Fungal Biology

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Antifungal Metabolites of Rhizobacteria for Sustainable Agriculture

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Antifungal Metabolites (AFM) for Sustainable Agriculture

Chapter 6
Biofungicidal Properties of Rhizobacteria for Plant Growth Promotion and Plant Disease Resistance

Rajashree B. Patwardhan, Pragati S. Abhyankar, Suneeti S. Gore, Saylee V. Kalekar, and Shriya P. Umrani

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6.1 Introduction

Microbes are an important part of living soil not only in transforming nutrients in the soil but also with several functions in influencing soil health. There are certain microbes which assist the plant to grow well in their presence by a variety of mechanisms (Basu et al. 2021). The rhizosphere is the narrow region of soil that is directly affected by root secretions and associated soil microorganisms known as the root microbiome. The plant growth-promoting rhizobacteria (PGPR) are a valuable group of soil bacteria that can reside plant roots and enhance plant growth and development. The concentration of microbes in the rhizosphere is 10 to 1000 times

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greater than that in soil. Hence, such plant growth-promoting rhizobacteria (PGPR) should be exploited and utilized for sustainable agriculture (Sagar et al. 2020). The PGPR include *Azotobacter*, *Azospirillum*, *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Pseudomonas*, *Serratia*, *Streptomyces*, *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Frankia*, *Thiobacillus*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* belonging to different genera *Acetobacter*, *Achromobacter*, *Exiguobacterium*, *Flavobacterium*, *Gluconacetobacter*, *Herbaspirillum*, *Methylobacterium*, *Paenibacillus*, and *Staphylococcus* (Kour et al. 2019).

Rhizosphere and soil bacteria are particularly important in nearly all biochemical cycles in terrestrial ecosystems and participate in maintaining health and productivity of soil in agriculturally managed systems. A considerable variation in relative abundances of bacterial communities at both phylum and genus level has been observed among different crop systems. Compared to conventional farming systems, organic farming system shows higher percentage of phylum Proteobacteria (many PGPR) and lower percentage of phylum *Actinobacteria* (Reddy et al. 2019). Over the last decades, world agriculture has experienced high increase in crop yield. This is a result of high input of inorganic fertilizers and pesticides, and mechanization driven by fossil fuel. Repeated use over the years has led to serious environmental problems such as depletion of soil quality and health, ocean and ground water pollution, and emergence of resistant pathogens. It is a big challenge to feed the increasing world population on decreasing farmland areas without damaging environment. It is well known that rhizosphere and PGPR play an important role in maintaining crop and soil health through versatile mechanisms like nutrient cycling and uptake, suppression of plant pathogens, induction of resistance in plant host, and direct stimulation of plant growth (Anith et al. 2004) (Haas and Défago 2005). To act efficiently, microbes should remain active under a large range of conditions, such as varying pH, temperature, and concentrations of different ions. Preserving biodiversity of PGPR in soil could be an essential component of environment-friendly sustainable agriculture policies (Kour et al. 2020). Some studies have demonstrated that agricultural practices affect the diversity and function of rhizosphere and soil microorganisms (Raut et al. 2017). Organic farming differs from conventional agriculture in the production process, and it relies on techniques such as crop rotation, green manure, and biological pest control to maintain the soil productivity instead of chemical fertilizer and pesticides (Liu et al. 2017).

Plants lack adaptive immunity. Instead, plants are dependent on a heritable, innate immunity based on the recognition by receptors of the presence of microbial triggers (cues) including effector proteins and microbe-associated molecular patterns (Jones and Dangl 2006). The perception of microbial cues leads to the induction of broad spectrum of plant defenses called systemic acquired resistance (SAR) (Patel et al. 2016; Bostock 2005). Until recently, SAR was thought to be limited to induction of plant defenses against foliar microbial pathogens. However, recent results suggest that plants can activate signal exchanges between aboveground (AG) and belowground (BG) responses (Raut et al. 2017).

Root colonization by beneficial microbe is a process which is required for all mechanisms of biocontrol. Microbes are attracted chemotactically by certain components secreted by root. Weapons used by beneficial microbes to attack the pathogen include lytic enzymes such as chitinase, protease, cellulose, and glucanase (Shaikh et al. 2018; Jadhav et al. 2017, 2020a, b) and the antibiotics (Zakaria et al. 2019) such as phenazines, 2,4-diacetylphloroglucinol, pyoluteorin, pyrrolnitrin, hydrogen cyanide, cyclic lipopeptides, 2-hexyl-5-propyl resorcinol, and d-gluconic acid (Lugtenberg and Kamilova 2009). Based on the growth promotion activity of selected bacteria and their abilities to produce siderophores (Nithyapriya et al. 2021; Sayyed et al. 2019), phytohormones, and phosphate solubilizing activity (Sharma and Sayyed 2013; Sharma et al. 2016), the rhizobacteria may have great potential to increase the yield, growth, and nutrition of various vegetable crops under greenhouse and field conditions (Zaman et al. 2021). Therefore, these can be utilized as biofertilizer and biological control agents for fruit and vegetable production in sustainable and ecological agricultural systems (Lamsal et al. 2012).

The use of PGPR supports plant nutrition, and this has led to the development of a specific research area and to applications in agriculture. More recent advancements in knowledge of biology of a variety of soil-dwelling species are leading to an increased interest for their potential application for both plant growth promotion and protection against pathogens and parasites. These can be inhibited through direct and indirect mechanisms, thus helping to maintain crop health and productivity. Studies targeting at screening bacterial strains with such characteristics not only rise our understanding of the ecological significance of bacterial community related with plant roots but also create concrete prospects of application in agricultural contexts, in line with the principles of integrated pest management and eco-sustainability (Ruiu 2020).

The various types of fungal infections include the black spot, downy mildew, powdery mildew, blight, rust, wilt, club root, and anthracnose. Due to harm caused by chemical pesticides, use of PGPR to inhibit fungal diseases has been boosting interest (Kumar et al. 2019; Sharma et al. 2020; Arora et al. 2021). This review is an update concerning the bio-fungicidal properties of Rhizobacteria for plant growth promotion and plant disease resistance.

6.2 Biodiversity of Rhizobacteria

Biodiversity is an important element of environmental protection and is vital to agriculture production. Most microbial diversity of soil ecosystem is confined to rhizosphere. Depositions made into soil through plant root exudates play a major role in defining resident microflora, which differs from that in remaining bulk of soil. Rhizobacterial diversity is influenced by both, plant type and soil type. Soil factors, plant root exudates, and agricultural management are the factors that determine the microbial community composition within rhizosphere.

Based on their relationship with plants, PGPR have been divided into two major groups: symbiotic and free-living (Mubeen et al. 2006; Dominguez et al. 2017). PGPR have three main features: (i) root colonization ability, (ii) high survivability and multiplicity in root surroundings helping in plant growth promotion, and (iii) inhibition of phytopathogens (Bloemberg et al. 2000; Sagar et al. 2022; Sukmawati et al. 2021). PGPR strains occur in various taxonomic groups, which may be present concurrently in each soil. This suggests that taxonomically different PGPR strains may coexist in soil and colonize same rhizosphere, along with all non-PGPR members of the bacterial community. The taxonomic status of bacterial isolates based on their positive effect on plant growth or health, their ability to inhibit phytopathogens, or occurrence of particular gene or property of relevance for PGPR effect is characterized (Belimov et al. 2001)

Functional group approaches can be implemented when specific genes are recognized. For instance, nitrogen fixers can be screened using the *nifH* gene, which encodes dinitrogenase subunit of the nitrogenase. Its sequence is well conserved within the functional group, and it is commonly used as marker to monitor the size and diversity of diazotrophic community (Oldroyd and Dixon 2014). Some of these PGPR functional groups are taxonomically confined, such as the *Pseudomonas* 2,4-diacetylphloroglucinol (DAPG) producers (Vinay et al. 2016a, b; Reshma et al. 2018). In contrast, others show high diversity; certain bacterial functional groups may also comprise both PGPR and non-PGPR strains. For example, nitrogen fixers comprise PGPR as well as mutualistic symbionts and even a few pathogens.

