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Plant perceptions of plant growth-promoting *Pseudomonas*

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Plant-associated *Pseudomonas* live as saprophytes and parasites on plant surfaces and inside plant tissues. Many plant-associated *Pseudomonas* promote plant growth by suppressing pathogenic micro-organisms, synthesizing growth-stimulating plant hormones and promoting increased plant disease resistance. Others inhibit plant growth and cause disease symptoms ranging from rot and necrosis through to developmental dystrophies such as galls. It is not easy to draw a clear distinction between pathogenic and plant growth-promoting *Pseudomonas*. They colonize the same ecological niches and possess similar mechanisms for plant colonization. Pathogenic, saprophytic and plant growth-promoting strains are often found within the same species, and the incidence and severity of *Pseudomonas* diseases are affected by environmental factors and host-specific interactions. Plants are faced with the challenge of how to recognize and exclude pathogens that pose a genuine threat, while tolerating more benign organisms. This review examines *Pseudomonas* from a plant perspective, focusing in particular on the question of how plants perceive and are affected by saprophytic and plant growth-promoting *Pseudomonas* (PGPP), in contrast to their interactions with plant pathogenic *Pseudomonas*. A better understanding of the molecular basis of plant–PGPP interactions and of the key differences between pathogens and PGPP will enable researchers to make more informed decisions in designing integrated disease-control strategies and in selecting, modifying and using PGPP for plant growth promotion, bioremediation and biocontrol.

Keywords: *Pseudomonas*; plant growth-promoting rhizobacteria; type III secretion system; induced systemic resistance

1. INTRODUCTION

> '…the best type go all over the world, fitting in so perfectly with their background that not even the inhabitants notice they are strangers; in other words they achieve the highest accomplishment possible.'
>
> Emily Post (1922, ch. 37)

Plant-associated bacteria colonize the foliage and roots of plants, living on nutrients obtained from plant cells. The 'highest accomplishment' for plant-colonizing bacteria lies in 'fitting in' to the host environment and being able to tolerate, manipulate or evade plant defence responses. Success for plants lies in being able to distinguish harmless saprophytes and beneficial symbionts from pathogenic parasites, and in using induced defence responses to repel dangerous pathogens at minimum cost. However, the distinction between saprophytes, pathogens and beneficial bacteria is not always clear-cut. Many pathogens have non-pathogenic, plant-associated relatives that share many of the same attributes. Both live on plant surfaces and inside plant tissues, and these common habitats provide frequent opportunities for recombination and horizontal gene transfer, facilitating the evolution and acquisition of common plant colonization mechanisms (Beattie & Lindow 1995, 1999; Bjorklof *et al.* 2000; Lindow & Brandl 2003). Nevertheless, the high level of immunity and disease-resistance in most plants to most bacteria suggests that plants are able to effectively recognize and protect themselves against most bacteria they encounter, while retaining the ability to form mutually beneficial symbioses with beneficial bacteria such as nitrogen-fixing rhizobia.

The signalling interactions involved in nitrogen-fixing symbioses, and the arms race between pathogens and disease-resistant plants have been extensively reviewed (Oke & Long 1999; Gage & Margolin 2000; Perret *et al.* 2000; Nimchuk *et al.* 2001; Schneider 2002; Holt *et al.* 2003). This review examines a less well-characterized aspect of plant–bacteria interactions: plant perception of non-pathogenic and plant growth-promoting bacteria. I have mainly focused on selected examples from one genus of bacteria, *Pseudomonas*, which contains animal pathogens, plant pathogens and plant growth-promoting bacteria (Thomashow 1996; Preston *et al.* 1998; Preston 2000; Plotnikova *et al.* 2000; Cao *et al.* 2001; Lugtenberg *et al.* 2001; Bloemberg & Lugtenberg 2001; Persello-Cartieaux *et al.* 2003). Comparative analyses of *Pseudomonas* offer the possibility of understanding not only how plants distinguish between closely related bacteria with different pathogenic potential, but also of understanding the factors that affect the evolution of pathogenic and beneficial relationships between animals, plants and bacteria (Preston *et al.* 1998). Individual *Pseudomonas* strains may have biocontrol activity, plant growth-promoting activity, the ability to induce systemic plant defence responses or the ability to act as pathogens. For this review I will use the term plant growth-promoting *Pseudomonas* (PGPP) as a blanket term for *Pseudomonas* strains that have a

beneficial effect on plant hosts, without specific reference to the mode of action of this effect, and without excluding the possibility that these strains may have deleterious effects on plants in certain contexts.

2. MEETING PLACES

Before examining the molecular interactions of plants and *Pseudomonas* in depth, it is important to have some understanding of the cellular and ecological contexts in which they take place. *Pseudomonas*–plant interactions can be considered to take place in four very broadly defined contact zones (figure 1):

(i) foliar surfaces colonized by epiphytic *Pseudomonas*;
(ii) root surfaces colonized by rhizosphere *Pseudomonas*;
(iii) intercellular spaces in leaves colonized by endophytic *Pseudomonas*; and
(iv) intercellular spaces in roots colonized by endophytic *Pseudomonas*.

These niches could be further subdivided, according to organs, tissue types, cell types, developmental stage, host-specific characteristics and so forth, but the study of plant–*Pseudomonas* interactions at this level of detail is still in its infancy. Each of the four zones outlined is broadly defined by the physiological properties of the relevant tissue, subject to diurnal variation and environmental conditions, and by biochemical and structural features that allow or restrict contact between *Pseudomonas* and host cells. I shall briefly summarize the most relevant aspects of each zone.

