Electron Microscopic Investigations on Root Colonization of *Lupinus albus* and *Pisum sativum* with Two Associative Plant Growth Promoting Rhizobacteria, *Pseudomonas fluorescens* and *Rhizobium leguminosarum* bv. *trifolii*

W. WIEHE[1], CH. HECHT-BUCHHOLZ[2] and G. HÖFLICH[1]

[1]*Zentrum für Agrarlandschafts- und Landnutzungsforschung, Institut für Ökophysiologie der Primärproduktion, Eberswalder Str. 84,*
D-15374 Müncheberg, Germany
[2]*Humboldt-Universität zu Berlin, Landwirtschaftlich-Gärtnerische Fakultät, Institut für Grundlagen der Pflanzenbauwissenschaften, Fachgebiet Pflanzenernährung, Lentzealle 55–57, D-14195 Berlin, Germany*
Tel. -49 33432 82268 Fax -49 33432 82212

Abstract

The two plant growth promoting rhizobacteria (PGPR), *Pseudomonas fluorescens* strain PsIA12 and *Rhizobium leguminosarum* bv. *trifolii* strain R39, stimulated the growth of *Lupinus albus* more than that of *Pisum sativum* in greenhouse experiments. Mean root colonization with strain PsIA12 was higher than with strain R 39 (log 7.2 versus log 6.3 cfu/cm$_{root}$). In monoxenic, nitrogen-free hydroponic culture the root colonization was up to ten-fold higher in lupin than in pea (mean values: log 7.1 versus log 6.5 cfu/cm$_{root}$). Surface sterilization indicated colonization of the interior root tissues of lupin. In agreement with these results electron microscopic observations showed only poor colonization of the pea rhizoplane by single bacterial cells. In contrast, the rhizoplane and the root tip mucigel of lupin was intensively colonized by both strains. We could observe strain PsIA12 in dense colonies in the intercellular spaces of the living cortex tissue.

1. Introduction

It is known that not only symbiotic bacteria, but also associative bacteria promote plant growth. While many symbiotic bacteria have a limited host range, the plant spectrum of associative bacteria is much wider. The mechanisms of plant growth stimulation by associative bacteria are mobilization of nutrients (Lifshitz et al., 1987; Zhoinska et al., 1992), stimulation of root growth by production of phytohormones (Müller et al., 1988; Bothe et al., 1992) or production of siderophores (Kloepper et al., 1980; Leong, 1986) and antibiotics (Haas et al., 1992). This enables associative bacteria to compete successfully with pathogens or other saprophytic bacteria (Weller, 1988).

The energetic basis for a "bacterial/plant growth community" is organic material secreted by the roots, especially mucigel, exudates and lysates (Foster et al., 1983; Curl and Truelove, 1986). But we assume that bacteria can only metabolize these substances in the rhizosphere in the case of extensive contact to the rhizoplane or other root tissues. Only under these conditions can the potential plant growth stimulating physiological characteristics be efficient.

The plant root – from the view of a bacterium – is not an uniform space, but is subdivided in many structurally and physiologically different compartments (Bolton et al. 1992). Bacteria are able to colonize in the intercellular spaces of the cortex or the xylem of the stele after overcoming the rhizodermis or the endodermis barrier (Kloepper et al., 1992).

The aim of our study is to analyse the root colonization of two associative plant growth promoting rhizosphere bacteria (PGPR), (1) *Pseudomonas fluorescens* strain PsIA12, (2) *Rhizobium leguminosarum* bv. *trifolii* strain R39 in white lupin and pea. The *Rhizobium* strain does not nodulate white lupin and pea, yet is able to promote the growth of non-host plants and to survive and establish in their rhizosphere. We therefore regard the *Rhizobium leguminosarum* bv. *trifolii* strain R39 as a PGPR according to Kloepper and Schroth (1978). The two strains are different in their physiological characteristics. They repeatedly stimulated the growth of cereals, legumes and other crops in greenhouse and field experiments (Höflich et al., 1992). Electron microscopic studies were used to analyse whether only the outer root surface was colonized or in addition, the inner root tissues. The results are discussed in relationship to physiological characteristics of plants and bacteria.

2. Materials and Methods
Plants and bacteria

Lupinus albus cv. Lublanc and *Pisum sativum* cv Grapis were used for

inoculation experiments with *Pseudomonas fluorescens* strain PsIA12 and *Rhizobium leguminosarum* bv. *trifolii* strain R39, in comparison to two symbiotic rhizobia strains, strain E164b in pea and strain lup84 in white lupin. Strain PsIA12 was isolated from wheat rhizosphere (Höflich, 1992), strain R39 from red clover nodules (Höflich and Weise, 1992). The symbiotic rhizobia strains were isolated from nodules of their host plants.

Greenhouse experiment

The influence of the selected bacteria strains PsIA12 and R39 was tested in a nonsterile greenhouse experiment on loamy sand with eight replicates (for soil parameters, see Höflich et al., 1992). The criteria for growth promotion were production of plant biomass, root length and number of laterial roots.

