Mini-Review

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Rhizobia and plant-pathogenic bacteria: common infection weapons

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Plant-interacting micro-organisms can establish either mutualistic or pathogenic associations. Although the outcome is completely different, common molecular mechanisms that mediate communication between the interacting partners seem to be involved. Specifically, nitrogen-fixing bacterial symbionts of legume plants, collectively termed rhizobia, and phytopathogenic bacteria have adopted similar strategies and genetic traits to colonize, invade and establish a chronic infection in the plant host. Quorum-sensing signals and identical two-component regulatory systems are used by these bacteria to coordinate, in a cell density-dependent manner or in response to changing environmental conditions, the expression of important factors for host colonization and infection. The success of invasion and survival within the host also requires that rhizobia and pathogens suppress and/or overcome plant defence responses triggered after microbial recognition, a process in which surface polysaccharides, antioxidant systems, ethylene biosynthesis inhibitors and virulence genes are involved.

Introduction

Soil bacteria belonging to various genera of the order Rhizobiales (collectively called rhizobia) are able to invade legume roots in nitrogen-limiting environments, leading to the formation of a highly specialized organ, the root nodule. Nodule formation is a complex process that requires a continuous and adequate signal exchange between the plant and the bacteria, of which we only have a fragmentary knowledge (for reviews see Perret *et al.*, 2000; Bartsev *et al.*, 2004b). Rhizobia are attracted by root exudates and colonize plant root surfaces. Flavonoids present in the exudates activate the expression of the bacterial nodulation (*nod*) genes involved in the synthesis and secretion of Nod-factors (NF), lipochito-oligosaccharides that are recognized by the plant (Table 1). Nod factors together with additional microbial signals such as polysaccharides and secreted

proteins allow bacteria attached to root hairs to penetrate the root through a tubular structure called the infection thread, which grows towards the root cortex where the nodule primordium is developing. When the thread reaches the primordium, the bacteria are released into the plant cytoplasm, where they differentiate into their endosymbiotic form, the bacteroids. These bacteroids are able to reduce nitrogen into ammonia, which is used by the plant. In return, the bacteria are supplied with carbohydrates in a protected environment.

Like rhizobia, pathogenic bacteria establishing compatible interactions with plants also obtain nutrients from the host upon colonization. This process, which often involves the participation of hydrolytic enzymes and toxins (Table 1), provokes plant injury, disease or even death. In spite of these different outcomes, accumulating evidence suggests that

Table 1. Specific rhizobial and phytopathogenic factors required for association with plants

Factor	Rhizobium specific	Plant pathogen specific	
	Nod factor	Hydrolytic enzymes	Toxins
Function	Root hair deformation, nodule primordium and preinfection thread formation, nodule-specific gene expression, control of plant defences	Nutrient acquisition and invasion	Interference with plant metabolism, control of plant defences
Phenotype of bacterial mutants	No nodules are formed	Reduction or loss of virulence	Reduction or loss of virulence

rhizobia and plant pathogens use similar 'weapons' to invade the host (Fig. 1). Here we review chemical signals, regulatory systems and virulence genes that play a role in the rhizobia-legume and plant-pathogenic interactions. We also describe common strategies and components used by rhizobia and phytopathogenic bacteria to suppress and/or overcome plant defence responses, requisites to establishing a chronic infection within the host.

Quorum sensing signals play several roles in plant-microbe interactions

Many bacteria are able to regulate gene expression in response to changes in the population density, a process known as quorum sensing (QS). QS is mediated by small,

diffusible signal molecules called autoinducers, which accumulate as the bacterial population increases (Miller & Bassler, 2001). The most common QS signals are *N*-acylhomoserine lactones (AHLs), containing a conserved homoserine ring connected to a variable acyl chain (Fig. 2a). These autoinducers are detected in the cytoplasm by LuxR-type transcriptional activators. Several AHLs have been identified in rhizobia and plant pathogens (reviewed by González & Marketon, 2003; von Bodman *et al.*, 2003) (Table 2). QS regulation in these bacteria is also mediated by non-AHL signals such as bradyoxetin in *Bradyrhizobium japonicum* (Loh *et al.*, 2002a), *Pseudomonas* quinolone signal (PQS) in *Pseudomonas aeruginosa* (Pesci *et al.*, 1999), 3-hydroxypalmitic acid methyl ester (3-OH PAME) in *Ralstonia solanacearum* (Flavier *et al.*, 1997), or the diffusible

