

# Chapter 8

## Plant Growth-Promoting Microbes: Contribution to Stress Management in Plant Hosts



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### Contents

Introduction.....	200
Conventional Methods to Deal with Biotic and Abiotic Stress.....	200
Traditional Agricultural Practices.....	201
Conventional Breeding Techniques.....	202
Conventional Biotechnological Interventions.....	202
Why the Need for Alternatives to Conventional Methods?.....	204
Plant Growth-Promoting Microbes.....	205
Role of PGPM in Dealing with Plant Stress.....	207
How Do PGPM Help in Fighting Biotic Stress?.....	207
How Do PGPM Help in Fighting Abiotic Stress?.....	222
Other Strategies of Dealing with Abiotic Stress.....	226
Genetic Modification to Improve PGPM.....	228
Future Prospects and Need for More Intensive Utilization of PGPM.....	229
References.....	231

**Abstract** Plants encounter various challenges that impact on growth and development. In the agricultural scenario, any limiting condition can transform into serious economic losses. Conventional methods employed to deal with biotic and abiotic stresses, including chemical methods, plant breeding, genetic engineering and other modern practices, present a variety of practical concerns. For example, transgenic plants can lead to selection pressure on the parasites thus providing a means to develop resistance. Hence a shift towards exploring the potentialities in plant growth-promoting microbes (PGPM) as a part of mainstream agricultural practices is imperative. In this review, we focus on PGPM (inclusive term for plant growth-

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promoting rhizobacteria and fungi), which, apart from their plant growth-promoting activities, also play a role in plant diseases control as well as in alleviating the impact of abiotic stresses. A deeper understanding of the mechanisms by which PGPM modify plant stress responses to boost their resistance and the nuances of the PGPM-host interactions would lead to increased acceptance of PGPM in agricultural applications.

**Keywords** Plant growth-promoting microbes (PGPM) · Biotic stress · Abiotic stress · Biotechnological interventions · ISR · SAR · Genetically modified PGPM

## Introduction

The chapter begins with a discussion on the conventional methods used to manage/mitigate stress in plants, either through selective breeding, hybridization, selection or through molecular biotechnology involving recombinant DNA technology and protein engineering, to develop genetically modified plants (Balconi et al. 2012). Certain operational bottlenecks in these strategies prompt the need to explore more competent alternatives. Plant-associated microbes with plant growth-enhancing effect can serve as promising alternatives for stress management in plants. Current chapter presents various mechanisms through which PGPM are capable of modulating plant responses to biotic or abiotic stressors, promoting stress resistance and/or tolerance in plants (Bach et al. 2016). The chapter further explores the possibility, whether the ability of PGPM to play a central role in nutrient recycling can alleviate stress effects in the soil micro-environment (Sarathambal et al. 2014; Santoyo et al. 2016). PGPM can improve soil fertility by participating in nitrogen fixation, phosphate solubilization, sequestering iron and modulating phytohormone levels (cytokinins, gibberellins, indoleacetic acid, ethylene, etc.), and this ability of theirs can prove useful to plants being grown in nutrient-deficient soils or soils where nutrients are present but are in unavailable form and hence inaccessible for rhizosphere functions (Porcel et al. 2014; Pontes et al. 2015). A synergistic action expressed by certain PGPM is discussed where they elicit cross protection, a phenomenon by which common effector molecules can offer protection against seemingly unrelated stressors. The chapter concludes with a note on how PGPM can be improved by genetic modification and how a system like tripartite approach of strengthening 'host-microbe-soil' as a unit and not as individual entities can help is discussed in context with their prospective role in future plant stress management biology.

## Conventional Methods to Deal with Biotic and Abiotic Stress

Plant productivity can be improved by mitigating stress effects, which can be achieved by employing strategies like improving plant material through breeding for tolerance/resistance and/or genetic engineering; improving root health by

methods like crop rotation, tillage of soil, control of soil-borne disease; good irrigation practices like ensuring optimal water quality and availability; and by protecting plants against airborne diseases (Balconi et al. 2012). These plant stress management approaches can be broadly classified as: agricultural-based practices, plant breeding-/hybridization-based and genetic engineering-based or biotechnological interventions.

### ***Traditional Agricultural Practices***

Good farming practices minimize soil disturbance and contribute to the preservation and improvement of overall soil health. Field rotation is another method that enhances crop nutrition and improves soil health by allowing replenishment of its resources. Maintenance of a protective organic cover on soil surface, by using cover crops or crop residues, is another strategy that not only protects the soil surface but also conserves water and nutrients while promoting biological activity in soil. Use of fertilizers and pesticides also enhances crop yield by their role in management of pathogens via nutrient balancing and biological control properties, respectively (Hobbs et al. 2008). In the recent times, India has shifted from healthy traditional practices and has become more and more reliant on modern systems of agriculture that include unstructured irrigation, usage of chemical fertilizers and harmful pesticides in excess. Some irrigation methods used include strip irrigation, check basin method, furrow irrigation and basin irrigation method. Such irrigation methods are not suitable for all types of crops. Apart from this, gallons of water wasted due to over-irrigation or irrigation run-off not only moves into the drain system, but it also carries off top layers of organic soil (humus) and crop chemicals. Furrow irrigation method particularly involves digging furrows. This requires extra labour, and due to the digging, the salts come up to the surface, increasing the overall salt concentration (Silva et al. 2007). Usage of chemical fertilizers is primarily intended to enhance the yield and reduce the attack of pests on the crop. However, once the crop is harvested, these chemical residues are left in the soil and are not readily degradable and hence become harmful to plants, cattle and human health. Additionally, due to their excessive presence, the soil fertility is decreased, and the chemical composition of the soil is also altered. The biggest negative impact of using chemical fertilizers is the groundwater contamination. Nitrates are produced from nitrogen fertilizers which easily seep into the soil and reach the groundwater. Being insoluble in water, they can stay there for decades (Viets and Lunin 2009). Thus, improper agricultural practices and high use of external inputs like fertilizers/pesticides, over a period of time, can result in soil and environmental degradation.

Majority of agricultural and soil conservation techniques focus on providing nutrients and water to satisfy the basic needs of plants. Such techniques do not have much to do with understanding the soil as a living system that has a dynamic nature. Lack of such integrated understanding has led to decreased levels of soil organic matter and further increased the use of chemical inputs.

## ***Conventional Breeding Techniques***

Introducing genetic resistance in plants by selective breeding has the advantage of negligible maintenance cost once cultivars are developed. However, the risk of these cultivars placing a selection pressure on parasite populations to develop resistance cannot be overruled. For example, R (resistance) genes have traditionally been used in conventional resistance breeding programmes as one R gene has the potential to provide complete resistance to one or more than one strain of a specific pathogen, when transferred to a previously susceptible plant of the same species. Unfortunately, co-evolving pathogens can quickly defeat R genes. Moreover, several R genes also lack durability as they can be nullified by even one, loss-of-function mutation, in corresponding Avr (avirulence) gene. Traditional breeding strategies use R genes in a 'one at a time' manner which exerts strong selection pressure for mutation of the relevant Avr gene, thereby increasing vulnerability to the emergent pathogen. Alternatively, multiple R genes (pyramids) can be bred into individual plant lines which would require the pathogen to accumulate mutations in multiple Avr genes to escape detection (Balconi et al. 2012). But this strategy requires multiple cycles of breeding and rigorous selection norms to be able to arrive at desirable cultivar with multiple R genes incorporated stably. This could be very time-consuming and many a times have unpredictable yield impacts. Particularly, such efforts to breed abiotic stress tolerance in plants gave some survival benefits to plants but exhibited their own set of limitations. Also, such strategies do not play a significant role in increasing the yield. Breeding for such traits generally employs a trade-off at the cost of yield potential, hence making it irrelevant in agricultural scenarios.

## ***Conventional Biotechnological Interventions***

Exogenous application of various organic compounds and plant hormones has shown increase in growth and yield in certain host plants that do not exhibit an inherent defence mechanism against stress conditions (Spoel and Dong 2008). In tomato plants, it was reported that GA (gibberellic acid) application decreases stomatal resistance and increases crop growth and yield under saline condition. Under stress, metabolic activities can get disturbed due to altered hormonal balance, and exogenous application of growth hormones might be a useful strategy for stress tolerance (Fahad et al. 2015). Exogenous application of SA (salicylic acid) was found to ameliorate the damage caused by cadmium toxicity in maize and barley. It also conferred tolerance to *Cassia tora* plants exposed to aluminium toxicity, augmented drought tolerance in tomato and bean plants and enhanced tolerance to high temperatures in *Agrostis stolonifera* by preventing oxidative damage. It was also found to relieve the damaging effects of low temperatures in rice, wheat, bean and banana and damaging effects of UV-B radiation in Kentucky bluegrass and tall fescue sod grass. Exogenous application of BRs (brassinosteroids) was found to

ameliorate adverse effects of salt stress on seed germination, elongation of roots and subsequent growth of rice plants (Fahad et al. 2015). As per research reports of Duque et al.(2013), increased concentration of CKs (cytokinins) in xylem and their exogenous application can decrease stomatal sensitivity to abscisic acid (ABA), which in return can help in obtaining a better yield from plants experiencing mild drought conditions. CK up-regulation can be achieved by reducing expression of a gene encoding cytokinin oxidase, an enzyme which degrades CKs.

Transgenic crop varieties were also successfully used in combating biotic stressors like viruses. Attempts to introduce a gene coding for whole viral protein or part of a viral protein into the host plants by transformation were successful (Boualem et al. 2015). Virus-resistant plants can be obtained by transferring genes from the pathogen itself into the plant (pathogen-derived resistance), by making transgenic plants expressing viral coat proteins (expression of viral genes disrupt viral infection or its symptoms) or by post-transcriptional gene silencing employing viral replicase genes or RNA-dependent RNA polymerase genes. The later method has been reported to confer resistance to potato leaf roll virus, barley yellow dwarf virus, cucumber mosaic virus and wheat streak mosaic virus in potato, oats, tomato and wheat, respectively. It also induced resistance to rice tungro spherical virus in rice. RIPs (ribosome-inactivating proteins) expression in transgenic plants is also used to protect plants against multiple viruses as RIPs inhibit protein synthesis. Depending on the plant species producing them, they exhibit varying toxicity levels against different pathogens. Another common approach is using antibodies directed against the virus coat proteins that can neutralize virus infection by interacting with newly synthesized coat protein and disrupting viral particle formation in the pathogen.

Stabilization of the functional conformation of proteins is a major concern in plant stress metabolism. Biotechnological approaches for improving abiotic stress response in plants include protein engineering approaches. This involves selection of protein mutants which increase protein stability by strategies such as random mutagenesis and high-throughput screening, functional screens or comparing homologous proteins. There has been a strong research focus on understanding the stabilization of hydrophobic core and internal structural elements of proteins. Protein surfaces also influence stability, and surface residues are generally more flexible. The protein surface structures have free movement than the compact core; therefore, mutations in the protein surface largely affect protein stability and enhance protein stability. Further information is required to understand the rules for protein folding stability and dynamics with the aim to improve protein stability and stress tolerance in plants (Ortbauer 2013).

