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Induced Systemic Resistance Mediated by Plant Growth-Promoting Rhizobacteria (PGPR) and Fungi (PGPF)

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10.1 Definitions of PGPR and PGPF

10.1.1 PGPR

Plant growth-promoting rhizobacteria, or PGPR, are a heterogenous group of non-pathogenic bacteria that are associated with plant roots (colonizing either the root itself, or the rhizosphere), and mediate improvements in plant growth or health. While it is quite possible for a bacterium to benefit plant growth or health while colonizing the phyllosphere (e.g., Bashan and de-Bashan, 2002), and the phyllosphere colonizers could in theory influence plant defense responses, this discussion will be restricted to soil-inhabiting rhizobacteria.

False impressions can easily be generated by considering any PGPR as part of a uniform group of organisms interacting similarly with plants. The classification of different bacteria as "PGPR" *does not* reflect a biological similarity between these bacteria: PGPR vary from one another quite radically in taxonomy, in physiology, and in their interactions with plants. When attempting to compare literature reports, it is invaluable to take into account whether the organisms under study are in any way biologically similar (e.g., producing a similar compound, or belonging to the same phylogenetic group). It is very frustrating to read reports in which the authors assume that, since one bacterium classified as a PGPR produces a particular plant response, that *all* bacteria classified as PGPR must effect this same response.

In reality, PGPR interact with their host plants by a variety of mechanisms, and most PGPR probably employ more than one of these mechanisms, either simultaneously, or at different times under different conditions. Also, despite the name, PGPR do not always promote plant growth. A bacterium that promotes the growth of one plant may have no effect, or a deleterious effect, upon the growth of other plants, and a bacterium that promotes the growth of a given plant under one set of environmental conditions may have no effect, or a deleterious effect, on

the same plant under different conditions (Tuzun and Bent, 1999, and references therein). The term "PGPR" should therefore be considered an operational rather than an absolute term, which describes the effect of a bacterium on a given range of plant hosts under a given range of environmental conditions only, as previously suggested in Bent and Chanway (1998).

Organisms identified as PGPR have diverse taxonomy (Glick, 1995), and include Firmicutes or Gram-positive bacteria (e.g., members of the Actinomycetales, including *Frankia* and *Streptomyces*, and Bacilli, including *Bacillus* and *Paenibacillus*), as well as Gram-negative organisms in various subdivisions of the Proteobacteria: Rhizobiaceae (*Rhizobium*, *Bradyrhizobium*), Rhodospirillaceae (*Azospirillum*), and Acetobacteraceae (*Acetobacter*) in the α-Proteobacteria; members of the Burkholderia group (*Burkholderia*) in the β-Proteobacteria, and members of the Enterobacteriaceae (*Enterobacter, Pantoea, Serratia*) and Pseudomonaceae (*Pseudomonas, Flavimonas*) in the γ-Proteobacteria.

Some PGPR form symbiotic structures with plants (e.g., rhizobial or actinorhizal nodules) while others are "associative", and live freely in the rhizosphere soil, the root surface, or even the interior of the root itself (Glick, 1995; Sturz et al., 2000).

10.1.2 PGPF

The defintion of plant growth-promoting fungi, or PGPF, is similar to that of PGPR except that the organisms in question are fungi (here including true fungi as well as oomycetes) rather than bacteria. While mycorrhizal fungi are known to improve the growth of plants and affect the expression of plant defense responses (Lambais and Mehdy, 1995; Peterson and Farquhar, 1994; Ruiz-Lozano et al., 1999; Sirrenberg et al., 1995), a comprehensive discussion of the interactions between mycorrhizal fungi and plants is beyond the scope of this chapter. Our definition of PGPF, therefore, is limited to nonsymbiotic saprotrophic fungi that live freely in rhizosphere soil or on the plant root surface.

The same caveats identified above for PGPR hold for PGPF: not every organism identified as a PGPF will improve plant growth under all conditions, or in association with all plant hosts (e.g., Ousley et al., 1993). The term "PGPF" is a convenient but artificial category, not an indication of any real biological similarity between organisms classified as PGPF, and when comparing the results of different studies, the fact that the organisms under question may be radically different from one another, or differ in their interactions with plants, must always be kept in mind. As with PGPR, it is helpful to keep in mind any phylogenetic or taxonomic similarities between PGPF reported in the literature when comparing reports.

Characterized fungi reported in the literature as PGPF primarily include ascomycetes (*Penicillium, Trichoderma, Fusarium, Phoma, Gliocladium*) and oomycetes (*Pythium, Phythophthora*). Interestingly, some reported PGPF are nonpathogenic or hypovirulent strains of phytopathogenic fungi (Table 10.2).

10.2 How PGPR and PGPF Interact with Plants to Improve Growth

There are a variety of ways in which PGPR and PGPF, here discussed together, may improve the growth or health of plants. A detailed discussion of each of these mechanisms is beyond the scope of this chapter, and the reader is referred to reviews by Buchenauer (1998), Glick (1995), and Whipps (2001) for more information.

Mechanisms of plant growth promotion include increasing plant nutrient acquisition, modification of plant growth and development, modification of the soil environment to promote plant growth, and biocontrol of plant pathogens. Biocontrol can be via direct mechanisms, where the pathogen itself is attacked, or via indirect ones, where plant defense responses against the pathogen are induced.

10.2.1 Plant Growth Promotion

Nitrogen-fixing rhizobial and actinorhizal nodules can increase plant uptake of nitrogen, and PGPR that assist in the formation of rhizobial nodules and the vigor of activity within them have also been identified (Srinivasan et al., 1996; Tokala et al., 2002). Free-living nitrogen-fixing bacteria that colonize the rhizosphere or interior tissues of plants may also improve plant growth by providing nitrogen (Sevilla et al., 2001), and this may be especially important in nutrient-limiting environments. Mycorrhizal infection can improve plant uptake of water as well as nutrients, phosphorus in particular (Peterson and Farquhar, 1994), and PGPR have been identified which assist mycorrhizal fungi in colonizing plants (Garbaye, 1994). Siderophore-overproducing mutants of a metal-tolerant soil bacterium were found to help plants overcome growth inhibition by heavy metals in soil, most likely by providing the plant with iron (Burd et al., 2000). Saprophytic PGPF can improve the nutrient supply also: for example, phosphate-solubilizing fungi have been identified which promote plant growth (Whitelaw et al., 1999).

Plant growth-altering hormones such as auxin, cytokinins, or giberellins, which can alter root morphology and stimulate growth, are known to be produced by rhizobacteria (Costacurta and Vanderleyden, 1995; Patten and Glick, 1996, 2002) as well as rhizofungi (Furukawa et al., 1996). PGPR may also produce enzymes that degrade the precursors of plant growth-inhibiting hormones such as ethylene, indirectly enhancing plant growth (Glick, 1995).

PGPR and PGPF may also improve plant growth indirectly, via alterations to the structure of rhizosphere soil, which benefit the plant. Exopolysaccharide-producing PGPR have been found to significantly increase rhizosphere soil aggregation and the volume of soil macropores, resulting in increased water and fertilizer availability to inoculated sunflowers (Alami et al., 2000). Desertified soils, in which the soil structure has been degraded, contain a greater number of hydrostable soil aggregates after inoculation with PGPR and fungi, and this improvement in soil structure may assist natural plant communities in recolonizing these soils (Requena et al., 2001).