Mutation is another vital component creating microbial variations that can interact diversely with plant host. Mutations influencing virulence-associated genes have striking results for advancement of destructiveness in wide evolution of pathogens (Sokurenko et al. 1999; McCann and Guttman 2008). Comparative phylogenetic analysis of DNA sequences of cloned 16S rRNA genes has shown that members of four major phylogenetic groups are ubiquitous to almost all soil types: class α-proteobacteria and phyla *Actinobacteria*, *Acidobacteria*, and *Verrucomicrobia*. These four groups are represented in >7% of 16S rRNA gene clone library studies of soil bacterial communities (Hugenholtz et al. 1998). Other classes of phylum Proteobacteria and phyla Firmicutes and Planctomycetes are detected in 25–75% of studies (Hugenholtz et al. 1998). Phyla *Proteobacteria*, *Cytophagales*, *Actinobacteria*, and Firmicutes are properly exemplified by cultivated microorganisms, and these four phyla account for 90% of all cultivated bacteria characterized by16S rRNA sequences from cultivated organisms in ARB database. The development of nif D and nif H specific primers has also proved extremely useful in screening diazotrophic strains.

The lack of information about the diversity of bacteria specifically isolated from rhizosphere of various plants needs to be filled up for our understanding of an important niche in microbial ecology of grasses such as rice. Some of standard rhizospheric and putative rhizospheric diazotrophs have been noticed to be naturally present in rhizosphere of the graminaceous plants like rice, sugarcane, wheat, kallar grass, etc. *Herbaspirillum* was first reported by (Olivares et al. 1996) as N2-fixing bacterium associated with roots of rice, maize, and sorghum. *Herbaspirillum seropedicae* as well as *Herbaspirillum rubrisubalbicans* (formerly identified as *Pseudomonas rubrisabalbicans*) are the confirmed diazotrophic bacteria. Another diazotrophic species *Herbaspirillum frisingense* has been isolated from C-4 fiber plants (Kirchhof et al. 2001)

Azospirillum spp. are known to be the most efficient diazotrophic bacteria isolated from rhizosphere of various plants. It is generally regarded as a rhizospheric bacterium and has often been reported to give best results upon inoculation to crop plants. Certain strains penetrate the roots suggesting that some strains of *Azospirillum* may also colonize within wheat tissues (Naiman et al. 2009). Till date several rhizospheric diazotrophic *Azospirillum* species including *Azospirillum lipoferum*, *A. amazonense*, *A. halopreferens*, *A. irakense*, and *A. doebereinerae* spp. have been reported (Khammas et al. 1989). *Klebsiella* spp., *Pseudomonas* spp., *Serratia* spp., *Azotobacter* spp., *Burkholderia* spp., *Alcaligenes* spp., *Enterobacter* spp., *Bacillus polymyxa*, *Gluconacetobacter* spp., *Azoarcus* spp., *Paenibacillus* spp., *Bacillus subtilis*, *B. licheniformis*, *B. pumilus*, *Brevibacterium halotolerans*, and *Pseudomonas putida* are plant associated bacteria reported from different plants.

6.3 Mechanisms of Plant Growth Promotion by Rhizobacteria

The primary goal of farming is production of high-quality, reliable, and reasonably priced food for an ever-increasing worldwide population. The widespread use of chemicals has harmful effects on environment, adverse effects on non-target organisms, carcinogenicity on living beings, and number of ecological problems. PGPR can promote plant growth by number of different mechanisms (Zope et al. 2019). They carry out the alteration of the whole microbial community in rhizosphere niche through production of secondary metabolites like hydrogen cyanide (Table 6.1),

Table 6.1 Role of secondary metabolites secreted by PGPR

Secondary metabolite	Inhibitory effect	Additional role
Hydrogen cyanide	Inhibits cytochrome C oxidase	Chelation of metals and cellular signalling
Volatile organic compounds	Antifungal and antibacterial	Plant growth promotion
Phenazines	Inhibition of conidial germination and mycelial growth, formation of reactive oxygen species (ROS) and reactive oxygen species (RNS)	Reduction in exopolysaccharide formation
Pyrrolnitrin	Affects electron transport and oxidative phosphorylation	Interferes with osmotic signal transduction
Pyoluteorin	Cell membrane destruction	Autoinduction and acts as a signal molecule
2,4-diacetylphloroglucinol	Cell membrane destruction and reduction on disease symptoms	Intra and intracellular signals and co-PLT, antiglycation activity, and beneficial effects on plant growth
Cyclic Lipopeptides	Formation of ion channel in cell membrane, disruption of cell wall and cell membrane	Swarming movement and biosurfactants properties

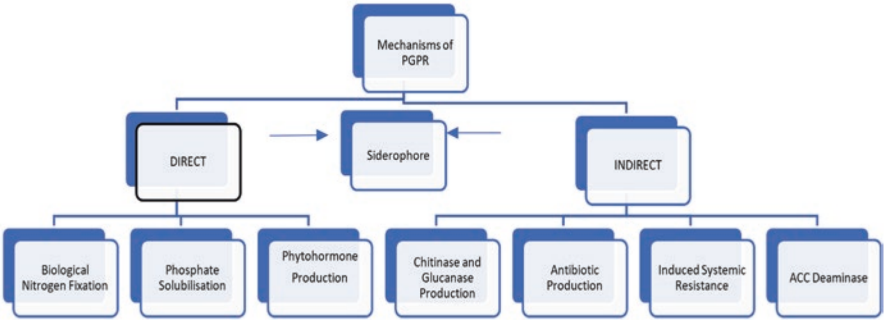


Fig. 6.1 Mechanisms of plant growth promotion

lytic enzymes, antibiotics, siderophores, auxins like indole acetic acid, gibberellins, ACC deaminase, etc. (Kusale et al. 2021a). Directly PGPR promote the plant growth by enabling resource gain (nitrogen, phosphorus, and essential minerals) (Kusale et al. 2021b) or regulating plant hormone levels (Fig. 6.1).

6.3.1 Alteration of the Whole Microbial Community in Rhizosphere Niche Through the Production of Various Substances

6.3.1.1 Hydrogen Cyanide Production

Several PGPR release hydrogen cyanide, a volatile compound that employs moderate biocontrol activity and improve effect of bacterial antibiotics acting synergistically (Beneduzi et al. 2012). PGPB that can produce HCN also synthesize some antibiotics or cell wall degrading enzymes (Ramette et al. 2006). The low level of HCN synthesized by bacterium improves effectiveness of antifungal compound directed against fungal pathogens thereby ensuring that fungi do not develop resistance to antifungal in question. Thus, HCN synthesized by PGPB act synergistically with antibiotics.

HCN toxicity is attributed to its ability to inhibit cytochrome-C oxidase as well as other important metallo-enzymes (Nandi et al. 2017). Many bacterial genera such as *Rhizobium*, *Pseudomonas*, *Alcaligenes*, *Bacillus*, and *Aeromonas* have shown to be HCN producers (Ahmad et al. 2008; Zachow et al. 2017). Inhibition of tomato root knot disease caused by *Meloidogyne javanica* have been attributed to the effect of HCN (Siddiqui et al. 2006) as well as control of *Odontoter mesobesus*, a crop pest in India.

