Factors affecting adsorption of *Azospirillum brasilense* Cd to root hairs as compared with root surface of wheat

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Electron microscopy of wheat (*Triticum aestivum*) roots inoculated with *Azospirillum brasilense* Cd revealed massive adsorption of bacterial cells to the root surface and less adsorption to root hairs. Quantitative analysis of *A. brasilense* Cd adsorption to root surface and to root hairs, confirmed qualitatively by light microscopy observations, revealed a bacterial adsorption ratio of 5 (±2) : 1 (root surface : root hairs). Extreme bacterial adsorption ratios were recorded when bacteria were previously grown in the presence of KNO_3 (27:1) or when bacterial cells were inoculated under hydroponic plant growth conditions (80:1). Adsorption of *A. brasilense* Cd to roots was directly related to the bacterial growth phase, with logarithmic phase cultures demonstrating a greater adsorption than stationary phase cultures. Adsorption to root hairs was dependent mainly on the number of root hairs developed under certain growth conditions. When very few root hairs had developed, most of the bacterial cells were adsorbed to the root surface. Factors such as starvation, bacteria grown in culture in the presence of KNO_3, addition of several nutrients, and protease or NaEDTA treatments of bacterial cells before the adsorption assay decreased bacterial adsorption to root hairs. Other factors such as microaerophilic growth conditions, addition of several bacterial chemoattractants, and cellulase-treated root hairs enhanced bacterial adsorption. It is proposed that although *A. brasilense* Cd adsorbed to every part of the root system, more cells adsorbed to the root surface of wheat than to the root hairs.

Key words: associative bacteria, *Azospirillum*, bacterial adsorption, beneficial bacteria, rhizosphere bacteria, root-hair colonization.

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L'examen en microscopie électronique de racines de blé (*Triticum aestivum*) inoculées avec de l'*Azospirillum brasilense* Cd a permis d'observer une adsorption massive des cellules bactériennes à la surface des racines et une adsorption moindre aux poils absorbants. Une analyse quantitative de l'adsorption d'*A. brasilense* Cd aux surfaces racinaires et aux poils absorbants, confirmée qualitativement par la microscopie optique, a révélé un ratio d'adsorption bactérienne de 5 (±2) : 1 (surface racinaire : poils absorbants). Des ratios extrêmes d'adsorption bactérienne ont été notés lorsque les bactéries avaient été cultivées antérieurement en présence de KNO_3 (27:1) ou lorsque les bactéries avaient été inoculées dans des conditions hydroponiques de croissance des plantes (80:1). L'adsorption de l'*A. brasilense* Cd aux racines s'est avérée être reliée aux phases de croissance bactérienne; les cultures en phase logarithmique ont présenté une plus grande adsorption que celles qui étaient en phase stationnaire. L'adsorption aux poils absorbants a été surtout dépendante du nombre de poils absorbants et des conditions dans lesquelles ceux-ci se sont développés. Lorsque très peu de poils absorbants se sont développés, la plupart des cellules bactériennes ont été adsorbées à la surface des racines. Des facteurs tels qu'une privation en nutriments, la culture des bactéries en présence de KNO_3, l'ajout de plusieurs nutriments, des traitements des cellules bactériennes à la protéase ou au NaEDTA avant les essais d'adsorption ont réduit l'adsorption aux poils absorbants. D'autres facteurs tels la croissance dans des conditions microaérophiles, l'ajout de plusieurs chimioattractants bactériens et les poils absorbants traités à la cellulase ont rehaussé l'adsorption bactérienne. La proposition est avancée que, bien que l'*A. brasilense* Cd soit adsorbé par toutes les parties du système racinaire, la majorité des cellules ont été adsorbées à la surface des racines du blé plurôt qu'aux poils absorbants.

Mots clés : bactéries associatives, *Azospirillum*, adsorption bactérienne, bactéries bénéfiques, bactéries de la rhizosphère, colonisation des poils absorbants.

[Traduit par la revue]

Introduction

Root colonization is the key factor in the successful interaction of plants with *Azospirillum*, which is known to positively affect plant growth (Bashan 1986*a*; Okon 1985). *Azospirillum* species are known to colonize root surfaces of several plant species (Bashan and Levanony 1987, 1988*c*; Bashan et al. 1987, 1989*a*; Gafni et al. 1986; Patriquin et al. 1983; Umali-Garcia et al. 1980) as well as the interior cortex of cereal roots (Bashan and Levanony 1988*d*; Levanony et al. 1989; Patriquin and Döbereiner 1978; Patriquin et al. 1983). Generally, *Azospirillum* cells can be found everywhere along inoculated root systems, but they are concentrated mainly in the elongation and the root-hair zones (Bashan et al. 1986; Okon and Kapulnik 1986). Inoculation of roots usually resulted in improved development (size and number) of the root hairs (Kapulnik et al. 1985*c*), as well as in formation of deformed root hairs (Jain and Patriquin 1984, 1985). These changes have been proposed to improve mineral and water uptake by the inoculated plant (Kapulnik et al. 1985*b*; Sarig et al. 1988). Despite this, the role of root hairs in the initiation of the primary interaction of *Azospirillum* with plant roots is still in question.

Unlike rhizobia, *Azospirillum* forms neither the known infection pattern through root hairs nor any external structures on the infected root system. Preliminary microscopic observations have indicated that *Azospirillum* is able to adsorb and later to colonize root hairs as well as the root surface. How-

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ever, the number of bacteria adsorbed to each of the root parts was not defined (Y. Bashan, unpublished; Kapulnik et al. 1985*c*). Therefore, it is of importance to define exactly where most of the *Azospirillum* population is located.

