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Review article

Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases

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Abstract

Plant growth promoting rhizobacteria (PGPR) belonging to *Pseudomonas* spp. are being exploited commercially for plant protection to induce systemic resistance against various pests and diseases. Mixtures of different PGPR strains have resulted in increased efficacy by inducing systemic resistance against several pathogens attacking the same crop. Seed-treatment with PGPR causes cell wall structural modifications and biochemical/physiological changes leading to the synthesis of proteins and chemicals involved in plant defense mechanisms. Lipopolysaccharides, siderophores and salicylic acid are the major determinants of PGPR-mediated ISR. The performance of PGPR has been successful against certain pathogens, insect and nematode pests under field conditions.

Keywords: Induced systemic resistance; PGPR; Plant pathogens; Insect pests

Contents

1. Introduction

Induced protection of plants against various pathogens by biotic or abiotic agents has been reported since 1930s when Chester (1933) proposed the term "acquired physiological immunity". Since then several terms have been used to describe the phenomenon of induced resistance such as "systemic acquired resistance" (Ross, 1961), "translocated resistance" (Hurbert and Helton, 1967) and "plant immunization" (Tuzun and Kuc, 1991).

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 induced systemic resistance (ISR) or systemic acquired resistance (SAR) (Hammerschmidt and Kuc, 1995). The induction of systemic resistance by rhizobacteria is referred as ISR, whereas that by other agencies is called SAR (Van Loon et al., 1998). SAR is expressed to a maximum level when the inducing organism causes necrosis (Cameron et al., 1994) whereas ISR by PGPR typically do not cause any necrotic symptoms on the host plants (Van Loon et al., 1998). Both SAR and ISR are the activation of latent resistant mechanisms that are

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expressed upon subsequent or challenge inoculation with a pathogen (Van Loon, 1997).

The biotic inducers of SAR include virulent pathogens, non-pathogens and elicitors of fungal cell wall metabolites. Abiotic agents are salicylic acid (SA), ethylene, dichloro-isonicotinic acid and benzothiadiazole (Gorlach et al., 1996; Sticher et al., 1997). The utilization of pathogenic organisms as inducing agents has not been successful under field conditions. Generally, the duration of protection is less following induction of a pathogen than that with PGPR-mediated ISR and prior inoculation of pathogen may provide a source of secondary inoculum (Wei et al., 1991). The virulent pathogen might suppress or not activate the expression of SAR during successful infection (Daly, 1972; Gras et al., 1979; Heath, 1982). For successful disease management, it is important to find more effective, practical and economical ways to protect plants from various pests or diseases. PGPR are root-colonizing bacteria with beneficial effects including plant growth promotion and biological disease control. In recent years, the use of PGPR as an inducer of systemic resistance in crop plants against different pathogens has been demonstrated under field conditions (Wei et al., 1991; 1996; Vidhyasekaran and Muthamilan, 1999; Viswanathan, 1999; Viswanathan and Samiyappan, 1999a). The utilization of natural PGPR strains as inducers of plant defence responses may increase the chance of their applicability and offer a practical way to deliver immunization. This paper reviews PGPR-mediated ISR against plant pathogens and insect pests, mechanisms of ISR and traits of PGPR in determining the ISR in crop plants.

2. Induction of systemic resistance by PGPR against diseases, insect and nematode pests

PGPR induce resistance in plants against fungal, bacterial and viral diseases (Liu et al., 1995a, b; Maurhofer et al., 1998), and insect (Zehnder et al., 1997) and nematode pests (Sikora, 1988). Induction of systemic resistance by selected strains of PGPR against plant diseases and insect pests has been proved by spatially separating the pathogen and PGPR in the plants (Van Peer et al., 1991).

