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ROOT-ASSOCIATED BACTERIA INDUCING SYSTEMIC RESISTANCE

Abstract. We describe the nature of rhizobacterially-induced systemic resistance (ISR) and distinquish ISR from the induced resistance triggered by limited pathogen infection, SAR. The progress made in the elucidation of induction, signaling and expression of ISR is documented for *Arabidopsis thaliana*, the model plant species and several other plants. The results confirm that, in general, ISR is not dependent on salycilic acid (SA), but indicate that, instead, it has a variable requirement or dependence on jasmonic acid (JA) and ethylene signaling. Evidence emerging from recent gene expression studies have suggested that plants can be in the state of ISR without expressing any changes in gene transcripts of leaves in such induced state. As usual, only upon a challenge inoculation, defense responses get boosted. This phenomenon points to the importance of priming of resistance mechanisms in ISR. Such an enhanced defensive capacity is suggested as the main characteristic of rhizobaterially-induced systemic resistance.

1. THE NATURE OF RHIZOBACTERIALLY-INDUCED SYSTEMIC RESISTANCE

When growing into the soil, plant roots become quickly colonized by a highly diverse microflora of bacteria and fungi that may have both beneficial and deleterious effects on plant growth (Lavelle and Spain, 2001). More than 80% of all land plants enter into a symbiosis with mycorrhizal fungi, which aid in the uptake of water and minerals, notably phosphate, and offer protection against abiotic and biotic stresses (Barea et al., 2002). Legumes engage in a symbiosis with rhizobium bacteria, which induce root nodules in which they fix atmospheric nitrogen (Albrecht et al., 1999). The interactions between rhizobia and their host plants are extensively studied and have yielded a fascinating picture of mutual exchanges of molecular signals between both partners for root nodule formation and nitrogen fixation to become established. The obligate nature of the symbiosis between mycorrhizal fungi and plants has slowed progress into the elucidation of the mechanisms involved in this type of interaction, but mutant and gene expression analyses are indicating a similar intricate interplay between the plant and its symbiont (Gianinazzi-Pearson, 1996; Mulder et al., 2005). Moreover, some plant genes have been identified that control initial stages of both the rhizobial nodulation response and of vesicular-arbuscular mycorrhizal development (Harrison, 1998; Stracke et al., 2002). In both of these interactions, the microorganisms invade plant roots and appear to suppress host defense reactions (Gianinazzi-Pearson et al., 1996). Thus, initially the plant seems to recognize the foreign invader and activates resistance mechanisms to limit spread and tissue colonization, but subsequently yields at least partially to the fungus or bacterium in order to allow an effective symbiosis.

Plant pathogenic fungi and bacteria exploit the host plant unilaterally by circumventing to trigger defense reactions or by evading the effects of activated defenses sufficiently to be able to grow and cause disease. Yet, plants do recognize the

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invader and start mounting a defensive response, but this is either too weak or too slow, or both, to limit the pathogen sufficiently to prevent it from colonizing the plant (Thordal-Christensen, 2003). However, when resistance reactions are triggered sufficiently early, the plant can restrict the pathogen to certain tissues, and when highly effective, to only a few cells around the site of attack (Kuc, 1982). If the plant overcomes the infection and damage is limited, an enhanced defensive capacity is acquired. This so-called induced resistance enables the plant to react more quickly and strongly to a subsequent attacker, and is variously denoted as systemic acquired resistance (SAR) or induced systemic resistance (ISR) (Van Loon, 2000). In the last decade it has become clear that mechanistically different types of induced resistance exist that are effective against different attackers (Ton et al., 2002a,c). To avoid confusion, the term SAR has been adopted predominantly for the type of induced resistance that is triggered by limited pathogen infection (Ryals et al., 1996; Sticher et al., 1997). This type of resistance is induced optimally when the plant withstands the first invader by developing a hypersensitive reaction. Although tissue necrosis contributes to the level of induced resistance attained, activation of defense mechanisms resulting in limitation of the primary infection seems sufficient to elicit induced resistance (Hammerschmidt and Kuc, 1995). ISR encompasses a broader range of induced resistance phenomena elicited mainly by non-pathogenic organisms (Van Loon et al., 1998). Generally, induced resistance is systemic, in that not only in the primary infected plant parts, but also in non-infected distant tissues the defensive capacity is increased. However, induced resistance is not always expressed systemically: localized acquired resistance (LAR) occurs when only tissues exposed to the primary invader become more resistant (Ross, 1961). Phenotypically, localized and systemically induced resistance are similar in that they are effective against various types of pathogens, but in LAR a signal appears to be lacking that is necessary to propagate the enhanced defensive capacity throughout the plant. The nature of the mobile signal in SAR is unknown (Vernooij et al., 1994) but, at least in Arabidopsis and tobacco, the expression of induced resistance is dependent on the accumulation of salicylic acid (SA) (Gaffney et al., 1993), and its level is modulated by ethylene and jasmonic acid (JA) (Pieterse et al., 1998; Verberne et al., 2003). Through the enhancement of the general defensive capacity of the plant, induced resistance is a protective mechanism that increases the basal level of resistance against all types of pathogens. It limits the effects of a subsequent infection by reducing disease incidence and/or severity, but it only rarely prevents disease development.

Like symbiotic and pathogenic microorganisms, other fungi and bacteria are attracted to plant roots by their release of exudates and lysates (Whipps, 2001; Persello-Cartieaux, 2003). Because of this nutrient supply, they grow and multiply in the rhizosphere, and many appear to establish mutualistic interactions with plants. Most rhizobacteria remain confined to the root surface but some enter the root interior and behave as endophytes. Several of these rhizobacteria have been found to increase plant growth and are therefore called "plant growth-promoting rhizobacteria" (PGPR)

(Kloepper et al., 1980). The mechanisms of growth promotion by these rhizobacteria are complex and usually taken to comprise increasing the availability and uptake of mineral nutrients and modulating plant hormone status (Glick et al., 1999; Van Loon and Glick, 2004). In addition, growth promotion can result from suppression of pathogenic fungi and bacteria through microbial antagonism, i.e. competition for nutrients, particularly iron, production of antibiotics, and/or secretion of lytic enzymes such as chitinases, glucanases and proteases (Handelsman and Stabb, 1996; Van Loon and Bakker, 2003). Some of these mechanisms may also affect the plant. At least one strain of non-pathogenic rhizobacteria has been shown to possess a type III secretion system (Preston et al., 2001). This secretory system is used by pathogenic bacteria to inject effector proteins into eukaryotic cells to induce disease (Collmer et al., 2000). In the case of e.g. the pathogenic leaf bacterium *Pseudomonas syringae*, various proteins have been characterized that play a role in virulence and suppression of plant defenses in susceptible hosts and that are recognized as foreign, and initiate defense responses in resistant hosts (He et al., 2002). The presence of a similar type of secretion apparatus in certain non-pathogenic *Pseudomonas* isolates with growth-promoting properties suggests that more intricate interactions between rhizobacteria and host plants may exist.

The expression of virulence factors by pathogenic bacteria is regulated by quorum sensing (Whitehead et al., 2001). Also non-pathogenic rhizobacteria can rely on quorum sensing for the production of e.g. factors for efficient root colonization or antibiotics to antagonize competing microorganisms (Somers et al., 2004). Thus, non-pathogenic rhizobacteria share with pathogens certain molecular mechanisms that can aid in competition in the rhizosphere and in the colonization of plant roots. Hence, it may not be too surprising that, like pathogens, selected strains of non-pathogenic, root-colonizing bacteria can interact with plant roots in ways that result in induced systemic resistance against pathogens. Induction of systemic resistance by rhizobacteria was first demonstrated independently by Van Peer et al. (1991) against fusarium wilt in carnation, and by Wei et al. (1991) against anthracnose in cucumber. Since then, it has been established in several plant species against different types of pathogens by various research groups using different rhizobacterial strains (e.g. Van Loon et al., 1998). It is not entirely clear in how far the plant reacts to inducing rhizobacteria in a way that resembles a defense response against a pathogen, and it cannot be excluded that some rhizobacteria that are considered non-pathogenic on the host species under study, are actually minor pathogens on other plant species (Hynes et al., 1994). Yet, root colonization by resistance-inducing bacteria does not normally cause any visible symptoms and often promotes plant growth. Thus, these resistance-inducing bacteria seem to be of substantial benefit to the plant and help it to defend itself against not only soil-borne, but also foliar pathogens by boosting its capacity to resist a range of pathogenic attackers.

Because rhizobacteria that trigger ISR can at the same time be antagonistic to the pathogen, disease suppression seen upon their application may result from more than one mechanism. Concluding that ISR is involved rather than a direct effect of the

bacterium on the pathogen requires rigorous demonstration that disease suppression is plant-mediated. Many studies in which induced resistance is considered as the mechanism responsible for disease reduction, have not specifically addressed this point, leaving doubts that other mechanisms may (also) be involved. In the presence of inducing rhizobacteria that may also have antagonistic properties, local protection as a result of induced resistance is difficult to verify experimentally. Mutants of the pathogen that are insensitive to the antagonism may aid in resolving this issue. Because rhizobacteria are present on the roots, systemic protection against root pathogens must be demonstrated by applying the inducing bacteria to one part of the root system and the challenging pathogen to another part, for instance by making use of split-root systems. Testing for protection against foliar pathogens is easier, because those are naturally separated from the rhizobacteria. However, rhizobacteria applied to seeds, or to soil into which seeds are sown or seedlings are transplanted, can move into the interior of aerial plant tissues and to some extent maintain themselves on the exterior of aerial surfaces (Kluepfel, 1993; Lamb et al., 1996). Thus, in order to prove that resistance is induced and that it is truly systemic, it must be shown that inducing rhizobacteria are absent from the site of challenge with the pathogen and no contact between the two is established.

Direct antagonistic effects of metabolites of the inducing bacterium on the pathogen should also be excluded. This pertains particularly to the production of antibiotics, and makes it very difficult to establish ISR when the bacterium produces an antimicrobial factor that acts directly on the pathogen. Even when the inducing organism itself is shown not to be present at the site of challenge with the pathogen, the toxic factor may have been produced and transported through the plant, contacting the pathogen directly. This does not mean that induced resistance could not be involved, or even that the toxic factor itself did not (also) induce resistance. Some antibiotics produced by rhizobacteria, such as 2,4-diacetylphloroglucinol (DAPG) or phenazine-1-carboxylic acid (PCA), in high concentrations have some toxicity to plants (Maurhofer et al., 1995), and could induce systemic resistance in the same way as a pathogen causing localized necrosis. Under such circumstances, induced resistance remains difficult to prove, and best evidence is obtained when the rhizobacterium does not antagonize the pathogen in in vitro culture. In Arabidopsis, mutants that cannot express ISR can be used to differentiate between antagonism and induced resistance (Van Loon and Bakker, 2005). If such mutants are not available, doubts can remain because conditions in the rhizosphere are different from those on artificial media, and an antibiotic produced exclusively in planta may go undetected. Determining the presence of an antibiotic in situ and comparing levels to effective doses in vitro can indicate whether microbial antagonism could explain disease reduction.

2. INDUCED SYSTEMIC RESISTANCE IN DIFFERENT PLANT SPECIES

2.1 Arabidopsis

Progress in the elucidation of the induction, signalling and expression of rhizobacterially-induced systemic resistance has been greatly aided by the adoption of *Arabidopsis thaliana* as a model plant species. ISR in Arabidopsis was first established against the vascular wilt fungus *Fusarium oxysporum* f.sp. *raphani* (For) upon application of the rhizobacterial strain *Pseudomonas fluorescens* WCS417r (Pieterse et al., 1996), using a bioassay involving rock wool wetted with nutrient solution (Leeman et al., 1995a). Two- to 3-weeks-old seedlings were placed horizontally on rock wool cubes with the distal part of the roots on cubes contained in a plastic bag, adjacent to another bag with cubes supporting the proximal part of the root system. The roots were laid down through an incision in the bags. The distal part of the roots was then treated with a bacterial suspension in talcum emulsion. Three days later the proximal part of the root system was inoculated with the fungus in a peat mixture. Disease started developing after 14 days and leaf yellowing and wilting symptoms were scored routinely for over 3 weeks after inoculation. After challenge with For, symptom development was delayed and disease severity reduced. Bacterial colonization of the root remained confined to the distal part and no fungus was recoverable from this part, demonstrating that the bacteria remained spatially separated from the pathogen for the duration of the experiments (Pieterse et al., 1996).

A simpler and more convenient bioassay was developed by using the foliar pathogen *Pseudomonas syringae* pv. *tomato* strain DC3000 (Pst) for challenge. Two-weeks-old Arabidopsis seedlings were transplanted into autoclaved potting soil into which the rhizobacterial strain was mixed. Three weeks later the plants were challenged by dipping the leaves in a suspension of the pathogen. Typical symptoms of bacterial speck disease, spreading chlorosis progressing into necrosis, developed within a few days. ISR against Pst was expressed as a reduction in the percentage of leaves with symptoms. In addition, growth of the pathogenic bacterium in the infected leaves was strongly inhibited. Colonization by the rhizobacteria remained confined to the roots and no contact between the inducing strain and the pathogen was demonstrable (Pieterse et al., 1996; Van Wees et al., 1997).

Of three rhizobacterial strains tested, *Pseudomonas putida* WCS358r, *Pseudomonas fluorescens* WCS374r and *P. fluorescens* WCS417r, WCS358r and WCS417r triggered ISR to similar extents, whereas WCS374r was ineffective (Van Wees et al., 1997). The level of resistance attained by application of either WCS417r or WCS358r was mostly similar to that of SAR induced by virulent or avirulent Pst, or by the chemical inducers SA or 2,6-dichloroisonicotinic acid (INA) (Pieterse et al., 1996; Van Wees et al., 1997). Development of SAR in response to limited infection by a pathogen is dependent on endogenous signalling through SA and associated with local and systemic expression of defense genes encoding pathogenesis-related proteins (PRs). Several of these PRs have potential antimicrobial activities and are taken to contribute to the enhanced resistance of induced plants (Van Loon, 1997). In Arabidopsis, development of SAR in response to Pst or treatment with SA or INA is associated with accumulation of PR-1, -2, and –5 mRNAs (Uknes et al., 1992).

However, in plants with ISR triggered by WCS417r or WCS358r no *PR*-gene expression or accumulation of PR-proteins was detectable (Pieterse et al., 1996). Therefore, the induction of ISR by WCS417r or WCS358r in Arabidopsis was not coupled to the activation of *PR* genes.

As shown by Zhang et al. (1998), a biocontrol agent-fortified compost mix suppressive to several diseases caused by soil-borne pathogens, induced resistance against the foliar pathogen *Pseudomonas syringae* pv. *maculicola* (Psm), as evidenced by a reduction in both the severity of bacterial speck symptoms en population densities of the pathogen in the leaves. Autoclaving destroyed the effect of the compost mix, and the activity apparently resided in a water extract of the compost. Transgenic Arabidopsis plants containing a fusion protein construct consisting of the *PR-2* (ß-1,3-glucanase) promoter and the ß-glucuronidase (*GUS*) open reading frame as a reporter did express GUS activity after spraying with the compost water extract. Although the disease suppression is reminiscent of ISR, the mechanism involved seems different from that induced by *P. fluorescens* WCS417r or *P. putida* WCS358r, and a minor toxic action of the water extract leading to activation of *PR*-gene expression cannot be excluded.

To investigate whether the induction of ISR by WCS417r or WCS358r was dependent on SA accumulation in the plant, tests were conducted using transgenic NahG plants. These plants harbour the *NahG* gene from *P. putida*, which encodes salicylate hydroxylase (Delaney et al., 1994). As a result, SA applied to, or synthesized by, the plant is converted into the inactive compound catechol. Since SA is required for the expression of both PRs and SAR, NahG plants no longer accumulate PRs or become induced upon a primary infection. In NahG plants, WCS417r and WCS358r were as effective in inducing ISR against Pst as in untransformed plants, demonstrating that these rhizobacteria activate a signalling pathway different from the one that controls SAR (Pieterse et al., 1996), even though WCS417r is capable of producing SA in vitro under iron-limiting conditions (Leeman et al., 1996). Strikingly, *P. fluorescens* WCS374r, a strain capable of producing a large amount of SA in vitro (Leeman et al., 1996; Mercado-Blanco et al., 2001), did not induce resistance in Arabidopsis, whereas WCS358r, incapable of producing SA, did (Van Wees et al., 1997). The latter result provided direct proof that bacterially-produced SA did not play a role in triggering ISR in Arabidopsis. Moreover, the finding that WCS358r was equally capable of inducing systemic resistance in untransformed and in NahG Arabidopsis demonstrated that also plant-derived SA was not involved. Similar to WCS417r and WCS358r, *P. fluorescens* CHA0r, a PGPR strain with multiple mechanisms of disease suppression, and *Pseudomonas aeruginosa* strain 7NSK2, both capable of producing SA in vitro under iron-limited conditions, induced resistance in NahG *Arabidopsis* (Ran et al., 2005b). It must be concluded that SA does not play a role in the ISR triggered by these strains.

Arabidopsis mutant *npr1* was identified as a non-expresser of *PR* genes, and unable to exhibit SAR (Cao et al., 1994). Because rhizobacterially-mediated ISR was found to be independent of SA and not associated with PRs, it was expected that ISR would still be expressed in this mutant. However, *npr1* did not express WCS417r-mediated ISR (Pieterse et al., 1998). This finding implied that NPR1 functions beyond the expression of *PR* genes and is required for both pathogen-dependent and rhizobacterially-mediated induced systemic resistance. The *npr1* gene is allelic to *nim1* (no immunity) (Delaney et al., 1995) and *sai* (SA-insensitive) (Shah et al., 1997) and encodes an ankyrin-repeat family protein structurally resembling the inhibitor IF-κB, which plays a role in animal innate immunity (Cao et al., 1997). Under the influence of SA, a redox change causes oligomers of NPR1 in the cytoplasm to be reduced to monomers. The monomers are transported into the nucleus, where they interact with specific TGA transcription factors to activate the expression of *PR* genes (Mou et al., 2003). The non-responsiveness of the Arabidopsis *npr1* mutant to rhizobacteria demonstrated that SAR and ISR converge at the last part of the signalling pathway. Yet, concomitant with SAR PRs are induced, whereas activation of their genes is not part of the pathway leading to ISR in Arabidopsis. Apparently, NPR1 regulates defense responses in ISR differently from SAR.

