MINIREVIEW

Attachment of bacteria to the roots of higher plants

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Abstract

Attachment of soil bacteria to plant cells is supposedly the very early step required in plant–microbe interactions. Attachment also is an initial step for the formation of microbial biofilms on plant roots. For the rhizobia–legume symbiosis, various mechanisms and diverse surface molecules of both partners have been proposed to mediate in this process. The first phase of attachment is a weak, reversible, and unspecific binding in which plant lectins, a Ca^{+2}-binding bacterial protein (rhicadhesin), and bacterial surface polysaccharide appear to be involved. The second attachment step requires the synthesis of bacterial cellulose fibrils that cause a tight and irreversible binding of the bacteria to the roots. Cyclic glucans, capsular polysaccharide, and cellulose fibrils also appear to be involved in the attachment of *Agrobacterium* to plant cells. Attachment of *Azospirillum brasilense* to cereals roots also can be divided in two different steps. Bacterial surface proteins, capsular polysaccharide and flagella appear to govern the first binding step while extracellular polysaccharide is involved in the second step. Outer cell surface proteins and pili are implicated in the adherence of *Pseudomonas* species to plant roots.

Introduction

Biofilms are the common life strategy for bacteria in natural environments. Biofilms are composed of populations or communities of microorganisms embedded in self-produced polymeric matrix (mainly extracellular polysaccharides) that have adhered to environmental surfaces in which sufficient moisture is present (Costerton *et al*., 1995). These three-dimensional microbial communities may be formed in all environments colonized by bacteria, such as on solid substrates in contact with moisture or on tissue surfaces in living organisms. The mutualistic association between microbial communities and plant roots, the so-called rhizosphere, form an environment that fulfils the requisites for biofilm formation: sufficient moisture and a supply of nutrients, which are provided by the plant.

Most researchers working with rhizospheric bacteria have not described the formation of biofilms on plant roots. In the past, however, different reports have indicated that rhizospheric bacteria (such as *Rhizobium, Azospirillum* and *Pseudomonas*) associated with root surfaces are embedded in the root mucigel and might also be encased in a self-produced extracellular matrix. Transmission electron microscopy has shown the presence of fibrillar material around rhizobia attached to the root surface (Fujishige *et al*., 2006). These observations further support the proposal that root-colonizing bacteria are capable of forming biofilms. It is reasonable to suppose that the molecular mechanisms operating in bacterial attachment to roots also might be relevant for biofilm development.

In this review, different aspects of the attachment mechanisms used by rhizospheric bacteria to attach to plant cells that enable them to act as biofertilizers, plant-growth promoters, or phytopathogens (*Rhizobium*, *Azospirillum*, *Pseudomonas*, and *Agrobacterium*) will be discussed.

Attachment of rhizobia and agrobacteria to plant cells

Attachment of rhizobia (*Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium*, and other related genera) to host roots is supposedly the very early step required for

infection and nodulation. This initial adsorption of rhizobia to the root surface of legumes constitutes one very early step in the symbiont's interaction in the complex host-specific infection process. Early reports suggested that *host-specificity*, one of the well-known characteristics of legume root-nodule bacteria, is already expressed during adsorption (Dazzo *et al.*, 1984; Dazzo *et al.*, 1976; Caetano-Anollés & Favelukes, 1986). Other studies, however, indicated that a nonhost-specific adsorption mechanism, independent of the symbiotic properties, may contribute to root attachment of rhizobia (Badenoch-Jones *et al.*, 1985; Mills & Bauer, 1985; Vesper & Bauer, 1985; Smit *et al.*, 1986; Villacieros *et al.*, 2003; Albareda *et al.*, 2006).

Various mechanisms and diverse surface molecules of both rhizobia and host plants have been proposed to mediate in this process (Fig. 1). It is generally accepted that, among the different plant factors that might be involved in this attachment, plant lectins may play an important role since they could serve as receptors for bacterial surface polysaccharides (Ridge *et al.*, 1998; Hirsch, 1999; Rudiger & Gabius, 2001). Plant lectins are proteins that have at least one noncatalytic domain that binds reversibly to mono- or oligosaccharides. The best characterized group of lectins is that found in leguminous plants. Legume lectins have been detected in more than 600 species, in which large amounts of lectin, up to 10% of the total protein content, are present in mature seeds. Lectins are also detected in other parts of the plant, such as leaves, stems, and roots (van Eijsden, 1994).