(a) *Foliar surfaces*

These are the initial contact zone for many plant pathogenic *Pseudomonas*. Surfaces are covered in a waxy cuticle, restricting water loss from the leaf and contact between *Pseudomonas* and host cells. Bacteria live as saprotrophs on nutrients exuded from the plant, or organic matter deposited on surfaces, and are subject to high levels of fluctuating environmental stress such as temperature, dehydration and UV light. Bacteria can only enter plant tissues through natural openings such as wounds, stomata or hydathodes, but some *Pseudomonas* increase the incidence of damage to host tissues through ice nucleation (Wisniewski *et al.* 1997; Beattie & Lindow 1999; Lindow & Brandl 2003).

(b) *Root surfaces*

In contrast to leaf surfaces, roots are designed for water uptake, and present a large surface area that is not covered with a hydrophobic cutin layer. The lack of a cutin layer may offer greater potential for direct signalling between *Pseudomonas* and epidermal cells than on foliar surfaces. Roots release substantial quantities of root exudates, which are rich in sugars, dicarboxylic acids, amino acids and sloughed off root border cells, and which support a complex microflora and microfauna of saprotrophs, symbionts and predators (see Gilroy & Jones 2000; Hawes *et al.* 2000). Roots also produce significant levels of secondary metabolites, many of which have anti-microbial activity (Flores *et al.* 1999). In addition to direct interactions with plant cells, root-colonizing *Pseudomonas* can affect plant physiology through interactions with other rhizosphere organisms, such as mycorrhizal fungi, soil-borne plant pathogens, and nitrogen-fixing and nitrogen-cycling bacteria (Lugtenberg *et al.* 2001).

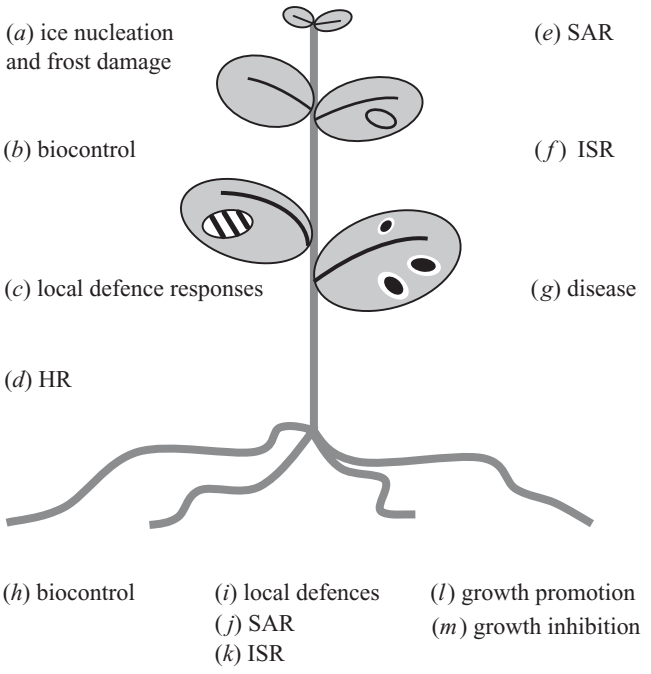

Figure 1. *Pseudomonas*–plant interactions. Many *Pseudomonas* are able to live as epiphytes on the surface of leaves. Ice-nucleating strains of *Pseudomonas* promote frost damage, but epiphytic *Pseudomonas* can also act as biocontrol agents that suppress foliar pathogens by competition, exclusion and antibiosis (*a*,*b*). *Pseudomonas* invade leaves through wounds and natural openings to establish endophytic populations. Recognition of generic and host-specific elicitors produced by endophytic *Pseudomonas* primes and induces local defence responses, and can elicit the hypersensitive response (HR) and systemic defence responses (*c*–*f*). Successful pathogens are able to evade or suppress recognition and cause disease symptoms at high bacterial densities (*g*). Damage caused by pathogens can also elicit systemic defences such as systemic acquired resistance (SAR) in roots and leaves (*e*). Many root-colonizing *Pseudomonas* also have the capacity to suppress pathogens (*h*), but some also prime and elicit local and systemic defence responses such as induced systemic resistance (ISR) in roots and leaves (*i*–*k*). The net effect of *Pseudomonas*–plant interactions, including modulation and biosynthesis of plant hormones, can result in plant growth promotion or inhibition of plant growth (*l*,*m*), and is influenced by environmental and host factors, such as temperature, water availability, host genotype and plant health.

(c) *Foliar interior*

Endophytic *Pseudomonas* live on nutrients present in the apoplast of host cells, the acidic, non-living continuum provided by the continuous matrix of cell walls, or on nutrients released from dead cells during pathogenesis. Signal exchange between *Pseudomonas* and plant cells generally occurs across the barrier of the plant cell wall, rather than in the context of close contact between bacterial and host membranes as in many animal–bacteria interactions (for clear images of endophytic interactions see Bestwick *et al.* 1997; Brown *et al.* 2001).

(d) *Root interior*

The properties of roots as habitats for endophytic *Pseudomonas* are poorly understood, although *Pseudomonas fluorescens* and *Pseudomonas putida* are frequently isolated as endophytes from roots and tubers. *Pseudomonas* enter roots through wounds and natural openings, such as the point of emergence of lateral roots. The interior of roots may have features in common with leaves, but they are also characterized by lack of photosynthetic tissue, less exposed surface area for gas exchange and synthesis of a wide range of anti-microbial secondary metabolites. Differences in the physiology of photosynthetic and non-photosynthetic tissue may have a substantial impact on the physiology of plant responses to *Pseudomonas*.

3. FIRST IMPRESSIONS

The first cellular symptoms of infection by plant pathogens such as *Pseudomonas syringae* can be observed within 5 hours after inoculation (Bestwick *et al.* 1997), but plant perception of *Pseudomonas* begins much earlier. Changes in plant signal transduction are observed within 2 minutes after exposure to bacterial elicitors and changes in plant gene expression are observed within as little as 15 minutes after infection (Gómez-Gómez *et al.* 1999; de Torres *et al.* 2003).