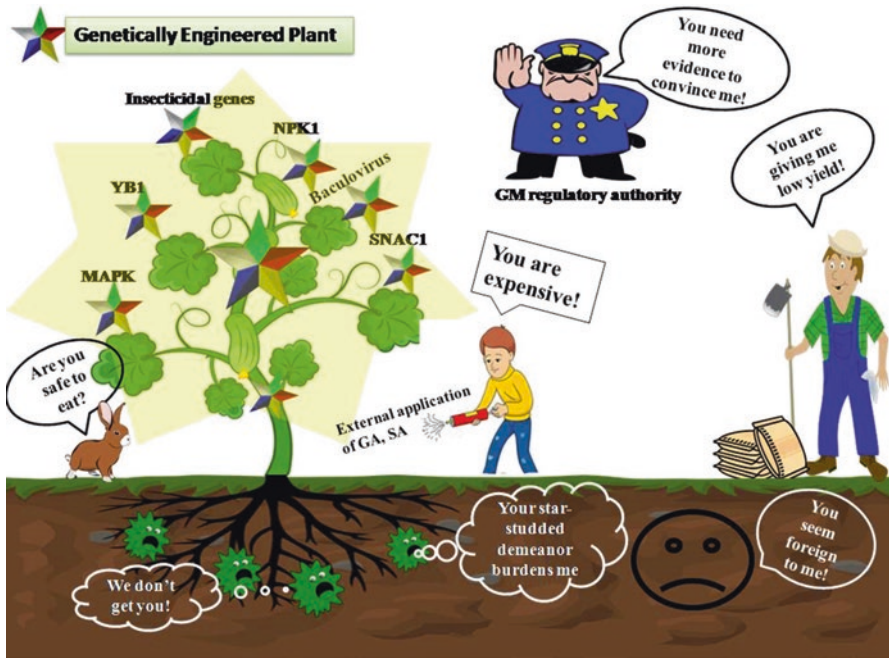
Insect-resistant transgenic crops are widely used; greater than 30 million hectares of land worldwide is planted with crops expressing Bt (*Bacillus thuringiensis*) d-endotoxins. About 140 genes have been characterized for the Bt d-endotoxins affective against lepidopterans, coleopterans and dipterans, and they are also target specific. Hence, they provide safe alternatives to chemical control agents. Apart from Bt Cry genes, other candidate genes, such as protease inhibitors, alpha-amylase inhibitors, vegetative insecticidal proteins from Bt, cholesterol oxidases and toxins from predators such as mites and scorpions, are also used to make insect-resistant

transgenics. Studies have shown that transgenic tobacco plants expressing chitinase show increased resistance to lepidopterans. Development of artificial resistance, via introduction of effector genes into the host plant, was reported to provide a viable molecular strategy for expressing nematode resistance. These effector genes can encode enzymatic inhibitors that block physiological processes within the nematodes, degrading enzymes (e.g. collagenases, chitinases), ingestible toxic compounds (cytotoxins), molecule binding compounds (e.g. lectins, monoclonal antibodies), enzymes which interact with nematodes and substances causing breakdown of particular feeding structures (cytotoxins).

Even though genetically modified (GM) plants have been the centre of attraction of plants researchers, there have been different limitations for the same. First, GM plants are not the natural way of cultivation; hence they pose unexplained and at times perceived threat to the environment. Second, the genes inserted in the plant genome add an extra burden to the host plant itself as it has to partition its metabolic energy resources to fuel these non-native functions, and this reallocation of resources might reflect in decreased crop yield. Such alterations add an extra burden to the roots and result in less plant biomass. GM plants may also influence abundance of soil organisms including the rhizobacteria. The chances of cross contamination are also high when one is dealing with GM plants. Even though there are not many significant facts reported, overall root-plant-soil relationships are perceivably disturbed. There has not been any extensive research about GM plants and their influence on this tri-partite (GM plant \_ rhizosphere microbes – soil micro-environment) due to ethical concerns, lack of evidence and apprehension from the market (Domingo and Bordonaba 2011). Figure 8.1 is a pictorial representation of all such aspects and challenges faced by host-soil-microbe systems.

## Why the Need for Alternatives to Conventional Methods?

All the methods discussed above in section ‘[Conventional methods to deal with biotic and abiotic stress](#)’ have their own strengths and weaknesses. Primarily, the effect on nontarget species, invasiveness, horizontal gene transfer of transgenes and adverse effects on natural soil biota are causes of great concern. Introduction of insecticide resistance can challenge natural ecosystems with unknown impacts on their associated complex network of nontarget organisms (Cramer et al. 2011). As global decline of biodiversity is a major issue, proactive measures are necessary, and consideration of the likely effects of transgenics on plant and insect biodiversity is essential (Downey 2003). Though engineering genes encoding insecticidal proteins into crop plants have several benefits, researchers expressed this technology could disrupt natural biological control by causing side effects of the plant on the fitness and behaviour of pests. Interactions between transgenic plants and beneficial insects were also taken up to assess issues of incompatibility (Schuler et al. 1999). There are two major concerns regarding the use of Bt transgenic crops: the effect on



**Fig. 8.1** Genetically engineered plant and its response to various biotic/abiotic stressors

nontarget organisms and the possibility of resistance development in target insects to the Bt protein. Satellite RNA can also be used to increase resistance against cucumber mosaic virus. Attempts using this approach have been made in tomato but are controversial as a single point mutation in the satellite RNA can transform it into a harmful necrogenic form.

To satisfy the increasing global demand for food, a re-evaluation of existing agricultural practices (including the use of chemical fertilizers, herbicides, fungicides and insecticides) is also required. To this end, there is a shift in agricultural practices towards approaches that are sustainable, as well as environmental friendly. One such beneficial approach with no discernible toxic implications happens to be the application of PGM in mainstream agriculture (Glick 2012).

## Plant Growth-Promoting Microbes

In nature, beneficial relationships between plants and microorganisms are present and defined under many types such as mutualism, symbiosis, cohabitation, commensalism, co-metabolism, biofilms, endophytes and so on. They generally occur in the rhizosphere and aid in improving plant growth or help the plant to cope with biotic and abiotic stress (Zamioudis and Pieterse 2012). Among the diverse range of



microscopic life forms found in soil, bacteria are by far the most common (i.e. 95%). However, they are generally not homogeneously distributed in the soil, i.e. greater concentration of bacteria would be found in the rhizosphere than in the rest of the soil. This suggests that plants can shape their microbiome by root exudates comprising of nutrients such as sugars, amino acids, organic acids and other small molecules, which account for up to a third of the carbon fixed by a plant. Considering the plant perspective, the interaction between soil bacteria and plants may either be beneficial or harmful or neutral. Also, the effect of particular bacteria on a plant will vary with changing conditions. For example, a bacteria facilitating plant growth by providing fixed nitrogen or phosphorus (which are generally present in limited quantities in soils) are unlikely to prove beneficial to plants when significant amounts of chemical fertilizer are added to the soil. Moreover, a particular bacterium can affect different plants disparately (Glick 2012). Plant growth-promoting rhizobacteria (PGPR) have been the most extensively studied plant growth promoting bacteria (PGPB) (Compant et al. 2005). Depending on plant interactions, PGPR can be divided into two groups: symbiotic bacteria (which live inside plants and exchange metabolites with them directly) and free-living rhizobacteria (which live outside plant cells). Typically symbiotic bacteria live in the intercellular spaces of the plant host, but some bacteria form truly mutualistic interactions and penetrate plant cells. Additionally, some of them integrate their physiology with the plant, leading to formation of specialized structures. Rhizobia are one of the most studied mutualistic bacteria, which live symbiotically with leguminous crop plants and fix atmospheric nitrogen for the plant in root structures called nodules. Other examples of mutualistic bacteria include *Frankia*, which forms nodules wherein it fixes nitrogen in actinorhizic plants such as *Alnus* trees. Several PGPR are used worldwide as biofertilizers, contributing to increased crop yields and soil fertility, and hence hold the potential to contribute to sustainable agriculture and forestry (García-Fraile et al. 2015). *Bacillus mycoides* B38V, *Burkholderia cepacia* 89 and *Paenibacillus riograndensis* SBR5 were studied for their plant growth-promoting characteristics. Bach et al. (2016) evaluated the biocontrol potential and rhizosphere competence of two PGPB. The study was with different cultivars of wheat, and PGPB were added to the substrate. They have recorded remarkable antifungal activity upon inoculation with PGPB in addition to improved growth characteristics in host plants. This and many such examples recommend that PGPB could be successfully used as bioinoculants once host-microbe optimization studies are completed. Regardless of the differences between these bacteria, they all utilize similar mechanisms of support (Glick 2012), as explained in the section ‘[Role of PGPM in dealing with plant stress](#)’ of the chapter.

Endophytic fungi have been widely studied in several geographic and climatic zones and are ubiquitously found within plant tissues and have rich species diversity. They play an important role in providing nutrients to host in exchange for photosynthates, adapting them to their environments, defending them from environmental stresses and promoting biodiversity of plant community (Zhou et al. 2014). Certain rhizospheric fungi belonging to the genera *Penicillium*, *Fusarium*, *Trichoderma* and *Phoma* are involved in promotion of plant growth and development. They impact growth and health of plants by direct and/or indirect mechanisms.



They can affect indirectly by strategies such as antibiotics and siderophore production and directly via solubilization of minerals, etc. They also stimulate plant growth partly by the production of secondary metabolites such as IAA (indoleacetic acid), CK, GAs, ET (ethylene) and other plant growth-promoting substances. They can also protect against pathogens by the production of phytohormones and also through production of molecules which affect hormone homeostasis within the plants (Salas-Marina et al. 2011). P (phosphate)-solubilizing and N (nitrogen)-fixing bacteria synergistically interact with AM (arbuscular mycorrhizal) fungi, increasing P and N availability to the plant and promoting its growth and biotic stress resistance (Alizadeh et al. 2013).

Beneficial associations of PGPM can stimulate plant growth through degradation of soil pollutants and production of phytostimulators (Zamioudis and Pieterse 2012). It has been reported that the PGPM strains of *Pseudomonas alcaligenes*, *B. polymyxa* and *Mycobacterium phlei* promote plant growth significantly when inoculated into the nutrient-deficient soils. PGPM can also foster plant's nutrient uptake efficiency under poor soil conditions. In addition, PGPM can induce plant resistance to phytopathogens, insect pests and nematodes. Since nutrients and PGPM all remain in the soil environment, the soil property could also affect their interactions (Sripontan et al. 2014).

## Role of PGPM in Dealing with Plant Stress

PGPM can contribute to plant growth by enhancing its resistance to biotic/abiotic stress or by protecting/priming them against the same. However, to establish this plant-PGPM association, mutual recognition and coordination are needed between them. Substantial evidence indicates that initially plants identify beneficial microbes as potential invaders, thereby triggering an immune response. But at later stages of such interactions, mutualists outwit plant defence responses to successfully colonize host roots (Zamioudis and Pieterse 2012).