6.3.1.2 Production of Lytic Enzymes

Several PGPR produce enzymes chitinases, cellulases, −1,3 glucanases, proteases, and lipases that can lyse a portion of the cell walls of several pathogenic fungi. PGPB that synthesize one or more of these enzymes have been observed to have biocontrol activity against a range of pathogenic fungi including *Botrytis cinerea*, *Sclerotium rolfsii*, *Fusarium oxysporum*, *Phytophthora* spp., *Rhizoctonia solani*, and *Pythium ultimum* (Kim et al. 2008). A wide variety of microbial lytic enzymes have been studied, some of which are cellulases, glucanases, proteases, and chitinases. These enzymes efficiently prevent the proliferation of pathogenic fungi by hydrolyzing different components of their cell walls. PGPR produce extracellular hydrolytic enzymes that are engaged in hydrolysis of fungal cell wall components such as chitin, proteins, cellulose, hemicellulose, and DNA; these hydrolytic enzymes have ability of hindering fungal pathogens (Pal 2006). The antagonistic properties of hydrolytic enzymes against various phytopathogens play a major role in biocontrol (Kim et al. 2003) (Shaikh and Sayyed 2015). Hydrolytic enzymes can break down glycosidic bonds in chitin. *Stenotrophomonas*, *Pseudomonas*, and *Alcaligenes* inhabiting in soil produce chitinase to lyse the cell wall of phytopathogenic fungi (Shaikh et al. 2018). Proteases play a substantial role in cell wall lysis of phytopathogenic fungi because chitin and/or fibrils of β-glucan are inserted into protein matrix. Several *Bacillus* species are known to produce protease (Beg and Gupta 2003; Gerze et al. 2005). Some of the proteases produced by *Trichoderma* spp. are involved in inactivating extracellular enzymes of phytopathogenic fungi (Kredics et al. 2005). Application of efficient rhizobacterial strains secreting various hydrolytic enzymes helps to reduce the abundant use and doses of agrochemicals which is the most important prospect in PGPR research.

6.3.1.3 Production of Antibiotics

It is one of the major biocontrol mechanisms of PGPR in nature. The diffusible compounds produced by PGPR are known to inhibit plant pathogens. A broad spectrum of antibiotics such as polyenes, macrolides, aminoglycosides, nucleosides, and benzoquinones are stated to be produced by PGPR. *Actinobacteria* are the major producer of antibiotics. An antibiotic that is recognized to control a pathogen to prevent plant damage from that pathogen might not be as effective against another pathogen on the same plant, and the antibiotic synthesizing PGPB may show varying differences in its actions under different field conditions. Many antibiotics have been derived from bacteria of the genera *Bacillus* and *Pseudomonas*. They produce a variety of metabolites which serve as antifungal, antibacterial, anti-helminthic, antiviral, antimicrobial, phytotoxic, antioxidant, cytotoxic, and antitumor agents. Fluorescent pseudomonads have been reported for pyoluteorin antibiotic production (Vinay et al. 2016a, b). At minimal concentrations, PGPR-secreted antibiotics are efficient at inhibiting the growth of other bacteria and fungi.

The problem on use of antibiotic-producing bacteria as biocontrol agents is, with increased use of these strains, some phytopathogens may develop resistance to specific antibiotics. Biocontrol strains synthesizing hydrogen cyanide and antibiotics can be used to overcome this problem. While hydrogen cyanide may not have much biocontrol activity by itself, it appears to act synergistically with bacterially encoded antibiotics (Raaijmakers et al. 2002).

6.3.1.4 Siderophore Production

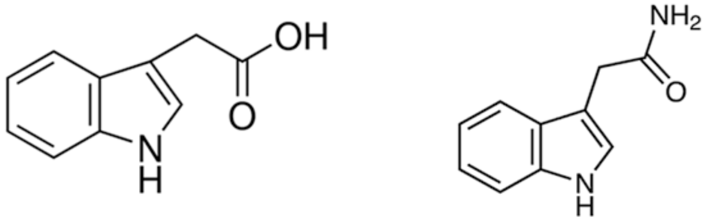
Siderophores are low-molecular-weight, iron-scavenging ligands produced by a wide variety of microorganisms to combat iron deficiency (Sayyed et al. 2013). They are tiny peptide molecules that have side chains and functional groups to which ferric ions can attach. Deficiency of iron in crop results in iron chlorosis, making them micronutrient deficient and hence susceptible to microbial diseases. Siderophore-producing rhizobacteria have been recognized as potential biocontrol agents for controlling plant diseases (Sairam et al. 2013). The ability of *Achromobacter* obtained from groundnut rhizosphere to produce siderophore in the presence of moderate/high levels of various metal ions can be exploited in bioremediation of metal contaminated soils (Sayyed et al. 2019). PGPR present in close vicinity to plant roots or its surface play a vital role in transporting iron nutrition to the crops, thereby endorsing plant health/growth as well as suppressing major phytopathogen, and have been seen as sustainable and eco-friendly substitute to chemical manures and chemical insecticides. PGPR serve as first defense against root-invading organisms and aid in removing toxic metals from polluted soil.

Siderophore-producing microbes can prevent pathogen proliferation by reducing amount of iron that is available to pathogen (Shen et al. 2013). PGPB synthesizing siderophores prevent proliferation of phytopathogens by secreting siderophores with an extremely high affinity for iron. These siderophores bind tightly to most of the Fe3 PLUS_SPI that is present in the rhizosphere of the host plant taking up bound iron either into PGPB or host plant. This avoids any fungal and bacterial pathogens in the host plant rhizosphere, where the biocontrol PGPB is bound, from acquiring sufficient iron for their growth. Thus, the pathogens are unable to proliferate because of lack of iron, causing them to lose ability to act as pathogens. Siderophore-producing rhizobacteria signify a promising option to chemical fertilizers by simultaneously tackling salt-stress impacts and Fe limitation in saline soils (Ferreira et al. 2019). Statistical optimization of siderophore production by *P. aeruginosa* RZS9 by applying Plackett–Burman design and response surface methodology (RSM) using central composite design (CCD) has been reported (Wani et al. 2016).

6.3.1.5 Production of Auxins

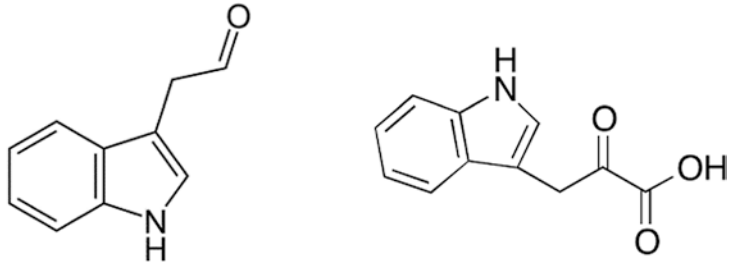
The most described mechanism primarily used to explain the positive PGPB effects on plant growth is their capability to produce auxin. Microbial auxin production is the major factor not only responsible for strengthening the plant-microbe relationship, but it also promotes plant growth and development (Ahmed and Hasnain 2014). The compounds that have been reported to have auxin activity include IAA and its derivatives (Fig. 6.2) like indole-3-acetamide, indole-3-pyruvate, indole-3-acetaldehyde, etc. The scientific literature considers auxin and IAA to be interchangeable terms (Spaepen et al. 2007).