10.2.2 Disease Control

Perhaps the most research on plant-growth promoting microorganisms has been devoted to determining how they can be used to protect plants from disease. Pathogen control by PGPR may involve the production of antimicrobial enzymes, antibiotics, predation, or it may occur via the systemic induction of plant defense responses (ISR) (Buchenauer, 1998; Whipps, 2001). Phyllosphere as well as rhizosphere bacteria have also been shown to successfully control pathogens via niche exclusion (Bashan and de-Bashan, 2002; Buchenauer, 1998). Bacteria may employ more than one mechanism simultaneously to control pathogens.

Pathogen control by PGPF may also occur via niche exclusion, antibiosis, predation, mycoparasitism, and ISR induction (Shivanna et al., 1996; Mauchline et al., 2002; Whipps, 2001). Hypovirulent pathogen isolates containing double-stranded RNA (dsRNA) may also control more virulent isolates via anastomosis, in which dsRNA conferring hypovirulence is transferred to the virulent isolate (e.g., Batten et al., 2000).

Fungi may employ more than one control mechanism simultaneously. For example, a nonpathogenic strain of *Fusaruim oxysporum* was found to control *Pythium ultimum* via a combination of ISR, antibiosis, and mycoparasitism (Benhamou et al., 2002), and *Trichoderma* isolates, known to act directly on pathogens as biocontrol agents, have been also found capable of inducing systemic resistance (de Meyer et al., 1998).

10.3 The Difference Between ISR and Direct Biological Control

It is important to draw a clear distinction between direct mechanisms of biological control, in which the PGPR/F acts directly upon the pathogen, and indirect mechanisms that require the induction of plant defense responses. This distinction is not always understood: there have been recent reports in which the authors conclude that biocontrol agents acted via induction of systemic resistance in plants, when alternate explanations for the reduction in disease symptoms of inoculated plants, such as antibiosis or niche exclusion, were not tested. The criteria for distinguishing between biocontrol agents that act via direct or via indirect (ISR) mechanisms have been thoroughly described elsewhere (van Loon et al., 1998).

It is also important to distinguish between ISR and race-specific, gene-for-gene types of interactions. ISR can be a nonspecific phenomenon, in which a variety of nonspecific elicitors can stimulate the plant's innate, and already existing, defenses. The plant does not acquire new defense mechanisms during the process of stimulation; ISR makes use of the plant's existing set of defense responses. Ton et al. (1999) provide an excellent illustration of this principle: ecotypes of *Arabidopsis thaliana* which exhibited greater innate, or "basal" susceptibility to *P. syringae* pv. *tomato*, also failed to develop ISR after treatment with *P. fluorescens* WCS417r, a bacterium known to elicit this response in other ecotypes of *A. thaliana*. A genetic

association was observed between basal resistance and ability to develop ISR, supporting the idea that plants with a more effective set of resistance responses (or greater basal resistance) will be able to muster a more effective ISR response than plants with a less effective set of resistance responses. This pattern was also observed in cucumbers inoculated with *Pseudomonas* isolates (Arndt et al., 1998), and in a variety of other plant systems where pathogenesis-related (PR) proteins are constitutively expressed at higher levels in cultivars expressing greater basal resistance to a given pathogen (Tuzun and Bent, 1999, Vleeshouwers, 2000).

10.3.1 Plant Nutrition and Improved Resistance to Disease

Since the defensive mechanisms activated in plants by a variety of plant-beneficial microorganisms are still largely unknown (e.g., van Wees et al., 1999) or remain unstudied (Tables 10.1, 10.2), the following possibility should be mentioned. In determining whether a PGPR/F inoculant can induce systemic resistance in a plant, direct biocontrol interactions between PGPR/F and the pathogens used must be ruled out, but there is no requirement to directly observe the induction of a plant defense mechanism. It is sufficient to observe that the inoculated plants have improved resistance to the disease, and that this effect cannot be explained by alternate biocontrol mechanisms, for the phenomenon to be labeled “ISR”. Plant-beneficial microorganisms, by improving plant nutrition or the rate or extent of plant growth, might improve plant resistance to or tolerance of pathogens or herbivores *without* the direct induction of any known plant defensive response. Fertilization of plants is known to improve plant tolerance of disease and herbivory (Goncalves et al., 2000; Matichenkov et al., 2000), and induced resistance to herbivory in soybean by an arbuscular mycorrhizal fungus was attributed to improved plant nutrition, rather than induction of any plant defense mechanisms (Borowicz, 1997). The effect of improved fertilization on disease resistance may be pathogen specific, however, and not provide consistent results against different pathogens (Ellis et al., 2000).

10.4 PGPR and PGPF-Mediated ISR

10.4.1 Explanation of Terminology Used in this Chapter

There are many reports of ISR/SAR induced by rhizosphere organisms in which the defensive mechanism for the resistance is unknown (Tables 10.1, 10.2). Recent research also indicates that there are more than two biochemical pathways by which induced resistance can be activated (e.g., Bostock et al., 2001; Dong and Beer, 2000; Mayda et al., 2000a,b; Zimmerli et al., 2000, Ryu et al., 2003). Moreover, since the mechanisms by which many PGPR or PGPF mediate ISR have never actually been studied, I find it would be impossible to discuss this topic without some generic term that means only resistance in plants which is inducible and systemic.

I will use “ISR” as the generic term. To distinguish between different established or hypothetical mechanisms that produce ISR, I will use a prefix suggesting a

TABLE 10.1. Overview of literature reporting PGPR, which induce systemic resistance in plants.

PGPR designation	Plant host(s)	Challenge organism(s)	Defense response in plant[a]	Reference
Pseudomonas fluorescens 89-B-61, *Serratia marcesens* 90-166	Cucumber (*Cucumis sativus*), tobacco (*Nicotania tabacum*)	*Colletotrichum orbiculare*, *Fusarium oxysporum* f. sp. *cucumerinum*, *Pseudomonas syringae* pv. *tabaci* (tobacco)		Liu et al. (1995a,b); Press et al. (1997, 2001)
P. fluorescens 89-B-61, *S. marcesens* 90-166, *Bacillus pumilis* SE34, T4, *B. pasteurii* C-9	Tobacco (*N. tabacum*)	*Peronospora tabacina*		Zhang et al. (2002)
P. fluorescens 89-B-61, *S. marcesens* 90-166, *Bacillus pumilis* SE34, T4	Thale cress (*Arabidopsis thaliana*)	*Pseudomonas syringae* pv. *tomato, P. syringae* pv. *maulicola*		Ryu et al. (2003)
P. fluorescens WCS374, WCS417	Radish (*Raphanus sativus*)	*F. oxysporum* f. sp. *raphani*		Leeman et al. (1995, 1996)
P. fluorescens WCS374r, WCS417r	Thale cress (*A. thaliana*)	*P. syringae* pv. *tomato* DC3000	Primes conversion of ACC to ethylene (ethylene could be involved in defense responses?)	Hase et al. (2003)
P. fluorescens WCS417r	Carnation (*Dianthus caryophyllus*)	*F. oxysporum* f. sp. *dianthi*	Phytoalexin accumulation	van Peer et al. (1991)
P. fluorescens WCS417r	Radish (*R.. sativus*), carnation (*D. caryophyllus*)	*F. oxysporum* f. sp. *raphani, F. oxysporum* f. sp. *dianthi*	Changes in cell wall composition	Steijl et al. (1999)
P. fluorescens WCS417r, WCS358r	Thale cress (*A. thaliana*)	*F. oxysporum* f. sp. *raphani*, *P. syringae* pv. *tomato*		van Wees et al., 1997, 1999; Pieterse et al., 1996
P. fluorescens WCS417r	Thale cress (*A. thaliana*)	*Alternaria brassicicola, Xanthomonas campestris* pv. *armoraciae, P. syringae* pv.*tomato*		Ton et al (1999, 2001, 2002)
P. fluorescens WCS417r	Tomato (*Lycoperscion esculentum*)	*F. oxysporum* f. sp. *lycopersici*		Duijff et al. (1998)

