MPMI Vol. 8, No. 6, 1995, pp. 800–810.

Current Review

The Role of Bacterial Motility, Chemotaxis, and Attachment in Bacteria-Plant Interactions

Ann Vande Broek and Jos Vanderleyden

F. A. Janssens Laboratory of Genetics, W. De Croylaan 42, B-3001 Heverlee, Belgium
Received 1 February 1995. Accepted 24 July 1995.

Soil bacteria that interact with plants largely differ in the effects that they exert on their host plant. The association between *Rhizobium*, *Azospirillum*, or many pseudomonads and their host plant(s) is beneficial for plant growth while the interaction of *Agrobacterium* with its host is pathogenic and results in plant disease. Despite these differences, it seems that, at least in some cases, common mechanisms are involved in the initial phases of the interaction processes.

It is generally assumed that successful interactions are preceded by proliferation of the appropriate bacteria in the rhizosphere of the plant host (for example Sprent 1989; Bull et al. 1991). Since most rhizobacteria carry the complex machinery for motility and chemotaxis, bacterial motility, either random or chemotactic, is likely to play a role in this early stage of interaction. Many substances present in root exudates, including simple sugars and amino acids as well as more specific nonmetabolizable compounds, have been identified as chemoattractants for different plant-associated bacteria. Once bacteria are in the vicinity of the root or the seed, attachment to target cells on the plant surface can occur. In some interactions, such as the *Agrobacterium* tumorigenesis, bacterial adherence to the plant surface has been shown to be a prerequisite for the further establishment of the interaction (Matthysse 1987). In contrast, some phytopathogenic bacteria appear to avoid attachment in compatible interactions (i.e., interactions resulting in disease) in order not to trigger plant defense responses (Sequeira et al. 1977; Young et al. 1986).

In this review, the role of bacterial motility, chemotaxis, and attachment in four types of bacterium-plant interactions i.e., in the *Agrobacterium* pathogenesis, in the *Rhizobium*-legume symbiosis, in the *Azospirillum*-plant root association and in the interaction of beneficial fluorescent pseudomonads with plants, will be discussed.

MOTILITY, CHEMOTAXIS, AND ATTACHMENT IN THE *AGROBACTERIUM*–PLANT PATHOGENESIS

Role of chemotaxis and motility in tumor formation.

The Gram-negative soil bacterium *Agrobacterium tumefaciens* causes the tumorigenic disease crown gall on a wide range of dicotyledonous plants. Plant tumorigenesis results from the expression of a discrete segment of bacterial DNA, the T-DNA, that is transferred from the Ti plasmid into the nuclei of host plant cells. Processing and transfer of this T-DNA is mediated by another portion of the Ti plasmid, the *vir* region, that is synergistically induced by a family of phenolic compounds exudated by plant wounds, such as acetosyringone and hydroxyacetosyringone, together with specific monosaccharides (for a review see Kado 1991; Winans 1992; Ankenbauer and Nester 1993).

Agrobacterium spp. are peritrichous motile bacteria and possess a highly sensitive chemotaxis system which responds to a wide range of amino acids and sugars. Some of these sugars galactose, glucose, arabinose, fucose, and xylose have also been identified as *vir* gene inducers (Loake et al. 1988; Cangelosi et al. 1990a). The genes involved in the general chemotactic response towards amino acids and sugars are located on the chromosome (Loake et al. 1988, Cangelosi et al. 1990a). In addition, Ashby et al. (1988) reported that *A. tumefaciens* C58 also exhibits chemotaxis towards acetosyringone, one of the major plant phenolic *vir* inducers. This attraction requires the presence of *virA* and *virG* genes, located on the Ti plasmid, and occurs at concentrations well below those necessary for *vir* gene induction. The authors suggested that migration towards acetosyringone may constitute the first step in the specific recognition between *A. tumefaciens* and its host plant in the soil. Therefore, specific chemotactic attraction may guide the bacteria towards plant wounds where the concentration of inducer is sufficiently high to switch on the expression of the *vir* genes. However, these results pointing to acetosyringone as a positive chemoattractant for *A. tumefaciens*, were disputed by others. Parke et al. (1987) reported chemotaxis of *A. tumefaciens* A348 towards a number of other aromatic *vir* inducers such as catechol, gallate, *p*-hydroxybenzoate, protocatechuate, and β-resorcylate but failed to observe migration to acetosyringone at any concentration. In this case, identical results were obtained with the wild-type strain and a Ti-cured derivative. From these results it was concluded that the Ti plasmid was not required for chemotaxis to the aromatic attractants. Moreover, chemotaxis towards aromatic compounds was found to be constitutively expressed. Since both groups used different strains in their experiments, the chemotactic response of *A. tumefaciens* towards acetosyringone appears to be strain-specific and is therefore probably not essential for virulence.

Only a few investigators have studied the involvement of chemotaxis and motility in virulence of *A. tumefaciens* by

Corresponding author: J. Vanderleyden;
E-mail: janssens@agr.kuleuven.ac.be

using specific mutants. Using resistance to flagella-specific bacteriophages as a selection procedure, Bradley et al. (1984) isolated nonmotile mutants of *A. tumefaciens*. These mutants retained both virulence and the ability to attach to plant cells under laboratory conditions. Hawes et al. (1988) developed an assay to detect migration of *A. tumefaciens* cells towards excised root tips and isolated root cap cells as sources of wound and nonwound exudates, respectively. Chemotaxis to both exudates was shown to be independent of the Ti plasmid. Using this assay, motility and chemotaxis mutants were isolated. To assay the significance of these processes in crown gall pathogenesis, virulence of these mutants was tested under a variety of experimental conditions (Hawes and Smith 1989). Virulence was shown to depend on the soil type and on the inoculation procedure. When inoculated either directly in sand or soil or indirectly in sand (i.e., inoculated sand was allowed to dry and then used for growing plants), virulence of the motility- and chemotaxis-deficient mutants was indistinguishable from the wild-type strain. Only in indirect inoculation assays in soil, the motility and chemotaxis mutants were avirulent. These results indicate that chemotaxis and motility of *A. tumefaciens* towards its host can be critical for virulence in some environments but are superfluous in others.

Role of attachment in tumor formation.

Site-specific attachment of *A. tumefaciens* to the plant cell surface as an essential step in the tumorigenicity, was first proposed by Lippincott and Lippincott (1969). This statement was based on the observation that avirulent *A. tumefaciens* strains, which were still able to attach, inhibited initiation of tumor formation by virulent strains, presumably by occupying all available binding sites on the plant cell surface. In addition, specific receptors on the *A. tumefaciens* cell surface were supposed to be involved since tumorigenesis could not be blocked by inoculation with heterologous bacteria such as *Rhizobium meliloti* and *Pseudomonas aeruginosa* (Lippincott and Lippincott 1969).

During the last two decades, evidence has accumulated that attachment of *A. tumefaciens* to plant cells is a two-step process (Matthysse et al. 1981). In the first step *A. tumefaciens* adheres to the plant cell surface as a single cell. In the second phase, in response to plant factors, *A. tumefaciens* elaborates cellulose fibrils that entrap other bacteria resulting in the formation of bacterial aggregates (Matthysse et al. 1981). These fibrils also cause the bacteria to bind very tightly to the plant cell surface.

Mutants unable to synthesize cellulose attach to the surface of their host cells as individual cells but do not form aggregates. These mutants are still virulent but are more susceptible to being washed off the plant cells (Matthysse 1983), indicating that the fibrils per se are not essential for tumor formation but can be advantageous to the bacteria under certain environmental conditions.

To date, four genetic loci, *chvA, chvB, pscA* (*exoC*), and *att*, have been identified as playing a role in the initial attachment of *A. tumefaciens* to plant cells. All four loci are located on the chromosome. Mutations within these loci cause an avirulent bacterial phenotype, pointing to an essential role of the initial binding of the bacteria in virulence.

chvA and *chvB* genes were initially identified by Garfinkel and Nester (1980) in a search for avirulent *A. tumefaciens* mutants. These mutants were subsequently demonstrated to be affected in their ability to bind to plant cells (Douglas et al. 1982). Mutations in *chvA* and *chvB* are pleiotropic: The mutants lack flagella resulting in nonmotility and resistance to some bacteriophages (Bradley et al. 1984) and are defective in the production (*chvB*, Zorreguita and Ugalde 1986) or secretion (*chvA*, Cangelosi et al. 1989) of the cellular and extracellular polysaccharide β-1,2-D-glucan. Since most flagella-deficient *A. tumefaciens* mutants are virulent and unaffected in the binding to plant roots under laboratory conditions (Bradley et al. 1984; Hawes and Smith 1989), the lack of periplasmic β-1,2-glucan was proposed to account for the inability of the *chvA* and *chvB* mutants to attach and to form tumors.

The important role of β-1,2-glucans in bacterial virulence was further supported by the finding that another avirulent, attachment-defective mutant, a *pscA* or *exoC* mutant, also lacks β-1,2-glucan. *pscA* encodes a phosphoglucomutase (Uttaro et al. 1990), an enzyme that is essential for the biosynthesis of UDP-glucose. Since UDP-glucose is a primary component in the synthesis of several polysaccharides, *pscA* mutants are even more pleiotropic than the *chvA* and *chvB* mutants. Besides a defective β-1,2-glucan production, *pscA* mutants are affected in the biosynthesis of cellulose and succinoglycan, the major acidic extracellular polysaccharide. However, the latter two phenotypes are probably not responsible for the avirulence of the *pscA* mutant since several other cellulose-negative mutants (see also above; Matthysse 1983) as well as several mutants deficient in the synthesis of succinoglycan (Cangelosi et al. 1987) have been proven to be fully virulent under laboratory conditions.