Fig. 1. Common strategies used by plant-interacting bacteria to establish compatible associations with their hosts. (a) Coordination of gene expression for host colonization and invasion mediated by quorum sensing (QS) signals and two-component regulatory (2-CR) systems. Detection of *N*-acylhomoserine lactones (AHL, loop and tail) by cytoplasmic LuxR-type transcriptional activators (black oval), and non-AHL (black triangles) by 2-CR systems (white and black squares), allow plant-interacting bacteria to coordinate the expression of important genes for host colonization and invasion in response to cell density. AHLs play an additional role in plant signalling (see text for details). Regulation of bacterial factors required during the infection process is also accomplished in plant-interacting bacteria by 2-CR systems (white and grey hexagons) which are activated by environmental conditions usually encountered during the invasion process. Common rhizobial and pathogenic bacterial responses are shown by bold arrows whereas responses observed only in one or the other are represented by dotted arrows. (b) Bacterial components used to control plant defence responses. Surface polysaccharides (SPS) are able to suppress microbial-induced defence reactions and/or to act as shields protecting the bacterium against toxic compounds. Additionally, active suppression of the defence reaction is achieved with ethylene (ET) inhibitors (ETin) and virulence factors such as type III and IV secretion systems (T3 and T4). Antioxidant systems protect bacteria against reactive oxygen species (ROS).

Fig. 2. Structures of autoinducers produced by plant-interacting bacteria. (a) *N*-Acylhomoserine lactones (AHLs). The general structure of AHLs is shown in bold. R₁, -H, -OH or =O; R₂, -CH₃, -(CH₂)₂₋₁₄CH₃ or -(CH₂)₅CH=CH(CH₂)₅CH₃. The structure of the 3-oxohexanoylhomoserine lactone (3-oxo-C₆-HSL) produced by several plant pathogens and by *Sinorhizobium meliloti* is represented below the general structure of AHL. (b) Non-AHL signals: bradyoxetin (2-[4-[[4-(3-aminooxetan-2-yl)phenyl]-(imino)methyl]phenyl]oxetan-3-ylamine), identified in *Bradyrhizobium japonicum*; PQS (*Pseudomonas* quinolone signal, 2-heptyl-3-hydroxy-4-quinolone); PAME (3-hydroxypalmitic acid methyl ester) produced by *Ralstonia solanacearum*; DSF (diffusible signal factor, *cis*-11-methyl-2-dodecenoic acid) of *Xanthomonas campestris*.

signal factor (DSF) of *Xanthomonas campestris* pv. *campestris* reported as *cis*-11-methyl-2-dodecenoic acid (Wang *et al.*, 2004a) (Fig. 2b, Table 2). In these cases the signals seem to be detected through two-component regulatory systems.

Deficiencies in QS lead to the reduction or loss of virulence in phytopathogenic bacteria, and to altered nodulation and nitrogen fixation efficiencies in rhizobia (Loh *et al.*, 2002b; González & Marketon, 2003; von Bodman *et al.*, 2003). QS regulation contributes in different ways to the establishment of a compatible interaction with the plant (Fig. 1a, Table 2).

Cell-cell communication within bacterial populations. QS enables bacterial cells within a population to coordinate the expression of genes important for the colonization and infection of their plant hosts. Some important processes regulated by QS signals include the following.

(i) Transition from a saprophytic lifestyle to that of a plant-interacting microbe. In plant-pathogenic bacteria such as *Ralstonia solanacearum* and *Pseudomonas syringae* pv. *syringae*, and in the mutualist *Sinorhizobium meliloti*, QS is involved in turning off behaviours suited to free-living survival such as motility, and turning on those required for host colonization and invasion such as production of extracellular polysaccharides (EPSs) (Clough *et al.*, 1997; Hoang *et al.*, 2004; Gao *et al.*, 2005; Quiñones *et al.*, 2005).