The earliest stages of plant recognition do not require bacterial gene expression and can be observed in response to heat-killed bacteria. Plants, like animals, have evolved the capacity to recognize and respond to a wide range of generic microbial molecules (Gómez-Gómez & Boller 2002). In animals, recognition of these pathogen-associated molecular patterns (PAMPs) elicits inflammatory and pro-inflammatory responses that contribute to innate immunity (Magor & Magor 2001; Nurnberger & Brunner 2002; Gómez-Gómez & Boller 2002). There is increasing evidence that functionally equivalent defence responses are elicited by general elicitors in plants. PAMP-elicited defence responses may contribute to restriction of endophytic growth by non-pathogenic and non-host bacteria, and to the systemic induced resistance elicited by PGPP. Two of the most widely studied PAMPs produced by *Pseudomonas* are flagellins, subunits of the polar flagella produced by motile *Pseudomonas* and lipopolysaccharides (LPSs), constituents of the bacterial envelope.

Flagellin recognition in plants is mediated by FLS2, a membrane-associated kinase with an extracellular leucine-rich repeat (LRR) domain. FLS2 is a member of the Toll family of receptor kinases, which have been linked to developmental signalling and innate immunity in animals, and pathogen recognition in plants. These parallels suggest that LRR kinases such as FLS2 may have evolved from an evolutionarily ancient recognition mechanism for general elicitors and pathogen-associated factors (Gómez-Gómez & Boller 2002). Purified flagellin from *P. syringae*, *Pseudomonas aeruginosa* or *P. fluorescens*, or a peptide consisting of 22 conserved amino acids (flg22), elicits an oxidative burst, callose deposition and synthesis of anti-microbial proteins in plant cells (Felix *et al.* 1999; Gómez-Gómez *et al.* 1999). *FLS2* is expressed in roots, but callose deposition in response to flg22 has only been reported for leaves, stems and cotyledons (Gómez-Gómez *et al.* 1999).

Flagellin recognition by plants is host and strain specific. The Ws-0 ecotype of *Arabidopsis* is insensitive to *Pseudomonas* flagellins, showing that flagellin recognition is not a universal characteristic of plants, even within a plant species (Gómez-Gómez *et al.* 1999). Purified flagellins from *P. syringae* pvs. *tomato* and *glycinea* elicit defence responses in tobacco, but flagellin from the tobacco pathogen *P. s.* pv. *tabaci* does not (Taguchi *et al.* 2003). At least some *Pseudomonas* use sequence variation and post-translational modification of flagellins to evade flagellin-mediated recognition (Taguchi *et al.* 2003). One unresolved question with regard to flagellin recognition is whether flagella are expressed at all stages of plant colonization. Flagella are important for initial colonization of roots and leaf surfaces, but not for endophytic multiplication (Haefele & Lindow 1987; Lugtenberg *et al.* 2001). Regulation of flagella expression could be an additional mechanism used to evade plant recognition of *Pseudomonas*.

A second commonly recognized factor is LPS. LPS recognition has mostly been studied in the context of plant pathogens, where it has been shown to induce plant synthesis of anti-microbial factors and to suppress the development of programmed cell death associated with the hypersensitive response (HR), an effect referred to as localized induced resistance or localized induced response (LIR; Dow *et al.* 2000; Newman *et al.* 2002). Variation in the composition and structure of LPS may contribute to evasion and suppression of plant defence responses by plant pathogenic *Pseudomonas*, although the core molecule required to elicit LIR is a lipid A-core oligosaccharide structure that is common to many bacteria.

Induction of LIR by PGPP LPS may enhance local defence responses to plant pathogens, but perhaps the most important role of LPS in PGPP–plant interactions may be in priming systemic expression of plant defence responses (Dow *et al.* 2000). Pathogen-induced expression of anti-microbial proteins is much stronger in plants pre-treated with LPS, and LPS may be a key signal in the induction of induced systemic resistance (ISR) by root-colonizing PGPP, as described in Conrath *et al.* (2002).

4. DAMAGE CONTROL

The PAMP or 'non-self' mechanism of innate immunity in animals is not the only mechanism that has been proposed to account for the innate immune response in animals. A second mechanism is the 'danger' mechanism (Matzinger 1994, 1998; Magor & Magor 2001). In this mechanism tissue damage, or cellular debris from necrotic cells, elicits the immune response. 'Danger' receptors recognize 'self' molecules that are displaced, degraded or incompletely processed. Saprophytic PGPP deploy an extensive array of degradative and catabolic activities that enable them to break down an exceptionally diverse selection of organic substrates. Many of the degradative and disruptive factors produced by PGPP, such as proteases, lipases and cell wall degrading enzymes have the potential to cause significant damage to plant cells. Damage recognition mechanisms do exist in plants, for example, plants recognize and respond to 10–12 unit pectate oligomers released as a consequence of cell wall degradation by pathogens (Dumville & Fry 2000). So even if degradative factors are only expressed at low levels, the consequent

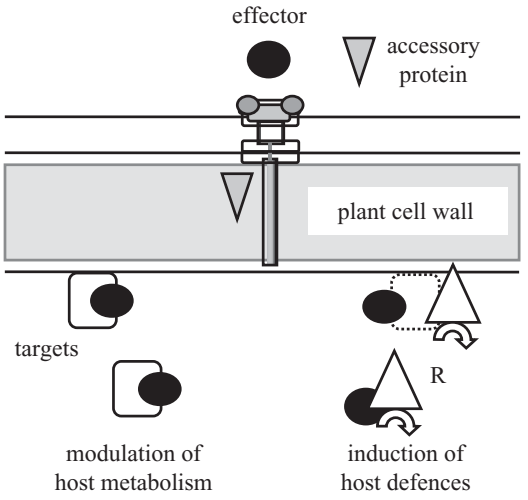

Figure 2. Type III secretion system. The TTSS delivers effector proteins (black ovals) across the plant cell wall. *Pseudomonas syringae* effector proteins are known to travel through a pilus assembled by the TTSS (He & Jin 2003). *Pseudomonas syringae* also secretes accessory proteins (grey triangles) that may facilitate the passage of effectors across the cell wall and plant cell membrane. Inside the plant cell, effector proteins act on host targets to alter plant signal transduction and promote plant growth (bottom left). In resistant plant cells, effectors, or the actions of effectors, are recognized by plant surveillance mechanisms in an R-protein-dependent manner (bottom right). Recognition of effectors elicits host defence responses. R, resistance.