IAA is a common product of L- tryptophan metabolism. IAA assists in the production of longer roots with enhanced number of root hairs and root laterals which



Indole- 3- acetic acid

Indole -3-aetamide



Indole-3-acetaldehyde

Indole-3-Pyruvic acid

Fig. 6.2 Some known derivatives of IAA

are engaged in nutrient uptake (Datta and Basu 2000). IAA accelerates cell elongation by modifying certain conditions like rise in osmotic contents of the cell, rise in permeability of water into cell, reduction in wall pressure, increase in cell wall synthesis, and inducing protein synthesis. Rhizobacterial IAA changes plant auxin pools, eventually increasing root length as well as surface area, and in the process increasing the amount of root exudates available for uptake by plants as demonstrated by Ali et al. (2020). IAA affects cell division in plant, extension, and differentiation; stimulates seed and tuber germination; rises the rate of xylem and root growth; controls processes of vegetative growth; initiates lateral and adventitious root development; mediates responses to light, gravity, and florescence; and affects photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to stressful conditions (Tsavkelova et al. 2006)

IAA released by rhizobacteria interferes with many plant developmental processes because the endogenous pool of plant IAA may be altered by acquisition of IAA that has been secreted by soil bacteria. In plant roots, endogenous IAA may be suboptimal or optimal for growth (Lucas García et al. 2004), and additional IAA that is taken up from bacteria could alter the IAA level to either optimal or supra-optimal, resulting in plant growth promotion or inhibition, respectively. PGPB can use any functional IAA biosynthesis pathways. Synthesis of IAA is important in life and functioning of bacterium. IAA synthesized by PGPR is required at different levels in plant-bacterial interactions. Plant growth promotion and root nodulation both require IAA (Hynes et al. 2008). Bacterial IAA enhances root surface area and length and delivers the plant greater access to soil nutrients. Additionally, bacterial IAA loosens plant cell walls and enables an increasing amount of root exudation which offers additional nutrient to support growth of rhizosphere bacteria.

6.3.1.6 Production of Gibberellins

Gibberellins are compounds which include a large group of tetracyclic diterpenoid carboxylic acids having either C20 or C19 carbon skeletons. Only 4 GAs have been identified in bacteria; GA1, GA3, GA4, and GA20 (Gupta et al. 2017), with GA1 and GA4 being most active (Sanghi et al. 2016). Gibberellins cause seed germination by breaking seed's dormancy and acting as chemical messenger. Gibberellins stimulate growth and activate important growth processes of plants including stem elongation, seed germination, flowering, fruit setting, improved photosynthesis rate, and chlorophyll content. PGPB production of GAs has been detected in *Achromobacter xylosoxidans*, *Gluconobacter diazotrophicus*, *Acinetobacter calcoaceticus*, *Rhizobia*, *Azotobacter* spp., *Bacillus* spp., *Herbaspirillum seropedicae*, and *Azospirillum* spp. (Joo et al. 2005).

6.3.2 By Enabling Source Gain (Nitrogen, Phosphorus, and Essential Minerals) or Regulating Plant Hormone Levels

6.3.2.1 Phosphate Solubilization

Bacteria that solubilize phosphorus are referred to as phosphate solubilizing bacteria (Alori et al. 2017). They supply phosphate in a more acceptable way to the plants and are not deleterious to environment. They transform insoluble organic and inorganic phosphate to a form which can be readily available to plants. Environmental conditions, plant and soil conditions, and bacterial strains all affect actions of phosphate solubilizers (Singh 2015). According to Banerjee et al. (2005), the most powerful phosphate solubilizers are from the genera *Bacillus*, *Rhizobium*, and *Pseudomonas*, as well as non-symbiont nitrogen fixers such as *Azotobacter* and *Azospirillum*. Organic acids produced together with their carboxyl and hydroxyl ions chelate cations or reduce the pH resulting in the release of phosphates (Khot et al. 2012). *Neurospora discreta* survives the stressed environment with high salinity and low precipitation rate and is reported as a powerful phosphate solubilizer (Sharma et al. 2016).

The main sources of organic phosphorus in the soil are organic materials in the form of inositol hexa-phosphate (phytate). Phytate is generally not biologically available to plants because plant roots produce incredibly low amount of phytase enzyme which breaks down phytate. However, many PGPB can solubilize phytate.

Phosphorus mineralization refers to the solubilization of organic phosphorus and degradation of remaining portions of the molecule which is triggered by unavailability of sufficient phosphate in the soil.

6.3.2.2 Nitrogen Fixation

Nitrogen is one of the important nutrients essential for growth of all living organisms including plants and bacteria. The observation of nitrogen shortage in soil has led to the use of large amounts of nitrogenous fertilizers to make up for the necessary plant requirements to accomplish maximum plant yield in most soils. Despite nitrogen's abundance in earth's atmosphere, about 78%, this form of gaseous nitrogen [N_2(g)] is not readily accessible to most organisms, i.e., it is unsuitable for plant assimilation until first converted to ammonia (Baas et al. 2014).

Broad range of nitrogen-fixing bacteria have been identified including organisms that fix nitrogen symbiotically with specific plants (mostly legumes). Examples of symbiotic nitrogen fixers are *Rhizobium*, *Sinorhizobium*, *Azorhizobium*, *Allorhizobium*, *Mesorhizobium*, *Bradyrhizobium*, *Frankia*, *Azoarcus*, *Achromobacter*, *Burkholderia*, and *Herbaspirillum* (Babalola 2010; Pérez-Montaño et al. 2014). Rhizobia are Gram-negative bacteria that form symbiotic relationships majorly with leguminous plants (Oldroyd and Dixon 2014). Rhizobial bacteria colonize plant root cells and initiate a complex trend of developmental changes that lead root nodule formation (Gage 2004).

One unsuitable side reaction in the activities of the nitrogenase enzyme in nitrogen fixation is the reduction of H^+ to H_2 (hydrogen gas) because the hydrogen gas produced is lost to atmosphere leading to a waste of ATP expended in its production. This side reaction significantly lowers the overall efficiency of the nitrogen-fixing process by approximately 30%. On the contrary, some strains of rhizobia have an enzyme called hydrogenase (Sotelo et al. 2021) that can retrieve the lost H_2 from the atmosphere and convert it back into H PLUS_SPI to produce ATP used for more nitrogen fixation. These strains assist to save energy while fixing nitrogen at the same time.

6.3.3 By Decreasing the Inhibitory Effects of Various Pathogens on Plant Growth and Development in the Forms of Biocontrol Agents

6.3.3.1 Use of Chemical Fungicide and Their Disadvantages

Synthetic fertilizers and pesticides are used to enhance the crop yield, which are thought to be the most favorable options to protect crops from fungi, pests, and weeds (Kumar et al. 2019). The major constraints in agriculture include depletion of nutrient supply in soils, gap between achievable and actual yields of the crop, and protection of crops from different types of phytopathogens. According to the FAO (Food and Agriculture Organization), 25% of global agricultural production is affected. People are deprived of getting adequate food (Mishra and Arora 2018). Fungal infection in plants is responsible for epidemics like late blight disease, cereal rusts, ergot, brown spot, coffee rust, Sigatoka disease of banana, chestnut blight, downy and powdery mildews, stem rusts, and leaf blight. Chemical control of fungal phytopathogens has immediate effect, easy application, and can control large variety of pests.

Various hazardous chemical families associated with fungicides are dithiocarbamates, nitriles, benzimidazoles, and phenylpyrrole. Possible pathways of environmental contamination due to field application of chemical fungicides are; leaching through soil causing groundwater contamination, evaporation into the surrounding environment which can cause contaminated rainfall, spray drift into the soil, drainage of pesticides from crops with rainfall, presence of pesticides in crop or food products.