Sterile plant culture and inoculation with bacteria

Seeds were germinated on water agar at $25\,^{\circ}$C after surface sterilization with 0.1% HgCl-solution. The seedlings were placed in glass flasks containing 150 ml of nitrogen-free nutrient solution of the following composition (μmol·l^{-1}): 400 K_2SO_4, 390 $KH_{P2}PO_4$, 340 $K_2HPO_4·3H_2O$, 300 KCl, 490 $MgSO_4·7H_2O$, 3200 $CaCl_2$, 16 Chelaplex-Fe-III, 2.8 H_3BO_3, 2.8 $MnSo_4·H_2O$, 0.55 $ZnSO_4·7H_2O$, 0.17 $CuSO_4·5H_2O$, 0.18 $Na_2MoO_4·2H_2O$, 2.0 $CoCl_2·6H_2O$. The sterile nutrient solution was adjusted to pH 7.0. The plants in the glass flasks were covered with Erlenmeyer flasks to prevent microbial contamination. The following day, the plants were inoculated with a bacteria suspension of log 8 cfu per plant. They were cultivated for 1 week in a growth chamber under the following conditions: 16 hr at 22 kLux and $16\,^{\circ}$C, 8 hr at $12\,^{\circ}$C.

Bacterial root colonization

For determination of the bacterial colonization 1 cm root segments of root tip, root hair zone and branching zone were macerated in 1 ml of a 0.3% NaCl solution, plated out on glycerol-peptone-agar (Hirte, 1961) and evaluated for number of colony forming units (cfu) after incubation for 7 days at $28\,^{\circ}$C. Surface sterilized root segments (8% sodium hypochloride for 30 sec) were prepared in the same way. Gnotobiotic conditions were controlled by testing the nutrient solution of ten plants per treatment at the end of the experiments; sterile control plants showed no infection by unknown bacteria. In all cases only the introduced bacterial strains were observed on nutrient-agar.

Electron microscopic preparation

For electron microscopy segments of root tip, root hair zone and branching zone of two plants per treatment were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 at 4 °C overnight. After gentle washing with buffer solution, segments were postfixed with 1% OsO_4 for 2 hr, dehydrated with acetone and embedded in epoxy resin (Spurr, 1969). First five specimens per root zone and plant were sectioned semithin for light microscopy. From these, two well-prepared specimens were selected for ultrathin sectioning. The sections were stained with uranylacetate/lead citrate and observed with ZEISS transmission electron microscope EM 9.

3. Results

Growth stimulation

In contrast to noninoculated plants, the inoculated associative strains PsIA12 and R39 stimulated the growth of the taproot and the branching density of the root system (Table 1). Both strains also promoted the development of root and shoot biomass. But the promotion with white lupin was more pronounced than that with pea.

Table 1. Growth stimulation of pea and white lupin roots inoculated with associative rhizosphere bacteria compared with non inoculated control plants, greenhouse experiment on loamy sand, 8 replicates

Plant	Bacteria strain	Main root length[1]	Number of laterals[1]	Dry matter[2] Shoot	Root
Pea	Control	100	100	100	100
		(9.6)[3]	(14.4)[4]	(2.70)[5]	(2.71)[5]
	R 39	104	109	117*	114
	PsIA12	104	111*	113	114
LSD α=5%		15	11	16	16
White lupin	Control	100	100	100	100
		(8.4)[3]	(10.1)[4]	(1.98)[5]	(0.68)[5]
	R 39	105	240*	117	122
	PsIA12	148*	340*	131*	124*
LSD α=5%		25	50	27	24

[1] 2 weeks after inoculation

[2] 6 weeks after inoculation

[3] cm·plant^{-1}

[4] number·plant^{-1}

[5] g·pot^{-1}

* significant value ($\alpha = 5\%$)

Bacterial colonization

All investigated strains colonized white lupin roots more extensively than pea roots (Table 2). Colonization by strain PsIA12 overlayed that of the rhizobia strains. The degree of colonization was higher in old root segments than in young ones. Surface sterilized white lupin roots, in contrast to those of pea, showed a noteworthy colonization of the root interior. The data were analysed by means of a three-way-ANOVA. In unsterilized root segments, all main effects (bacteria, plants, root fractions) and all interaction effects were highly significant ($p < 0.01$). If roots were surface sterilized, the only significant effects (again $p < 0.01$) were those of plants and root fractions as well as their interactions.

Electron microscopic investigations
Strain PsIA12

PsIA12 cells were found in all segments of white lupin roots as colonies. There was no orientation of the bacterial cells in relation to the plant cell

Table 2. Colonization of pea and white lupin roots by associative rhizobacteria, 1 week after inoculation in monoxenic hydroponic culture, 6 replicates

Bacteria		Plant		Colonization (log cfu·cm root^{-1})		
				Root tip α	Zone of root hairs α	Zone of lateral roots β
PsIA12		Pea	A	5.8	5.6	6.9
	c	W. lupin	B	6.7	6.8	7.6
R39		Pea	A	4.9	4.8	6.8
	a	W. lupin	B	6.0	6.3	6.3
E164	b	Pea	A	4.8	4.8	6.6
lup84	b	W. lupin	B	6.0	5.8	6.7
Surface sterilized roots:				Root tip α	Zone of root hairs α	Zone of lateral roots β
PsIA12		Pea	A	< 2.0	< 2.0	4.5
	a	W. lupin	B	4.6	2.1	6.0
R39		Pea	A	< 2.0	< 2.0	4.6
	a	W. lupin	B	2.8	4.6	6.0
E164	a	Pea	A	< 2.0	< 2.0	5.8
lup84	a	W. lupin	B	3.5	4.4	6.0

The Student-Newman-Keuls test was used for differences between treatments. Significant differences ($p < 0.05$) are indicated by letters: a, b, and c for differences between bacteria; A and B between plants, α and β for differences between root fractions.