Although β-1,2-glucans are likely involved in *A. tumefaciens* attachment, their role seems to be rather indirect, affecting important properties of the cell surface. *A. tumefaciens* β-1,2-glucans are involved in resistance of the bacteria to low osmotic pressure and their synthesis is osmoregulated (Miller et al. 1986). The periplasmic cyclic β-1,2-glucan is believed to maintain high osmolarity in the periplasm during growth of the bacteria on low-osmotic strength media. *chvA* and *chvB* mutants were indeed shown to grow more slowly than the parental strain under conditions of low osmolarity (Cangelosi et al. 1990b). Evidence for the indirect role of β-1,2-glucan in the initial binding of *A. tumefaciens* to plant cells was recently given by Swart et al. (1993 and 1994b). When grown in low-osmotic medium, the outer membrane of a *chvB*- mutant lacks an active 14-kDa Ca^{2+}-dependent outer membrane protein, named rhicadhesin (Swart et al. 1993). This protein had previously been demonstrated to be important for the attachment of *Rhizobium leguminosarum* bv. *viciae* cells to pea roots (Smit et al. 1989b; see also further). The lack of active rhicadhesin in the outer membrane of the *A. tumefaciens chvB* mutant was shown to cause the attachment-deficient and avirulence phenotypes. Motility, attachment to pea root hair tips, and virulence on *Kalanchoë daigremontiona* of *chvB* mutants of *A. tumefaciens* could be restored by growing the *chvB* mutants in medium of high osmolarity in the presence of Ca^{2+} (Swart et al. 1994b). The production of inactive rhicadhesin by the *chvA* and *chvB* mutants in low-osmotic medium and at plant wound sites is therefore thought to be due to improper osmoadaptation by the lack of periplasmic β-1,2-glucan, which in turn may cause aberrant posttransla-

tional processing of the rhicadhesin protein in the outer membrane (Swart et al. 1993). A few plant species (including, e.g., *Solanum tuberosum*), however, are infected by *A. tumefaciens chvB* mutants (Hooykaas and Schilperoort 1986). A higher osmolarity at the wound sites of these plant species might compensate for the lack of bacterial β-1,2-glucan.

By direct screening for mutants which failed to bind to carrot cells in a suspension culture, Matthysse (1987) isolated five additional attachment mutants. All mutants were avirulent. The mutations (*att*) were located on the chromosome and mapped in a region unlinked to the *chvA, chvB,* and *pscA* loci. Phenotypic characterization of the nonattaching mutants revealed no differences as compared to the parental strain in hydrophobicity, motility, flagella, fimbriae, size of lipopolysaccharide and production of cyclic β-1,2-glucan and cellulose. The *att* mutants however lacked a number of outer membrane proteins (33, 34, and 38 kDa). At present there is no evidence whether these proteins are directly involved in the binding of *A. tumefaciens* to the host cell surface.

The presence of specific receptor molecules at the plant cell surface to which *A. tumefaciens* cells bind has been postulated for a long time and is supported by the observations that (i) attachment of *A. tumefaciens* to the host cell surface is saturable (Neff and Binns 1985) and (ii) tumor formation by virulent strains can be blocked by preinoculation with avirulent but attaching strains (Lippincott and Lippincott 1969). In addition, tumor initiation of infectious *A. tumefaciens* cells is strongly reduced in the presence of externally added pectin and polygalacturonic acid (Rao et al. 1982). Similarly, Neff and Binns (1985) observed inhibition of *Agrobacterium* attachment to plant cells in a quantitative direct binding assay by a pectin-enriched extract of tomato cell walls. However, the inhibitory activity of the extract was found to be partially sensitive to a protease treatment, indicative for the involvement of a proteinaceous component. Therefore, the reduction of *Agrobacterium* attachment to the natural plant sites by pectin and polygalacturonic acid might be nonspecific. Recently, additional evidence for the involvement of a plant cell wall protein in *A. tumefaciens* binding was given by two different research groups. Indirect evidence for a vitronectin-like plant cell surface protein as receptor site for *A. tumefaciens* was given by Wagner and Matthyssse (1992) who reported inhibition of attachment of *A. tumefaciens* to carrot cells by vitronectin, a human serum spreading factor. Wild-type *A. tumefaciens* was found to bind radioactive-labeled vitronectin, while this ability was strongly reduced in the nonattaching *chvA, chvB, pscA,* and *att* mutants. Moreover, anti-vitronectin antibodies recognized polypeptides in a detergent extract of carrot cells. Extraction of carrot cells with dilute detergent as well as treatment of the cells with trypsin or other proteases had previously been shown to remove the bacterial binding-site from the carrot cell surface (Gurlitz et al. 1987). In a more direct approach, Swart et al. (1994a) isolated a putative plant receptor molecule for *A. tumefaciens* rhicadhesin from pea root cell walls using a bioassay based on the suppression of rhicadhesin activity. However, this molecule, a plant cell wall glycoprotein, is probably not a vitronectin-like protein. First of all, its molecular weight (32 kDa) is much lower than that from known vitronectins (see also further). Secondly, the glycoprotein was not recognized by a polyclonal antiserum directed against human vitronectin.

MOTILITY, CHEMOTAXIS, AND ATTACHMENT IN THE *RHIZOBIUM*–LEGUME SYMBIOSIS

Role of motility and chemotaxis in nodulation.

Symbiotic interactions between legumes and bacteria of the genera *Rhizobium, Bradyrhizobium,* and *Azorhizobium* result in the formation of nodular structures on the stems and/or roots of the host plant. Within these nodules, the rhizobia convert atmospheric nitrogen into ammonia; the plant assimilates the ammonia into amino acids and in return supplies the bacteria with organic carbon sources. The formation of root nodules requires the activation of the bacterial nodulation (*nod* and *nol*) genes. Transcription of most nodulation genes is activated by the transcriptional activator protein NodD in conjunction with specific flavonoids, exuded by the plant roots or seeds. In turn, the products of the nodulation genes participate in the production of substituted lipo-oligosaccharides, the Nod factors which are secreted by the bacteria and, when perceived by the host plant, induce several morphological changes including the formation of nodule primordia in the root of the host-plant (for a review see Long 1989; Carlson et al. 1994; Michiels and Vanderleyden 1994; van Rhijn and Vanderleyden 1995).

Rhizobia are motile, the flagellation being polar or subpolar in *Bradyrhizobium* and peritrichous in case of *Rhizobium* spp. (De Ley and Rassel 1965; Jordan 1984). The involvement of motility in nodule formation has been studied in several *Rhizobium* and *Bradyrhizobium* species. When tested under different, although sometimes highly artificial conditions (i.e., on agar slants, in Fahraeus glass slide assemblies, or in growth pouches using sterilized soil), nonmotile mutants of *R. meliloti* and *R. leguminosarum* bv. *trifolii* were generally found to infect and induce as many nodules on their host plants as the parental strains (Napoli and Albersheim 1980; Ames and Bergman 1981; Mellor et al. 1987; Malek 1992). However, in some of these experiments (Ames and Bergman 1981; Mellor et al. 1987; Malek 1992), the motile wild-type was always found to have a marked advantage when competing against equal numbers of the nonmotile isolates, suggesting that motility is a factor in the competition for nodule occupation under these experimental conditions. Using autoclaved soils of various textures, Soby and Bergman (1983) showed that efficient spreading of *R. meliloti* strictly necessitates motility and chemotaxis. However, their results indicate that the flagellar motility is dependent of the soil matric potential. A similar effect of the soil moisture tension on motility was described by Hambdi (1971). Catlow et al. (1990) observed a markedly reduced spread of a nonmotile mutant of *R. leguminosarum* bv. *trifolii*, when compared with the wild-type, through a steam-treated sandy soil but not in a nonsterile soil. In contrast, Liu et al. (1989) demonstrated that in a nonsterile silt loam soil a nonmotile mutant of *B. japonicum* was substantially less competitive than the wild-type.

The above-cited experiments indicate that, while rhizobial motility contributes to competiveness under some environmental conditions, it may not be of major importance under others.

Although the role of chemotaxis in nodulation remains unclear, several observations indicate positive chemotaxis of rhizobia towards the root surface. Several sugars and amino acids that are commonly present in root exudates act as

chemoattractants for rhizobia (Currier 1980; Gaworzewska and Carlile 1982; Bergman et al. 1988). In addition, analogous to the parallel effect of specific phenolic compounds on chemotaxis and *vir* gene induction in *A. tumefaciens* (Ashby et al. 1988; Parke et al. 1987), *R. meliloti* is chemotactically attracted towards luteolin, a potent flavonoid inducer of nodulation genes in this species (Caetano-Anollés et al. 1988).

Role of attachment in nodulation.