Only 0.1% of fungicides reach the target pathogens, while 99.9% leak into the surrounding environment responsible for harmful effects leading to water pollution, soil contamination, increased pathogen resistance, loss of biodiversity, and elimination of useful and beneficial species (e.g., bees). Pesticides affect pollinators like bees interfering with their homing ability, reproductive process, and foraging behavior. Some chemical fungicides reduce the visitation rates of pollinators and reduce the pollen collection, thus, reducing the pollination rate and affecting crop yield. Incorrect applications of pesticides cause health hazards, environmental pollution, and poisoning in humans. Exposure of humans to chemical pesticides can occur directly through oral, dermal route or by inhalation or indirectly due to occupation (Fig. 6.3). Exposure causes various harmful effects like allergic reactions, obesity, cancer, neurological disorders, endocrine problems, etc. High level exposure of chemical fungicide during gestation shows different types of birth defects to a range of 5 to 9% (Kumar et al. 2019).

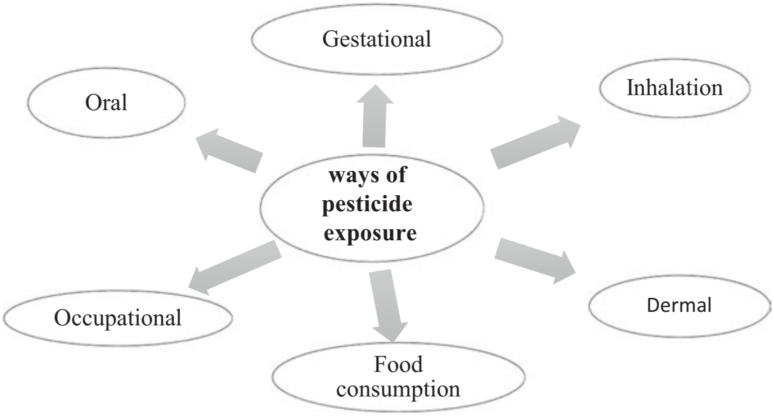


Fig. 6.3 Different ways of pesticide exposure to humans

Fungal pathogens cause devastating losses of crops and post-harvest fruits. Different chemical fungicides like anilinopyrimidine, benzimidazoles, demethylation inhibitors, dicarboximide, phenylpyrrole, Qo respiration inhibitors, and strobilurin are used to minimize the damage caused due to fungal pathogens. Due to damage caused by chemical pesticides, use of PGPR to prevent fungal diseases has been gaining interest. PGPR are considered as front-line defenses against soilborne pathogens.

6.3.3.2 Biological Control Agents (BCAs) as Alternatives to the Chemical Pesticides for Management of Pest and Diseases in Agriculture

PGPR replace the toxic and inefficient pesticides. PGPR are able to suppress bacterial, fungal, and nematode diseases in plants, and hence they have been used widely in integrated pest management programs (Sivasakthi et al. 2014). The strategies used by PGPR acting as competitors of fungal pathogens are nutrient competition, antibiosis, induced systemic resistance, parasitism, and production of hydrolytic enzymes.

6.3.3.3 PGPR as Biocontrol Agents

PGPR are involved in biocontrol of plant diseases. They colonize rhizosphere and compete with the harmful microorganisms. They also have different mechanisms to prevent plant diseases by controlling the growth of phytopathogens (Sivasakthi et al. 2014). Many PGPR have been used as biocontrol agents. This is an ecofriendly approach; however, they have not replaced the harmful chemicals due to their inconsistency, poor shelf life, and lack of knowledge of actual factors involved in biocontrol (Mishra and Arora 2018).

Properties of PGPR as biocontrol agents include rapid growth in vitro and rapid mass production, utilize root exudates and seed exudates, colonize and multiply faster in rhizosphere and plant tissues, produce different types of bioactive secondary metabolites, compete with phytopathogens, and rapid adaptability to the environmental stress (Sivasakthi et al. 2014). PGPR synthesize several secondary metabolites, which act against phytopathogens, hence can be used in different preparations (Table 6.2). These are phenazines, phloroglucinol, 2,4-diacetylphloroglucinol, pyoluteorin, pyrrolnitrin, and cyclic peptides.

Table 6.2 PGPRs showing biocontrol activity against phytopathogens

PGPRs	Crops	Diseases/pathogen	References
Bacillus amyloliquefaciens	Tomato	*Tomato mottle virus*	Murphy et al. (2000)
	Bell pepper	*Myzus persicae*	Herman et al. (2008)
Pseudomonas fluorescens	Tobacco	*Tobacco necrosis virus*	Park and Kloepper (2000)
Bacillus spp.	Cucumber	*Cotton aphids*	Stout et al. (2002)
Pseudomonas aeruginosa	Mung bean	*Root rot*	Siddiqui et al. (2001)

6.3.4 Bacteria to Bacteria Communication by PGPR in the Promotion of Plant Growth and Colonization

Rhizosphere is rich in microbial community which have beneficial effects on plant growth and plant health. Survival and activities in these niches are under the control of density-dependent mechanism called quorum sensing (Pierson et al. 1994). The communication includes intra as well as inter-species interactions.

6.3.4.1 Quorum Sensing Activity

Quorum sensing is a density-dependent mechanism of cell-to-cell interaction. A property like luminescence, production of certain chemical, is expressed only after achieving specific density. It is the basis of bacterial communication. Quorum sensing depends on the extracellular concentration of the signaling molecule. When an optimum concentration of this molecule is reached, it is detected by the group of bacteria and they respond to it. Quorum sensing is different in Gram-positive and Gram-negative bacteria due to differences in the signal molecule production. In Gram-negative bacteria they have a quorum sensing apparatus that consists of LuxI-type (I) protein, Lux R type (R) regulator that acts as signal receptor, and AHL (N-acyl homoserine lactone) synthase. At a low population density, bacteria produce basal level of AHL. When a threshold amount is reached, signaling molecule interacts with R protein forming R-AHL which interacts with the target promoters that induce expression of target gene. Gram-positive organisms have peptide auto inducers as signaling molecule (Dong and Zhang 2005). The phenomenon is also seen in PGPR which are present in the close vicinity of rhizosphere soil. Microbe-Microbe and Plant-Microbe interactions are highest in region and are responsible for various properties like hormone, antibiotic, siderophore. Etc. production.

6.3.5 Role of PGPR ACC Deaminase in Stress Agriculture

As plants are immobile, they are confronted to various kinds of stresses such as drought, flooding, salinity, heat, cold, exposure to heavy metals and nutrient deficiency, and phytopathogen and pests attack (Table 6.3) (Shen et al. 2013). The rise in synthesis of ethylene from its immediate precursor, ACC, is secreted by plants as root exudates. It has been found in almost all plants growing under stress conditions (Liu et al. 2015).

ACC deaminase has been widely reported in numerous microbial species of Gram-negative, Gram-positive bacteria, rhizobia, endophytes, and fungi (Jia et al. 1999). It is extensively studied in numerous species of PGPR like *Agrobacterium genomovars*, *Azospirillum lipoferum* (Blaha et al. 2006), *Alcaligenes*, *Bacillus* (Belimov et al. 2001), *Burkholderia* (Blaha et al. 2006) (Pandey et al. 2005),

