

Chapter 2

Plant Growth Promoting Rhizobacteria in Crop Protection and Challenges

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2.1 Introduction

Plant beneficial microorganisms are increasingly being used in sustainable agriculture. Beneficial microorganisms are used with the aim of improving crop yields by augmenting nutrient availability, enhancing plant growth and providing protection to plants from diseases and pests. The bacteria residing in the rhizosphere of plants and which bring about enhancement in growth and yield of crop plants are widely referred to as plant growth promoting rhizobacteria (PGPR).

PGPR can mediate plant growth by different direct and indirect mechanisms (Glick 1995). Some of the mechanisms commonly observed are (1) increased availability of nutrients due to solubilization/mobilization; (2) biological nitrogen fixation; (3) providing protection to plants from diseases and pests by producing antibiotics, siderophores, hydrogen cyanide, etc. (Medeiros et al. 2005; Keel and Maurhofer 2009); (4) production of plant hormones like IAA, cytokinins, gibberellic acid, etc.; (5) improving the tolerance to stresses like salinity, drought, etc.; (6) lowering of ethylene levels in plants by production of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Glick et al. 1999).

Over the years workers have added newer definitions of PGPR. According to Vessey (2003), numerous species of soil bacteria which flourish in the rhizosphere of plants, but which may grow in, on, or around plant tissues, and stimulate plant growth by a plethora of mechanisms are collectively known as PGPR. Gray and

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Smith (2005) went a step further and separated PGPR into extracellular (ePGPR) organisms, existing in the rhizosphere, on the rhizoplane, or in the spaces between cells of the root cortex, and intracellular (iPGPR), which exist inside root cells.

Several PGPR inoculants have been commercialized. These inoculants result in improvement of crop growth and yield or provide protection to the crop from pests and diseases. Several microbial inoculants are used as biofertilizers, which improve the uptake of nutrients like nitrogen, phosphorus, potassium, sulphur, iron, etc. The genera commonly used as biofertilizers are *Rhizobium*, *Bacillus*, *Pseudomonas*, etc. The genera commonly used as biocontrol agents are *Pseudomonas*, *Bacillus*, *Burkholderia*, *Agrobacterium*, *Streptomyces*, etc. These organisms suppress plant disease by production of antibiotics, siderophores, or by induction of systemic resistance or any other mechanism (Tenuta 2003). Biofertilizers have been an alternative to mineral fertilizers to increase the yield and plant growth in sustainable agriculture (Canbolat et al. 2006). The current trend is the development of a consortium of beneficial microorganisms which will offer multiple beneficial effects including growth promotion, yield enhancement and protection from diseases and pests. Understanding the interaction between consortium of microbial inoculants and plant systems will pave way to harness more benefits from microbial inoculants for improving plant growth and yield (Raja et al. 2006).

2.2 PGPR as Biocontrol Agents of Plant Diseases

There are several mechanisms by which PGPR bring about control of plant diseases. The most commonly used methods are competition and production of metabolites. The metabolites include antibiotics, siderophores, HCN, cell wall-degrading enzymes, etc. (Enebak et al. 1998; Kloepper 1993). Many mechanisms may simultaneously act in a single strain towards providing biocontrol of diseases. Kloepper et al. (1992) mentioned about two types of resistances in plants. Induced systemic resistance (ISR) or systemic acquired resistance (SAR) is defined as the activation of chemical and physical defenses of the plant host by an inducer which could be a chemical or a microorganism, leading to the control of several pathogens.

There are several reports of antagonism of pathogenic fungi by PGPR (Table 2.1). *Pseudomonas* strains MRS23 and CRP55b inhibited the growth of pathogenic fungi, i.e. *Aspergillus* sp., *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia solani* under culture condition (Goel et al. 2002).

There are several reports of reduction of disease incidences by application of PGPR. *Bacillus* spp. isolated from healthy cabbage, kale, and radish reduced black rot incidence in kale and cabbage caused by *Xanthomonas campestris* pv. *campestris* (Xcc), in greenhouse and field experiments (Assis et al. 1996). Later, Monteiro et al. (2005) reported that four of these *Bacillus* strains produced lipopeptides active against Xcc during its late growth phase. Lipopeptides can also stimulate ISR in plants, probably by interacting with plant cell membranes and inducing temporary

Table 2.1 PGPR having potential biocontrol properties

| PGPR | Target pathogen | Disease | Crop |
|--|---|---|--------------------------------------|
| <i>Pseudomonas fluorescens</i> F113, Pf-5, Q2-87, CHA0, etc. | <i>Pythium ultimum</i> , <i>Pythium aphanidermatum</i> , and <i>Pythium</i> sp. <i>Rhizoctonia solani</i> <i>Fusarium oxysporum</i> | Damping off Damping off Damping off Root rot | Cotton Tomato Tomato Cotton |
| <i>Pseudomonas fluorescens</i> strain PfA 506 | <i>Erwinia amylovora</i> strain 153nal super(R) | Fire blight | Apple |
| <i>Agrobacterium radiobacter</i> | <i>Agrobacterium tumefaciens</i> | Crown gall | Dicot plants |
| <i>Bacillus subtilis</i> AU195 | <i>Aspergillus flavus</i> | Aflatoxin contamination | Groundnut |
| <i>Bacillus amyloliquefaciens</i> FZB42 | <i>Fusarium oxysporum</i> | Wilt | Tomato |
| <i>Bacillus subtilis</i> 168 | <i>Aspergillus niger</i> | collar rot | Groundnut |
| <i>Bacillus subtilis</i> QST713 | <i>Botrytis cinerea</i> , <i>Rhizoctonia solani</i> | Damping off | Grape, cotton |
| <i>Bacillus subtilis</i> BBG100 | <i>Pythium aphanidermatum</i> | Damping off | Papaya |
| <i>P. fluorescens</i> HV37aR2 | <i>Pythium ultimum</i> | Damping off | Cotton |
| <i>P. fluorescens</i> HV37aR2 | <i>Pythium ultimum</i> | Damping off | Cotton |
| <i>Pseudomonas fluorescens</i> 2-79, 30-84 | <i>Gaeumannomyces graminis</i> var. <i>tritici</i> | Take-all | Wheat |
| <i>Pseudomonas fluorescens</i> Pf-5 | <i>Pythium ultimum</i> , <i>Rhizoctonia solani</i> | Damping off | Cotton |
| <i>P. cepacia</i> | <i>R. solani</i> and <i>Pycularia oryzae</i> | Damping off and rice blast | Cotton, rice |
| <i>Bacillus cereus</i> UW85 | <i>Phytophthora medicaginis</i> , <i>Pythium aphanidermatum</i> | Damping off | Alfalfa |
| <i>Pseudomonas fluorescens</i> strain 97 | <i>Pseudomonas syringae</i> pv. <i>phaseolicola</i> | Halo blight | Beans |
| <i>Pseudomonas cepacea</i> | <i>Sclerotium rolfsii</i> | Stem rot | Beans |
| <i>Bacillus subtilis</i> | <i>Blumeria graminis</i> f. sp. <i>hordei</i> | Powdery mildew | Barley |
| <i>Pseudomonas</i> sp. (WCS 417r) | <i>Burkholderia caryophylli</i> | Fusarium wilt | Carnation |
| <i>Pseudomonas fluorescens</i> | <i>Pythium ultimum</i> | Damping off | Cotton |
| <i>Bacillus subtilis</i> | <i>Meloidogyne incogita</i> | Root knot | Cotton |
| <i>Pseudomonas cepacea</i> | <i>Rhizoctonia solani</i> | Damping off | Cotton |