Initially, the hypothesis that host-specificity in *(Brady)rhizobium*–legume symbioses results from specific attachment of homologous rhizobial partners to the host root surface was put forward. Specific adherence of compatible rhizobia was proposed to be mediated by specific binding of unique polysaccharide moieties present on the bacterial cell surface to host plant lectins (Hamblin and Kent 1973; Bohlool and Schmidt 1974) (= lectin recognition hypothesis). In accordance with this hypothesis, Dazzo and Hubbell (1975) and Zurkowski (1980) observed host-specific attachment of *R. leguminosarum* bv. *trifolii* to root hairs of white clover. During this attachment, the clover lectin, trifoliin A, was thought to act as a bridge-forming unit between *R. leguminosarum* bv. *trifolii* and clover roots by recognizing unique polysaccharide receptors on both the rhizobial and root hair cell surface (Dazzo et al. 1978). Adherence of *R. leguminosarum* bv. *trifolii* to clover roots was found to be strongly reduced in the presence of the trifoliin A-haptenic monosaccharide: 2-deoxy-D-glucose (Dazzo et al. 1978). Similar results, showing lectin-mediated host-specific attachment, were obtained in case of the *R. leguminosarum* bv. *viciae*-pea and *Bradyrhizobium*-soybean interactions (Kato et al. 1980; Stacey et al. 1980). Lectin binding sites were found in the extracellular and capsular polysaccharides (Mort and Bauer 1980; Abe et al. 1984) as well as in the *O*-antigenic part of the lipopolysaccharide of rhizobial outer membranes (Wolpert and Albersheim 1976; Hrabak et al. 1981). However, in contrast to these reports, other investigators failed to demonstrate any degree of host specificity at the attachment level. Heterologous rhizobia were shown to adhere equally well to nonhost root hairs as did the homologous isolates (Pueppke 1984; Mills and Bauer 1985; Smit et al. 1989b). Smit et al. (1986) and Kijne et al. (1988) observed that the conditions under which the rhizobia were grown strongly influence the attachment capacity of *R. leguminosarum* bv. *viciae* to pea root hair tips. Optimal attachment was always found to coincide with nutrient limitation and the type of limitation determines whether host lectins are involved or not (Kijne et al. 1988). *R. leguminosarum* bv. *viciae* cells grown under carbon-limiting conditions adhere to root hairs in a nonhost specific way (Smit et al. 1986), while manganese limitation as well as other limitations (e.g., limitations for oxygen and nitrogen) leads to an attachment mechanism in which host lectins are involved (Kijne et al. 1988).

In their study on the adhesion capacity of *R. leguminosarum* bv. *viciae* to pea root tips under various physiological conditions, Smit et al. (1987) demonstrated that *Rhizobium* attachment to the legume root hair is a two-steps process, similar to the attachment process described for *Agrobacterium*. In the first step *R. leguminosarum* bv. *viciae* binds loosely as single cells directly to the root hair surface. In a second step additional bacteria accumulate at the adhesion site forming bacterial aggregates. The latter process is referred to as cap formation. Two bacterial components were identified as being involved in these processes: a proteinaceous adhesin, called rhicadhesin and cellulose fibrils.

The initial binding of rhizobial cells to the root hair surface is nonhost specific and is mediated by the rhizobial Ca^{2+}-dependent surface protein rhicadhesin (rhizobial calcium-dependent adhesin, Smit et al. 1987). The involvement of a Ca^{2+}-dependent adhesin was initially suggested by the observations that (i) rhizobia, grown under Ca^{2+} limitation, elicit a strongly reduced binding capacity to pea roots (Smit et al. 1987) and (ii) although Ca^{2+} limitation affects several cell surface features such as the loss of the *O*-antigenic part of lipopolysaccharides and the loss of flagella, these characteristics did not account for the attachment deficient phenotype (Smit et al. 1989a). Rhicadhesin has been purified using its capacity to block competitively the adherence of rhizobia to pea root hairs (Smit et al. 1989b). It is a 14-kDa Ca^{2+}-binding protein that requires Ca^{2+} for its anchoring in the bacterial cell surface (Smit et al. 1991). When rhizobia are grown under conditions of low Ca^{2+} concentration, rhicadhesin is released from the bacteria into the growth medium explaining the poor attachment capability of rhizobia grown under Ca^{2+}-limiting conditions. Rhicadhesin activity has been detected in all members of the *Rhizobiaceae* family (including *B. japonicum* and *A. tumefaciens*, see also above), but not in other bacteria such as *E. coli* and *Pseudomonas putida* (Smit et al. 1989b). Furthermore, rhicadhesin-mediated adhesion of *Rhizobiaceae* cells is not restricted to pea root hair tips but occurs with a number of other plants, including nonleguminous dicotylodenous and monocotylodenous species (Smit et al. 1989b). These results indicate that rhicadhesin-mediated attachment is a common initial binding mechanism in *Rhizobiaceae*-plant interactions and requires a common plant surface component as a receptor molecule. Recently Swart et al. (1994b) reported the purification and partial characterization of a putative plant receptor molecule for rhicadhesin. This molecule, a 32-kDa glycoprotein, was purified from cell walls of pea roots on the basis of its ability to suppress inhibition of attachment of *R. leguminosarum* bv. *viciae* to pea roots by rhicadhesin. Rhicadhesin was isolated either from *R. leguminosarum* bv. *viciae* or *A. tumefaciens* (see also above). Since an Arg-Gly-Asp (RGD) containing hexapeptide is also able to suppress inhibition of attachment by rhicadhesin, the authors suggest that binding of the putative receptor molecule to rhicadhesin occurs, as is the case for a variety of animal adhesive proteins (Pytela et al. 1987) through an RGD attachment site.

The second step in the attachment of rhizobia to root hair tips involves the adherence of additional bacteria to the root hair bound bacterial cells and the formation of aggregates at the attachment site (cap formation). In the *R. leguminosarum* bv. *viciae*-pea interaction, bacterial cellulose fibrils together with the pea lectin Psl (*Pisum sativum* lectin) are involved in this step (Smit et al. 1987; Kijne et al. 1988). Fibril overproducing mutants of *R. leguminosarum* bv. *viciae* exhibit an increased aggregation ability as compared to the parental strain and form larger caps at the root hair tips. On the other hand, cellulose-minus mutants completely lack the capacity to form aggregates and attach as individual cells (Smit et al. 1987). Optimal cap formation by *R. leguminosarum* bv. *viciae* al-

ways coincides with nutrient limitation, that is limitation for carbon, nitrogen, oxygen, manganese, or other components. Among these, carbon deficiency is the only limitation that leads to cap formation in which the pea lectin is not involved (Smit et al. 1987). When compared to cells grown under other physiological conditions, the accumulation of carbon-limited cells at the root hair tips is strongly delayed while the initial binding is not affected (Kijne et al. 1988). Apparently, the lectin accelerates accumulation of additional bacteria immediately after the primary binding step to the root hair surface. Since rhizobial lectin receptors are found in various polysaccharide fractions (exopolysaccharides, capsular polyssacharides and lipopolyssacharides) (Mort and Bauer 1980; Abe et al. 1984), the failure of carbon-limited *R. leguminosarum* bv. *viciae* cells to recognize root lectin may be due to the fact that the production of surface polysaccharides is probably seriously restricted under these conditions. Furthermore, carbon-limited cells were also found to be less infective, suggesting an important role of lectins not only in rhizobial attachment but also in infection thread formation (Kijne et al. 1988). The hypothesis that lectins are required for infectivity is consistent with the work of Diaz et al. (1989). These authors constructed transgenic clover plants carrying the pea lectin (*psl*) gene and demonstrated that, in contrast to wild-type clover roots, the transgenic roots could be nodulated by *R. leguminosarum* bv. *viciae*, the normal symbiont of pea. Since *R. leguminosarum* bv. *viciae* was previously shown to attach to clover root hairs, to induce root hair curling but not to form infection threads (Yao and Vincent 1976; Smit et al. 1986), these results clearly demonstrate an additional and primary role of lectins in an infection step following root hair curling, most probably in the initiation of infection thread formation.

In case of *B. japonicum*, pili (fimbriae) have been proposed to mediate firm attachment to soybean roots (Vesper and Bauer 1986). In support of this hypothesis a correlation was found between the proportion of piliated cells in a bacterial culture and their attachment capacity to hydrophobic plastic surfaces and soybean roots. Addition of anti-pilus antibodies was shown to completely block firm adherence of *B. japonicum* to soybean roots and pilus-minus mutants were reported to have a severely impaired adhesion ability (Vesper and Bauer 1986; Vesper et al. 1987; Vesper and Bhuvaneswari 1988). Moreover, the presence of galactose seemed to specifically inhibit pili-mediated binding of *B. japonicum* to soybean roots, suggesting the involvement of pili-associated lectin-like proteins (Vesper and Bauer 1986). More recently, Ho et al. (1990) isolated a 38-kDa galactose-specific lectin (BJ38) from the *B. japonicum* cell surface, which appeared to be involved in adhesion of *B. japonicum* to soybean roots. Using BJ38-specific antibodies and immunomicroscopy, BJ38 was shown to be located on one pole of the bacteria. Polar attachment of *B. japonicum* to soybean roots was mediated by the BJ38-containing pole of the bacterium (Loh et al. 1993). However, BJ38 was not found to be associated with pili or any filamentous structure.

As described, rhicadhesin is involved in the initial binding step of rhizobia to root hairs while cellulose fibrils and lectins determine the second step. In case of *B. japonicum*, pili and a galactose specific lectin were found to control firm attachment. Cellulose-minus mutants of *R. leguminosarum* bv. *viciae* are not affected in nodulation of pea under laboratory conditions. However, since these mutants are affected in firm adherence to root hairs, it can be speculated that under certain environmental conditions, nodulation by these mutants in the field is more severely affected than the wild-type strain, as it is the case in *A. tumefaciens* (see also above). Similarly, nodulation by a *B. japonicum* field isolate lacking pili and affected in firm adherence is reduced on roots which were exposed to the bacteria for only 1 h prior to planting in growth pouches as compared to roots which were contacted by the bacteria for the entire infectible period of 4 to 6 h (Vesper and Bhuvaneswari 1988). So far, no *Rhizobium* mutants that lack rhicadhesin have been described. However, in view of the central role of the initial binding step in the *A. tumefaciens* tumorigenesis, rhicadhesin-mediated binding of *Rhizobium* to root hairs might be a prerequisite for successful nodulation.