disruption to membrane signalling and integrity, or the release of elicitor-active peptide and pectate fragments from plant cell walls, could have a significant impact on plant signal transduction. However, the impact of biodegradative enzymes on plant–PGPP interactions remains unclear. The primary substrates used as nutrients by PGPP appear to be simple sugars, organic acids and amino acids (Rainey 1999; Lugtenberg *et al.* 2001; Lindow & Brandl 2003), and many degradative factors may only be produced under particular environmental conditions, in the presence of high levels of inducing molecules, or when other sources of nutrients are limiting.

Degradative enzymes such as lipases and pectate lyases have been identified as pathogenicity and virulence factors in *Pseudomonas* pathogenesis (Preston 2000; Cao *et al.* 2001). As these factors are common to both pathogens and PGPP the distinction between pathogenic and plant growth-promoting interactions must lie in other factors. Such factors may include the regulation, specificity and combination of extracellular factors produced by the bacterium; environmental factors such as temperature and water availability; host genotype and physiology, and perhaps most importantly in the ability of the bacterium to evade or suppress host recognition by 'innate' or 'specific' immune responses and overcome natural barriers to infection.

5. SUBVERSION AND STIMULATION OF HOST DEFENCES

(a) *Type III protein secretion*

One system that can play an important role in modulation of host defence responses by pathogens and PGPP is the type III protein secretion system (TTSS; figure 2). Pathogens such as *P. syringae* and *P. aeruginosa* use TTSSs to deliver 'effector' proteins into the cytoplasm of host cells (Buttner & Bonas 2002; Greenberg & Vinatzer 2003). TTSS effectors are highly diverse, but their collective function appears to be to render the host more susceptible to infection, and to promote bacterial multiplication in host tissues (Gabriel 1999; Kjemtrup *et al.* 2000; Shao *et al.* 2002; Greenberg & Vinatzer 2003; Abramovitch *et al.* 2003). Intriguingly, recent studies suggest that many of these diverse TTSS effectors act on a few key defence-related proteins. For example, at least three structurally unrelated effectors from *P. syringae* affect the *Arabidopsis* RIN4 protein (Axtell & Staskawicz 2003; Mackey *et al.* 2003). Plants have responded to the threat of bacterial hijacking by evolving surveillance mechanisms that detect the presence and activities of effector proteins. Recognition of effectors triggers a pre-emptive defence response known as the HR during the early stages of infection, which generally manifests as localized programmed cell death and accumulation of anti-microbial compounds (Dangl *et al.* 1996). Effectors that elicit the HR are referred to as Avr (avirulence) proteins. Recognition of Avrs is generally conditioned by a single host protein, an R protein (Nimchuk *et al.* 2001; Schneider 2002; Holt *et al.* 2003).

Owing to the clear links between TTSS activity and pathogenesis, many studies have used TTSS genes as molecular markers of pathogenic potential, or highlighted the TTSS as a target for intervention (Stuber *et al.* 2003). However, TTSSs have also been identified in beneficial symbionts of plants and animals, such as the nitrogen-fixing bacterium *Rhizobium*, and in PGPP (Marie *et al.* 2001; Preston *et al.* 2001; ffrench-Constant *et al.* 2003). The role of TTSSs in rhizobial symbioses appears to be similar to their role in pathogenesis: to modulate host defences and promote growth in plant tissues. But, as in pathogenic interactions, *Rhizobium* TTSSs promote nodulation and endophytic growth at the cost of limiting host range (Marie *et al.* 2001).

My own studies have shown that TTSS genes are present in many plant-colonizing and plant growth-promoting *P. fluorescens* and *P. putida* strains, whereas other studies have shown that the TTSS-secreted ADP-ribosyltransferase ExoS is present and expressed at high levels in soil populations of *P. aeruginosa* (Preston *et al.* 2001; Ferguson *et al.* 2001). The requirement for a eukaryotic cofactor for ExoS activity strongly suggests that soil isolates of *P. aeruginosa* use ExoS to establish parasitic or commensal relationships with eukaryotes, and the similarity between the *P. fluorescens* and *P. syringae* systems suggests a common interaction with plants. However, it is possible that the widespread distribution and expression of TTSSs in PGPP may reflect the importance of TTSSs in bacterial interactions with soil eukaryotes such as invertebrates, fungi and protozoa rather than exclusive interactions with plants. *Pseudomonas aeruginosa* has been shown to colonize organisms ranging from humans and mice, through to insects, nematodes, plants, fungi and amoebae (Mahajan-Miklos *et al.* 1999, 2000; Lyczak *et al.* 2000; Cao *et al.* 2001; Pukatzki *et al.* 2002; Rabin & Hauser 2003), and the *P. aeruginosa* TTSS has been shown to have a role in animal and fungal models of infection (Lyczak *et al.* 2000; Roy-Burman *et al.* 2001; Saliba

et al. 2002; Rabin & Hauser 2003). Nevertheless, another ADP-ribosyltransferase, the type-II-secreted protein exotoxin A, has been shown to increase plant colonization by *P. aeruginosa*, which suggests that this class of proteins can affect bacteria–plant interactions (Cao *et al.* 2001).