P. aeruginosa 7NSK2	Bean (*Phaseolus vulgaris*)	*Botrytis cinerea*	Induction of phenylalanine ammonia-lyase, SA accumulation	de Meyer et al. (1999)
P. fluorescens WCS417, *P. aeruginosa* KMPCH	Bean (*P. vulgaris*)	*Colletotrichum lindemnthianum*		Bigirimana and Hofte (2002)
P. aeruginosa KMPCH	Bean (*P. vulgaris*)	*B. cinerea*		de Meyer et al. (1998)
Nine *Pseudomonas* sp. strains and two *Serratia* sp. strains	Bean (*P. vulgaris*)	Fusarium solani pv. phaseoli	HR and phytoalexin accumulation	Hynes et al. (1994)
P. fluorescens S97	Bean (*P. vulgaris*)	*Pseudomonas syringae* pv. *syringae*		Alström (1991)
Pseudomonas. putida BTP1, M3	Cucumber (*C. sativus*)	*Pythium aphanidermatum*	Phytoalexin accumulation	Ongena et al. (2000)
Pseudomonas WB1, WB15, WB52	Cucumber (*C. sativus*)	*Pythium ultimum, Rhizoctonia solani*	Callose deposition	Arndt et al. (1998)
P. fluorescens CHA0	Tobacco (*N. tabacum*)	Tobacco necrosis virus	Accumulation of PR proteins	Maurhofer et al. (1994)
P. fluorescens CHA0, EP1, Pf1, *P. putida* KKM1	Sugarcane (*Saccharum* sp. hybrids)	*Colletotrichum falcatum*	Enhanced lignification and activity of defense proteins	Viswanathan and Samiyappan (2002a,b)
P. fluorescens PF1, FP7	Rice (*Oryza sativa*)	*R. solani*	Accumulation of chitinase and peroxidase isozymes	Nandakumar et al. (2001)
P. fluorescens 7-14, *P. putida* Vl4i	Rice (*O. sativa*)	*Magnaporthe grisea*	Accumulation of SA	Krishnamurthy and Gnanamanickam (1998)
P. fluorescens WR8-3, WR9-11, *P. putida* WR9-16	Watermelon (*Citrullus lanatus* cv. Geumchon)	*Didymella byroniae*		Lee et al. (2001)
P. fluorescens Blight Ban A506, *Pantoea agglomerans* C9-1	Apple (*Malus domestica*)	*Erwinia amylovora*		Momol et al. (1999)
Pantoea agglomerans E278Ar	Radish (*Raphanus sativus*)	*X. campestris* pv. *armoraciae*		Han et al. (2000)
Bacillus subtilis FZB-G	Tomato (*Lycoperscion esculentum*)	*Fusarium oxysporum* f. sp. *lycopersici*		Gupta et al., (2000)
B. subtilis AF 1	Groundnut (*Arachis hypogaea*)	*Aspergillus niger*	Increased lipoxygenase activities, production of antifungal hydroperoxides	Sailaja et al. (1997)
B. pumilis SE 34, *Serratia plymuthica* R1GC4, *P. fluorescens* 63-28	tomato (*L. esculentum*), pea (*Pisum sativum*), cucumber (*C. sativus*)	*Fusarium oxysporum* f. sp. *lycopersici*, *P. ultimum*	Callose and cellulose deposition, accumulation of phytoalexins and PR proteins	Benhamou et al. (1996, 1998, 2000); M'Piga et al. (1997)

TABLE 10.1. (*Continued*)

PGPR designation	Plant host(s)	Challenge organism(s)	Defense response in plant[a]	Reference
P. fluorescens 63-28, *P. corrugata* 13	Cucumber (*C. sativus*)	*Pythium aphanidermatum*	Induction of peroxidases and enzymes involved in lignification	Chen et al. (1998, 2000)
B. pumilis SE 34, SE 52, INR7, *S. marcesens* 90-166	Loblolly pine (*Pinus taeda*)	*Cronartium quercum* f. sp. *fusiforme*		Enebak and Carey (2000)
Paenibacillus polymyxa B2	thale cress (*A. thaliana*)	Erwinia carotovora	Induced changes in drought stress, SA-ISR and JA-ISR-related genes	Timmusk and Wagner (1999)
Bacillus thuringiensis Berliner	coffee (*Coffea arabica*)	*Hemileia vastatrix*	Accumulation of PR proteins	Guzzo and Martins (1996)
B. pumilis INR7, *B. subtilis* GB03, *Curtobacterium flaccumfaciens* ME1, alone or in mixtures	Cucumber (*C. sativus*)	*C. orbiculare, P. syringae* pv. *lachrymans, Erwinia tracheiphila*, alone or in mixtures		Raupach and Kloepper (1998)
Bacillus amyloliquefaciens EXTN-1	tobacco (*N. tabacum*), thale cress (*A. thaliana*), cucumber (*C. sativus*)	Pepper mild mottle virus (tobacco, cress), *C. orbiculare* (cucumber)	Accumulation of PR and defense-related gene transcripts (tobacco, cress), callose deposition (cucumber)	Ahn et al. (2002); Jeun et al. (2001)
B. pumilis SE34, *B. subtilis* IN937b, *B. amyloliquifaciens* IN937a, *Kluyvera cryocrescens* IN114	Tomato (*L. esculentum*)	Cucumber mosaic cucumovirus, tomato mottle virus (not IN114)		Zehnder et al. (2000); Murphy et al. (2000)
B. pumilis INR-7, T4, *Flavomonas oryzihabitans* INR-5, *P. putida* 89-B-61, *S. marcesens* 90-166	Cucumber (*C. sativus*)	Cucumber beetle (*Acalymma vittata*), *Erwinia tracheiphila* (T4, 90-166 only)	Decreased levels of cucurbitacin (INR-7, INR-5)	Zhender et al. (1997a,b, 2000)
Rhizobium elti G12, *Agrobacterium radiobacter* G12, *Bacillus sphaericus* B43	Potato (*Solanum tuberosum*)	Potato cyst nematode (*Globodera pallida*)		Reitz et al. (2000); Hasky-Günther et al. (1998)
Pseudomonas-like sp. P29, P80, *Bacillus cereus* B1	White clover (*Trifolium repens*)	Clover cyst nematode (*Heterodera trifolii*)		Kempster et al. (2001)

[a] This column summarizes positive reports of plant defense-related reactions that were induced or augmented by PGPR inoculation. Negative results (i.e., reports that a substance or reaction is *not* involved), or reports of optimal conditions or plant or bacterial genotypes required for ISR, are not included.

TABLE 10.2. Overview of literature reporting PGPF (fungi and oomycetes) which induce systemic resistance in plants.