MOTILITY AND ATTACHMENT IN THE INTERACTION OF ASSOCIATIVE PLANT-GROWTH-PROMOTING RHIZOSPHERE BACTERIA WITH PLANT ROOTS

Plant-growth promoting fluorescent pseudomonads.

Certain isolates of *Pseudomonas fluorescens* and *Pseudomonas putida* have the potential to promote plant growth and to protect plants against microbial pathogens. These beneficial traits have been attributed to the bacterial production of siderophores, antibiotics, hydrogen cyanide, and plant growth hormones (for a review see Dowling and O'Gara 1994).

Extensive colonization of the root surface by pseudomonads is essential for an efficient biocontrol activity (Bull et al. 1991). The *O*-antigenic part of the lipopolyssacharide was demonstrated to be essential for efficient colonization of potato roots by *P. fluorescens* and *P. putida* but is not involved in adhesion of the bacteria to the root surface (de Weger et al. 1989; de Weger et al. 1991). Flagella were found to be important for extensive colonization of *P. putida* on potato roots (de Weger et al. 1987) but not for the colonization of wheat by *P. fluorescens* (Howie et al. 1987). The reason why bacterial motility had such large effects in one experiment but not in the other is not clear. Based on their results, Howie et al. (1987) concluded that the main mechanism for dispersal of pseudomonads over root surfaces is caused by a passive carriage downward as the root extends through the soil. However, the experiments of De Weger et al. (1987) indicate that the basic colonization pattern resulting from passive dispersal additionally can be modified by active bacterial motility and/or chemotaxis.

Several bacterial surface molecules have been implicated in *Pseudomonas*-plant root adhesion. However, distinct interactions probably require different mechanisms and the surface components involved depend on both the bacterial and the plant species. Fimbriae were found to increase the attachment of *P. fluorescens* to corn roots (Vesper 1987). De Mot et al. (1991) identified a root adhesin in the outer membrane of *P. fluorescens*, displaying in vitro adsorption to seedling roots of wheat, barley, maize, bean, and sunflower. Sequence analysis of the structural gene revealed strong homology of the *P. fluorescens* adhesin with the amino- and carboxy terminal regions of the porin F proteins (OprF) of *P. aeruginosa* and *P. syringae* (De Mot et al. 1992). A *P. fluorescens oprF* mutant was constructed by reversed genetics and the effect of this muta-

tion on *P. fluorescens*-root attachment was investigated (De Mot et al., abstract of E.C. Meeting on Microbial Ecology, Granada, Spain, 24 to 27 October 1993). The adhesion capacity of the *oprF* mutant was found to be impaired at low inoculum levels but was not completely abolished. The authors therefore suggest that besides the OprF protein of *P. fluorescens*, also additional surface components must be functional in the *P. fluorescens*-root adsorption. The *E. coli* maltoporin, LamB, has been reported to carry a specific binding-site for malto-oligosaccharides (Nakae et al. 1986). A similar protein-carbohydrate binding might also be responsible for the adhesion ability of the *P. fluorescens* porin OprF protein to plant roots (De Mot, personal communication). Anderson (1983) described the agglutination of *P. putida* cells by a bean root surface glycoprotein, termed agglutinin. *P. putida* mutants deficient in the agglutination phenotype (Agg^-) showed a reduced ability to adhere to plant roots, to colonize roots from a seed inoculum and to suppress *Fusarium* attack (Anderson et al. 1988; Tari and Anderson 1988). The bacterial component that interacts with the plant agglutinin remains to be determined. Since no differences were identified between the Agg^- mutants and the parental strain regarding the cell surface features pili, flagella, and lipopolysacharides and since, secondly, the agglutination phenotype is sensitive to protease treatment, proteinaceous surface molecules are thought to be involved (Buell et al. 1993). A genetic locus, termed *aggA*, essential for the agglutination-mediated attachment of *P. putida* to plant roots has been identified (Buell and Anderson 1992). *aggA* encodes a 48-kDa periplasmic protein and has been shown to be expressed on plant root surfaces (Buell and Anderson 1993). The exact function of the periplasmic AggA protein in the agglutination phenotype, however, remains to be elucidated.

***Azospirillum* spp.**

Bacteria of the genus *Azospirillum* are diazotrophs that closely associate with the roots of many grasses including important agricultural crops such as maize, rice, and wheat. Plant growth promotion by *Azospirillum* has been demonstrated in field and greenhouse experiments (Okon and Labandera-Gonzalez 1995; Corich et al. 1995) and attributed to several mechanisms including nitrogen fixation and auxin production (for a review see Okon 1994; Vande Broek and Vanderleyden 1995). To date five species have been identified within the genus *Azospirillum: A. brasilense, A. lipoferum, A. amazonense, A. halopraeferans,* and *A. irakense* (Tarrand et al. 1978; Magalhaes et al. 1983; Reinhold et al. 1987; Khammas et al. 1985).

Azospirillum spp. possess a mixed pattern of flagellation: During growth in liquid media, a single polar flagellum is synthesized while, in addition to the polar flagellum, peritrichous flagella are induced when growing on solidified media (Hall and Krieg 1983). *Azospirillum* strains were demonstrated to exhibit positive chemotaxis in vitro towards several attractants, including sugars, amino acids, organic acids (Okon et al. 1980; Barak et al. 1982; Reinhold et al. 1985), aromatic compounds (Lopez-De-Victoria et al. 1993) as well as towards root exudates (Heinrich and Hess 1985) and root mucilage (Mandimba et al. 1986). Furthermore, migration of *A. brasilense* cells towards the roots of wheat seedlings has been shown in sand cultures and in a wet soil (Bashan 1986). Aromatic compounds were found to be chemotactically detected at concentrations similar to those found in the soil and in the plant rhizosphere (Lopez-De-Victoria et al. 1993). Genetics of *Azospirillum* flagellation and chemotaxis is so far restricted to the species *A. brasilense. A. brasilense* genes required for motility in liquid medium, for motility on solid surfaces and for general chemotaxis were found to be located on the chromosome as well as to reside on a 90-MDa megaplasmid (van Rhijn et al. 1990; Croes et al. 1993).

Using two short-term in vitro binding assays, Michiels et al. (1991) demonstrated that attachment of *Azospirillum* to wheat roots proceeds in two distinct steps, similar to what has been described for *Agrobacterium* and *Rhizobium.* The first step (the adsorption step), consists of a rapid, loose, and reversible binding of *Azospirillum* to the wheat root, while in the second phase (the anchoring phase), the bacteria become irreversibly bound to the root surface. Recently, Croes et al. (1993), presented evidence that the *A. brasilense* polar flagellum or a component thereof functions as an adhesin, mediating adsorption of *Azospirillum* to root surfaces. Three lines of evidence were given. Firstly, dissociation of the flagella by heat or acid was shown to strongly reduce adsorption to wheat roots by *A. brasilense.* Secondly, purified polar flagella were demonstrated to adsorb to wheat roots while purified lateral flagella did not. Thirdly, five genetically distinct *Azospirillum* mutants lacking the polar flagellum were isolated. All showed a severely reduced adsorption capacity to wheat roots as compared to the wild-type.

A yet unidentified bacterial Calcofluor-binding polysaccharide (PS) was found to be responsible for the anchoring of *Azospirillum* at the root surface in the second attachment step (Michiels et al. 1991). By means of this Calcofluor-binding PS, adsorbed azospirilla become firmly attached and additional free bacteria are entrapped, forming large aggregates at the attachment site. *A. brasilense* Cal^- mutants that are affected in the production of the Calcofluor-binding PS were shown to have lost the ability to form flocs in liquid cultures (Michiels et al. 1990) and to exhibit a strongly reduced anchoring capacity to wheat roots (Michiels et al. 1991). Adsorption, however, was not affected in these Cal^- mutants.

Mainly because of the lack of an easily scorable plant phenotype reflecting a succesful plant interaction, not many studies, using specific *Azospirillum* mutants, have yet been conducted to evaluate a role of bacterial motility, chemotaxis and attachment in the establishment of the plant association. Recently, the use of the GUS reporter system has been described to study *Azospirillum*-plant associations (Vande Broek et al. 1993). Using *A. brasilense* strains expressing constitutively the *E. coli gusA* gene, the main colonization sites of *Azospirillum* on wheat roots could be visualized. During the first days of the association, *Azospirillum* was shown to specifically colonize the sites of lateral root emergence and the root hair zones of the primary as well as the secundary roots. Further proliferation of *Azospirillum* on the root surface was found to be dependent on the nitrogen status of the rooting medium. In a subsequent study (Vande Broek 1994), the effect of specific mutations in the *Azospirillum* genome on the colonization ability of wheat roots was investigated. *A. brasilense* mutants lacking the polar and lateral flagella showed a severely reduced colonization capacity, whereas mutants defective in the production of the Calco-

fluor-binding PS displayed a wild-type colonization phenotype. Whether the reduced colonization capacity of the flagella-deficient mutants is caused by a defective plant root attachment due to the loss of the bacterial adhesin on the polar flagellum or/and by a hampered motility of these mutants towards the roots, however, still remains to be demonstrated.