Table 3. Localization of inoculated, associative and symbiotic bacteria on pea and white
lupin roots, electron microscopic investigations

Bacteria	Root tip	Zone of root hairs	Zone of lateral roots
Pea:			
PsIA12	Single bacteria on mucigel	Single bacteria on rhizoplane	Single bacteria on rhizoplane, single bacteria in xylem vessels
R 39	No bacteria	Single bacteria attached to epidermal cell walls	No bacteria
E 164	No bacteria	Single bacteria attached to epidermal cell walls	Single bacteria attached to epidermal and cortical cell walls
White lupin:			
PsIA12	Colonies of bacteria on/in mucigel	Colonies of bacteria on rhizoplane, orientated to sloughed root cap cells	Colonies of bacteria on rhizoplane and in intercellular spaces of the vital cortex
R 39	Colonies of bacteria in mucigel	Single bacteria attached to epidermal cell walls	Colonies of bacteria on/in lysed cells
lup84	Single bacteria on mucigel	Single bacteria attached to epidermal cell walls	Colonies of bacteria, partly attached to epidermal cell walls

walls. PsIA12 was embedded in the mucigel matrix of the root tip. Around the
single bacteria we observed an electron-transparent halo of bacterial capsule
material or lysed plant mucigel (Fig. 1). In the root hair zone, bacteria were
found near lysed root cap cells, which were still adhering to the root epidermis.
The bacteria were distinctly oriented to the root cap cells and not to the living
epidermis (Fig. 2).

Some different habitats of PsIA12 were observed in the zone of branching.
The niches in or on lysed epidermal cells (Fig. 3) and the rupture between cor-
tex of the main root and the emerging lateral roots were colonized by PsIA12.
This strain also sporadically colonized (down to the 7th layer of the cortex)
intercellular spaces of the living and intact cortex. These colonies showed a
high density (Figs. 6 and 7). The bacteria were embedded in a fine fibrillious
matrix. It is assumed that this material was secreted by the plant because it
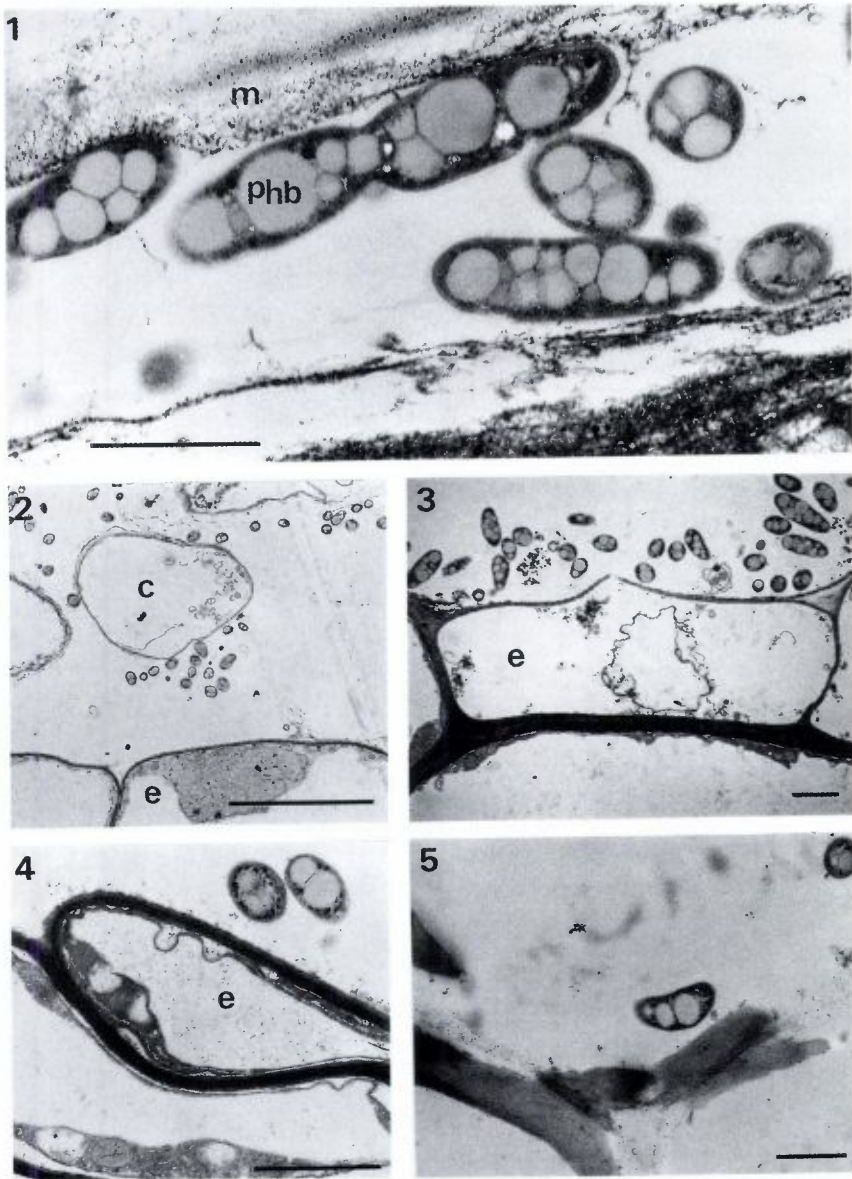was also found in intercellular spaces without bacterial colonization (Fig. 8).

Figure 1–3. Colonization of white lupin roots by *Pseudomonas fluorescens* PsIA12.
Fig. 1. Colony of PsIA12 embedded in the root tip mucigel (m), bacteria with large amounts of poly-β-hydroxybutyrate (PHB). bar = 1 μm.
Fig. 2. Colonies of PsIA12 around lysed root cap cells (c) in the root hair zone, (e) epidermal cells, bar = 10 μm.
Fig. 3. Colony of PsIA12 on a lysed epidermal cell (e) in the zone of branching, bar = 1 μm.
Figure 4–5. Colonization of pea roots with strain PsIA12, bar = 1 μm. Single cells of PsIA12 on the epidermis (e) (Fig. 4) and in a xylem vessel (Fig. 5) in the zone of branching.