Because SA is essential for the expression of SAR but ISR does not involve SA, the signalling pathway of ISR before NPR1 must be different from that of SAR. Ethylene is an important signalling compound in plant defense responses (Van Loon et al., 2005). It enhances the sensitivity of Arabidopsis to SA (Lawton et al., 1994) and activates several defense-related genes, including specific *PRs* (Brederode et al., 1991). Moreover, during a virus-induced hypersensitive reaction, transgenic ethylene-insensitive tobacco plants were fully capable of elevating SA levels and expressing induced resistance locally but, when used as rootstock, failed to transmit the mobile signal to a wild-type scion. As a result, no systemically induced resistance was evident (Verberne et al., 2003). To investigate the role of ethylene in the expression of ISR, the ethylene-insensitive Arabidopsis mutant *etr1,* as well as the ethylene signalling mutants *ein2 – ein7* and *axr1-12,* and the ethylene-overproducing mutant *eto1-1* were tested (Pieterse et al., 1998; Knoester et al., 1999). WCS417r was not capable of inducing resistance against Pst in these mutants, whereas pathogen-induced SAR was essentially unimpaired. Because WCS417r colonized the roots of the mutants to the same extent as those of wild-type seedlings, these observations implicate ethylene perception as a specific and essential step in the signal-transduction pathway leading to ISR. Indeed, application of the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), to wild-type seedlings induced resistance against Pst to the same level as the rhizobacterial strains, not only in untransformed but also in SA non-accumulating NahG Arabidopsis (Pieterse et al., 1998). However, no significant increase in ethylene production was measurable in plants treated with resistance-inducing WCS417r bacteria (Pieterse et al., 2000).

The Arabidopsis mutant *eir1* is insensitive to ethylene in the roots, but not in the shoots (Roman et al., 1995). In accordance with the requirement for ethylene

sensitivity, in bioassays the *eir1* mutant did not express ISR upon application of WCS417r to the roots, while it did exhibit ISR when the inducing bacteria were infiltrated into the leaves (Knoester et al., 1999). This result demonstrated that for the induction of ISR in Arabidopsis by WCS417r, ethylene responsiveness is required at the site of application of the inducing rhizobacteria.

In a screening for natural variation within Arabidopsis to express rhizobacteria-mediated ISR, accessions RLD and Ws-0 were found to not develop ISR after root colonization by WCS417r, in contrast to eight other accessions tested. All ten accessions were capable of expressing pathogen-induced SAR, indicating that RLD and Ws-0 were impaired specifically in ISR expression. When RLD and Ws-0 were crossed, no complementation occurred, indicating that in both accessions the same trait was involved (Ton et al., 1999). The non-responsive phenotype was associated with an enhanced susceptibility to infection by Pst, suggesting that induced resistance represents an enhancement of an extant defensive capacity. Both traits – inability to express ISR and enhanced disease susceptibility – were inherited monogenically and genetically linked. The corresponding locus was designated *Isr1* and appears to encode a so far unidentified component of the ethylene response that is required both for basal resistance against Pst and for the expression of ISR upon root bacterization with WCS417r (Ton et al., 2001). All these observations are suggestive of a triggering of ethylene production or modulated perception by the plant in the presence of the inducing bacteria.

The enhanced disease susceptibility phenotype of RLD and Ws-0 resembles that of Arabidopsis *eds* mutants, that have been described to possess an enhanced susceptibility to Psm or the powdery mildew fungus *Erysiphe orontii* (Glazebrook et al., 1996; Volko et al., 1998). Out of 11 of these *eds* mutants tested, three proved non-responsive to expression of ISR upon root colonization by WCS417r, while pathogen-induced SAR was unimpaired. Of the three, *eds4-1* showed reduced sensitivity to ethylene, whereas *eds8-1* was non-responsive to methyl jasmonate (MeJA) (Ton et al., 2002b). The Arabidopsis JA response mutant *jar1* likewise exhibited wild-type levels of pathogen-induced SAR but failed to express rhizobacterially-mediated ISR (Pieterse et al., 1998). JA levels were not increased in plants treated with resistance-inducing WCS417r bacteria (Pieterse et al., 2000). Yet, on wild-type plants, application of MeJA induced resistance, as did ACC. However, MeJA did not induce resistance in the *etr1* mutant, whereas ACC did induce resistance in the *jar1* mutant, indicating that perception of JA is dependent on components of the ethylene response. Neither JA, nor ACC induced resistance in the *npr1* mutant, indicating an action prior to NPR1 (Pieterse et al., 1998). How perception of JA and ethylene are coupled to ISR is still unclear.

The development of ISR against the downy mildew oomycete *Peronospora parasitica* in Arabidopsis in response to root inoculation with *P. aeruginosa* strain CHA0 has likewise been shown to require JAR1, EIR1 and NPR1, and not SA.

However, mutants *etr1-1* and *ein2-1* still expressed ISR against this pathogen (Iavicoli et al., 2003), suggesting that the requirements for ISR against *P. parasitica* overlap only partly with those defined for ISR against Pst, as induced by WCS417r. On the other hand, ISR activated by *Bacillus subtilis* GB03 was found to be independent of SA and dependent on ethylene, yet did apparently not require JA (Ryu et al., 2004b). ISR elicited in Arabidopsis against Pst or Psm by four other rhizobacterial strains was reported to be variably dependent on ethylene and JA (Ryu et al., 2003a). These results confirm that, in general, ISR is not dependent on SA, but indicate that, instead, ISR has a variable requirement for JA and ethylene signalling (Table 1). The latter does not need to be problematic, because hormone sensitivity is still poorly understood and may vary depending on experimental conditions. In a given situation, either sensitivity to JA or to ethylene, or both, might be limiting.

The lack of expression of WCS417r-elicited ISR in accessions RLD and Ws-0, as well as in the *jar1*, ethylene-response and *npr1* mutants, rules out that rhizobacterially-produced antibiotics might have been responsible for, or contributed to, the induced resistance, in agreement with findings that in vitro no antagonistic effects between the rhizobacteria WCS417r and WCS358r and the pathogen Pst were observed (Van Wees et al., 1997). By requiring JA and ethylene signalling, but not SA, the signalling pathway triggered by these resistance-inducing rhizobacteria is different from SAR (Pieterse et al., 1998) (Fig. 1).

Whereas both SAR and ISR in Arabidopsis are effective against different types of pathogens, their effects vary both qualitatively and quantitatively. Activation of ISR provides a significant level of protection against the leaf spotting fungus *Alternaria alternata* and the grey mould fungus *Botrytis cinerea*, whereas SAR is ineffective against these pathogens. Conversely, activation of SAR results in a high level of protection against turnip crinckle virus, whereas ISR reduces neither virus multiplication, nor symptom severity. Compared to SAR, protection by ISR proved less effective against the downy mildew oomycete *Peronospora parasitica*, but almost equally effective against Pst, and at least as effective against For and *Xanthomonas campestris* pv. *armoraciae* (Xca) (Ton et al., 2002a). These relative effectiveness of SAR and ISR correlate with an enhanced susceptibility of Arabidopsis mutants that are impaired in the accumulation of SA, and sensitivity to JA or ethylene, respectively (Thomma et al., 1998, 2001; Oliver and Ipcho, 2004). Thus, SAR is effective against pathogens that in non-induced plants are resisted through SA-dependent basal resistance responses, whereas ISR is effective against pathogens that in non-induced plants are resisted through JA/ethylene-dependent basal resistance mechanisms, supporting the notion that induced resistance represents an enhancement of extant defensive capacity (Van Loon, 1997). In the assays used, treatment of Arabidopsis seedlings with inducing rhizobacteria occurred for a few days to a few weeks before challenge inoculation, and spatial separation between the rhizobacteria and the challenging pathogen was verified.

Table 1. Dependence of rhizobacteria-induced systemic resistance in Arabidopsis on signalling components [a].

Bacterial strain	*Pathogen*	*SA*	*JA*	*Ethylene*	*NPR1*
Bacillus amyloliquefaciens IN937a	*Erwinia carotovora* subsp. *carotovora*	-	-	-	-
Bacillus pumilus SE34	*Pseudomonas syringae* pv. *maculicola*	-	+	+	+
	Pseudomonas syringae pv. *tomato*	+	-	-	+
Bacillus pumilus T4	*Pseudomonas syringae* pv. *maculicola*	-	+	-	-
	Pseudomonas syringae pv. *tomato*	-	-	-	-
Bacillus subtilis GB03	*Erwinia carotovora* subsp. *carotovora*	-	-	+	-
Pseudomonas fluorescens CHA0	*Peronospora parasitica*	-	+	-/+	+
Pseudomonas fluorescens WCS417	*Pseudomonas syringae* pv. *tomato*	-	+	+	+
Pseudomonas fluorescens 89B-61	*Pseudomonas syringae* pv. *maculicola*	-	-	+	+
	Pseudomonas syringae pv. *tomato*	-	-	+	+
Serratia marcescens 90-166	Cucumber mosaic virus	-	+	n.d.	-
	Pseudomonas syringae pv. *maculicola*	-	+	+	+
	Pseudomonas syringae pv. *tomato*	-	-	+	+

[a] +: required: when impaired ISR is abolished; -: not required: when impaired, ISR is not abolished; -/+: variably required as deduced from different mutants; n.d.: not determined. Data compiled from Iavicoli et al., 2003; Pieterse et al., 1998; Ryu et al., 2003a, 2004a,b.

Whereas *P. fluorescens* WCS417r-triggered ISR was not effective against a virus, *Bacillus amyloliquefaciens* IN937a, *B. pumilus* SE34, *P. fluorescens* 89B-61 and *S. marcescens* 90-166 substantially reduced symptom severity upon infection with cucumber mosaic virus (CMV). In plants treated with strains SE34, 89B-61 and 90-166 CMV accumulation in systemically infected leaves was significantly less than in non-bacterized control plants. Reductions as a result of treatments with strains SE34 or 90-166 were maintained in NahG plants. A SA^- mutant of the SA-producing strain 90-166 still reduced CMV symptom severity and virus accumulation in NahG plants, ruling out induction of resistance through a SA-dependent mechanism. A siderophore $(sid)^-$ mutant of strain 90-166 was likewise unimpaired in its inducing action. Resistance induction was also maintained in *npr1* mutant plants. In contrast, no reduced CMV accumulation occurred in strain 90-166-treated triple-mutant *fad3,7,8* plants, that are unable to accumulate JA. These data indicated that strain 90-166 elicited a SA-independent, JA-dependent ISR against CMV that was independent of NPR1 (Ryu et al., 2004a).

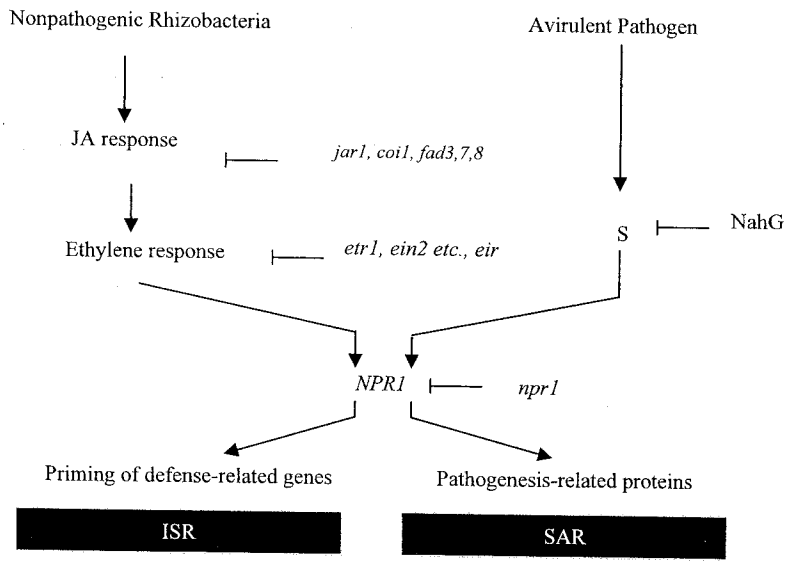

Fig. 1. Signal-transduction pathways leading to pathogen-induced systemic acquired resistance (SAR) and rhizobacterially induced systemic resistance (ISR) (Adapted from Pieterse et al., 1998).

Whereas SAR is associated with accumulation of PRs throughout the plant, no major changes in gene expression occurred either locally in the roots or systemically in the leaves upon induction of ISR by WCS417r (Van Wees et al., 1999). As SA is not involved in WCS417r-mediated ISR, it was not surprising to find that, contrary to SAR, SA-responsive PRs, such as PR-1, PR-2 and PR-5, were not induced. Also the ethylene-inducible hevein gene *Hel*, the ethylene- and JA-responsive genes *ChiB*, encoding a basic chitinase, and *Pdf1.2* (defensin), and the JA-inducible genes *Vsp* (vegetative storage protein), *Lox1*, *Lox2* (lipoxygenase 1 and 2), *Pal1* (phenylalanine ammonia-lyase 1) and *Pin2* (proteinase inhibitor 2) were not expressed, in spite of the involvement of both hormones in ISR. However, pathogen challenge of ISR-expressing plants led to an enhanced level of *Vsp* transcript accumulation.

In contrast, planting Arabidopsis seedlings in soil containing *B. amyloliquefaciens* strain EXTN-1 triggered activation of both *PR-1* and *Pdf1.2* genes. The activation of *PR-1* was abolished in transgenic NahG, and reduced in mutant *npr1* plants, while induction of *Pdf1.2* mRNA was absent in the *jar1*, and

reduced in the *etr1* mutants. These observations implied activation of both SA- and JA-dependent signalling pathways, resembling the result of infection by an avirulent pathogen. However, expression of systemically induced resistance was not verified in this study (Ahn et al., 2002).

Recently, transcriptome analyses by cDNA microarrays, RNA differential display, or subtractive hybridization of cDNA libraries have confirmed the notion that rhizobacteria influence plant gene expression to only limited extents. Analysis of the expression of over 8000 genes of Arabidopsis plants with ISR elicited by WCS417r revealed changes in the expression of 102 genes in the roots on which the bacteria were present. In contrast, systemically in the leaves, none of the genes that were expressed to sufficiently high levels to be reliably detected showed a consistent change, in spite of the fact that, when challenge inoculated, these leaves showed a significant ISR response (Verhagen et al., 2004). Clearly, the roots reacted locally to colonization by the bacteria. Within the first week transient changes were observed in the expression of hundreds of genes, but these were not associated with the persistent state of ISR. Of the 102 genes whose expression was changed over a longer period, 39 were up-regulated and 63 were down-regulated. In view of the ethylene dependency of ISR, an increase in expression of a putative ACC oxidase and down-regulation of ethylene response factor 1 (*Erf1*) and ethylene-responsive element binding factors 1 (*Erebp1*) and 2 (*Erebp2*) are particularly interesting.

Upon challenge inoculation of Arabidopsis plants with Pst, SAR-induced plants showed an augmented expression of SA-dependent *PR-1* mRNA, whereas plants with ISR accumulated mRNA of the JA-inducible gene *Vsp* to higher levels than non-induced plants (Van Wees et al., 1999). This "priming" effect indicated that induced plants activate defense-related gene expression earlier and to a greater extent than non-induced plants (Conrath et al., 2002). Indeed, cDNA microarray analysis of WCS417r-induced plants revealed 81 genes showing an augmented expression pattern in ISR-expressing leaves after challenge inoculation with Pst (Verhagen et al., 2004). Of these, 51 genes were expressed at at least 1.5 times higher levels, including *Vsp*, the JA- and ethylene-responsive gene *Pdf1.2*, a thaumatin-like gene, a chitinase gene, and a gene encoding EREBP2. The other 30 genes showed a Pst-induced change in WCS417r-treated plants only, and appear to be ISR-specific. These included genes that are presumably involved in regulating gene transcription and signal transduction. The majority of the genes were predicted to be regulated by JA or ethylene. Thus, the requirement for JA and ethylene sensitivity in ISR seems to be related to the priming action of defense-related gene expression after challenge inoculation of induced plants.

An independent analysis of Arabidopsis plants with induced resistance against Pst as a result of root colonization by *Pseudomonas thivervalensis* led to contrasting results (Cartieaux et al., 2003). cDNA microarray analysis of

approximately 14300 Arabidopsis genes revealed that the transcript levels in colonized roots were hardly changed relative to axenic control plants, and none were elevated. In contrast, in shoots the levels of 63 transcripts were modified, including 42 genes that were upregulated. Except for a putative chitinase, no indication of increased *PR* gene expression was evident. An intermediate situation seems to apply to Arabidopsis colonized endophytically by *P. fluorescens* FPT9601-T5, which caused transient suppression of disease symptoms of Pst (Wang et al., 2005). FPT9601-T5 suppressed plant growth in early stages of colonization, but promoted it in later stages. Of 22800 Arabidopsis genes, 95 and 105 genes were up- and down-regulated, respectively, in leaves of bacterized plants as compared to control plants. These up-regulated genes are involved in metabolism, signal transduction and stress responses, whereas genes involved in ethylene signalling were suppressed, including EREBP 1 and 2. Thus, both Verhagen et al. (2004) and Wang et al. (2005) observed that the onset of ISR is associated with a reduction in ethylene signaling, indicating that ISR triggered by FPT9601-T5 may partly shares signalling pathways with ISR elicited by strain WCS417r. Induction of resistance against *Erwinia carotovora* in Arabidopsis by *Paenibacillus polymyxa* was associated with increased tolerance to drought and changes in the abundance of mRNAs encoding drought stress- and biotic stress-responsive proteins, consistent with a mild pathogenic effect of *P. polymyxa* on Arabidopsis (Timmusk and Wagner, 1999). These alterations do not seem typical of ISR.

There is comparatively little information on the bacterial determinants that trigger ISR (Bakker et al., 2003). In searches for the bacterial factor(s) that trigger ISR in Arabidopsis, dead bacterial cells of *P. fluorescens* WCS417r were effective in inducing resistance against Pst, be it to a significantly lower level than live cells (Van Wees et al., 1997). Dead cells of a bacterial mutant that lacked the O-antigenic side-chain (OA^-) of the outer membrane lipopolysaccharide (LPS) were ineffective. These results suggested that the OA of the LPS of WCS417r contributes to elicitation of ISR, but may not be sufficient for full induction. More information is available on the determinants of ISR-eliciting *P. putida* WCS358r (Meziane et al., 2005). As shown by mutant analysis and application of isolated components, at least three factors can be active in eliciting ISR: LPS, the pseudobactin siderophore, and flagella. Due to the redundancy of these inducing factors, their relative importance in the induction of live bacteria was not assessed. *P. fluorescens* WCS374 does not induce systemic resistance in Arabidopsis when grown at 28°C. However, when grown at 33°C or 36°C, it triggered ISR in both wild-type and NahG plants, but not in ethylene-insensitive *ein2* or in non-PR-expressing *npr1* mutant plants, irrespective of the growth temperature of the bacteria (Ran et al., 2005b). These results demonstrate that, when grown at elevated temperatures, WCS374 expresses a, so far unidentified, determinant that triggers the ISR pathway in Arabidopsis.

Other rhizobacteria that trigger ISR in Arabidopsis have been shown to act through other determinants. Both *P. fluorescens* CHA0 and *P. fluorescens* Q2-87

elicit ISR through the production of the antibiotic DAPG (Iavicoli et al., 2003; Weller et al., 2004). Siderophores and antibiotics are important for microbial antagonism in the rhizosphere. These factors can thus function in disease suppression through restriction of growth or activity of soilborne pathogens, as well as in the induction of systemic resistance against both soilborne and foliar pathogens. Recently, it was found that certain bacilli, i.e. *B. amyloliquefaciens* IN937a and *B. subtilis* GB03, trigger ISR through a volatile compound, 2,3-butanediol, which is also active in promoting plant growth (Ryu et al., 2003b, 2004b). Probably, related compounds are also active. These results demonstrate that several different components present on, or released by, rhizobacteria can elicit ISR in Arabidopsis.