A Ca^{2+}-binding bacterial protein called rhicadhesin also appears to be involved in bacterial attachment to legume root hairs. *Rhizobium leguminosarum* bv. *viciae* cells grown under low Ca^{2+} conditions show reduced attachment capacity to pea (*Pisum sativum*) root hair surfaces. Under low Ca^{2+} conditions, rhicadhesin is released from the bacterial surface into the growth medium. Calcium ions are not involved in binding of rhicadhesin to the root surface. Instead, Ca^{2+} appears to be involved in anchoring rhicadhesin to the rhizobial cell surface (Smit *et al.*, 1991). Although the rhicadhesin protein has been purified from *R. leguminosarum* (Smit *et al.*, 1989), the corresponding gene has not been identified. An *R. l.* bv. *trifolii*-adhering protein, called RapA1, shares many of the properties that were previously described for the *R. l.* bv. *viciae* rhicadhesin. Both are secreted proteins that bind calcium, bind at bacterial cell poles and to root hairs, and mediate calcium-dependent agglutination (Gage, 2004).

It is generally believed that, in addition to rhicadhesin and plant lectins, bacterial surface polysaccharides are also involved in the first attachment step. Legume lectins located at the root-hair tip would recognize and bind to specific carbohydrate structures that are present in the bacterial surface. This early first step of attachment, mediated by lectins and/or rhicadhesin, is rather weak and also reversible (Matthysse & Kijne, 1998). The second binding step would require the synthesis of bacterial cellulose fibrils, produced either before or after the bacterial attachment to the plant cell surface. These cellulose fibrils would cause a tight and irreversible binding and the formation of bacterial aggregates on the host surface (Robertson *et al.*, 1988). Lectins also could act in the second binding step. At least for *R. l.* bv. *viciae* it has been shown that the initial rhicadhesin-mediated attachment step is required before fibril- and lectin-mediated attachment can take place (Smit *et al.*, 1992).

The first evidence indicating that lectins are involved in rhizobia attachment to legume roots as a specific step in the

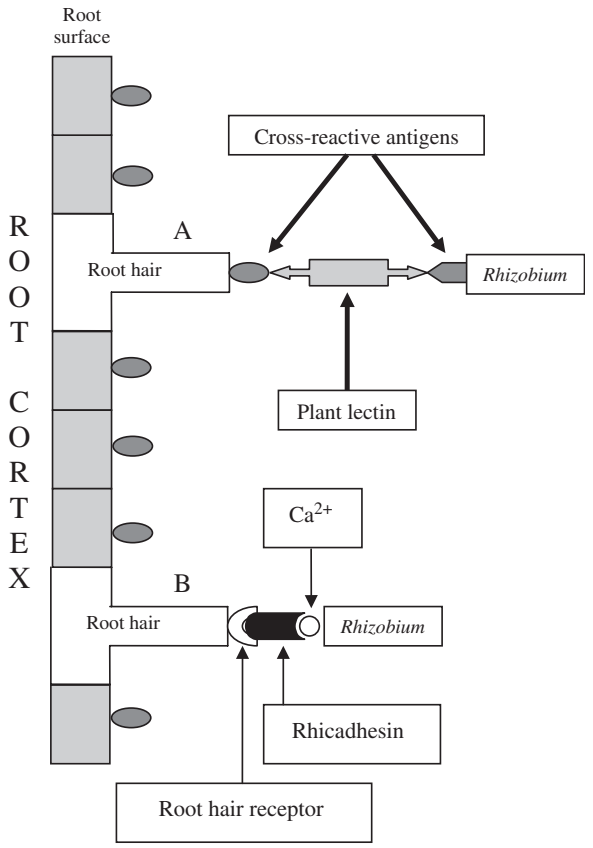


Fig. 1. The two attachment models proposed for the attachment of *Rhizobium leguminosarum* to root hairs. A: Lectin-mediated attachment. Plant lectins would act as a bridge between the bacteria and the plant by recognizing cross-reactive antigens that would be present in both symbionts. Plant cross-reactive antigens would be uniformly distributed along the whole root surface while lectins would be mainly localized in root-hair tips. B: Rhicadhesin-mediated attachment. The bacterial rhicadhesin would have two binding domains, one for a receptor situated on the bacteria cell surface and the other for a molecule of the root-hair surface. Calcium ions would anchor rhicadhesin to the bacterial surface. (Dazzo & Hubbell 1975; Dazzo & Brill, 1979; Laus *et al.*, 2006).

recognition between both symbionts had their experimental origin in the early works of Hamblin & Kent (1973), Bohlool & Schmidt (1974), and Dazzo & Hubbell (1975). The lectin-recognition hypothesis stated that plant lectins are involved in the binding of rhizobia to the host root surface and that they are determinants of host specificity because of the strong correlation between the nodulation specificity of rhizobial strains and their capacity to bind to those lectins produced by their specific legume hosts. For instance, soybean lectin (SBL) bound and aggregated 22 out of 25 strains of *Bradyrhizobium japonicum* that nodulate soybeans. Twenty-three other bradyrhizobial strains that were not soybean symbionts failed to bind SBL (Bohlool & Schmidt, 1974).