Table 6.3 Role of bacterial ACC deaminase in stress agriculture

Type of stress	Role of ACC deaminase	References
Salinity stress	The plants inoculated with PGPR containing AC deaminase were able to thrive better through the salinity stress while demonstrating a normal growth pattern as salinity-induced ethylene is successfully reduced and it could decrease the negative impact of salinity onto plant growth	Raut et al. (2017) O'Donnell et al. (1996) Mayak et al. (2004)
Drought stress	Drought stimulates enhanced ethylene production in plant tissues which leads to unusual growth of a plant. ACC deaminase PGPR reduces the production of ethylene exposed to water stress	Mayak et al. (2004)
Waterlogging stress	In flooding, ACC, synthesized in roots, is transported to plant shoots where it is converted to ethylene by ACC oxidase. ACC deaminase PGPR showed substantial tolerance to flooding stress implying that bacterial ACC deaminase lowered the effects of stress induced ethylene	Mamoona et al. (2021)
Temperature stress	A fluctuation in temperature leads to hormonal imbalances in plants, and thus their growth is significantly affected. Accelerated ethylene production under high and chilling temperatures has widely been reported in plant tissues. Plants with ACC deaminase expression can overcome this unfavorable situation by lowering ethylene level	Choi et al. (2021)
Pathogenicity stress	The broadly recognized mechanisms of biocontrol mediated by PGPR are competition for an ecological niche or a substrate, generating inhibitory allelochemicals, and stimulating systemic resistance (ISR) in host plants to a broad spectrum of pathogens. ACC deaminase rhizobacteria have antagonistic consequences against microbial pathogens	Dobbelaere et al. (2003) Bloemberg et al. (2000) Lugtenberg and Kamilova (2009) Wang et al. (2000) Pandey et al. (2005) Blaha et al. (2006) Belimov et al. (2001)
Heavy metals stress	When present in excess, heavy metals may act as toxicants and suppress the plants growth. The application of PGPR containing ACC deaminase activity in phytoremediation of heavy metal contaminated soil environment has been proved	Belimov et al. (2001) Arshad et al. (2008)
Organic contaminants stress	Organic pollutants in the soil environment, if present above permissible limits, hinder plant growth via several mechanisms including abnormal growth of affected plant species. The significance of PGPR containing ACC deaminase activity in improving the growth of plants in the presence of organic contaminants has been recently reviewed	Khalid et al. (2004)

(continued)

Table 6.3 (continued)

Type of stress	Role of ACC deaminase	References
Air pollutants stress	Air pollution, in addition to harming plants, prevents many enzyme systems and metabolic processes of plants. It is likely that PGPR can be utilized as a gene source for genetic modification of plants expressing the enzyme ACC deaminase against plant harm by air pollutants	Vahala et al. (2003) Wang et al. (2000)
Wilting of flowers	The wilting of ornamental flowers caused by ethylene production is a major impediment in the success of flowering business. Shelf life of flowers could be increased to manifold by treating them with suspensions of PGPR involving ACC deaminase action	Felske et al. (2000)
Nodulation	It has been well proven that ethylene and its precursor ACC are the destructive regulator of nodulation in numerous plant species. PGPR containing ACC deaminase activity endorses nodulation in legumes through inhibition of ethylene biosynthesis, and consequently, they augment symbiosis and nitrogen fixation in plants	Belimov et al. (2001) Belimov et al. (2001) Jia et al. (1999)

Enterobacter (Penrose and Glick 2001), *Pseudomonas* (Blaha et al. 2006), and *Rhizobium* (Ma et al. 2003; Uchiumi et al. 2004).

6.3.5.1 Mode of Action of Bacterial ACC Deaminase

The bacterial ACC deaminase having a low affinity for ACC can effectively compete with plant enzyme, ACC oxidase, which has high affinity for the same substrate. This results in the reduction of the plant's endogenous ethylene concentration. The biological activity of PGPR relates to the relative amounts of ACC deaminase and ACC oxidase in the system under consideration (Nayani et al. 1998). For PGPR to be able to lower plant ethylene levels, the ACC deaminase level should be at least 100–1000-fold greater than the ACC oxidase level.

PGPR synthesize and secrete indole-3-acetic acid (IAA), which gets adsorbed on the seed or root surface of plants from tryptophan and other small molecules present in seed or root exudates. Some of the newly synthesized IAA is taken up by the plant, and in conjunction with the endogenous plant IAA can further stimulate plant cell proliferation and elongation. IAA stimulates the activity of the enzyme ACC synthetase (Kende 1993).

A significant portion of ACC is exuded from plant roots or seeds and taken up by the soil microbes or hydrolyzed by the ACC deaminase to yield ammonia and α-keto-butyrate. The uptake and subsequent hydrolysis of ACC by microbes decreases the amount of ACC outside the plant (Mayak et al. 2004). The balance between the internal and the external ACC levels is retained through exudation of more ACC into the rhizosphere. Soil microbial communities containing ACC deaminase activity trigger plants to produce more ACC than the plant would otherwise require. They stimulate ACC exudation from plant roots, while supplying microorganisms with a distinctive source of nitrogen (ACC). The growth of microorganism containing ACC deaminase is enhanced in the close vicinities of plant roots as compared to the other soil microbes. By doing so, not only the ACC level is lowered within the plant but also the biosynthesis of the stress hormone ethylene is inhibited. Thus, a plant inoculated with bacteria containing ACC deaminase exhibits more root growth.

6.3.5.2 Recent Advances at Molecular Level

Recently, efforts have been made to introduce specific genes into plants to enable them to cope with complex environmental stresses. Introduction of specific genes responsible for expression of enzymes like ACC deaminase from microbial species directly into crop plants has received great attention in the last few decades. Genetic manipulation of ACC deaminase trait in bacteria has not been much attempted. This is most likely due to (1) ACC deaminase trait is widely found among soil indigenous microbial species and (2) survival and functioning of wild-type microbial species containing ACC deaminase is better than genetically engineered microorganism expressing ACC deaminase genes (Nadeem et al. 2006). However, the genetically modified bacteria could be helpful for improving better understanding of processes responsible for induction of tolerance in plants inoculated with ACC deaminase bacteria against both biotic and abiotic stresses. The use of PGPR containing ACC deaminase activity along with other innovations could prove to be a cost-effective and environment-friendly strategy to ensure sustainable agriculture.

6.3.6 Formation of Biofilm by PGPR

Success of the plant microbe interaction depends on effective root colonization and subsequent biofilm formation. PGPR exhibit regulatory mechanisms like quorum sensing making them stable entities in biofilms (Sanghi et al. 2016). Biofilms are microbial communities associated with a surface which are enclosed in a matrix of polysaccharides allowing growth and survival even in adverse conditions. Biofilms are not washed off easily. Biofilms are formed in a specific manner showing initial surface attachment, microcolony formation, three-dimensional community structure, maturation, and detachment (Fig. 6.4) (Hall-Stoodley et al. 2004). Certain bacteria produce exopolysaccharides which is an important factor in biofilm formation. Essential nutrients are circulated to plants properly by effective root colonization by exopolysaccharides (EPS) producing microbes. Functions performed by these biofilms include protection against abiotic stress and plant defense response.

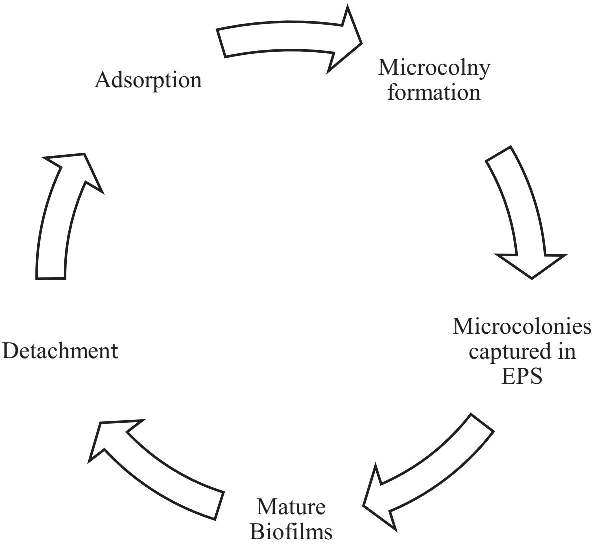


Fig. 6.4 Steps involved in biofilm formation

6.3.6.1 PGPR Biofilms in Plant Microbe Interaction

Bacteria exhibit different properties when in biofilms than planktonic form. They perform well in inhibiting other competing organisms and outgrow in number, nutrition uptake, and adaptation to changing environments. Cells in biofilm show modulations in their metabolic function. EPS acts as barrier for antimicrobial agents and toxins produced by soil microbiota. Some metal ions are also sequestered in the process. Presence of water in the biofilm allows nutrient uptake, exchange of metabolites, and removal of toxins. The most common groups associated with plant roots and leaves are species of *Pseudomonas*, *P. putida* and *P.* fluorescens, and *Bacillus* which form biofilms on plant surfaces. Various symbiotic (species of *Rhizobium*) and non-symbiotic (Species of *Azospirillum*) nitrogen fixing bacteria have ability to form biofilm. Endospore-forming *Bacillus* produce antimicrobial substances in a biofilm. It was shown in a study that plant polysaccharides such as xylan, pectin, and arabinogalactan were stimulated at initiation stage of biofilm formation (Raut et al. 2017).