2.2 Banana

The causal agent of Panama disease, *Fusarium oxysporum* f.sp. *cubense*, is a major problem in banana cultivation and difficult to control. *Pseudomonas fluorescens* strain IIHRPf12 inhibited growth of the fungus in vitro and, when applied as a soil drench 3 days before inoculation of banana tissue culture explants with the pathogen, reduced colonization of the roots by *F. oxysporum* by 72%. This result could be fully explained by antibiosis, but electron microscopy of treated root parts revealed structural changes in the cortical cells, mainly consisting of densely stained amorphous material and cell wall appositions that were associated with enhanced restriction of fungal growth (Mohandas et al., 2004). In a similar study (Thangavelu et al., 2003), *P. fluorescens* strain Pf10 increased peroxidase, PAL, chitinase and β-1,3-glucanase activities and phenolic content in banana leaves, indicating that at least some defense-related activities were enhanced systemically as a result of bacterial application. Moreover, upon challenge inoculation with the pathogen, these defense-related activities were stimulated to a larger extent in bacterized than in non-bacterized plants. These results suggest that part of the protection might be ascribed to ISR.

2.3 Bean and other legumes

An early report describes how bean seeds were subjected to treatments with suspensions of *P. fluorescens* strain S97 and the emerging seedlings were grown for 3-4 weeks (Alström, 1991). The first trifoliate leaf was then challenged with *P. syringae* pv. *phaseolicola* and lesions due to halo blight were recorded in the third trifoliate leaf. The number of lesions decreased with increasing concentration of the induction inoculum, 4.6×10^8 cfu.ml^{-1} reducing the number to 17% of that in non-treated controls. Protection was eliminated when the bacterial suspension was autoclaved, indicating a need for live bacteria for protection to be achieved. S97 exhibited bacteriostatic activity against the bean pathogen and it was suggested that the rhizobacterium might produce substances already during seed germination that were later translocated to the foliage. These inhibitory compounds might accumulate around the site of bacterial multiplication and contribute to their restricted growth.

Thus, antibiosis rather than ISR may have been responsible for the reduction in disease symptoms. Absence of the antagonistic bacteria from the aerial parts of the plants was not checked. However, ISR could have been involved (Alström, 1995). Reduced levels of the challenging pathogen in protected leaves and in the intercellular washing fluid (IWF) from those leaves were correlated with a general rise in protein content in the IWF, and associated with accumulation of certain phenolic compounds. Hence, changes in plant metabolism did occur as a result of prior seed bacterization, but a causal relationship with disease suppression was not established.

Indications for the induction of plant defense responses were also obtained upon root colonization by other fluorescent *Pseudomonas* spp. A *P. aureofaciens* strain induced symptoms of a hypersensitive response on bean cotyledons and in bean suspension cultures, and induced defense proteins resembling the plant's response to pathogens inducing SAR (Zdor and Anderson, 1992). Isolates of *P. tolaasii* and *P. putida* induced only certain acid-soluble proteins. Upon inoculation on cotyledons some accumulation of phytoalexins and phenolics was associated with a slight browning reaction, indicating that the bean plants responded defensively to foliar application of these rhizobacterial species. However, upon root treatment no metabolic changes were apparent, suggesting that the rhizobacteria had only very minor effects on root metabolism. These observations are important in showing that at least some rhizobacterial species have limited pathogenic activity when applied to leaves, and initiate defense reactions also observed in leaves exhibiting SAR. Unfortunately, no attempt was made in this study to relate the metabolic effects seen to protection against pathogens.

The rhizobacterium *P. aeruginosa* 7NSK2 induces resistance to leaf infection by the gray mould fungus *Botrytis cinerea* (De Meyer and Höfte, 1997). Bean seeds were soaked in a bacterial suspension for 5 min prior to planting in soil mixed with the bacterial inoculum also. After plant emergence leaves were wounded by touching with a red hot pinhead to allow *Botrytis* to infect and produce a spreading lesion. Inducing bacteria were never detected in stem and cotyledon extracts of challenged bean leaves, but were present occasionally in extracts of first leaves at levels below 50 cfu.leaf $^{-1}$. 7NSK2 reduced the number of spreading lesions by about one half when suspensions were prepared from iron-limited King's medium B, but not from iron-rich LB medium. Under iron limitation, 7NSK2 produces three siderophores: pyoverdin, pyochelin and SA. By using bacterial mutants deficient in either one or two of these siderophores, it was demonstrated that SA production was essential for the induction of resistance (De Meyer and Höfte, 1997). Because SA is a precursor of pyochelin, a role for pyochelin could not be excluded. Indeed, pyochelin has been shown to be required for 7NSK2-elicited ISR in tomato (Audenaert et al., 2002). 7NSK2-induced resistance was expressed not only against *B. cinerea*, but also against the anthracnose fungus *Colletotrichum lindemuthianum* (Bigirimana and Höfte, 2002). A bacterial mutant, KMPCH, that lacked pyoverdin and pyochelin but still produced SA, induced resistance against anthracnose in a moderately resistant, but not in a susceptible bean cultivar. KMPCH-567, a SA$^-$ mutant of KMPCH, failed to induce resistance,

demonstrating that SA is important for the induction of systemic resistance by 7NSK2. Production of SA and pyochelin was detected during 7NSK2 colonization of bean, indicating that iron limitation occurs in the rhizosphere. These results demonstrate that induction of systemic resistance in bean by 7NSK2 is dependent on iron nutritional state and confirm that specific siderophores function not only in the acquisition of iron by the bacteria, but also in the induction of systemic resistance in the plant.

In a model system with plants grown in perlite, 7NSK2-induced resistance was equivalent to the inclusion of 1 nM SA in the nutrient solution (De Meyer et al., 1999b). Hydroponic feeding of 1 nM SA solution induced PAL activity in roots and increased free SA levels in leaves. In bacterized roots of soil-grown plants no such increases were associated with the elicitation of ISR by 7NSK2. In contrast, KMPCH was as effective as wild-type 7NSK2 in triggering ISR but did stimulate PAL activity. Thus, although these bacteria can stimulate PAL activity in bean roots, an increase in PAL activity is unlikely to be essential for ISR. Neither increased PAL activity, nor elevated SA levels were found in bean plants in which ISR was elicited by seed treatment with the non-pathogenic *P. putida* strain BTP1. Furthermore, no enhanced fungitoxicity was detected in methanolic leaf extracts, suggesting that accumulation of bean phytoalexins was not part of the stimulated defense mechanisms against *B. cinerea*. However, lipoxygenase and hydroperoxide lyase, two key enzymes in the biosynthetic pathway of JA, were significantly stimulated during the first four days after challenging BTP1-treated plants with the pathogen, suggesting that priming of JA-dependent defenses occurred upon challenge inoculation (Ongena et al., 2004). Pyoverdin, pyochelin or SA were not involved in the systemic resistance triggered by BTP1. Nevertheless, most of the resistance-eliciting activity was present in culture fluid when cells were grown under iron-limited conditions, and not associated with killed bacterial cells. However, the nature of the inducing fraction was not determined (Ongena et al., 2002).

Like 7NSK2, *P. putida* WCS358 and *P. fluorescens* WCS417 have been demonstrated to trigger ISR in bean against gray mould and anthracnose, and anthracnose, respectively (Bigirimana and Höfte, 2002; Meziane et al., 2005). Of strain WCS358, both the LPS and the pseudobactin siderophore were effective in eliciting ISR, whereas flagella were not. In agreement with more than one factor acting as inducing determinant, bacterial mutants lacking either pseudobactin or flagellin were still capable of eliciting ISR. However, the OA^- mutant was not, suggesting an interference with siderophore production.

Few data are available for other legume species. An electron microscopic study showed that infection of in vitro grown, *Agrobacterium rhizogenes* T-DNA-transformed hairy pea roots by *Fusarium oxysporum* f.sp. *pisi* was restricted to the outer root cortex when pretreated with *P. fluorescens* strain 63-28 (Benhamou et al., 1996a) or *B. pumilus* strain SE34 (Benhamou et al., 1996b). Lack of ingress of the

fungus toward the vascular stele was associated with deposition onto the inner surface of cell walls of callose-enriched appositions and accumulation of phenolic compounds. Apparently, the rhizobacterial strains enhanced plant defense reactions culminating in the elaboration of physical barriers to fungal spread.

A similar enhancement of the accumulation of defense-related phenolic compounds was observed in pea plants grown from seed bacterized with *P. fluorescens* strain Pf4 or a strain of *P. aeruginosa* and, when 20-25 days old, inoculated with conidia of the powdery mildew fungus *Erysiphe pisi* (Singh et al., 2002). These observations are suggestive of the rhizobacteria acting through a stimulation of a plant defense-related response upon challenge. However, spatial separation of the rhizobacterial inducers and the challenging pathogen and lack of antibiosis between those were not checked. The same two strains, alone or in combination, were also reported to protect chickpea against collar rot caused by the soilborne fungus *Sclerotium rolfsii* (Singh et al., 2003). Protection was associated with increases in specific phenolic acids, including SA, in roots and leaves. Soil drenching with culture filtrate of *S. rolfsii* had similar effects, and a combination of both rhizobacterial strains and culture filtrate induced additive increases. The interpretation of these findings is difficult, but the rhizobacterial strains may have acted as minor pathogens, inducing stress-related phenolic compounds systemically. Seed treatments with seven other *P. fluorescens* strains were likewise found to protect chickpea against collar rot in association with increased accumulation of phenolic acids in the plant (Sarma et al., 2002).

Protection of chickpea against charcoal rot disease caused by the fungus *Macrophomona phaseolina* was obtained upon soil mixing, drenching or root dip with a suspension of *P. fluorescens* isolate 4-92 (Srivastava et al., 2001). Two days were required between induction treatment and challenge inoculation for protection to be manifested. Bacterial treatments increased root glucanase and chitinase activities up to 7-fold after 5 days. Effects were evident already upon bacterization with 10^4 cfu ml^{-1}, and reached a plateau at 10^5 cfu ml^{-1}, similar to the induction of ISR in e.g. radish (Raaijmakers et al., 1995).

Soil drench of 3-4 weeks old white clover plants with 10^5-10^6 cfu ml^{-1} of fluorescent *Pseudomonas* spp. strains P29 and P80, or *B. cereus* strain B1, 1-3 days prior to inoculation with the clover cyst nematode *Heterodera trifolii*, caused morhological abnormalities and reduced fecundity of the nematodes (Kempster et al., 2001). Both live and dead cells of P29, but not cell-free culture filtrate, induced these effects, suggesting that some type of ISR was involved. On the basis of the significantly improved performance of the plants, the induced resistance was taken to be expressed also systemically against the blue-green aphid, *Acyrthosiphon kondoi*, in the while clover plants, as well as in annual medic (*Medicago trunculata*) (Kempster et al., 2002), but the mechanism involved remains unclear.

2.4 Carnation

When *P. fluorescens* WCS417r and the soil-borne fungus *Fusarium oxysporum* f.sp. *dianthi* (Fod) were applied to different parts of carnation plants by treating the roots with the bacterium and introducing the fungus one week later in the stem by slashing, a significant reduction in wilting symptoms was obtained (Van Peer et al., 1991). Bacteria and fungus were confirmed to remain spatially separated, indicating that WCS417r protected carnation against Fod by induction of systemic resistance. In contrast, *P. putida* WCS358 did not enhance resistance of carnation against Fod when the fungus was inoculated in the stem.

Resistance of carnation to Fod is polygenic and associated with the accumulation of phytoalexins (Baayen and Niemann, 1989). No increase in phytoalexins was detectable upon induction of systemic resistance by WCS417r prior to challenge, but upon subsequent inoculation with Fod production of phytoalexins was enhanced (Van Peer et al., 1991). Disease incidence was reduced substantially in the moderately resistant carnation cultivar Pallas and less consistently in the susceptible cultivar Lena. These results indicate that genetically determined differences in the level of protection occur in different cultivars, and confirm that priming of the extant defensive capacity is at the basis of the induced resistance expressed.

In the systemic protection of carnation against Fod by WCS417r, heat-killed bacteria or the purified LPS were as effective in inducing resistance as were live bacteria (Van Peer and Schippers, 1992), thus confirming that the protective effect is plant-mediated. Moreover, these results indicated that the bacterial LPS acts as a determinant of resistance induction by WCS417r in carnation.

2.5 Cucumber

Ninety-four rhizobacterial strains previously shown to reduce disease incidence caused by *Pythium ultimum* or *Rhizoctonia solani*, or to promote seedling emergence and crop yield, were screened for induction of systemic resistance in cucumber against the fungus *Colletotrichum orbiculare*, the causal agent of leaf anthracnose (Wei et al., 1991). Bacteria were coated on the seeds and colonized roots to high levels, and none of the strains was recovered from surface-disinfested petioles when the second true leaf of each plant was challenge inoculated 21 days after planting. Out of the 94 strains, six reduced lesion size after challenge inoculation with the fungus by 33-75% relative to the non-bacterized control, compared to a reduction of 84% when SAR was induced by the pathogen itself. Five of the bacterial strains also significantly reduced lesion numbers. Antagonism in vitro to *C. orbiculare* on three media was generally absent with five of the strains, and weak with one strain. Using spontaneous rifampicin-resistant (rif^r) mutants for treating seeds, some strains were recovered from inside surface-disinfested roots. However, none of the inducing strains was found in leaves challenged with *C. orbiculare*. Thus, while some bacteria which induced systemic resistance may have limited internal colonization and behave as endophytes, they did not appear to translocate to a significant extent or colonize challenged leaves,

suggesting that neither competition nor antibiosis were involved in the observed protection (Wei et al., 1991, 1992).

Subsequently, seed treatment with two different rhizobacterial strains, *P. putida* 89B-27 and *Serratia marcescens* 90-166 was shown to induce systemic resistance against vascular wilt disease, caused *F. oxysporum* f.sp. *cucumerinum* (Foc) (Liu et al., 1995a), angular leaf spot due to the foliar bacterium *P. syringae* pv. *lachrymans* (Psl) (Liu et al., 1995b), bacterial wilt provoked by *Erwinia tracheiphila* (Kloepper et al., 1993), systemic mosaic disease-inducing cucumber mosaic virus (CMV) (Raupach et al., 1996), and *C. orbiculare* (Liu et al., 1995c). In the study on the protection against fusarium wilt, a split-root assay was used in which the inducing bacteria and the pathogen were inoculated on separate halves of seedling roots at the same time, and then planted in separate pots. ISR was expressed by delayed disease symptom development, lesser disease severity and reduced number of dead plants compared to non-bacterized controls, reflecting reduced spread of the pathogen in the plant. Movement of inducing bacteria was monitored with a bioluminescent derivative of 89B-27, which was detected with a charge-coupled device camera. The bacteria showed only limited movement within inoculated pots and did not migrate to the pots in which the pathogen was inoculated, demonstrating that the bacteria and the pathogen remained spatially separated (Liu et al., 1995a). Upon elicitation of ISR by *P. fluorescens* 89B-61 or *S. marcescens* 90-166 and challenge inoculation with *C. orbiculare*, cytological studies using fluorescence microscopy revealed a higher frequency of autofluorescent cells, which are related to accumulation of phenolic compounds at the sites of fungal penetration. Callose deposition was also often observed in epidermal cells. These observations are suggestive of an impediment of fungal penetration in the ISR-expressing plants (Jeun et al., 2004).

Treatment of seeds or cotyledons with either *P. putida* 89B-27 or *Serratia marcescens* 90-166 also resulted in substantial decreases in the number and size of lesions upon challenge inoculation of the second leaves with the bacterium Psl. Upon injection of cotyledons with 0.1 ml of 10^{10} cfu.ml^{-1}, the inducing bacterial strain reached population densities of 10^{11} or 10^{12} cfu.cotyledon^{-1} 3 days later. However, neither bacterial strain was recovered from stems 1 or 2 cm above or below the inoculated cotyledons at any sampling time. Associated with reductions of about 60% in total Psl lesion area pathogen populations in inoculated leaves also were significantly reduced in the rhizobacterial treatments. The level of protection afforded was similar to that upon induction of SAR by the pathogens *C. orbiculare* or Psl itself (Liu et al., 1995b). In field trials involving bacterized seeds, naturally occurring bacterial wilt, caused by *E. tracheiphila*, was also significantly reduced (Kloepper et al., 1993).

Testing for ISR against CMV revealed that both rhizobacterial strains reduced the number of plants with symptoms. Protected plants did not develop mosaic symptoms throughout the experimental period, whereas pathogen-induced SAR was expressed as a delay in symptom development (Bergstrom et al., 1982). No viral antigen was detected by enzyme-linked immunosorbent assay in any

asymptomatic bacteria-treated plants (Raupach et al., 1996), indicating that due to the inducing bacteria the plant had become refractory to viral infection.

When strains 89B-27 or 90-166 were inoculated on seeds prior to planting and plants were thereupon challenged with *C. orbiculare*, 89B-27 reduced mean total lesion diameter up to 60%, whereas 90-166 was often significantly less effective. Populations of 89B-27 declined at a consistent rate over time, dropping from over 10^8 cfu.g^{-1} root one week after planting to 10^3 cfu.g^{-1} 3 weeks later. ISR was evident at the first-leaf stage, increased over time, and was maintained at least to the fifth-leaf stage more than 5 weeks after seeding. Strain 90-166 showed population dynamics similar to 89B-27. ISR was somewhat more variable but also generally observed at all leaf stages tested. The decline in population densities of the two strains on the roots did not match the extent of ISR, supporting the notion that once induced, systemic resistance in the plant is maintained (Liu et al., 1995c).

Strain 90-166-mediated ISR was dependent on low-iron conditions, but independent of SA, as four different SA$^-$ mutants were as effective in inducing ISR against *C. orbiculare* as wild-type bacteria, whereas an ISR$^-$ mutant still produced SA (Press et al., 1997). In contrast, a Tn*5-phoA* insertion into the *entA* gene, which encodes an enzyme in the biosynthetic pathway of catechol siderophores, eliminated the ability of strain 90-166 to elicit ISR. Although total population densities of 90-166 and its mutant on cucumber roots were similar, the internal population size of the mutant was significantly lower (Press et al., 2001). However, it is questionable whether internal colonization is a decisive factor in the elicitation of ISR. Rather, the data support a role for a catechol siderophore as the bacterial determinant responsible for triggering ISR in cucumber.

Cultivar specificity of ISR was studied in one resistant and three susceptible cucumber cultivars. Strain 89B-27 induced systemic resistance in all three susceptible cultivars, whereas 90-166 did so in only two. Both strains failed to increase resistance in the resistant cultivar. No cultivar specificity in root colonization patterns by the two strains was observed, ruling out the possibility that failure of the strains to enhance protection was due to impaired root colonization. Apparently, protection in the genetically highly resistant cultivar could not be further enhanced by the rhizobacterium (Liu et al., 1995c). These data confirm that rhizobacterial strains differ in their inducing properties and that plant cultivars differ in inducibility.