In contrast to these data, other reports did not demonstrate specific lectin-mediated binding of homologous rhizobia to legume root hairs (Smit *et al*., 1986; Vesper & Bauer, 1986). Latter reports showed that variations in bacterial attachment capacity could be at least partially explained by strain-specific differences, bacterial growth conditions, and many other physiological factors. Culture age, growth medium, pretreatments of bacteria and/or roots, bacterial chemotaxis, motility, and cellular hydrophobicity are factors that are known to affect bacterial attachment.

Among these factors controlling the adsorption process, pH of the assay media and the presence of divalent cations are of critical importance and may greatly vary in different symbiotic associations. Caetano-Anollés *et al.* (1989) showed that adhesion of *Sinorhizobium meliloti* to alfalfa (*Medicago sativa*) roots required neutral pH and was proportional to Ca^{2+} and/or Mg^{2+} concentrations up to 1.5 mM. At pH lower than 6.0, higher amounts of Ca^{2+} are required to attain similar adsorption levels. In other systems, such as soybean–bradyrhizobia, the effect of Ca^{2+} was dependent on the concentration. Calcium concentrations between 2 and 10 mM were inhibitory compared with 50–100 mM, while the level of Mg^{2+} ions apparently have no effect on bradyrhizobia attachment (Smith & Wollum, 1993). However, in other bradyrhizobia–legume associations low levels of Ca^{2+} appear to be required for an optimal attachment. For instance, *Bradyrhizobium* sp. (*Lupinus*) showed increased attachment capacity to white lupin roots at low Ca^{2+} concentration (Wisniewski & Delmotte, 1996). Similarly, the attachment of *Bradyrhizobium* sp. to peanut (*Arachys hypogaea*) roots was unaffected at pH values ranging from 7 to 5, although a significant increase was observed at pH 5.0 and 50 μM of Ca^{2+} when compared with higher (500 μM) Ca^{2+} concentrations (Macció *et al*., 2002).

It is worth pointing out that in most of the studies mentioned above only a small proportion (0.4–3.5%) of the bacterial population apparently attached to the host roots. Attachment levels as high as 15% were reported for the bradyrhizobia–soybean symbiosis, although only 4.6% of the population were tightly attached (Vesper & Bauer, 1985). In a recent study, Albareda *et al*. (2006) reported that the proportion of tightly attached homologous rhizobia strains and different rhizospheric bacteria to bean and soybean roots occurred in a very small proportion, $< 1\%$ of the added inoculum.

The lectin-recognition hypothesis for the clover system (*Trifolium-R. l.* bv. *trifolii*) is based largely on the existence of 'cross-reactive antigens' on the surface of both macro- and microsymbionts (Dazzo & Hubbell, 1975; Graham, 1981). In this model, clover lectin would act as a bridge between the legume and the bacteria by binding (recognizing) to these cross-reactive antigens at the initial stages of the bacterial–plant interaction. In clover, this cross-reactive antigen would be uniformly distributed on the root surface while the clover lectin would be concentrated on the root-hair tip, the place to which rhizobial cells bind.

Some rhizobial strains, such as *R. l.* bv. *trifolii*, carrying an *Agrobacterium tumefaciens* pTi plasmid are able to induce plant tumour formation. *Vice versa*, transfer of nodulation genes to agrobacterial strains confers to these bacteria the capacity to induce nodule formation. These results were considered as an indication that rhizobia and agrobacteria might share the same two-step mechanism for attachment to plant cells, although there are reasons to believe that the first step might be different (Matthysse & Kijne, 1998).

Rhizobia and agrobacteria are typical Gram-negative bacteria with a cytoplasmic and an outer membrane separated by a periplasmic space. At least four different polysaccharides are known to play a role in symbiosis: (1) Cyclic glucans (CG), which are mainly found in the bacterial periplasmic space, and also in culture supernatants; (2) exopolysaccharides, weakly associated with the outer membrane or totally released into the extracellular medium; (3) lipopolysaccharides, which are structural components of the outer membrane; and (4) capsular polysaccharides (CPS and/or KPS), which are usually bound to the outer membrane. Bacterial surface polysaccharides have also been proposed to play a role in the attachment of agrobacteria to plant–host cells. Figure 2 shows the position of all these polysaccharides on the surface of rhizobial cells.