The aim of this study is the quantification of *Azospirillum brasilense* Cd cells located on the root surfaces and on the root hairs in order to determine the proportion of total bacterial adsorption of wheat roots attributable to root-hair adsorption.

Materials and methods

Organisms and growth conditions

The rhizosphere bacteria *Azospirillum brasilense* Cd (ATCC 29710) and wheat seedlings (*Triticum aestivum*, cv. Deganit (Zeraim Gedera Co., Israel)) were used as model organisms. Bacteria were grown and prepared for inoculation as described earlier (Bashan 1986*b*; Bashan and Levanony 1985). For testing the effects of starvation, KNO_3, and chemoattractant and nonattractant substances on bacterial adsorption, *A. brasilense* Cd was grown in nitrogen-free medium (Bashan and Levanony 1985) (control cultures) or media supplemented (separately, final concentration) with 10 mM glycine, aspartic acid, arabinose, or galactose (attractants) or with 5 mM KNO_3 and 10 mM glucose, lactose, sodium pyruvate, or sodium lactate (nonattractants and nutrients) (Barak et al. 1983). Starved bacteria were obtained by maintaining double-washed cells in 0.06 M potassium phosphate buffer supplemented with 0.15 M NaCl (PBS) for 24 h at 30 ± 1°C. To avoid self-aggregation of bacterial cells, the cultures were grown for 16 h in Erlenmeyer flasks fitted with shallow grooves and placed on a shaker operated at 250 rpm. This procedure allowed harvesting of nonaggregated bacteria in the logarithmic phase of growth.

Wheat seedlings were grown by one of the following methods and incubated in the dark for 3–4 days at 22 ± 1°C. (*i*) Disinfected seeds (1% NaOCl, 3 min, thoroughly washed with sterile tap water (Bashan 1986*b*)) were carefully placed on the surface of 0.75% water agar in Petri plates (9 cm diam., five per plate). The plates were sealed with Parafilm to avoid agar dryness during germination. (*ii*) Germinating seedlings were grown hydroponically by placing seeds gently on the surface of Fåhraeus nutrient solution (Fåhraeus 1957) supplemented with 0.06% agar (to form semisolid medium; 10 mL medium per 10-mL test tube). (*iii*) Seedlings were grown under microaerophilic conditions by a procedure similar to procedure *ii* except that the 15 mL nutrient solution completely filled the test tube, and after first leaf emergence, the tubes were sealed with Parafilm, leaving the leaf out of the tube through a slit. (*iv*) Seedlings were grown in large test tubes (200 mL) containing quartz sand at field capacity (2% v/v) supplemented with Fåhraeus medium. (*v*) Seedlings were grown aeroponically on wet gauze placed on a miniature stainless-steel stand in high glass Petri plates (9 cm diam., 7 cm depth). Water (0.5-cm layer) was placed on the bottom of the plate, and wet Whatman No. 1 filter paper was attached to the cover of the Petri plate. The plates were sealed with Parafilm, forming a high relative humidity growth chamber.

Removal of seedling root hairs

Unless otherwise stated, germinated seedlings from aeroponic growth conditions with a large amount of root hairs were selected using a stereoscopic microscope. Detached root hairs were obtained by the ultralow-temperature procedure described by Gerhold et al. (1985). Alternatively, in a few cases, root hairs were obtained by mechanical cutting. Root hairs were viewed under a stereoscopic microscope and removed with a sharp razor blade, cutting as close to the root surface as possible. Both roots and root hairs were then immediately transferred to PBS.

Adsorption assays

After removal of root hairs by the ultralow-temperature technique, roots (three per replicate) and the equivalent root hairs were transferred (separately) into Eppendorf minitubes containing 1 mL freshly prepared nonaggregated *A. brasilense* Cd (1×10^4 colony-forming units (cfu) per millilitre) suspended in 0.06 M PBS pH 6.1 (Gafni et al. 1986) and were lightly shaken in a miniature shaker for 90 min at 30 ± 2°C. Whole roots, i.e., containing intact untreated root hairs, were inoculated in an identical manner. The number of *A. brasilense* Cd cells adsorbed to the whole root, to the root surface, and to the root hairs was determined. Higher inoculation levels (1×10^6 – 1×10^7 cfu/mL) have the disadvantage of aggregated adsorption (Y. Bashan and H. Levanony, unpublished), which results in greater numerical variation in bacterial counts described later. Therefore, low inoculum levels containing the nonaggregated fraction of the bacterial culture were used throughout the study. Adsorption under microaerophilic conditions was done by transfer of roots grown under these conditions into Eppendorf tubes filled with bacterial solution maintained in helium-filled anaerobic jars. The amount of oxygen in the jars was adjusted to 3 ± 1 μM dissolved O_2 (Hurek et al. 1987).

Bacterial counts

Bacteria were counted on each root part by one of the following procedures. (*i*) In a direct counting method, roots and root hairs were removed from the adsorption solution and were placed in 2.5 mL PBS. *Azospirillum brasilense* Cd cells attached to these parts of the roots were released into the buffer by sonic vibration of the sample (Sonifier B-12 for 3 min at 10 W, Branson Sonic Power Co., CN). This sonication separated the bacterial cells from the root parts and broke the bacterial aggregates but did not affect the multiplication ability of *A. brasilense* Cd (Bashan and Levanony 1989). The suspension was plated on nitrogen-free medium (Bashan and Levanony 1985) by using a spiral plater (Spiral Systems, Cincinnati, OH), incubated for 72–96 h at 30 ± 2°C, and counted. The release of nearly all *A. brasilense* Cd cells from the root parts (lower limit of 10–30 cells) was checked by liquid enrichment of bacteria remaining in these root parts in nitrogen-free semisolid medium for 48 h (Bashan and Levanony 1985; Y. Bashan, G. Mitiku, O. Ziv-Vecht, and H. Levanony, unpublished). Results obtained from root or root-hair samples that tested positive (presence of remaining *A. brasilense* Cd cells in these root parts) were discarded. (*ii*) In an indirect method, the initial number of *A. brasilense* Cd in the inoculum solution was determined by a plate count method on nutrient agar (Difco) medium, then root parts were added. After adsorption was completed, roots and root hairs were removed (mechanically for roots or by a low-speed centrifugation (150–200 × *g*), which pelleted only root hairs) and the number of bacteria remaining in the solution was redetermined. Adsorption of bacteria was calculated by subtracting the bacteria remaining in the solution from the initial bacterial number.