The observations that in cucumber two different fungi, the root-infecting Foc and the foliar pathogen *C. orbiculare*, and two leaf-spotting bacteria, Psl and *E. tracheiphila*, were controlled by the same bacterial strains through the induction of systemic resistance indicates that rhizobacterially-mediated ISR in cucumber is effective against different pathogens in a similar way as in Arabidopsis. Moreover, ISR appeared to also reduce insect attack. Cucumber plants growing in the field

from seeds treated with *B. pumilus* strain INR7, *Flavomonas oryzihabitans* strain INR5, *P. putida* 89B-61 or *S. marcescens* 90-166 supported lower numbers of the spotted cucumber beetle, *Diabrotica undecimpunctata*, and the striped cucumber beetle, *Acalymna vittatum* (Zehnder et al., 1997a,b). As a result cucumber plant growth and yield were significantly greater. Bacterial treatment also resulted in greater beetle control than weekly applications of insecticide. The inducing bacteria appeared to reduce the level of the secondary compound cucurbitacin in the plants, which acts as a feeding stimulant to the beetles. This observation is particularly interesting because it shows that induction of systemic resistance by these rhizobacterial strains is associated with a defined change in plant metabolism.

Three different rhizobacterial strains, *P. corrugata* 13, *P. fluorescens* C15 and *P. aureofaciens* 63-28 were shown to induce a systemic resistance in cucumber against crown rot-causing *Pythium aphanidermatum* (Zhou and Paulitz, 1994; Chen et al., 1998). The strains were applied to one half of the root system spatially separated from the pathogen-inoculated root part by splitting the root systems in rock wool in two separate pots. Bacterial population densities were maintained at about 10^5 cfu.g^{-1} dry rockwool in the bacterized pots. Systemic resistance was expressed as a significant decrease in the numbers of attaching and germinating zoospores. Occurrence of stem rot was delayed for 4-6 days and disease index was reduced, but application of the bacteria to one pot one week before inoculation of the other pot with the fungus did not reduce disease to the same degree as when both were applied at the same time. This result is puzzling in view of the common observation that time is needed to achieve the induced state. The possibility that the rhizobacteria produced antifungal metabolites that were transported to the opposite root system was discounted on the grounds that these bacterial isolates did not inhibit mycelial growth of *P. aphanidermatum* in vitro. In the split-root system, both *P. corrugata* strain 13 and *P. aureofaciens* strain 63-28 increased PAL, peroxidase and polyphenoloxidase activity both locally and systemically. Moreover, additional peroxidase isoenzymes were induced, indicating that the cucumber roots reacted to colonization by these rhizobacterial strains with increases in defense-related enzymes (Chen et al., 2000).

Both strains were shown to produce SA in liquid medium and also induce cucumber roots to accumulate endogenous SA one day after bacterial treatment. More SA accumulated in roots treated with bacteria than in distant roots on the opposite site of the root system in the first two days, but this difference disappeared after 3-4 days (Chen et al., 1999). It appears unlikely that this SA accumulation was involved in ISR, because exogenously applied SA failed to induce local or systemic resistance against challenging *P. aphanidermatum*, and the mechanisms involved have remained unclear.

P. putida BTP1, as well as a derivative impaired in siderophore production likewise protected cucumber against *P. aphanidermatum* root rot. In analyses of root extracts from split-root experiments, this protection was found to be associated with a systemic accumulation of several phytoalexin-like compounds. The nature

of these compounds appeared to be different in leaves as compared to roots, suggesting that the defense response to *Pythium* induced by this strain is not based on a single antifungal factor (Ongena et al., 1999, 2000). These reports are among the few that provide evidence for a defined induced response that is active against the pathogen involved.

In a similar split-root system the suppressive activity of three different types of compost on root rot caused by *P. ultimum* or *P. aphanidermatum* was analysed (Zhang et al., 1996). Mean root rot severity was significantly less for split roots in fungus-infested peat mix paired with compost-amended mixes than in infested peat mix paired with non-infested peat mix without compost. Moreover, 3 weeks after planting, anthracnose due to inoculation of the second leaf with *C. orbiculare* was significantly less severe on plants grown in compost-amended mixes than in peat mix. Plants grown in the compost-amended mixes showed enhanced leaf peroxidase activity, indicative of a stress response. Inoculation with *C. orbiculare* dramatically increased the activity of the major peroxidase isoenzyme in plants grown in peat mix, but this increase was even greater in plants grown in the compost-amended mixes. Initially, β-glucanase activity was low, but when infected with *C. orbiculare*, this activity was induced to significantly higher levels in plants grown in the compost mix than in plants grown in the peat mix (Zhang et al., 1998). This situation is similar to the priming action of defense responses in challenge-inoculated ISR-expressing plants.

ISR triggered by *Pseudomonas chlororaphis* strain O6 upon root colonization of cucumber against target leaf spot, caused by *Corynespora cassiicola*, was associated with a faster and stronger accumulation of transcripts of six distinct genes upon challenge inoculation, as revealed through subtractive hybridization (Kim et al., 2004). Expression of these genes was not induced by O6 colonization alone, and became apparent only after challenge with the pathogen. These results are in line with the priming of defense responses upon challenge inoculation of induced plants, as also observed in e.g. Arabidopsis and carnation. A similar amplification of defense reactions was seen after challenge inoculation with *P. ultimum* of cucumber plants that were endophytically colonized by *Serratia plymuthica*. Light and transmission electron microscopy of root tissues revealed that invading hyphae of the pathogen were trapped in a phenolic-enriched material, which infiltrated and filled moribund hyphae. Cell wall appositions containing callose, pectin, cellulose and phenolics at sites of potential pathogen invasion were suggestive of a coordinated induction of structural and biochemical barriers which adversely affect pathogen growth and development (Benhamou et al., 2000).

Several *Bacillus* spp. have been demonstrated to elicit ISR in cucumber. *B. pumilus* INR7 was reported to elicit ISR against anthracnose and angular leaf spot (Raupach and Kloepper, 1998). When applied as a seed coating singly or in combination with *B. subtilis* GB03 and *Curtobacter flaccumfaciens* ME1 under

greenhouse conditions, there was a general trend towards greater suppression and enhanced consistency of protection against anthracnose, angular leaf spot and cucubit wilt when using strain mixtures. In how far ISR contributed to disease suppression was not determined, however. Seed treatments with *B. amyloliquefaciens* IN937a or *B. pumilus* IN937b suppressed symptoms of CMV in cotyledons of challenged plants, suggestive of induced resistance. Mixtures of the two strains or with strains *B. pumilus* INR7, SE34, SE49 or T4, or *B. sphaericus* SE56 were equally or even more effective under both greenhouse (Jetiyanon and Kloepper, 2002) and field conditions (Jetiyanon et al., 2003).

2.6 Pepper

Eighteen isolates of *P. fluorescens* and two of *P. putida* were evaluated for their ability to control damping off caused by *Pythium apanidermatum* in hot pepper. Seeds bacterized with the effective *P. fluorescens* isolate Pf1 and sown in sterilized potting medium mixed with pathogen inoculum developed into substantially more vigorous plants than non-bacterized controls. Disease was reduced by 60%. Earlier and increased activities of PAL, peroxidase and polyphenol oxidase were observed in Pf1-pretreated plants. Moreover, higher accumulation of phenolics was noticed (Ramamoorthy et al., 2002a). These results were interpreted as evidence for ISR, but no spatial separation between the bacterium in the seed coating and the pathogenic fungus was imposed. The increased vigour of the bacterized plants was referred to as increased plant growth promotion, and may have resulted from an action of Pf1 unrelated to disease suppression, or due to suppression of *P. aphanidermatum* through microbial antagonism. The latter was not checked. The increase in the defense-related enzyme activities may have been the consequence of the improved plant development, or of a specific stimulation by the biocontrol agent that may, or may not, be related to ISR. This study exemplifies the difficulties encountered when interpreting mechanisms from studies aimed at the identification of effective disease-suppressing rhizobacterial isolates that were not designed to specifically study involvement of ISR. However, similar results were reported associated with the reduction of chilli fruit rot and die-back incited by *Colletotrichum capsici* as a result of treatments with strain Pf1 or an isolate of *Bacillus subtilis* (Bharathi et al., 2004). Bacteria were applied to the soil, whereas conidia of the pathogen were sprayed on the leaves, suggesting that the effect might indeed be due to ISR.

At least absence of in vitro antibiosis was checked in a study on systemic suppression of pepper anthracnose, caused by *Colletotrichum gloeosporioides*, by seed bacterization with several *Bacillus* spp. Treatments with seven individual strains and eighteen mixtures all caused significant reduction of lesion development on fruits, resulting in smaller lesions than on nontreated control plants (Jetyanon and Kloepper, 2002). Although absence of biocontrol bacteria on the fruits was not checked, it seems likely that in this case ISR was involved.

2.7 Pines and other tree species

Seed treatment of loblolly pine with *Bacillus pumilus* strains INR7, SE34 or SE52, or *Serratia marcescens* 90-166 increased the capacity to resist gall formation by the causal agent of fusiform rust, *Cronartium quercuus* f.sp. *fusiforme*. Averaged over two years, 31% of control seedlings inoculated with the pathogen one month after sowing were infected, whereas treatment with the bacterial strains reduced infection to 15, 13, 16 and 14%, respectively. Although spatial separation of the bacteria and the rust was not checked, it appears that systemic resistance was induced (Enebak and Carey, 2000). This report is of interest because it shows protection in a gymnosperm tree.

Induction of systemic resistance in seedlings of *Eucalyptus urophylla* was found when *P. putida* WCS358 or *P. fluorescens* WCS374 were infiltrated into two lower leaves and the causal agent of bacterial wilt, the soilborne bacterium *Ralstonia solanacearum*, was inoculated 3-7 days later on the decapitated stem. ISR was evident as a reduction in the length of the stem from the top that blackened and decayed (Ran et al., 2005a). However, no resistance was induced when the biocontrol strains were applied to the soil and, thus, ISR was evident only under an unnatural condition.

2.8 Potato

In spite of the importance of potato as a food crop, no data are available on rhizobacteria-mediated ISR against pathogenic fungi, bacteria or viruses. However, for nematode control, in split-root experiments *Bacillus sphaericus* strain B43 and *Rhizobium etli* G12 were found to reduce penetration by the cyst nematode *Globodera pallida* up to 37% (Hasky-Günther et al., 1998; Reitz et al., 2000). Heat-killed bacterial cells were equally active. The ISR-eliciting factor of B43 was present in culture filtrate, wheras G12 was shown to trigger ISR through its LPS. Oligosaccharides of the core region of the LPS were the main factor in triggering ISR (Reitz et al., 2002). ISR was not associated with enhanced accumulation of PRs or increased lignification of root cells (Reitz et al., 2001), leaving the resistance mechanism(s) involved to be determined.

2.9 Radish

Out of 538 rhizobacterial strains isolated from roots of radish seedlings grown in compost-amended potting mixes, eleven suppressed bacterial leaf spot caused by Xca, when applied individually as inoculum in non-inducing potting mixes. Reduction in symptom severity varied between 8 and 22% and was assumed to be the result of ISR (Krause et al., 2003).

Using the two-compartment, separate inoculation rockwool system described for Arabidopsis, *P. fluorescens* strains WCS374 and WCS417 induced ISR against For, reducing disease incidence by up to 50%. ISR was expressed primarily as a reduction in the percentage of diseased plants, apparently resulting from a more frequent failure of the fungus to reach or colonize the vascular tissue (Hoffland et al., 1995). Unlike in

Arabidopsis, *P. putida* WCS358 was not active, but another *P. putida* strain, RE8, was as effective as *P. fluorescens* WCS417 (De Boer et al., 1999). Strain WCS374 induced a similar level of resistance in six cultivars ranging from highly susceptible to relatively resistant, provided fungal inoculum pressure was adjusted to cause between 40 and 80% of the plants in non-bacterized controls to become diseased. Apparently, at higher inoculum doses the resistance induced was insufficient to block disease development, whereas at low disease incidence the effect of the inducing bacteria was too small to be significant (Leeman et al., 1995a). Differences in inducibility would have been recorded, though, if all cultivars had been inoculated with the same dose of the fungus.

At least one day was necessary after application of at least 10^5 cfu.g^{-1} talcum powder for significant resistance to be induced. No increase in resistance was evident when the dosage of bacteria was increased. Root colonization of the bacteria was determined in root macerates using immunofluorescence colony-staining, and found to be maintained at levels above 10^5 cfu.g^{-1} root for at least 3 weeks (Leeman et al., 1995a). A minimum of 10^5 cfu.g^{-1} root were required for effective protection, and no disease suppression was evident when colonization of the roots did not reach this level. However, no relationship was apparent between the remaining rhizosphere population density of WCS374 and disease incidence at later stages, when protection was evident (Raaijmakers et al., 1995). Thus, the initial triggering of the plant leads to its induced state and, once this has been reached, further protection appears independent of the remaining population of the inducing bacterium in the rhizosphere.

Resistance was effective not only against For, but also against avirulent Pst, *Alternaria brassicicola* and a different isolate of *F. oxysporum* (Hoffland et al., 1996). The same results were obtained when the inducing rhizobacterium was applied spatially separated on the roots, or to a single leaf different from the ones used for challenge inoculation. The level of protection achieved was at least as high as that afforded by SAR induced by the pathogen Pst itself. The rhizobacteria remained confined to the induction site and resistance was expressed while bacterial numbers on the induction site decreased with time (Hoffland et al., 1996).

The bacterial LPS appeared to be the main trait responsible for resistance induction (Leeman et al., 1995b). Cell wall extracts of WCS374 or WCS417, or purified LPS were as effective as live bacteria when applied to radish roots. Bacterial OA$^-$ mutants, as well as their cell wall extracts, were ineffective. These results rule out any protective effects resulting from bacterial metabolism and show that the specificity within the structure of the O-antigenic side chain of the LPS determines the induction of systemic resistance by these rhizobacteria in radish. The resistance-inducing OA of WCS374 was effective not only on roots, but also when applied to the cotyledons. Hence, the plant is able to perceive this bacterial determinant not only at the root, but also at the leaf surface.

When similar experiments were conducted under iron-limiting conditions by adjusting the composition of the nutrient solution, the OA$^-$ mutants of WCS374 and

WCS417 were found not to be impaired in their ability to induce resistance (Leeman et al., 1996). Indeed, ISR reached levels equivalent to those induced by the wild type strains. These results indicated that (an)other bacterial determinant(s) expressed only under low-iron conditions induced resistance. Since siderophores are produced by the bacteria under these conditions, the pyoverdin-type pseudobactins of the strains were applied to radish roots. The purified siderophore of WCS374 induced resistance to the same level as the bacterial LPS. In contrast, the siderophore of WCS417 did not. Moreover, the sid$^-$ mutants of both WCS374 and WCS417 induced resistance under conditions of iron deficiency as well as in the presence of excess iron, and again to a level similar to that reached upon application of the wild type bacteria. Although the siderophore of WCS374 could induce resistance, it did not seem to be responsible for the induction under low-iron conditions. Thus, (an)other iron-regulated metabolite(s) appeared to be involved. Apparently, different bacterial determinants are equally able to induce systemic resistance in radish, but these effects seem complementary rather than additive and full induction by one factor masks any contribution by the others.

Unlike *P. aeruginosa* 7NSK2 (De Meyer and Höfte, 1997), WCS374 and WCS417 do not produce an additional siderophore of the pyochelin type. However, at low iron availablity they do produce SA in vitro, amounting to about 50 and 10 μg SA ml^{-1} standard succinate medium (SSM) for WCS374 and WCS417, respectively (Leeman et al., 1996). The production of SA decreased rapidly with increasing iron concentration. WCS374 is able to produce a second, SA-containing siderophore, pseudomonine (Mercado-Blanco et al., 2001), and it is possible that SA produced in the rhizosphere is channelled into pseudomonine, and pseudomonine could act as the additional iron-dependent inducing determinant. However, this hypothesis has not been tested so far.

The non-SA-producing strain *P. putida* WCS358 did not induce resistance under low-iron conditions. Commercial SA induced resistance in radish against Fod when applied to roots at concentrations as low as 100 $fg.g^{-1}$ talcum emulsion. It cannot be excluded, therefore, that low levels of SA released by bacteria in the rhizosphere induce the systemic resistance. Moreover, a fair correlation was found to exist between the capacity of WCS374 and WCS417 to produce SA in vitro and the ability of these strains to trigger ISR in vivo under low-iron conditions (Leeman et al., 1996).

Treatment of radish leaves with avirulent pathogens or millimolar concentrations of SA induced SAR and PR-homologues of the families PR-1, -2 and -5 (Hoffland et al., 1995). However, neither root-applied low doses of SA, nor the rhizobacterial strains triggering ISR induced PRs under either high- or low-iron conditions (Hoffland et al., 1995, 1996). While strong resistance was induced by the rhizobacteria and their OA$^-$-mutants under low-iron conditions, SA was apparently not present in sufficient quantities to induce PRs. Under high-iron conditions, SA is even less likely to be produced and resistance is induced by the wild-type strains only through their LPS.

Therefore, the role of SA in the induction of systemic resistance by these rhizobacterial strains in radish remains to be determined.

2.10 Rice and other monocots

Grain crops are the most widely cultivated plants for world food supply but very little research on ISR in monocots has been documented so far. In rice, two *P. fluorescens* strains, Pf1 and FP7, were reported to inhibit mycelial growth of the sheath blight fungus *Rhizoctonia solani* (Radjacommare et al., 2004) and to increase seedling vigour when applied as a seed coating, by root dipping, by mixing through the soil, or as a foliar spray (Nandakumar et al., 2001). Inoculation with *R. solani* was performed by placing sclerotia in the sheath of the rice plants 40 days after planting. Treatments by the single strains or their combination effectively reduced sheath blight incidence, promoted plant growth, and ultimately increased yield, under glasshouse or field conditions, comparable to the fungicide carbendazim. In those experiments in which the bacteria were applied by root dipping or mixing through the soil, it is possible that the reduction in disease incidence could be ascribed to ISR. In treated plants chitinase and peroxidase activities were enhanced and increased more strongly upon challenge inoculation than in non-treated plants (Nandakumar et al., 2001; Radjacommare et al., 2004). However, the level of increase varied between treatments, *Pseudomonas* strains used and treatment duration, even though disease index was reduced to similar extents. Absence of bacteria on the plants was not checked, the increased vigour of treated plants may have reduced infection by the fungus, and antibiotics produced by the bacteria may have contributed to disease reduction. This makes it difficult to interpret the significance of ISR for the decrease in sheath blight incidence. Similar results were obtained by Madhaiyan et al. (2004) by imbibing rice seeds in a suspension of *Methylobacterium* sp. strain PPFM-Os-7. This bacterium increased plant phenolics content, PAL, chitinase, β-1,3-glucanase and peroxidase activities when sprayed upon the leaves. These conditions are artefactual and should be considered local effects rather than caused by induction of systemically induced resistance.