CONCLUSIONS

Most rhizosphere bacteria studied so far are motile. Although chemotaxis of plant-interacting bacteria towards root exudates has been often demonstrated in in vitro experiments (Heinrich and Hess 1985; Mandimba et al. 1986; Hawes et al. 1988; Caetano-Anollés 1988), it is not easy to demonstrate the necessity of bacterial motility and chemotaxis for a successful interaction with the host plant. Experimental conditions such as the inoculation procedures or the soil type often determine whether or not bacterial motility and chemotaxis are essential for the establishment of a plant-bacteria interaction. The influence of the experimental conditions used is well documented in case of the *A. tumefaciens* pathogenesis and the *Rhizobium*–legume symbiosis. By comparing the virulence of nonmotile and chemotaxis-deficient *A. tumefaciens* mutants with that of wild-type stains in sterilized sand or soil, it was demonstrated that only under conditions whereby soil is used and whereby bacteria are not directly inoculated on the plant, motility and chemotaxis mutants are completely avirulent (Hawes and Smith 1989). When pathogenesis was tested under other experimental conditions (i.e., in sterilized sand or by direct inoculation) no difference in the virulence of these mutants as compared to the wild-type could be found. Under laboratory conditions, *Rhizobium* mutants affected in motility have been reported to induce as many nodules as the wild-type strains, at least in the absence of competition with wild-type strains (for instance, Ames and Bergman 1981; Malek 1992). When coinoculated with the wild type, however, the nonmotile isolates appeared to be strongly disadvantaged in comparison with the parental strains, indicating that bacterial motility can play an essential role in competition for nodule occupancy. In contrast, other experiments using autoclaved soils as well as nonsterilized native soils showed that under the tested conditions flagellar motility is not a major factor in rhizobial competiveness (Hambdi 1971; Soby and Bergman 1983; Catlow et al. 1990). In these soils, rhizobia were shown to remain restricted to their microsites, irrespective of motility and chemotaxis. This is probably caused by the absence of continuous water films between soil pores and/or adsorptive effects of clay and soil colloids. In accordance with these results, studies with other soil bacteria showed that bacteria can only spread over measurable distances in certain native soils when the soil is at saturation with water or at near saturation conditions, providing water bridges between the large soil pores (Wong and Griffin 1976; Bashan 1986; Howie et al. 1987). Under these conditions, it is likely that the seedling root encounters bacteria when it advances through these soils, rather that bacteria move actively towards the root. Once on the root surface, the bacteria can be passively carried downward by the roots elongating into the soil. In addition, at this stage, flagellar motility and chemotaxis can become important for the localized spread of bacteria in the rhizoplane where root mucilage provides a continuous water film. Obviously, field releases of well-characterized nonmotile mutants and isogenic wild-type strains in different soil types at normal soil moistures are essential to further assess the relative importance of flagellar locomotion for the establishment of plant interactions under natural conditions. In this respect, attention has not only to focus on the spread of bacteria over the root surface but also on aerial parts. In addition, the question has to be raised whether motility mediated by flagella is the only mechanism of active movement by plant-associated bacteria. Alternative flagellum-independent surface translocations have been described in other bacteria. Two recently studied examples are the 'twitching motility,' mediated by retractile polar type IV pili in *Pseudomonas aeruginosa* (Bradley 1980; Henrichsen 1983; Russell and Darzins 1994) and the surface spreading, requiring cell surface polysaccharides and lipids as reported for nonflagellated gliding bacteria (Godchaux et al. 1991) and the enteric bacterium *Serratia marcescens* (Matsuyama et al. 1995). Noteworthy in this respect is the recent isolation of type IV pilus genes (*pil* genes) in *P. putida* (De Groot et al. 1994). These *P. putida pil* genes were shown to be expressed and functional in *P. aeruginosa*, as concluded from heterologous expression studies and the restoration of *P. aeruginosa pil* mutants for piliation and twitching motility. However, electron microscopic analyses of *P. putida* cells grown under different growth conditions, did so far not reveal the presence of polar pili on the *P. putida* cell surface.

That bacterial adherence to plant roots is important for a successful plant interaction has so far been demonstrated unequivocally only in the case of the *A. tumefaciens* tumorigenesis and for the interaction of fluorescent pseudomonads with plant roots. *A. tumefaciens* mutants that are unable to bind to plant cells, were all shown to be completely avirulent (Douglas et al. 1982; Cangelosi et al. 1987; Matthysse 1987). *P. putida* Agg$^-$ mutants, defective in agglutinability by a bean root surface glycoprotein and in adherence to plant roots were demonstrated to possess a severely reduced capacity to colonize plant roots and to suppress fungal attack (Anderson et al. 1988; Tari and Anderson 1988).

At this stage it appears that a variety of bacterial surface components, depending of the bacterial system studied, are involved in plant root attachment. However, until the molecular structures of these surface components have been further analyzed in detail, it is not clear whether these different components also represent different binding domains for plant interaction. It might well be that the same binding domains are exposed by different structures depending on the bacterial species studied.

It is striking to observe that in three totally different types of bacterial plant interactions, namely in the *A. tumefaciens* pathogenesis, the *R. leguminosarum* bv. *viciae*–pea symbiosis and the associative interaction of *A. brasilense* with plants, attachment to plant roots proceeds through a common mechanism, consisting of two consecutive steps. In the first step, mediated by a proteinaceous bacterial adhesin, i.e., rhicadhesin in the case of *A. tumefaciens* and *R. leguminosarum* bv. *viciae* (Smit et al. 1989b) and a yet unidentified protein located on the polar flagellum in case of *A. brasilense* (Croes et al. 1993), the bacteria adhere loosely as single cells to the plant root surface. *A. tumefaciens chvB* mutants affected in rhicadhesin biosynthesis were shown to be completely aviru-

lent, pointing to the essential role of the initial attachment step in tumorigenicity (Douglas et al. 1982; Swart et al. 1993). Similarly, *A. brasilense* mutants lacking the polar flagellum were demonstrated to be severely impaired in wheat root colonization (Vande Broek 1994). However, it is still unclear whether the reduced colonization ability of these mutants is caused by a deficient initial plant root binding or by an impaired motility. In the second attachment step, the bacteria become more firmly attached to the plant roots and additional free bacteria are entrapped resulting in the formation of large bacterial clusters at the attachment site. Bacterial polysaccharides were found to be responsible for this strong adherence and agglutination (Matthysse et al. 1981; Smit et al. 1987; Michiels et al. 1991). In the case of *R. leguminosarum* bv. *viciae*, bacterial growth conditions and especially nutrient limitations were shown to strongly affect the characteristics of this second attachment step. To evaluate the significance of these biochemical data, it will be essential to characterize the environmental conditions present at the legume root hairs. The availibility of nutrients in the microbial habitat may be assessed using a so-called biological sensor whereby a reporter gene is fused to a promoter responsive to a chemical signal (De Weger et al. 1994; Loper and Lindow 1994). Interestingly, for the three types of bacterial plant interactions, the second binding step was found not to be a prerequisite for a succesful plant interaction under normal laboratory conditions. This raises the question why this second binding step is conserved among different plant-bacteria interactions. As demonstrated for the *A. tumefaciens* pathogenesis, the fibril-mediated adhesion may become essential under certain adverse environmental conditions, such as flushing of the wounds by water, strictly requiring a firm anchoring of the bacteria to the root surface (Matthysse 1983). In addition, under natural conditions the bacterial appendages might also be important by enabling the bacteria to colonize plant tissues before specific interaction. Clearly, field experiments are essential to test this putative role of fibril-mediated root adhesion in the bacterial colonization capacity and to examine the significance of firm anchoring in the establishment of the plant association under native soil conditions.

ACKNOWLEDGMENTS

We acknowledge the financial support of the Flemish Government (GOA 93 Vanderleyden) and from the European Union (IMPACT EEG BIO2-CT93-0053). A.V.B. is a recipient of a postdoctoral fellowship of the 'Onderzoeksfonds K.U.Leuven'. The authors also wish to thank René De Mot for critical reading of the manuscript.

LITERATURE CITED

Abe, M., Sherwood, J. E., Hollingsworth, R. I., and Dazzo, F. B. 1984. Stimulation of clover root hair infection by lectin-binding oligosaccharides from the capsular and extracellular polysaccharides of *Rhizobium trifolii*. J. Bacteriol. 160:517-520.

Anderson, A. J. 1983. Isolation from root and shoot surfaces of agglutinins that show specificity for saprophytic pseudomonads. Can. J. Bot. 61:3438-3443.

Anderson, A. J., Habibzadegah-Tari, P., and Tepper, C. S. 1988. Molecular studies on the role of a root surface agglutinin in adherence and colonization by *Pseudomonas putida*. Appl. Environ. Microbiol. 54:375-380.

Ankenbauer, R. G., and Nester, E. W. 1993. The *Agrobacterium* Ti plasmid and crown gall tumorigenesis: A model for signal transduction in host-pathogen interactions. Pages 67-104 in: Signal Transduction. J. Kurjan and B. L. Taylor, eds. Academic Press, Inc., San Diego.

Ames, P., and Bergman, K. 1981. Competitive advantage provided by bacterial motility in the formation of nodules by *Rhizobium meliloti*. J. Bacteriol. 148:728-729.