(continued)

Table 2.1 (continued)

| PGPR | Target pathogen | Disease | Crop |
|---|--|--------------------|------------|
| <i>Pseudomonas putida</i> (89B-27) | <i>Colletotrichum lagenarium</i> | Anthracnose | Cucumber |
| <i>Pseudomonas cepacea</i> | <i>Pythium ultimum</i> | Damping off | Cucumber |
| <i>Pseudomonas</i> sp. | <i>Aspergillus</i> sp., <i>Curvularia</i> sp., <i>Fusarium oxysporum</i> , <i>Rhizoctonia solani</i> | Wilt | Green gram |
| <i>Pseudomonas aeruginosa</i> , <i>Bacillus subtilis</i> | <i>Meloidogyne javanica</i> | Root knot | Mung bean |
| <i>Pseudomonas fluorescens</i> , <i>Burkholderia</i> sp. | <i>Rhizoctonia solani</i> | Rice sheath blight | Rice |
| <i>Pseudomonas fluorescens</i> strain Pf1 and Fp7 | <i>Rhizoctonia solani</i> | Rice sheath blight | Rice |
| <i>S. marcescens</i> 90-1, <i>Bacillus pumilus</i> SE34 | <i>Peronospora tabacina</i> | Blue mold | Rice |
| <i>Aeromonas caviae</i> | <i>Rhizoctonia solani</i> and <i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i> | | Cotton |
| <i>Enterobacter agglomerans</i> , <i>Bacillus cereus</i> | <i>Rhizoctonia solani</i> | | Bean |
| <i>Paenibacillus illinoisensis</i> | <i>Rhizoctonia solani</i> | | Cotton |
| <i>Serratia marcescens</i> | <i>Sclerotium minor</i> | | Cucumber |
| <i>Bacillus</i> spp. | <i>Sclerotium sclerotiorum</i> | | Lettuce |

Source: Data from: Pal and Gardener (2006) and Bouizgame (2013)

alterations in the plasma membrane which could raise plant defenses (Ongena et al. 2009). Phenaminomethylacetic acid produced by *Bacillus methylotrophicus* BC79 was reported to be a new kind of substance never found in *Bacillus methylotrophicus* (Shan et al. 2013). Culture filtrate of BC79 showed biocontrol efficiency against rice blast.

Vegetatively propagated crops like plantation and horticultural crops are often susceptible to soil-borne diseases which are difficult to control. The Fusarium wilt of banana caused by *Fusarium oxysporum* f. sp. *cubense* is a very destructive disease worldwide (Figueiredo et al. 2010). Application of endophytic and epiphytic bacteria, single culture or in mixtures, as root or substrate treatments, significantly improved the growth of micropropagated banana plantlets and controlled fusarium wilt (Mariano et al. 2004). *Bacillus amyloliquefaciens* Ba33 was used as a soil disinfectant and an antiviral agent against tobacco mosaic virus (TMV) (Shen et al. 2012). Application of mixture of PGPR, more than one genera or species, is more desirable and effective means for controlling plant diseases, as compared to single cultures. The different members in a mixture will have additive or synergistic effects and therefore will result in better control of diseases.

Some bacteria reside in arbuscular and ectomycorrhizal systems and either assist mycorrhiza formation or promote the functioning of their symbiosis (Figueiredo et al. 2010). These bacteria are known as mycorrhiza helper bacteria (MHB). MHB present three significant functions: nutrient mobilization from soil minerals, fixation of atmospheric nitrogen, and plant protection against root pathogens (Frey-Klett et al. 2007). The MHB mentioned by this group were *Pseudomonas fluorescens*, *P. monteilii*, *Bacillus coagulans*, *B. subtilis*, *Paenibacillus brasiliensis*, *Rhizobium leguminosarum*, and *Bradyrhizobium japonicum*.

Several workers have successfully tried using biocontrol agents along with synthetic pesticides for disease control and yield enhancement. These treatments may reduce the application of chemical pesticides to crop plants. Corn seeds when bacterized with *Paenibacillus macerans* along with the seed-treatment with fludioxonil and metalaxyl M reduced incidences of pathogens, promoted germination and grain yield (Luz 2003). Similarly, Bugg et al. (2009) used *Bacillus*-based treatments along with seed-treatment practices.

Biocontrol agents need to be formulated if they have to be commercialized. The formulation should be cheap and should not pose any threat to human, animal or plant life or to the environment. Screening for new agents should consider the biology and ecology of the pathosystem, as well as agricultural practices associated with the crop (Fravel 2007). Raj et al. (2003a, b) studied the comparative performance of formulations of PGPR in growth promotion and suppression of downy mildew in pearl millet. The formulations contained two different strains of bacilli with chitosan as a carrier. Formulations LS256 and LS257 besides being the best growth promoters were also the most efficient resistance inducers. Among the application methods tested, soil amendment was found to be the most suitable and desirable way of delivering the formulations. The study demonstrates a potential role for plant growth promoting rhizobacterial formulations in downy mildew

management. A few examples of PGPR and biocontrol products are: *Agrobacterium radiobacter* K1026 (Nogall®), *Bacillus pumilus* QST 2808 (Sonata® TM), *B. pumilus* GB34 (YieldShield®), *B. subtilis* GBO3 (Kodiak®), *Pantoea agglomerans* C9-1 (BlightBan C9-1®), *P. agglomerans* E325 (Bloomtime®), *Pseudomonas aureofaciens* Tx-1 (Spot-Less®T), *P. syringae* ESC-10 and ESC-11 (Bio-save®), *P. fluorescens* A506 (BlightBan®), *P. chlororaphis* MA 342 (Cedomon®), *Streptomyces griseoviridis* K61 (Mycostop®) and *S. lydicus* WYEC 108 (Actinovate®) (Figueiredo et al. 2010). *B. subtilis* has great potential for use in agriculture and has been used in the formulation of commercial products for agricultural use in several countries (Lazzaretti and Bettiol 1997). Several substances have been used in experimental formulations such as lactose, peptone, gum Arabic, xanthan, cellulose and others (Schisler et al. 2004). Formulations based on *Bacillus* are widely available because of their longer shelf life and tolerance to heat and desiccation.