Although rhizobial exopolysaccharides are important for eliciting infection thread formation in legumes forming indeterminate nodules (such as alfalfa or clovers), they are not ligands for their respective legume host lectins (Hirsch, 1999). However, van Workum *et al*. (1998) showed that the number of infection sites on vetch (*Vicia sativa*) roots inoculated with an exopolysaccharides-deficient *R. l.* bv. *viciae* mutant was severely reduced in comparison with wild-type inoculated roots. These results indicate that the exopolysaccharides (or a CPS) could enhance bacterial binding to growing root hairs during the first attachment step.

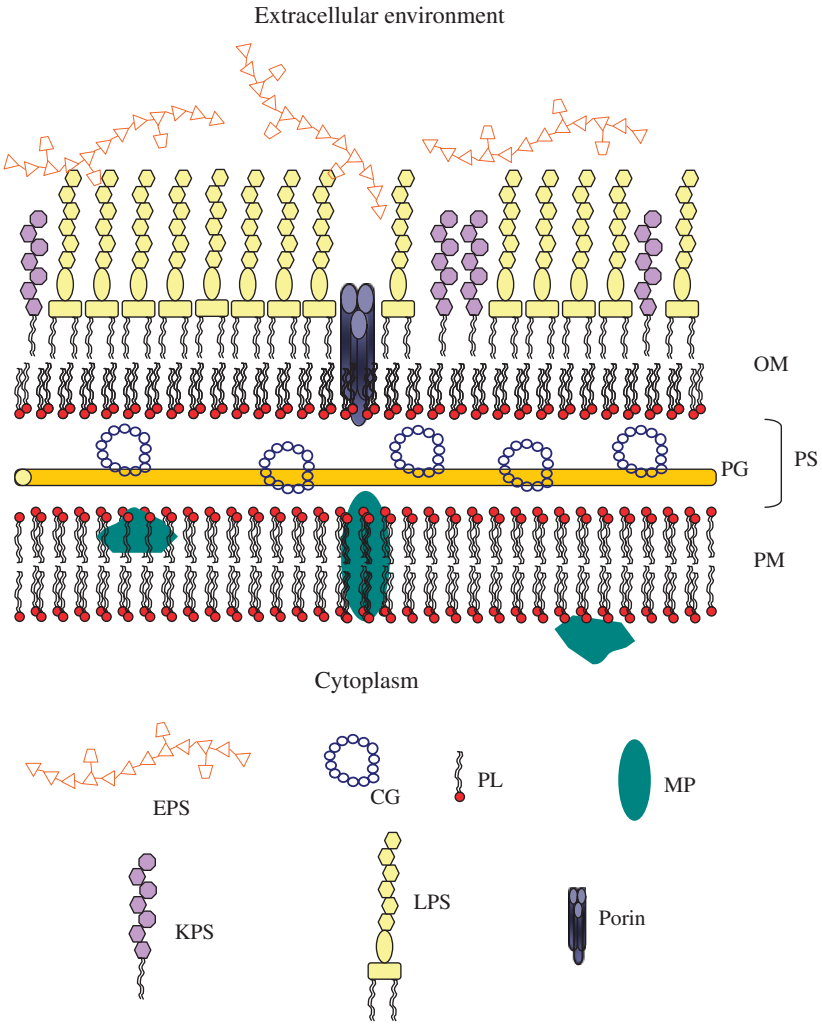
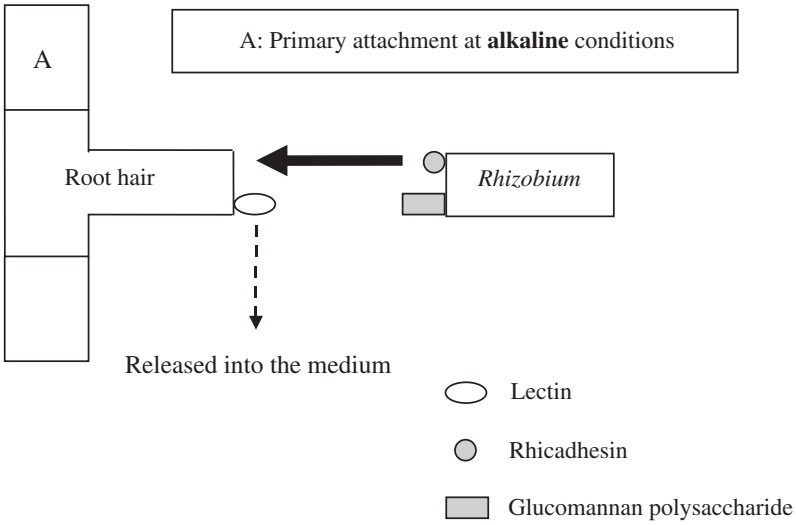