Ashby, A. M., Watson, M. D., Loake, G. J., and Shaw, C. H.. 1988. Ti plasmid-specified chemotaxis of *Agrobacterium tumefaciens* C58C[1] toward *vir*-inducing phenolic compounds and soluble factors from monocotyledonous and dicotyledonous plants. J. Bacteriol. 170:4181-4187.

Baldani, V. L. D., Baldani, J. I., and Döbereiner, J. 1987. Inoculation of field-grown wheat (*Triticum aestivum*) with *Azospirillum* spp. in Brasil. Biol. Fertil. Soils 4: 37-40.

Barak, R., Nur, I., Okon, Y., and Henis, Y. 1982. Aerotactic response of *Azospirillum brasilense*. J. Bacteriol. 152:643-649.

Bashan, Y. 1986. Migration of the rhizosphere bacteria *Azospirillum brasilense* and *Pseudomonas fluorescens* towards wheat roots in the soil. J. Gen. Microbiol. 132:3407-3414.

Bergman, K., Gulash-Hoffee, M., Hovestadt, R. E., Larosiliere, R. C., Ronco II, P. G., and Su, L. 1988. Physiology of behavioral mutants of *Rhizobium meliloti*: Evidence for a dual chemotaxis pathway. J. Bacteriol. 170:3249-3254.

Bohlool, B. B., and Schmidt, E. L. 1974. Lectins: A possible basis for specificity in the *Rhizobium*-legume root nodule symbiosis. Science 185:269-271.

Bradley, D. E. 1980. A function of *Pseudomonas aeruginosa* PAO polar pili: Twitching motility. Can. J. Microbiol. 26:146-154.

Bradley, D. E., Douglas, C. J., and Peshon, J. 1984. Flagella-specific bacteriophages of *Agrobacterium tumefaciens*: Demonstration of virulence of nonmotile mutants. Can. J. Microbiol. 30:676-681.

Buell, C. R., and Anderson, A. J. 1992. Genetic analysis of the *aggA* locus involved in agglutination and adherence of *Pseudomonas putida*, a beneficial fluorescent pseudomonad. Mol. Plant-Microbe Interact. 5:154-162.

Buell, C. R., and Anderson, A. J. 1993. Expression of the *aggA* locus of *Pseudomonas putida in vitro* and *in planta* as detected by the reporter gene, *xylE*. Mol. Plant-Microbe Interact. 6:331-340.

Buell, C. R., Whetton, R., Tari, P., and Anderson, A. J. 1993. Characterization of cell surface properties in agglutinable and nonagglutinable mutants of *Pseudomonas putida*. Can. J. Microbiol. 39:787-794.

Bull, C. T., Weller, D. M., and Thomashow, L. S.. 1991. Relationship between root colonization and suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* strain 2-79. Phytopathology 81:954-959.

Caetano-Anollés, G., Crist-Estes, D. K., and Bauer, W. D. 1988. Chemotaxis of *Rhizobium meliloti* to the plant flavone luteolin requires functional nodulation genes. J. Bacteriol. 170:3164-3169.

Cangelosi, G. A., Hung, L., Puvanesarajah, V., Stacey, G., Ozga, D. A., Leigh, J. A., and Nester, E. W. 1987. Common loci for *Agrobacterium tumefaciens* and *Rhizobium meliloti* exopolysaccharide synthesis and their roles in plant interactions. J. Bacteriol. 169:2086-2091.

Cangelosi, G. A., Martinetti, G., Leigh, J. A., Chang Lee, C., Theines, C., and Nester, E. W. 1989. Role for *Agrobacterium tumefaciens* ChvA protein in export of β-1,2-glucan. J. Bacteriol. 171:1609-1615.

Cangelosi, G. A., Ankenbauer, R. G., and Nester, E. W. 1990a. Sugars induce the *Agrobacterium* virulence genes through a periplasmic binding protein and a transmembrane signal protein. Proc. Natl. Acad. Sci USA 87:6708-6712.

Cangelosi, G. A., Martinetti, G., and Nester, E. W. 1990b. Osmosensitivity phenotypes of *Agrobacterium tumefaciens* mutants that lack periplasmic β-1,2-glucan. J. Bacteriol. 172:2172-2174.

Carlson, R. W., Price, N. P. J., and Stacey, G. 1994. The biosynthesis of rhizobial lipo-oligosaccharide nodulation signal molecules. Mol. Plant-Microbe Interact. 7:684-695.

Catlow, H. Y., Glenn, A. R., and Dilworth, M. J. 1990. The use of transposon-induced nonmotile mutants in assessing the significance of motility of *Rhizobium leguminosarum* biovar *trifolii* for movement in soils. Soil Biol. Biochem. 22:331-336.

Croes, C., Moens, S., Van Bastelaere, E., Vanderleyden, J., and Michiels, K. 1993. The polar flagellum mediates *Azospirillum brasilense* adsorption to wheat roots. J. Gen. Microbiol. 139:2261-2269.

Currier, W. W. 1980. Chemotaxis of a birdsfoot trefoil strain of *Rhizobium* to simple sugars. FEMS Microbiol. Lett. 8:43-46.

Dazzo, F. B., and Hubell, D. H. 1975. Cross-reactive antigens and lectins

as determinants of symbiotic specificity in the *Rhizobium*-clover association. Appl. Microbiol. 30:1018-1033.

Dazzo, F. B., Yanke, W. E., and Brill, W. J. 1978. Trifoliin: A *Rhizobium* recognition protein from white clover. Biochim. Biophys. Acta 539:276-286.

De Groot, A., Heijnen, I., de Cock, H., Filloux, A., and Tomassen, J. 1994. Characterization of type IV pilus genes in plant-growth promoting *Pseudomonas putida* WCS358. J. Bacteriol. 176:642-650.

De Ley, J., and Rassel, A. 1965. DNA base composition, flagellation and taxonomy of the genus *Rhizobium*. J. Gen. Microbiol. 41:85-91.

De Mot, R., and Vanderleyden, J. 1991. Purification of a root-adhesive outer membrane protein of root-colonizing *Pseudomonas fluorescens*. FEMS Microbiol. Lett. 81:323-328.

De Mot, R., Proost, P., Van Damme, J., and Vanderleyden, J. 1992. Homology of the root adhesin of *Pseudomonas fluorescens* OE 28.3 with porin F of *P. aeruginosa* and *P. syringae*. Mol. Gen. Genet. 231:489-493.

de Weger, L. A., van der Vlugt, C. I. M., Wijfjes, A. H. M., Bakker, P. A. H. M., Shippers, B., and Lugtenberg, B. 1987. Flagella of a plant-growth-stimulating *Pseudomonas fluorescens* strain are required for colonization of potato roots. J. Bacteriol. 169:2769-2773.

de Weger, L. A., van Loosdrecht, M. C. M., Klaasen, H. E., and Lugtenberg, B. 1989. Mutational changes in physicochemical surface properties of plant-growth-stimulating *Pseudomonas* spp. do not influence the attachment properties of the cells. J. Bacteriol. 171:2756-2761.

de Weger, L. A., Bakker, P. A. H. M., Schippers, B., van Loosdrecht, M. C. M., and Lugtenberg, B. 1991. Mutational changes in the O-antigenic side chains of the lipopolysaccharides of *Pseudomonas* spp. affect colonization of but not adhesion to potato roots. Page 306 in: The Rhizosphere and Plant Growth. D. L. Keister and P. B. Cregan, eds. Kluwer Academic Publishers, Dordrecht.

De Weger, L. A., Dekkers, L. C., van der Bij, A. J., and Lugtenberg, B. J. J. 1994. Use of phosphate reporter bacteria to study phosphate limitation in the rhizosphere and in bulk soil. Mol. Plant-Microbe Interact. 7:32-38.

Douglas, C., Halperin, W., and Nester, E. W. 1982. *Agrobacterium tumefaciens* affected in attachment to plant cells. J. Bacteriol. 152:1265-1275.

Dowling, D. N., and O'Gara F. 1994. Metabolites of *Pseudomonas* involved in the biocontrol of plant disease. Trends Biotech. 12:133-141.

Garfinkel, D. J., and Nester, E. W. 1980. *A. tumefaciens* mutants affected in crown gall tumorigenesis and octopine catabolism. J. Bacteriol. 144:732-743.

Gaskins, M. H., Umali-Garcia, M., Tien, T. M., and Hubbell, D. H. 1977. Nitrogen fixation and growth substance produced by *Spirillum lipoferum* and their effects on plant growth. Plant Physiol. 59 (Suppl.):128.

Gaworzewska, E. T., and Carlile, M. J. 1982. Positive chemotaxis of *Rhizobium leguminosarum* and other bacteria towards root exudates from legumes and other plants. J. Gen. Microbiol. 128:1179-1188.

Godchaux III, W., Lynes, M. A., and Leadbetter, E. R. 1991. Defects in gliding motility in mutants of *Cytophaga johnsonae* lacking a high-molecular-weight cell surface polysaccharide. J. Bacteriol. 173:7607-7614.

Gurlitz, R. H. G., Lamb, P. W., and Matthysse, A. G. 1987. Involvement of carrot cell surface proteins in attachment of *Agrobacterium tumefaciens*. Plant Physiol. 83:564-568.

Hall, P. G., and Krieg, N. R. 1983. Swarming of *Azospirillum brasilense* on solid media. Can. J. Microbiol. 29:1592-1594.

Hambdi, Y. A. 1971. Soil-water tension and the movement of rhizobia. Soil Biol. Biochem. 3:121-126.