Seed treatment or root dipping with strains Pf1 and FP7, singly and even more so in combination, also protected rice against damage due to feeding by larvae of the leaffolder *Cnaphalocrocis medinalis*. Larval and pupal weight were reduced and larval mortality was increased. Moreover, increased populations of hymenopterous parasitoids and predatory spider mites acting as natural enemies of the leaffolder, were noticed in *Pseudomonas*-treated plots under field conditions (Radja Commare et al., 2002). Thus, the bacterized plants appeared to express both enhanced direct and indirect defenses against this insect pest.

LacZY-marked *P. fluorescens* strain 7-14 and *P. putida* strain V14i were applied either as a seed treatment or as a root dip and found to suppress rice blast disease, caused by the fungus *Magnaporthe grisea*, by about 25%. Based on the expression of the marker gene, bacteria remained spatially separated from the pathogen, indicating that disease reduction was caused by ISR (Krisnamurthy and Gnanamanickam, 1998).

Similarly, seed treatment, root dipping or soil amendment with either one out of four strains of *P. fluorescens* or various combinations of two out of five *Bacillus* spp. with chitosan as a carrier promoted growth and advanced time of flowering of pearl millet and reduced the incidence of downy mildew caused by *Sclerospora graminicola* under greenhouse and field conditions. Resistance induced by the most effective *P. fluorescens* isolate required a minimum of three days to build up and was sustained throughout the plant's life (Niranjan Raj et al., 2003a,b).

Induced resistance was likewise found to be a mechanism in tall fescue (*Festuca arundinacea*) elicited by the bacterium *Lysobacter enzymogenes* strain C3 and effective against leaf spot development caused by *Bipolaris sorokiniana* and leaf blight caused by *R. solani*. Heat-inactivated cells were likewise active, ruling out an effect of antibiosis. Application of live or heat-killed cells to leaves resulted only in localized resistance, but treatment of roots elicited systemic resistance expressed in the foliage. Both live and heat-killed cells, when applied to the roots, increased peroxidase activity to similar extents in the foliage, demonstrating that the plant reacted systemically to the local application of bacterial components (Kilic-Ekici and Yuen, 2003). Two other strains of *L. enzymogenes* were ineffective in triggering ISR, whereas *Bacillus pumilus* INR7 and *P. fluorescens* 89B-61 and WCS417r elicited ISR against leaf spot rapidly, with reductions being apparent one day after root treatment. *Bacillus* spp. strains SE34, IN937a and IN937b were only slightly active, while strain T4 was ineffective. All bacterial strains colonized tall fescue roots to a similar degree. Increases in peroxidase activity in leaves upon root treatment were too variable to allow any conclusion about a possible correlation between ISR and peroxidase activity (Kilic-Ekici and Yuen, 2004).

In field experiments with sugarcane, seed treatment during planting and two following soil applications with various strains of *P. fluorescens* (Pf1, EP1, VTP4, VTP10, CHA0) or *P. putida* strain KKM1 reduced red rot disease when the pathogen *Colletotrichum falcatum* was inoculated at the third internode from the base of the stalk some time later. Yield of cane and sugar were significantly enhanced. Protection was higher in susceptible sugarcane cultivars than in moderately resistant ones. Four strains were also tested and found suppressive to natural infection under field conditions (Viswanathan and Samiyappan, 2002a). Treated canes had enhanced peroxidase and PAL activities and lowered catalase activity, particularly upon challenge inoculation, and had a higher lignin content (Viswanathan and Samiyappan, 2002b). In addition, increases in chitinases, β-1,3-glucanases and thaumatin-like proteins upon bacterization with strain KKM1 were also recorded (Viswanathan et al., 2003), suggesting that the bacterium induced a systemic resistance associated with the induction of PRs. However, it is not clear whether these differences were related to ISR or to the enhanced growth and development of the plants.

2.11 Tobacco

At least eight of the ten major PRs induced in tobacco in response to pathogens causing hypersensitive necrosis, were found in the IWF of leaves of plants grown in autoclaved soil in the presence of *P. fluorescens* strain CHA0 (Maurhofer et al., 1994). Strain CHA0 was reisolated from the roots of the plants but could not be detected in stems or leaves. Six weeks after the bacteria had been added to the soil, plants were challenged by inoculation with tobacco necrosis virus (TNV). Both numbers of viral lesions and lesion diameter were reduced to the same extent as in plants with SAR due to a previous infection with TNV itself. Strain CHA400, a sid$^-$ mutant of CHA0, was still able to induce PRs but showed only partial resistance to TNV, implicating the involvement of the pyoverdin siderophore of CHA0 in the induction of resistance against TNV. Root colonization of the plants with CHA0 or CHA400, as well as leaf infection with TNV, caused up to five-fold increases in SA in the leaves. These observations suggested that SA was involved in the ISR elicited by CHA0 in tobacco, with induction being at least partly determined by the siderophore of CHA0. However, the transposon insertion generating the sid$^-$ mutation in CHA400 was not localized, and it is not clear whether the loss of pyoverdin is the only mutation in CHA400.

Besides pyoverdin, CHA0 also produces SA under low-iron conditions (Meyer et al., 1992). Therefore, it is not clear whether the increase in SA in the bacterized plants was the result of induction by the bacteria or of synthesis of SA by the plant, or whether the plant takes up bacterial SA and translocates it to the leaves. CHA0 behaves as an endophyte and produces several toxic metabolites, among which are DAPG, pyoluteorin and hydrogen cyanide (Maurhofer et al., 1995). Because tobacco plants are sensitive to these antibiotics, these can cause stress to the plant, resulting in the induction of stress-related defense responses. A bacterial transformant over-producing DAPG and pyoluteorin protected tobacco roots significantly better than the wild type against the black root rot-causing fungus *Thielaviopsis basicola*, but at the same time drastically reduced the growth of the plants. Because CHA0 was seldom found in contact with the mycelium of *T. basicola* despite reducing the extent of black root rot, and the physical integrity of the fungal hyphae in direct contact with cells of CHA0 were not affected, it was inferred that secondary metabolites involved in the antagonism to the fungal pathogen also induced resistance in the roots (Troxler et al., 1997). Although the antibiotics suppressed black root rot through inhibition of fungal growth, there was no correlation between the sensitivity of various pathogens to the synthetic antibiotics and the degree of disease suppression by the transformants, suggesting that the plants exhibited an antibiotic stress-induced resistance resembling SAR (Maurhofer et al., 1995; Troxler et al., 1997). However, protection against TNV and against *T. basicola* may not be caused by the same mechanism. Introduction of the SA biosynthetic gene cluster *pchDCBA* from *P. aeruginosa* PAO1 (Serino et al., 1995) under a constitutive promoter into strain CHA0 did not increase its suppression of TNV or black root rot in tobacco. However, introduction into the non-SA-producing strain P3, leading to the production of 0.8 μg SA.10^9 cfu^{-1}, made this poor biocontrol agent an effective suppressor of TNV lesion formation. In contrast, suppression of black root rot was not improved (Maurhofer et al., 1998).

The SA-producing rhizobacterial strain *S. marcescens* 90-166 induced resistance to the wildfire bacterium *P. syringae* pv. *tabaci*, but mini-*Tn5 phoA* mutants, which did not produce detectable amounts of SA, induced resistance to the same level (Press et al., 1997). Thus, bacterial SA did not appear to be involved in the ISR induced by this strain in tobacco against the wildfire bacterium. Yet, when tested in a microtiter plate assay, strain 90-166 induced the *PR-1a* promoter in transgenic tobacco seedlings containing the *GUS* gene as a reporter, similar to SA (Park and Kloepper, 2000). In this assay, surface-sterilized tobacco seeds were germinated in Murashige & Skoog medium and 7 days later treated with bacteria, effectively bathing the seedlings in the bacterial suspension. *Enterobacter asburiae* strain JM-22, *P. putida* 89B-61 and *Burkholderia gladioli* IN-26 induced *GUS* expression likewise, while *Clavibacter michiganensis* TE-5, *Bacillus pumilus* strains T4, SE-34, SE-49, SE76 and INR7 and *P. fluorescens* 89B-027 did not. Infiltration of greenhouse-grown tobacco leaves with the same strains resulted in significant increases in GUS activity after treatment with all of the tested strains, including T4 and TE5, although the level of GUS activity in plants treated with the latter two strains was lower than in plants treated with strains JM-22, 89B-61, 90-166 and IN-26. When bacteria were applied as a root drench, only T4 and JM-22 induced significant GUS activity in leaves. These data demonstrate that local application of ISR-eliciting bacteria to leaves can induce PRs, whereas ISR elicited by treatment of the roots is not consistently associated with *PR*-gene activation. Indeed, tobacco was significantly protected against *P. syringae* pv. *tabaci* by root treatments with strains T4, JM-22, 89B-61, 90-166 or IN-26. However, these strains did induce stronger GUS expression than non-inducing strains in the microtiter plate assays (Park and Kloepper, 2000).

The relationship between SA and ISR was explored further by assaying strains 90-166, SE34, 89B-61, T4 and *B. pasteurii* C-9 for ISR against blue mould of tobacco, caused by *Peronospora tabacina*, in microtiter plates and detached leaf assays, as well as greenhouse tests. ISR was confirmed by checking for absence of bacteria on challenged leaves. Elicitation of ISR among strains varied in the different types of assays and on different tobacco cultivars, stressing the need for reliable assays for assessing ISR (Zhang et al., 2002a). In the microtiter plate assay, levels of endogenous free SA in tobacco seedlings treated with strains 90-166, 89B-61 and SE 34 increased significantly during the first week after bacterization, but in the second week were substantially lower than in non-treated seedlings. Upon challenge inoculation of plants treated with SE34, levels of SA peaked strongly one day after challenge. Such an increase was absent upon challenge of plants treated with 90-166 or 89B-61. Moreover, the three strains were similarly effective in reducing blue mould severity in both Xanthi-nc and transgenic NahG tobacco. Since SA is effectively broken down in NahG plants, these results indicate that the ISR against *P. tabacina* was SA-independent (Zhang et al., 2002b). In such a case, SA-inducible PRs would not be expected to occur in ISR-expressing plants and the induction of the *PR-1a* promoter by some of the strains remains to be explained.

Abundant induction of PR-like proteins was described in tobacco plants that were treated with LPS from an endophytic strain of *Burkholderia cepacia*, associated with a protective effect against black shank disease, caused by the oomycete *Phytophthora nicotianae*. As a result of the treatment with the LPS, cell permeability was slightly increased and viability decreased, suggesting that at the high concentration used (100 $\mu g\ ml^{-1}$) toxic side effects may have contributed (Coventry and Dubery, 2001). The LPS triggered transient phosphorylation of a 43-kDa (ERK-like) mitogen-activated protein (MAP) kinase within a few minutes, marking a rapid response associated with elicitation (Piater et al., 2004). MAP kinase phosphorylation cascades mediate innate immunity responses in animals, suggesting parallels between induction of defense responses in animals and plants.

No PR-1 expression was associated with ISR against tobacco mosaic virus (TMV) induced by *P. aeruginosa* strain 7NSK2, in spite of the demonstration that resistance was abolished in NahG plants (De Meyer et al., 1999a). A reduction in TMV lesion diameter was also evident after root application of mutant KMPCH, the pyoverdin- and pyochelin-deficient derivative of 7NSK2, ruling out an involvement of the pseudobactin or the SA-containing pyochelin siderophores in triggering ISR. ISR was no longer observed for mutant MPFMI-569, which is additionally deficient in SA production. Thus, SA production is essential for 7NSK2-elicited ISR against TMV and appears to be the only determinant for ISR in KMPCH. However, in grafting experiments in which NahG tobacco was used as rootstock to which bacteria were applied, and non-transformed tobacco plants served as scion for testing ISR, 7NSK2 and KMPCH did elicit ISR against TMV. It must be assumed that any SA produced by the bacteria was converted to inactive catechol by the SA-hydroxylase in NahG plants and, thus, the absence of expression of ISR in intact NahG plants must be due to a dependency of 7NSK2-elicited ISR on plant-derived SA accumulation. The amount of SA produced seems to be sufficient for inducing resistance but too low to induce detectable levels of PR-1 (De Meyer et al., 1999a). Nevertheless, the results remain puzzling and suggest that induced resistance against viruses is expressed differently from induced resistance against fungi and bacteria.

ISR triggered by *B. amyloliquefaciens* EXTN-1 against pepper mild mottle virus was manifested by strong reductions in systemic mosaic symptoms and viral RNA accumulation. Protection was associated with increased transcript levels of *PR-1a*, *PAL* and 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGR*). Upon challenge inoculation with the virus, expression of these genes was enhanced in both inoculated and non-inoculated leaves of induced plants (Ahn et al., 2002), but a dependence on SA was not checked. Together, these data suggest that tobacco is more prone to react to ISR-eliciting rhizobacteria by expressing PRs than Arabidopsis. However, systemic protection induced by *Pseudomonas chlororaphis* strain O6 against *P. syringae* pv. *tabaci* and the soft rot bacterium *Erwinia carotovora* subsp. *carotovora* was maintained in NahG plants (Spencer et al., 2003). In leaves of bacterized plants no induction of PR-1a occurred, but transcripts of JA- and ethylene-inducible *PR-1g*, *Hmgr* and *Lox* accumulated, indicating that the bacterium activated the JA/

ethylene-dependent response pathway. Accordingly, ethylene-insensitive tobacco transformants did not display ISR against *E. carotovora*. However, resistance against *P. syringae* pv. *tabaci* was maintained in this genotype. Hence, the requirements for the expression of ISR against different pathogens are clearly different.

2.12 Tomato

In tomato, many strains of rhizobacteria have been tested for induction of ISR against various diseases. Using a split-root system, *P. fluorescens* WCS417r was found to induce resistance against *F. oxysporum* f.sp. *lycopersici* (Fol) (Duijff et al., 1998), the level of which appeared related to the presence of the OA of the LPS. ISR was associated with a thickening of cortical cell walls in tomato roots if epidermal or hypodermal cells were colonized densely by the bacterium (Duijff et al., 1997).

P. fluorescens 89B-27 and *S. marcescens* 90-166, when inoculated onto seeds, reduced disease severity of CMV challenge inoculated on the first pair of leaves three weeks after planting (Raupach et al., 1996). Combination of *B. subtilis* GB03 and either one of the *B. pumilus* strains SE34, INR7 or T4, or *B. amyloliquefaciens* IN937a or *B. subtilis* IN937b, formulated with the carrier chitosan and mixed through potting medium, all substantially slowed CMV accumulation and reduced the number of plants developing symptoms as well as symptom severity, and promoted plant growth and yield in greenhouse experiments (Murphy et al., 2003). Seed bacterization with three strains (SE34, IN937a and IN937b) was likewise effective under field conditions (Zehnder et al., 2000). Under these conditions, development of tomato mottle virus symptoms was also retarded (Murphy et al., 2000). In view of the intimate relationship between viral multiplication and plant metabolism, it is unlikely that these reductions in viral diseases were caused by a mechanism other than ISR, even though SE34 was reported to move upwards from the soil and colonize the phyllosphere (Yan et al., 2002, 2003).

The three strains, alone or in combination, were also effective in suppressing bacterial wilt, caused by *Ralstonia solanacearum* (Jetiyanon and Kloepper, 2002) and variably reduced southern blight caused by *Sclerotium rolfsii* (Jetiyanon et al., 2003). No in vitro antibiosis was observed, suggesting the mechanism to be ISR. Bacterial wilt was likewise suppressed when transplants at the time of seeding and one week prior to challenge were treated with *P. putida* 89B-61 or a combination of strains IN937a and *B. subtilis* GB03 ("BioYield") (Anith et al., 2004).

When incorporated into soilless growth media, strains 89B-61 as well as SE34 were also active in eliciting ISR against late blight, caused by the oomycete *Phytophthora infestans*. By testing NahG tomato, the ethylene-insensitive mutant *Nr*, and the JA-insensitive mutant *def1*, it was established that the induced protection by both strains was SA-independent, but ethylene- and JA-dependent (Yan et al., 2002). 89B-61 was not detected on leaves of bacterized plants, implying that systemic protection was due to ISR. In contrast, SE34 colonized the leaves to a population

density of 10^6 cfu g^{-1}. Presumably, antibiosis was not involved because the bacteria and *P. infestans* did not antagonize each other in vitro. Plant-mediated protection appears to apply because the resistance was abolished in the *Nr* and *def1* genotypes. However, because SE34 was present at high densities on the leaves, protection could have resulted from localized rather than systemically induced resistance (Yan et al., 2003).

Bacterization of tomato seeds with a strain of *Bacillus cereus* retarded development of late blight, early blight (*Alternaria solani*) and septoria leaf spot (*Septoria lycopersici*) under field conditions. Disease severity was reduced by a marginal 13, 19 and 18%, respectively, and fruit yield was increased (Silva et al., 2004b). ISR was inferred, but not proven. Other bacterial strains (*B. cereus* B 101 R and B 212 K and the actinomycete A 068 R) were reported to elicit ISR against Pst, and in greenhouse bioassays reduced lesion formation by *A. solani*, *Corynespora cassiicola* (foliar blight), *Oidium lycopersici* (powdery mildew), *Stemphylium solani* (leaf spot) and *Xanthomonas campestris* pv. *vesicatoria* (Xcv) (bacterial spot) to different extents. Increases in peroxidase and lipoxygenase activities were detected in foliar extracts of bacterized plants, both before and after challenge, whereas no significant increases in PAL activity were evident (Silva et al., 2004a). Another isolate, *B. cereus* UFV101, when grown in modified Simmon's liquid culture produced a factor in the medium that did not inhibit *A. solani*, Pst, *C. cassiicola*, Xcv, *P. syringae* pv. *syringae*, *Pseudomonas corrugata*, *R. solanacearum* or *Clavibacter michiganensis* in vitro, yet when applied to tomato roots, reduced lesion numbers of Pst, Xcv, *A. solani* or *C. cassiicola* inoculated on the leaves (Romeiro et al., 2005). The nature of the elicitor(s) present was not clarified, however.

Earlier and increased activities of PAL, peroxidase and polyphenoloxidase activity, as well as high accumulation of phenolics were recorded in roots from tomato plants that were seed-treated with *P. fluorescens* strain Pf1 and challenged with *Pythium aphanidermatum* or Fol. Similarly, β-1,3-glucanase and chitinase were increased and thaumatin-like proteins accumulated at higher levels in bacterized plants and after challenge with Fol. No spatial separation between the bacteria and the pathogen was present and Pf1 antagonized mycelial growth of *P. aphanidermatum* in vitro. Although bacterized roots reacted more strongly to the pathogen than control roots, the evidence appears insufficient to claim that ISR was involved, as antibiotics can have some toxicity to plant roots. However, a priming effect on defense-related enzyme activities after challenge was apparent and may, or may not, be responsible for the reductions in damping-off and vascular wilt afforded by the bacterial treatment (Ramamoorthy et al., 2002a,b).