The adequacy of these methods to quantify the actual bacterial numbers adsorbed to the root parts was determined by evaluating factors affecting the adsorption of *A. brasilense* Cd in these tests, e.g., adsorption of bacteria to the Eppendorf tubes in the absence of roots (<3.8%); agitation of root surface on mechanical lifting out of the solution (<0.5%); centrifugation (<1.2%); and variation due to spiral plating technique and equipment (<2.7%). Comparison of the two count methods revealed only a slight difference between the two (for example, compare Figs. 2A and 2B with 2C and 2D); therefore the indirect method was chosen as the preferred counting method because it was simpler to perform.

Electron microscopy and immuno-gold labeling

Samples for scanning electron microscopy were obtained from inoculated (1×10^4 – 5×10^5 cfu/mL) as well as noninoculated roots (10 root pieces for each treatment) and were prepared according to the method of Bashan et al. (1986). Specific antibodies against *A. brasilense* Cd were raised as previously described (Levanony et al. 1987). Samples for transmission electron microscopy labeled by immuno-gold were prepared according to Levanony and Bashan (1989) and Levanony et al. (1989).

Root exudates

Root exudates were collected as described by Gafni et al. (1986).

Pretreatments with NaEDTA, protease, cellulase, and root exudates

Double-washed bacterial cells or roots were incubated separately in

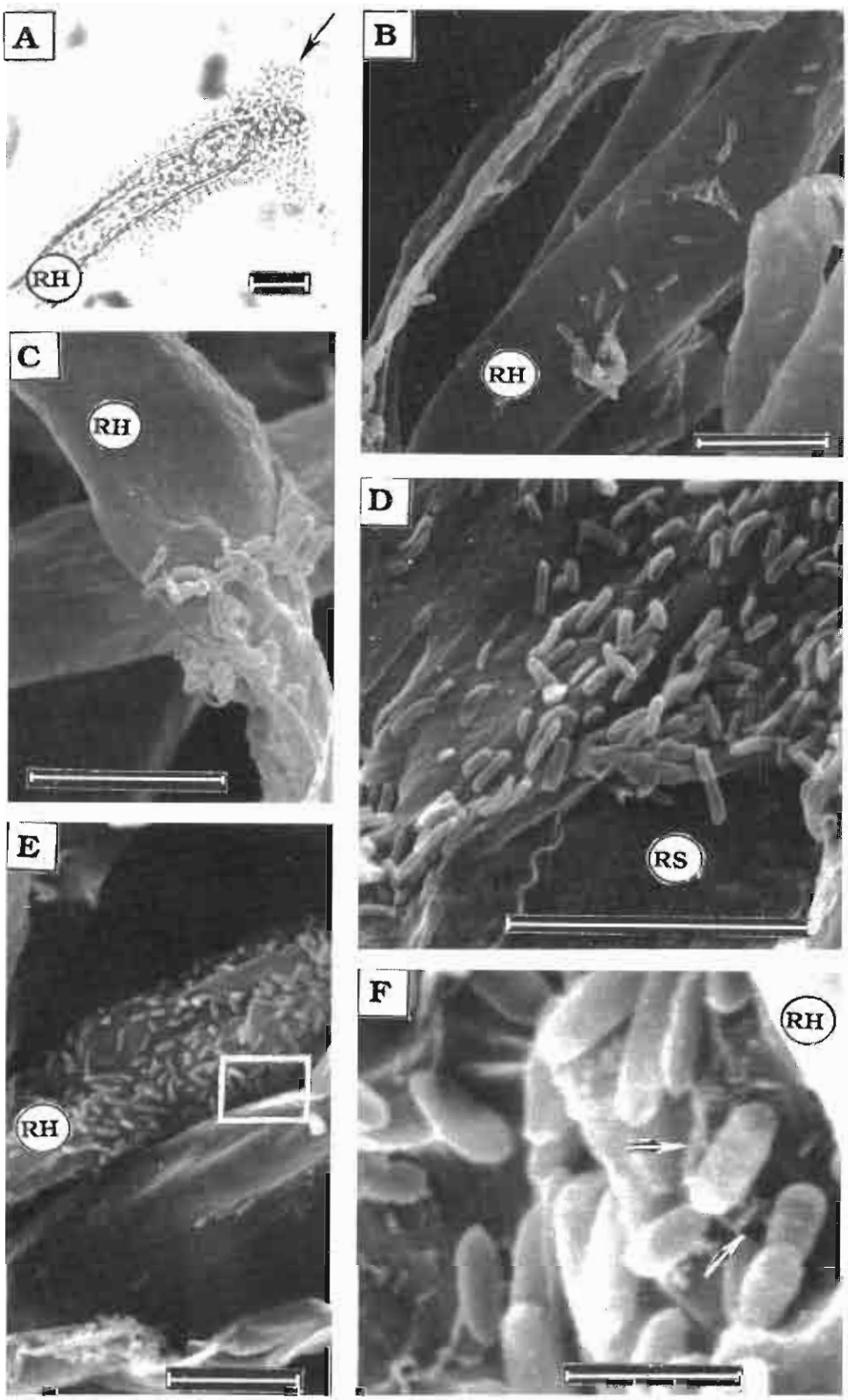

FIG. 1. Wheat root-hair colonization by *A. brasilense* Cd. (A) Light microscopy of a bacterial aggregate near root-hair tip (arrow). (B–F) Scanning electron microscopy of typical root-hair colonization. (B) Single cells randomly dispersed along the root hair. (C) Small microcolony. (D) Root surface at the root-hair zone showing massive colonization. (E) Massive colonization of collapsed root hair. (F) Magnification of boxed-in area of Fig. 1E, showing bacteria attached to the root hair by fibrillar material (arrows). Figs. 1A–1E. Bars represent 10 μm. Fig. 1F. Bar represents 1 μm. RH, root hair; RS, root surface.