(ii) The development of biofilms on plant surfaces, a process that is probably important in plant-microbe associations, is controlled by QS in pathogens such as *X. campestris* pv. *campestris* (Dow *et al.*, 2003) and *Pantoea stewartii* (Koutsoudis *et al.*, 2006), and this could also be the case in rhizobia (Wang *et al.*, 2004b).

(iii) Expression of factors involved in plant invasion. Important pathogenicity determinants like EPS, degradative

exoenzymes and effector secretion are controlled in a cell-density-dependent manner in several plant pathogens (von Bodman *et al.*, 2003; Table 2). Within the rhizobia, QS regulation controls the expression of *nod* and rhizosphere-expressed (*rhi*) genes that influence nodulation in *B. japonicum* and *Rhizobium leguminosarum* bv. *viciae*, respectively (Cubo *et al.*, 1992; Loh *et al.*, 2002a), the production of EPSs involved in nodule invasion by *S. meliloti* (Marketon *et al.*, 2003; Hoang *et al.*, 2004; Gao *et al.*, 2005), and bacteroid differentiation in *Rhizobium etli* (Daniels *et al.*, 2002). QS control of these determinants prevents early production of factors like EPS which could interfere with other important processes that precede invasion, such as adhesion. QS mutants of the plant pathogen *Pantoea stewartii* subsp. *stewartii* (Koutsoudis *et al.*, 2006) and of *Mesorhizobium tianshanense* (Zheng *et al.*, 2006) show altered adhesion to surfaces. Moreover, QS regulation avoids early or overexpression of virulence/symbiotic factors which could be detrimental to the bacteria if plant defence responses are alerted before the population reaches a density that ensures productive infection.

Cross-kingdom signalling between bacteria and their plant hosts. Mathesius *et al.* (2003) demonstrated that the model legume *Medicago truncatula* can detect and respond to AHLs produced by mutualistic and pathogenic bacteria. Although many of the plant responses to the two different types of AHL tested were common, specific changes were also detected depending on the concentration and structure of the AHL, suggesting that plants may discriminate between AHLs from different bacteria. In addition to changes in root protein levels, exposure of plant roots to bacterial AHLs induces changes in the secretion of compounds of unknown chemical structure that mimic QS signals and potentially could interfere with bacterial behaviours regulated by QS. AHL structure also affects the changes in the QS-mimics secreted by the plant.

Table 2. Quorum-sensing signals detected in plant-interacting bacteria and regulated functions

Abbreviations: AHL, *N*-acylhomoserine lactone; DSF, diffusible signal factor; EPS, extracellular polysaccharide; HSL, homoserine lactone; PAME, palmitic acid methyl ester; PQS, pseudomonas quinolone signal; ROS, reactive oxygen species; T3SS, type III secretion system.