Hamblin, J., and Kent, S. P. 1973. Possible role of phytohemagglutinin in *Phaseolus vulgaris* L. Nature New Biol. 254:28-30.

Hawes, M. C., Smith, L. Y., and Howarth, A. J. 1988. *Agrobacterium tumefaciens* mutants deficient in chemotaxis to root exudates. Mol. Plant-Microbe Interact. 1:182-186.

Hawes, M. C., and Smith, L. Y. 1989. Requirement for chemotaxis in pathogenicity of *Agrobacterium tumefaciens* on roots of soil-grown pea plants. J. Bacteriol. 171:5668-5671.

Heinrich, D., and Hess, D. 1985. Chemotactic attraction of *Azospirillum lipoferum* by wheat roots and characterization of some attractants. Can. J. Microbiol. 31:26-31.

Henrichsen, J.1983. Twitching motility. Annu. Rev. Microbiol. 37:81-93.

Ho, S. C., Schindler, M., and Wang, J. L. 1990. Carbohydrate binding activities of *Bradyrhizobium japonicum*. II. Isolation and characterization of a galactose-specific lectin. J. Cell Biol. 111:1639-1643.

Hooykaas, P. J. J., and Schilperoort, R. A. 1986. The molecular basis of the *Agrobacterium*-plant interaction: Characteristics of *Agrobacterium* virulence genes and their possible occurrence in other plant-associated bacteria. Pages 189-202 in: Recognition in Microbe-Plant Symbiotic and Pathogenic Interactions. B. Lugtenberg, ed. Springer-Verlag, Berlin.

Howie, W. J., Cook, R. J., and Weller, D. M. 1987. Effects of soil matric potential and cell motility on wheat root colonization by fluorescent pseudomonads suppressive to take-all. Phytopathology 77:286-292.

Hrabak, E. M., Urbano, M. R., and Dazzo, F. B. 1981. Growth-phase-dependent immunodeterminants of *Rhizobium trifolii* lipopolyssacharide which bind trifoliin A, a white clover lectin. J. Bacteriol. 148:697-711.

Jordan, D. C. 1984. Pages 234-254 in: Bergey's Manual Systematic Bacteriology. Vol. 1. N. R. Krieg and J. G. Holt, eds. Williams and Wilkins, Baltimore, MD.

Kado, C. I. 1991. Molecular mechanisms of crown gall tumorigenesis. Crit. Rev. Plant Sci. 10:1-32.

Kato, G., Maruyama, Y., and Nakamura, M. 1980. Role of bacterial polysaccharides in the adsorption process of the *Rhizobium*-pea symbiosis. Agric. Biol. Chem. 44:2843-2855.

Khammas, K. M., Ageron, E., Grimont, P. A. D., and Kaiser, P. 1989. *Azospirillum irakense* sp. nov. a nitrogen fixing bacterium associated with rice roots and rhizosphere soil. Res. Microbiol. 140:679-693.

Kijne, J. W., Smit, G., Diaz, C. L., and Lugtenberg, B. J. J. 1988. Lectin-enhanced accumulation of manganese-limited *Rhizobium leguminosarum* cells on pea root hair tips. J. Bacteriol. 170:2994-3000.

Leigh, J. A., Signer, E. R., and Walker, G. C. 1985. Exopolysaccharide-deficient mutants of *Rhizobium meliloti* that form ineffective nodules. Proc. Natl. Acad. Sci. USA 82:6231-6235.

Lerouge, P., Roche, P., Faucher, C., Maillet, G., Truchet, G., Promé, J. C., and Dénarié, J. 1990. Symbiotic host-specificity of *Rhizobium meliloti* is determined by a sulphated and acylated glucosamine oligosaccharide. Nature 344:781-784.

Lippincott, B. B., and Lippincott, J. A. 1969. Bacterial attachment to a specific wound site as an essential stage in tumor initiation by *Agrobacterium tumefaciens*. J. Bacteriol. 97:620-628.

Liu, R., Tran, V. M., and Schmidt, E. L. 1989. Nodulating competitiveness of a nonmotile Tn*7* mutant of *Bradyrhizobium japonicum* in nonsterile soil. Appl. Environ. Microbiol. 55:1895-1900.

Loake, G. J., Ashby, A. M., and Shaw, C. M. 1988. Attraction of *Agrobacterium tumefaciens* C58C^1 towards sugars involves a highly sensitive chemotaxis system. J. Gen. Microbiol. 134:1427-1432.

Loh, J. T., Ho, S. C., de Feijter, A. W., Wang, J. L., and Schindler, M. 1993. Carbohydrate binding activities of *Bradyrhizobium japonicum*: Unipolar localization of the lectin BJ38 on the bacterial cell surface. Proc. Natl. Acad. Sci. USA 90:3033-3037.

Loper, J. E., and Lindow, S. E. 1994. A biological sensor for iron available to bacteria in their habitats on plant surfaces. Appl. Environ. Microbiol. 60:1934-1941.

Lopez-de-Victoria, G., and Lovell, C. R. 1993. Chemotactic behavior of *Azospirillum* species to aromatic compounds. Appl. Environ. Microbiol. 59:2951-2955.

Long, S. R. 1989. *Rhizobium*-legume symbiosis: Life together in the underground. Cell 56:203-214.

Magalhaes, F. M. M., Baldani, J. I., Souto, S. M., Kuykendall, J. R., and Döbereiner, J. 1983. A new acid tolerant *Azospirillum* species. Ann. Acad. Bras. Cienc. 55:417-430.

Malek, W. 1992. The role of motility in the efficiency of nodulation by *Rhizobium meliloti*. Arch. Microbiol. 158:26-28.

Mandimba, G., Heulin, T., Bally, R., Guckert, A., and Balandreau, J. 1986. Chemotaxis of free-living bacteria towards maize mucilage. Plant Soil 90:129-136.

Matsuyama, T., Bhasin, A., and Harshey, R. M. 1995. Mutational analysis of flagellum-independent surface spreading of *Serratia marcescens* 274 on a low agar medium. J. Bacteriol. 177:987-991.

Matthysse, A. G., Holmes, K. V., and Gurlitz, R. H. G. 1981. Elaboration of cellulose fibrils by *Agrobacterium tumefaciens* during attachment to carrot cells. J. Bacteriol. 145:583-595.

Matthysse, A. G. 1983. Role of bacterial cellulose fibrils in *Agrobacterium tumefaciens* infection. J. Bacteriol. 154:906-915.