PGPF designation	Plant host(s)	Challenge organism(s)	Defense response in plant[a]	Reference
Trichoderma GT3-2, *Fusarium* GF18-3, *Penicillium* GP17-2, *Phoma* GS8-2, sterile fungus GU23-3	Cucumber (*Cucumis sativus*)	*Colletotrichum orbiculare, Fusarium oxysporum* f. sp. *cucumerinum, Pseudomonas syringae* pv. *lachrymans*	Superoxide generation, induction of lignification	Koike et al. (2001)
Phoma GS8-2, sterile fungus GU23-3, and uncharacterized PGPF GS8-1, GS8-2, GU21-2	Cucumber (*C. sativus*)	*C. orbiculare*		Meera et al. (1995)
Nonpathogenic *Fusarium oxysporum* Fo47	Cucumber (*C. sativus*)	*Pythium ultimum*	Production of physical barriers to infection, production of antimicrobial compounds	Benhamou et al. (2002)
Nonpathogenic *F. oxysporum* isolates	Watermelon (*Citrullus lanatus*), tomato (*Lycoperscion esculentum*)	F. oxysporum f. sp. niveum, *F. oxysporum* f. sp. *lycopersici*		Larkin et al. (1996); Larkin and Fravel. (1999)
Nonpathogenic *F. oxysporum* isolates	Asparagus (*Asparagus officinalis*)	*Fusarium oxysporum* f. sp. *asparagi*	Hypersensitive response, lignification, increased defense protein production, production of antifungal metabolites	He et al. (2002)
Penicillium janczewskii	Cotton (*Gossypium barbadeuse*), melon (*Cucumis melo* L. var. *reticulatus*)	*Rhizoctonia solani*	Hypersensitive response and elevated defense protein activity (melon), increased peroxidase activity (both)	Madi and Katan (1998)
Trichoderma harizanum T39	Tomato (*L. esculentum*), lettuce (*Lactuca sativa*), pepper (*Capsicum annuum*), bean (*Phaseolus vulgaris*), tobacco (*Nicotania tabacum*)	*Botrytis cinerea*		De Meyer et al. (1998)
Nonpathogenic *Alternaria cucumarina, Cladosporium fulvum*	Cucumber (*C. sativus*)	*Sphaerotheca fuliginea*		Reuveni and Reuveni (2000)
Nonpathogenic, binucleate *Rhizoctonia sp.*	Bean (*P. vulgaris*)	*Rhizoctonia solani, Colletotrichum lindemuthianum*	Systemic increase in PR proteins, accumulation of phenolics, increased lignification	Xue et al. (1998)
Nonpathogenic *Phytophthora cryptogea*	Tomato (*L. esculentum*)	*F. oxysporum* f. sp. *lycopersici*		Attitalla et al. (2001)
Aureobasidium pullulans	Apple (*Malus domestica* cv. Red Delicious)	*Botrytis cinerea, Penicillium expansum*	Increases in PR proteins (but control may be due to biocontrol rather than ISR)	Ippolito et al. (2000)

[a] This column summarizes positive reports of plant defense-related reactions that were induced or augmented by PGPF inoculation. Reports of optimal conditions, or plant or bacterial genotypes required for ISR, are not included.

compound involved in the biochemical response. I feel this scheme is simple to understand and allows for the discussion of many different potential mechanisms, as well as reports of ISR where the biochemical pathway or mechanism involved in induction is unknown.

10.4.2 Overview of Known Pathways Involved in Microbially-Stimulated ISR

There are at least three, and potentially more, interconnected biochemical mechanisms by which ISR can be activated in plants. I will focus here on those mechanisms, which are or could be stimulated by rhizosphere microorganisms. By "mechanism" I refer to the entire biochemical pathway involved in recognition of the stimulus and generation of the response in the plant. It should be noted that multiple mechanisms can share part of the same biochemical "pathway" if receptors for different elicitors activate the same signaling cascades at some point. The pathways involved in the ISR phenomenon and their interconnections are reviewed by Nawrath et al. and Pieterse et al. (Chapter 7 and 8 of this volume).

It should also be noted that these mechanisms may not function in every plant species or variety. ISR in peanut (*Arachis hypogaea* L.), for example, was induced by β-aminobutyric acid (BABA) but not by a variety of PGPR inoculants or commonly used chemical elicitors, including salicylic acid, methyl jasmonate, and ethylene (Zhang et al., 2001).

Salicylate-mediated, salicylate-dependent or "classical" ISR (here defined as SA-ISR; also sometimes defined in recent works as "systemic acquired resistance" or "SAR") was the first mechanism identified. It is sometimes also referred to as "pathogen-mediated" ISR, since the phenomenon was first observed on plants inoculated with plant pathogens, but nonpathogenic organisms may also stimulate SA-ISR (see Section 10.4.4). SA-ISR typically involves the accumulation of pathogenesis-related (PR) proteins and the induction of a hypersensitive response.

Jasmonate-mediated or jasmonate-dependent ISR (JA-ISR) is less well characterized; it is not associated with PR protein accumulation or a hypersensitive response, but appears to involve changes to plant secondary metabolism, resulting in the accumulation of phytoalexins in at least some plants. Since the first organisms discovered to induce this set of responses were rhizobacteria, JA-ISR has generally been associated with PGPR, but this association is misleading since it gives the impression that *all* PGPR that elicit ISR do so via the JA-ISR mechanism exclusively, which is not the case. This mechanism is discussed further in Section 10.4.5.

The above example of peanut plants in which ISR cannot be elicited by jasmonate or salicylate, but instead by BABA (Zhang et al., 2001), illustrate the existence of a third ISR mechanism. This mechanism is induced by aminobutyric acids (Jakab et al., 2001; Zimmerli et al., 2000). I am not aware of any evidence that plant-beneficial microorganisms use this mechanism to activate ISR in plants,

although this is possible in theory. I will therefore not discuss this mechanism further.

Neither jasmonate, salycilate, nor aminobutyric acids appear to be involved in a fourth, potentially distinct, mechanism of ISR, in which the associated responses differ from those seen in JA-ISR and SA-ISR. This mechanism appears to be induced by the onset of cellular insensitivity to auxin (Mayda et al., 2000a,b). The potential for involvement of auxin in microbially mediated ISR is discussed in Section 10.4.6.

There are additional reports of ISR mechanisms induced by riboflavin (Dong and Beer, 2000), a modified antiviral protein (Zoubenko et al., 2000) or ceramides (Bostock et al., 2001), via which microorganisms could in theory induce disease resistance should they produce a sufficiently similar inducing substance, but it is beyond the scope of this chapter to discuss these.

10.4.3 Specificity and Induction of More than One ISR Mechanism by Microorganisms

There may be some specificity in the ability of PGPR to induce an ISR response, although the basis for such specificity is currently unknown: for example, different strains of *Pseudomonas fluorescens* were found to induce ISR in radish or in *Arabidopsis*, but not both plants (van Wees et al., 1997), and while a variety of PGPR (*Bacillus pumilis, Serratia marcesens*, and *Pseudomonas fluorescens*) were found to induce resistance in *NahG* (salicylate-deficient) plants, some of these same strains were also found to function via pathways deficient in jasmonic acid, or ethylene signaling, or in plants deficient in *npr1*, previously thought to be required for ISR mediated by nonpathogenic rhizobacteria (Ryu et al., 2003). There is also no theoretical reason why a single organism (or, as happens more often in nature, a consortia of organisms) cannot induce resistance via *more* than one ISR pathway, either by stimulating different responses in different plant hosts, or by stimulating different responses in the same plant under varying conditions. An ISR-inducing PGPR was found to induce changes in *Arabidopsis* drought stress-related genes, as well as genes relating to the SA-ISR and JA-ISR pathways (Timmusk and Wagner, 1999), suggesting that biotic and abiotic stress responses may be linked, and that one organism may possess the ability to induce more than one ISR pathway.

10.4.4 PGPR that Activate SA-ISR

SA-ISR is still commonly thought to be restricted to necrotrophic phytopathogenic fungi and bacteria, despite the fact that there are several reports of PGPR that induce systemic resistance by SA-ISR (van Loon et al., 1998; Table 10.1). Because of the strong linkage between this pathway and the presence of necrotic pathogens, or metabolites of necrotic pathogens, that induce an oxidative burst and a hypersensitive response, it has been hypothesized that the nonpathogenic bacteria that do induce plant defenses via this pathway may have evolved, or acquired genes from pathogenic organisms (Tuzun and Bent, 1999). This is not a unique idea, Arndt

et al. (1998) described an ISR-inducing strain of *Pseudomonas* that could "imitate infections of soilborne pathogens" in tomato, and Reitz et al. (2000) suggest that the induction of PR proteins by PGPR strain *P. fluorescens* CHA0 is due to "stress" caused by this organism on the plant.