### *How Do PGPM Help in Fighting Biotic Stress?*

PGPB can confer induced systemic resistance (ISR) in plants, a phenomenon which resembles systemic acquired resistance (SAR) in phenotypic aspects. Plants exhibiting ISR are called primed (Glick 2012), and biopriming of plants with certain PGPB provides systemic resistance against a broad spectrum of plant pathogens and diseases. ISR is not very specific in its targets and can rather control diseases caused by a variety of pathogens (Compant et al. 2005). The PGPM-mediated ISR is important for disease control under conditions where the PGPM and pathogens are spatially separated. The systemic resistance induction process leads to increases in peroxidase (PO) and phenoloxidase (PPO) activities, which are involved in

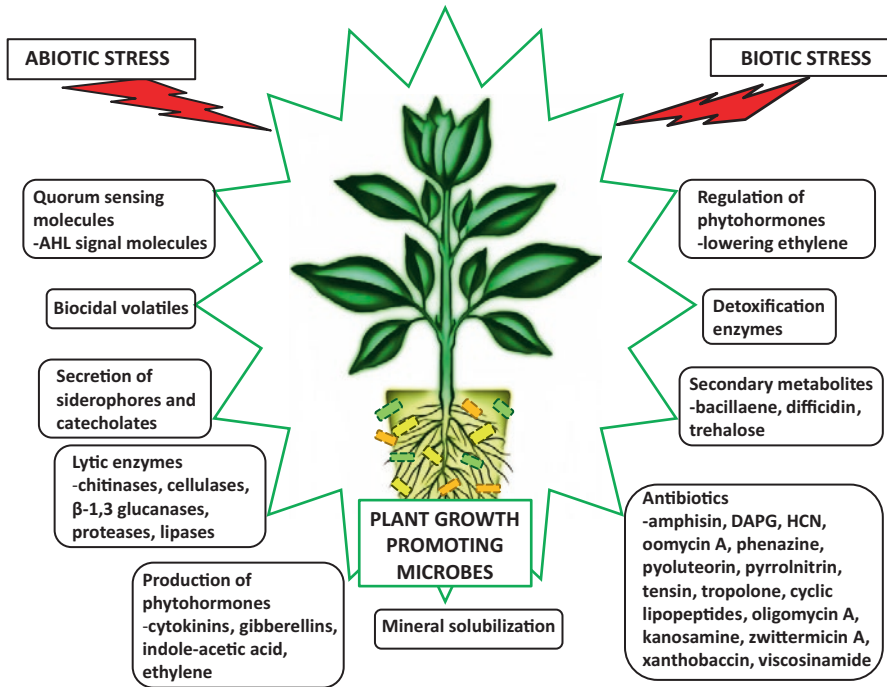


Fig. 8.2 Strategies by which PGPM protect plants against biotic and abiotic stress

catalysing lignin formation and phenyl ammonia lyase (PAL), for the biosynthesis of phytoalexin and phenol (Filippi et al. 2011). Direct interaction between resistance inducing PGPB and pathogen is not required in ISR. Apart from ethylene (ET) and jasmonate, bacterial molecules like O-antigenic side chain of the bacterial outer membrane protein lipopolysaccharide, pyoverdine, flagellar proteins, chitin,  $\beta$ -glucans, cyclic lipopeptide surfactants and salicylic acid (SA), also act as signaling molecules for ISR (Glick 2012). SA can cause an ISR even when present in nanogram amounts (Bloemberg and Lugtenberg 2001). A brief overview of different strategies employed by PGPM to protect plants against biotic and abiotic stressors is given in Fig. 8.2.

The first instances of PGPB-induced ISR were reported in carnation (*Dianthus caryophyllus*) and in cucumber (*Cucumis sativus*) which exhibited reduced susceptibility to wilt caused by *Fusarium* sp. and foliar disease caused by *Colletotrichum orbiculare*, respectively. The combination of host plant and bacterial strain decides manifestation of ISR. Even though most reports of PGPB-induced ISR involve free-living rhizobacterial strains, endophytic bacteria were also found to exhibit ISR activity. *Pseudomonas fluorescens* EPI triggers ISR against red rot caused by *Colletotrichum falcatum* on sugarcane. *Burkholderia phytofirmans* PsJN induces ISR against *Botrytis cinerea* on grapevine and *Verticillium dahliae* on tomato. *Pseudomonas denitrificans* and *Pseudomonas putida* confer resistance against *Ceratocystis fagacearum* in oak, while *P. fluorescens* does the same against

*Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato and *Pythium ultimum* and also against *F. oxysporum* f. sp. *pisi* in pea roots. *Bacillus pumilus* SE34 provides ISR against *F. oxysporum* f. sp. *pisi* in pea roots and against *F. oxysporum* f. sp. *vasinfectum* in cotton roots. Both rhizobacteria and bacterial endophytes were observed to have the ability to act as bioprotectants via ISR. Bacterial traits such as flagellation, production of siderophores, lipopolysaccharides, etc. were proposed to trigger ISR. However, no solid evidence exists for an overall ISR signal produced by bacteria. Even though some PGPB trigger an SA-dependent signalling pathway in the rhizosphere, majority of ISR inducing PGPB were shown to utilize a SA-independent pathway involving jasmonate and ET signals. Rather than increasing the production of these hormones, ISR was implicated to increase sensitivity towards them as the former could lead to the activation of a partially different set of defence genes. When PGPB triggers ISR, it was also observed to fortify the strength of the plant cell wall and alter the physiology and metabolic responses of the host to enhance synthesis of plant defence chemicals. After inoculating tomatoes with endophytic *P. fluorescens* WCS417r, the cortical cell walls were found to be thickened upon colonization of epidermal or hypodermal cells. During endophytic colonization by *B. phytofirmans* PsJN in grapevine, accumulation of phenolic compounds and strengthening of the cell walls of exodermis and other cortical cell layers were reported. Plant ISR response also includes formation of structural barriers, like thickening of cell wall papillae by deposition of callose and accumulation of phenolic compounds at the site of attack. Biochemical or physiological changes in plants include accumulation of pathogenesis-related (PR) proteins like PR-1, PR-2, chitinases and some peroxidases. Instead of inducing PR proteins, certain PGPB were known to induce accumulation of phytoalexins, peroxidases, polyphenol oxidase, phenylalanine ammonia lyase and/or chalcone synthase. Production of some of these compounds (e.g. chalcone synthase) in plant defence might be triggered by the same N-acyl homoserine lactones which bacteria also use for intraspecific signalling (Compant et al. 2005).

The list of rhizobacterial *Pseudomonas* species known to induce ISR is rapidly growing as many researchers have worked on the genus. There exists a dependency on plant genotype in generation of ISR as reported in literature. Detailed review of the plant factors involved in the ISR and SAR pathways has shown that induced disease resistance can be increased by simultaneous activation of these two pathways (Bloemberg and Lugtenberg 2001). PGPM were shown to induce ISR in several crops like *Arabidopsis*, cucumber, tomato, potato and so on against fungal, bacteria, nematode and viral pathogens. Studies have reported early and enhanced levels of peroxidase being stimulated in rice plants by seed treatment and seedling root dipping. In the PGPR-treated rice plants inoculated with sheath blight pathogen, *Rhizoctonia solani*, two isoforms of peroxidase were induced. Chilli plants treated with *P. fluorescens* Pf1, when challenged with *Colletotrichum capsici*, reportedly showed higher levels of expression of peroxidases. Similarly, tomato plants treated with PGPR exhibited increased activity of polyphenol peroxidase upon challenging with *F. oxysporum* f. sp. *lycopersici*. *Pseudomonas* strains treated plants have registered higher levels of phenylalanine ammonia lyase as compared to control.

Seedlings dipped in talc-based formulation of *P. fluorescens* were observed to increase the activity of phenylalanine ammonia lyase in finger millet leaves against blast disease. In an experiment, researchers inoculated PGPR strains *P. putida* 89B-27 and *Serratia marcescens* 90-166 with *F. oxysporum* f. sp. *cucumerinum* on two separate halves of roots of cucumber seedlings and have reported induction of systemic resistance against *Fusarium* wilt. They observed delayed development of disease symptoms and reduced number of dead plants. The same PGPR strains were also seen to induce systemic resistance against *P. syringae* pv. *lachrymans* which causes bacterial angular leaf spot in cucumber (Liu et al. 1995). Treatment of maize seeds with *P. fluorescens* witnessed higher activity of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase in the host plant against pathogen *R. solani*. Seeds bactericized with *P. fluorescens* led to the accumulation of higher phenolic compounds and greater activity of polyphenol peroxidase, peroxidase and phenylalanine ammonia lyase, compounds that are known to have a role in multiple defence mechanisms in plants against pathogen (Sivakumar and Sharma 2003). PGPR-induced systemic resistance also controlled diseases caused by nematodes in tomato and bell pepper, and ISR was accredited for a reduction of 42% in nematode penetration (Siddiqui and Shaikat 2002). The experimental set-up consisted of an in vitro split root system, where one half of the split root system was challenged with nematodes, while PGPR strains were applied in the other half of the system (in tomato). A study involving application of PGPR by seed, root and foliar spray treatments separately in different combinations in field revealed that among the different PGPR strains tested, highest activity was by *B. subtilis* strain GB3, in terms of suppressing bacterial spots and increased activity of defence-related enzymes like peroxidase and phenylalanine ammonia lyase. PGPR which were found effective in greenhouse against bacterial spots also showed sustained ability to induce resistance in tomato under field conditions. Symbiotic association of *Glomus mosseae* with clover plants crop variety Sonja totally prevented infection by *P. ultimum*. Also, disease symptoms induced were systemically reduced even in non-mycorrhizal roots of plants which were grown in split root systems inoculated with AM fungi. Systemic regulation of pathogens induced by AM colonization indicates establishment of ISR. In plants colonized by AM species with biocontrol activities, higher concentrations of ISR-related compounds such as phenolic acids and new isoforms of superoxide dismutases, peroxidases and PR-1 proteins (pathogenesis-related proteins type 1) were detected. Rhizobacteria-mediated ISR in mycorrhizal roots is associated with accumulation of JA (jasmonic acid) which might be related to the systemic pathogen biocontrol. Additionally, local cell wall modifications like callose accumulation were identified around arbuscule-containing cortical cells of tomato roots (Alizadeh et al. 2013).

PGPB are able to colonize and retain their niches in the rhizosphere by production of bacterial allelochemicals, like siderophores, biocidal volatiles, antibiotics and lytic and detoxification enzymes (Compant et al. 2005). Secondary plant metabolites also play a vital role in stress management and plant growth-promoting activities. For example, *Bacillus amyloliquefaciens* FZB42 (a plant-associated bacteria) simultaneously promotes plant growth while producing secondary metabolites like

polyketides bacillaene and difficidin which aid in suppression of soil-borne plant pathogens (Chen et al. 2007). Furthermore, colonization of basil plants by *Glomus mosseae* (which protects against *F. oxysporum*) did not increase the concentration of defence-related compounds such as rosmarinic and caffeic acids, phenolics and essential oils, highlighting the role of mechanisms other than the stimulation of systemic and localized plant defence mechanisms in AM-mediated biocontrol (Alizadeh et al. 2013).