The single bacterial cells in the intercellular space were surrounded by an electron-transparent zone, capsule material or lysed fibrillar material of the plant.

In contrast to white lupin, the colonization with inoculated bacteria on the rhizoplane of pea roots was very poor in all segments. Only single bacterial cells were observed (Fig. 4). In one case, single bacteria were recorded in xylem vessels (Fig. 5).

All bacteria cells showed numerous large, grey contrasted inclusions, presumably of poly-β-hydroxybutyrate (PHB), in their central regions. Dense, presumably polyphosphate granules were observed in their peripheral regions (Fig. 1).

Strain R39 was found as single cells, one or a few bacteria per epidermis cell, on the epidermis of the pea root hair zone. They were attached in a polar fashion to the plant cell wall.

R39 colonized the mucigel layer of the white lupin root tip very extensively. As described for PsIA12, the bacterial cells of R39 also were surrounded by an electron-transparent halo of capsular material or lysed mucigel (Fig. 9). In contrast to single cells attached to the root hair zone or on the outer layers of the mucigel, the cell walls of bacteria living in colonies were finely sculptured (Fig. 10).

The bacterial cells were attached to the plant cell walls on the epidermis of the root hair zone (Fig. 11). Electron-dense material was observed between cell wall and bacterial cell (Fig. 13). Bacterial division was not observed. In the zone of branching, the habitat of R39 was mainly the rupture between the cortex of the main root and the emerging lateral root. In this habitat, the intercellular spaces surrounding cortex and lysed epidermal cells were well accessible for the bacteria and densely colonized (Fig. 14). Single bacteria were attached to the cell walls of epidermal and cortex walls.

In contrast to the *Pseudomonas* strain, the rhizobia PHB inclusions could be differentiated into two shapes. (1) In solitary cells, the inclusions were completely electron-transparent except, for a sickle-shaped more electron-dense border. (2) Cells in colonies showed PHB inclusions which were contrasted uniformly grey. The explanation for this might be that the electron-transparent inclusions are not stained with heavy metals, whereas in the grey PHB inclusions, probably a protein component binds heavy metals from the staining solutions. The occurrence of differentially stained PHB inclusions was striking in the root tip (Figs. 10, 12). Polyphosphate granules were observed in all cells.
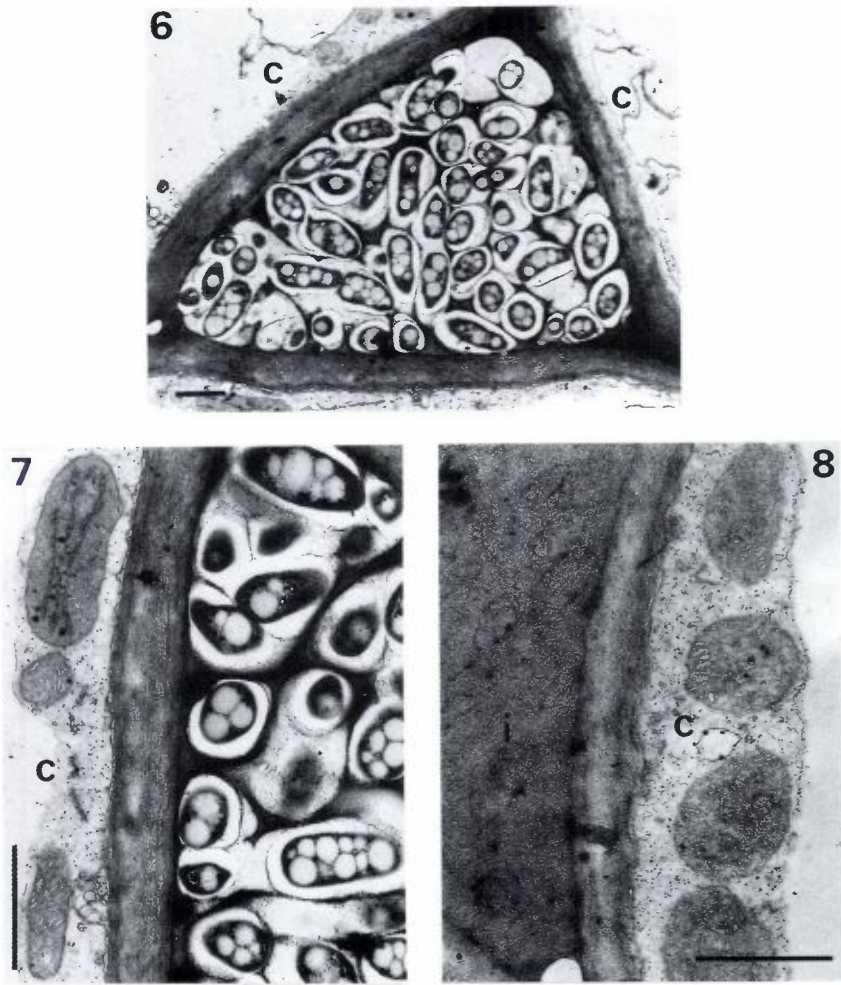
Figure 6–8. Intercellular spaces of the cortex in the zone of branching, white lupin, bar = 1 μm.