Typical hallmarks of SA-ISR include the systemic accumulation of salicylic acid and a variety of PR protein isoforms, including chitinases, β-1,3-glucanases, and thaumatin-like proteins, increased lignification or callose deposition, the production of phytoalexins or phenolic antimicrobial secondary compounds, and increased expression of enzymes associated with active oxygen species, lignification, or plant secondary metabolism (Kobayashi et al., 1995; Hammerschmidt and Smith-Becker, 1999).

Accumulation of salicylate or PR proteins in response to PGPR inoculation has been described in several plant-PGPR systems (Zdor and Anderson, 1992; Table 10.1) along with the strengthening of physical barriers to infection and the accumulation of antifungal substances (Table 10.1). The latter responses may be temporally separated from the onset of PR protein induction and occur prior to PR protein accumulation (Benhamou et al., 1996, 1998, 2000; M'Piga et al., 1997).

Harpins produced by bacterial plant pathogens are known to elicit SA-ISR (Dong et al., 1999; Strobel et al., 1996). Tuzun and Bent (1999) speculated that nonpathogenic rhizobacteria that induce ISR may express harpin-like proteins that cause microscopic necrotic lesions and so stimulate the SA-ISR response. Since then, conserved type III secretion system genes, similar to the *hrp* cluster in plant pathogens, have been reported in PGPR, including *Rhizobium* sp. and *Pseudomonas fluorescens* (Preston et al., 2001). The nature of hypersensitive responses mediated by PGPR and pathogens seems to differ, which may explain why some PGPR can induce resistance via SA-ISR yet do not cause symptoms of disease: HR mediated by *P. fluorescens* were slower, required at least tenfold more cells, and were induced differently in different tissues, compared to HR mediated by the pathogen *P. syringae* (Preston et al., 2001). Preston et al. (2001) speculated that type III secretion systems may play broadly conserved roles in plant-microbe interactions, and may help nonpathogens as well as pathogens to live intimately with plants.

A variety of stress conditions, including exposure to salicylate, inhibits the production of the OmpF porin in *Escherichia coli* (Ramani and Boyake, 2001). OmpC and OmpF porins function as nonselective pores in the outer membrane of *E. coli* through which small hydrophilic molecules can diffuse, with the channel diameter of OmpC being slightly smaller. A decrease in OmpF expression would therefore result in generally smaller channels available for nonselective diffusion, and increased protection against the entry of larger molecules that are more likely to be toxic to the cell. It has been suggested that the ability of a bacterium to colonize plant tissues and the rhizosphere is influenced by its sensitivity to phytoalexins (Hynes et al., 1994), and it is tempting to speculate that regulation of porin size in response to plant defense signals may help Gram negative ISR-inducing PGPR to survive plant defense responses.

10.4.5 PGPR that Activate JA-ISR

JA-ISR is elicited by jasmonic acid and its derivatives, as well as by ethylene, and is implicated in systemic wound responses (Staswick and Lehman, 1999). Plant responsiveness to jasmonate and ethylene is required for the JA-ISR response to be generated in *Arabidopsis thaliana* (Knoester et al., 1999; Ton et al., 2001, Pieterse et al., 1998). Ethylene is not produced by at least some of the PGPR that are known to induce the JA-ISR response, and ethylene levels in the vicinity of induced plants do not always rise (Knoester et al., 1999). However, *Arabidopsis* roots show an increased ability to convert 1-aminocyclopropane-1-carboxylate (ACC) to ethylene after treatment with *Pseudomonas fluorescens* (both strains inducing ISR and not), suggesting that strains of this bacterium may prime plants to produce greater quantities of ethylene upon pathogen infection (Hase et al., 2003). The phytopathogenic fungus *Botrytis cinerea* also produces ethylene, both in vitro and on tomato fruit (Cristescu et al., 2002), and it is possible that nonpathogenic organisms may also produce ethylene. Rather than acting as an elicitor, ethylene may play a regulatory role, and modify plant defense responses (those regulated by JA, and others) according to particular circumstances. Salicylate, for example, was shown to enhance the expression of genes regulated by both ethylene and jasmonic acid in *Arabidopsis*, while suppressing the expression of genes regulated by jasmonic acid alone (Norman-Setterblad et al., 2000).

Ethylene production in higher plants requires ACC synthase activity, and ACC synthase expression is induced by auxin (Yi et al., 1999). As described previously, rhizobacterial inoculation has been shown to result in enhanced ability of *Arabidopsis* to convert ACC to ethylene, although the mechanism by which this occurs is unclear (Hase et al., 2003). Ethylene also regulates auxin levels: nitrilase is a key enzyme involved in auxin biosynthesis, and a gene encoding a nitrilase-like protein was found to strongly bind an ethylene-responsive element-binding protein (Xu et al., 1998). Interestingly, the expression of two ACC synthase genes in lupin increased in response to wounding (Bekman et al., 2000) and the expression of a particular ACC synthase gene in mung bean also increased continuously in response to 24-epibrassinolide (BR), an active brassinosteroid, until 24 hours after treatment (Yi et al., 1999). BR is known to promote auxin-induced ethylene production (Yi et al., 1999). Are brassiniosteroids and auxins part of a JA-ISR defense mechanism, controlling the level of expression and timing of this particular response in different plant tissues via their regulation of ethylene production? Many PGPR have been identified which produce or degrade auxin or auxin precursors, or affect auxin levels within plants (Patten and Glick, 1996). In addition, the ability of microorganisms to produce auxins in the rhizosphere will vary with environmental factors (e.g., available tryptophan levels), leaving open the possibility that microbially-mediated plant defense induction that requires the auxin production may not function in all environments or on all plant types. The elicitation of JA-ISR by strains of *Pseudomonas fluorescens* has been linked to the production of lipopolysaccharides and siderophores, but these elicitors do not fully account for ISR elicitation by these strains (van Wees et al., 1997). It would be interesting

to determine if rhizobacteria that stimulate JA-ISR can also produce auxin, and under what circumstances.

The only plant defense responses known to be activated by JA-ISR are increases in phytoalexin production (van Peer et al., 1991) and alterations of the composition of lignin that seem to retard pathogen ingress (Steijl et al., 1999). No pathogenesis-related proteins appear to be induced, and there is apparently no hypersensitive response nor, to our knowledge, induction of enzymes related to lignification or plant secondary metabolism, although this possibility may not have been adequately explored (Pieterse et al., 1996; Reitz et al., 2001; van Wees et al., 1999). The transient accumulation of a single jasmonate-inducible transcript (*Atvsp)* has been noted in *Arabidopsis* treated with a PGPR known to activate JA-ISR (van Wees et al., 1999), but it is not clear that this is a defense response. AtVsp is a vegetative storage protein (VSP) in *Arabidopsis*; such proteins accumulate in the vacuoles of young leaves and developing reproductive structures, and serve a nutritional function by acting as a storage form for amino acids. VSPs are induced in older plant parts upon wounding (Berger et al., 1995), but do not appear to have any direct role in plant defenses. It should be possible for rhizobacteria that stimulate even transient accumulations of ethylene in plant tissues to also elicit the expression of at least some PR proteins, however, since ethylene can increase the expression of a variety of defense-related genes, including osmotin, chitinases, β-1,3-glucanases, thaumatin-like proteins, and protein kinases (del Campillo and Lewis, 1992; Xu et al., 1998).