Over the past two decades, there has been an increase in understanding of anti-biosis being employed as biocontrol mechanism by PGPB. Detailed studies of several antibiotics along with their specificity and mode of action have been done, identifying the contribution of compounds like amphisin, DAPG (2,4-diacetylphloroglucinol), HCN (hydrogen cyanide), oomycin A, phenazine, pyoluteorin, pyrrolnitrin, tensin, tropolone and cyclic lipopeptides produced by Pseudomonads. *Bacillus*, *Streptomyces* and *Stenotrophomonas* spp. were also reported to produce oligomycin A, kanosamine, zwittermicin A and xanthobaccin. DAPG is a polyketide compound with broad-spectrum activity against fungi, bacteria and helminths. Phenazines are heterocyclic pigments which contain nitrogen and are synthesized by *Pseudomonas*, *Streptomyces*, *Burkholderia* and *Brevibacterium* species. Pyrrolnitrin is a broad-spectrum antifungal metabolite which can persist actively in the soil for a minimum of 30 days. Pyoluteorin, an aromatic polyketide antibiotic, inhibits oomycetous fungi and has strong activity against *P. ultimum* upon application to seeds, leading to decreased severity of *Pythium* damping off. *P. fluorescens* strain CHAO and its antibiotic over-expressing derivative CHAO/PME 3424 reduce *Meloidogyne incognita* galling in primary growth stages of crops such as tomato and brinjal. There exists a strong negative correlation between rhizobacteria colonization and nematode invasion as reported by Alizadeh et al. (2013). Different PGPR isolates from weedy grass have been used to control rice plant pathogens such as *Pyricularia oryzae*, *R. solani* and *Sarocladium oryzae* (Sarathambal et al. 2014). Many strains of *Pseudomonas* produce AFM (antifungal metabolites) out of which phenazines, pyrrolnitrin, DAPG and pyoluteorin are most common, but new AFMs like viscosinamide and tensin have also been reported. Studies have shown that viscosinamide prevents *P. ultimum* infection in sugar beet. Interestingly, AFM production is also observed in *Pseudomonas* where its biosynthesis happens under complex global regulation and quorum sensing. Global regulators like *gacS/gacA* genes regulate AFMs (and other extracellular products like protease, HCN) by encoding a two-component regulatory system. *GacA* has recently been shown to indirectly control the HCN synthase genes (*hcnABC*) and the protease gene *aprA* in *P. fluorescens* CHAO via a post-transcriptional mechanism involving a distinct recognition site overlapping the ribosomal binding site. It has recently been established that plants can recognize AHLs (N-acyl homoserine lactones) and their gene expression in roots and shoots can be altered by them. AHLs can also regulate the defence and cell growth responses of plant (Ortiz-Castro et al. 2009). An AHL synthase such as *LuxI* produces AHL signal molecules, which are believed to be involved in quorum sensing. It was reported that when AHL is at a threshold concentration (depending on density of

bacterial cells), it binds to and activates LuxR, a transcriptional regulator. This activated form of the transcriptional regulator further stimulates gene expression (Bloembergen and Lugtenberg 2001).

Moreover, some PGPB-produced antibiotics are being tested for their utility as pharmaceuticals which can be used to tackle the increasing menace of multidrug resistance among human pathogenic bacteria. It is reported that their regulatory cascades involve global regulators GacA/GacS or GrrA/GrrS, the sigma factors RpoD and RpoS and quorum-sensing autoinducers such as AHL derivatives and are under positive autoregulation. Antibiotic synthesis is closely linked to the overall metabolic status of the cell, and the metabolic status is shaped by nutrient availability and other environmental factors. Trace elements specifically zinc and carbon source levels affect the capacity of secondary metabolite producing PGPM by influencing the genetic stability/instability of microbes. It is pertinent that several strains produce pellet of secondary antimicrobial metabolites and that conditions which favour one compound might not favour another. Therefore, the wide variety of biocontrol strains can enable suppression of pathogens under a wide range of environmental conditions. This was illustrated by the reports that the presence of glucose as a carbon source in *P. fluorescens* stimulates CHAO biosynthesis of DAPG and represses pyoluteorin (bacterial aromatic polyketide antibiotic). However, as glucose levels get depleted, pyoluteorin levels increase, and it becomes the more abundant antimicrobial compound produced by this strain which ensures that the antagonist has flexibility when dealing with different or changing environment. Biotic conditions also influence antibiotic biosynthesis. For instance, bacterial metabolites-salicylates and pyoluteorin affect DAPG production by *P. fluorescens*. Additionally, plant growth and development also have an impact on production of antibiotic compounds as biological activity of DAPG producers is induced not by the exudates of young plant roots but by the exudates of older plants. This leads to the creation of selective pressure against other microorganisms in the rhizosphere. Another feature that influences disease-suppressive interaction of plant with a microbial biocontrol agent is the host genotype itself (Compant et al. 2005).

Too much dependence on antibiotic-producing bacteria as biocontrol agents poses the complication of resistance development in phytopathogens against specific antibiotics. To overcome this shortcoming, researchers are utilizing biocontrol strains which synthesize HCN along with one or more antibiotics. This is an effective strategy as even though HCN may not have much biocontrol activity individually, it acts synergistically with bacterially encoded antibiotics (Glick 2012). The cyanide ion is exhaled as HCN and is metabolized further into other compounds. The mechanism through which HCN exerts its biocidal action is by inhibiting electron transport and disrupting the energy supply to the cell leading to the death of the organism. It also disrupts functioning of enzymes and natural receptors that can reverse its impact and inhibits action of cytochrome oxidase. HCN is reportedly produced by several rhizobacteria. HCN has broad-spectrum antimicrobial activity and is involved in biocontrol of several root diseases by many plant-associated fluorescent pseudomonads (Alizadeh et al. 2013). The ability of *P. fluorescens* strain CHAOs to suppress black root rot of tobacco and take-all of wheat was attributed to the production of HCN, and the same isolate was also shown to inhibit in vitro



mycelial growth of *Pythium* and suppression of *F. oxysporum* f. sp. *radicis-lycopersici* in tomato. The cyanide producing strain CHAO was also observed to stimulate the formation of root hair presumably by inducing and altering plant physiological activities. In a particular study, four out of six PGPR strains were recognized for inducing systemic resistance in cucumber against *C. orbiculare* through production of HCN. Fluorescent *Pseudomonas* strain RRS1 isolated from Rajnigandha (tuberose) was studied to be positive for HCN production and also contributed to improved seed germination and root length. It has been reported that low oxygen levels are essential for the activity of ANR, a transcription factor responsible for positively regulating HCN biosynthesis.

Several microorganisms exhibit hyper-parasitic activity and attack pathogens by secretion of cell wall hydrolases. PGPB which can synthesize enzymes capable of lysing a portion of the cell walls of pathogenic fungi, such as chitinases, cellulases,  $\beta$ -1,3 glucanases, proteases and lipases, can exhibit biocontrol activity against wide spectrum of pathogenic fungi including *B. cinerea*, *Sclerotium rolfisii*, *F. oxysporum*, *Phytophthora* spp., *R. solani* and *P. ultimum* (Glick 2012). *Serratia plymuthica* C48 produces chitinase to inhibit spore germination and germ-tube elongation in *Botrytis cinerea*. The same enzyme was also responsible for its antagonistic activity against *S. rolfisii*. Suppression of *F. oxysporum* f. sp. *cucumerinum* by *Paenibacillus* sp. 300 and *Streptomyces* sp. strain 385 was also attributed to their ability to produce chitinase (Compant et al. 2005). Extracellular chitinases and laminarinases from *Pseudomonas stutzeri* could digest and lyse mycelia of *F. solani*. In PGPB *S. plymuthica* IC14 suppression of *Sclerotinia sclerotiorum* and *B. cinerea* was due to synthesis of proteases and other biocontrol traits. The  $\beta$ -1,3-glucanases synthesized by both *Paenibacillus* sp. (strain 300) and *Streptomyces* sp. (strain 385) were seen to lyse fungal cell walls of *F. oxysporum* f. sp. *cucumerinum*. The same enzyme synthesized by *B. cepacia* was found to damage the integrity of *R. solani*, *S. rolfisii* and *P. ultimum* cell walls (Compant et al. 2005). Constitutive and additional isoforms of defence-related enzymes were also reported in mycorrhizal roots (Alizadeh et al. 2013).

Iron is a vital element for growth of all living organisms, but the scarcity of bio-available iron can foment great competition in soil habitats and on plant surfaces. Bacterial strains which don't possess or employ other means of biocontrol can use their capability of producing siderophores to establish themselves as biocontrol agents. Under iron-limiting conditions, PGPB-produced siderophores can help them to efficiently outcompete pathogens in competitively acquiring ferric ion. In fact siderophores from PGPB limit the proliferation of pathogenic fungi by depriving them of iron, an essential element to carry out many metabolic functions. Some PGPB strains can even derive iron from heterologous siderophores being produced by other microorganisms in their vicinity. Biosynthesis of siderophores is under strict regulation of iron-sensitive Fur proteins; GacS and GacA; sigma factors RpoS, PvdS, FpvI and N-acyl homoserine lactone; and site-specific recombinases. However, there are contradictory opinions on this as some studies do not support the involvement of these global regulators in siderophore production. For instance, GacS or RpoS had no significant effect on the level of siderophores synthesized by *Enterobacter cloacae* CAL2 and UW4. Similarly, non-involvement of RpoS of *P.*

*putida* strain WCS358, and preferential involvement of GrrA/GrrS over GacS/GacA of *S. plymuthica* strain IC1270 in regulation of siderophore synthesis, indicates evolution of genes in the siderophore-producing bacteria. Furthermore various environmental factors were also evidenced to modulate siderophore synthesis, viz. pH, iron levels, form of iron ions, presence or absence of other trace elements and optimal supply of major nutrients like phosphorus, nitrogen and carbon (Compant et al. 2005). Iron depletion in the rhizosphere by siderophores produced by the PGPB does not affect the growth of plants as most plants can thrive at much lower iron concentrations than most microorganisms. Moreover, several plants can bind, take up and utilize the iron-siderophore complexes generated by these PGPB. Evidence for involvement of bacterial siderophores in biocontrol of fungal pathogens comes from various studies. Certain studies used mutants defective in production of siderophores and found them less effective at protecting plants against fungal pathogens than the wild-type strains. Also, another study found that mutants which overproduce siderophores show greater effectiveness in protecting plants against fungal attacks (Glick 2012). Siderophores from endophytic bacteria were reported to limit the growth of *Streptomyces scabies* and *Xanthomonas campestris* in vitro. Compant et al. (2005) proved with their experiment the tissue type and tissue site-specific activities of siderophores by employing endophytic bacteria isolated from potato tubers. A study by Tiwari and Thrimurthy (2007) utilized 21 isolates of siderophore producing *P. fluorescens* and has concluded that isolates, PFR 1 and PFR 2, were superior over others in increasing shoot and root length of rice cv. Bamleshwari. An in vitro evaluation of the *P. fluorescens* isolates confirmed their antagonistic ability against *Pyricularia grisea* and *R. solani*. Pure culture of *Pseudomonas aeruginosa* were also studied for siderophore production, and their antifungal activity was tested against *Fusarium moniliformae*, *Alternaria solani* and *Helminthosporium halodes*, and it was seen that *P. aeruginosa* inhibits these fungal pathogens by production of antifungal secondary metabolites (Alizadeh et al. 2013). Apart from having high affinity for iron, siderophores may show affinity for other metals too. Excretion of catecholate compounds has been reported in *Azotobacter vinelandii*. These were earlier identified as siderophores, and they bind to metal cofactors of nitrogenase (Mo, V and Fe) enzyme (Pontes et al. 2015).