Fig. 6. Colonization of cortex intercellular spaces with *Pseudomonas fluorescens* PsIA12, cortex cells (c).

Fig. 7. Detail of Fig. 6. Note the intact cell plasma of the cortex cell with proplastid (above), mitochondria (m) (below) and vesicles indicating a high physiological activity.

Fig. 8. Living cortex cell (c) with mitochondria next to an intercellular space (i) filled with electron-dense fibrous material.
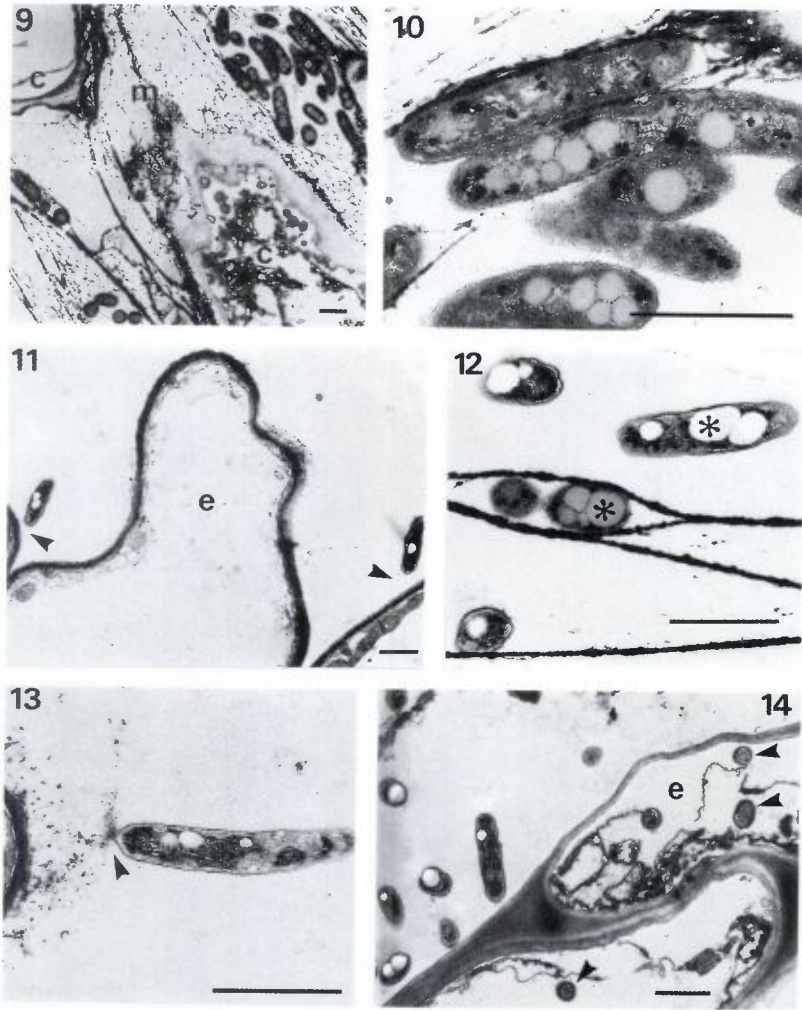
Figure 9–14. Colonization of white lupin roots with *Rhizobium leguminosarum* bv. *trifolii* R39, bar = 1 μm.

Fig. 9. Colonization of the root tip mucigel (m) between root cap cells (c).

Fig. 10. Detail of Fig. 9 with dividing bacterial cells.

Fig. 11. Two single rhizobia cells attached to the thin mucigel layer on the epidermis (e) of the root hair zone.

Fig. 12. Bacterial cells at the border of mucigel with differentially contrasted PHB inclusions (*).

Fig. 13. *Rhizobium* cell attached to the mucigel layer of the root hair zone epidermis.