Bacterium	QS signals	Regulated functions*	References
Pathogens			
<i>P. stewartii</i> subsp. <i>stewartii</i>	3-oxo-C ₆ -HSL 3-oxo-C ₈ -HSL	Expression of EPS and T3SS, biofilm development, adhesion, host colonization	von Bodman <i>et al.</i> (2003); Koutsoudis <i>et al.</i> (2006)
<i>E. carotovora</i> subsp. <i>carotovora</i>	3-oxo-C ₆ -HSL	Expression of extracellular enzymes and T3SS	von Bodman <i>et al.</i> (2003)
<i>P. syringae</i> pv. <i>syringae</i>	3-oxo-C ₆ -HSL	Expression of EPS, motility, ROS resistance	Quñones <i>et al.</i> (2005)
<i>P. aeruginosa</i>	3-oxo-C ₁₂ -HSL C ₄ -HSL PQS	Expression of extracellular enzymes, ROS resistance, plant responses	Pesci <i>et al.</i> (1999); Mathesius <i>et al.</i> (2003); von Bodman <i>et al.</i> (2003)
<i>R. solanacearum</i>	3-OH-PAME	Expression of EPS and extracellular enzymes, motility	Clough <i>et al.</i> (1997); von Bodman <i>et al.</i> (2003)
<i>X. campestris</i> pv. <i>campestris</i>	DSF	Expression of EPS and extracellular enzymes, biofilm development	Dow <i>et al.</i> (2003); Wang <i>et al.</i> (2004a)
Rhizobia			
<i>S. meliloti</i>	C ₆ -HSL, 3-oxo-C ₆ -HSL C ₈ -HSL, C ₁₂ -HSL C ₁₄ -HSL, 3-oxo-C ₁₄ -HSL, C ₁₆ -HSL, 3-oxo-C ₁₆ -HSL, C _{16:1} -HSL 3-oxo-C _{16:1} -HSL, C ₁₈ -HSL	Expression of EPSII, motility, regulation of nitrogen fixation, rhizopine utilization, plant responses	González & Marketon (2003); Marketon <i>et al.</i> (2003); Mathesius <i>et al.</i> (2003); Hoang <i>et al.</i> (2004); Gao <i>et al.</i> (2005)
<i>R. leguminosarum</i> bv. <i>viciae</i>	C ₆ -HSL, C ₇ -HSL, C ₈ -HSL, 3-oxo-C ₈ -HSL, 3-OH-C _{14:1} -HSL	Expression of <i>rhi</i> genes	Cubo <i>et al.</i> (1992); González & Marketon (2003)
<i>R. etli</i>	Hydroxylated-long-chain AHL, short-chain AHL	Bacteroid differentiation	Daniels <i>et al.</i> (2002)
<i>B. japonicum</i>	Bradyoxetin	Regulation of <i>nod</i> genes	Loh <i>et al.</i> (2002a)
<i>M. huakuii</i>	Unidentified AHL	Biofilm development	Wang <i>et al.</i> (2004b)
<i>M. tianshanense</i>	Unidentified AHL	Root hair adhesion, expression of essential nodulation factors	Zheng <i>et al.</i> (2006)

*Only functions with a direct role in association with the host are described.

An area of future research will be directed to understanding how plants respond not only to AHLs but to bacterial QS signals in general, to identify the different QS-mimics produced by plants in response to particular bacteria and their roles in controlling bacterial behaviours.

Identical two-component regulatory systems identified in rhizobia and phytopathogenic bacteria

Generally consisting of a sensor protein kinase and a response regulator, these systems allow bacteria to regulate gene expression in response to environmental changes, enabling them to rapidly adapt to new conditions. Identical two-component regulatory (2-CR) systems have been identified in plant pathogens and rhizobia that are essential for the successful interaction with their plant hosts (Fig. 1a). This is the case for the ChvG/ChvI system of *Agrobacterium tumefaciens*, and its orthologue ExoS/ChvI in *S. meliloti*

(Cheng & Walker, 1998). In *A. tumefaciens*, mutations in either *chvI* or *chvG* abolish tumour-forming ability and lead to altered surface properties. ChvG has been proposed to be a general sensor of low pH controlling the expression of acid-inducible genes (Li *et al.*, 2002). In *S. meliloti* the signal(s) sensed by ExoS is/are yet unknown but ExoS and ChvI participate in the regulation of two surface components: succinoglycan (EPS I) and flagella (Cheng & Walker, 1998; Yao *et al.*, 2004). A *S. meliloti* *exoS* mutant overproduces EPSI and does not synthesize flagella, and is significantly impaired in colonization of curled root hairs of alfalfa. As described above, the coordinated regulation of flagella and invasion factors mediated by QS and/or 2-CR systems plays an important role in the establishment of an effective symbiosis.