2.1. Diseases

PGPR provide different mechanisms for suppressing plant pathogens. They include competition for nutrients and space (Elad and Baker, 1985; Elad and Chet, 1987), antibiosis by producing antibiotics viz., pyrrolnitrin, pyocyanine, 2,4-diacetyl phloroglucinol (Pierson and Thomashow, 1992) and production of siderophores (fluorescent yellow green pigment), viz., pseudobactin which limits the availability of iron necessary for the growth of pathogens (Kloepper et al., 1980; Lemanceau et al., 1992). Other important mechanisms include production of lytic enzymes such as chitinases and β-1,3-glucanases which degrade chitin and glucan present in the cell wall of fungi (Frindlender et al., 1993; Lim et al., 1991; Potgieter and Alexander, 1996; Velazhahan et al., 1999), HCN production (Defago et al., 1990) and degradation of toxin produced by pathogen (Borowitz et al., 1992; Duffy and Defago, 1997).

Several studies have been carried out to elicit ISR by PGPR in plants. In carnation, application of *Pseudomonas* sp. strain WCS 417r protected plants systemically against *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *dianthi* (Van Peer et al., 1991). PGPR strains applied as a seed-treatment resulted in a significant reduction in anthracnose disease caused by *Colletotrichum orbiculare* in cucumber (Wei et al., 1991, 1996). Similarly, 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). PGPR as a seed-treatment alone or as seed-treatment plus soil-drenching has protected cucumber plants against anthracnose disease (Wei et al., 1996). In rice seed-treatment followed by root-dipping and a foliar spray with *P. fluorescens* strains Pf1 and FP7 showed higher induction of ISR against the sheath blight pathogen, *Rhizoctonia solani* (Vidhyasekaran and Muthamilan, 1999). Similarly, in sugarcane, Viswanathan and Samiyappan (1999a) established PGPR-mediated ISR against *Colletotrichum falcatum* causing red rot disease.

PGPR can also induce systemic protection against bacterial diseases. Seed treated with *P. fluorescens* strain 97 protected beans against halo blight disease caused by *Pseudomonas syringae* pv. *phaseolicola* (Alstrom, 1991), while treatment of cucumber seed with *P. putida* strain 89B-27 and *S. marcescens* strain 90-166 decreased the incidence of bacterial wilt disease (Kloepper et al., 1993). Similarly seed-treatment of cucumber with *P. putida* strain 89B-27, *Flavomonas oryzihabitans* 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).

Induction of systemic resistance by PGPR against viral diseases has been reported in cucumber and tobacco plants. Seed-treatment with *P. fluorescens* strain 89B-27 and *S. marcescens* strain 90-166 has consistently reduced the number of cucumber mosaic virus-infected plants (CMV) and delayed the development of symptoms in cucumber and tomato (Raupach et al., 1996). Soil-application of *P. fluorescens* strain CHAO has induced systemic protection against inoculation with tobacco necrosis virus (TNV) in tobacco (Maurhofer et al., 1994, 1998). These experiments show that PGPR strains

initiate ISR against a wide array of plant pathogens causing fungal, bacterial and viral diseases.

2.2. Insect pests

Reports on PGPR-mediated ISR against insects are restricted to very few crops. Generally, fluorescent pseudomonads influence the growth and development of insects at all stages of their growth. *Pseudomonas maltophila* affects the growth of larval stage of *Helicoverpa zea*, the corn earworm, 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). Similarly, the relative growth rate, consumption rate and digestibility of feed by *Helicoverpa armigera* have been affected when larvae fed on cotton plants treated with *Pseudomonas gladioli* due to an increase in their polyphenol and terpenoid content (Qingwen et al., 1998). Induction of systemic resistance by PGPR strains, viz., *P. putida* strain 89B-27, *S. marcescens* strain 90-166, *Flavomonas oryzihabitans* strain INR-5 and *Bacillus pumilus* strain INR-7 have significantly reduced populations of the striped cucumber beetle, *Acalyma vittatum* and the spotted cucumber beetle, *Diabrotica undecimpunctata howardi* on cucumber. Among these strains, *S. marcescens* strain 90-166 was more effective in reducing the population of both the beetles and its efficacy was better than application of the insecticide esfenvalerate (Zehnder et al., 1997). As certain fluorescent pseudomonads are effective rhizosphere colonizers and are endophytic in nature in the plant system, attempts have been made to transfer the insecticidal crystal protein from *Bacillus thuringiensis* to *P. fluorescens*. *P. fluorescens*, thus genetically engineered was effective against a lepidopteran insect pest. Transgenic *P. cepacia* strain 526 with the crystal protein gene has consistently shown insecticidal activity against tobacco hornworm (Stock et al., 1990). Thus, PGPR treatment of crops can be effective for insect pest management and has a great potential for future use.

