in the scale of our ability to process genomic and metagenomic samples for data analysis. Indeed, the complexity, extent, and measure of cross talks in biological systems are huge, but simultaneously, we need to become more knowledgeable and able to start addressing significant issues of global agriculture and environment.

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