What is the role of TTSSs in PGPP? The regulatory, structural and effector genes of *P. fluorescens* and *P. putida* TTSSs are closely related to those of *P. syringae*, whereas plant growth-promoting *P. aeruginosa* strains probably possess TTSSs and effectors similar to those described for animal pathogenic *P. aeruginosa* (Preston *et al.* 2001; Wolfgang *et al.* 2003). It therefore seems likely that PGPP TTSSs promote colonization of susceptible hosts in much the same way as in plant and animal pathogens. Modulation of host responses or host-specific recognition of effectors secreted by PGPP could have a significant impact on induction of local and systemic defence mechanisms by PGPP and on the ability of PGPP to live endophytically in plant tissues. However, it is possible that the use of TTSS effectors imposes host-specificity on plant–PGPP interactions, as has been observed for pathogens and *Rhizobium*, and the fact that the distribution of TTSSs in plant-associated *Pseudomonas* is by no means universal, suggests that for many bacteria, the costs outweigh the benefits. Current evidence clearly suggests that plant cells can and do receive TTSS-secreted effectors from a wide range of plant-colonizing bacteria, including PGPP, but extensive further analyses are needed to address the role of TTSSs in the ecology of plant-colonizing bacteria.

(b) *Priming plant defences: induced resistance*

Many recent studies of plant–PGPP interactions have focused on the ability of PGPP to induce systemic defence responses such as ISR or systemic acquired resistance (SAR) in host plants. Induced resistance is defined as active resistance dependent on the host plant's physical or chemical barriers, activated by biotic or abiotic agents (Press *et al.* 1997). Jasmonate (JA) and ethylene (ET) are key signal intermediates in the expression of ISR, whereas salicylic acid (SA) is a key signal intermediate in the induction of SAR (McCloud & Baldwin 1997; Bi *et al.* 1997; Van Loon *et al.* 1998; Moran & Thompson 2001; Conrath *et al.* 2002). Induced resistance arising from plant–PGPP interactions may be linked to ISR, SAR or both, in combination with other effects of PGPP on plants and plant-associated microbes. There is substantial communication between the two pathways, and they have been observed to act synergistically or antagonistically (Conrath *et al.* 2002; Kunkel & Brooks 2002). Most studies of ISR and SAR have used use foliar pathogens as the challenging pathogen, but induced resistance also operates in roots. The systemic nature of ISR and SAR in roots can be shown using split root systems, which demonstrate that treatment of one section of a root system with bacteria induces resistance to soil-borne pathogens in untreated roots (Chen *et al.* 1999, 2000).

SAR is primarily observed in response to pathogen-induced necrosis of plant cells. PGPP may induce SAR directly by synthesizing SAR signalling intermediates such as SA and reactive oxygen species (ROS), or by causing necrosis of host cells through the action of toxins, enzymes or elicitation of the HR. Bacteria produce SA as an intermediate in the biosynthesis of iron-chelating siderophores such as pyochelin, and in the catabolism of naphthalene (Press *et al.*1997). However, SA-deficient PGPP generally retain the ability to induce some degree of induced resistance, which suggests that multiple factors or multiple resistance pathways are involved (De Meyer *et al.* 1999).

ISR expression operates through the wound signalling intermediates JA and ET. ISR is not associated with local and systemic changes in the production of these signal molecules, but rather with an increased sensitivity to these hormones. ISR-expressing plants are primed to react faster and more strongly to JA and ET produced as a result of pathogen infection (Van Loon *et al.* 1998; Van Wees *et al.* 1999; Conrath *et al.* 2002). Bacterial signals involved in priming and eliciting ISR are poorly defined, although LPSs (specifically the O-antigen of LPS) and iron-chelating siderophores have been identified as potential inducers (Van Loon *et al.* 1998; Persello-Cartieaux *et al.* 2003). The ability of plants to express ISR is influenced by the plant genotype. Some ecotypes of *Arabidopsis* display a strong ISR response to *P. fluorescens* WCS417r, whereas others do not (Van Wees *et al.* 1997; Ton *et al.* 1999). Cultivar-specific induction of ISR has also been reported for other plants, including carnation and cucumber (Van Peer *et al.* 1991; Liu *et al.* 1995). The key difference between the ISR-inducible and ISR-non-inducible phenotype of *Arabidopsis* has been mapped to a single locus, *ISR1*, which encodes a component of the ET response. This suggests that the key difference between these plants lies in the signal transduction cascade rather than in recognition, and provides no clear evidence as to whether ISR is elicited by single or multiple signals (Ton *et al.* 2001; Conrath *et al.* 2002).

As ISR expression increases sensitivity to ET and JA, bacterial production of ET and JA analogues could also affect the expression of ISR in primed plants. Several plant pathogenic *Pseudomonas* produce ET and analogues of JA, and this ability may also be present in some PGPP. *Pseudomonas syringae* uses ET and the JA analogue coronatine to promote infection and endophytic growth (Mittal & Davis 1995; Weingart & Volksch 1997; Weingart *et al.* 1999; Bender *et al.* 1999; Budde & Ullrich 2000).

Plants display priming-like responses to a range of abiotic stresses, including cold, salt and drought, and multiple chemical and biophysical signals may affect the ISR phenotype (Conrath *et al.* 2002; Kunkel & Brooks 2002). Cross-talk between stress response pathways means that PGPP–plant interactions affect and are affected by many aspects of stress tolerance in plants. For example, plants inoculated with the PGPR *Paenibacillus polymyxa* show increased resistance to both pathogens and to drought stress (Timmusk & Wagner 1999), whereas disease resistance and ISR in *Arabidopsis* are strongly affected by plant age and environmental conditions (Ton *et al.* 2002*a*,*b*; Kus *et al.* 2002).