2.3. Nematodes

PGPR also induce systemic resistance against nematode pests (Oostendorp and Sikora, 1990; Sikora, 1992; Sikora and Hoffmann-Hergarten, 1992). *P. fluorescens* has induced systemic resistance and inhibited early root penetration of *Heterodera schachtii*, the cyst nematode in sugar beet (Oostendorp and Sikora, 1989, 1990). Similarly, *B. subtilis* has induced protection against *Meloidogyne incognita* and *M. arenaria* in cotton (Sikora, 1988). Though attempts to use PGPR for nematode control are limited, the use of PGPR as biological control agents of plant parasitic nematodes especially for sugar beet and potato cyst nematode has been reported as a successful strategy in management of these nematodes (Sikora, 1992). Treatment of rice seed with PGPR alone or in combination with chitin and neem cake has reduced the root and soil population of the rice root nematode, *Hirschmanniella oryzae* (Swarnakumari and Lakshmanan, 1999; Swarnakumari et al., 1999). The level of infestation of root-knot nematode *M. 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). Similarly, application of the bacterium, *P. chitinolytica* reduced the root-knot nematode infection in tomato crop (Spiegel et al., 1991).

These experiments show that PGPR-mediated ISR is effective in both dicotyledonous plants, viz., *Arabidopsis*, bean, carnation, cucumber, radish, tobacco and tomato and certain monocotyledonous plants, viz., rice, maize and sugarcane.

3. Synergistic effect of PGPR strain mixtures

It is likely that most of naturally occurring biological control results from mixtures of antagonists rather than from high populations of a single antagonist. Similarly, application of a mixture of introduced biocontrol agents would more closely mimic the natural situation and might broaden the spectrum of biocontrol activity and enhance the efficacy and reliability of control (Duffy and Weller, 1995). Application of a mixture of three PGPR strains viz., *Bacillus pumilus* strain INR 7, *B. subtilis* strain GB03 and *Curtobacterium flaccumfaciens* strain ME1 as a seed-treatment has resulted in much more intensive plant growth promotion and disease reduction when compared to strains tested singly. This might be due to different mechanisms of action for each PGPR strain (Raupach and Kloepper, 1998). Use of 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 incited by *Rhizoctonia solani* (Sung and Chung, 1997). Application of a mixture 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 incited by *F. oxysporum* f. sp. *cucumerinum* (Singh et al., 1999). Similarly, mixtures of *Pseudomonas* spp. have suppressed take-all disease of wheat (*Gaeumannomyces graminis* var *tritici*) than treatment using a single *Pseudomonas* sp. (Pierson and Weller, 1994; Duffy and Weller, 1995). Combination of *P. fluorescens* strains, viz., Pf1 and FP7 gave effective control of rice sheath blight disease when compared to each strain applied singly (Nandakumar, 1998).

However, in certain cases, mixtures of different strains have no synergistic effect. Further, a mixture that improves efficacy under one set of conditions or on one host may not perform under different conditions and also on different hosts (Schisler et al., 1997). From the economic point of view, a biocontrol product composed of a mixture of strains may be more expensive than a product containing a single strain. In developing mixtures of strains, greater emphasis must be given on their efficacy against disease suppression by ISR.