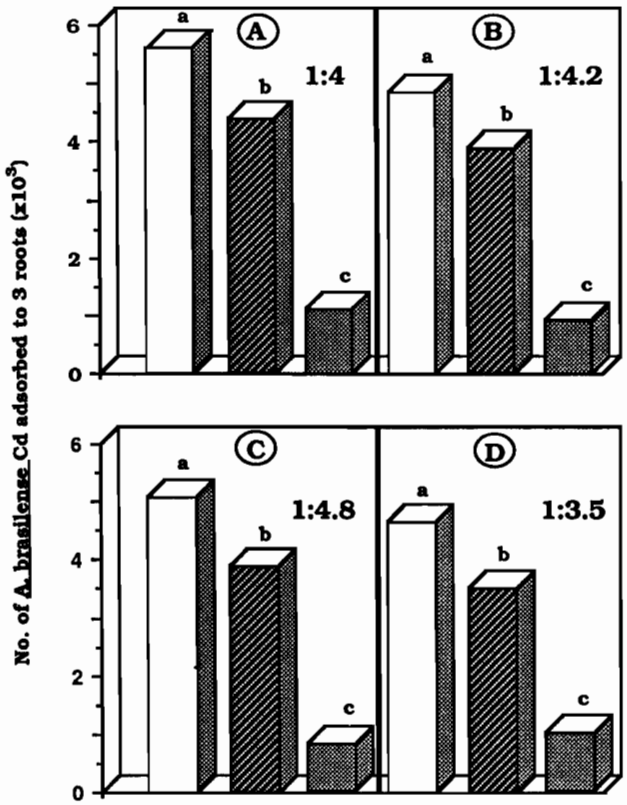

FIG. 2. Adsorption of *A. brasilense* Cd to whole wheat roots (□); to root hairs (▩); and to root surfaces after removal of the root hairs (▨). (A, C) Mechanical cutting of root hairs. (B, D) Ultralow-temperature extraction of root hairs. Ratios given in Figs. 2A–2D are the ratio of adsorption of *A. brasilense* Cd between root hairs and root surface. The number of *A. brasilense* Cd cells was detected by the direct (A, B) and indirect (C, D) method. Columns denoted by a different lower case letter differ significantly at $P \leq 0.05$.

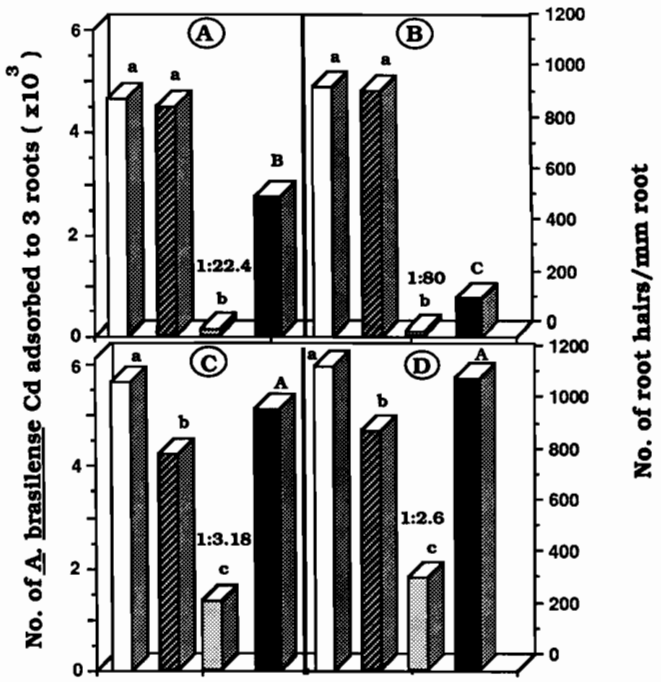

FIG. 3. Adsorption of *A. brasilense* Cd to root surface and to root hairs in different plant growth conditions. (A) Solid water agar surface; (B) semisolid hydroponic; (C) quartz sand; (D) aeroponic. Adsorption to whole wheat root (□); to root hairs (▩); and to root surfaces after removal of root hairs (▨); and the number of root hairs developed under these conditions (■). Ratios given in Figs. 3A–3D are the ratio of adsorption of *A. brasilense* Cd between root hairs and root surface. Columns denoted by a different lower case letter, for each figure section separately, differ significantly at $P \leq 0.05$, whereas columns denoted by a different capital letter, for all sections, differ significantly at $P \leq 0.05$.

the presence of the following substances: 1 mM NaEDTA, 1% trypsin (Sigma), 1% cellulase, and 100 mg/mL root exudates dissolved in PBS for 3 h. These pretreated bacterial cells or roots were then subjected to adsorption experiments.

Experimental design and statistical analysis

All experiments were conducted with five replicates per treatment. A replicate consisted of three main roots in adsorption assays and two roots for electron microscopy observations. All experiments were repeated two or three times. Results are given from one of the experiments in each case. Significance is given by $P \leq 0.05$ in Duncan's multiple-range test.