JA-ISR induces responses within plants that appear to activate only a subset of available plant defenses (i.e., phytoalexin accumulation and lignification). This appears to explain why JA-ISR has been found to be a less effective mechanism for protection against disease in *Arabidopsis* than SA-ISR, which elicits a broad array of defenses (Ton et al., 2002). The presence of multiple disease resistance mechanisms in plants may reflect plant defenses geared toward different pathogen strategies, as well as some measure of functional redundancy.

10.4.6 Can PGPR Stimulate ISR by Modifying Auxin Levels?

Mayda et al. (2000a,b) have described an interesting model for another, apparently independently regulated, defense response induction pathway, which may help explain how induced resistance in plants against viruses occurs in some plant–virus interactions. The tomato *CEV-1* gene is an anionic peroxidase induced during compatible viral infections, but not during incompatible infections. *CEV-1* expression is also not induced by salicylate, methyl jasmonate, or ethylene, or wounding, and is therefore unlikely to be involved in the typical SA-ISR or JA-ISR responses. *CEV-1* is rapidly induced when connections between plant cells are broken in normal plants, and is also induced in auxin-insensitive tomato mutants. It is hypothesized that *CEV-1* is up-regulated via the induction of plant cell insensitivity to auxin, imposed upon plants during compatible viral infections, and that auxin itself does not induce this gene (Mayda et al., 2000a). A *CEV-1* recessive mutant (*dth9*) was found to be more susceptible to fungal and bacterial infections, although salicylic acid

metabolism and expression of PR genes remained normal, and was insensitive to exogenously applied auxin (Mayda et al., 2000b). *CEV-1*, and similarly-regulated genes, could participate in a defense response that is controlled by changes in the ability of plant cells to perceive auxin, a phenomenon which is linked to auxin homeostatic mechanisms (Leyser, 2002).

One mechanism for auxin signal transduction involves the targeted degradation of transcriptional regulators that participate in complex and competing systems, modulating the expression of a wide variety of genes (Leyser, 2002), including, probably, defense-related genes. Links between auxin metabolism and plant defense responses have already been identified earlier in this review, the most obvious of these being the link between ethylene and auxin. Auxin and ethylene each regulate levels of the other in plant tissues (Xu et al., 1998; Yi et al., 1999), and ethylene, as outlined previously, is known to be involved in JA-ISR. Auxin-activated gene transcripts were also found to accumulate in tobacco upon inoculation with compatible and incompatible bacterial pathogens (Froissard et al., 1994), and auxins negatively regulated the expression of defense-related genes in tobacco and carrot (Jouanneau et al., 1991; Ozeki et al., 1990). In pepper, an auxin-repressed protein was among a variety of genes induced by pathogen infection (Jung and Hwang, 2000).

Could there be more than one ISR mechanism in plants that is controlled by auxins, one where the role of auxin is to control ethylene levels, which, in turn, control the expression of defense-related genes, and other(s) where auxins themselves control these responses, perhaps serving as negative regulators? If there are multiple, auxin-regulated ISR mechanisms (which may or may not interact, although it seems probable that they would), is it possible for bacterial strains which produce or degrade auxins or their precursors—either from a location exterior to the plant, such as in the rhizosphere, or from a location *within* the plant, as in the case of naturally occurring, nonpathogenic endophytic microorganisms—to manipulate these mechanisms? It has often been noted that treatment of plants with biological or chemical elicitors of ISR can produce, in addition to resistance, significant increases in plant growth and yield (Tuzun and Bent, 1999). Given that plants must make an energy investment in their plant defenses, this result is counterintuitive. However, if known plant growth stimulants such as auxins are involved in plant defense responses, increases in plant growth under most conditions would be expected.

10.4.7 PGPF-Mediated ISR

Most of the PGPF studied seem to stimulate the SA-ISR pathway in plants, judging solely from the reports of the activation of defense responses (e.g., PR protein induction) normally linked to this pathway (Table 10.2). As this pathway is most closely related to ISR induced by pathogens, and many of the PGPF found to induce systemic resistance are themselves nonvirulent forms of plant pathogens (Table 10.2), this is perhaps to be expected. It is a mistake to link SA-ISR responses with fungi or with pathogens only, however, SA-ISR can also be induced

by nonpathogenic, mycorrhizal fungi (Cordier et al., 1998; Lambais and Mehdy, 1995), bacterial pathogens (Preston et al., 2001), and PGPR (Table 10.1). It is also important to realize that not all necrotrophic pathogens are able to induce ISR, even when they induce defense reactions (Govrin and Levine, 2002). If the defense reactions induced by these pathogens are, for whatever reason, wrongly timed or of insufficient extent to contain a particular pathogen, there will of course be disease.

Given that many fungi produce auxins, or auxin precursors, it is possible that PGPF could stimulate plant defenses via an auxin-regulated ISR pathway. Interestingly, the PGPF *Penicillium janczewskii* and its sterile culture filtrate were both able to induce ISR and to alter cotton root development (Madi and Katan, 1998). Whether auxin or auxin precursors were involved in these phenomena was not determined, but the alterations in root development were consistent with the observed effects of microbially-supplied auxin (Patten and Glick, 2002).

10.5 Microbial Elicitors of PGPR- and PGPF-Mediated ISR

10.5.1 Elicitor Production by Microorganisms May Vary with their Physiological Status

Whether a bacterium can function to induce systemic resistance in plants will probably relate to its physiological status. Different phase culture filtrates from *Bacillus subtilis* strain FZB-G differed in their ability to activate ISR against *Fusarium oxysporum* f.sp. *radicis-lycopersici* in tomato: stationary phase filtrates were fungitoxic, and did not induce resistance, while the opposite was observed for transition phase filtrates (Gupta et al., 2000). The effect of *B. subtilis* FZB-G on tomato resistance will therefore depend upon the physiological state of the bacterial cells, a variable often overlooked by researchers studying PGPR-plant interactions. The composition of the growth medium used to produce or deliver a bacterial or fungal biocontrol inoculant, and therefore the physiological state of the inoculant, can affect its ability to control pathogens (Fuchs et al., 2000; Hoitink and Boehm, 1999; Ousley et al., 1993), and it is not inconceivable that PGPR/F that induce ISR are similarly influenced.

10.5.2 Avr Elicitors

A variety of established or putative elicitors of ISR are produced by bacteria and fungi. Specific defense response elicitors are those involved in gene-for-gene interactions, where an inducing organism (i.e., an incompatible pathogen) expresses an avirulence (*avr*) gene, the product of which is detected by a plant possessing a resistance (*R*) gene, triggering a defense response. The same defensive responses are presumably triggered by specific as by nonspecific elicitors, but it is not clear how ISR and Avr-R interactions are related. Avr products do not seem to act in the same fashion as known ISR elicitors, since the elicitor Avr9 did not induce

systemic resistance, and sometimes enhanced pathogen growth, when applied to tomato or transgenic canola expressing the *Cf-9* resistance gene (Hennin et al., 2001).