4. Spectrum of protection by PGPR

Recently, work on the spectrum of PGPR-mediated ISR against different pathogens in different crop plants has been gaining importance (Hoffland et al., 1996, 1997; Wei et al., 1996; Kloepper et al., 1997; Ramamoorthy and Samiyappan, 1999). Seed-treatment with *P. fluorescens* strain WCS 417 has protected radish through induction of systemic resistance not only against the fungal root pathogen *F. oxysporum* f. sp. *raphani*, but also against the avirulent bacterial leaf pathogen *P. syringae* pv. *tomato* and fungal leaf pathogens *Alternaria brassicola* and *F. oxysporum* (Hoffland et al., 1996). This implies that the same PGPR strain can induce resistance against multiple pathogens in the same crop. ISR by *P. putida* strain 89 B - 27 and *S. marcescens* strain 90-166 against anthracnose of cucumber was established by Wei et al. (1991). Later studies showed that the same PGPR strains induced systemic protection against angular leaf spot caused by *P. syringae* pv. *lachrymans* (Liu et al., 1993a), *Fusarium* wilt incited by *F. oxysporum* f.sp. *cucumerinum* (Liu et al., 1993b) and cucurbit wilt caused by *Erwinia tracheiphila* (Kloepper et al., 1993). Seed-treatment of *S. marcescens* strain 90-166 has shown ISR in cucumber against anthracnose, cucumber mosaic virus, bacterial angular leaf spot and cucurbit wilt diseases (Kloepper et al., 1993; Liu et al., 1995a, b). In addition, the same bacterial strain has also been effective in controlling the striped cucumber beetle, *Acalyma vittatum* and spotted cucumber beetle, *Diabrotica undecimpunctata howardi* (Zehnder et al., 1997). Parallel experiments have shown that the *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). The broad spectrum of PGPR-mediated ISR is more rewarding than narrow spectrum of disease protection. Hence selecting a suitable strain having potential to induce systemic resistance against multiple pathogens and pests is the most important task in the delivery of microbial agents to the field.

5. PGPR as endophytes

Endophytic bacteria are defined as bacteria which reside within the living plant tissues without doing substantive harm or gaining benefit other than residency (Kado, 1992). In addition to rhizosphere and rhizoplane colonization, certain PGPR are reported to be endophytes localized in the intercellular spaces of the root epidermal cells and vascular tissue (Chen et al., 1995; Benhamou et al., 1996a, b; Hallmann et al., 1997; M'Piga et al., 1997). Several factors favour endophytic bacteria as potential agents of ISR. Endophytes have a natural and intimate association with plants. The internal tissues of plants provide a relatively uniform and protected environment when compared with the rhizosphere and rhizoplane (Chen et al., 1995), where ectophytic bacteria must compete for nutrients with other microbes and endure fluctuations of temperature and moisture, as well as exposure to ultraviolet radiation on above ground surfaces. In spite of these advantages, the potential of bacterial endophytes has only been explored to a limited extent. Application of endophytic bacteria by stem injection in cotton plants reduced root rot caused by *Rhizoctonia solani* and vascular wilt caused *by F. oxysporum* f. sp. *vasinfectum* (Chen et al., 1995). These bacteria move upward and downward from the point of application and by colonizing the internal tissues, can exclude the entry of a pathogen in the vascular stele. Endophytic bacteria have brought about significant control against *F. solani* in cotton and *Sclerotium rolfsii* in beans (Pleban et al., 1995). In pea, colonization of epidermis, cortex and vascular tissue in roots by endophytic bacteria prevented entry of mycelial growth of fungus or restricted the growth of mycelium to the epidermis. (Benhamou et al., 1996a, b). 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). Similarly application of *P. fluorescens* strain 63-28 restricted the growth of *Pythium ultimum* in pea (Benhamou et al., 1996a, b) and *F. oxysporum* f. sp. *radicis-lycopersici* in tomato (M'Piga et al., 1997).

The use of an endophytic strain for inducing systemic resistance is more beneficial in vegetatively-propagated crops, like banana, sugarcane, and tapioca, for example Viswanathan (1999) and 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 (*Colletotrichum falcatum*). Similarly prophylactic inoculation of

oak trees with *Pseudomonas denitrificans* strain 1-15 and *P. putida* strain 5-48 protected the trees against *Ceratocystis fagacearum* (Brooks et al., 1994). Further, when the endophytic bacteria are introduced into the vegetatively propagated seed, the bacteria survives and moves in the vegetative part and subsequently the propagative seed will also have the introduced bacteria, thus minimizing the need for frequent application of bacterial strains.

6. Mechanism of ISR-mediated by PGPR

PGPR bring about ISR through fortifying the physical and mechanical strength of the cell wall as well as changing the physiological and biochemical reaction of the host leading to the synthesis of defence chemicals against the challenge pathogen.