Results

Light, scanning electron, and gold-labeling transmission electron microscopy of root-hair adsorption

Light microscopy observation of wheat root hairs inoculated by *A. brasilense* Cd revealed that only a few bacterial cells adsorbed to most root hairs. In a few cases bacterial aggregates were found near the root-hair tips (Fig. 1A) as well as along the root hair without particular preference to any part of the root hair. Scanning electron microscopy of the root hairs revealed a single-cell pattern of adsorption, randomly dispersed on the root hairs (Fig. 1B). In several cases, small bacterial aggregates were formed (Fig. 1C). Heavy adsorption of *A. brasilense* Cd was detected on the basal portion of the root hairs, as well as on the root surface in the root-hair zone (Fig. 1D). In a few cases, massive adsorption to only collapsed root hairs was detected (Figs. 1E, 1F). The bacteria adsorbed to root hairs were specifically identified as *A. brasilense* Cd by immuno-gold labeling. Few *A. brasilense* Cd were identified within the root hairs (data not shown).

Adsorption of A. brasilense *Cd to root surface and to root hairs*

Generally, about half of all the inoculated cells were adsorbed to wheat root. Separation of root hairs from the roots revealed that the adsorption ratio was about 4:1 (root surface : root hairs) (Fig. 2). No significant difference was found between adsorption of bacteria to live (Fig. 2A) or to thawed frozen roots (Fig. 2B) or between the detection methods (Figs. 2C, 2D). Light microscopy observation of *A. brasilense* Cd attachment to root surfaces or to root hairs in each sample confirmed clear attachment of bacterial cells to root surface and to the root hairs as well (see also Fig. 1).

Adsorption of A. brasilense *Cd to root surface and to root hairs in relation to plant growth conditions*

Growth conditions greatly affected development of root hairs by the roots. Minimal numbers of root hairs were developed in semisolid hydroponic conditions (Fig. 3B) and maximal numbers of root hairs were detected in either aeroponic growth conditions (Fig. 3D) or in quartz sand (Fig. 3C). Generally, adsorption of *A. brasilense* Cd to root hairs was

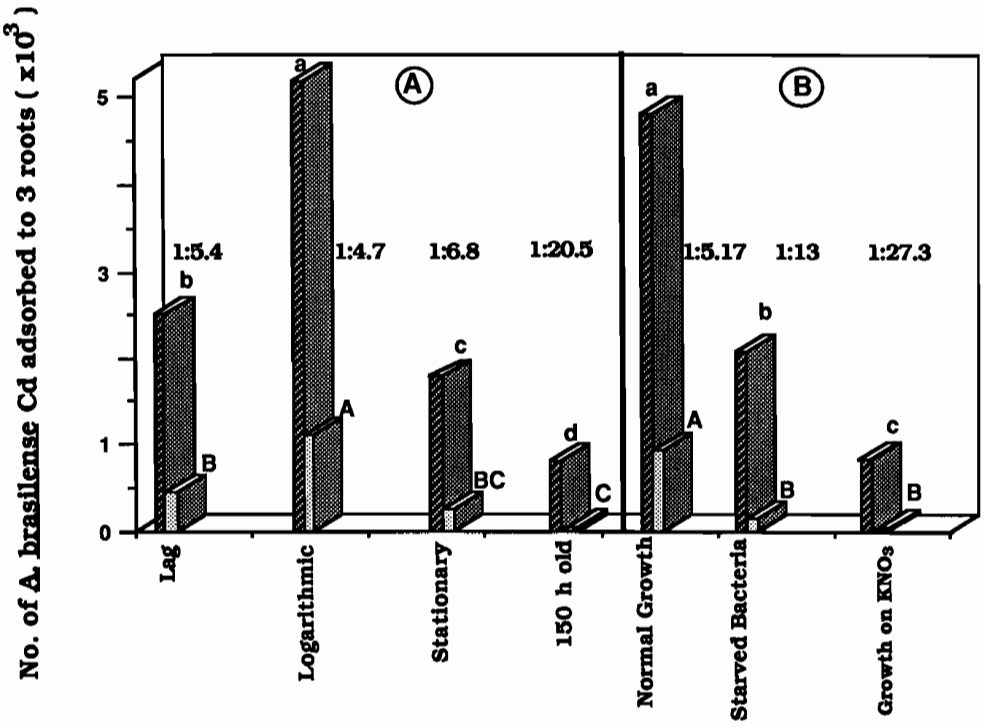

FIG. 4. Adsorption of *A. brasilense* Cd to root surfaces and to root hairs in relation to (A) bacterial age and (B) bacterial growth conditions. Adsorption to root hairs (▨) and to root surfaces after removal of the root hairs (▨). Ratios given are the ratio of adsorption of *A. brasilense* Cd between root hairs and root surface. Columns denoted by a different lower case or capital letter, for each figure section separately, differ significantly at $P \leq 0.05$.

mainly affected by the number of root hairs developed (Figs. 3A–3D). Adsorption of *A. brasilense* Cd to roots and to root hairs was maximal in aeroponic and sand growth conditions (Figs. 3C, 3D). In any plant growth condition tested, the number of *A. brasilense* Cd adsorbed to root hairs was always significantly lower than the number of bacterial cells adsorbed to root surfaces. An increase in the number of root hairs developed under different growth conditions was correlated with an increase in the number of *A. brasilense* Cd cells adsorbed to a single root hair (from 0.3 in agar to 1.67 cells/root hair in aeroponic medium). However, when fewer root hairs were developed (agar and hydroponic media), most of the bacterial cells were adsorbed to the surfaces of the roots, creating extreme ratios of adsorption between root surface and root hairs (Figs. 3A, 3B).