2.3 PGPR Induced Systemic Resistance in Crop Plants Against Pests and Diseases

Plants have developed various strategies to combat aggressors (Van Loon et al. 1998). One of these strategies is the initiation of a defense reaction at the site of infection, which spreads throughout the plant resulting in the development of resistance. Induced resistance is defined as an enhancement of the plant's defensive capacity against a broad spectrum of pathogens and pests that is acquired after appropriate stimulation. The resulting elevated resistance due to an inducing agent upon infection by a pathogen is called ISR or SAR (Hammerschmidt and Kuc 1995). The induction of systemic resistance by rhizobacteria, which are nonpathogenic, is referred as ISR, whereas that by other agents is called SAR (Van Loon et al. 1998). SAR is commonly triggered by the elicitors of avirulent pathogens, such as microbial-associated molecular patterns (MAMPs) (Abramovitch et al. 2006), but it can also be induced by biological (nonmicrobial) and chemical compounds. Typically the ISR by PGPR do not cause any necrotic symptoms on the host plants, whereas SAR is expressed to a maximum level when the inducing organism causes necrosis (Cameron et al. 1994). The expression of induced resistance can be local or systemic when it is expressed at sites not directly exposed to the inducers agent (Stadnik 2000). ISR is quite similar to SAR, making the plant resistant to subsequent attacks of pathogenic organisms, such as viruses, bacteria and fungi (Bakker et al. 2007). SAR or ISR do not provide complete resistance to any particular pathogen, but provide substantial protection to plants for a long time to a broad range of pathogens. Some chemicals, such as SA or analogues [benzothiadiazole (BTH) and its derivatives, e.g. 2,6-dichloronicotinic acid], are known to induce SAR (Table 2.2) and have been successfully used in the field to control diseases (Vallad and Goodman 2004).

Table 2.2 Effect of some SAR elicitors on disease suppression potential

| Crop | Pathogen | Disease | SAR elicitors | % Disease reduction |
|-----------------|---|--------------------|---------------|---------------------|
| Monocots | | | | |
| Maize | <i>Peronosclerospora sorghi</i> | Downy mildew | BTH | –35 |
| Wheat | <i>Blumeria graminis</i> f. sp. <i>tritici</i> | Powdery mildew | BTH | –64 |
| Dicots | | | | |
| Tobacco | <i>Pseudomonas syringae</i> pv. <i>tabaci</i> (tox+) | Bacterial wildfire | BTH | –99 |
| Tomato | <i>Pseudomonas syringae</i> pv. <i>tomato</i> | Bacterial speck | BTH | –47 |
| Pepper | <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> | Bacterial spot | BTH | –64 |
| Soybean | <i>Sclerotinia sclerotiarum</i> | White mold | INA | –46 |
| Cotton | <i>Xanthomonas campestris</i> pv. <i>malvacearum</i> | bacterial blight | BTH | –42 |
| Leguminous bean | <i>Uromyces appendiculatus</i> | rust | INA | –42 |
| Peanut | <i>Cercosporidium personatum</i> | late leaf spot | INA | +52 |
| Apple | <i>Erwinia amylovora</i> | fire blight | BTH | –73 |

Source: Data from: Vallad and Goodman (2004)

BTH benzo (1,2,3) thiadiazole-7-carbothiolic acid S-methylester, INA 2,6-dichloro isonicotinic acid

2.3.1 Induction of Systemic Resistance by PGPR Against Diseases and Pests

The use of PGPR for inducing systemic resistance against diseases has been demonstrated in field conditions (Vidhyasekaran and Muthamilan 1999; Viswanathan 1999). PGPR have been reported to induce resistance in plants against bacterial, fungal and viral diseases (Liu et al. 1995a, b; Maurhofer et al. 1998; Raj et al. 2003a, b; Halfeld-Vieira et al. 2006), and insect (Zehnder et al. 1997) and nematode pests (Sikora 1988). This type of induced resistance shows advantages such as: effectiveness against various pathogens; stability due to the action of different mechanisms of resistance, systemicity, energy economy; and metabolic utilization of genetic potential for resistance in all susceptible plants (Bonaldo et al. 2005). *Bacillus* and *Pseudomonas* are among the most studied genera of PGPR. Induced resistance was first analyzed in 1961 by pre-inoculation of tobacco plants with TMV (Ross 1961). This procedure protected the plant against other viruses and resulted in the conception of “Systemic Acquired Resistance” (SAR). The activation of defense mechanisms induced by fungi, bacteria, viruses, and nematodes can be achieved by different routes, which may occur alone or concomitantly (Bonaldo et al. 2005). The induction of resistance to disease is an added advantage to the promotion of plant growth and yield by the application of PGPR. The presence of the PGPR in the rhizosphere makes the entire plant, including the shoot, more resistant to pathogens (Figueiredo et al. 2010).

2.3.1.1 Diseases

PGPR have been reported to provide protection to plants from diseases by employing different mechanisms. These mechanisms include production of antibiotics like pyocyanine, pyrrolnitrin, 2,4- diacetylphloroglucinol (Pierson and Thomashow 1992); production of siderophores (Kloepper et al. 1980); competition for nutrients and space (Elad and Chet 1987); production of lytic enzymes like chitinases and β -1,3-glucanases (Potgieter and Alexander 1996; Velazhahan et al. 1999); HCN production (Defago et al. 1990), fluorescent pigments, etc.

The role of ISR in controlling diseases in plants has been demonstrated by many studies (Ramamoorthy et al. 2001). Application of PGPR strains as a seed-treatment resulted in a significant reduction in anthracnose disease caused by *Colletotrichum orbiculare* in cucumber (Wei et al. 1991, 1996). They showed that this plant could be used as a model for ISR. Induction of systemic resistance by *Pseudomonas putida* strain 89B-27 and *Serratia marcescens* strain 90-166 reduced *Fusarium* wilt of cucumber incited by *Fusarium oxysporum* f. sp. *cucumerinum* (Liu et al. 1995a). In sugarcane, Viswanathan and Samiyappan (1999a) established PGPR-mediated ISR against *Colletotrichum falcatum* causing red rot disease. *Pseudomonas fluorescens* 1-94 (Pf1-94) and *Pseudomonas fluorescens* (Pf4-92) strains isolated from rhizosphere soil of chickpea showed ISR against fusarium wilt of chickpea and charcoal rot (Srivastava et al. 2001).

PGPR induce systemic resistance against bacterial diseases as well. Treatment of cucumber seed with *Pseudomonas putida* strain 89B-27 and *Serratia marcescens* strain 90-166 decreased the incidence of bacterial wilt disease (Kloepper et al. 1993). Seed-treatment of cucumber with *P. putida* strain 89B-27, *Flavomonas ory- zihabitans* strain INR-5, *S. marcescens* strain 90-166 and *Bacillus pumilus* strain INR-7 provided systemic protection against angular leaf spot caused by *Pseudomonas syringae* pv. *lachrymans* by reducing total lesion diameter compared with non-treated plants (Liu et al. 1995b; Wei et al. 1996).

There are reports of induction of systemic resistance in plants against viral diseases by PGPR. Seed-treatment with *P. fluorescens* strain 89B-27 and *S. marcescens* strain 90-166 reduced the number of cucumber mosaic virus (CMV)-infected plants and delayed the development of symptoms in cucumber and tomato (Raupach et al. 1996). Soil application also proved beneficial. Soil application of *P. fluorescens* strain CHAO resulted in induced systemic protection against inoculation with tobacco necrosis virus (TNV) in tobacco (Maurhofer et al. 1998). Thus, there are ample reports of PGPR ISRs in plants against bacterial, fungal and viral diseases.