Matthysse, A. G. 1987. Characterization of nonattaching mutants of

Agrobacterium tumefaciens. J. Bacteriol. 169:313-323.
Mellor, H. Y., Glenn, A. R., Arwas, R., and Dilworth, M. J. 1987. Symbiotic and competitive properties of motility mutants of *Rhizobium trifolii* TA1. Arch. Microbiol. 148:34-39.
Michiels, J., and Vanderleyden, J. 1994. Molecular basis of the establishment and functioning of a N_2-fixing root nodule. World J. Microbiol. Biotechn. 10:612-630.
Michiels, K., Verreth, C., and Vanderleyden, J. 1990. *Azospirillum lipoferum* and *Azospirillum brasilense* surface polysaccharide mutants that are affected in flocculation. J. Appl. Bacteriol. 69:705-711.
Michiels, K., Croes, C., and Vanderleyden, J. 1991. Two different modes of attachment of *Azospirillum brasilense* Sp7 to wheat roots. J. Gen. Microbiol. 137:2241-2246.
Miller, K. J., Kennedy, E. P., and Reinhold, V. N. 1986. Osmotic adaptation by gram-negative bacteria: Possible role for periplasmic oligosaccharides. Science 231:48-51.
Mills, K. M., and Bauer, W. D. 1985. *Rhizobium* attachment to clover roots. J. Cell Sci. Suppl. 2:333-345.
Mort, A. J., and Bauer, W. D. 1980. Composition of the capsular and extracellular polysaccharides of *Rhizobium japonicum*: Changes with culture age and correlation with binding of soybean seed lectin to the bacteria. Plant Physiol. 66:158-163.
Nakae, T., Ishii, I., and Ferenci, T. 1986. The role of the maltodextrin-binding site in determining transport properties of the LamB protein. J. Biol. Chem. 261:622-626.
Napoli, C., and Albersheim, P. 1980. Infection and nodulation of clover by nonmotile *Rhizobium trifolii*. J. Bacteriol. 141:979-980.
Neff, N. T., and Binns, A. N. 1985. *Agrobacterium tumefaciens* interaction with suspension-cultured tomato cells. Plant Physiol. 77:35-42.
Okon, Y., Gakmakgi, L., Nur, I., and Chet, I. 1980. Aerotaxis and chemotaxis of *Azospirillum brasilense*. Microbiol. Ecol. 6:277-280.
Okon, Y. 1994. *Azospirillum* Associations. CRC Press Inc., Boca Raton, FL.
Okon, Y., and Labandera-Gonzalez, C. 1995. Agronomic applications of *Azospirillum*: An evaluation of 20 years worldwide field inoculation. Soil Biol. Biochem. In press.
Parke, D., Ornston, L. N., and Nester, E. W. 1987. Chemotaxis to plant phenolic inducers of virulence genes is constitutively expressed in the abscence of the Ti plasmid in *Agrobacterium tumefaciens*. J. Bacteriol. 169:5336-5338.
Pueppke, S. G. 1984. Adsorption of slow- and fast-growing rhizobia to soybean and cowpea roots. Plant Physiol. 75:924-928.
Pytela, R., Pierschbacher, M. D., Argraves, S., Suzuki, S., and Ruoslahti, E. 1987. Arginine-Glycine-Aspartic acid adhesion receptor. Meth. Enzymol. 144:475-489.
Rao, S. S., Lippincott, B. B., and Lippincott, J. A. 1982. *Agrobacterium* adherence involves the pectic portion of the host cell wall and is sensitive to the degree of pectic methylation. Physiol. Plant. 56:374-380.
Reinhold, B., Hurek, T., and Fendrik, I. 1985. r-specific chemotaxis of *Azospirillum* spp. J. Bacteriol. 162:190-195.
Reinhold, B., Hurek, T., Fendrik, I., Pot, B., Gillis, M., Kersters, K., Thielemans, S., and De Ley, J. 1987. *Azospirillum halopraeferans* sp. nov., a nitrogen-fixing organism associated with roots of Kallar grass (*Leptochloa fusca* (L.) Kunth.). Int. J. Syst. Bacteriol. 43:574-584.
Russell, M. A., and Darzins, A. 1994. The *pilE* gene product of *Pseudomonas aeruginosa*, required for pilus biogenesis, shares amino acid sequence identity with the *N*-termini of type 4 prepilin proteins. Mol. Microbiol. 13:973-985.
Sequeira, L., Gaard, G., and Soeten, G. A. 1977. Interaction of bacteria and host cell walls:its relation to mechanisms of induced resistance. Physiol. Plant Pathol. 10:43-50.
Smit, G., Kijne, J. W., and Lugtenberg, B. J. J. 1986. Correlation between extracellular fibrils and attachment of *Rhizobium leguminosarum* to pea root hair tips. J. Bacteriol. 168:821-827.
Smit, G. , Kijne, J. W., and Lugtenberg, J. J. 1987. Both cellulose fibrils and a Ca^{+2}-dependent adhesin are involved in the attachment of *Rhizobium leguminosarum* to pea root hair tips. J. Bacteriol. 169:4294-4301.
Smit, G., Kijne, J. W., and Lugtenberg, B. 1989a. Roles of flagella, lipopolysaccharide and a Ca^{+2}-dependent cell surface protein in attachment of *Rhizobium leguminosarum* biovar *viciae* to pea root hair tips. J. Bacteriol. 171:569-572.
Smit, G., Logman, T. J. J., Boerrigter, M. E. T. I., Kijne, J. W., and Lugtenberg, B. J. J. 1989b. Purification and partial characterization of the Ca^{+2}-dependent adhesin from *Rhizobium leguminosarum* biovar *viciae*, which mediates the first step in attachment of *Rhizobiaceae* cells to plant root hair tips. J. Bacteriol. 171:4054-4062.
Smit, G., Tubbing, D. M. J., Kijne, J. W., and Lugtenberg, B. J. J. 1991. Role of Ca^{+2} in the activity of rhicadhesin from *Rhizobium leguminosarum* biovar *viciae*, which mediates the first step in attachment of *Rhizobiaceae* cells to plant root hair tips. Arch. Microbiol. 155:278-283.
Soby, S., and Bergman, K. 1983. Motility and chemotaxis of *Rhizobium meliloti* in soil. Appl. Environ. Microbiol. 46:995-998.
Spaink, H. P., Sheeley, D. M., van Brussel, A. A. N., Glushka, J., York, W. S., Tak, T., Geiger, O., Kennedy, E. P., Reinhold, V. N., and Lugtenberg, B. J. J. 1991. A novel highly unsaturated fatty acid moiety of lipo-oligosaccharide signals determines host specificity of *Rhizobium*. Nature 354:125-130.
Sprent, J. I. 1989. Which steps are essential for the formation of functional legume nodules? New Phytol. 111:129-153.
Stacey, G., Paau, A. S., and Brill, W. J. 1980. Host recognition in the *Rhizobium*-soybean symbiosis. Plant Physiol. 66:609-614.
Swart, S., Smit, G., Lugtenberg, B. J. J., and Kijne, J. W. 1993. Restoration of attachment, virulence and nodulation of *Agrobacterium tumefaciens chvB* mutants by rhicadhesin. Mol. Microbiol. 10:597-605.
Swart, S., Logman, T. J. J., Smit, G., Lugtenberg, B. J. J., and Kijne, J. W. 1994a. Purification and partial characterization of a glycoprotein from pea (*Pisum sativum*) with receptor activity for rhicadhesin, an attachment protein of Rhizobiaceae. Plant Mol. Biol. 24:171-183.
Swart, S., Lugtenberg, B. J. J., Smit, G., and Kijne, J. W. 1994b. Rhicadhesin-mediated attachment and virulence of an *Agrobacterium tumefaciens chvB* mutant can be restored by growth in a highly osmotic medium. J. Bacteriol. 176:3816-3819.
Tari, P. H., and Anderson, A. J. 1988. *Fusarium* wilt suppression and agglutinability of *Pseudomonas putida*. Appl. Environ. Microbiol. 54:2037-2041.
Tarrand, J. J., Krieg, N. R., and Döbereiner, J. 1978. A taxonomic study of the *Spirillum lipoferum* group, with description of a new genus, *Azospirillum* gen. nov., and two species *Azospirillum lipoferum* (Beijerinck) sp. nov., and *Azospirillum brasilense* sp. nov. Can. J. Microbiol. 24:967-980.
Truchet, G., Roche, P., Lerouge, P., Vasse, J., Camut, S., De Billy, F., Promé, J. C., and Dénarié, J. 1991. Sulphated lipo-oligosaccharide signals of *Rhizobium meliloti* elicit root nodule organogenesis on the host *Medicago sativa*. Nature 351:670-673.
Uttaro, A. D., Cangelosi, G. A., Geremia, R. A., Nester, E. W., and Ugalde, R. A. 1990. Biochemical characterization of avirulent *exoC* mutants of *Agrobacterium tumefaciens*. J. Bacteriol. 172:1640-1646.
Vande Broek, A., Michiels, J., Van Gool, A., and Vanderleyden, J. 1993. Spatial-temporal colonization patterns of *Azospirillum brasilense* on the wheat root surface and expression of the bacterial *nifH* gene during association. Mol. Plant-Microbe Interact. 6:592-600.
Vande Broek, A. 1994. Histochemical and genetic analysis of the *Azospirillum brasilense*-wheat root association. Dissertationes de agricultura Nr. 267, PhD thesis, Catholic University of Leuven, Belgium.
Vande Broek, A., and Vanderleyden, J. 1995. Genetics of the *Azospirillum*-plant root association. Crit. Rev. Plant Sci. 14:445-466.
van Rhijn, P., Vanstockem, M., Vanderleyden, J., and De Mot, R. 1990. Isolation of behavioral mutants of *Azospirillum brasilense* by using Tn*5-lacZ*. Appl. Environ. Microbiol. 4:990-996.
van Rhijn, P., and Vanderleyden, J. 1995. The *Rhizobium*-plant symbiosis. Microbiol. Rev. 59:124-142.
Vesper, S. J., and Bauer, W. D. 1986. Role of pili (fimbriae) in attachment of *Bradyrhizobium japonicum* to soybean roots. Appl. Environ. Microbiol. 52:134-141.
Vesper, S. J. 1987. Production of pili (fimbriae) by *Pseudomonas fluorescens* and correlation with attachment to corn roots. Appl. Environ. Microbiol. 53:1397-1405.
Vesper, S. J., Malik, S. A., and Bauer, W. D. 1987. Transposon mutants of *Bradyrhizobium japonicum* altered in attachment to host roots. Appl. Envrion. Microbiol. 53:1959-1961.
Vesper, S. J., and Bhuvaneswari, T. V. 1988. Nodulation of soybean roots by an isolate of *Bradyrhizobium japonicum* with reduced firm attachment capability. Arch. Microbiol. 150:15-19.
Wagner, V. T., and Matthysse, A. G. 1992. Involvement of a vitronectin-like protein in attachment of *Agrobacterium tumefaciens* to carrot suspension culture cells. J. Bacteriol. 174:5999-6003.

Winans, S. C. 1992. Two way signalling in *Agrobacterium*-plant interactions. Microbiol. Rev. 56:12-31.

Wolpert, J. S., and Albersheim, P. 1976. Host symbiont interactions. I. The lectins of legumes interact with the *O*-antigen containing lipopolysaccharides of their symbiont rhizobia. Biochem. Biophys. Res. Commun. 70:729-737.

Wong, P. T. W., and Griffin, D. M. 1976. Bacterial movement at high matric potentials. I. In artificial and natural soils. Soil Biol. Biochem. 8:214-218.

Yao, P. Y., and Vincent, J. M. 1976. Factors responsible for the curling and branching of clover root hairs by *Rhizobium*. Plant Soil 45:1-16.

Young, D. H., and Sequeira, L. 1986. Binding of *Pseudomonas solanacearum* fimbriae to tobacco leaf cell walls and its inhibition by bacterial extracellular polysaccharides. Physiol. Mol. Plant Pathol. 28:393-402.

Zorreguieta, A., and Ugalde, R. A. 1986. Formation in *Rhizobium* and *Agrobacterium* spp. of a 235 kilodalton protein intermediate in β-D(1,2)-glucan synthesis. J. Bacteriol. 167:947-951.

Zurkowski, W. 1980. Specific adsorption of bacteria to clover root hairs, related to the presence of plasmid pWZ2 in cells of *Rhizobium trifolii*. Microbios 27:27-32.