(c) *Signalling and plant stress: reactive oxygen*

One important stress signal used by plants is the generation of ROS. ROS are generated in a diverse array of plant processes, including photosynthesis, development, PCD, senescence, induction of anti-microbial defences and abiotic stress responses, and are important factors in many aspects of plant–PGPP interactions. ROS affect

plant cells in two main ways, as a cause of stress, through oxidative damage to plant molecules, and as signalling intermediates (Finkel 2003; Foreman *et al.* 2003). ROS may also act directly as anti-microbial factors when they are produced during plant defence responses, as pathogens and symbionts impaired in their ability to detoxify ROS are frequently impaired in their ability to colonize plants (Santos *et al.* 2001; Jamet *et al.* 2003; Venisse *et al.* 2003). However, it is possible that active ROS detoxification by microbes also alters stress signalling by ROS.

The role of plant-derived ROS in plant–microbe interactions has been studied for many years. However, it has only recently become clear that bacteria-derived ROS may also affect plant–PGPP interactions. De Meyer *et al.* (1999) showed that *P. aeruginosa* 7NSK2 induces systemic resistance, and suggested that this could be owing to SA biosynthesis. However, Audenaert *et al.* (2002) have subsequently shown that the generation of ROS by the interaction of the *Pseudomonas*-derived peptides Fe-pyochelin and pyocyanin may make an equally important contribution towards the induced resistance observed in this interaction. Phenazines such as pyocyanin have also been shown to have toxic effects in animal and plant models of pathogenic infection by *P. aeruginosa*, which may also be caused by the generation of ROS such as superoxide and hydrogen peroxide (Mahajan-Miklos *et al.* 1999; Cao *et al.* 2001).

(d) *Eavesdropping on bacterial conversations: autoinducers*

One recent, and still controversial, question about plant–bacteria interactions is the role and impact of acylated homoserine lactones (AHLs). Many *Pseudomonas* use AHLs to monitor the external environment and the proximity of other bacteria (Loh *et al.* 2002; von Bodman *et al.* 2003). AHLs from *P. aeruginosa* have been shown to have immunomodulatory effects on mammalian cells, suggesting that AHLs may act as targets for host recognition or as virulence factors (Mathesius *et al.* 2003). Mathesius *et al.* (2003) used proteomics to show that the model legume *Medicago trunculata* responds to AHLs produced by both pathogenic (*P. aeruginosa*) and symbiotic bacteria (*Sinorhizobium meliloti*), and showed that AHL treatment modulates the production of AHL signal-mimics by *Medicago*. The red alga *Delisea pulchra* has been shown to produce halogenated furanones that inhibit AHL-mediated gene expression by displacing the AHL signal from its receptor protein (Manefield *et al.* 1999). AHL mimics produced by *Medicago* and other plants may disrupt or unbalance AHL signalling in bacteria (Bauer & Robinson 2002). However, the jury is still out on whether AHLs constitute another class of molecule involved in modulating local and systemic plant responses to bacteria.

6. MODULATION OF PLANT DEVELOPMENT AND PLANT PHYSIOLOGY

In addition to modulating plant defence responses, PGPP also produce chemicals that act on other aspects of plant development and plant physiology. Plant hormones produced by *Pseudomonas* include auxin (indole acetic acid, IAA) and cytokinins, as well as volatile signals such as ethylene 2,3 butanediol and acetonin (Lambrecht *et al.* 2000; Persello-Cartieaux *et al.* 2003; Ryu *et al.* 2003). *Pseudomonas* may also have indirect effects on hormones and signalling intermediates, for example by secreting cell wall degrading enzymes that release peptides and oligosaccharides that subsequently affect plant development and plant signal transduction (Dumville & Fry 2000), or by disrupting the balance of normal hormone synthesis in plant cells (Glick *et al.* 1994). In some plant–pathogen interactions hormone synthesis induces the development of galls and other dystrophies (Lindow & Brandl 2003; Persello-Cartieaux *et al.* 2003). One role of pathogen-induced galls appears to be the redirection of host metabolism and development to favour nutrient transport to the point of infection. Plant hormone synthesis may serve a similar but less disruptive role in PGPP, which have frequently been shown to stimulate root growth and proliferation (Persello-Cartieaux *et al.* 2003). Hormone-producing bacteria may also benefit from IAA and cytokinin-stimulated release of saccharides and methanol from the plant cell wall as a local nutrient source, and from the effects of phytohormones on wound and defence signal transduction (Lindow & Brandl 2003).

7. PLANT GROWTH-PROMOTING *PSEUDOMONAS*: BENIGN BACTERIA OR NECESSARY EVILS

Iron-chelating and anti-microbial peptides produced by PGPP can have direct (ROS generation) or indirect (suppression of pathogens) effects on plants. Many of the beneficial effects of PGPP, such as their interactions with other plant-associated organisms, fall into the indirect category and are beyond the scope of this article. However, some factors that have been extensively studied in the context of beneficial PGPP activities, such as antagonism of plant pathogenic fungi, can also have direct and sometimes deleterious effects on plants (Nehl *et al.* 1997). PGPP and other root-colonizing bacteria produce many molecules that are potentially toxic or inhibitory to both micro-organisms and plant cells, including pore-forming toxins and hydrogen cyanide (Bender *et al.* 1999; Blumer & Haas 2000). Some cyanogenic soil bacteria have even been touted as potential bioherbicides (Kremer & Souissi 2001).

A plant–bacteria interaction may be categorized as beneficial if the net benefit (suppression of pathogens, promotion of plant growth and disease resistance) outweighs the net cost (phytotoxicity and parasitism by PGPP). The potential negative effects of any single factor are strongly affected by the genetic and ecological context. For example, many beneficial root-colonizing PGPP and non-pathogenic *P. syringae* produce cyclic lipopeptides with surfactant and anti-fungal properties that help these bacteria to spread across plant surfaces and suppress competing micro-organisms (see Thrane *et al.* 1999; Nielsen *et al.* 1999; Nielsen & Sorensen 2003; Lindow & Brandl 2003). However, similar lipopeptides are also linked to the spread of *P. fluorescens* wet rot across waxy plant surfaces (Braun *et al.* 2001), and account for many of the pore-forming toxins and surfactants identified as virulence factors in *P. syringae* (Bender *et al.* 1999).