10.5.3 Oligosaccharides and Peptides

Nonspecific elicitors are perhaps more interesting, in that they are broadly conserved and less likely to be overcome by pathogen mutation, and that all the elicitors implicated in PGPR- and PGPF-mediated ISR seem to be of this type. Oligosaccharide and peptide elicitors derived from fungal cell walls can elicit plant defense responses, as reviewed by Hahn (1996). These elicitors need not be derived from virulent phytopathogenic fungi: sterile culture filtrates of the PGPF *Penicillium janczewskii* induced ISR in melon, cotton, and tobacco (Madi and Katan, 1998), and filtrates in which various PGPF were grown contained a high molecular weight ($>$12,000 Da) elicitor able to induce defense responses in cucumber and tobacco (Koike et al., 2001). Chitin, which is present not only in fungal cell walls but in arthropod exoskeletons and nematode egg membranes, has long been known to stimulate ISR (El Ghaouth et al., 1994) and has been used by itself or in combination with agricultural inoculants for this purpose.

10.5.4 Lipopolysaccharides

Lipopolysaccharide (LPS) is present on the outer membrane of Gram-negative bacteria, and it consists of a lipid moiety linked to a polysaccharide that contains a conserved core region and a variable antigenic (*o*-antigen) region (Freer, 1985). Gram-positive organisms do not produce LPS. Bacterial LPS is known to affect plant defense responses, including the expression of PR protein genes, synthesis of antimicrobial compounds, and the hypersensitive response (Dow et al., 2000). Crude cell wall extracts as well as purified LPS from *P. fluorescens* strains WCS374 and WCS417 were able to induce resistance in radish while similar preparations from *P. putida* WCS358, or from mutants of WCS374 and WCS417 lacking an o-antigenic side chain, did not (Leeman et al., 1995). In contrast, outer membrane fragments derived from *P. fluorescens* WCS417r have been shown to induce resistance in radish and carnation, but this resistance was also observed when fragments were prepared from a mutant of WCS417r lacking an o-antigenic side chain (van Wees et al., 1997). LPS from *Rhizobium elti* strain G12 was also found to induce resistance in potato to potato cyst nematode (Reitz et al., 2000).

10.5.5 Siderophores

Siderophores are low molecular weight, iron-sequestering compounds produced by bacteria under iron-limiting conditions. It was thought for some time that control of pathogens by several PGPR depended upon competition for iron, and that PGPR-produced siderophores reduced the amount of iron available to pathogens (de Weger et al., 1988). It has since been demonstrated that ISR mediated by at least

some PGPR depends upon siderophore production, or the level of iron availability in the rhizosphere (de Meyer and Hofte, 1998; Leeman et al., 1996). ISR has been induced in radish by purified pseudobactins (fluorescent siderophores), as well as by concentrations of SA as low as 1 ng (de Meyer et al., 1999), which may also be produced under low-iron conditions and used as a siderophore by *Pseudomonas* (de Meyer and Hofte, 1997; Leeman et al., 1996). For *P. aeruginosa* 7NSK2, SA production is essential for ISR induction (de Meyer and Hofte, 1998), but for *Serratia marcesens* strain 90-166, SA or pseudobactin production is not required for ISR induction (Press et al., 1997). However, siderophores may help protect *S. marcesens* 90-166 against activated oxygen species and facilitate its colonization of the root interior (Press et al., 2001).

10.5.6 Flagellins, Harpins, and Other Bacterial Proteins

Bacterial flagellin is also a potent elicitor of plant defense responses in *Arabidopsis thaliana* (Gómez- Gómez et al, 1999) and tomato (Felix et al., 1999). Plants may be able to distinguish between flagellin from different sources: peptides corresponding to conserved eubacterial flagellin domains produced a response (oxidative burst, callose deposition, and production of PR proteins) in *A. thaliana*, while peptides corresponding to these regions from *Agrobacterium tumefaciens* and *Rhizobium meliloti* were inactive (Gómez- Gómez et al., 1999). Heat-killed cells and culture filtrates of *Bacillus sphaericus* were found to induce resistance in potato to potato cyst nematode (Hasky-Günther et al., 1998), and it is possible that peptidoglycan or flagellin, sheared from cell surfaces during centrifugation, are responsible for this.

Other bacterial proteins, including those encoded by *hrp* clusters in both pathogenic and nonpathogenic strains of bacteria (Dong et al., 1999; Preston et al., 2001; Strobel et al., 1996) have been found to elicit ISR. A bacterial proton pump from *Halobacterium halobium* that was constitutively expressed in potatoes also elicited ISR, but this was probably due to effects on cell membrane polarization, which mimicked early events in plant defense responses (Abad et al., 1997).

10.5.7 Elicitins and Mycotoxins

Elicitins are low molecular weight peptides produced by oomycete fungi, including all analyzed species of the plant pathogen *Phytophthora* (Keller et al., 1996) and the mycoparasite *Pythium oligandrum* (Benhamou et al., 2001). Elicitins are not virulence factors, but rather avirulence factors, since the most virulent organisms are those which produce little or no elicitin (Keller et al., 1996). Elicitins can induce ISR, and different elicitins may vary in their ability to stimulate plant defense reactions. For example, resistance induced by a basic elicitin, cryptogein, induced both necrosis in tobacco leaves and the transcription of a variety of defense-related genes, while resistance induced by an acidic elicitin, capsicein, was not accompanied by visible necrosis but still induced the transcription (albeit to a lesser extent) of the same genes (Keller et al., 1996). Cryptogein was also found to increase the extent of apoplastic RNase activity, which, in turn, was found to be

sufficient to reduce infection of tobacco by *Phytophthora parasitica* (Galiana et al., 1997). Proteinaceous ISR elicitors may also be produced by non-oomycete fungi, for example, a *Fusarium oxysporum* 24 kDa protein was found to induce ethylene production and varying defense responses in different weed species (Jennings et al., 2000).

Mycotoxins are produced by virulent fungi, and include the AAL-toxins and fumonisins, groups of structurally related sphingosine analogs that are produced by *Alternaria alternata* f. sp. *lycopersici* and *Fusarium moniliforme*, respectively. Both kinds of toxins have been found to induce cell death in plants, apparently by disrupting ceramide synthesis (Bostock et al., 2001). Treatment of plant roots with ceramide has been found to induce ceramide accumulation in leaves, as well as systemic resistance (Bostock et al., 2001).

10.5.8 Detection of Nonspecific Elicitors by Plants

Plant receptor-like kinases that bind peptidoglycan, a polymer found in the cell walls of bacteria, or chitin have been identified (Shiu and Bleecker, 2001), as well as plant plasma membrane proteins that have a high binding affinity for chitin fragments (Okada et al., 2002), plant proteins that are rapidly phosphorylated in response to flagellin or chitin (Peck et al., 2001), and calmodulin isoforms that are activated by nonspecific fungal elicitors (Heo et al., 1999). These discoveries may help explain how the detection of nonspecific elicitors can stimulate a plant defense response.

10.6 Spectrum of PGPR- and PGPF-Mediated ISR Activity

PGPR-induced systemic resistance has been observed on a wide variety of plants, including monocots, dicots, and gymnosperms, in response to several types of pathogens or herbivores (Table 10.1). I am not aware of any studies performed with plants that belong outside these categories (e.g., mosses and ferns), although it would be interesting from an evolutionary perspective to know if the more ancient plant forms can express induced systemic resistance.

The majority of reports I was able to find focus on plant pathogenic fungi or bacteria, but PGPR-induced resistance to viruses, nematodes, and herbivorous insects has also been reported (Table 10.1). For example, *Bacillus sphaericus*, *Agrobacterium radiobacter*, and *Rhizobium elti* can induce ISR against the potato cyst nematode, *Globodera pallida* (Hasky-Günther et al., 1998; Reitz et al., 2001). Inoculation with *Bacillus pumilis* strain INR-7 can decrease levels of the feeding stimulant cucurbitacin in cucumber leaves, resulting in reduced feeding on these plants by cucumber beetles (Zhender et al., 1997a).