Split root trials were performed to demonstrate ISR against damping-off caused by *Rhizoctonia solani* upon root-dip treatment or soil drench with *P. aeruginosa* IE-65 or *P. fluorescens* CHA0. Root infection by *R. solani* as a result of inoculation of the soil one week after bacterial treatment was about halved. Dead bacterial cells were similarly active. Culture filtrate was likewise active, suggesting that the bacterial cells released inducing compounds into the medium. These data

provide good proof of ISR being responsible for the disease reduction and imply induction by bacterial components that still need to be defined (Siddiqui and Shaukat, 2002b). Both rhizobacterial strains also induced resistance against the root knot nematode *Meloidogyne javanica* (Siddiqui and Shaukat, 2002a). The culture filtrate of CHA0, as well as of *P. aeruginosa* 7NSK2, caused significant mortality of juveniles of the nematode (Siddiqui and Shaukat, 2004), suggesting that the effect was due to a toxic compound. However, in split-root systems application of the bacterial cell suspension to one half of the root system lowered the population of the root knot nematode in the non-bacterized, nematode-treated other half, indicating that both rhizobacterial strains elicited ISR against attack by the root knot nematode. A similar level of ISR was attained in wild-type and in NahG tomato, confirming observations that mutants of 7NSK2 and CHA0 that lacked or overproduced SA did not influence bacterial efficacy to cause nematode death, and indicating that SA-independent ISR was involved (Siddiqui and Shaukat, 2004). A derivative of CHA0 that overexpressed the antibiotics DAPG and pyoluteorin triggered ISR more strongly than wild-type bacteria, whereas a *GacA* mutant deficient in antibiotic production, did not elicit ISR at all (Siddiqui and Shaukat, 2003). These results implicate antibiotic production by CHA0 as the mechanism of induction of systemic resistance in tomato against the nematode *M. javanica*, similar to the ISR triggered by this strain in Arabidopsis against *Peronospora parasitica* and Pst (Iavicoli et al., 2003; Weller et al., 2004).

In contrast, ISR triggered by 7NSK2 against *Botrytis cinerea* was lost in NahG plants and SA-lacking bacterial mutants were no longer capable of eliciting ISR in tomato (Audenaert et al., 2002). Mutant KMPCH, a producer of SA but not of the SA-containing siderophore pyochelin, induced PAL activity, whereas wild-type 7NSK2 did not, suggesting that in the wild type SA is converted into pyochelin. Pyochelin by itself did not elicit ISR. A mutant lacking the ability to produce the antibiotic pyocyanin was likewise impaired in triggering ISR. However, when the latter mutant and the SA- and pyochelin-lacking 7NSK2-562 were combined, the combination of both mutants did induce ISR. These results indicated that 7NSK2 elicited ISR through the combined action of the SA-containing siderophore pyochelin and the antibiotic pyocyanin, probably through a toxic action of reactive oxygen species (Audenaert et al., 2002). Strain *P. putida* WCS358 also elicited ISR in tomato against *B. cinerea*. Through mutant analysis and application of purified components it was established that this strain triggered ISR through its pseudobactin siderophore and its LPS (Meziane et al., 2005).

Except for the occasional increases in defense-related proteins and enzymes, little information is available on the mechanisms that are responsible for the expression of ISR against different pathogens in tomato. Mpiga et al. (1997) reported that tomato plants treated with *P. fluorescens* strain 63-28 allowed less ingress of *Fusarium oxysporum* f.sp. *radicis-lycopersici*, the causal agent of tomato

crown and root rot. Light and electron microscopic analyses showed a restriction of the fungus to the outer root tissues with preferential localization in the intercellular spaces, accumulation of electron-dense material in epidermal and cortical cells and elaboration of callose-enriched wall appositions at sites of attempted fungal penetration. These features indicate a priming of defense responses against the fungus, which appears characteristic of induced resistance. Enhanced defenses against other pathogens in ISR-expressing tomato may similarly depend on priming of basal resistance responses against the various pathogens that have been tested.

3. CONCLUDING REMARKS

In the last few years, many studies have reported on ISR triggered by various root-colonizing bacteria in several dicotyledonous and some monocotyledonous plant species. Due to the diversity of plant species, rhizobacterial strains and pathogens used, it is difficult to draw general conclusions, except that triggering of ISR is not uncommon. This raises the question why, if all plants in nature host high numbers of bacteria on their roots, plants do not seem to be naturally induced already (Tuzun and Kloepper, 1995). However, only selected strains of rhizobacteria appear to be able to trigger ISR. Moreover, in those cases where dose-response relationships for ISR induction have been established, a minimum of 10^{5} cfu g^{-1} root appears to be necessary for triggering ISR (Raaijmakers et al., 1995). Because of the enormous diversity of microorganisms on plant roots, this number is unlikely to be reached by any individual strain under natural conditions.

Strains seem to differ considerably in the determinants that are recognized by plant roots and give rise to ISR (Preston, 2004). Depending on bacterial strain and plant species, LPS, different types of siderophores, flagella, antibiotics or volatile alcohols have been shown to be capable of eliciting ISR, sometimes even in a plant species-specific manner (Van Loon and Bakker, 2005). This diversity implies that plants must possess intricate mechanisms for recognizing specific bacterial components. In Arabidopsis, a receptor-like kinase with a structure similar to that of several major resistance (*R*) genes has been shown to bind a common domain in bacterial flagellins (Gómez-Gómez, 2004), but its linkage to systemically induced resistance has not been well established. For none of the other bacterial inducing determinants is it known how perception is achieved.

Some rhizobacterial strains have been shown to elicit ISR in several plant species, others in some but not all species that have been studied. It may be that strains triggering ISR in several plant species possess more than a single determinant that can be recognized, i.e. *P. putida* WCS358r is recognized through at least its LPS, pseudobactin siderophore and flagella in Arabidopsis, bean and tomato (Meziane et al., 2005), but does not elicit ISR in carnation and radish. Otherwise, widely effective strains may possess an evolutionarily conserved determinant that is strongly recognized by many plant species. LPS is often considered to act as such a determinant and, indeed, has been found for many strains to be an important inducing

Table 2. Demonstrated effectiveness of specific rhizobacterial strains in induction of systemic resistance in at least three plant species[a].

Bacterial strain	*Plant species*
Bacillus amyloliquefaciens IN937a	Arabidopsis, cucumber, pearl millet, pepper, tomato
Bacillus pumilus INR7	Cucumber, tall fescue, tomato
Bacillus pumilus SE34	Arabidopsis, cucumber, pearl millet, pepper, pine, tobacco, tomato
Bacillus pumilus T4	Arabidopsis, cucumber, pearl millet, pepper, tobacco, tomato
Bacillus subtilis GB03	Arabidopsis, cucumber, pearl millet, tomato
Bacillus subtilis IN937b	Cucumber, pearl millet, pepper, tomato
Pseudomonas aeruginosa 7NSK2	Arabidopsis, bean, tobacco, tomato
Pseudomonas fluorescens CHA0	Arabidopsis, sugarcane, tobacco, tomato
Pseudomonas fluorescens Pf1	Pepper, rice, sugarcane, tomato
Pseudomonas fluorescens WCS417	Arabidopsis, bean, carnation, radish, tall fescue, tomato
Pseudomonas fluorescens 89B-61	Arabidopsis, cucumber, tall fescue, tobacco, tomato
Pseudomonas putida WCS358	Arabidopsis, bean, tomato
Serratia marcescens 90-166	Arabidopsis, cucumber, pine, tobacco

[a] See main text for references.

factor in more than one plant species. Yet, the structure of the LPS can vary substantially, and strains possessing LPS that is recognized by one plant species but not by another, do occur, e.g. *P. fluorescens* WCS374 elicits ISR in radish through its LPS but under the same conditions does not elicit ISR in Arabidopsis (Van Wees et al., 1997). A listing of strains that have been found to be effective in several plant species is presented in Table 2.

In those systems in which the signal-transduction pathway of ISR has been studied by making use of mutant and transgenic plants, almost all inducing bacteria have been shown to elicit ISR through a SA-independent route (Van Loon and Bakker, 2005). Thus, rhizobacterially-induced ISR is different from SA-dependent SAR. Indeed, whereas SAR is usually most effective against biotrophic pathogens, ISR is often more effective against necrotrophic pathogens, and the combination of SAR and ISR can afford greater protection against a wider range of pathogens (Van Wees et al., 2000; Ton et al., 2002a). Only ISR triggered by *P. aeruginosa* strain 7NSK2 has been demonstrated to be dependent on SA. For ISR in tomato, SA is required for the production of the siderophore pyochelin, which, in conjunction with the antibiotic pyocyanin, triggers ISR (Audenaert et al., 2002). Moreover, results from tobacco indicate that 7NSK2-elicted ISR involves SA accumulation in planta (De Meyer et al., 1999a). So far, these results have not been confirmed for other plant species, and the mechanisms involved remain to be clarified. SA-independent ISR in different rhizobacterium-plant-pathogen combinations has been shown to be variably dependent on ethylene and/or JA perception, and these variations also need to be investigated in more detail.

Recent gene expression studies have revealed a remarkable variation in the number of genes that are activated in plants upon bacterization by different rhizobacterial strains. As shown by Verhagen et al. (2004), Arabidopsis plants can be in the state of ISR without expressing any changes in gene transcripts in induced leaves. Thus, one should be cautious not to link any alterations in gene expression, protein accumulation or enzyme activities to ISR unless a causal connection can be firmly established. Only after challenge inoculation, defense responses are boosted. This has been observed in several studies and points to the importance of priming of resistance mechanisms in ISR. In this way, invading pathogens can be resisted earlier and to a greater extent than in non-induced plants. This enhanced defensive capacity appears the main characteristic of rhizobacterially induced systemic resistance (Conrath et al., 2002). Elucidation of the priming phenomenon will provide further insight into how the recognition of various bacterial determinants by plant roots is channeled through a largely common JA- and ethylene-dependent signalling pathway towards the state of attentive alert in the plant.

4. REFERENCES

Ahn, I.P., Park, K. and Kim, C.H., 2002. Rhizobacteria-induced resistance perturbs viral disease progress and triggers defense-related gene expression. Mol. Cells 13:302-308.

Albrecht, C., Geurts, R. and Bisseling, T., 1999. Legume nodulation and mycorrhizae formation; two extremes in host specificity meet. EMBO J. 18:281-288.

Alström, S., 1991. Induction of disease resistance in common bean susceptible to halo blight bacterial pathogen after seed bacterization with rhizosphere pseudomonads. J. Gen. Appl. Microbiol. 37:495-501.

Alström, S., 1995. Evidence of disease resistance induced by rhizosphere pseudomonad against *Pseudomonas syringae* pv. *phaseolicola*. J. Gen. Appl. Microbiol. 41:315-325.

Anith, K.N., Momol, M.T., Kloepper, J.W., Marois, J.J., Olson, S.M. and Jones, J.B., 2004. Efficacy of plant growth-promoting rhizobacteria, acibenzolar-S-methyl, and soil amendment for integrated management of bacterial wilt on tomato. Plant Dis. 88:669-673.

Audenaert, K., Pattery, T., Cornelis, P. and Höfte, M., 2002. Induction of systemic resistance to *Botrytis cinerea* in tomato by *Pseudomonas aeruginosa* 7NSK2: role of salicylic acid, pyochelin, and pyocyanin. Mol. Plant-Microbe Interact. 15:1147-1156.

Baayen, R.P. and Niemann, G.J., 1989. Correlations between accumulation of dianthramides, dianthalexin and unknown compounds, and partial resistance to *Fusarium oxysporum* f.sp. *dianthi* in eleven carnation cultivars. J. Phytopathol. 126:281-292.

Bakker, P.A.H.M., Ran, L.X., Pieterse, C.M.J. and Van Loon, L.C., 2003. Understanding the involvement of rhizobacteria-mediated induction of systemic resistance in biocontrol of plant diseases. *Can. J. Plant Pathol.* 25:5-9.

Barea, J.M., Azcon, R. and Azcon-Aguilar, C., 2002. Mycorrhizosphere interactions to improve plant fitness and soil quality. Antonie van Leeuwenhoek Int. J. Gen. Mol. Microbiol. 81:343-351.

Benhamou, N., Bélanger, R.R. and Paulitz, T.C., 1996a. Induction of differential host responses by *Pseudomonas fluorescens* in *Ri* T-DNA-transformed pea roots after challenge with *Fusarium oxysporum* f.sp. *pisi* and *Pythium ultimum*. Phytopathology 86:1174-1185.

Benhamou, N., Kloepper, J.W., Quadt-Hallman, A. and Tuzun, S., 1996b. Induction of defense-related ultrastructural modifications in pea root tissues inoculated with endophytic bacteria. Plant Physiol. 112:919-929.

Benhamou, N., Gagné, S., Le Quéré, D. and Dehbi, L., 2000. Bacterial-mediated induced resistance in cucumber: beneficial effect of the endophytic bacterium *Serratia plymuthica* on the protection against infection by *Pythium ultimum*. Phytopathology 90:45-56.

Bergstrom, G.C., Johnson, M.C. and Kuc, J., 1982. Effects of local infection of cucumber by *Colletotrichum lagenarium*, *Pseudomonas lachrymans*, or tobacco necrosis virus on systemic resistance to cucumber mosaic virus. Phytopathology 72:922-926.

Bharathi, R., Vivekananthan, R., Harish, S., Ramanathan, A. and Samiyappan, R., 2004. Rhizobacteria-based bio-formulations for the management of fruit rot infection in chillies. Crop Prot. 23:835-843.

Bigirimana, J. and Höfte, M., 2002. Induction of systemic resistance to *Colletotrichum lindemuthianum* in bean by a benzothiadiazole derivative and rhizobacteria. Phytoparasitica 30:159-168.

Brederode, F.T., Linthorst, H.J.M. and Bol, J.F., 1991. Differential induction of acquired resistance and PR gene expression in tobacco by virus infection, ethephon treatment, UV light and wounding. Plant Mol. Biol. 17:1117-1125.

Cao, H., Bowling, S.A., Gordon, A.S. and Dong, X., 1994. Characterization of an *Arabidopsis* mutant that is nonresponsive to inducers of systemic acquired resistance. Plant Cell 6:1583-1592.

Cao, H., Glazebrook, J., Clarke, J.D., Volko, S. and Dong, X.N., 1997. The Arabidopsis *NPR1* gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. Cell 88:57-63.

Cartieaux, F., Thibaud, M.C., Zimmerli, L., Lessard, P., Sarrobert, C., David, P., Gerbaud, A., Robaglia, C., Somerville, S. and Nussaume, L., 2003. Transcriptome analysis of *Arabidopsis* colonized by a plant-growth promoting rhizobacterium reveals a general effect on disease resistance. Plant J. 36:177-188.

Chen, C., Bélanger, R.R., Benhamou, N. and Paulitz, T.C., 1998. Induced systemic resistance (ISR) by *Pseudomonas* spp. impairs pre- and post-infection development of *Pythium aphanidermatum* on cucumber roots. Eur. J. Plant Pathol. 104:877-886.

Chen, C., Bélanger, R.R., Benhamou, N. and Paulitz, T.C., 1999. Role of salicylic acid in systemic resistance induced by *Pseudomonas* spp. against *Pythium aphanidermatum* in cucumber roots. Eur. J. Plant Pathol. 105:477-486.

Chen, C., Bélanger, R.R., Benhamou, N. and Paulitz, T.C., 2000. Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. Physiol. Mol. Plant Pathol. 56:13-23.

Collmer, A., Badel, J.L., Charkowski, A.O., Deng, W.L., Fouts, D.E., Ramos, A.R., Rehm, A.H., Anderson, D.M., Schneewind, O., Van Dijk, K. and Alfano, J.R., 2000. *Pseudomonas syringae* Hrp type III secretion system and effector proteins. Proc. Natl. Acad. Sci. USA 97:8770-8777.

Conrath, U., Pieterse, C.M.J. and Mauch-Mani, B., 2002. Priming in plant-pathogen interactions. Trends Plant Sci. 7:210-216.

Coventry, H.S. and Dubery, I.A., 2001. Lipopolysaccharides from *Burkholderia cepacia* contribute to an enhanced defensive capacity and the induction of pathogenesis-related proteins in *Nicotianae tabacum*. Physiol. Mol. Plant Pathol. 58:149-158.

Delaney, T.P., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gut-Rella, M., Kessmann, H., Ward, E. and Ryals, J., 1994. A central role of salicylic acid in plant disease resistance. Science 266:1247-1250.

De Boer, M., Van der Sluis, I., Van Loon, L.C. and Bakker, P.A.H.M., 1999. Combining fluorescent *Pseudomonas* spp. strains to enhance suppression of fusarium wilt of radish. Eur. J. Plant Pathol. 105:201-219.

De Meyer, G. and Höfte, M., 1997. Salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 induces resistance to leaf infection by *Botrytis cinerea* on bean. Phytopathology 87:588-593.

De Meyer, G., Audenaert, K. and Höfte, M., 1999a. *Pseudomonas aeruginosa* 7NSK2-induced systemic resistance in tobacco depends on *in planta* salicylic acid accumulation but is not associated with PR1a expression. Eur. J. Plant Pathol. 105:513-517.

De Meyer, G., Capieau, K., Audenaert, K., Buchala, A., Métraux, J.-P. and Höfte, M. 1999b. Nanogram amounts of salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 activate the systemic acquired resistance pathway in bean. Mol. Plant-Microbe Interact. 12:450-458.

Delaney, T., Friedrich, L. and Ryals, J., 1995. *Arabidopsis* signal transduction mutant defective in chemically and biologically induced disease resistance. Proc. Natl. Acad. Sci. USA 92:6602-6606.

Duijff, B.J., Gianinazzi-Pearson, V. and Lemanceau, P., 1997. Involvement of the outer membrane lipopolysaccharides in the endophytic colonization of tomato roots by biocontrol *Pseudomonas fluorescens* strain WCS417r. New Phytol. 135:325-334.

Duijff, B.J., Pouhair, D., Olivain, C., Alabouvette, C. and Lemanceau, P., 1998. Implication of systemic induced resistance in the suppression of fusarium wilt of tomato by *Pseudomonas fluorescens* WCS417r and by nonpathogenic *Fusarium oxysporum* Fo47. Eur. J. Plant Pathol. 104:903-910.

Enebak, S.A. and Carey, W.A., 2000. Evidence for induced systemic protection to fusiform rust in loblolly pine by plant growth-promoting rhizobacteria. Plant Dis. 84:306-308.

Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H. and Ryals, J., 1993. Requirement of salicylic acid for the induction of systemic acquired resistance. Science 261:754-756.

Gianinazzi-Pearson, V., 1996. Plant cell responses to arbuscular mycorrhizal fungi: getting to the roots of the symbiosis. Plant Cell 8:1871-1883.

Gianinazzi-Pearson, V., Dumas-Gaudot, E., Gollotte, A, Tahiri-Alaoui, A. and Gianinazzi, S., 1996. Cellular and molecular defence-related root responses to invasion by arbuscular mycorrhizal fungi. New Phytol. 133:45-57.

Glazebrook, J., Rogers, E.E. and Ausubel, F.M., 1996. Isolation of *Arabidopsis* mutants with enhanced disease susceptibility by direct screening. Genetics 143:973-982.

Glick, B.R., Patten, C.L., Holguin, G. and Penrose, D.M., 1999. Biochemical and Genetic Mechanisms Used by Plant Growth Promoting Bacteria. Imperial College Press, London.