6.1. Structural and ultra-structural cell wall modifications in the host plants

The success of a plant in warding off invading pathogens relies primarily on its ability to build a line of defence rapidly for protecting cell walls against the spread of a pathogen (Benhamou et al., 1996a). It is well known that PGPR induces cell wall structural modification in response to pathogenic attack (Benhamou et al., 1996b, 1998; M'Piga et al., 1997). Seed-treatment of PGPR in bean induces the lignification of cell wall (Anderson and Guerra, 1985). Treatment of pea seeds with *P. fluorescens* strain 63-28 has 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). In tomato, cell wall thickening, deposition of phenolic compounds and formation of callose restricted growth of *F. oxysporum* f. sp. *radicis-lycopersici* to the epidermal cell and outer cortex in the root system in the treated plants (M'Piga et al., 1997). Similarly, seed-treatment using *Bacillus pumilus* strain SE 34 has also induced strengthening of cell walls in tomato against *F. oxysporum* f. sp. *radicis-lycopersici* (Benhamou et al., 1998). Such a rapid defence reaction at sites of fungal entry delays the infection process and allows sufficient time for the host to build up other defence reactions to restrict pathogen growth to the outer most layer of root tissue.

6.2. Biochemical/Physiological changes in the host plants

Application of PGPR results in biochemical or physiological changes in the plants. Normally ISR by PGPR is associated with the accumulation of PR proteins (pathogenesis-related proteins) (Maurhofer et al., 1994; Benhamou et al., 1996a; M'Piga et al., 1997; Viswanathan and Samiyappan, 1999b), synthesis of phytoalexin and other secondary metabolites (Van Peer et al., 1991; Zdor and Anderson, 1992). Colonization of bean root by fluorescent bacteria is correlated with induction of PR proteins and systemic resistance against *Botrytis cinerea* (Zdor and Anderson, 1992). Maurhofer et al. (1994) reported that ISR by *P. fluorescens* strain CHAO against tobacco necrosis virus (TNV) in tobacco was associated with accumulation of PR proteins namely β-1,3 glucanases and endochitinases. They also established that ineffective strain P3 did not accumulate PR proteins indicating involvement of PR proteins in induction of resistance. In pea, seed treatment with *P. fluorescens* strain 63-28 has produced hydrolytic enzymes such as chitinases and β-1,3 glucanases. These host lytic-enzymes accumulate at the site of penetration of the fungus, *F. oxysporum* f. sp. *pisi* resulting in the degradation of fungal cell wall (Benhamou et al., 1996b). Inoculation of tomato plants with the same strain has similarly induced the production of plant chitinases when challenged with the wilt pathogen, *F. oxysporum* f. sp. *radicis-lycopersici* (M'Piga et al., 1997). ISR has been correlated with a two-fold increase in activity of pathogenesis-related peroxidase and chitinase proteins. Two peroxidase and one chitinase (35 kDa) isoforms have been induced in the PGPR-treated plant inoculated with the rice sheath blight pathogen, *Rhizoctonia solani* (Nandakumar, 1998). Similarly, in sugarcane, PGPR-mediated ISR against *C. falcatum*, enhanced levels of chitinase and peroxidase were noticed and specific induction of two new chitinase isoforms were found when inoculated with *C. falcatum* (Viswanathan and Samiyappan, 1999a, b).

Defense chemicals other than PR proteins are also induced in ISR by PGPR in certain crops. PR proteins are not accumulated in radish plant expressing ISR elicited by *P. fluorescens* strain WCS417r against *F. oxysporum* f. sp. *raphani* (Hoffland et al., 1995, 1996). In *Arabidopsis*, ISR induced by *P. fluorescens* strain WCS 417r is not associated with PR gene activation (Pieterse et al., 1996). Similarly, strains WCS 417r and WCS 358r have induced protection in both wild type *Arabidopsis* and transgened-*Arabidopsis* with NahG-gene (NahG-gene encodes salicylate hydrolase) without activating PR gene expression (Van Wees et al., 1997). These experiments indicate that the PR protein accumulation is not the only defence chemical involved in the induction of systemic resistance in these hosts and there is possibility of involvement of other defence compounds in ISR. Accumulation of phytoalexin in response to *Pseudomonas* sp. strain WCS 417r treatment in carnation results in protection of carnation from wilt disease incidence (Van Peer et al., 1991). Increased peroxidase activity as well as an increase in the level of mRNAs encoding for phenylalanine ammonia lyase (PAL) and chalcone synthase (which lead to synthesis of phytoalexin) are recorded in the early stages of interaction between bean

roots and various bacterial endophytes (Zdor and Anderson, 1992).