PGPF-induced resistance has likewise been observed on a variety of angiosperms in response to various plant pathogens (Table 10.2). Mycorrhizal fungi, although not included in the definition of PGPF, have also been observed to induce defense responses in angiosperms (Cordier et al., 1998; Lambais and Mehdy,

1995) and gymnosperms (Sylvia and Sinclair, 1983; Strobel and Sinclair, 1991; Salzer et al., 1996).

10.7 Effects of the Environment and Other Microorganisms on PGPR- and PGPF-Mediated ISR

In a natural environment, PGPR and PGPF exist in the midst of a wide variety of other micro- and macroorganisms, some of which may themselves exert effects on plant defense responses, and all of which can be influenced by the host plant, soil- and climate-related factors, and other nearby vegetation. These factors can only be briefly outlined here.

Microbial rhizosphere communities have been observed to vary between soil and plant types (Catellan et al., 1998; Weland et al., 2001; Latour et al., 1996; Kuske et al., 2002; Timonen et al., 1998), in response to crop rotations (Vargas-Ayala et al., 2000) and the addition of organic soil amendments (Zhang et al., 1996; Bent and Topp, unpublished observations). The microbial rhizosphere community can vary with depth (Kuske et al., 2002), location along the root surface, and the nutritional status of the plant, which will affect the composition of root exudates upon which microorganisms feed (Yang and Crowley, 2000). Precipitation will affect the distribution of microorganisms in the rhizosphere, as percolating water in the soil can flush bacteria off roots and down into the soil (Mawdsley and Burns, 1994). Earthworms have been shown to facilitate the movement of bacteria within soils, and by providing an environment in which plasmid transfer between bacteria can readily occur, may increase the rate of gene transfer between bacteria in natural soils (Daane et al., 1997). The physicochemical properties of a soil will also have a profound influence on microbial metabolism, and the ability of a microorganism to produce compounds by which it interacts with plants or with other microorganisms. For example, the production of siderophores and antibiotics by *Pseudomonas fluorescens* CHA0 was found to be modulated by such factors as phosphate availability, the ratio of carbon sources to nutrients, the presence of soluble cobalt, molybdenum or zinc, and the composition of available carbon sources (Duffy and Defago, 1999).

It is not always easy to identify which microorganisms in the environment are affecting plant growth or metabolism. "Non-culturable" soil microorganisms that cannot be cultured using traditional techniques appear to make up a majority of the organisms present in soils, based upon analyses of rDNA extracted from soils (Amann et al., 1995). Such non-culturable organisms may be so because they are only able to grow in mixed cultures, as has recently been demonstrated for non-culturable marine bacteria (Kaeberlein et al., 2002), and an obligately biotrophic mycorrhizal fungus that requires a bacterium to grow in vitro (Hildebrandt et al, 2002). The presence of other bacterial rhizosphere colonists has been shown to improve the ability of some bacterial strains to colonize specific root microsites (Bent et al., 2002), and microorganisms in soil or on plant roots will naturally exist in biofilms, which change in chemical and microbial composition over time.

Direct metabolic interactions between members of a biofilm community have been reported (Moller et al., 1998). The production of quorum sensing signal molecules that regulate and coordinate the activity of individuals within a given bacterial species has been known for some time (Pierson et al., 1998). Bacteria can respond to quorum sensing-like molecules produced by other rhizobacteria (Steidle et al., 2001), by plants (Teplitski et al., 2000), and even destroy the quorum-sensing molecules produced by other bacterial species (Dong et al., 2002). Bacterial activity in the rhizosphere can therefore be altered directly by plants or other microorganisms via quorum-sensing molecules.

Mycorrhizal fungi are known to induce plant defense responses directly, as outlined in the previous section. The presence of nonhost plants, or their root exudates, can sometimes prevent mycorrhizal fungi from colonizing plants they would otherwise be able to infect (Fontenla et al., 1999), and root exudates containing allelopathic, phytotoxic compounds can prevent other plant species from establishing in the vicinity of the producer (e.g., Yamane et al., 1992). The infection of roots with mycorrhizal fungi will alter the composition of root exudates, and therefore the community of rhizosphere microorganisms (Belimov et al., 1999), and can even increase the number of rhizosphere protozoa (Jentscke et al., 1995). Protozoa feed upon rhizosphere bacteria and can alter their spatial distribution upon surfaces (Lawrence and Snyder, 1998), their taxonomic and functional diversity (Bonkowski, 2002) and appear to induce physiological responses in the bacteria, which remain uneaten (Kandeler et al., 1999). Protozoa can also influence plant growth directly via a mechanism that is unrelated to the release of nutrients from bacteria during grazing (Jentscke et al., 1995).

Grazing of roots or foliage by herbivores can also alter the composition of the microbial rhizosphere community, by altering the quantity or quality of root exudates or plant litter (Bardgett et al., 1998; Denton et al., 1999).

PGPR field trials can be quite variable in their results, and it is consistently hypothesized that other soil microorganisms may be interfering with the ability of the PGPR inoculants to adequately colonize plant roots or interact with plants (Bent and Chanway, 1998; Bent et al., 2000, and references therein). This interference may be due to an inability to adequately colonize the plant root, or alternatively, due to the alteration of growth-promoting or signaling molecules produced by PGPR and PGPF by other rhizosphere microorganisms. The effects of PGPR or PGPF on plant defense responses under natural conditions are therefore very likely to be affected by the presence of other organisms in the plant's environment, which may include other plants, protists, earthworms, insects, herbivorous animals, fungi, and bacteria. This is in addition to climactic and soil factors that can alter the physiology of plants, and potentially affect interactions between PGPR or PGPF and their host plants.

Still, it may be possible to manipulate soil microbial communities so that plant defenses are stimulated and plant growth and health improved. Disease-suppressive soils may contain microorganisms that disrupt the disease cycle in a variety of ways, including direct attacks on the pathogen or utilization of substrates the pathogen requires to locate host roots (Yin et al., in press). While ISR has not been clearly

identified with a disease-suppressive soil to our knowledge, ISR can be induced by compost amendments (Hoitink and Boehm, 1999; Zhang et al., 1996, 1998), as well as aqueous extracts of compost, or autoclaved compost amended with a biocontrol agent (Hoitink and Boehm, 1999; Zhang et al., 1998). In each of these cases, the induction of systemic resistance was attributed to the presence of compost-related microorganisms.

10.8 Conclusion

PGPR and PGPF interact with plants in complex and numerous ways, especially under natural conditions where each organism is part of a dynamic consortium that fluctuates in response to environmental biotic and abiotic stimuli. Systemic resistance in plants is induced via several different mechanisms by PGPR, and possibly by several in PGPF, although less research has been conducted on PGPF-mediated ISR. These mechanisms are likely to interact at some point with the host plant's hormonal balances, especially since both bacteria and fungi are capable of synthesis of various plant hormones such as auxin. More details of these interesting plant–microbe interactions will be elucidated as research progresses, and I hope to see more work in the near future conducted on the mechanisms governing microbially-mediated ISR as well as the role(s) of typically growth-related phytohormones (auxins, cytokinins) in the ISR phenomenon. Emerging technology, such as oligonucleotide fingerprinting of rRNA genes (OFRG; Valinsky et al., 2002a,b) will enable the detailed study of rhizosphere and endophytic consortia in natural soils and in the interior of the plant. Using OFRG, it will become possible to determine the conditions under which natural or artificially produced microbial consortia tend to flourish and induce ISR in particular plants. It may one day be possible to engineer ISR-stimulating soils containing stable populations of ISR-inducing microbial consortia for particular crops, via the strategic addition of substrates, inocula, or other soil treatments.

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