2.3.1.2 Insect Pests

There are few reports on the induction of systemic resistance in crop plants against insect pests. Fluorescent pseudomonads have been found to influence the growth and development of different stages of insects. *Pseudomonas maltophilia* affected

the growth of larval stage of *Helicoverpa zea*, leading to more than 60 % reduction in adult emergence while pupae and adults that emerged from bacteria-infected larvae were smaller (Bong and Sikorowski 1991). Certain PGPR strains activate octadecanoid, shikimate and terpenoid pathways. This in turn alters the production of volatiles in the host plant leading to the attraction of natural enemies (Bell and Muller 1993). Qingwen et al. (1998) reported an increase in polyphenol and terpenoid content in cotton plants treated with *Pseudomonas gladioli*, which affected the relative growth rate, consumption rate and digestibility of feed by *Helicoverpa armigera*. *Serratia marcescens* strain 90-166 was found quite effective in reducing the populations of striped cucumber beetle, *Acalyma vittatum* and the spotted cucumber beetle, *Diabrotica undecimpunctata howardi* on cucumber and its efficacy was better than application of the insecticide esfenvalerate (Zehnder et al. 1997). Attempts have been made to transfer the insecticidal crystal protein from *Bacillus thuringiensis* to fluorescent pseudomonads, keeping in view the efficient root colonization ability and endophytic nature of some fluorescent pseudomonads. Transgenic *P. cepacia* strain 526 with the crystal protein gene has consistently shown insecticidal activity against tobacco hornworm (Stock et al. 1990). PGPR formulations comprising of bacterial strain mixtures having the capability to induce chitinase in plant play an important role in hydrolyzing chitin, the structural component in gut linings of insects and would lead to better control of insect pest (Broadway et al. 1998). Identification of entomopathogenic PGPR strains that have the capability to colonize phylloplane in a stable manner will be a breakthrough in the management of foliar pests (Otsu et al. 2004). *Pseudomonas fluorescens* CHA0 is a root-associated PGPR that suppresses soil-borne fungal diseases of crops. Remarkably, the pseudomonad is also endowed with systemic and oral activity against pest insects which depends on the production of the insecticidal Fit toxin (Pechy-Tarr et al. 2013). The toxin gene (*fitD*) is part of a virulence cassette encoding three regulators (FitF, FitG, FitH) and a type I secretion system (FitABC-E). *P. fluorescens* CHA0 hence can actively induce insect toxin production in response to the host environment, and FitH and FitG are key regulators in this mechanism. Thus, application of PGPR may be useful for management of insect pests as well.

2.3.1.3 Nematodes

Though studies on induction of systemic resistance by PGPR against nematode pests in crop plants are few, PGPR strains have been used successfully as biological control agents for sugar beet and potato cyst nematode (Sikora 1992). *P. fluorescens* induced systemic resistance against *Heterodera schachtii* and inhibited early root penetration in sugar beet (Oostendorp and Sikora 1990). Application of the bacterium *P. chitinolytica* reduced the root-knot nematode infection in tomato crop (Spiegel et al. 1991), while the level of infestation of root-knot nematode *Meloidogyne incognita* on tomato was reduced with fewer galls and egg masses in the soil following root-dipping with *P. fluorescens* strain Pf1 (Santhi and Sivakumar 1995).

2.4 Application of PGPR Mixtures

Application of mixed cultures are often better suited as biological control agents as compared to single ones. The mixed cultures closely mimic the natural environment and might broaden the spectrum of biocontrol activity and enhance the efficacy and reliability of control (Duffy and Weller 1995). The enhancement in biological control abilities of mixed cultures may be due to different mechanisms of action and synergism between the PGPR cultures. Chitinase-producing *Streptomyces* spp. and *Bacillus cereus* isolates used in combination with antibiotic-producing *P. fluorescens* and *Burkholderia (Pseudomonas) cepacia* isolates have shown a synergistic effect on the suppression of rice sheath blight caused by *Rhizoctonia solani* (Sung and Chung 1997). Similarly, combination of *P. fluorescens* strains Pf1 and FP7 gave effective control of rice sheath blight disease when compared to each strain applied singly (Nandakumar 1998). A combination of two chitinolytic bacterial strains viz., *Paenibacillus* sp. 300 and *Streptomyces* sp. 385 in the ratio of 1:1 or 4:1 was more effective than when they were applied individually for the control of *Fusarium* wilt of cucumber caused by *F. oxysporum* f. sp. *cucumerinum* (Singh et al. 1999). Biocontrol mixtures should be formulated very carefully. The individual strains in the mixture should be compatible with each other and should not inhibit the other strains.

2.5 Broad Spectrum of PGPR Activity

Literature shows many instances of PGPR ISR against a broad range of diseases and pests. Same PGPR strain may induce resistances against many bacterial and fungal diseases and sometimes against insect pests as well in the same crop. Seed-treatment with *P. fluorescens* strain WCS 417 protected radish through induction of systemic resistance against the fungal root pathogen *F. oxysporum* f. sp. *raphani*, avirulent bacterial leaf pathogen *P. syringae* pv. *tomato* and fungal leaf pathogens *Alternaria brassicicola* and *F. oxysporum* (Hoffland et al. 1996). Seed-treatment of *S. marcescens* strain 90-166 showed ISR in cucumber against anthracnose, CMV, bacterial angular leaf spot and cucurbit wilt diseases (Kloepper et al. 1993; Liu et al. 1995a, b). The same strain was also reported to be effective in controlling the striped cucumber beetle, *Acalyma vittatum* and spotted cucumber beetle, *Diabrotica undecimpunctata howardi* (Zehnder et al. 1997). PGPR can also induce ISR against different pathogens in different crops. *P. fluorescens* strain Pf1 induces resistance against different pathogens in different crops, viz., *Rhizoctonia solani* (Nandakumar 1998), *Colletotrichum falcatum* in sugarcane (Viswanathan 1999) and *Pythium aphanidermatum* in tomato (Ramamoorthy et al. 1999). Thus, it would be prudent to select a PGPR having a broad spectrum of activity involving plant growth promotion and induction of resistance against multiple diseases and pests.

2.6 Induction of ISR by Endophytic PGPR

Apart from the colonization of rhizosphere and rhizoplane, some PGPR colonize the internal tissues of plants and are reported to be endophytes. Endophytic bacteria reside within the living plant tissues without doing substantive harm or gaining benefit other than residency (Kado 1992). Endophytic bacteria have the advantage of the protected environment inside the living plant tissues and are potential candidates for inducing ISR in plants. Endophytic bacteria brought about significant control against *F. solani* in cotton and *Sclerotium rolfsii* in beans (Pleban et al. 1995). Seed-treatment of tomato with endophytic bacterium *Bacillus pumilus* strain SE 34 prevented the entry of vascular wilt fungus *F. oxysporum* f. sp. *radicis-lycopersici* into the vascular stele and the mycelial growth was restricted to the epidermis and outer root cortex (Benhamou et al. 1998). Two endophytic tomato root colonizing strains, *Bacillus amyloliquefaciens* CM-2 and T-5 enhanced the growth of tomato seedlings along with the biocontrol of tomato bacterial wilt caused by *Ralstonia solanacearum* (Tan et al. 2013). Biological control of wheat stripe rust by an endophytic *Bacillus subtilis* strain E1R-j in greenhouse and field trials was reported by Li et al. (2013). The biocontrol agent inhibited the germination of urediniospore and reduced the rate of diseased leaves. The use of endophytic PGPR for induction of resistance will be more useful in vegetatively propagated crops like sugarcane, banana, etc. Viswanathan and Samiyappan (1999a) revealed the utility of endophytic *P. fluorescens* strain EP1 isolated from stalk tissues of sugarcane in inducing systemic resistance against red rot caused by *Colletotrichum falcatum*. The endophytic bacteria survives in the vegetatively propagated plant parts and move from one crop to the succeeding crop through vegetative propagation.