In summary, induction of systemic resistance by PGPR especially against soil-borne pathogens is associated with ultra-structural cell wall modification that prevents the ingress of mycelium of the pathogen in the vascular stele followed by the biochemical changes, viz., accumulation of PR proteins and/or phytoalexins.

Defence mechanisms induced against insect pests in cucumber plants are different from that against pathogens. PGPR do not kill insects, but application of PGPR brings about some physiological changes in the host plant that prevents the insects from feeding as has been demonstrated in cucumber against cucumber beetles (Zehnder et al., 1997). Normally *Diabrotica* beetles are attracted to volatiles, cucurbitacins (triterpenoids occur mainly in cucurbitaceae), coming from cucurbit blossoms and probably use these olfactory clues in long-range host finding. The cucurbitacin causes locomotory arrest and compulsive feeding of *Diabrotica* beetles (Anderson and Metcalf, 1986; Lewis et al., 1990). Unless a feeding stimulant is detected on the cucumber plant, the beetles may leave the host within 1–2 min (Lewis et al., 1990). Due to PGPR treatment, there is a shift in the metabolic pathway in cucumber plants away from the cucurbitacin synthesis and towards that of other plant defence compounds, resulting in fewer beetles being attracted (Zehnder et al., 1997).

In nematode control, 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). In rice, seed treatment with PGPR strains increases the chitinase enzyme activity and phenolic content and this is correlated with the reduced nematode infestation (Swarnakumari, 1996).

7. PGPR determinants in ISR

There are several bacterial determinants involved in the induction of systemic resistance by PGPR the most important being lipopolysaccharides present in the outer membrane of bacterial cells, siderophore and salicylic acid production (Van Loon et al., 1998).

7.1. Lipopolysaccharides (*LPS*)

Lipopolysaccharides present in the outer membrane of PGPR are the major determinants of ISR in certain PGPR strains. LPS of *P. fluorescens* strain WCS 417 have induced systemic resistance in carnation against *Fusarium* wilt caused by *F. oxysporum* f. sp. *dianthi* (Van Peer and Schippers, 1992). Similarly, LPS of *P. fluorescens* strains WCS 374 and WCS 417 have induced systemic resistance in radish against *F. oxysporum* f. sp. *raphani* (Leeman et al., 1995). They also established that mutant of *P. fluorescens* strain WCS 417, lacking the O-antigen side chain of the LPS, has not induced resistance in radish indicating the O-antigen side chain of the LPS might have served as a signal or trigger in the induction of defence mechanism in plants. In contrast, LPS of *P. putida* strain WCS 358 having o-antigen side chain do not induce systemic resistance in radish. In another study, LPS of WCS 417r and mutant of WCS 417r lacking O-antigen side chain of LPS elicit defence mechanism in *Arabidopsis* (Van Wees et al., 1997). This indicates that ISR by LPS of PGPR varies with different host plant and lipopolysaccharide is not the only trait in determining the ISR. Other traits of PGPR are also involved in ISR.

7.2. Siderophores

Siderophores are produced by PGPR under iron-limited conditions. Leeman et al. (1996) reported that LPS of *P. fluorescens* strains WCS 374 and WCS 417 are the major determinants of ISR under iron-replete conditions but under iron-limited conditions, LPS of these bacteria were not involved in ISR in radish against *Fusarium* wilt. They also found that pyoverdin-type pseudobactin, siderophore, produced by these bacteria was responsible for ISR. Application of purified pseudobactin alone, isolated from strain WCS 374, to the roots of radish induced resistance. Thus, different bacterial determinants in inducing systemic resistance in radish vary depending upon iron availability. Induction of ISR by LPS and siderophores seems to be complementary rather than additive and full induction of resistance by one determinant masks contributions by other(s).