PGPM also employ detoxification of pathogen virulence factors as a mechanism of biocontrol. For example, *Xanthomonas albilineans* produces an albicidin toxin which is detoxified by certain biocontrol agents. In *Klebsiella oxytoca* and *Alcaligenes denitrificans*, this detoxification mechanism involves the production of a protein which reversibly binds the toxin, while *Pantoea dispersa* produces an esterase which irreversibly detoxifies albicidin. *Fusarium* species produce fusaric acid, a phytotoxin which is hydrolysed by many different microorganisms including *B. cepacia* and *Ralstonia solanacearum* strains. However, most pathogen toxins have a broad-spectrum activity capable of suppressing growth of microbial competitors, or as self-defence against biocontrol agents, they can detoxify the antibiotics they produce. PGPB can quench pathogen quorum-sensing capacity by degrading the autoinducer signals which would effectively block the expression of several virulence genes. A majority of plant bacterial pathogens depend on

autoinducer-mediated quorum-sensing to activate their gene cascades coding for virulence factors, and this mechanism holds promise for relieving/curing disease even after the onset of infection. Both free-living rhizobacteria and endophytic bacteria share some biocontrol mechanisms. For instance, both are capable of synthesizing metabolites with antagonistic activity towards plant pathogens (Compant et al. 2005).

Lowering a plant's ET response to pathogens can ameliorate the extent of damage caused to plants by phytopathogens. This can be done by treating plants (generally the roots or seeds) with PGPB containing ACC (1-aminocyclopropane-1-carboxylate) deaminase. This technique has lowered the damage in cucumber, potato, castor bean, tomato, carrot and soybean plants, caused by various phytopathogens including *P. ultimum*, *F. oxysporum*, *Erwinia carotovora*, *Agrobacterium tumefaciens*, *Allorhizobium vitis*, *S. rolfsii* and *R. solani*, in both greenhouse and growth chamber experiments (Glick 2014). Transgenic plants expressing bacterial ACC deaminase are protected from damage caused by various phytopathogens to a significant level. Another study by Gamalero et al. (2010) supported the beneficial nature of PGPR for plant growth even under stress conditions. Interaction between ACC (1-aminocyclopropane-1-carboxylate) deaminase-producing bacterial strains and an arbuscular mycorrhizal fungus (AMF) was studied, and the effect of this interaction on cucumber growth was examined under saline conditions. Seeds of cucumber plant were treated with ACC deaminase-producing strain *P. putida* UW4 (Acds<sup>+</sup>). Inoculation with *P. putida* was shown to have a positive effect on various parameters of assessment including root length, photosynthetic activity and overall plant growth. Inoculation of peas with *Pseudomonas* spp. containing ACC deaminase witnessed a similar growth-promoting effect and also contributed significantly to decrease the adverse effects of drought stress on growth, grain yield and ripening of pea (*Pisum sativum* L.) (Arshad et al. 2008). Salinity has negative effects on growth, but root colonization by ACC deaminase-producing bacteria or AMF can improve the tolerance of plant for such stressful conditions. This study was not only relevant from an ecological point of view, but also it has tremendous application as well. While PGPR stimulate growth rates in wild-type plants, it inhibits growth in ABA-deficient mutant plants. It has also been shown to induce accumulation of ET in ABA-deficient plants correlating with increased expression of the pathogenesis-related gene SI-PR1b. Such results suggest that in ABA-deficient mutant plants, over-accumulation of ET corresponds with increased expression of SI-PR1b indicating that maintenance of normal plant endogenous ABA levels might be essential for promotion of growth caused by *Bacillus megaterium* (Porcel et al. 2014). Goel et al. (2008) has elaborated on various type III effector proteins from *P. syringae* strains that act as virulence factors in the host cells. The virulence factors, Pma M6CΔE and HopAM1, were discussed for their role in enhanced nutrient uptake in plants that are grown under drought stress and adaptation to water availability. In *Arabidopsis*, HopAM1 was shown to induce hypersensitivity to ABA, causing stomatal closure and germination arrest. Although a discussion about all the PGPM currently in use is beyond the scope of this chapter, Table 8.1 provides a brief list of some PGPM which are presently being explored/used to protect plants against biotic stress.

**Table 8.1** A brief list of PGPM reported to protect plants from biotic stress

S. no	PGPM	Action against	Host	Mechanism	References
1	<i>Acetobacter</i> sp., <i>Xanthomonas</i>	<i>Rhizoctonia solani</i>	Soybean	Antagonistic	Dalal and Kulkarni (2013)
2	<i>Aureobacterium anophageum</i> , <i>Bacillus pumilus</i> , <i>Phyllobacterium rubiaccearum</i> , <i>Pseudomonas putida</i> and <i>Burkholderia solanacearum</i>	Vascular wilt of cotton caused by <i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Cotton	Biological control potential	Chen et al. (1995)
3	<i>Bacillus subtilis</i> 281 (B1) strain and <i>Bacillus pumilus</i> 293 (B2)	Watermelon mosaic poty virus (WMV)	Pumpkin ( <i>Cucurbita maxima</i> L.)	Induce systemic resistance	Elbeshehy et al. (2015)
4	<i>Bacillus</i> sp., <i>Burkholderia</i> sp.	<i>Sclerotium rolfsii</i> , <i>Fusarium oxysporum</i> , <i>Rhizoctonia solani</i>	Soybean	Antagonistic	Dalal and Kulkarni (2013)
5	<i>Klebsiella</i> sp., <i>Xanthomonas</i>	<i>Fusarium oxysporum</i>	Soybean	Antagonistic	
6	<i>Bacillus</i> sp.	<i>Colletotrichum truncatum</i> , <i>Macrophomina phaseolina</i>	Soybean	Antagonistic	
7	<i>Bacillus simplex</i> 30N-5	<i>Fusarium</i>	Cowpea plant	Antagonism	Schwartz et al. (2013)
8	<i>Bacillus subtilis</i>	Cowpea fungal pathogens <i>Fusarium verticillioides</i> , <i>F. equiseti</i> and <i>Rhizoctonia solani</i>	Cowpea plant	Antibiosis by antagonism	Killani et al. (2011)
9	<i>Bacillus thuringiensis</i>	Silences <i>Erwinia carotovora</i> which causes potato soft rot	In vitro on potato tubers	Antagonism and signal interference. Production of AHL-degrading enzyme (AHL-lactonase)	Dong et al. (2004)
10	<i>Beauveria bassiana</i> , <i>Clonostachys solani</i> , <i>Metarhizium anisopliae</i> , <i>Trichoderma atroviride</i> , <i>T. koningtopsis</i> and <i>T. gamsii</i>	<i>Delta radicum</i> L. or cabbage maggot	Cabbage	Rhizosphere competence	Razinger et al. (2014)
11	<i>Cladosporium</i> sp. and <i>Ampelomyces</i> sp.	<i>Pseudomonas syringae</i> pv. tomato DC3000	<i>Arabidopsis thaliana</i>	SA- and JA-signalling pathways employed in ISR	Naznin et al. (2014)

12	<i>Fusarium equiseti</i> GF183	Biocontrol of <i>Fusarium</i> wilt of spinach	Spinach	-	Horinouchi et al. (2010)
	<i>Klebsiella</i> sp., <i>Xanthomonas</i>	<i>Fusarium oxysporum</i>	Soybean	Antagonistic	Dalal and Kulkarni (2013)
13	<i>Lysobacter enzymogenes</i> C3	<i>Bipolaris sorokiniana</i>	Tall fescue	ISR	Kilic-Ekici and Yuen (2004)
14	<i>Paenibacillus</i> sp. 300 and <i>Streptomyces</i> sp. 385	<i>Fusarium</i> wilt caused by <i>Fusarium oxysporum</i> f. sp. <i>cucumerinum</i>	Cucumber ( <i>Cucumis sativus</i> )	-	Singh et al. (1999)
15	<i>Pseudomonas</i> sp. DSMZ 13134	Barley leaf pathogen <i>Rhynchosporium secalis</i> and barley root pathogen <i>Gaeumannomyces graminis</i>	Barley plant	ISR	Fröhlich et al. (2012)
16	<i>Pseudomonas</i> sp. (strain PsJN)	Grey mould caused by <i>Botrytis cinerea</i>	Grapevine ( <i>Vitis vinifera</i> L.)	ISR	Barka et al. (2000)
17	<i>Pseudomonas</i>	<i>Alternaria alternata</i> , <i>Colletotrichum truncatum</i> , <i>Rhizoctonia solani</i> , <i>Sclerotium rolfsii</i> , <i>Fusarium oxysporum</i>	Soybean	Antagonistic	Dalal and Kulkarni (2013)
18	<i>Pseudomonas fluorescens</i> WCS417r	<i>Bipolaris sorokiniana</i>	Tall fescue	ISR	Kilic-Ekici and Yuen (2004)
19	<i>Pseudomonas cepacia</i>	<i>Rhizoctonia solani</i> , <i>Sclerotium rolfsii</i> and <i>Pythium ultimum</i>		Chitinolytic activity	Fridlender et al. (1993)
20	<i>Penicillium</i> sp. (UOM PGPF 27) and <i>Pythium</i> sp. (UOM PGPF 41)	Pearl millet downy mildew disease	Pearl millet	-	Murali et al. (2012)
21	<i>Phoma</i> sp. (GS6-1, GS7-4 and GU23-3) (non-sporulating fungus)	<i>Gaeumannomyces graminis</i> var. <i>tritici</i> (Gg) and <i>Cochliobolus sativus</i> (Cs). GS6-1, GS7-4 ( <i>Phoma</i> sp.)	Barley kernels	Competitive root colonization	Shivanna et al. (1996)

(continued)

Table 8.1 (continued)

S. no	PGPM	Action against	Host	Mechanism	References
22	<i>Penicillium</i> GP17-2, <i>Phoma</i> GS8-2 and <i>sterilefungus</i> GU23-3, <i>Fusarium</i> GF18-3, <i>Penicillium simplicissimum</i> (isolate GP17-2)	<i>Colletotrichum orbiculare</i>	Cucumber plant	ISR	Koike et al. (2001) and Chandamie et al. (2006)
23	<i>Penicillium</i> GP17-2, <i>Phoma</i> GS8-2, <i>sterilefungus</i> GU23-3	Bacterial angular leaf spot	Cucumber plant	IR	
24	<i>Phoma</i> GS8-2, <i>sterilefungus</i> GU23-3, <i>Fusarium</i> GF19-2	<i>Fusarium</i> wilt	Cucumber plant	IR	
25	<i>Verticillium albo-atrum</i> and <i>Diplodia scrobiculata</i>	<i>Diplodia pinea</i>	Aleppo pine ( <i>Pinus halepensis</i> )	ISR	Muñoz et al. (2008)
26	<i>Penicillium</i> spp. GP15-1 ( <i>Penicillium neoehinulatum</i> or <i>Penicillium viridicatum</i> )	Damping-off disease by <i>Rhizoctonia solani</i>	Cucumber plant	ISR	Hossain et al. (2014)
27	<i>Bacillus pumilus</i> (PGPR strain SE 34)	<i>Fusarium oxysporum</i> f. sp. <i>radicis-lycopersici</i>	Tomato ( <i>Lycopersicon esculentum</i> Mill.)	–	Benhamou et al. (1998)
28	<i>Pseudomonas denitrificans</i> 1–15	<i>Ceratocystis fagacearum</i>	Live oaks ( <i>Quercus fusiformis</i> )	–	Brooks et al. (1994)
29	<i>Pseudomonas</i> (Ps.) <i>chlororaphis</i> Tx-1	<i>Pythium aphanidermatum</i> and <i>Pythium dissotocum</i> and controls root rot	Sweet peppers	–	Chatterton et al. (2004)
30	<i>Pseudomonas aeruginosa</i> 7NSK2	Leaf infection by <i>Botrytis cinerea</i> and resistance to tobacco mosaic virus	Bean and in tobacco	ISR by production and accumulation of salicylic acid	De Meyer and Hofte (1997); Audenaert et al. (1999)
31	<i>Pseudomonas fluorescens</i> PB27	Aflatoxigenic fungus <i>Aspergillus flavus</i>	–	Antibiosis by producing chitinase	Akocak et al. (2015)