2.7 Mechanisms of ISR by PGPR

The PGPR employ several mechanisms for bringing about ISR in plants. These mechanisms may involve strengthening or fortification of the cell wall or elicitation of chemicals for defense against the invasion of disease causing agents.

2.7.1 Structural Modification of Cell Wall in Plants

Plant growth promoting rhizobacteria induce structural modification of the cell wall in response to pathogenic attack (Benhamou et al. 1996b; M’Piga et al. 1997). Treatment of pea seeds with *P. fluorescens* strain 63-28 resulted in formation of structural barriers, viz., cell wall apposition (papillae) and deposition of newly formed callose and accumulation of phenolic compounds at the site of penetration

of invading hyphae of *Pythium ultimum* and *F. oxysporum* f. sp. *pisi* (Benhamou et al. 1996a). Seed-treatment of tomato using *Bacillus pumilus* strain SE 34 also induced strengthening of cell walls in tomato against *F. oxysporum* f. sp. *radicis-lycopersici* (Benhamou et al. 1998). This type of rapid defense reaction does not allow the pathogen to invade and also offers the host plant sufficient time to employ other defense mechanisms to fight the pathogens.

2.7.2 PGPR-Mediated Biochemical Changes in the Host Plants

Biochemical and physiological changes have been reported in plants upon application of PGPR. ISR may be due to accumulation of pathogenesis-related (PR) proteins (M'Piga et al. 1997), synthesis of phytoalexin and other secondary metabolites (Zdor and Anderson 1992). ISR by *P. fluorescens* strain CHAO against TNV in tobacco was associated with accumulation of PR proteins namely β -1,3 glucanases and endochitinases (Maurhofer et al. 1994). Involvement of these lytic enzymes was reported by Benhamou et al. (1996b) in the induction of resistance by *P. fluorescens* strain 63-28. These lytic enzymes accumulated at the site of penetration of the fungus, *F. oxysporum* f. sp. *pisi* resulting in the degradation of fungal cell wall. Pathogenesis-related peroxidase and chitinase proteins have been found to induce systemic resistance. In sugarcane, PGPR-mediated ISR against *C. falcatum*, enhanced levels of chitinase and peroxidase and specific induction of two new chitinase isoforms were found when inoculated with *C. falcatum* (Viswanathan and Samiyappan 1999a, b).

PGPR induce systemic resistance in plants through means other than the production of PR proteins also (Pieterse et al. 1996). The plants produce other enzymes of the defense including peroxidases, phenylalanine ammonia-lyase (PAL), and polyphenol-oxidase (PPO). While peroxidase and PPO are catalysts in the formation of lignin, PAL and other enzymes are involved in the formation of phytoalexins (Figueiredo et al. 2010). The phytoalexins are secondary metabolites, antibiotics of low molecular weight produced by plants in response to physical, chemical, or biological stress. They are able to prevent or reduce the activity of pathogens, the rate of production dependent on the genotypes of host and/or pathogen (Daniel and Purkayastha 1995). *P. fluorescens* strains WCS 417r and WCS 358r induced protection in both wild type *Arabidopsis* and transgenic *Arabidopsis* with NahG-gene (coding for salicylate hydrolase) without activating PR gene expression (Van Wees et al. 1997). Accumulation of phytoalexin in response to *Pseudomonas* sp. strain WCS 417r treatment in carnation resulted in protection of carnation from wilt disease (Van Peer et al. 1991). Zdor and Anderson (1992) recorded increased peroxidase activity as well as an increase in the level of mRNAs encoding for phenylalanine ammonia-lyase (PAL) and chalcone synthase in the early stages of interaction between bean roots and various bacterial endophytes. The enzymes produced by antagonistic strains have a crucial role to play in disease resistance. The production of enzymes related to pathogenesis (PR proteins) by strains of rhizobacteria is con-

sidered as one of the most important property of the antagonistic strains (Saikia et al. 2004). These enzymes are chitinases, lipoxigenases, peroxidases, and glucanases. Plants express the activity of peroxidase during pathogen–host interaction (Saikia et al. 2006). Peroxidase enzyme has been implicated in the oxidation of phenols, lignification (Saparrat and Guillen 2005), plant protection (Hammerschmidt et al. 1982), and elongation of plant cells (Goldberg et al. 1986). Similarly, another enzyme lipoxigenase also contributes to the defense reactions involving the inhibition of growth of the pathogen and induction of phytoalexins (Li et al. 1991). The extent of activity and accumulation of these enzymes depends mainly on the inducing agent, besides the genotype of the plant, physiological conditions, and the pathogen (Tuzun 2001). Certain proteins involved in plant growth and development were up-regulated, such as xyloglucan endotransglycosylase (Wang et al. 2013). Proteins involved in defense were also up-regulated, including peroxidases, glutathione S-transferases and kinases. These proteins associated with disease resistance characteristics were induced in rice plants after exposure to *Bacillus cereus* NMSL88. There are reports of induction of disease resistance by rhizobia also. Hemissi et al. (2013) reported enhanced defense responses of chickpea plants against *Rhizoctonia solani* by pre-inoculation with Rhizobium strains Pch Azm and Pch S.Nsir2. The reduction in infection was accompanied by enhanced level of defense-related enzymes, PAL and peroxidase (POX). An increased level of phenol content was also recorded in the roots of bacterized plants grown in the presence of pathogen.

The defense mechanisms induced by PGPR against insect pests are different. Treatment with PGPR brings about some physiological changes in the host plant that prevent the insects from feeding. Due to PGPR treatment, there was a shift in the metabolic pathway in cucumber plants away from the cucurbitacin synthesis and towards that of other plant defense compounds, resulting in fewer beetles being attracted (Zehnder et al. 1997). In controlling nematodes, PGPR induce resistance by altering root exudates or inducing the host to produce repellents that affect nematode attraction or recognition of the host (Oostendorp and Sikora 1990) and altering the syncytial development or sex ratio in the root tissue (Wyss 1989). Seed-treatment with PGPR strains resulted in increased chitinase enzyme activity and phenolic content in rice, which correlated with the reduced nematode infestation (Swarnakumari 1996). The application of PGPR can thus form an important component of integrated pest management practices in agriculture.