Fig. 2. Scheme of the rhizobial cell surface showing the position of surface polysaccharides that might be involved in rhizobial attachment to legume roots. OM, outer membrane; PS, periplasmic space; PG, peptideglycan layer; PM, cytoplasmic membrane; EPS, Exopolysaccharide; CG, cyclic glucan; PL, phospholipid; MP, membrane protein; KPS, capsular polysaccharide (K-antigens); LPS, lipopolysaccharide.

CG could be involved in bacterial attachment by interacting with plant lectins (Hirsch, 1999). *Agrobacterium tumefaciens chvB* and *Sinorhizobium meliloti ndvB* mutants are unable to produce CG and show reduced binding capacity to their respective host plants (Dylan *et al.*, 1990; Brencic & Winans, 2005). However, this reduction in the bacterial binding capacity is not the ultimate reason why these mutants are unable to form tumours or nitrogen-fixing nodules. *Sinorhizobium meliloti ndv* mutants also are severely impaired for motility and unable to invade alfalfa roots, so that nitrogen-fixing nodules are not formed. Instead, white unoccupied pseudonodules are formed. Pseudorevertants selected for restoration of motility showed enhanced attachment capacity to alfalfa roots but were only slightly restored symbiotically. On the other hand, pseudorevertants selected on alfalfa for restoration of symbiosis showed little or no attachment capability, indicating that the level of attachment capacity exhibited by the wild-type strain is not strictly required for the invasion of alfalfa roots. Neither motile nor symbiotic pseudorevertants regained the capacity to produce CG. These facts suggest that, although the symbiotic properties of *S. meliloti ndvB* mutants are severely impaired, CG are not strictly required for nodule development (Dylan *et al.*, 1990). Mutations that abolish the production of CG generate rhizobial pleiotropic mutants that show alterations of different bacterial traits, including an increase of the amount of exopolysaccharide produced. Because of this, and although infection site initiation requires efficient attachment, it is difficult to ascertain whether CG production, attachment to plant cells, and the establishment of a successful interaction (symbiotic or pathogenic) are all directly or indirectly related.

Lectins from alfalfa or peanut can bind to the lipopolysaccharides of alfalfa- or peanut-specific rhizobia but not to lipopolysaccharides from other rhizobial strains that do not nodulate with these legumes (Hirsch, 1999). Early reports suggested that agrobacterial lipopolysaccharides might be involved in bacterial attachment to host plant cells. Later



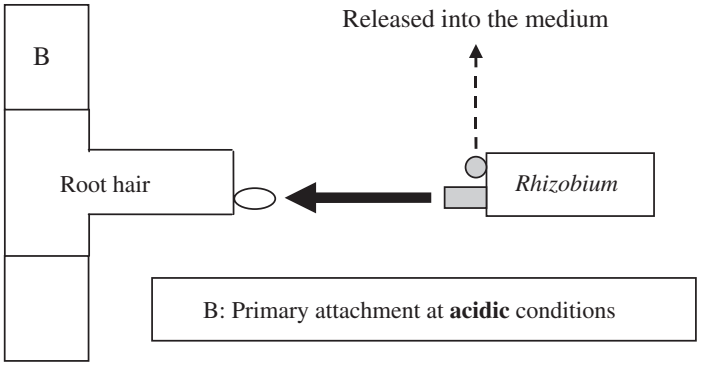


Fig. 3. The two attachment mechanisms proposed for the primary attachment of *Rhizobium leguminosarum* to pea and vetch root hairs under alkaline and acidic conditions (Laus *et al*., 2006). A: Under alkaline conditions lectins would be released from the root-hair tips. The rhizobial rhicadhesin would be the main molecule mediating bacterial attachment to plant roots. B: Under acidic conditions lectins would remain anchored to the root-hair tip while rhicadhesin would be released from the bacterial surface. Plant lectins and bacterial glucomannan would be the main molecules involved in bacterial attachment to the plant.

reports, however, posed serious doubts about the actual role of agrobacterial lipopolysaccharides in bacteria–plant cell attachment: (1) binding to tissue culture plant cells appeared to be unaffected by agrobacterial lipopolysaccharides addition; (2) binding to tissue culture cells was also unaffected by treatment of the bacteria with an inhibitor of the biosynthesis of smooth lipopolysaccharides (complete lipopolysaccharides molecules), although rough lipopolysaccharides (molecules devoid of the O-antigen chain) is still produced (Matthysse & Kijne, 1998).