Adsorption of A. brasilense *Cd to root surfaces and to root hairs in relation to bacterial growth phase and bacterial growth conditions*

Adsorption of *A. brasilense* Cd to root surfaces and to root hairs was dependent on the bacterial growth phase. Active logarithmic growth phase bacteria adsorbed to roots in highest numbers. Stationary or lag phase cells adsorbed less, whereas old cultures adsorbed only slightly. Similar adsorption patterns were observed both on root surfaces and on root hairs. However, the total number of *A. brasilense* Cd adsorbed to root hairs was significantly lower than the number of bacterial cells adsorbed to root surfaces (about 1:5) (Fig. 4A). Starved bacteria or bacteria grown in the presence of KNO_3 adsorbed in smaller numbers compared with bacteria grown in nitrogen-free medium (Fig. 4B). Since the adsorption ratio had been higher, it indicates that fewer bacteria were adsorbed to the root hairs in these growth conditions.

Adsorption of A. brasilense *Cd to root hairs under microaerophilic conditions*

Microaerophilic growth conditions significantly decreased root-hair development of wheat seedlings. However, these conditions significantly increased bacterial attachment to the root hairs. Both the total number of attached bacteria and the number of bacteria attached per root hair increased. The ratio between attachment of *A. brasilense* Cd to root hairs under normal growth conditions and under microaerophilic conditions was 1:6.13 (Fig. 5). The ratio of adsorption to root surface under normal growth conditions as compared with that under microaerophilic conditions was 1:1.53, while the ratio between adsorption to root surface and adsorption to root hairs under microaerophilic conditions was 5.1:1.

Adsorption of A. brasilense *Cd to root hairs in the presence of chemoattractants and nonattractants*

Addition of chemoattractants to the adsorption solution moderately but significantly increased *A. brasilense* Cd adsorption to root hairs. No significant difference was found between the effect of the four attractants tested. Addition of five nonattractant substances, known to be taken up by *Azospirillum*, significantly reduced bacterial adsorption to root hairs. All substances tested had a similar reducing effect on adsorption (Fig. 6).

Effect of pretreatment of A. brasilense *Cd cells or root hairs with NaEDTA, protease, cellulase, and root exudates on bacterial adsorption to root hairs*

Addition of *A. brasilense* Cd cells pretreated with either protease or NaEDTA significantly decreased bacterial adsorption to root hairs, whereas cellulase-treated cells moderately decreased adsorption. On the other hand, bacterial pretreat-

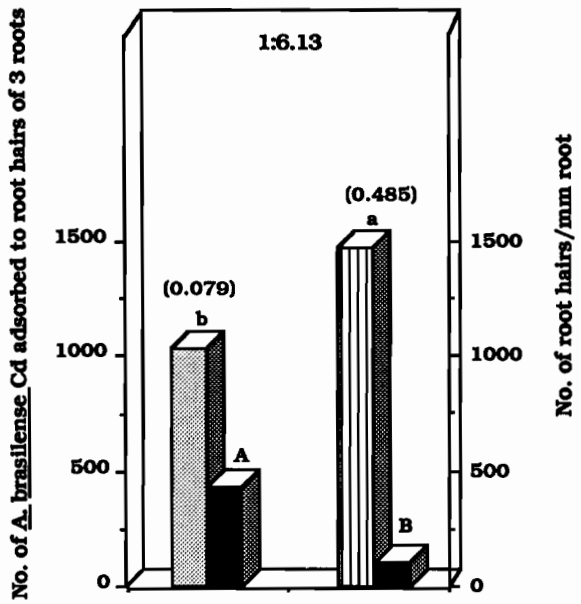

FIG. 5. Adsorption of *A. brasilense* Cd to root hairs under microaerophilic conditions. ▨, Adsorption under regular growth conditions; ▥, adsorption under microaerophilic conditions; ▮, the number of root hairs developed in each condition. Columns denoted by a different lower case or capital letter differ significantly at $P \leq 0.05$. Numbers in parentheses indicate the calculated number of adsorbed bacteria per root hair (30-mm root segments) and the ratio reflects the adsorption ratio to root hairs between normal and microaerophilic growth conditions.

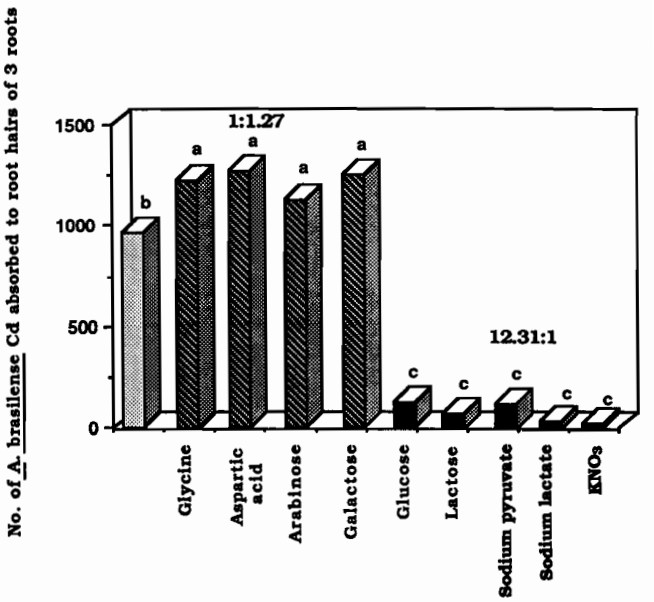

FIG. 6. Adsorption of *A. brasilense* Cd to root hairs in the presence of bacterial attractants and nonattractants. ▨, adsorption under regular growth conditions; ▩, adsorption in the presence of attractants; ▮, adsorption in the presence of nonattractants. Columns denoted by a different letter differ significantly at $P \leq 0.05$. The ratios (after combining data from all substances in each case) reflect the increase or decrease in adsorption to root hairs.

ment with root exudates significantly increased bacterial adsorption to root hairs (Fig. 7A). When root hairs were treated with these substances, only cellulase-treated root hairs significantly increase *A. brasilense* Cd adsorption (Fig. 7B).