2.7.3 Pathogen-Associated Molecular Patterns

To cause a disease, the invading pathogen must access to the plant interior. But in the process plant also can sense the presence of the pathogens by recognizing the several bio-molecules of pathogens called pathogens associated molecular patterns (PAMPs). Once pathogen penetrates the rigid cell wall of the plant, it comes in touch with the host plasma membrane wherein they encounter the plant extracellular surface receptors which in turns recognizes the PAMPs. On the onset of this reception, activation of plant defenses against the invading pathogens starts with a

dramatic cellular reprogramming and initiate PAMP triggered immunity (PTI). This PTI helps the plant to gain a hold over the pathogen and restricts their further proliferation. Thus, to cause disease, the pathogenic microbes must suppress PTI, activated in the plant system. To do so, the pathogens start interfering with the recognition at the plasma membrane or by secreting the effector proteins into the plant cell cytosol that alters the signaling processes leading to manifestation of disease symptoms. However, if microbes succeeded in subverting the PTI, plant develops more specialized mechanisms to detect and inactivate invading microbes called effector-triggered immunity (ETI) (Chisholm et al. 2006). In this mechanism, plant resistance (R) proteins recognize the bacterial proteins, directly or indirectly, involved in subverting the PTI system activated earlier. It has been discovered that there is remarkable similarities between the molecular mode of PAMP perception in animal and plants. Over the last decades, a number of PAMPs has been identified including lipopolysaccharides (LPS), harpin and flagellin in Gram-negative bacteria; cold shock protein in both Gram-negative and -positive bacteria; transglutaminase, elicitor, β -glucans in Oomycetes; invertase in yeast; chitin and ergosterol in all fungi; xylanase in *Trichoderma*, etc. (Numberger et al. 2004). The role of plasma membrane receptor proteins in recognizing the PAMPs and subsequent immunity has been studied in details. It has been proposed that PTI is induced on recognition of the microbial PAMPs and subsequent induction in the transcription of the pathogen-responsive genes, transcription of MAP kinase, production of reactive oxygen species along with the deposition of callose at the site of infection (Numberger et al. 2004).

The recognition of flagellin (protein present in flagella) as PAMP by plant has been studied in details. Though the central region of the flagellin is variable, the highly conserved regions at N and C terminals across eubacterial species facilitated it to become an excellent PAMP. In *Arabidopsis*, a 22 amino acid peptide (flg22) of the highly conserved N terminus region triggered the PTI. The flagellin receptor protein in *Arabidopsis*, FLS2, is a receptor like kinase (RLK) and mutant plant lacking this receptor is insensitive to flagellin which demonstrates the importance of receptors. Besides, flagellin, protein elongation factor Tu (EF-Tu) is one of the most abundant proteins and acts as PAMP in many plants (Chisholm et al. 2006). The possible mechanisms of PAMP-mediated disease suppression is shown in Fig. 2.1.

Once pathogenic microbes could overcome the PTI of plant, it secretes the effector molecules into the cytosol and thereby suppresses the PAMP triggered immunity. In bacteria, type III secretion system (TTSS) is present and it can directly deliver the effector protein into the plant cell. A number of effector proteins in different microbes have been identified. In *Pseudomonas syringae*, 20-30 effector proteins, including AvrRpt2 (protease), AvrB, AvrRpm1, HopPtoD2 (protein phosphatase) and AvrPtoB (E3 ligase), have been found during development of disease symptoms. These effector molecules inhibit the host defense responses initiated by PAMP recognition process (Fig. 2.1).

There are three plant-signaling molecules; salicylate (SA), jasmonate (JA) and ethylene; which regulate the plant defense against the invading microbes. The SA and JA defense pathways are mutually antagonistic and the bacterial pathogen takes advantage of this and overcome the SA-mediated defense responses.

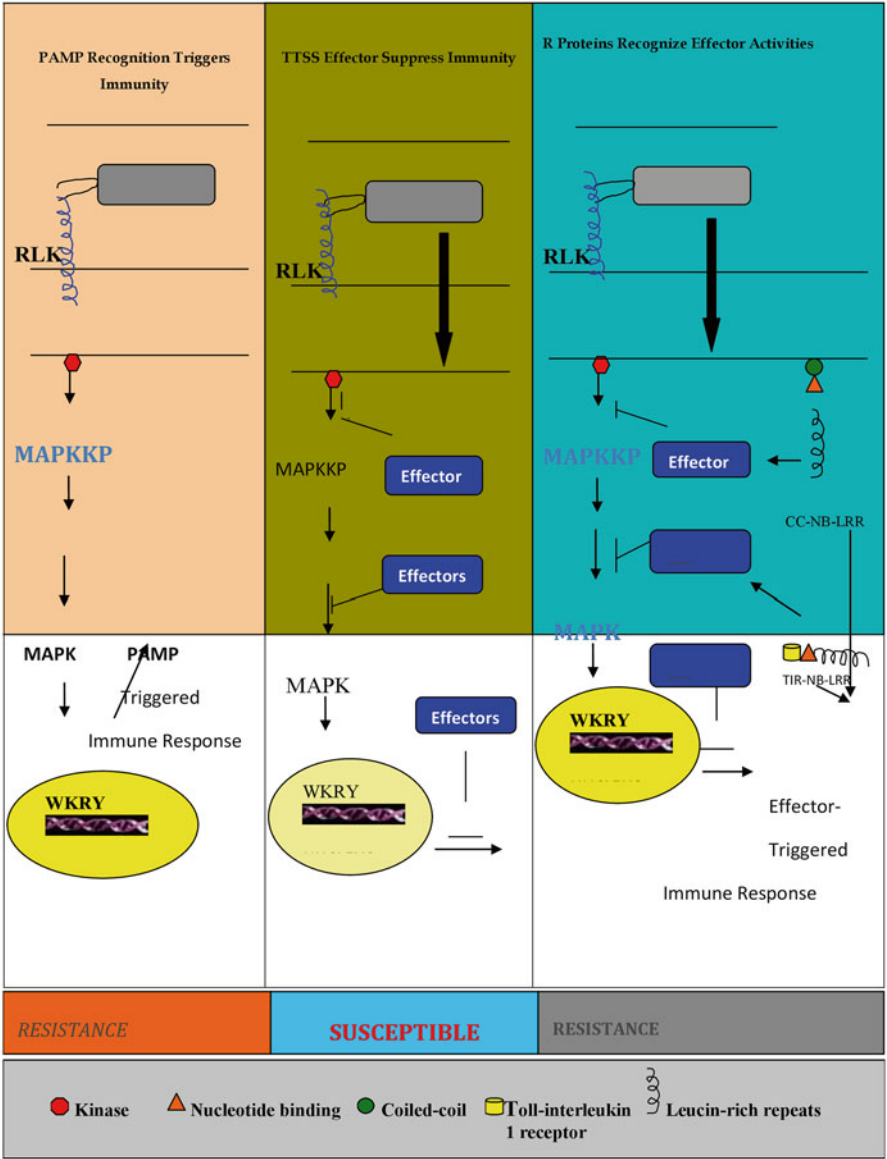


Fig. 2.1 Proposed model for the evolution of bacterial resistance in plants. (Source: Data from: Chisholm et al. 2006)

During infection, *Pseudomonas* pathogen produces coronatine which is similar to JA and thus overcome the SA pathway (He et al. 2004). Multiple effector proteins are found to be involved to manipulate the jasmonate pathway in *Pseudomonas syringae*. Majority of the effectors possess enzymatic activity and thus modify a number of host proteins to induce bacterial virulence. Besides, bacterial effectors,

effectors molecule have also been found in fungal and viral pathogenesis like in Oomycetes pathogen *Phytophthora infestans*.

The major focus in future would be on identification of novel plant receptors which would recognize the pathogen effector proteins and inactivate them as a disease control strategies.

2.8 Determinants of PGPR Imparting ISR

2.8.1 Lipopolysaccharides

The LPS present in the outer membrane of bacterial cells are important determinants of ISR in many PGPR strains (Table 2.3). The LPS of *P. fluorescens* strains WCS 374 and WCS 417 induced systemic resistance in radish against *F. oxysporum* f. sp.