Another interesting example of a 2-CR system shared by plant pathogens and rhizobia is the VirA/VirG system. In *A. tumefaciens* it is involved in controlling the expression of the

vir (virulence) region necessary for T-DNA transfer to the plant (Winans, 1992) in response to small phenolic compounds released by wounded plants. A highly similar system is present in the symbiosis island of *Mesorhizobium loti* R7A linked to a *virB* operon encoding a type IV secretion system (T4SS) (Hubber *et al.*, 2004). Although the signal sensed by VirA/VirG in *M. loti* is unknown, upstream of *virA* there is a *nod* box typical of rhizobial *nod* genes, which suggests the participation of plant-derived compounds and the transcriptional activator NodD. *M. loti virA* and *virG* mutants show symbiotic phenotypes identical to mutants affected in the structural *virB* genes, i.e. reduced nodule formation efficiencies in a host plant or formation of nitrogen-fixing nodules in a non-host plant. This suggests that VirA/VirG play a role in symbiosis, regulating the expression of a T4SS that influences the outcome of the interaction with the host.

Future investigations should unveil commonalities among the regulons controlled by these 2-CR systems in phytopathogenic and mutualistic bacteria.

Control of plant defence responses

In response to microbial invaders, plants can mount complex defence responses mediated by signalling molecules such as salicylic acid, reactive oxygen species (ROS), nitric oxide, jasmonic acid or ethylene (Dong, 1998). These molecules have also been detected in legumes in response to rhizobial inoculation (reviewed by Ferguson & Mathesius, 2003). To establish a chronic infection within the plant, rhizobia and pathogens must be able to control or avoid the host defence mechanisms. Bacterial components with a role in the control of the plant defence response have been identified. In some cases, very specialized strategies adapted to the infection mode have been acquired (Table 1). Thus, rhizobial Nod factors are able to suppress salicylic acid accumulation and ROS production when recognized by the cognate legume host (Martínez-Abarca *et al.*, 1998; Bueno *et al.*, 2001; Shaw & Long, 2003). On the other hand, several pathovars of *P. syringae* produce the phytotoxin coronatin, which suppresses salicylic-acid-dependent plant defences by inducing the jasmonic acid signalling pathway (Abramovitch & Martin, 2004). Besides these specific strategies, rhizobia and plant pathogens make use of similar components to overcome or actively suppress plant defences such as surface polysaccharides (SPSs), antioxidant systems, ethylene inhibitors and specific virulence factors (Fig. 1b).

Surface polysaccharides. SPSs are considered as either signalling molecules in the plant–microbe cross-talk, and/or compounds that protect against plant defences. Rhizobial mutants defective in the synthesis of EPS, lipopolysaccharide (LPS) or cyclic β -glucans are unable to infect the host successfully and/or to form nitrogen-fixing nodules. Isolated low-molecular-mass EPS I, LPS or only the lipid A substructure of the LPS from *S. meliloti*, or bradyrhizobial cyclic β -glucans, are able to suppress elicitor-induced typical defence reactions in host plants

(reviewed by Fraysse *et al.*, 2003; Scheidle *et al.*, 2005). Additionally, all or some of these SPSs could act as shields protecting the bacterium from an early plant defence response. Thus, bradyrhizobial mutants defective in cyclic glucans are more sensitive to oxidative burst and to phytoalexins (Mithöfer *et al.*, 2001), whereas EPSs from *Azorhizobium caulinodans* prevent the entry of toxic H₂O₂ during the early stages of invasion (D'Haeze *et al.*, 2004). Phytopathogenic bacteria defective in SPSs are generally affected in their virulence. Although this aspect is less investigated than for rhizobia, there are data correlating SPSs with increased resistance to environmental stresses and toxic molecules, suggesting that SPSs could also protect bacterial pathogens against plant defences (Keith & Bender, 1999; Yu *et al.*, 1999).