32	<i>Pseudozyma aphidis</i>	<i>Clavibacter michiganensis</i>	Tomato plant	Secretes extracellular metabolites. Activates pathogenesis-related genes. Triggers SA-independent IR response	Barda et al. (2015)
33	<i>Pseudomonas</i> sp. (pf 12)	Basal rot disease by <i>Fusarium oxysporum</i> Schlechtend: fr. f. sp. <i>cepae</i>	Onion	Reduction of mycelial growth	Malathi (2015)
34	<i>Serratia plymuthica</i>	<i>Botrytis cinerea</i> , grey mould and <i>Sclerotinia sclerotiorum</i> , white mould diseases of leaves	Cucumber	Antagonism	Kamensky et al. (2003)
35	<i>Streptomyces</i> NRRL 30562	<i>Rhizoctonia solani</i> , <i>Aspergillus</i> sp., <i>Fusarium oxysporum</i> , <i>Botrytis allii</i> and <i>Alternaria helianthi</i> , <i>Pythium ultimum</i> , <i>Phytophthora infestans</i> , <i>Penicillium</i> sp., <i>Sclerotinia sclerotiorum</i> , <i>Erwinia carotovora</i> and <i>Cochliobolus carbonum</i>	Isolated from stems of <i>Kennedia nigricans</i>	Antibiosis. Wide-spectrum antibiotics named Munumbicins	Castillo et al. (2002)
36	<i>Serratia marcescens</i> strain 90-166 and <i>Bacillus pumilus</i> strain SE34	Cucumber mosaic virus (CMV)	<i>Arabidopsis thaliana</i>	ISR by salicylic acid and NPR1-independent and JA-dependent signalling pathway	Ryu et al. (2004)
37	<i>Streptomyces</i> sp. (CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90)	Fusarium wilt disease	Chickpea		Gopalakrishnan et al. (2015)
38	<i>Streptomyces</i> sp. MT7	Wood-rotting <i>Basidiomycetes</i>	–	Cell wall lytic enzymes: chitinase, $\beta$ -1,3-glucanase and protease; siderophores	Nagpure et al. (2013)

(continued)

Table 8.1 (continued)

S. no	PGPM	Action against	Host	Mechanism	References
39	<i>Streptomyces violaceusniger</i> MTCC 3959	<i>Basidiomycetes</i> which causes white rot and brown rot fungi	–	Antagonism, mycolytic enzymes: extracellular chitinase, protease, $\beta$ -1,3-glucanase	Nagpure et al. (2014)
40	<i>Trichoderma virens</i> and <i>T. atroviride</i>	<i>Alternaria solani</i> , <i>Botrytis cinerea</i> and <i>Pseudomonas syringae</i> pv. <i>tomato</i> (Pst DC3000)	Tomato plant	Induce both ISR and SAR	Salas-Marina et al. (2015)
41	<i>Trichoderma hamatum</i> 382	Bacterial spot of tomato by <i>Xanthomonas euvesicatoria</i> I10c	Tomato plant	ISR	Alfano et al. (2007)
42	<i>Trichoderma asperellum</i> T203	<i>Pseudomonas syringae</i> pv. <i>Lachrymans</i>	Cucumber plant	ISR	Shoreesh et al. (2005)
43	<i>Trichoderma</i>	<i>Botrytis cinerea</i>	–	ISR, antagonism, competition for nutrients and space	Vos et al. (2015)
44	<i>Trichoderma</i> sp., <i>T. harzianum</i> (TH 3)	Basal rot disease caused by <i>Fusarium oxysporum</i> f. sp. <i>cepae</i>	Onion	–	Malathi (2015)

Disease incidence and severity can be minimized when competition between pathogens and PGPB occurs. For example, non-pathogenic soil microbes can rapidly colonize plant surfaces and use up the available nutrients making it difficult for pathogens to grow. Treatment of plants with *Sphingomonas* sp. has been reported to prevent the bacterial pathogen *P. syringae* pv. tomato from causing disease symptoms (Glick 2012). However, poor rhizosphere competence leading to inconsistent performance hampers application of PGPB in field tests. Rhizosphere competence of biocontrol agents is determined by effective root colonization and ability to survive/proliferate along with growing plant roots over a significant time period, in the presence of native microflora. As rhizosphere competence is of utmost importance for effective biological control, detailed understanding of genetic and environmental regulation of root-microbe interaction can considerably help to improve the efficacy of these biocontrol agents. Root colonization is important in competing for root niches and bacterial determinants. Root surface and its surrounding rhizosphere are significant sink for carbon. Photosynthate allocation to this zone can be up to 40%. PGPB compete for these nutrients and niches in order to protect plants from phytopathogens. They reach root surfaces chemotactically, facilitated by flagella. Root exudates contain chemical attractants like organic acids, amino acids and specific sugars. Some of these exudates are also effective as antimicrobial agents providing an ecological niche advantage to organisms that have the ability to detoxify them via adequate enzymatic machinery. Genetic and environmental factors control the quantity and composition of chemoattractants and antimicrobials exuded by plant roots. Thus, it can be deduced that PGPB competence depends heavily upon their ability to take advantage of a specific environment and/or adapt to changing conditions. For example, sugars, amino acids and organic acids induce chemotaxis in *Azospirillum*. However, different strains vary in their degree of chemotactic response to each of these compounds. Rice exudates were shown to induce stronger chemotactic responses of endophytic bacteria compared to non-PGPB present in the rice rhizosphere, suggesting that PGPB are uniquely equipped to sense chemoattractants. Bacterial LPS (lipopolysaccharides) are also involved in root colonization, but their importance in colonization may be strain dependent as the LPS O-antigenic side chain of *P. fluorescens* WCS374 doesn't play a part in potato root adhesion, while on the other hand, the O-antigen chain of *P. fluorescens* PCL1205 does contribute in tomato root colonization. Moreover, the O-antigenic aspect of LPS was shown to have no contribution in rhizoplane colonization of tomato by the PGPB *P. fluorescens* WCS417r, whereas this bacterial determinant did contribute in its endophytic colonization of roots. Properties like high rate of bacterial growth, ability to synthesize vitamin B1 and exuding NADH dehydrogenases enable PGPB to colonize plants. They also indulge in root colonization via type IV pili which are known for their role in the adhesion of bacteria to eukaryotic cells and are also involved in plant colonization by endophytic bacteria. Efficient root colonization in certain PGPB is linked to the ability of secreting even a site-specific recombinase. Transfer of a site-specific recombinase gene from a rhizosphere-competent *P. fluorescens* into a rhizosphere-incompetent *Pseudomonas* strain reportedly increased its

ability to colonize root tips (Compant et al. 2005). In field experiments, inadequate biocontrol is often correlated with poor root colonization. A screen for mutants of the rhizobacterial strain *P. putida* KT2440 was able to identify set of putative surface and membrane proteins that have a role in attachment to corn seeds. Some such proteins were homologs of a calcium-binding protein, a hemolysin and a potential multidrug efflux pump. A study used in vivo expression technology to identify *P. fluorescens* genes that are specifically expressed in the rhizosphere (i.e. rhi genes) and found greater than 20 rhi genes. Out of these, 14 were shown to have significant homology to genes involved in nutrient acquisition, stress response or secretion. Various root colonization genes and traits were also observed in the *Pseudomonas* species with biocontrol properties and suggested for use in improving colonization of wild-type *Pseudomonas* strains. The competitiveness of these strains is also increased by their ability to produce siderophores. Authors observed that ability to utilize organic acids is the nutritional basis of tomato rhizosphere colonization where a defect/inability in the utilization of the raid organic acids has led to decreased competitive colonization of the tomato rhizosphere. On the other hand, a defect in sugar utilization found to have no impact on colonization (Bloemberg and Lugtenberg 2001). AMF have to compete with soil-borne pathogens in order to acquire space and nutrients. Plant pathogens may obstruct mycorrhizal colonization if they are present in very large numbers. To avoid this competitive inhibition and have better biocontrol efficiency, AMF pre-inoculation and pre-host treatment are always favoured. Dual inoculation of AMF with rhizobacteria has a synergistic or additive effect on its control of plant growth suggesting that biocontrol properties would depend on the combination of bacterial/fungal species used, soil's nutritional status and other environmental factors (Alizadeh et al. 2013).

### ***How Do PGPM Help in Fighting Abiotic Stress?***

Typically, plant growth involves periods of maximum growth interjected randomly with various levels of no growth or growth inhibition periods triggered by external stress stimuli. Upon addition, PGPM can employ any one or more of several different strategies described below, in order to overcome the growth inhibition caused by environmental stress or biotic and abiotic.

ACC deaminase-containing PGPB can be employed to ameliorate abiotic stresses like temperature extremes, metal toxicity, flooding, drought, hypoxia, salt and organic contamination (Glick 2012). Several PGPR containing ACC deaminase are present in the soil which aid in improvement of plant growth, especially under unfavourable environmental conditions. Abiotic stresses trigger increase of ET in plants which is directly related to the concentration of ACC in plant tissues. These bacteria may improve the survival of seedlings in the first few days post-sowing by decreasing ET levels, which helps in longer root formation. Plants with decreased level of ET react better when facing different environmental stresses such as salinity, drought and metal toxicity (Fahad et al. 2015). Both endogenous and exogenous

ACC deaminase genes have been shown to increase the symbiotic performance of several rhizobial strains. In case of flooding, plant roots are typically subjected to hypoxic or oxygen-limiting conditions which lead to increased production of ACC synthase enzyme and other stress proteins. The stressed plant subsequently synthesizes more ACC in its roots, but the newly made ACC cannot be converted to ET in the roots (ET synthesis requires oxygen), hence ACC has to be transported to the shoots where there is an aerobic environment. There ACC gets converted to ET, and production of ET by flooded plants results in epinasty (wilting), chlorosis, necrosis and reduced growth. Treating plants with ACC deaminase-producing PGPB can protect plants from majority of damage caused by flooding. A study reported that PGP bacteria that are endemic to sites of limited rainfall prove better in protecting plant growth against drought stress as compared to similar bacteria from sites of water abundance. Researchers have proved the efficacy of ACC deaminase-containing PGPB in protecting a wide variety of plants against drought-induced growth inhibition. Lowering ET levels using ACC deaminase-containing PGPB might also afford protection against salinity stress as reported in studies by Glick (2014) and Sarathambal et al. (2014). The presence of organic contaminants in the soil would also result in increase in stress-induced ET synthesis. As a number of ACC deaminase-containing PGPB are able to protect plants from a wide range of abiotic stresses, this technology can be explored to become a technology (marketable product) with commercial use in the field. However, certain inhibitions about the use of bacteria on a large scale in agriculture exist as the biosafety level of such PGPB needs confirmation (Glick 2012, 2014).