Gómez-Gómez, L., 2004. Plant perception systems for pathogen recognition and defence. Mol. Immunol. 41:1055-1062.

Hammerschmidt, R. and Kuc, J., 1995. Induced Resistance to Disease in Plants. Kluwer Acad. Publ., Dordrecht.

Handelsman, J. and Stabb, E.V., 1996. Biocontrol of soilborne plant pathogens. Plant Cell 8:1855-1869.

Harrison, M.J., 1998. Development of the arbuscular mycorrhizal symbiosis. Curr. Opin. Plant Biol. 1:360-365.

Hasky-Günther, K., Hoffmann-Hergarten, S. and Sikora, R.A., 1998. Resistance against the potato cyst nematode *Globodera pallida* systemically induced by the rhizobacteria *Agrobacterium radiobacter* (G12) and *Bacillus sphaericus* (B43). Fundam. Appl. Nematol. 21:511-517.

He, S.Y., Jin, Q.-L., Thilmony, R., Nomura, K., Zwiesler-Vollick, J., Bandyopadhyay, S., Hauck, P., Hu, W., Plovanich-Jones, A., Lu, M. and Bray, E., 2002. The molecular basis of *Pseudomonas syringae* pathogenesis in susceptible *Arabidopsis thaliana* plants. In: Biology of Plant-Microbe Interactions, Vol. 3, Leong, S.A., Allem, C. and Triplett, E.W. (eds), Int. Soc. Mol. Plant-Microbe Interact., St. Paul, MN, pp. 129-134.

Hoffland, E., Pieterse, C.M.J., Bik, L. and Van Pelt, J.A., 1995. Induced systemic resistance in radish is not associated with accumulation of pathogenesis-related proteins. Physiol. Mol. Plant Pathol. 46:309-320.

Hoffland, E., Hakulinen, J. and Van Pelt, J.A., 1996. Comparison of systemic resistance induced by avirulent and non-pathogenic *Pseudomonas* species. Phytopathology 86:757-762.

Hynes, R.K., Hill, J., Reddy, M.S. and Lazarovits, G., 1994. Phytoalexin production by wounded white bean (*Phaseolus vulgaris*) cotyledons and hypocotyls in response to inoculation with rhizobacteria. Can. J. Microbiol. 40:548-554.

Iavicoli, A., Boutet, E., Buchala, A. and Métraux, J.-P., 2003. Induced systemic resistance in *Arabidopsis thaliana* in response to root inoculation with *Pseudomonas fluorescens* CHA0. Mol. Plant-Microbe Interact. 16:851-858.

Jetiyanon, K. and Kloepper, J.W., 2002. Mixtures of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. Biol. Control 24:285-291.

Jetiyanon, K., Fowler, W.D. and Kloepper, J.W., 2003. Broad-spectrum protection against several pathogens by PGPR mixtures under field conditions in Thailand. Plant Dis. 87:1390-1394.

Jeun, Y.C., Park, K.S., Kim, C.H., Fowler, W.D. and Kloepper, J.W., 2004. Cytological observations of cucumber plants during induced resistance elicited by rhizobacteria. Biol. Control 29:34-42.

Kempster, V.N., Davies, K.A. and Scott, E.S., 2001. Chemical and biological induction of resistance to the clover cyst nematode (*Heterodera trifolii*) in white clover (*Trifolium repens*). Nematology 3:35-43.

Kempster, V.N., Scott, E.S. and Davies, K.A., 2002. Evidence for systemic, cross-resistance in white clover (*Trifolium repens*) and annual medic (*Medicago trunculata* var *trunculata*) induced by biological and chemical agents. Biocontrol Sci. Technol. 12:615-623.

Kilic-Ekici, O. and Yuen, G.Y., 2003. Induced resistance as a mechanism of biological control by *Lysobacter enzymogenes* strain C3. Phytopathology 93:1103-1110.

Kilic-Ekici, O. and Yuen, G.Y., 2004. Comparison of strains of *Lysobacter enzymogenes* and PGPR for induction of resistance against *Bipolaris sorokiniana* in tall fescue. Biol. Control 30:446-455.

Kim, M.S., Kim, Y.C. and Cho, B.H., 2004. Gene expression analysis in cucumber leaves primed by root colonization with *Pseudomonas chlororaphis* O6 upon challenge-inoculation with *Corynespora cassiicola*. Plant Biol. 6:105-108.

Kloepper, J.W., Tuzun, S., Liu, L. and Wei, G., 1993. Plant growth-promoting rhizobacteria as inducers of systemic disease resistance. In: Pest Management: Biologically Based Technologies, Lumsden, R.D. and Vaughn, J.L. (eds), Amer. Chem. Soc, Washington DC, pp. 156-165.

Kloepper, J.W., Leong, J., Teintze, M. and Schroth, M.N., 1980. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286:885-886.

Kluepfel, D.A., 1993. The behavior and tracking of bacteria in the rhizosphere. Annu. Rev. Phytopathol. 31:441-472.

Knoester, M., Pieterse, C.M. J., Bol, J.F. and Van Loon, L.C., 1999. Systemic resistance in Arabidopsis induced by rhizobacteria requires ethylene-dependent signaling at the site of application. Mol. Plant-Microbe Interact. 12:720-727.

Krause, M.S., De Ceuster, T.J.J., Tiquia, S.M., Michel Jr., F.C., Madden, L.V. and Hoitink, H.A.J., 2003. Isolation and characterization of rhizobacteria from composts that suppress the severity of bacterial leaf spot of radish. Phytopathology 93:1292-1300.

Krishnamurthy, K. and Gnanamanickam, S.S., 1998. Induction of systemic resistance and salicylic acid accumulation in *Oryza sativa*, L. in the biological suppression of rice blast caused by treatments with *Pseudomonas* spp. World J. Microbiol. Biotechnol. 14:935-937.

Kuc, J., 1982. Induced immunity to plant disease. Bioscience 32:854-860.

Lamb, T.G., Tonkyn, D.W. and Kluepfel, D.A., 1996. Movement of *Pseudomonas aureofaciens* from the rhizosphere to aerial plant tissue. Can. J. Microbiol. 42:1112-1120.

Lavelle, P. and Spain, A.V., 2001. Soil Ecology, Kluwer Acad. Publ., Dordrecht.

Lawton, K.A., Potter, S.L., Uknes, S. and Ryals, J., 1994. Acquired resistance signal transduction in *Arabidopsis* is ethylene independent. Plant Cell 6:581-588.

Leeman, M., Van Pelt, J.A., Den Ouden, F.M., Heinsbroek, M., Bakker, P.A.H.M. and Schippers, B., 1995a. Induction of systemic resistance by *Pseudomonas fluorescens* in radish cultivars differing in susceptibility to fusarium wilt, using a novel bioassay. Eur. J. Plant Pathol. 101:655-664.

Leeman, M., Van Pelt, J.A., Den Ouden, F.M., Heinsbroek, M., Bakker, P.A.H.M. and Schippers, B., 1995b. Induction of systemic resistance against fusarium wilt of radish by lipopolysaccharides of *Pseudomonas fluorescens*. Phytopathology 85:1021-1027.

Leeman, M., Den Ouden, F.M., Van Pelt, J.A., Dirkx, F.P.M., Steijl, H., Bakker, P.A.H.M. and Schippers, B., 1996. Iron availability affects induction of systemic resistance against fusarium wilt of radish by *Pseudomonas fluorescens*. Phytopathology 86:149-155.

Liu, L., Kloepper, J.W. and Tuzun, S., 1995a. Induction of systemic resistance in cucumber against fusarium wilt by plant growth-promoting rhizobacteria. Phytopathology 85:695-698.

Liu, L., Kloepper, J.W. and Tuzun, S., 1995b. Induction of systemic resistance in cucumber against angular leaf spot by plant growth-promoting rhizobacteria. Phytopathology 85:843-847.

Liu, L., Kloepper, J.W. and Tuzun, S., 1995c. Induction of systemic resistance in cucumber by plant growth-promoting rhizobacteria: duration of protection and effect of host resistance on protection and root colonization. Phytopathology 85:1064-1068.

M'piga, P., Bélanger, R.R., Paulitz, T.C. and Benhamou, N., 1997. Increased resistance to *Fusarium oxysporum* f.sp. *radicis-lycopersici* in tomato plants treated with the endophytic bacterium *Pseudomonas fluorescens* strain 63-28. Physiol. Mol. Plant Pathol. 50:301-320.

Madhaiyan, M., Poonguzhali, S., Senthilkumar, M., Seshadri, S., Chung, H., Yang, J., Sundaram, S. and Sa, T., 2004. Growth promotion and induction of systemic resistance in rice cultivar Co-47 (*Oryza sativa* L.) by *Methylobacterium* spp. Bot. Bull. Acad. Sin. 45:315-324.

Maurhofer, M., Hase, C., Meuwly, P., Métraux, J.-P. and Défago, G., 1994. Induction of systemic resistance of tobacco to tobacco necrosis virus by the root-colonizing *Pseudomonas fluorescens* strain CHA0: influence of the *gacA* gene and of pyoverdine production. Phytopathology 84:139-146.

Maurhofer, M., Keel, C., Haas, D. and Défago, G., 1995. Influence of plant species on disease suppression by *Pseudomonas fluorescens* strain CHA0 with enhanced antibiotic production. Plant Pathol. 44:40-50.

Maurhofer, M., Reimmann, C., Schmidli-Sacherer, P., Heeb, S., Haas, D. and Défago, G., 1998. Salicylic acid biosynthetic genes expressed in *Pseudomonas fluorescens* strain P3 improve the induction of systemic resistance in tobacco against tobacco necrosis virus. Phytopathology 88:678-684.

Mercado-Blanco, J., Van der Drift, K.M.G.M., Olsson, P.E., Thomas-Oates, J.E., Van Loon, L.C. and Bakker, P.A.H.M., 2001. Analysis of the *pmsCEAB* gene cluster involved in biosynthesis of salicylic acid and the siderophore pseudomonine in the biocontrol strain *Pseudomonas fluorescens* WCS374. J. Bacteriol. 183:1909-1920.

Meyer, J.M., Azelvandre, P. and Georges, C., 1992. Iron metabolism in *Pseudomonas*: salicylic acid, a siderophore of *Pseudomonas fluorescens* CHA0. BioFactors 4:23-27.

Meziane, H., Van der Sluis, I., Van Loon, L.C., Höfte, M. and Bakker, P.A.H.M., 2005. Determinants of *Pseudomonas putida* WCS358 involved in inducing systemic resistance in plants. Mol. Plant Pathol. 6:177-185.

Mohandas, S., Manamohan, M., Rawal, R.D., Chakraborty, S., Sreekantappa, H., Manjula, R. and Lakshmikantha, H.C., 2004. Interaction of *Fusarium oxysporum* f.sp. *cubense* with *Pseudomonas fluorescens* precolonized to banana roots. World J. Microbiol. Biotechnol. 20:651-655.

Mou, Z., Fan, W.H. and Dong, X.N., 2003. Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. Cell 113:935-944.

Mulder, L., Hogg, B., Bersoult, A. and Cullimore, J.V., 2005. Integration of signalling pathways in the establishment of the legume-rhizobia symbiosis. Physiol. Plant. 123:207-218.

Murphy, J.F., Zehnder, G.W., Schuster, D.J., Sikora, E.J., Polston, J.E. and Kloepper, J.W., 2000. Plant growth-promoting rhizobacterial mediated protection in tomato against *tomato mottle virus*. Plant Dis. 84:779-784.

Murphy, J.F., Reddy, M.S., Ryu, C.-M., Kloepper, J.W. and Li, R., 2003. Rhizobacteria-mediated growth promotion of tomato leads to protection against *cucumber mosaic virus*. Phytopathology 93:1301-1307.

Nandakumar, R., Babu, S., Viswanathan, R., Raguchander, T. and Samiyappan, R., 2001. Induction of systemic resistance in rice against sheath blight disease by *Pseudomonas fluorescens*. Soil Biol. Biochem. 33:603-612.

Niranjan Raj, S., Deepak, S.A., Basavaraju, P., Shetty, H.S., Reddy, M.S. and Kloepper, J.W., 2003a. Comparative performance of formulations of plant growth promoting rhizobacteria in growth promotion and suppression of downy mildew in pearl millet. Crop Prot. 22:579-588.

Niranjan Raj, S., Shetty, N.P. and Shetty, H.S., 2003b. Seed bio-priming with *Pseudomonas fluorescens* isolates enhances growth of pearl millet plants and induces resistance against downy mildew. Int. J. Pest Manag. 50:41-49.

Oliver, R.P. and Ipcho, S.V.S., 2004. *Arabidopsis* pathology breathes new life into the necrotrophs-vs. biotrophs classification of fungal pathogens. Mol. Plant Pathol. 5:347-352.

Ongena, M., Giger, A., Jacques, P., Dommes, J. and Thonart, P., 2002. Study of bacterial determinants involved in the induction of systemic resistance in bean by *Pseudomonas putida* BTP1. Eur. J. Plant Pathol. 108:187-196.

Ongena, M., Daayf, F., Jacques, P., Thonart, P., Benhamou, N., Paulitz, T.C., Cornélis, P., Koedam, N. and Bélanger, R.R., 1999. Protection of cucumber against Pythium root rot by fluorescent pseudomonads: predominant role of induced resistance over siderophores and antibiosis. Plant Pathol. 48:66-76.

Ongena, M., Daayf, F., Jacques, P., Thonart, P., Benhamou, N., Paulitz, T.C. and Bélanger, R.R., 2000. Systemic induction of phytoalexins in cucumber in response to treatments with fluorescent pseudomonads. Plant Pathol. 49:523-530.

Ongena, M., Duby, F., Rossignol, F., Fauconnier, M.-L., Dommes, J. and Thonart, P., 2004. Stimulation of the lipoxygenase pathway is associated with systemic resistance induced in bean by a nonpathogenic *Pseudomonas* strain. Mol. Plant-Microbe Interact. 17:1009-1018.

Park, K.S. and Kloepper, J.W., 2000. Activation of PR-1a promoter by rhizobacteria that induce systemic resistance in tobacco against *Pseudomonas syringae* pv. *tabaci*. Biol. Control 18:2-9.

Persello-Cartieaux, F., Nussaume, L. and Robaglia, C., 2003. Tales from the underground: molecular plant-rhizobacteria interactions. Plant Cell Environ. 26:189-199.

Piater, L.A., Nürnberger, T. and Dubery, I.A., 2004. Identification of a lipopolysaccharide responsive erk-like MAP kinase in tobacco leaf tissue. Mol. Plant Pathol. 5:331-341.

Pieterse, C.M.J., Van Wees, S.C.M., Hoffland, E., Van Pelt, J.A. and Van Loon, L.C., 1996. Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. Plant Cell 8:1225-1237.

Pieterse, C.M.J., Van Wees, S.C.M., Van Pelt, J.A., Knoester, M., Laan, R., Gerrits, H., Weisbeek, P.J. and Van Loon, L.C., 1998. A novel signaling pathway controlling induced systemic resistance in Arabidopsis. Plant Cell 10:1571-1580.

Pieterse, C.M.J., Van Pelt, J.A., Ton, J., Parchmann, S., Mueller, M.J., Buchala, A.J., Métraux, J.-P. and Van Loon, L.C., 2000. Rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis* requires sensitivity to jasmonate and ethylene but is not accompanied by an increase in their production. Physiol. Mol. Plant Pathol. 57:123-134.

Press, C.M., Wilson, M., Tuzun, S. and Kloepper, J.W., 1997. Salicylic acid produced by *Serratia marcescens* 90-166 is not the primary determinant of induced systemic resistance in cucumber or tobacco. Mol. Plant-Microbe Interact. 10:761-768.

Press, C.M., Loper, J.E. and Kloepper, J.W., 2001. Role of iron in rhizobacteria-mediated induced systemic resistance of cucumber. Phytopathology 91:593-598.

Preston, G.M., 2004. Plant perceptions of plant growth-promoting Pseudomonas. Phil. Trans. Roy. Soc. London B 359:907-918.

Preston, G.M., Bertrand, N. and Rainey, P.B., 2001. Type III secretion in plant growth-promoting *Pseudomonas fluorescens* SBW25. Mol. Microbiol. 41:999-1014.

Raaijmakers, J.M., Leeman, M., Van Oorschot, M.P.M., Van der Sluis, I., Schippers, B. and Bakker, P.A.H.M., 1995. Dose-response relationships in biological control of fusarium wilt of radish by *Pseudomonas* spp. Phytopathology 85:1075-1081.

Radja Commare, R., Nandakumar, R., Kandan, A., Suresh, S., Bharathi, M., Raguchander, T. and Samiyappan, R., 2002. *Pseudomonas fluorescens* based bio-formulation for the management of sheath blight disease and leaffolder insect in rice. Crop Prot. 21:671-677.

Radjacommare, R., Kandan, A., Nandakumar, R. and Samiyappan, R., 2004. Association of the hydrolytic enzyme chitinase against *Rhizoctonia solani* in rhizobacteria-treated rice plants. J. Phytopathol. 152:365-370.

Ramamoorthy, V., Raguchander, T. and Samiyappan, R., 2002a. Enhancing resistance of tomato and hot pepper to Pythium diseases by seed treatment with fluorescent pseudomonads. Eur. J. Plant Pathol. 108:429-441.

Ramamoorthy, V., Raguchander, T. and Samiyappan, R., 2002b. Induction of defense-related proteins in tomato roots treated with *Pseudomonas fluorescens* Pf1 and *Fusarium oxysporum* f.sp. *lycopersici*. Plant Soil 239:55-68.

Ran, L.X., Li, Z.N., Wu, G.J., Van Loon, L.C. and Bakker, P.A.H.M., 2005. Induction of systemic resistance against bacterial wilt in *Eucalyptus urophylla* by fluorescent *Pseudomonas* spp. Eur. J. Plant Pathol., in press.

Ran, L.X., Van Loon, L.C. and Bakker, P.A.H.M., 2005b. No role for bacterially produced salicylic acid in rhizobacterial induction of systemic resistance in *Arabidopsis*. Phytopathology, in press.

Raupach, G.S. and Kloepper, J.W., 1998. Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology 88:1158-1164.

Raupach, G.S., Liu, L., Murphy, J.F., Tuzun, S. and Kloepper, J.W., 1996. Induced systemic resistance in cucumber and tomato against cucumber mosaic cucumovirus using plant growth-promoting rhizobacteria (PGPR). Plant Dis. 80:891-894.

Reitz, M., Rudolph, K., Schröder, I., Hoffmann-Hergarten, S., Hallmann, J. and Sikora, R.A., 2000. Lipopolysaccharides of *Rhizobium etli* strain G12 act in potato roots as an inducing agent of systemic resistance to infection by the cyst nematode *Globodera pallida*. Appl. Environ. Microbiol. 66:3515-3518.

Reitz, M., Hoffmann-Hergarten, S., Hallmann, J. and Sikora, R.A., 2001. Induction of systemic resistance in potato by rhizobacterium *Rhizobium etli* strain G12 is not associated with accumulation of pathogenesis-related proteins and enhanced lignin biosynthesis. Z. Pflanzenkrankh. Pflanzenschutz 108:11-20.