Antioxidant systems. Mechanisms to scavenge ROS are common among pathogens and rhizobia, and catalases and superoxide dismutases (SOD) are encoded in the genomes of these plant-interacting microbes (Van Sluys *et al.*, 2002). SOD and catalase, enzymes responsible for the inactivation of O₂^{•−} and H₂O₂, respectively, are virulence factors for some phytopathogenic bacteria (Xu & Pan, 2000; Santos *et al.*, 2001). Similarly, antioxidant defence systems are crucial for the establishment and the maintenance of the symbiosis. *S. meliloti* mutants defective in ROS-scavenging enzymes show deficiencies in nodulation and nitrogen fixation (reviewed by Hérouart *et al.*, 2002). Moreover, the inability to synthesize the tripeptide glutathione (γ -glutamyl-L-cysteinyglycine), an important antioxidant, leads to important symbiotic defects in *S. meliloti* and *Rhizobium tropici* (Ricciolo *et al.*, 2000; Harrison *et al.*, 2005).

Ethylene inhibitors. Strategies to limit the synthesis of ethylene by the plant in response to microbial infection have also been adopted by some plant pathogens and rhizobia. *Bradyrhizobium elkanii* and the plant pathogen *Burkholderia andropogonis* produce rhizobitoxine [2-amino-4-(2-amino-3-hydropropoxy)-*trans*-but-3-enoic acid], an inhibitor of ethylene synthesis (Mitchell *et al.*, 1986; Ma *et al.*, 2002), and several rhizobia produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which degrades the immediate precursor of ethylene (Ma *et al.*, 2002). In rhizobia, either strategy leads to increased nodule formation efficiencies.

Virulence genes present in rhizobia

Analysis of available rhizobial genomes reveals the presence of hundreds of genes homologous to the virulence factors of pathogens (Van Sluys *et al.*, 2002). Interestingly, the functional characterization of some of them, such as those encoding type III and type IV secretion systems (T3SS and T4SS, respectively) indicate a likely role in the mutualistic rhizobia–legume interaction.

Type III secretion systems. Encoded by the hypersensitive response and pathogenicity (*hrp*) gene cluster, these

systems are essential for virulence of phytopathogenic bacteria in susceptible hosts and for the induction of the hypersensitive response (HR) in resistant and non-host plants. These genes are expressed in response to environmental factors that usually correspond to the conditions encountered during the infection of a host (Hueck, 1998). As a molecular syringe, and upon contact with host cells, the T3SS injects bacterial proteins or effectors directly into the plant cell. In a susceptible host, T3S effectors counter plant defences and modulate plant physiology to sustain the growth of the pathogen, whereas in resistant plants they have an avirulence activity that triggers the plant immune system. Some T3S effectors show enzymic activity and are able to interfere with the regulation of host proteins by mimicking the activity of plant proteases and phosphatases (Mudgett, 2005).

T3SS-like genes have been identified in several rhizobial species, usually linked to symbiotic genes in plasmids or chromosomal islands (Marie *et al.*, 2001; Bartsev *et al.*, 2004b), but they are absent in two species, *S. meliloti* and *R. leguminosarum* bv. *viciae*, and in some strains of *M. loti*. As in plant pathogens, the expression of rhizobial T3SS genes is induced by plant signals, similar to those controlling *nod* gene expression. Recognition of plant flavonoids by NodD activates transcription of the regulator *ttsI*, which in turn modulates expression of T3SS genes, allowing for secretion of proteins (Marie *et al.*, 2004). Clearly in rhizobia T3SSs help to modulate the host range as in plant pathogens. Blockage of T3 secretion has various effects on the rhizobia-legume interaction which are plant-host specific (Marie *et al.*, 2001; Bartsev *et al.*, 2004b). T3S has little influence on the symbiosis with some plant species whereas it is important for optimal or efficient nodulation in others. Negative effects by the secreted proteins have also been observed; thus, blockage of T3S can improve nodulation or convert pseudonodules to nitrogen-fixing nodules.