PGPB that do not contain ACC deaminase nevertheless protect plants from the harmful effects of abiotic stresses by providing IAA to the plant which directly stimulates plant growth, even if other inhibitory compounds are present. However, bacteria producing both IAA and ACC deaminase can be considered effective in protecting plants against a wide range of stresses. The synergistic effect of IAA and ACC deaminase in plant growth promotion can be explained as follows: PGPB bound to plant roots utilize tryptophan (exuded by plant roots) to convert it into IAA. This IAA produced is secreted out by the bacteria in the rhizosphere and is taken up by plant cells. Once there, it joins the plant's IAA to stimulate an auxin signal transduction pathway, which includes various auxin response factors. As a result, growth and proliferation of plant cells occur. Simultaneously, some of the IAA promotes transcription of the ACC synthase-encoding gene which increases concentration of ACC. As ACC oxidase catalyses ACC into ET, the ET levels also shoot up. Various biotic and abiotic stresses can also increase IAA synthesis or stimulate transcription of ACC synthase gene. In the absence of bacterial ACC deaminase, ET-induced cell growth and proliferation limitation take place due to decreased transcription of auxin response factors. It also limits IAA stimulation of the synthesis of more ET. In the presence of ACC deaminase, lesser ET is formed, and therefore in its presence, transcription of auxin response factors is not inhibited, and IAA stimulates cell growth and proliferation without causing a build-up of ET. Therefore, both in the presence and absence of stress, ACC deaminase can decrease inhibition of plant growth by ET and allows IAA to promote plant growth (Glick 2012).

CKs are compounds with structural resemblance to adenine and are named so because of their ability to promote cytokinesis or cell division in plants. Various plants, some yeast strains and a number of soil bacteria including PGPB produce CKs. Transgenic plants which can overproduce CKs under abiotic stress were found to have withstand the harmful effects of stress. However, there aren't any conclusive studies showing that bacterially produced CKs can protect plants from abiotic stresses. A comparative study concentrating on the activity of CK-producing PGPB as against that of CK minus mutants may help in understanding the role of PGPB in CK of those bacteria is needed (Glick 2012).

Gibberellins are plant hormones regulating growth and development processes like germination of seeds, emergence of seedling, floral induction, growth of roots, stems, leaves, fruits and flowers and senescence. In majority of such processes, GAs play a complementary role with other phytohormones and regulatory factors and integrate the signalling pathways (Bottini et al. 2004). It was reported that drought resistance can be acquired by inhibition of GA synthesis (Waqas et al. 2012). Under drought, synthesis of GA and ABA was found to be reduced in maize seedlings, and it was shown that prior inoculation with *Azospirillum lipoferum* promoted growth of both roots and shoots. It has also been reported to relieve water stress effects on wheat, at least in part by GA synthesis (Bottini et al. 2004). It has also been reported that two endophytic fungi, *Phoma glomerata* LWL2 and *Penicillium* sp. LWL3, alleviate drought and salt stress by secreting phytohormones like gibberellins and IAA along with promoting plant growth in cucumber plants (Waqas et al. 2012).

Trehalose is a non-reducing storage disaccharide and is ubiquitous in nature. Increased levels of trehalose act as protectant against various abiotic stresses like drought, high salt and temperature extremes. Trehalose is a highly stable molecule resistant to both acid and high temperature. It forms a gel phase as cells dehydrate, by replacing water and, thereby, decreases drought and salt damage. Additionally, trehalose also prevents protein degradation and aggregation which occur under extreme temperature stresses. PGPM engineered to overproduce trehalose were observed to make plants tolerant against drought and other stresses. Treatment of bean plants with the genetically engineered symbiotic bacteria *Rhizobium etli* (an overproducer of trehalose) has reportedly led to formation of more nodules, more nitrogen fixation, increase in biomass and increased recovery from drought stress as compared to plants inoculated with wild-type *R. etli*. Similarly, treatment of maize plants with the PGPB *Azospirillum brasilense* (modified for overproduction of trehalose) made the plants more resistant to drought, producing more biomass than plants which were treated with wild-type *A. brasilense*. Even though plants can also be directly engineered to overproduce trehalose, it is easier to use genetically modified PGPB to attain the same end. Also, one engineered bacterial strain can be effective in protecting many different crop plants (Glick 2012).

PGPR can also enhance stress resistance by solubilizing minerals and nutrients in the plant-soil system and enhance release of these nutrients into soil solution (Sarathambal et al. 2014). Nitrogen-fixing bacteria (NFB) can play a vital role in plant establishment and development as they provide the limiting nutrient (nitrogen) to plants. The relationships of symbiotic bacteria (like *Rhizobium* and *Frankia*) with



leguminous crops have been studied extensively, and its total coverage is beyond the scope of this chapter. It has been reported that diazotrophs, such as *Acetobacter* and *Herbaspirillum*, might be beneficial for plant growth promotion in nonlegumes (Grandlic 2008). Non-rhizobial N<sub>2</sub>-fixing bacteria were shown to exhibit endophytic growth in several grasses. *Pseudomonas* species were the most dominant class of nifH (the nitrogen fixation gene) carrying bacteria in the rhizosphere of perennial grasses of South Australia. The nifH gene is present in many non-*Frankia* actinobacteria like *Agromyces*, *Microbacterium*, *Corynebacterium* and *Micromonospora*. Nitrogen fixation is also reported in *Prosopis* sp. under natural conditions (Sarathambal et al. 2014). Other well-known NFB include *Azospirillum*, *Bradyrhizobium*, *Burkholderia*, *Sinorhizobium*, *Mesorhizobium* and *Azorhizobium* (Grandlic 2008). It has been reasonably hypothesized that optimally nitrogen-nourished plants have survival advantage over nitrogen malnourished ones, when challenged by abiotic stresses (Arora 2004). Certain species of *Rhizobium* have been reported to form effective (N<sub>2</sub>-fixing) symbioses with legumes under various stresses (salt, heat, acid stress, heavy metal stress) (Zahran 1999).

Some microorganisms are capable of solubilizing the inorganic P (phosphorus) present in the soil and increase its availability to plants. Plant-associated microbes have been reported to solubilize P in various instances. This group comprises of bacteria, fungi and some actinomycetes, and they solubilize unavailable forms of organic P such as tricalcium, iron, aluminium and rock phosphates into soluble forms by releasing various organic acids like malic, fumaric, succinic, citric, glyoxylic and gluconic acids (Pontes et al. 2015). In conditions wherein phosphate is present in an insoluble form, phosphate-solubilizing bacteria (PSB) like strains belonging to the genus *Cedecea* and *Microbacterium* have been reported to promote the growth of barley plants (Sarathambal et al. 2014). The hyphae of AMF were noted to absorb poor mobility nutrients like P from soil beyond the zones that are depleted by roots particularly when nutrient availability is low. Mycorrhizal fungi increase plant growth by enhanced phosphorus uptake, and some AMF aid in enhancing plant resistance towards salinity stress by either modulating the hormonal balance of the host plant or by enhancing water uptake (Alizadeh et al. 2013). At a particular deserted site with low nutrient and organic matter content, a native mycorrhiza, *Geastrum coronatum*, was shown to be an important microbe in plant establishment. In addition, *G. coronatum* was most effective at heightening nitrogen and P content in plants when used together with *Rhizobium* sp. Another mycorrhizal species, *Glomus intraradices*, showed greater affectivity at promoting plant growth with a completely different strain of *Rhizobium* (Grandlic 2008).

Apart from phosphorous, most soils are deficient in micronutrients, such as: Zn, Fe and Mn with Zn registering as foremost nutrient that is deficient across the world. Hence zinc solubilizing bacteria (ZSB) based bioinoculants are highly beneficial for countries like India in which there is high incidence of zinc deficiency (greater than 70%). These ZSB are capable of solubilizing insoluble zinc compounds/minerals in agar plates as well as in the soil. Potassium-solubilizing bacteria like *Bacillus mucilaginosus* and *Bacillus edaphicus* are used in bioinoculants and are capable of solubilizing potassium rock by production and secretion of organic acids. The

bacteria being heterotrophic and aerobic can obtain energy and carbon from existing organic sources. Hence, they can also improve soil structure by contributing to the formation and stabilization of water-stable soil aggregates. A study by Sarathambal et al. (2014) reported that *B. subtilis*, *Azospirillum* sp., *A. brasilense* and *Bacillus* sp. have the ability to solubilize minerals such as P, potassium and Zn. The metal-resistant PGPB *Pseudomonas* sp. and *Pseudomonas jessenii* were shown to aid in metal sequestering (nickel, copper and Zn) *Ricinus communis* when associated with host as well as phosphate solubilization and IAA production. These aforementioned abilities could be highly useful for promoting plant growth to counter abiotic stress polluted soils in metal-stressed soils (Rajkumar and Helena 2008). Sheng et al. (2008) isolated and characterized endophytic lead (Pb)-resistant bacteria (*P. fluorescens* G10 and *Microbacterium* sp. G16) from rape roots of plants grown in heavy metal-contaminated soils. These two strains were shown to possess various heavy metal and antibiotic resistance characteristics and can increase water-soluble Pb (in solution) in Pb-added soil while simultaneously showing plant growth promotion.

For in-field effectiveness, a PGPB should show persistence and proliferation ability in the environment. Some countries have a common spring temperature of 5–10 °C, and PGPB should be functional in these cool soil temperatures and should be able to survive repeated freeze-thaw cycles common during the winter season in several places. Moreover, as many fungal pathogens are most destructive in cold and temperate climates, cold-tolerant (psychrotrophic) PGPB can be expected to show better biocontrol activity in such climates than mesophilic biocontrol strains. It has been studied that in some psychrophilic and psychrotrophic PGPB, exudation of antifreeze proteins into surrounding area takes place when grown at low temperatures. Bacterial antifreeze proteins would regulate the formation of ice crystals outside the bacterium, protecting cell walls of the bacteria from the lethal piercing damage which might be caused by the formation of large crystals at freezing temperatures. Additionally, some of them were also found to possess ice nucleation activity. Even though there have been various studies aiming at isolation and characterization of bacterial antifreeze proteins, none of these studies focused on the possibility of utilizing this activity to enable PGPB functionality in environments that include cold temperatures (Glick 2012).

### ***Other Strategies of Dealing with Abiotic Stress***

Recent researches in the field of PGPM have shown tremendous opportunities that have a positive impact on the growth and health of plants. Also, applying PGPM for the remediation of contaminated soils has opened newer possibilities of research. For instance, combining PGPM with contaminant-degrading bacteria can help to eradicate contaminants present in the soil. Rhizobacteria can also be used to increase the uptake of specific metal pollutants from soil. Exploiting and manipulating genetic engineering technologies in this line can be proven to help bioremediation

(Zhuang et al. 2007). Phytodegradation and rhizoremediation are remediation strategies that utilize rhizospheric bacteria to degrade persistent organic compounds. It is well known that ET is integral for initiation of senescence in flowers. Many cut flowers (e.g. carnations and lilies) are treated with silver thiosulfate (an ET inhibitor) prior to their sale. However, high silver thiosulfate levels can be phytotoxic and cause environmental problems. Using ACC deaminase-containing plant PGPR (with ability to limit ET production) for treating cut flowers can provide an environmentally friendly alternative. In the first instance of using ACC deaminase-containing PGPB for metal phytoremediation, it was reported that a nickel-resistant bacteria could alleviate nickel toxicity in canola plants. Since then, there have been several accounts of metal phytoextraction using PGPB including a wide variety of plants, different metals, soils and bacteria. In many of those studies, bacteria were first selected based on their resistance to the toxic metal(s) and then tested for ACC deaminase activity, IAA and siderophore synthesis. Additionally, some other bacterial traits might be involved in metal phytoremediation. Certain bacteria facilitating phytoremediation were shown to have the ability to solubilize phosphate and were proposed to assist in metal uptake. Another study reported the production of biosurfactants in the bacterial strain aiding in phytoremediation which might be involved in increasing the bioavailability of metals. Strategy of using bacteria in combination with phytoremediation was suggested to be effective in removing and/or degrading organic contaminants from impacted soils, both in the lab and under field conditions (Glick 2014).