Fig. 14. Strain R39 in the zone of branching, colonization of the rhizoplane and lysed epidermal cells (e).
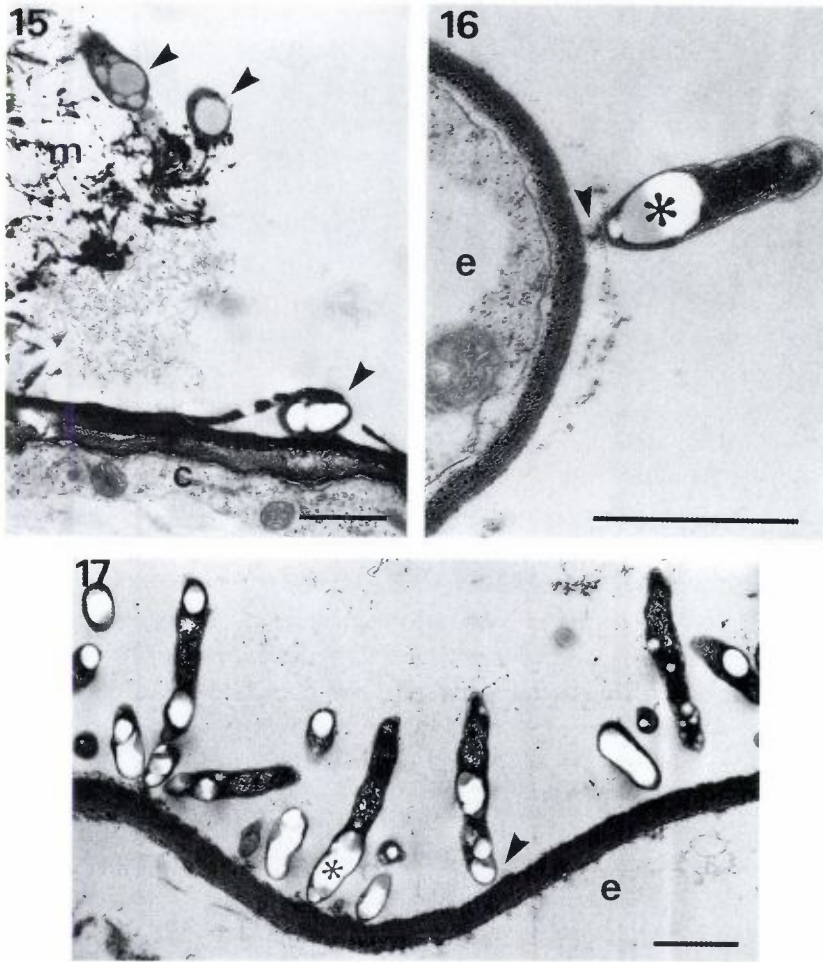
Figure 15–17. *Rhizobium* strain lup84 in the rhizosphere of white lupin, bar = 1 μm.
   Fig. 15. Single cells on the root tip mucigel (arrowhead).
   Fig. 16. Single cell of lup84 attached to an epidermal cell (e) in the zone of root hairs; note the large PHB inclusion (*).
   Fig. 17. Colonization with lup84 on a lysed epidermal cell in the zone of branching; note the attachment of the *Rhizobium* cells (arrowhead) and the large electron-transparent PHB inclusions (*).
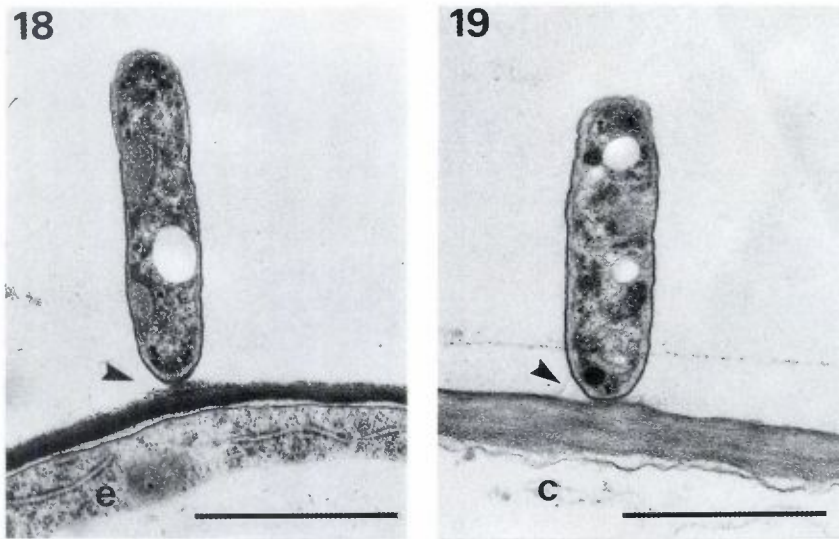
Figure 18–19. *Rhizobium* strain E164 in the rhizosphere of pea, bar = 1 μm. Single *Rhizobium* cell in the root hair zone attached to an epidermal cell (e) (Fig. 18) and an intercellular space attached to the cortex cell (c) wall, zone of branching (Fig. 19).

Symbiotic rhizobia strains E164 and lup84

Both strains were polar-attached on the epidermis of the root hair zone of pea and white lupin (Figs. 16, 18). No nodulation or infection stage exceeding the described attachment was observed 1 week after bacterial inoculation. The pea root tip was not colonized by strain E164, but white lupin root tips were colonized very sparsely by some single cells of lup84 (Fig. 15). In the lateral root zone of pea, some single cells of E164 were attached to cell walls of the epidermis or lysed cortex cells in the rhizobia-typical polar orientation (Fig. 19). In the same root segment of white lupin, we could observe numerous attached bacteria oriented like a "lawn" (Fig. 17). PHB inclusions of strain E164 and lup84 showed the electron-transparent form.

4. Discussion

The present study shows that the inoculated plant growth-promoting rhizosphere bacteria, *Pseudomonas fluorescens* PsIA12 and *Rhizobium leguminosarum* bv. *trifolii* R39, colonized white lupin roots more intensively than

pea roots under monoxenic conditions. First results from non sterile green-house and field experiments with strain PsIA2 and partly with strain R39 on white lupin and pea confirm this (Wiehe, Höflich and Schloter, not published).

The plant/bacteria-culture was prepared so, that the only source for carbon and nitrogen were root depositions, simulating a nitrogen limitation of the rhizosphere (Breland and Bakken, 1991, Liljroth et al., 1990). It is assumed that the differences in bacterial colonization capacity of white lupin and pea are due to differences in rhizodeposition. Our direct observations of colony formation and bacterial PHB inclusions on white lupin and pea roots and different habitats on/in root, lead to this conclusion.

However, the associative strains PsIA12 and R39 do not colonize the rhizo-plane, as the symbiotic rhizobia strains E164 and lup84 do, but also mucigel-filled intercellular spaces of the root cap of white lupin. The root tip is generally sparsely colonized by microorganisms in contrast to older root zones. One ex-planation for that might be that the dominant constituents of the mucigel are pectins and hemicelluloses and only 0.1% of rhizosphere bacteria are able to metabolize cellulose and only 2% of them pectin in contrast to the metaboliz-ing capacity of fungi or bacteria of root-free soil (Newman, 1985). First results showed that strain PsIA12, less so strain R39 are able to metabolize pectin and carboxymethylcellulose (not published). Another reason for the sparse colonization of root tips is the fast growth of this zone. Microorganisms which are able to colonize this zone are often diazotrophs (Jagnow et al., 1991). This may be related to N-limitation in this root zone.