Attachment of *A. tumefaciens* C58 to carrot suspension cells is dependent on the presence of a cell-associated acetylated bacterial polysaccharide (Reuhs *et al*., 1997). This polysaccharide contains glucose, glucosamine, and a deoxysugar and might be related to sinorhizobial acidic KPS, which are structurally analogous to the group II K antigens of *Escherichia coli* (Reuhs *et al*., 1993). To the authors' knowledge, there are no reports describing whether KPS from *S. meliloti* or *Sinorhizobium fredii* are able to bind lectins.

Recently, a novel high molecular weight surface-polysaccharide from *R. l.* bv. *viciae* RBL5523 was isolated (Laus *et al*., 2006). This polysaccharide, which is mainly composed of glucose and mannose and minor amounts of galactose and rhamnose, show high binding affinity for pea and vetch (*Vicia sativa*) lectins. Other surface polysaccharides produced by this strain (exopolysaccharides, lipopolysaccharides, CG, and CPS) do not bind to pea and vetch lectins. Experiments using labelled pea lectin showed that the glucomannan polysaccharide is only located on the bacterial pole, which is involved in the attachment to the root surface. Laus *et al*. (2006) have proposed that *R. leguminosarum* RBL5523 can use at least two mechanisms for primary attachment to pea and vetch root hairs. One of them would be mediated by rhicadhesin, the other by the interaction of the bacterial glucomannan and the plant lectins. This model also predicts which one of the two mechanisms will operate for bacterial attachment to the plant at acidic and alkaline conditions (Fig. 3). Under slightly alkaline conditions (pH 7.4), lectins located in root-hair tips would be released into the rhizosphere medium as a result of an increase of their solubility. In these circumstances, bacterial attachment would be carried out through the rhicadhesin-mediated mechanism. In contrast, under acidic conditions (such as

pH 5.6) lectins would not become soluble and would be retained on the root-hair tip while rhicadhesin would be released from the bacterial surface. Consequently, under acidic conditions bacteria would use the lectin–glucomannan-mediated mechanism to attach to the plant.

The general structure of rhizobial Nod factors (lipochitin oligosaccharides) indicates that these bacterial signal molecules might not act as ligands for typical legume lectins such as SBL (from *Glycine max*) or PSL (from *Pisum sativum*). However, a root lectin isolated from *Dolichos bifloros* binds to the Nod factors produced by rhizobial strains that nodulate this legume. Interestingly, the aminoacid sequence of the lectin isolated from *D. bifloros* is different from that of the legume lectins currently studied (Etzler *et al.*, 1999).

The lectin recognition hypothesis received strong support when it was demonstrated that transgenic white clover roots expressing the pea lectin gained the capacity to nodulate with *R. l.* bv. *viciae*, a pea symbiont that does not (or extremely poorly) nodulate clovers (Díaz *et al.*, 1989). It was later demonstrated that this heterologous bacterial–plant interaction was actually mediated by the pea lectin because alterations of the PSL carbohydrate-binding domain abolished the capacity of transgenic clover roots to nodulate with *R. l.* bv. *viciae* (van Eijsden *et al.*, 1995). Similarly, transgenic *Lotus corniculatus* roots expressing the SBL gained the capacity to form (ineffective) nodules with *B. japonicum* (van Rhijn *et al.*, 1998). All these results indicate that, although Nod factor-production and recognition are considered as the primary determinants of host plant specificity in the *Rhizobium*–legume interaction, the lectin-mediated recognition process also plays a significant role in rhizobia–legume interactions.

As mentioned before, cellulose fibrils are involved in the second attachment step of rhizobia and agrobacteria to plant cells. Agrobacteria genes (*cel*) required for cellulose syntheses are located on the bacterial chromosome near other genes (*att*) involved in attachment. Tumour induction by cellulose-minus mutants requires the use of very high bacterial populations, a clear indication that their virulence capacity is attenuated (Matthysse & Mcmahan, 1998). Nevertheless, the fact that *A. tumefaciens* and *R. leguminosarum* mutants unable to produce cellulose fibrils are still able to induce tumours and nodules on their respective host plants acted as a discouraging factor for carrying out further studies about the role of cellulose fibrils in the bacteria–plant interaction (Smit *et al.*, 1987; Matthysse *et al.*, 1995).