6.3.6.2 PGPR Biofilms in Advanced Agriculture

Current concerns regarding hazardous effects of pesticides have led to search for alternative strategies which include use of biofertilizers and biocontrol agents. They consist of plant growth-promoting rhizobacteria having beneficial effects on plant growth as well as protect plant against plant pathogens. However, inability to establish themselves in rhizosphere along with indigenous species limits their commercial use in field applications. This problem can be overcome by application of PGPR in biofilm form giving high output yield, improved soil fertility, and enhanced production.

6.3.6.2.1 PGPR Biofilms as Biofertilizers

Biofertilizers can be used as an alternative to chemical fertilizer for improved soil fertility and crop yield without any pollution and health hazards. Some of the PGPR provide nutrients to plants in a form that can be easily assimilated. Nitrogen fixing bacteria inoculants of *Burkholderia* spp., *Azotobacter* spp., *Bacillus polymyxa*, and *Azospirillum* spp. are commercialized biofertilizers (Vessey 2003). Phosphorus is a limiting nutrient which is not easily accessible to plants. Phosphate solubilizing microorganisms produce organic acids and help in solubilizing this P usually complexed with other minerals. Beneficial biofilms which can be developed in vitro and applied as biofertilizers effectively are called biofilm biofertilizers. Biofilms of *Pseudomonas*, *Trichoderma*, *Penicillium*, and *Bradyrhizobium* showed increased IAA production, phosphate solubilization, siderophore production, nitrogen fixation rates, etc. (Bais et al. 2004). Application of biofilm inocula results in cotton seed germination, soyabean dry weight, increased wheat root, and shoot length. Biofilm formation was observed on the roots of *Arabidopsis thaliana* improving plant productivity and growth (Elshahat et al. 2016).

6.3.6.2.2 PGPR Biofilms as Plant Growth-Promoting Agents

Efficacy of the bioinoculants depends on successful root colonization and environmental factors. Rhizospheric colonization is thus considered as a critical step in the application of microorganisms for valuable purposes. The organisms in the biofilm are responsible for production of IAA and hydrogen cyanide. There is increase in root and shoot dry weight. Some attributes of photosynthesis like transpiration rate, stomatal conductance, SPAD chlorophyll value, and photosynthesis rate improve in a mixed culture biofilm (Ali et al. 2020).

6.3.6.2.3 PGPR Biofilms as Biocontrolling Agents

B. subtilis is utilized as a biocontrol agent against *F. oxysporum*, the causative agent of tomato wilt disease. Studies revealed that *B. subtilis* produced lipopeptide and inhibited phytopathogenic fungi by antibiosis mechanism. Usage of consortium of PGPR may often have more impact on biological control and plant growth than a single strain (Krishnamurthy and Gnanamanickam 1998). Antibiotics produced by these PGPR biofilms act as biocontrol agents. Biofilms are usually formed on root tips making plants less sensitive to infection (Bais et al. 2004). *Pseudomonas florescence* coats plant roots by biofilm formation and protects plants against various bacterial and fungal pathogens. *Bacillus amyloliquefaciens* is used as a biocontrol agent against *Rhizoctonia solani. Bacillu*s spp. inocula on cotton plants showed increased production of jasmonic acid reducing larval feeding. *Bacillus pumilus* biofilm played important role in prevention of pine seedlings damping off disease caused by a *Rhizoctonia solani* (Raut et al. 2017). PGPR biofilms have proved their

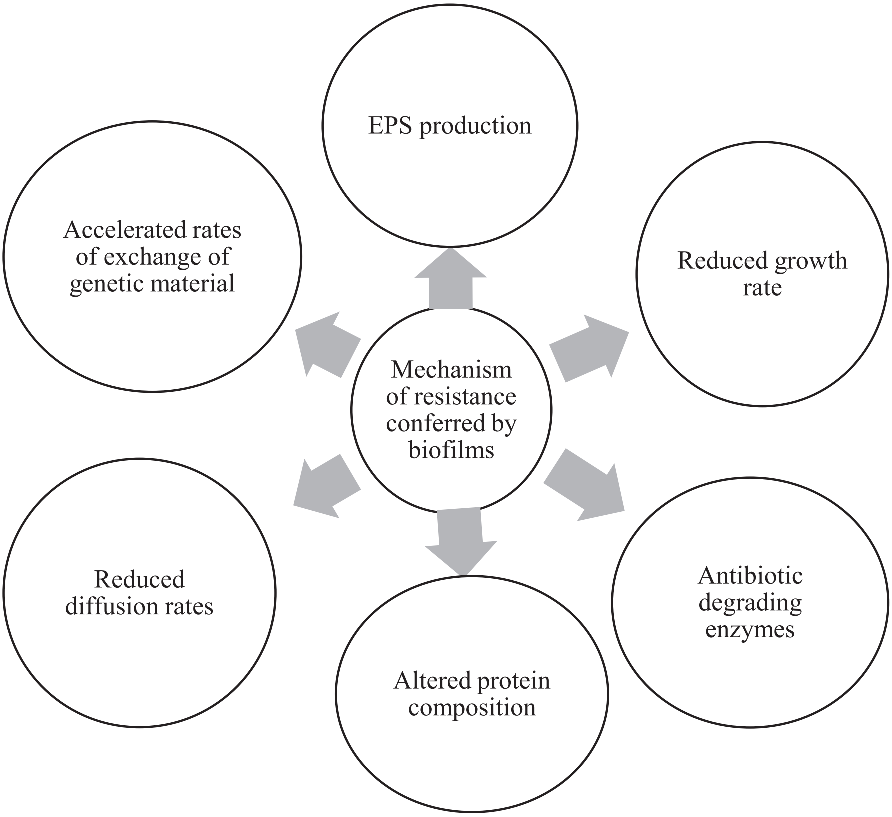


Fig. 6.5 Mechanism of resistance by biofilms

potential as biocontrol agents with increased microbial activity and hence have better prospects in modern agriculture (Fig. 6.5).

6.4 Fungal Diseases Associated with Plants

The phytopathogens include bacteria, fungi, viruses, viroid, and protozoa. Most of the phytopathogenic fungi belong to the Ascomycetes and the Basidiomycetes. The fungicides and other agriculture practices are used to control the fungal diseases. However, new species of fungi often emerge which are resistant to various fungicides. Biotrophic fungal pathogens colonize living plant tissue and get nutrients from living host cells. Necrotrophic fungal pathogens infect and kill host tissue and obtain nutrients from the dead host cells. The different types of fungal infections include the black spot, downy mildew, powdery mildew, blight, rust, wilt, club root, and anthracnose (Table 6.4).