Discussion

One of the most remarkable phenomena of inoculation with rhizosphere bacteria on root development is their effect on root-hair development in many plant species (Bowen and Rovira 1961; Rovira 1963). In particular, inoculation with *Azospirillum* species has a significant visible effect on cereal root-hair development. The number, the density, and the length of the root hairs, as well as the number of deformed root hairs, increased dramatically as compared with those of noninoculated plants (Jain and Patriquin 1984; Kapulnik et al. 1985*b*, 1985*c*; Morgenstern and Okon 1987; Okon and Kapulnik 1986; Tien et al. 1979; Umali-Garcia et al. 1980). Although a few studies have suggested that *Azospirillum* has some affinity to root hairs and colonizes them efficiently (Lakshmi et al. 1977; Murty and Ladha 1987; Patriquin et al. 1983; Umali-Garcia et al. 1980), others have shown that root-hair colonization was minimal (Kapulnik et al. 1985*c*; Okon and Kapulnik 1986). It is also known that adsorption of bacteria to root hairs may depend on the plant species (Rovira 1956). Therefore, this study focuses on adsorption of *Azospirillum* to root hairs in comparison with adsorption to root surface in the same root area, i.e., the root-hair zone.

Microscopic observations of wheat roots inoculated with *Azospirillum* revealed massive colonization of the elongation zone, root-hair zone (Bashan et al. 1986), root tip (Bashan and Levanony 1989), isolated plant cells (Eyers et al. 1988), and the bases of root hairs (Kapulnik et al. 1985*c*). In contrast, *Azospirillum*-inoculated pearl-millet plants had heavily colonized root hairs (Umali-Garcia et al. 1980). Using three different microscopic techniques, this study revealed that *A. brasilense* Cd adsorbed poorly to the root hairs of wheat. Only collapsed root hairs showed dense adsorption. However, as in *Azospirillum* adsorption on pearl-millet root hairs (Umali-Garcia et al. 1980), *A. brasilense* Cd cells were connected to plant surfaces by a network of fibrillar material. That most *A. brasilense* Cd cells adsorbed to the root surface at the elongation zone and the root-hair junction, as seen microscopically, may be because such growing regions are constantly exuding compounds that attract *Azospirillum* cells and provide nutrients for this metabolically active association (Bashan et al. 1986). Quantitative verification of these observations revealed that in wheat, more *A. brasilense* Cd cells adsorbed to the root surfaces than to the root hairs, although it affects their development. These results should take into account that removal of root hairs may induce a wound response on the root surface. This may result in the exudation of several compounds that may induce a chemotactic response, which ultimately results in enhanced binding to such areas.

In soil, under normal growth conditions, the rhizosphere bacteria are randomly distributed along root surfaces of cereals; however, most root surfaces are usually free of bacteria (Dart 1971; Foster and Bowen 1982; Newman and Bowen 1974; Rovira and Campbell 1974). Inoculation with *Azospirillum* resulted in much denser root colonization (for a review, see Patriquin et al. 1983). We suggest that since plant growth conditions greatly affected root-hair development, they also affect root-hair adsorption by *Azospirillum*. Despite an increase in *A. brasilense* Cd adsorption per root hair when higher numbers of root hairs were developed, the total adsorption of bacterial cells to the root hairs was lower than to the root surfaces. These data describing low root-hair adsorption

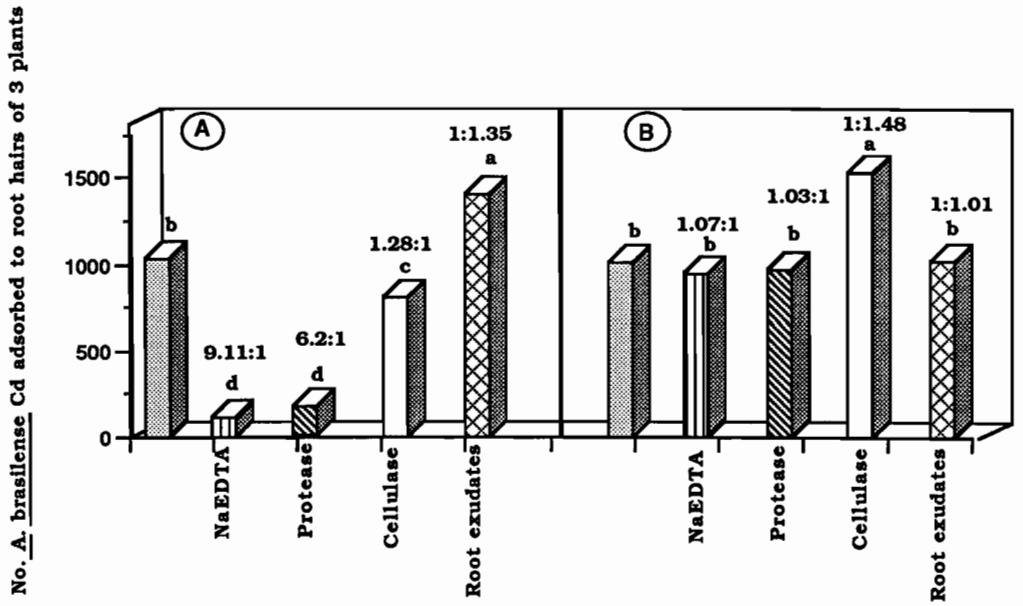

FIG. 7. Effect of pretreatment of (A) *A. brasilense* Cd or (B) root hairs with NaEDTA, protease, cellulase, and root exudates on bacterial adsorption to root hairs. ▨, adsorption under regular growth conditions. Columns denoted by a different lower case letter differ significantly to root hairs at $P \leq 0.05$. Ratios reflect the adsorption relative to nontreated bacteria or root hairs.

by *A. brasilense* Cd differ from the high affinity of *Rhizobium* to legume root hairs, which was calculated to be within the range of 20–40 cells/root hair for *R. trifolii* and *R. meliloti* (Dazzo et al. 1976).