However, Laus *et al.* (2005) have recently provided new insights about a possible role of cellulose fibrils that could partially explain the well-known incapacity of *R. leguminosarum* exopolysaccharides mutants to colonize infection threads. These authors have hypothesized that the failure of *R. leguminosarum* exopolysaccharides-deficient mutants to carry out early infection-thread colonization is probably caused by cellulose-mediated agglutination of the bacterial cell in the root-hair curl. This hypothesis implies that, in a wild-type situation, exopolysaccharides would prevent bacterial agglutination by masking the cellulose fibrils in the root-hair curl. In fact, an *R. leguminosarum* double mutant, unable to produce exopolysaccharides and cellulose fibrils, did not show bacterial agglutination in *V. sativa* infection threads.

Bacterial attachment to host tissues also plays a role in the pathogenesis of many plant and animal pathogens. Numerous reports have shown that necrogenic plant pathogens produce a variety of potential adhesins, including fimbriae by *Erwinia rapontici*, *Erwinia carotovora*, *Pseudomonas syringae, Xanthomonas campestris*, and *Ralstonia solanacearum*, type IV pili by *P. syringae*, and adhesive factors such as lipopolysaccharide by *R. solanacearum* (Rojas *et al.*, 2002). Similarly, bacteria attach to mammalian cells using proteins, glycoproteins, and glycolipids located on the mammalian cell membrane. In many cases this adhesion is mediated by lectins produced by the pathogenic microorganism. As carbohydrates are involved in bacterial attachment to animal cells, the use of short-chain oligosaccharides to inhibit attachment has been proposed as a potential therapeutic strategy that could be used to reduce, or as an alternative of, the use of antibiotics in infectious diseases (Sharon & Ofek, 2002; Thomas & Brooks, 2006).

Attachment of plant growth-promoting rhizobacteria (PGPR) to plant cells

PGPR are soil bacteria that have the ability to colonize roots and stimulate plant growth. PGPR activity has been reported for strains belonging to many different genera such as *Azoarcus*, *Azospirillum*, *Azotobacter*, *Arthrobacter*, *Bacillus*, *Clostridium*, *Enterobacter*, *Gluconoacetobacter*, *Pseudomonas*, and *Serratia* (Somers *et al.*, 2004). *Rhizobium* also can be considered as a soil bacteria with PGPR activity. Root colonization and growth promotion of rice, cereals, and other nonlegumes has been reported (Chabot *et al.*, 1996). Plant growth-promoting capacity has been related with different physiological activities: (1) synthesis of phytohormones, such as cytokinins, gibberellins, and auxines; (2) enhancement of factors affecting mineral nutrition, such as phosphorous solubilization; (3) protection of plants against phytopathogens (Persello-Cartieaux *et al.*, 2003; Somers *et al.*, 2004).

A variety of compounds, such as surface proteins and polysaccharides, have been implicated in adherence of several PGPRs to plant roots. The importance of bacterial attachment in PGPRs–plant interactions has been intensively studied in *Azospirillum* and *Pseudomonas*.

It is generally believed that the main mechanism by which *Azospirillum* enhances plant growth is by the production of

plant hormones (Steenhoudt & Vanderleyden, 2000). These growth-promoting substances stimulated the density and length of root hairs and root surface area, improving the utilization of water and mineral nutrients.

Similar to the *Rhizobium*–legume symbiosis, attachment of *Azospirillum brasilense* cells to wheat (*Triticum aestivum*) roots also can be divided in two different steps (Michiels *et al*., 1991). The first phase is a weak, reversible, and unspecific binding governed by bacterial surface proteins, CPSs, and flagella. Polar flagella of *A. brasilense* contain an adhesin component that is involved in bacterial attachment to wheat roots. The second attachment phase appears to be irreversible. It occurs 8–16 h after inoculation and is mediated by a bacterial surface polysaccharide. *Azospirillum irakense* cells are mainly associated with rice root hairs, whereas *A. brasilense* cells are mainly located on root surfaces (Zhu *et al*., 2002). These differences in spatial distribution are the reason why these two species do not compete for root colonization.