In addition, a high percentage of pathogenic fungi and bacteria may be able to penetrate into the root interior via the tip. If this root zone is colonized by beneficial bacteria with high plant affinity, as the two described strains, pathogens may not be able to penetrate this ecological niche (Kloepper et al., 1989). The ability of PGPR to produce siderophores or antibiotics plays a prominent role in pathogen defense (Weller, 1988). In contrast to R39, PsIA12 is able to reduce growth of pathogenic fungi *in vitro* (Höflich, 1992). We as-sume that a siderophore of the pseudobactin/pyoverdine-group produced by this strain *in vitro* is responsible for this (not published). The intensive colo-nization of the root tip may be a potential source of further colonization of the older root zones, which is a consequence of the type of root growth and the passive transport of bacteria by sloughed root cap cells. The C:N ratio and the wide chemical spectrum of lysis products from sloughed root cap cells are favourable for bacterial growth (Griffin et al., 1976). Strain PsIA12 colonized sloughed root cells more than the living epidermis cells, in contrast to strain R39, which attached itself in polar orientation to the epidermis cells. The lat-ter is a noteworthy, but nonspecific early step of the nodulation process (Smith

and Wollum, 1993; Dart and Mercer, 1964). *Rhizobium* cells were always found in this zone in only a few individuals per epidermal cell. The rhizoplane in the zone of branching is colonized by both strains. PsIA12 colonization is also described for other rhizosphere bacteria, often diazotrophs such as *Azospirillum* (Umali-Garcia et al., 1978; Bashan and Levanony, 1988), *Pantoea* (Ruppel et al., 1993) and some strains of *Pseudomonas* (Aström et al., 1993) in association with cereals. However, it is not always clearly described, whether the cortex is still alive or already lysed. The colonization of the living cortex is an active process. Preconditions are: pectinolytic activity as described for *Azospirillum* to dissolve the middle lamella of the plant cell walls (Umali-Garcia et al., 1978), as well as the overcoming of chemical defenses of the plant. It is assumed that the observed electron-dense matrix in the intercellular cortex space of white lupine is secreted by the plant to enclose the bacterial invader. Bashan and Levanony (1988), Ruppel et al. (1992) described similar electron-dense materials between cortex cell wall and bacteria. Electron microscopic pictures of Foster et al. (1983) showed a very dense colonization of the cortex interspace, similar to our observations, but with bacterial lysis, interpreted as a strong host reaction against pathogenic bacteria. We can not decide now, whether colonization of interspaces of the living cortex by the strain PsIA12 is a factor of plant growth promotion or rather a deleterious effect.

Numerous bacteria of strain PsIA12 and strain R39 adhered or attached to cell walls near the rupture in the cortex when lateral roots emerged. This is in agreement with Reinhold and Hurek (1989), who described this for *Azospirillum* and Kallar grass. The rupture is possibly a way for bacteria to colonize the endorhizosphere and the shoot via xylem migration (Charlton, 1991; Kloepper et al., 1992).

One hypothesis for plant promotion by introduced bacteria is the presence of phytohormones produced by bacteria, predominantly auxin and cytokinin (Müller et al., 1988; Bothe et al., 1992). These external hormones may influence the growth and the branching intensity of the root. Our observations showed that the introduced auxin and cytokinin producing stains R39 and PsIA12 settled near by the growing apex of the root and intensively colonized the zone of branching (Höflich et al., 1992). The potential external phytohormone gradient may be responsible for the observed root promotion of pea and white lupin. Whether the higher growth stimulation of white lupin compared to pea is directly correlated with a higher root colonization, will be the subject of further research.

Acknowledgements

REFERENCES

Aström, B., Gustafsson, A., and Gerhardson, B. 1993. Characteristics of a plant deleterious rhizosphere pseudomonad and its inhibitory metabolite(s). *J. Appl. Bacteriol.* **74**: 20–28.

Bashan, Y. and Levanony, H. 1988. Migration, colonization and adsorption of *Azospirillum brasiliense* to wheat roots, *Lectins-Bio. Biochem. Clinical Biochem.* **6**: 69–83.

Bolton, H., Fredrickson, J.K., and Elliott, L.F. 1992. Microbial ecology of the rhizosphere. In: *Soil Microbial Ecology.* F.B. Metting, ed. Dekker, New York, pp. 27–63.

Bothe, H., Körsgen, H., Lehmacher, T., and Hundeshagen, 1992. Differential effects of *Azospirillum*, auxin and combined nitrogen on growth of the roots of wheat. *Symbiosis* **13**: 167–179.

Breland, T.A. and Bakken, L.R. 1991. Microbial growth and nitrogen immobolization in the root zone of barley (*Hordeum vulgare* L.), Italian ryegrass (*Lolium multiflorum* Lam.), and white clover (*Trifolium repens* L.). *Biol. Fertil. Soils* **12**: 154–160.

Charlton, W.A. 1991. Lateral root initiation. In: *Plant Roots, the Hidden Half.* Y. Waisel, A. Eshel and U. Kafkafi, eds. Dekker, New York, pp. 103–128.

Curl, E.A. and Truelove, B. 1986. *The Rhizosphere.* Springer-Verlag, Berlin.

Dart, P.J. and Mercer, F.V. 1964. The legume rhizosphere. *Arch. Mikrobiol.* **47**: 344–378.