Table 2.3 Bacterial determinants and types of host resistance induced by biocontrol agents

| Bacterial strain | Plant species | Bacterial determinant | Type |
|--|--------------------|--|------|
| <i>Pseudomonas aeruginosa</i> strain 7NSK2 | Tobacco | Salicylic acid | SAR |
| | Bean | Salicylic acid | SAR |
| | Tomato | Phenazine and Salicylic acid | SAR |
| <i>Bacillus amyloliquifaciens</i> | Sugar beet | Lipopolysaccharide | ISR |
| <i>Pseudomonas fluorescens</i> | Tomato | Massetolide A | ISR |
| <i>P. fluorescens</i> strain P3 | Tobacco | Salicylic acid | ISR |
| <i>Pseudomonas fluorescens</i> CHAO | Tobacco | Siderophore | SAR |
| | Arabidopsis | Antibiotics (DAPG) | ISR |
| WCS374 | Radish | Lipopolysaccharide | ISR |
| | | Siderophore | ISR |
| | | Iron regulated factor | ISR |
| | | | |
| WCS417 | Carnation | Lipopolysaccharide | ISR |
| | <i>Arabidopsis</i> | Lipopolysaccharide | ISR |
| | Radish | Lipopolysaccharide | ISR |
| | | Iron regulated factor | ISR |
| | Tomato | Lipopolysaccharide | ISR |
| <i>Pseudomonas putida</i> WCS 358 | <i>Arabidopsis</i> | Lipopolysaccharide | ISR |
| | | Siderophore | ISR |
| <i>Pseudomonas putida</i> BTP1 | Bean | Iron regulated metabolite Cx | ISR |
| <i>Serratia marcescens</i> 90-166 | Cucumber | Siderophore | ISR |
| <i>Bacillus mycoides</i> strain Bac J | Sugar beet | Peroxidase, chitinase and β -1,3-glucanase | ISR |
| <i>Bacillus pumilus</i> 203-6 and 203-7 | Sugar beet | Peroxidase, chitinase and β -1,3-glucanase | ISR |
| <i>Bacillus subtilis</i> GB03, IN937a | <i>Arabidopsis</i> | 2,3-butanediol | ISR |
| <i>Pseudomonas putida</i> | Bean | Hexenal | ISR |

Source: Data from: Pal and Gardener (2006)

raphani (Leeman et al. 1995). They further explained that the O-antigen side chain of the LPS might have triggered the induction of defense mechanism in plants. However, the LPS of *P. putida* strain WCS 358 having O-antigen side chain did not induce systemic resistance in radish. Van Wees et al. (1997) also obtained similar results where he reported that LPS of WCS 417r and mutant of WCS 417r lacking O-antigen side chain of LPS elicit defense mechanism in *Arabidopsis*. These studies indicated that LPS was not the only determining factor in ISR but other factors were also involved and also elicitation of ISR by LPS was different in different host plants.

2.8.2 Lipopeptides

Some lipopeptides that are produced by bacteria, especially by plant growth promoting rhizobacteria, have been found to induce systemic resistance in plants. Desoignies et al. (2013) investigated the putative action of *Bacillus amyloliquifaciens* lipopeptides in achieving rhizoctonia biocontrol through the control of the virus vector *Polymyxa betae*. Lipopeptides were shown to effectively induce systemic resistance in both the roots and leaves of sugar beet, resulting in a significant reduction in *P. betae* infection. Two classes of bacterial biosurfactant were found to be elicitors of ISR: rhamnolipids and cyclic lipopeptides (cLPs). Massetolide A from *Pseudomonas fluorescens* elicited ISR and enabled *Phytophthora infestans* on tomato to be controlled (Tran et al. 2007). The ISR activity of surfactin was associated, in treated plants, with the accumulation of antifungal compounds (phytoalexins) (Adam 2008) and with the stimulation of the lipoxygenase pathway, leading to the synthesis of fungitoxic oxylipins (Ongena et al. 2007). The induction of systemic resistance by cLPs is not yet clear, but a study by Henry et al. (2011) strongly suggests that the plant cell recognition of surfactin is mediated through interaction with lipids at the plasma membrane level, rather than through specific protein receptors

2.8.3 Siderophores

Siderophore production is an important feature in the suppression of plant pathogens. Siderophores are low molecular weight compounds produced by PGPR under iron-limited conditions. Siderophores act as determinants of ISR under iron starved conditions. The LPS of *P. fluorescens* strains WCS 374 and WCS 417 were the major determinants of ISR in radish against *Fusarium* wilt under iron-replete conditions but not under iron-limited conditions (Leeman et al. 1996). It was found that pyoverdinin-type pseudobactin siderophore produced by these bacteria was responsible for ISR. Press et al. (2001) reported the gene for catechol siderophore biosynthesis in *Serratia marcescens* 90-166 and associated it with induced resistance in cucumber against anthracnose. Thus, iron availability may determine the type of PGPR determinant responsible for ISR.

2.8.4 Salicylic Acid

Certain PGPR strains are capable of producing salicylic acid and are responsible for the induction of ISR in plants (Maurhofer et al. 1994). Introduction of *pchA* and *pchB* gene which encode for the synthesis of salicylic acid in *P. fluorescens* strain P3, rendered this strain capable of salicylic acid production and significantly improved its ability to induce systemic resistance in tobacco against TNV. Under conditions of iron limitation, *P. fluorescens* strain CHAO, naturally produced salicylic acid and also induced ISR in tobacco against TNV (Maurhofer et al. 1998).

Apart from these studies, contradictory observations have been also reported by workers. Mutants of *S. marcescens* strain 90-166 lacking in salicylic acid production were found to induce the same level of resistance in cucumber as the wild strain in cucumber and tobacco. Press et al. (1997) working with the salicylic acid producing strain 90-166 of *S. marcescens*, reported induction of resistance both in wild type tobacco and NahG-tobacco (tobacco plant transgened with NahG-gene encoding salicylic acid hydroxylase which converts salicylic acid to catechol). Van Wees et al. (1997) suggested that ISR induced by *P. fluorescens* strains WCS 417r and WCS 358r was independent of salicylic acid production in *Arabidopsis*.

These studies further emphasize the fact that different determinants of PGPR are involved in the induction of systemic resistance and this resistance varies with iron-limiting conditions, PGPR strains, host plants and their cultivars.

2.9 Formulation of PGPR

PGPR need to be formulated for large-scale application in crop fields. PGPR formulation helps in enhancing the shelf life, effective application and delivery of the bacterial cultures to the targeted site. Formulation also aids the packaging, transport and storage of the microbial product. Suslow (1980) reported the survival of PGPR in a dried formulation and the effectiveness of methyl cellulose in a powder formulation for coating sugar beet seed. The organic carriers used for formulation development include peat, talc, lignite, kaolinite, pyrophyllite, zeolite, montmorillonite, alginate, press mud, sawdust and vermiculite. Talc and Peat have been used as traditional carrier materials for effective formulations of PGPR. Vidhyasekaran and Muthamilan (1995) reported that the population of bacteria had been stable up to 240 days in talc-based and peat-based formulations. PGPR can be effectively formulated for systemic protection of crop plants against diseases. The most commonly used formulations of PGPR involve strains of *Pseudomonas fluorescens*, *P. aeruginosa*, *P. putida*, *Bacillus subtilis*, *B. amyloliquifaciens*, etc. *P. putida* strain 30 and 180 survived up to 6 months in talc-based formulations. The population load at the end of 6th month was 10^8 cfu/g of the product (Bora et al. 2004). Shelf life of *P. chlororaphis* (PA23) and *B. subtilis* (CBE4) in peat carriers was retained for more than 6 months (Nakkeeran et al. 2004).