7.3. Salicylic acid

White (1979) observed that treatment with salicylic acid decreased the disease development caused by tobacco mosaic virus in the tobacco cultivar Xanthi-nc. Kessmann et al. (1994) and Schneider et al. (1996) reviewed induction of systemic resistance by the application of 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).

Salicylic acid production by *P. aeruginosa* strain 7NSK2 is essential for induction of resistance to *Botrytis cinerea*. Mutants of the same strain lacking salicylic acid production have lost their ability to induce systemic resistance in bean (De Meyer and Hofte, 1997). Introduction of pch A and pch B gene which encode for the synthesis of salicylic acid in *P. fluorescens* strain P3, renders this strain capable of salicylic acid production and significantly improved its ability to induce systemic resistance in tobacco against TNV. *P. fluorescens* strain

CHAO naturally produces salicylic acid under conditions of iron limitation and also induces ISR in tobacco against TNV (Maurhofer et al., 1998).

In contrast to these examples, mutants of *S. marcescens* strain 90-166 lacking salicylic acid production induce the same level of resistance in cucumber as the wild strain of *S. marcescens* (salicylic acid producing strain) does in cucumber and tobacco. In another study, salicylic acid producing strain of *S. marcescens* 90-166 induce 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) (Press et al., 1997). Similarly, *P. fluorescens* strains WCS 417r and WCS 358r, which produce salicylic acid, induce resistance in both wild type *Arabidopsis* and NahG-gene transformed *Arabidopsis* plants (Van Wees et al., 1997). This suggests that ISR induced by *P. fluorescens* strains WCS 417r and WCS 358r is independent of salicylic acid production in *Arabidopsis*. Expression of ISR by *P. fluorescens* strain WCS 417r requires ethylene-dependent signaling pathway but not salicylic acid-dependent signaling at the site of application (Knoester et al., 1999).

All these experiments show that different determinants of PGPR are involved in the induction of systemic resistance and ISR by these bacterial determinants varies with iron-limiting conditions, bacterial strains, host plants, and their cultivars.

8. PGPR formulation and methods of application

An important area of microbiological research with regard to biocontrol is the development of formulations that would preserve microbial activity for a period long enough to enable delivery of an effective product for field application. *P. fluorescens* can be applied in the form of a bacterial suspension (Mew and Rosales, 1986; Thompson, 1996) and as a powder formulation (Kloepper and Schroth, 1981; Vidhyasekaran et al., 1997a, b). It is desirable from the consumer's perspective to formulate and package PGPR in ways similar to chemical pesticides. Mass multiplication of PGPR in a suitable medium and development of a powder formulation was first carried out in 1980. A dried powder formulation of PGPR is especially important for seed-treatment and soil-application. The survival of PGPR in a dried formulation and the effectiveness of methylcellulose in a powder formulation for coating sugar beet seed has been well documented (Suslow, 1980). Kloepper and Schroth (1981) developed a talc-based powder formulation of PGPR for inoculation of potato seed pieces. When the stability and efficacy of the product was tested under field conditions root colonization was better with the powder formulation than aqueous preparations. In assessing, the suitability of different carriers for the development of stable formulations in talc-based and peat-based formulations, the population of bacteria has been stable upto 240 days of storage period (Vidhyasekaran and Muthamilan, 1995). Seed-treatment followed by soil-application of talc-based powder formulation effectively checked chickpea wilt and pigeonpea wilt under field conditions and has increased the yield (Vidhyasekaran and Muthamilan, 1995; Vidhyasekaran et al., 1997b). A peat-based formulation has also effectively controlled the rice sheath blight disease under field conditions and increased the yield (Rabindran and Vidhyasekaran, 1996). Thus PGPR can be formulated and delivered effectively to the field for systemic protection of crop plants.

The methods of application of formulated product include seed-treatment (Rosales and Mew, 1997), root-dip (Maurhofer et al., 1994), sett-treatment in sugarcane (Viswanathan and Samiyappan, 1999a), sucker-treatment in banana (Raguchander et al., 1997), soil-application (Vidhyasekaran et al., 1997a, b) and foliar application (Mew and Rosales, 1986; Chatterjee et al., 1996). Combinations of different methods of application could be more effective in disease management than a single method of application (Vidhyasekaran et al., 1997a, b; Vidhyasekaran and Muthamilan, 1999).