Table 1 lists some of the main factors known or predicted to be involved in plant–*Pseudomonas* interactions.

Table 1. Summary of key PGPP factors with effects on plant signal transduction.

factor	function in PGPP	effect(s) on plants
flagellin	motility	elicits defence responses
LPS	protection; host interactions	elicits local and systemic defence responses; suppresses HR
exoenzymes	saprotrophy; pathogenesis?	damage plant cells; release of peptides and oligosaccharides may induce host defences
TTSS effectors	promote endophytic growth	elicit or suppress host defence responses; may affect a wide variety of cellular processes
SA	iron acquisition; catabolic intermediate	induces local and systemic defence responses
ROS (secreted peptides)	iron acquisition; antagonism; pathogenesis?	oxidative stress, oxidative signalling; induce resistance
plant hormones	modulation of plant physiology	induce or suppress plant defence responses; stimulate or inhibit plant growth and development
toxins and surfactants	surface colonization; antagonism; pathogenesis?	membrane dysfunction and necrosis; induce local and systemic plant defence responses; inhibit metabolism and growth

However, the outcome of a plant–*Pseudomonas* interaction cannot be simply predicted by adding up the factors listed in table 1. The net cost or benefit of interactions with PGPP is affected by the nutritional status of the soil, the toxic effects of the bacterium and the presence of fungal pathogens, further complicated by plant age, environmental factors, induced stress resistance and cross-talk between plant signal transduction pathways.

8. PROSPECTS AND CHALLENGES

Studies of plant–*Pseudomonas* interactions have identified several key factors involved in plant recognition of bacteria, and in bacterial modulation of host metabolism, that help to explain some of the effects of *Pseudomonas* on plants (table 1, figures 1–3). But are we any closer to understanding how plants perceive PGPP and pathogenic *Pseudomonas*? The existence of plant recognition mechanisms for common bacterial molecules such as flagellins and LPS, and the stresses bacteria impose on plant cells, suggest that few bacteria can avoid being 'noticed' by plants, although modification and regulation of some of these factors may reduce the overall conspicuousness of a bacterium. The key differences between successful and unsuccessful endophytes seem to lie in the dialogue between plant and bacterium, and in the ability of successful endophytes to suppress the expression of plant defences subsequent to this basic recognition, using hormones, toxins and TTSS effectors. Kang *et al.* (2003) recently showed that *Arabidopsis* plants with mutations in the *NHO1* locus are susceptible to infection by previously incompatible pathogens and non-pathogenic *Pseudomonas*. A virulent pathogen, *P. syringae* pv. *tomato* is able to suppress *NHO1* in wild-type plants. In considering the differences between pathogens and PGPP, it may be worth reframing the question in terms of bacteria that do or do not succeed in multiplying as endophytes inside plant tissue, and looking at the key differences between these interactions, regardless of whether they involve pathogens or PGPP. It seems likely that the true picture of plant–*Pseudomonas* interactions is closer to a continuum than a hard and fast divide, and there may be significant commonalities in plant colonization mechanisms used by endophytic *Pseudomonas*.

One important and largely unexplored area of PGPP research is the level of host specificity and host variation in PGPP–plant interactions, and how this affects endophytic and epiphytic populations of PGPP. Cultivar-specific variation in disease suppression, antibiotic production and colonization has been described for several PGPR–plant interactions, but few studies have addressed the genetic and mechanistic basis of this variation (Smith & Goodman 1999). I have briefly discussed the potential for host-specific recognition and exclusion of PGPP in the context of flagellins, LPS, type III secretion and ISR, but it is also conceivable that there is host-specific compatibility between PGPP and plants as a result of host-specific targets for TTSS effectors, or because of a specific PGPP's ability to catabolize host-specific chemicals.

To develop a cohesive model of plant–PGPP interactions it will be necessary to focus future experiments on a few model systems for which extensive resources are available. Many recent studies have used the model plant *Arabidopsis*. The availability of genome sequences for *Arabidopsis* and for pathogens and PGPR that are able to colonize this plant makes *Arabidopsis* an extremely valuable model for post-genomic analyses of plant–microbe interactions (The *Arabidopsis* Genome Initiative 2000; Preston 2000; Schenk *et al.* 2000; Nelson *et al.* 2002; Ramonell & Somerville 2002; Wan *et al.* 2002; de Torres *et al.* 2003; Buell *et al.* 2003). However, it should be noted that *Arabidopsis* is of limited value in understanding plant–PGPP interactions in the context of plant–mycorrhizal or plant–rhizobia associations. Alternative model plant hosts such as the legume *M. trunculata* will become increasingly important as we try to understand how plants manage simultaneous interactions with diverse organisms (Cook 1999).

In reviewing this area of research, it is important to stress that plant–PGPP interactions have been most extensively characterized in the context of interactions with plant roots, whereas most studies of plant interactions with pathogenic *Pseudomonas* have focused on leaves. Relatively few studies have looked at the differences and