The involvement of extracellular fibrils was demonstrated in the irreversible anchoring of *A. brasilense* (Michiels *et al*., 1991). The nature of this fibrillar material has not been determined yet. Extracellular polysaccharide production also has been related to the process of flocculation of *Azospirillum* cells and might be similar to the fibrillar material produced during root association (Burdman *et al*., 1998; Skvortsov & Ignatov, 1998).

The major outer membrane protein of *A. brasilense* appears to be involved in cell aggregation and the first step of attachment. This outer protein exhibits higher affinity to cereal roots than to those of other plants, which may explain why *Azospirillum* is mainly found associated with the rhizosphere of cereals.

Plant-associated *Pseudomonas* bacteria live as saprophytes but also as pathogenic parasites on plant surfaces and inside plant tissues. In addition, some *Pseudomonas* species show plant growth-promoting activity by suppressing the growth (biocontrol) of other phytopathogenic microorganisms, synthesizing growth-stimulating plant hormones and promoting plant mechanisms involved in disease resistance. The formation of biofilms has been reported in the phytopathogen *Pseudomonas aeruginosa* (Walker *et al*., 2004). Some *Psuedomonas fluorescens* strains form biofilms composed of cellulose and fibre at the air–liquid interface. The formation and strength of this biofilm results from the interactions between lipopolysaccharides and the cellulose matrix (Spiers & Rainey, 2005).

Initial attachment to surfaces, biotic or abiotic, leads to a global change in gene expression in *P. putida*. The isolation of genes involved in adhesion to abiotic surfaces and attachment to plant roots suggests that initial colonization of both abiotic and biotic surfaces proceeds via similar pathways (Sauer & Camper, 2001).

In *P. fluorescens*, adhesion to plant roots has been shown to involve pili (Vesper, 1987). Other cell-surface proteins that have been involved in the attachment of *Pseudomonas* spp. to plant roots include the outer membrane protein OprF of *P. fluorescens* OE 28.3 (de Mot *et al*., 1992), and an agglutinin of *Psuedomonas putida* strain Corvallis, which mediates bacterial agglutination to a plant-root glycoprotein (Anderson *et al*., 1988). This bacterial protein encoded by the *aggA* locus appears to be involved in the adherence to and in the colonization of roots of different plants such as bean, potato, tomato, and grass (Anderson *et al*., 1988; Buel & Anderson, 1992). Although agglutinin plays a major role in the adherence and colonization abilities of *P. putida* strain Corvallis to bean and cucumber, the role of agglutinins is not general for all biocontrol strains. No agglutination-dependent adherence and root colonization could be demonstrated for 30 different *Pseudomonas* isolates on tomato, potato, and grasses (Lugtenberg & Dekkers, 1999).

Outlook

Bacterial attachment to plant roots is considered one of the very early steps of plant root colonization by symbiotic, pathogenic, and other plant-associated microborganisms. Although the relationships between rhizospheric bacteria and plants can vary from mutualistic to pathogenic, the molecular mechanisms governing microbial attachment to plant cells share many similarities in their dynamic (phases) of binding and in the nature of the bacterial surface molecules involved in this process. This implies that knowledge acquired from the study of the attachment mechanisms governing a specific microbe–plant interaction (symbiotic or pathogenic) provides new insights for understanding those operating in another association.

The lectin-recognition hypothesis was the first model in which a possible molecular mechanism for bacterial attachment to plant roots was related with symbiotic specificity. This hypothesis has withstood the test of time and, although the exact role of lectins in bacterial attachment remains obscure, there is no doubt that these plant surface molecules are important in different microbe–plant interactions. Various bacterial surface polysaccharides have been postulated to be the ligand that interacts with plant lectins, although the nature of the bacterial polysaccharide that binds to the host-plant lectin varies in the different symbiotic systems investigated.

Microbial attachment capacity to plant cells probably acts as key factor in determining microbe competitiveness to colonize the root. Unfortunately, technical difficulties to purify and identify all possible bacterial surface molecules that could be involved in bacterial attachment have not been completely overcome. In addition, mutations that abolish the production of a particular surface molecule can also

influence the production and/or presence of other bacterial surface components, which makes it difficult to identify which particular surface molecule is directly responsible for the changes observed in bacterial attachment capacity. In combination with *omics* technologies, the structural determination of bacterial surface molecules and the construction of transgenic plants expressing receptors for bacterial surface components appear as the most promising way to investigate which molecules are actually involved in bacterial attachment, how important they are for successful root colonization under natural conditions, and how they are affected by environmental conditions.

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