9. Durability of ISR

Resistance mechanisms attain their maximum effectiveness at four to five days after the application of an inducing agent, but the level of persistence of resistance generally decreases over time. These criteria determine the number of applications of PGPR needed to maintain the resistance level in the crop plants (Dalisay and Kuc, 1995). In rice, seed-treatment with *P. fluorescens* strain Pf1 induces resistance which is observed upto 45 days after sowing. When a foliar spray is given at 45 days after sowing, the resistance is observed at 4 days after application and persists for 15 days in rice leaves (Vidhyasekaran et al., 1997a). Foliar sprays of *P. fluorescens* formulations should be given at every 15 days intervals for managing rice foliar diseases. A parallel experiment conducted by Nayar (1996) indicated that induction of defence mechanisms using *P. fluorescens* persisted upto 60 days by seed-treatment, 30 days by root-dipping and 15 days by foliar spray. In cucumber, PGPR-mediated ISR resulted in protection of cucumber from anthracnose disease for five weeks from the time of sowing. But the consistency of ISR over time varies with PGPR strains (Liu et al., 1995c). In sugarcane, induction of resistance by PGPR persists for 90 days of crop growth (Viswanathan, 1999). Generally, the durability of resistance by PGPR differs from crop to crop and also due to different bacterial strains.

10. PGPR-mediated ISR under field conditions

In most of the cases, ISR has been studied mainly in the laboratory and greenhouse. However, some reports indicate that ISR by PGPR can protect the crop plants under field conditions (Kloepper et al., 1993; Wei et al., 1996; Nandakumar, 1998). In a field trial, PGPR strains, viz., *P. putida* strain 89B-27, *S. marcescens* strain 90-166 and *Flavomonas oryzihabitans* strain INR-5 have caused systemic protection against angular leaf spot and bacterial wilt besides increasing the plant growth (Kloepper et al., 1993). Similarly, application of PGPR strains either as seed-treatment alone or as seed-treatment plus soil-drenching at the time of transplanting have protected cucumber plants inoculated with the *P. syringae* pv. *lachrymans*, the angular leaf spot pathogen and reduced the levels of anthracnose disease in the field (Wei et al., 1996). In rice, seed-treatment and root-dipping of rice seedling with PGPR strain mixtures, viz., *P. fluorescens* strains Pf1 and PB2 reduced rice sheath blight disease incidence and improved the grain yield under field conditions (Nandakumar, 1998). In sugarcane, application of PGPR as sett-treatment induced systemic resistance against *C. falcatum* in addition to enhanced sett germination, tillering and growth of the cane both under controlled conditions as well as field conditions. These studies clearly show that PGPR-mediated ISR and plant growth promotion can operate under field conditions.

11. Conclusions

The beneficial effects of PGPR include direct plant growth promotion, biological control and inducing systemic resistance in host plants. Specific PGPR strains bring about ISR against multiple pathogens attacking the same crop. In addition to disease suppression, application of PGPR also reduces the insect and nematode damage. The broad spectrum of control using PGPR strains can provide an effective, economical and practical way of plant protection. The endophytic nature of some PGPR make; them suitable for the use in vegetatively propagated crops because of their capability to colonize and persist in the intercellular space of epidermal cells thereby reducing the need for further application if the same vegetative parts are used as propagative material. Furthermore, certain PGPR strain mixtures have showed synergistic action in plant protection and growth promotion, indicating different mechanisms are involved in disease control. So, selecting such combinations of strains would be beneficial in crop production. Stable formulations using different carriers such as peat and talc have been developed for the delivery of the PGPR stains for field level application. Though the research on PGPR-mediated disease resistance originated several decades ago, its effectiveness has been demonstrated under field conditions only in the 1990s. It is concluded that instead of using single strain, it would be more effective to apply a mixture of strains showing synergistic action for broad spectrum activity against multiple pathogens and pests.

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