Adsorption of *Azospirillum* to roots is totally dependent on bacterial metabolism. Dead bacteria, a dead plant, or both eliminated adsorption to corn roots (Gafni et al. 1986). It was also shown that active metabolism of the root is less essential than bacterial metabolism in this adsorption (Bashan et al. 1986). This phenomenon is similar to adsorption of *Bradyrhizobium japonicum* to wheat roots (Shimshick and Hebert 1978, 1979). In this regard, the bacterial growth phase has a crucial role in adsorption to roots. Although it was previously shown that old cultures of *Azospirillum* adsorbed to roots in higher numbers (Kapulnik et al. 1985*c*), this study does not support this notion. Only highly active, nonstarved bacterial cells adsorbed in high numbers to the root surfaces as well as to the root hairs, whereas adsorption decreased with an increase in culture age. However, this study gave additional support to the inhibitory effect of nitrate on *Azospirillum* adsorption (Gafni et al. 1986; Patriquin et al. 1983; Umali-Garcia et al. 1980). It appears that this nitrogen source, which prevents nitrogen fixation by *Azospirillum*, also prevents *Azospirillum* adsorption to roots.

Microaerophilic conditions favour nitrogen fixation and multiplication of *Azospirillum* in semisolid medium (Patriquin et al. 1983) as well as increase the adsorption capability of the cells to soil particles (Bashan and Levanony 1988*a*). Although these conditions are not favourable for wheat cultivation, this study presents evidence that microaerophilic conditions also increase *A. brasilense* Cd adsorption to roots. This significant increase in bacterial adsorption to root hairs and to the root surface may have been caused by substances that accumulated during oxygen-limited growth of the seedlings and were released by root hairs and root surfaces during the adsorption period. Therefore, one may predict that bacterial nitrogen fixation and root adsorption, two essential features of *A. brasilense* Cd, may, in fact, be coupled.

The role of chemoattractants in root adsorption by *Azospirillum* is controversial. On one hand, this genus has a significant chemotaxis ability both *in vitro* (Barak et al. 1983) and in the soil (Bashan 1986*c*; Bashan and Levanony 1988*c*). However, since these attractants are not specific to *Azospirillum*, it is assumed that they attract other native rhizosphere bacteria as well. This study presents evidence that these attractants and also root exudates (which yield these attractants) play an additional vital role in enhancing adsorption to roots. These findings support evidence of enhancement of *Azospirillum* adsorption to pearl-millet root hairs by root exudates (Umali-Garcia et al. 1980) but are not in accordance with the findings of Gafni et al. (1986) showing that chemoattractants decreased adsorption of *A. brasilense* Cd to corn roots. However, both this study and Gafni et al. (1986) demonstrated that nutrients that are known to be taken up by *A. brasilense* Cd but which are not chemoattractants decrease adsorption. Therefore, it was concluded that the role of chemoattractants, especially when they are applied at high concentration and may saturate the chemoreceptors, is not simply that of a nutritional source after the bacterium cell reaches the targeted root. Probably, the change the attractants usually induce in the bacterium cell to start migration may play an additional role in the final colonization of the root.

Protease and EDTA treatments of *Azospirillum* cells are known to eliminate the aggregation characteristics of *Azospirillum* (L. Madi, M. Kessel, E. Sadovnik, and Y. Henis. 1986. Int. Symp. Microb. Ecol. 4th. p. 119. Abstr.) as well as to minimize active adsorption of *A. brasilense* Cd to sand particles by destruction of the protein bridges connecting the bacteria (Bashan and Levanony 1988*b*). Our present study demonstrates that in addition to these aspects, the fibrillar connections of *A. brasilense* Cd to wheat roots, previously described (Bashan et al. 1986), are also of proteinaceous

nature, originating mainly from the bacterial cell. In addition, the increase in adsorption as a result of cellulase-treated root hairs gives some support to the yet-unproven lectin binding theory proposed for this plant–bacteria interaction (Bashan and Levanony 1988*c*; Umali-Garcia et al. 1980).

A few studies have claimed that inoculation with *Azospirillum* yielded high frequencies of lysed and deformed root hairs of wheat (Patriquin et al. 1983) as well as penetration of *Azospirillum* through lysed pearl-millet root hairs (Umali-Garcia et al. 1980) or through unaffected rice root hairs (Lakshmi et al. 1977). Although carefully tested, this study neither supports nor denies this possibility, probably as a result of their very low frequency in our preparations. Nevertheless, recent study (Levanony et al. 1989) gave ultrastructural evidence that *A. brasilense* Cd heavily colonized cortex intercellular spaces in the absence of a significant number of root hairs.

In conclusion, this study suggests that of the total number of *A. brasilense* Cd cells adsorbed to the root after inoculation, most adsorbed to wheat root surface and few to the root hairs. Additionally, although the contribution of bacterial nitrogen fixation to plant growth is negligible (Kapulnik et al. 1985*a*; Bashan et al. 1989*b*), factors that affect this process in the bacteria also affect bacterial adsorption and colonization of roots.

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