Table 6.4 Fungal diseases associated with plants

Name of the disease	Plants affected	Symptoms	Causative agent
Black spot	Rose, oak, maple, sycamore, walnut	Black, gray, or brown spots formed on the leaves of the plant, causing them to drop	*Pseudomonas*, *Asterina*, *Asterinella*, *Diplotheca*, *Glomerella*, *Gnomonia*, *Schizothyrium*, *Placosphaeria*, *Stigmea*
Downy mildew	Sunflower, rosemary, primula, osteospermum, *Impatiens walleriana*, coleus, statice, verbena, ornamental cabbage, basil, and cineraria	Leaves and stems turn yellow, prevent flowering	*Plasmopara viticola*, *Bremia*, *Peronospora*, *Phytophthora*, *Plasmopara*, *Sclerospora*, *Pseudoperonospora*
Powdery mildew	Rose, grape, grass, wheat, barley, Onion, apple, pear	The powdery fungus grows on the upper surface of the plant leaves, white, yellow, or brown in color. It spreads below the leaves or stems	*Podosphaera xanthii*, *Sphaerotheca fuliginea*, *Erysiphe cichoracearum*
Blight	Tomato, potato, Apple, rice, wheat	Brown, discolored leaves tend to dry and curl inwards, in moist conditions shows a white fungal growth. Both tomatoes and potatoes develop brown patches turning into rotten sores	*Phytophthora infestans*, *Erwinia amylovora*, *Xanthomonas oryzae*, *Alternaria triticina*
Rust	Wheat, barley, rye, oats, beans, sugarcane	Raised spots below leaves and on the stem, covered with reddish orange spore masses; leaves turn yellow green and black and cause leaves drop	*Puccinia coronata*, *Phragmidium*, *Puccinia graminis*, *Uromyces appendiculatus*
Wilt	Chrysanthemum, pepper, tomato, potato, cucumber, muskmelon, pumpkin, gourds	The wilting leaves can turn yellow or brown	*Erwinia tracheiphila*, *Ralstonia solanacearum*, *Solanum tuberosum*, *Zingiber officinale*
Clubroot	Cabbage, cauliflower, Brussels sprouts, turnip, swede, wallflowers, *Saxifraga*, broccoli	Roots become swollen and distorted, stunted growth, and purplish, wilting foliage	*Plasmodiophora brassicae*
Anthracnose	Tomatoes, cucumbers, beans, melon, sycamore, ash, oak, and maple	Small, sunken spots that appear on fruits and pods, which have pinkish sores in the center	*Colletotrichum* or *Gloeosporium*

Table 6.5 Production antifungal metabolites by PGPR against phytopathogens

PGPR	Antifungal metabolite production by PGPR	Fungicidal activity against the phytopathogen
P. fluorescens BL915	Pyrrolnitrin	*Rhizoctonia solani*
Pseudomonas spp.	2, 4-DAPG	Membrane destruction of *Pythium* spp.
Pseudomonas spp.	Phenazine	Antagonistic activity against *fusarium oxysporum*
Bacillus spp.	Circulin, polymyxin, and colistin	Pathogenic fungi as well as gram-negative and gram-positive bacteria
Bacillus subtilis	Fengycin and iturins	*Podosphaera fusca*
E. coli	Colicins	Broad range of inhibition of fungal, yeast, gram-positive and gram-negative species
B. Megaterium	Megacins	
Enterobacter cloacae	Cloacins	
P. Pyogenes	Pyocins	
Serratia marcescens	*P. Pyogenes*	

6.5 Production of Antifungal Metabolites by PGPR

Application of PGPR for the purpose of controlling or lessening the harmful effects of phytopathogens is known as biological control. PGPRs control the destructive effects of pathogenic agents on plants by generating growth inhibitors, i.e., antibiotics, bacteriocins, siderophores, and lytic enzymes or by rising natural resistance of host plant (Table 6.5).

6.5.1 *PGPR-Mediated Induced Systemic Resistance (ISR) in Plants*

The plant defense mechanisms are triggered by some stimulus prior to infection by a plant pathogen; the possibility of the disease can be reduced. Interactions between plants and pathogens can lead either compatible response to a successful infection or incompatible response to resistance. Induced resistance is a state of enhanced defensive capacity developed by a plant when appropriately stimulated (de Laat and van Loon 1982). Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two forms of induced resistance. SAR can elicit a quick local reaction, or hypersensitive reaction; the pathogen is restricted to a small area of the site of infection. SAR is triggered by accumulation of pathogenesis-related proteins or salicylic acid (Gundlach et al. 1992).

Selected strains of PGPR repress diseases by antagonism between the bacteria and soilborne pathogens and by inducing a systemic resistance in plant against both root and foliar pathogens. Rhizobacteria mediated ISR look like that of pathogen induced systemic acquired resistance (SAR). Both types of induced resistance make uninfected plant parts more resistant to a broad spectrum of plant pathogens. Several rhizobacteria generate the salicylic acid (SA)-dependent SAR pathway by producing SA at the root surface while other rhizobacteria produce different signaling pathway independent of SA (Reshma et al. 2018).

6.6 Conclusion

Plant diseases or phytopathogens alter plants right from the planting stage up to the harvesting and storage of the crop. The plants have developed several different mechanisms by which they defend themselves. PGPR can promote plant growth by both direct and indirect mechanisms (Glick 1995). Direct mechanisms incorporate the production of auxin, ACC deaminase, cytokinin, gibberellin, nitrogen fixation, phosphorus solubilization, and sequestration of iron by bacterial siderophores. Indirect mechanisms include ACC deaminase, antibiotics, cell wall degrading enzymes, competition, hydrogen cyanide, induced systemic resistance, quorum quenching, and siderophores.

These biocontrol bacteria have shown root colonization properties, broad-spectrum antifungal activity, and the ability to promote plant growth. The profiles of antifungal compounds include 2,4-DAPG, phenazine, pyochelin, rhamnolipids, pyoverdines, surfactins, and AHLs which varied among different organisms. The plant inoculation study validates their innate biocontrol and biofertilizer potentials where in vitro antifungal activity shows positive correlation with in vivo disease suppression activity (Ali et al. 2020). PGPR strains launched into the rhizosphere might play vital roles in the transition of soil from a suppressive to favorable condition. Such strains can be used in sustainable agriculture (Wang et al. 2019).

The capacity of bacteria to elicit a defense response in plant, called induced systemic resistance (IRS), includes the induction of synthesis of defense metabolites, but without causing a disease itself, boosting the plant's defensive capacity. PGPR can be used as biocontrol agent and as ecological replacement to the use of agrochemicals (Salomon et al. 2017).

Many fungi play an important role in soil in increasing soil fertility. Some fungi like *Trichoderma*, *Penicillium*, *Aspergillus*, etc. show antagonistic effect against plant pathogens. These beneficial microorganisms are killed due to use of fungicides. Instead of fungicides use of biocontrol agents which show antimicrobial activity should be used.

ACC deaminase activity and quorum sensing play a key role in the expression of several rhizobacterial qualities as well as in plant–bacteria interactions by inducing systemic resistance and enhancing plant tolerance to stress. Several ecological and interdependent crucial characters of bacteria, like antibiotic, siderophore, or enzyme secretion, virulence factors of phytopathogens, as well as plant-microbe communications, are synchronized through quorum sensing (Altaf et al. 2017).

EPS released assists in biofilm formation that enhances plant growth and provides protection from pathogens.

Use of biocontrol agents which can promote growth and also elicit the expression of defense enzymes can be a suitable alternative to environmentally hazardous agrochemicals (Reshma et al. 2018).

The PGPR will require a systematic strategy designed to fully utilize all these beneficial factors, applying combinations of different mechanisms of action allowing crop yields to be maintained or even increased while chemical treatments are reduced (Beneduzi et al. 2012). Nature has a huge biodiversity of PGPR. The knowledge on the regulation of various strategies for bio-fungicidal properties of rhizobacteria for plant growth promotion and disease resistance will lead to development of PGPR with improved reliability and efficacy.

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