Foster, R.C., Rovira, A.D., and Cock, T.W. 1983. *Ultrastructure of the Root-Soil Interface.* The Am. Phytopathol. Soc., St. Paul, MI.

Griffin, G.J., Hale, M.G., and Shay, F.J. 1976. Nature and quantity of sloughed organic matter produced by roots of axenic peanut plants. *Soil Biol. Biochem.* **8**: 29–32.

Haas, D., Keel, C., Laville, J., Maurhofer, M., Oberhansli, T., Schnider, U., Voisard, C., Wuthrich, B., and Defago, G. 1992. Secondary metabolites of *Pseudomonas fluorescens* strain Cha0 involved in the suppression of root diseases. *Advances in Molecular Genetics of Plant-Microbe Interactions, Vol. 1, (Ser.: Curr. Plant Sc. Biotechnol. Agric.* **10**): 450–456.

Hirte, W.F. 1961. Glycerin-Pepton-Agar, ein vorteilhafter Nährboden für bodenbakteriologische Arbeiten. *Zbl. Bakt. Abt. II* **114**: 141–146.

Höflich, G. 1992. Interrelationships between phytoeffective *Pseudomonas* bacteria and the growth of crops. *Zbl. Mikrobiol.* **147**: 182–191.

Höflich, G. and Weise, I. 1989. Improvement of efficiency of a *Rhizobium* inoculation on pea through a combined inoculation of *Rhizobium leguminosarum* biovar. *viceae* with *R. leguminosarum* biovar. *trifolii*. *Zlb. Mikrobiol.* **147**: 378–387.

Höflich, G., Glante, F., Liste, H.-H., Weise, I., Ruppel, S., and Scholz-Seidel, C. 1992. Phytoeffective combination effects of symbiotic and associative microorganisms on legumes. *Symbiosis* **14**: 427–438.

Höflich, G., Glante, F., Kühn, G., and Hickisch, B. 1993. Phytoeffective symbiosis in pea. *Zbl. Mikrobiol.* **148**: 48–54.

Jagnow, G., Höflich, G., and Hoffmann, K.-H. 1991. Inoculation of non-symbiotic rhizosphere rhizosphere bacteria: possibilities of increasing and stabilizing yields. *Angew. Botanik* **65**: 97–126.

Kloepper, J.W. and Schroth, M.N. 1978. Plant growth promoting rhizobacteria on radishes. *Proc. 4th Int. Conf. Plant Path. Bact. Angers, France*, pp. 879–882.

Kloepper, J.W., Leong, J., Teintze, M., and Schroth, M.N. 1980. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature* **286**: 885–886.

Kloepper, J.W., Lifshitz, R., and Zablotowicz, R.M. 1989. Free-living bacterial inocula for enhancing productivity. *Tibtech* **7**: 39–44.

Kloepper, J.W., Schippers, B., and Bakker, P.A.H.M. 1992. Proposed elimination of the term endorhizosphere. *Phytopathology* **82**: 726–727.

Leong, J. 1986. Siderophores: their biochemistry and possible role in the biocontrol of plant pathogens. *Ann. Rev. Phytopathol.* **24**: 187–209.

Lifshitz, R., Kloepper, J.W., Kozlowski, M., Simonson, C., Carlson, J., Tipping, E.M., and Zaleska, I. 1987. Growth promotion of canola (rapeseed) seedlings by the strain *Pseudomonas putida* under gnotobiotic conditions. *Can. J. Microbiol.* **33**: 390–395.

Liljroth, E., Van Veen, J.A., and Miller, H.J. 1990. Assimilate translocation to the rhizosphere of two wheat lines and subsequent utilization by rhizosphere microorganisms of two soil nitrogen concentrations. *Soil Biol. Biochem.* **22**: 1015–1021.

Müller, M., Deigele, C., and Ziegler, H. 1988. Hormonal interactions in the rhizosphere of maiz (*Zea mays* L.) and their effects on plant development. *Z. Pflanzenernähr. Bodenk.* **152**: 247–254.

Newman, E.I. 1985. The rhizosphere: carbon sources and microbial population. In: *Ecological Interactions in Soil, Plants, Microbes and Animals.* A.H. Fitter, ed. Blackwell Scientific, Oxford, pp. 107–121.

Reinhold, B. and Hurek, T. 1989. Location of diazotrophs in the root interior with special attention to kallar grass association. In: *Nitrogen Fixation with Non-Legumes.* F.A. Skinner, ed. Kluwer Academic, Dordrecht, pp. 209–218.

Ruppel, S., Hecht-Buchholz, Ch., Remus, R., Ortmann, U., and Schmelzer, R. 1992. Settlement of the diazotrophic, phytoeffective bacterial strain *Pantoea agglomerans* on and within winter wheat: an investigation using ELISA and transmission electron microscopy. *Plant Soil* **145**: 261–273.

Smith, G.B. and Wollum, A.G. 1993. Physiochemical and d-galactose-mediated interactions in the attachment of *Bradyrhizobium japonicum* to roots of *Glycine max. Can. J. Microbiol.* **39**: 245–251.

Spurr, A.R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultastr. Res.* **26:** 31–43.

Umali-Garcia, M., Hubbell, D.H., and Gaskins, M.H. 1978. Process of infection of *Panicum maximum* by *Spirillum lipoferum. Ecol. Bull. (Stockholm)* **26:** 379–407.

Zboinska, E., Lejczak, B., and Kafarski, P. 1992. Organophosphate utilization by wild-type strain of *Pseudomonas fluorescens. Appl. Environ. Microbiol.* **58:** 2993–2999.