Timmusk and Wagner (1999) reported changes in plant gene expression up on inoculation with PGPR. The model plant chosen was *Arabidopsis thaliana*, and PGPR inoculated was *Paenibacillus polymyxa*. Abiotic and biotic stress was introduced where abiotic stress was induction of drought and biotic stress was infection by pathogen *E. carotovora*. The results showed that the plants inoculated with PGPR were more tolerant to stress and showed more resistance than the control plants. In a study by De Souza et al. (2015), *Pseudomonas* sp. FeS53a was isolated from rice roots from an area with a history of iron toxicity, and the bacterial genome was sequenced. Its genome was found to contain genes involved in auxin biosynthesis that can regulate metabolic processes and promote plant growth under abiotic stress conditions. This strain was also observed to encode superoxide dismutase and catalase, the enzymatic antioxidants which remove free radicals and prevent damage to cell membranes and DNA. Bacterioferritin genes that can be involved in iron storage systems and the ferric uptake regulation protein (Fur) were also detected in this isolate. In a recent study, Amaresan et al. (2016) isolated salt-tolerant isolates of *Bacillus* PGPR from Tsunami (India)-affected areas and reported the presence of isolates capable of growing even at 10% NaCl concentration. Out of these isolates, 14 showed phosphate solubilization activity, 13 produced siderophores and five produced IAA, while 16 isolates could produce at least one extracellular enzyme. Some of these isolates also exhibited antagonistic activity against *S. rolfsii*. It was hypothesized that such PGPR could be employed as bioinoculants for enhancing crop growth in Tsunami-stressed soils.

The plant-bacteria associations have been a topic for research since decades. But there is a lot more to explore about the mechanisms employed by PGPB. Currently it is known that the bacteria can be good for plant growth and health, whereas a significant factor to be considered is that the plant can also select their microbiome to have beneficial bacteria preferential to their systems (Marasco et al. 2012). This kind of an approach needs to be further explored to maximize the benefits of PGPM-host associations. Bacterial endophytes have been a topic for research due to their direct mechanism of plant growth-promoting capacity as well as for their indirect mechanism as biocontrol agents. The mechanism of rhizospheric bacteria and endophytes are almost similar; therefore much work has been done in the domain of rhizospheric bacteria, assuming a similar mechanism in endophytes. But the fine line that separates the two is the micro-environment in which they dwell. For instance, for rhizospheric bacteria, the variations in temperature, light, soil type and other abiotic factors play a key role. It is also possible that different plant growth-promoting mechanisms which are unexplored in rhizospheric bacteria might be discovered in bacterial endophytes (Santoyo et al. 2016).

## Genetic Modification to Improve PGPM

Identification of the genes involved in plant growth-promoting activity of rhizobacterial strains can be used to improve the performance of biocontrol strains and/or to design novel biocontrol strains by genetic modification. Rhizobacterial strains have been transformed using single genes or even complete operons, under control of regulatory genes or regulated by *tac* or *lac* promoters. Introduction of a mini-Tn5 vector including the complete operon for biosynthesis of PCA (phenazine-1-carboxylic acid), an antifungal metabolite, has been reported to enhance rhizosphere competence and suppression of fungal diseases by genetically engineered *P. fluorescens*. Similarly, introduction of the *phzH* gene from *Pseudomonas chlororaphis* PCL1391 was found to enhance the biocontrol ability of *Pseudomonas* strains producing PCA by increasing the production of phenazine-1-carboxamide, additionally. Effective control was seen against tomato foot and root rot. Other studies also reported improved biocontrol and/or plant growth promotion by introduction of such genes like Cry-toxin-encoding *cry1Ac7* gene of *B. thuringiensis*, chitinase-encoding *chiA* gene of *S. marcescens*, and ACC deaminase gene from *E. cloacae* into rhizobacterial strains. Also, it was shown that the transfer of *Sss* gene of *P. fluorescens* WCS365 could enhance the competitive colonization ability of other *P. fluorescens* strains (Bloemberg and Lugtenberg 2001).

The *Sss* recombinase genes, *ptsP* and *orfT*, are important in the interaction of *Pseudomonas* spp., with various hosts. The gene *ptsP* encodes a nitrogen-specific EI paralogue called EI<sup>Ntr</sup> that forms a regulatory PTS phosphoryl transfer chain. This chain is involved in sugar-dependent utilization of certain amino acids and is also linked to metabolism of carbon and nitrogen. *Sss* gene and *orfT* were hypothesized to have a contributory role in providing rhizosphere competence and phenotypic

variation in fluorescent *Pseudomonas* (Mavrodi et al. 2006). Two genetically modified derivatives of *P. putida* WCS358r carrying the *phz* biosynthetic gene locus of strain *P. fluorescens* 2–79 are known to constitutively produce the antifungal compound PCA, thus imparting improved antifungal activity. Glandorf et al. (2001) suggested that the gene responsible to produce PCA can be introduced into a plant growth-promoting bacterial strain, *P. putida* WCS358r, using the mini-Tn5 transposon system as a delivery vector. Pectin, a complex plant polysaccharide, when broken down to D-glucuronate and D-galacturonate, serves as a carbon source for bacterial growth and could potentially serve as a nutrient source for efficient root colonization of PGPR. Therefore, the presence of genes that enable D-galacturonate and D-glucuronate utilization could be advantageous for plant growth-promoting activity through efficient root colonization. These genes were present in *B. amyloliquefaciens* subsp. *plantarum* and can be genetically introduced into other species to improve their root colonization ability (Hossain et al. 2015).

## Future Prospects and Need for More Intensive Utilization of PGPM

The growing concern and awareness to protect the agriculture, environment and food safety issues has been significant enough to warrant a reduction in the usage of pesticides and other crop chemicals. Biological control through application of PGPB is highly suggested as a potential alternative in crop disease management. Plant productivity and other factors like quality and health have been evidently improved by direct application of PGPM to the soil and through seed inoculation as examined by many researchers and also elaborated in this chapter. A number of studies confirmed that microbial inoculants lead to higher microbial populations in the soil and promote plant growth through improved nutrient acquisition, suppression of plant diseases, increased levels of phytohormones and other growth metabolites and ISR in various crops including cereals (Nelson 2004; Rodriguez et al. 2007; Van Loon 2007; Umashankari and Sekar 2011; Yadav et al. 2011; Basja 2013). Detailed understanding of the mechanisms of plant growth promotion by PGPB has shed light on multiple facets of disease suppression by these biocontrol agents. However, most studies have focused on free-living rhizobacterial strains, especially *Pseudomonas* and *Bacillus*, and much remains to be understood from non-symbiotic endophytes about their unique associations and growth-promoting activity on host plants. Increased understanding of the mechanisms of PGPB action opens up new possibilities for designing strategies for improvement of efficacy of biocontrol agents. Identification of key antimicrobials, like DAPG produced by superior agents, can be exploited for efficient targeted selection of isolates carrying relevant biosynthetic genes. Determining the edaphic parameters that favour disease suppression, production of antibiotics and their activity can be advantageous for identifying target inoculants for soils to support biocontrol. Amending soils or



growth substrates with minerals like Zn or priming of inoculants with media amendments during fermentation can also be highly effective as per some scientific recommendations. Similarly, consortium of rhizosphere bacteria can be further augmented by soil aeration, hydrogenation, delivery of molasses, sugars and by apt crop rotations. Identification of the various mechanisms of action, facilitating strain combinations, like bacteria with bacteria or bacteria with fungi, to attack pathogens with a broader arsenal of microbial weapons, etc., can be more beneficial options. Biotechnology can also be applied to further improve strains which have prized qualities (like ease of formulation, stability or exceptional suitability to plant colonization) by creation of transgenic strains that combine multiple means of activity. Continued research on endophytic bacteria holds potential for the development of biocontrol agents which may self-perpetuate by colonizing hosts and getting transferred to progenies, like in the case of associative nitrogen-fixing PGPB on sugarcane. The importance and target-specific approach in plant genetic engineering is unquestionable to further the goals of modern day demands on agriculture systems. And most of the functional advantages that PGPM are offering can be traced back to the genes encoding these functions, and one can either directly engineer the plants with genes from PGPM to overproduce specific metabolites or can easily generate genetically modified PGPB to attain the same end. Also, as many of these PGPM are non-host specific and are predominantly rhizospheric (and some endophytic), the advantage of using PGPM over GM plants would be that one engineered PGPM strain can be effective in protecting many different crop plants. Figure 8.3 is

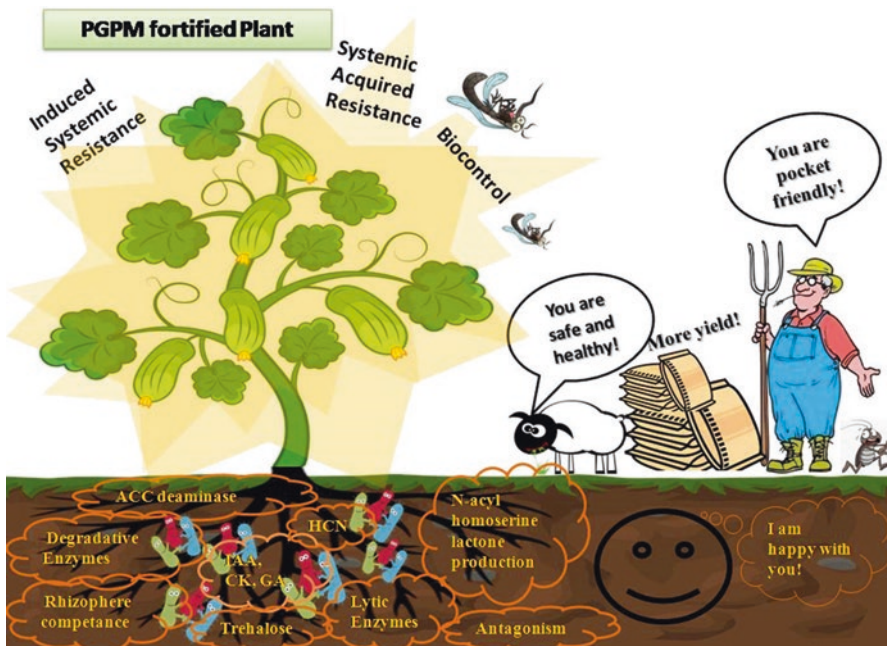


Fig. 8.3 PGPM fortified plant and its response to various biotic/abiotic stressors

a comprehensive pictorial representation of various means by which PGPM can benefit the plants and why they can be a preferred choice over GM plants.

Considering the fact that population growth is far exceeding the availability of food resources, it is imperative that new innovative technologies be developed and implemented to increase crop yield at the face of biotic and abiotic stressors. Plant-associated microorganisms can play a vital role in conferring resistance to several environmental stresses. Use of microbial inoculation for stress alleviation in plants can provide a cost feasible and environmentally sound method in lieu of plant breeding, genetic engineering or use of agricultural chemicals. Promoting the use of PGPM, initially in addition to and ultimately instead of agricultural chemicals currently in use, is important to achieve this aim.

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