Reitz, M., Oger, P., Meyer, A., Niehaus, K., Farrand, S.K., Hallmann, J. and Sikora, R.A., 2002. Importance of the O-antigen, core-region and lipid A of rhizobial lipopolysaccharides for the induction of systemic resistance in potato to *Globodera pallida*. Nematology 4:73-79.

Roman, G., Lubarsky, B., Kieber, J.J., Rothenberg, M., and Ecker, J.R., 1995. Genetic analysis of ethylene signal transduction in *Arabidopsis thaliana*: five novel mutant loci integrated into a stress response pathway. Genetics 139:1393-1409.

Romeiro, R.S., Filho, L., Vieira Junior, J.R., Silva, H.S.A., Baracat-Pereira, M.C. and Carvalho, M.G., 2005. Macromolecules released by a plant growth-promoting rhizobacterium as elicitors of systemic resistance in tomato to bacterial and fungal pathogens. J. Phytopathol. 153:120-123.

Ross, A.F., 1961. Localized acquired resistance to plant virus infection in hypersensitive hosts. Virology 14:329-339.

Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H.Y. and Hunt, M.D., 1996. Systemic acquired resistance. Plant Cell 8:1809-1819.

Ryu, C.-M., Hu, C.H., Reddy, M.S. and Kloepper, J.W., 2003a. Different signaling pathways of induced resistance by rhizobacteria in *Arabidopsis thaliana* against two pathovars of *Pseudomonas syringae*. New Phytol. 160:413-420.

Ryu, C.-M., Farag, M.A., Hu, C.H., Reddy, M.S., Wei, H.X., Paré, P.W. and Kloepper, J.W., 2003b. Bacterial volatiles promote growth in *Arabidopsis*. Proc. Natl. Acad. Sci. USA 100:4927-4932.

Ryu, C.-M., Murphy, J.F., Mysore, K.S. and Kloepper, J.W., 2004a. Plant growth-promoting rhizobacteria systemically protect *Arabidopsis thaliana* against *Cucumber mosaic virus* by a salicylic acid and NPR1-independent and jasmonic acid-dependent signaling pathway. Plant J. 39:381-392.

Ryu, C.-M., Farag, M.A., Hu, C.H., Reddy, M.S., Kloepper, J.W. and Paré, P.W., 2004b. Bacterial volatiles induce systemic resistance in Arabidopsis. Plant Physiol. 134:1017-1026.

Sarma, B.K., Singh, D.P., Mehta, S., Singh, H.B. and Singh, U.P., 2002. Plant growth-promoting rhizobacteria-elicited alterations in phenolic profile of chickpea (*Cicer arietinum*) infected by *Sclerotium rolfsii*. J. Phytopathol. 150:277-282.

Serino, L., Reimmann, C., Baur, H., Beyeler, M., Visca, P. and Haas, D., 1995. Structural genes for salicylate biosynthesis from chorismate in *Pseudomonas aeruginosa*. Mol. Gen. Genet. 249:217-228.

Shah, J., Tsui, F. and Klessig, D.F., 1997. Characterization of a salicylic acid-insensitive mutant (*sai1*) of *Arabidopsis thaliana*, identified in a selective screen utilizing the SA-inducible expression of the *tms2* gene. Mol. Plant-Microbe Interact. 10:69-78.

Siddiqui, I.A. and Shaukat, S.S., 2002a. Rhizobacteria-mediated induction of systemic resistance (ISR) in tomato against *Meloidogyne javanica*. J. Phytopathol. 150:469-473.

Siddiqui, I.A. and Shaukat, S.S., 2002b. Resistance against the damping-off fungus *Rhizoctonia solani* systemically induced by the plant-growth-promoting rhizobacteria *Pseudomonas aeruginosa* (IE-6S$^+$) and *P. fluorescens* (CHA0). J. Phytopathol. 150:500-506.

Siddiqui, I.A. and Shaukat, S.S., 2003. Suppression of root-knot disease by *Pseudomonas fluorescens* CHA0 in tomato: importance of bacterial secondary metabolite, 2,4-diacetylpholoroglucinol. Soil Biol. Biochem. 35:1615-1623.

Siddiqui, I.A. and Shaukat, S.S., 2004. Systemic resistance in tomato induced by biocontrol bacteria against the root-knot nematode, *Meloidogyne javanica* is independent of salicylic acid production. J. Phytopathol. 152:48-54.

Silva, H.S.A., Romeiro, R.S., Macagnan, D., Halfield-Vieira, B.A., Pereira, M.C.B. and Mounteer, A., 2004a. Rhizobacterial induction of systemic resistance in tomato plants: non-specific protection and increase in enzyme activities. Biol. Control 29:288-295.

Silva, H.S.A., Romeiro, R.S., Carrer Filho, R., Pereira, J.L.A., Mizubuti, E.S.G. and Mounteer, A., 2004b. Induction of systemic resistance by *Bacillus cereus* against tomato foliar diseases under field conditions. J. Phytopathol. 152:371-375.

Singh, U.P., Sarma, B.K., Singh, D.P. and Bahadur, A., 2002. Plant growth-promoting rhizobacteria-mediated induction of phenolics in pea (*Pisum sativum*) after infection with *Erysiphe pisi*. Curr. Microbiol. 44:396-400.

Singh, U.P., Sarma, B.K. and Singh, D.P., 2003. Effect of plant growth-promoting rhizobacteria and culture filtrate of *Sclerotium rolfsii* on phenolic and salicylic acid contents in chickpea (*Cicer arietinum*). Curr. Microbiol. 46:131-140.

Somers, E., Vanderleijden, J. and Srinivasan, M., 2004. Rhizosphere bacterial signalling: a love parade beneath our feet. Crit. Rev. Microbiol. 30:205-240.

Spencer, M., Ryu, C.-M., Yang, K.Y., Kim, Y.C., Kloepper, J.W. and Anderson, A.J., 2003. Induced defence in tobacco by *Pseudomonas chlororaphis* strain O6 involves at least the ethylene pathway. Physiol. Mol. Plant Pathol. 63:27-34.

Srivastava, A.K., Singh, T., Jana, T.K. and Arora, D.K., 2001. Induced resistance and control of charcoal rot in *Cicer arietinum* (chickpea) by *Pseudomonas fluorescens*. Can. J. Bot. 79:787-795.

Sticher, L., Mauch-Mani, B. and Métraux, J.-P., 1997. Systemic acquired resistance. Annu. Rev. Phytopathol. 35:235-270.

Stracke, S., Kistner, C., Yoshida, S., Mulder, L., Sato, S., Kaneko, T., Tabata, S., Sandal, N., Stougaard, J., Szczyglowski, K. and Parniske, M., 2002. A plant receptor-like kinase required for both bacterial and fungal symbiosis. Nature 417:959-962.

Thangavelu, R., Palaniswami, A., Doraiswamy, S. and Velazhahan, R., 2003. The effect of *Pseudomonas fluorescens* and *Fusarium oxysporum* f.sp. *cubense* on induction of defense enzymes and phenolics in banana. Biol. Plant. 46:107-112.

Thomma, B.P.H.J., Eggermont, K., Penninckx, I.A.M.A., Mauch-Mani, B., Vogelsang, R., Cammue, B.P.A. and Broekaert, W.F., 1998. Separate jasmonate-dependent and salicylate-dependent defense response pathways in Arabidopsis are essential for resistance to distinct microbial pathogens. Proc. Natl. Acad. Sci. USA 95:15107-15111.

Thomma, B.P.H.J., Tierens, K.F.M., Penninckx, I.A.M.A., Mauch-Mani, B., Broekaert, W.F. and Cammue, B.P.A., 2001. Different micro-organisms differentially induce Arabidopsis disease response pathways. Plant Physiol. Biochem. 39:673-680.

Thordal-Christensen, H., 2003. Fresh insights into processes of nonhost resistance. Curr. Opin. Plant Biol. 6:351-357.

Timmusk, S. and Wagner, E.G.H., 1999. The plant-growth-promoting rhizobacterium *Paenibacillus polymyxa* induces changes in *Arabidopsis thaliana* gene expression: a possible connection between biotic and abiotic stress responses. Mol. Plant-Microbe Interact. 12:951-959.

Ton, J., Pieterse, C.M.J. and Van Loon, L.C., 1999. Identification of a locus in Arabidopsis controlling both the expression of rhizobacteria-mediated induced systemic resistance (ISR) and basal resistance against *Pseudomonas syringae* pv. *tomato*. Mol. Plant-Microbe Interact. 12:911-918.

Ton, J., Davison, S., Van Wees, S.C.M., Van Loon, L.C. and Pieterse, C.M.J., 2001. The Arabidopsis *ISR1* locus controlling rhizobacteria-mediated induced systemic resistance is involved in ethylene signaling. Plant Physiol. 125:652-661.

Ton, J., Van Pelt, J.A., Van Loon, L.C. and Pieterse, C.M.J., 2002a. Differential effectiveness of salicylate-dependent and jasmonate/ethylene-dependent induced resistance in Arabidopsis. Mol. Plant-Microbe Interact. 15:27-34.

Ton, J., De Vos, M., Robben, C., Buchala, A.J., Métraux, J.-P., Van Loon, L.C. and Pieterse, C.M.J., 2002b. Characterisation of *Arabidopsis* enhanced disease susceptibility mutants that are affected in systemically induced resistance. Plant J. 29:11-21.

Ton, J., Van Pelt, J.A., Van Loon, L.C. and Pieterse, C.M.J., 2002c. The Arabidopsis *ISR1* locus is required for rhizobacteria-mediated induced systemic resistance against different pathogens. Plant Biol. 4:224-227.

Troxler, J., Berling, C.H., Moënne-Loccoz, Y., Keel, C. and Défago, G. 1997. Interactions between the biocontrol agent *Pseudomonas fluorescens* CHA0 and *Thielaviopsis basicola* in tobacco roots observed by immunofluorescence microscopy. Plant Pathol. 46:62-71.

Tuzun, S. and Kloepper, J., 1995. Practical application and implementation of induced resistance. In: Induced Resistance to Disease in Plants, Hammerschmidt, R. and Kuc, J. (eds), Kluwer Acad. Publ., Dordrecht, pp. 152-168.

Uknes, S., Mauch-Mani, B., Moyer, M., Potter, S., Williams, S., Dincher, S., Chandler, D., Slusarenko, A., Ward, E. and Ryals, J., 1992. Acquired resistance in *Arabidopsis*. Plant Cell 4: 645-656.

Van Loon, L.C., 1997. Induced resistance in plants and the role of pathogenesis-related proteins. Eur. J. Plant Pathol. 103:753-765.

Van Loon, L.C., 2000. Systemic induced resistance. In: Mechanisms of Resistance to Plant Diseases, Slusarenko, A.J., Fraser. R.S.S. and Van Loon, L.C. (eds), Kluwer Acad. Publ., Dordrecht, pp. 521-574.

Van Loon, L.C. and Bakker, P.A.H.M., 2003. Signalling in rhizobacteria-plant interactions. In: Root Ecology, De Kroon, H. and Visser, E. J. W. (eds), Springer-Verlag, Berlin Heidelberg, pp. 297-330.

Van Loon, L.C. and Bakker, P.A.H.M., 2005. Induced systemic resistance as a mechanism of disease suppression by rhizobacteria. In: PGPR: Biocontrol and Biofertilization, Siddiqui, Z.A. (ed.), Springer Science, Dordrecht, pp. 39-66.

Van Loon, L.C. and Glick, B.R., 2004. Increased plant fitness by rhizobacteria. In: Molecular Ecotoxicology of Plants, Sandermann, H. (ed.), Springer-Verlag, Berlin Heidelberg, pp 177-205.

Van Loon, L.C., Bakker, P.A.H.M., and Pieterse, C.M.J., 1998. Systemic resistance induced by rhizosphere bacteria. Annu. Rev. Phytopathol. 36:453-483.

Van Loon, L.C., Geraats, B.P.J. and Linthorst, H.J.M., 2005. Ethylene as a modulator of disease resistance in plants. Trends Plant Sci., in press.

Van Peer, R. and Schippers, B., 1992. Lipopolysaccharides of plant-growth promoting *Pseudomonas* sp. strain WCS417r induce resistance in carnation to fusarium wilt. Neth. J. Plant Pathol. 98:129-139.

Van Peer, R., Niemann, G.J. and Schippers, B., 1991. Induced resistance and phytoalexin accumulation in biological control of fusarium wilt of carnation by *Pseudomonas* sp. strain WCS417r. Phytopathology 81:728-734.

Van Wees, S.C.M., Pieterse, C.M.J., Trijssenaar, A., Van' t Westende, Y., Hartog, F. and Van Loon, L.C., 1997. Differential induction of systemic resistance in *Arabidopsis* by biocontrol bacteria. Mol. Plant-Microbe Interact. 10:716-724.

Van Wees, S.C.M., Luijendijk, M., Smoorenburg, I., Van Loon, L.C. and Pieterse, C.M.J., 1999. Rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis* is not associated with a direct effect on expression of known defense-related genes but stimulates the expression of the jasmonate-inducible gene *Atvsp* upon challenge. Plant Mol. Biol. 41:537-549.

Van Wees, S.C.M., De Swart, E.A.M., Van Pelt, J.A., Van Loon, L.C. and Pieterse, C.M.J., 2000. Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA 97:8711-8716.

Verberne, M.C., Hoekstra, J., Bol, J.F. and Linthorst, H.J.M., 2003. Signaling of systemic acquired resistance in tobacco depends on ethylene perception. Plant J. 35:27-32.

Verhagen, B.W.M., Glazebrook, J., Zhu, T., Chang, H.S., Van Loon, L.C. and Pieterse, C.M.J., 2004. The transcriptome of rhizobacteria-induced systemic resistance in Arabidopsis. Mol. Plant-Microbe Interact. 17:895-908.

Vernooij, B., Friedrich, L., Morse, A., Reist, R., Kolditz-Jawhar, R., Ward, E., Uknes, S., Kessmann, H. and Ryals, J., 1994. Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. Plant Cell 6: 959-965.

Viswanathan, R. and Samiyappan, R., 2002a. Induced systemic resistance by fluorescent pseudomonads against red rot disease of sugarcane caused by *Colletotrichum falcatum*. Crop Prot. 21:1-10.

Viswanathan, R. and Samiyappan, R., 2002b. Role of oxidative enzymes in the plant growth promoting rhizobacteria (PGPR) mediated induced systemic resistance in sugarcane against *Colletotrichum falcatum*. Z. Pflanzenkrankh. Pflanzenschutz 109:88-100.

Viswanathan, R., Nandakumar, R. and Samiyappan, R., 2003. Role of pathogenesis-related proteins in rhizobacteria-mediated induced systemic resistance against *Colletotrichum falcatum* in sugarcane. Z. Pflanzenkrankh. Pflanzenschutz 110:524-534.

Volko, S.M., Boller, T. and Ausubel, F.M., 1998. Isolation of new *Arabidopsis* mutants with enhanced disease susceptibility to *Pseudomonas syringae* by direct screening. Genetics 149:537-548.

Wang, Y., Ohara, Y., Nakayashiki, H., Tosa, Y. and Mayama, S., 2005. Microarray analysis of the gene expression profile induced by the endophytic plant growth-promoting rhizobacteria, *Pseudomonas fluorescens* FPT9601-T5 in *Arabidopsis*. Mol. Plant-Microbe Interact. 18:385-396.

Wei, G., Kloepper, J.W. and Tuzun, S., 1991. Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacteria. Phytopathology 81:1508-1512.

Wei, G., Kloepper, J.W. and Tuzun, S., 1992. Internal and external colonization of cucumber by bacteria which induce systemic resistance to *Colletotrichum orbiculare*. Phytopathology 82:1108.

Weller D.M., Van Pelt, J.A., Mavrodi, D.V., Pieterse, C.M.J., Bakker, P.A.H.M. and Van Loon, L.C., 2004. Induced systemic resistance (ISR) in *Arabidopsis* against *Pseudomonas syringae* pv. *tomato* by 2,4-diacetylphloroglucinol (DAPG)-producing *Pseudomonas fluorescens*. Phytopathology 94:S108.

Whitehead, N.A., Barnard, A.M.L., Slater, H., Simpson, N.J.L. and Salmond, G.P.C., 2001. Quorum-sensing in Gram-negative bacteria. FEMS Microbiol. Rev. 25:365-4004.

Whipps, J.M., 2001. Microbial interactions and biocontrol in the rhizosphere. J. Exp. Bot. 52:487-511.

Yan, Z., Reddy, M.S., Ryu, C.-M., McInroy, J.A., Wilson, M. and Kloepper, J.W., 2002. Induced systemic protection against tomato late blight elicited by plant growth-promoting rhizobacteria. Phytopathology 92:1329-1333.

Yan, Z., Reddy, M.S. and Kloepper, J.W., 2003. Survival and colonization of rhizobacteria in a tomato transplant system. Can. J. Microbiol. 49:383-389.

Zdor, R.E. and Anderson, A.J., 1992. Influence of root colonizing bacteria on the defense responses of bean. Plant Soil 140:99-107.

Zehnder, G., Kloepper, J., Tuzun, S., Yao, C., Wei, G., Chambliss, O. and Shelby, R., 1997a. Insect feeding on cucumber mediated by rhizobacteria-induced plant resistance. Entomol. Exp. Appl. 83:81-85.

Zehnder, G., Kloepper, J., Yao, C.B. and Wei, G., 1997b. Induction of systemic resistance in cucumber against cucumber beetles (Coleoptera: *Chrysomelidae*) by plant growth-promoting rhizobacteria. J. Econ. Entomol. 90:391-396.

Zehnder, G.W., Yao, C., Murphy, J.F., Sikora, E.R. and Kloepper, J.W., 2000. Induction of resistance in tomato against cucumber mosaic cucumovirus by plant growth-promoting rhizobacteria. BioControl 45:127-137.

Zhang, S., Reddy, M.S. and Kloepper, J.W., 2002a. Development of assays for assessing induced systemic resistance by plant growth-promoting rhizobacteria against blue mold of tobacco. Biol. Control 23:79-86.

Zhang, S., Moyne, A.-L., Reddy, M.S. and Kloepper, J.W., 2002b. The role of salicylic acid in induced systemic resistance elicited by plant growth-promoting rhizobacteria against blue mold of tobacco. Biol. Control 25:288-296.

Zhang, W., Dick, W.A. and Hoitink, H.A.J., 1996. Compost-induced systemic acquired resistance in cucumber to Pythium root rot and anthracnose. Phytopathology 86:1066-1070.

Zhang, W., Han, D.Y., Dick, W.A., Davis, K.R. and Hoitink, H.A.J., 1998. Compost and compost water extract-induced systemic acquired resistance in cucumber and Arabidopsis. Phytopathology 88:450-455.

Zhou, T. and Paulitz, T.C., 1994. Induced resistance in the biocontrol of *Pythium aphanidermatum* by *Pseudomonas* spp. on cucumber. J. Phytopathol. 142:51-63.

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