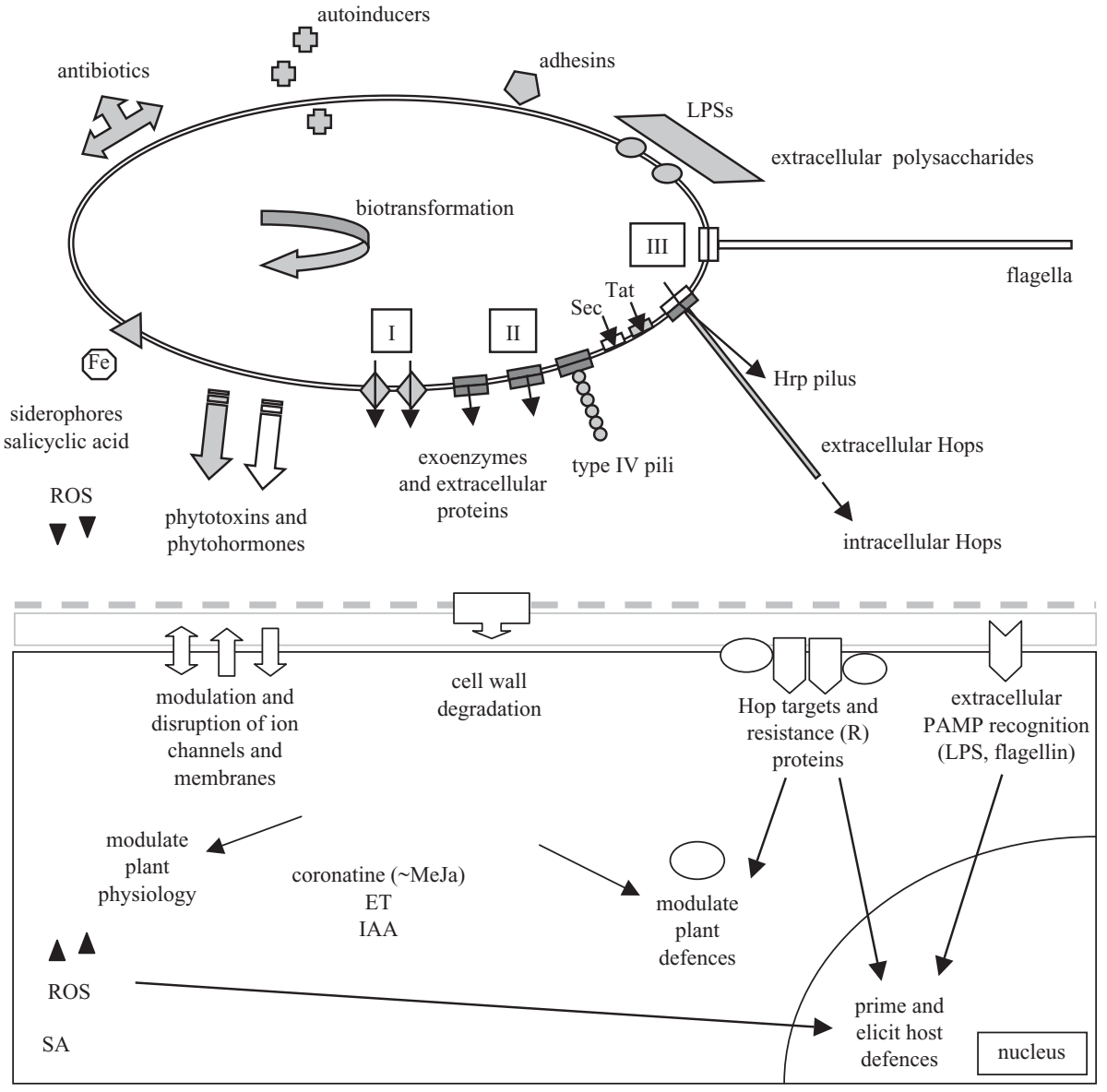

Figure 3. Overview of interactions between *Pseudomonas* and plant cells. The figure illustrates the range of factors produced by pathogenic and non-pathogenic *Pseudomonas* that can be recognized by, and have effects on, plant cells. Extracellular proteins are mainly secreted across the outer membrane of *Pseudomonas* through three routes, the types I, II and III secretion pathways, labelled in boxes as I, II and III (Thanassi & Hultgren 2000; Ma *et al.* 2003). Proteins are delivered to the terminal ends of the general secretory pathway (type II secretion, type IV pilus biogenesis) in a two-step process involving a periplasmic intermediate, which is translocated across the inner membrane by the Sec or Tat pathway. Type III secreted Hops are secreted directly from the cytoplasm to the outside of the cell and can be divided into two classes, extracellular effectors (Hops) that are secreted at high levels to the outside of the cell and intracellular Hops that are delivered directly into the cytoplasm of the plant cell via the Hrp pilus. Several well-characterized intracellular Hops have been shown to be targeted to the plant cell membrane, as shown, where they may act to modulate plant defence mechanisms (Greenberg & Vinatzer 2003). For simplicity, the complex signal transduction pathways that regulate plant responses to *Pseudomonas* have been reduced to a few key arrows that highlight important connections.

similarities between responses to bacteria in leaves and roots, or between specific cell types within plant tissues. Although ISR, SAR and local defence responses do operate in roots, it seems possible that some defence mechanisms that operate effectively against foliar pathogens may be less effective in the less confined arena of root interactions with soil-borne bacteria. It would not be surprising to discover that root epidermal cells, which are continually exposed to contact with a wide range of microorganisms, express different surveillance and defence mechanisms from those expressed by less exposed cells in the interior of leaves, roots and stems.

The versatile and physiologically robust nature of *Pseudomonas* means that they have the potential to provide biological solutions to important problems in industry, agriculture and the environment. However, current analyses of *Pseudomonas* are raising more questions than answers about the ecology and pathogenic potential of these organisms. Does the presence of type III secretion genes in some beneficial plant-colonizing *Pseudomonas*

reflect an underlying predisposition to pathogenicity? Do the biochemical activities of PGPP actively modulate plant metabolism and signal transduction to favour PGPP colonization? What are the key factors that promote pathogenesis by 'opportunistic' *Pseudomonas* and trigger the transition from saprotrophy to disease, or from living plant tissue to a substrate for decomposition? To what extent are interactions between plants and PGPP host specific, and what impact does host specificity have on PGPP populations? A better understanding of the factors that determine whether a plant identifies a bacterium as a partner or a threat, and the factors that give a bacterium the potential to be a pathogen or PGPP, will guide researchers in establishing a substantially more rational basis for selecting the *Pseudomonas* we choose to enlist as partners.

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