Rhizobial proteins secreted by T3SSs are known as Nops (nodulation outer proteins). Some Nops show a degree of sequence conservation with T3S effectors of bacterial pathogens, whereas others seem to be specific to rhizobia (Marie *et al.*, 2003; Skorpil *et al.*, 2005). Amongst the latter, some are present in all rhizobia whereas others may be strain or species specific. Yet it is not known how Nops affect nodule formation. Two of them, NopL and NopP, are likely effectors with a function within the plant cell. Both seem to be phosphorylated by plant protein kinases (Bartsev *et al.*, 2003; Skorpil *et al.*, 2005). Interestingly, and as is the case for many T3SS effectors of plant pathogens, NopL probably interferes with plant defence responses, because these are suppressed in transgenic plants expressing *nopL* (Bartsev *et al.*, 2004a). Rhizobia with T3SSs probably secrete a mix of Nops. In some legumes the effector mix can be recognized as avirulence factors and mounts a strong defence reaction against the bacterium that interferes with nodulation, whereas in other plants the lack of the corresponding

resistance proteins allows the effectors to fulfil their function. Future work should clarify the specific roles of the various effector proteins in nodulation, the plant cell types where they are injected and the plant programmes interfered with.

Type IV secretion systems. These multicomponent transporters of Gram-negative bacteria mediate secretion of proteins or nucleoproteins into virtually any cell type or into the milieu. A subgroup of T4SSs is formed by the bacterial conjugation machines mediating the spread of plasmids among bacterial populations. Several pathogens use T4SS to deliver effector molecules to eukaryotic target cells. The transported substrates can suppress defence reactions, facilitate intracellular growth and even induce the synthesis of nutrients that are beneficial to bacterial colonization (Cascales & Christie, 2003). The prototype of T4SSs involved in delivering macromolecules to eukaryotic cells is the VirB/D4 T4SS of *A. tumefaciens* that participates in delivering the oncogenic T-DNA into plant cells. *virB* clusters have been identified in the genomes of several nontumorigenic phytopathogens. Although functional analyses of these systems are still scarce, roles in virulence have been demonstrated for the T4SS of *Erwinia carotovora* subsp. *atroseptica* and *Burkholderia cenocepacia* (Bell *et al.*, 2004; Engledow *et al.*, 2004).

Among rhizobia, likely T4SSs have been identified in the genomes of *S. meliloti*, *R. etli* and several *M. loti* strains. A symbiotic role has been so far assigned to the VirB/D4 T4SS present in the symbiotic island of *M. loti* strain R7A (Hubber *et al.*, 2004). As for T3SS, the symbiotic role of this T4SS is host-specific, assisting or impeding nodulation depending on the legume species. The fact that *M. loti* T3SS mutants show the same symbiotic phenotype as *M. loti* T4SS mutants suggests that T4 and T3 secretion systems may be functionally interchangeable. Two genes, *msi059* and *msi061*, have been identified as encoding putative T4 effectors. Interestingly, the corresponding proteins show significant similarities to known effectors of the T3 and T4 secretion systems of plant pathogens, suggesting that they function inside plant cells. *Msi059* could have a role similar to that of the T3SS effector protein XopD of *X. campestris* pv. *vesicatoria* shown to exhibit plant-specific SUMO substrate protease activity, whereas *Msi061* could act as VirF of *A. tumefaciens*, targeting proteins for ubiquitination and subsequent degradation.

In *S. meliloti* and *R. etli* the genes encoding the T4SS are located in the respective symbiotic plasmids and closely resemble the conjugation system AvhB of the cryptic plasmid pAtC58 of *A. tumefaciens* (Chen *et al.*, 2002). Indeed the *virB* regions of these two rhizobia are required for the conjugal transfer of Sym plasmids (J. J. Oliva-García *et al.*, unpublished; D. Romero, personal communication). Future work should determine whether the *virB* operons of these species also have a role in symbiosis.

Conclusions

Similar efficient strategies have been acquired by pathogenic and mutualistic bacteria to establish compatible associations with their host plants (Fig. 1). Cell–cell communication within a bacterial population and the ability to respond to environmental conditions encountered during the infection process are critical for successful invasion of the plant. Equally important is adequate cross-talk between bacteria and plants mediated by chemical signals and secreted proteins to avoid strong plant defence responses. Knowledge acquired in these fields will allow in the future the design of specific strategies to create plants resistant to plant pathogens and rhizobial strains with improved symbiotic properties.

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