The formulated products can be delivered through different methods of application like seed-treatment, seed-priming, soil application, foliar application, root-dip, sett-treatment in sugarcane, sucker-treatment in banana. Drum priming of carrot and parsnip seeds with *P. fluorescens* Pf CHAO proliferated well on the seeds and could be explored for realistic scale up of PGPR (Wright et al. 2003). Root-dipping of seedlings has been found effective for the control of soil-borne pathogens in case of transplanted plants. Dipping of *Phyllanthus amarus* seedlings in talc-based formulation of *B. subtilis* (BSCBE4) or *P. chlororaphis* (PA23) for 30 min prior to transplanting reduced stem blight of *P. amarus* (Mathiyazhagan et al. 2004). Foliar application of PGPR formulations are used for controlling foliar diseases. However, the leaf surface microclimate is subjected to frequent changes and should be considered while designing spray schedules. Preharvest foliar application of talc-based fluorescent pseudomonads strain FP7 supplemented with chitin at fortnightly intervals (5 g/L; spray volume 20 L/tree) on to mango trees from pre-flowering to fruit maturity stage induced flowering to the maximum, reduced the latent infection by *Colletotrichum gloeosporioides* beside increasing the fruit yield and quality (Vivekananthan et al. 2004). Application of PGPR formulations with strain mixtures perform better than individual strains for the management of pest and diseases of crop plants, in addition to plant growth promotion (Nakkeeran et al. 2005). Combination of iron chelating pseudomonad strains and inducers of systemic resistance suppressed Fusarium wilt of radish better than the application of individual strains (de Boer et al. 2003).

Microencapsulation of rhizobacteria has been tried in recent years as a formulation. Microcapsules of rhizobacteria consist of a cross linked polymer deposited around a liquid phase, where bacteria are dispersed (Nakkeeran et al. 2005). The process of microencapsulation involves mixing of gelatin polyphosphate polymer pair (81:19 w/w) at acidic pH with rhizobacteria suspended in oil (Charpentier et al. 1999). The microencapsulation technique has not picked up in a big way. The cost factor could be a reason. This formulation needs to be tested in large-scale field trials in order to be adopted for commercial use.

2.9.1 Frequency of Application

The effectiveness of application of PGPR formulation remains for a certain time followed by a decline over time. This determines the number of applications of PGPR formulations needed to maintain the resistance levels in crop plants (Dalisyay and Kuc 1995). Different methods of application have different durability. Foliar sprays of *P. fluorescens* formulations should be given at every 15 days intervals for managing rice foliar diseases (Vidhyasekaran et al. 1997). Experiments conducted by Nayar (1996) indicated that induction of defense mechanisms using *P. fluorescens* persisted up to 60 days by seed-treatment, 30 days by root-dipping and 15 days by foliar spray. The duration of the induced resistance varies from crop to crop and strain to strain of PGPR. The induction of resistance by PGPR persisted for 90 days of crop growth in sugarcane (Viswanathan 1999).

2.10 Challenges

Though PGPR have a potential scope in commercialization, the threat of certain PGPR (*P. aeruginosa*, *P. cepacia* and *B. cereus*) to infect human beings as opportunistic pathogens has to be clarified before large-scale acceptance (Nakkeeran et al. 2005). Potential biocontrol agents have to pass through several tests in order to be commercially viable. After thorough, large-scale field testing at multiple locations, differing in soil and climatic conditions, these agents can be recommended for registration with the government agencies. The technology must be transferred to some firms which can take up the mass production of the product and finally it must be adopted by the end users i.e. the farming community. The biocontrol agent should not pose any threat to human and animal health and should not be an environmental hazard.

The knowledge of ecology of the introduced PGPR strains is sometimes lacking which may be a serious impediment to the establishment and multiplication of the PGPR strains. The interaction of the introduced strains with the native flora and fauna will also be a deciding factor in the success of the biocontrol agent.

PGPR formulations are usually produced at small entrepreneurial levels or at the fermentation units of research stations, but seldom at very large industrial firms. Hence, technologies for production of biofertilizers and biopesticides at very large levels are not suitably developed. Moreover, IPR issues have not been dealt with suitably in case of these bioproducts. Ambiguities prevail with respect to registration/licensing/patenting of these products with the law differing in different countries.

PGPR have been discovered and researched for last two-three decades, but till date widespread use of these products is yet to be seen. Availability of good quality biofertilizers and biopesticides to the farmers is still an issue along with lack of awareness about the products and their benefits. The available products have less shelf life and should be used properly because of the biological nature of the products. The issue of quality control should be dealt with stringency to ensure quality products to the end users. Very often, locally formulated products are available in the market in plenty but quality of those products cannot be ascertained along with tangible benefit by the farmers.

2.10.1 Constraints to Commercialization

The success of any biological agents depends on availability of quality formulation with good shelf life, marketing and perceived acceptability and demand of the end users. The factors limiting the successful commercialization of biological agents are as follows:

- Reliability and authenticity of the selection of the biocontrol agent.
- Concerns about the possible ecological consequences of the intended commercialization of the biocontrol PGPR.

- Lack of awareness about the biological agents and their target pathogens.
- Risk associated with the mass multiplication of the biocontrol agents in industrial scale fermenters.
- Concerns of inconsistent performance of PGPR biocontrol agents in managing disease and pests.
- Chances of mutation and loss of desirable traits in the biocontrol agents.
- Lack of awareness among the farmers about the potential of the biocontrol agents in managing diseases and pests.
- Competition from the spurious locally developed biocontrol agents.
- Procedural delays in registration of the products.
- Lack of proper delivery system for biocontrol PGPR.
- Concerns about stability and quality of the products.
- Stiff challenges from environment protection agencies and inherent difficulties in addressing their concerns.
- Perceived potential threats from few opportunistic human pathogens as bio-control agents.

2.11 Conclusions

PGPR are beneficial to crop plants in many ways. Inoculation with PGPR results in improvement of plant growth, control of diseases and induction of systemic resistance. Tikhonovich and Provorov (2011) argued that utilization of appropriate preparations of beneficial microorganisms is the most promising strategy for maintaining agricultural productivity whilst reducing the inputs of inorganic fertilizers, herbicides and pesticides and that ‘microbiology is the basis of sustainable agriculture’. Several strains of PGPR have broad spectrum activity against multiple diseases and also provide protection against insect and nematode pests. Endophytic PGPR have been found beneficial in growth promotion and disease control in vegetatively propagated crops. With the progress of agriculture towards sustainability, microbes will find greater use as biocontrol agents.

However, we should be realistic with cautions. Though tall claims have been made by researchers over the past several decades about the potential applications of a plethora of PGPR biocontrol agents in managing a number of disease and pests in many crop species, not much success has been achieved yet for commercialization and their application at field level. Concerted efforts will be required to demonstrate the benefits of the PGPR biocontrol agents to the farmers so that the eco-friendly agents can be popularized. Unless end users are convinced by the benefits of the biocontrol PGPRs by conducting trials of their own, the success stories will remain in the research laboratories only.

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