

Changes in membrane potential of intact soybean root elongation zone cells induced by *Azospirillum brasilense*¹

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Inoculation of soybean seedlings with *Azospirillum brasilense* significantly reduced the membrane potential of root cells and made it less negative. The effect was most pronounced in the cells of the elongation zone. Three *A. brasilense* strains reduced the membrane potential of elongation zone cells to a similar extent. Dead cells or cells of an associative nonbeneficial *Pseudomonas* species caused no effect. Reduction of membrane potential of root cells was directly related to the physiological status of the bacterial cells. Active bacteria reduced the membrane potential. Stressed bacteria, i.e., bacteria exposed to either starvation, anaerobic conditions, or a high level of streptomycin prior to inoculation, failed to affect membrane potential. Continuous perfusion of the roots immediately after inoculation, while maintaining the bacterial cells surrounding the roots, cancelled the effect of live bacteria on membrane potential. Regardless of bacterial cell treatment or effect on membrane potential of root cells, root colonization levels of all *A. brasilense* strains were similar throughout all experiments. In addition, all strains positively affect the growth of soybean seedlings. It is suggested that inoculation of soybean plants with live *A. brasilense* strains, possessing an active metabolism, reduced the membrane potential of root cells probably through the release of an, as yet, unidentified bacterial signal(s).

Key words: *Azospirillum*, beneficial bacteria, *Glycine max*, membrane potential, plant–bacteria interaction, rhizosphere bacteria, soybean.

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L'inoculation de jeunes plants de soja avec *Azospirillum brasiliense* réduit significativement le potentiel membranaire des cellules des racines en diminuant la charge négative. Cet effet est le plus marqué au niveau des cellules de la zone d'élongation. Trois souches d'*A. brasiliense* réduisaient à un niveau similaire le potentiel membranaire des cellules de la zone d'élongation. Des cellules mortes ou des cellules de *Pseudomonas* sp. d'association non bénéfique ne causaient aucun effet. La réduction du potentiel membranaire des cellules des racines était directement reliée à l'état physiologique des cellules bactériennes. Les bactéries actives réduisaient le potentiel membranaire. À l'opposé ce potentiel n'était pas affecté par des bactéries stressées, c.-à-d., privées de nourriture, ou placées en anaérobiose ou en présence d'une forte concentration de streptomycine avant leur inoculation. La perfusion en continu des cellules des racines immédiatement après l'inoculation contribue à maintenir les bactéries autour des racines, mais malgré tout elle annule l'effet des bactéries vivantes sur le potentiel membranaire. Dans tous nos essais expérimentaux, le niveau de colonisation de toutes les souches d'*A. brasiliense* est demeuré inchangé indépendamment du traitement infligé aux cellules bactériennes ou sur la différence de potentiel membranaire des cellules des racines. Toutes les souches ont favorisé la croissance des plants de soja. Nos observations nous permettent de croire que l'inoculation des plants de soja avec des souches vivantes d'*A. brasiliense* qui ont un métabolisme actif peuvent réduire le potentiel membranaire des cellules des racines probablement par l'intermédiaire d'un ou de signaux bactériens non identifiés.

Mots clés : *Azospirillum*, bactéries utiles, *Glycine max*, potentiel membranaire, soja, bactéries de la rhizosphère.

[Traduit par la rédaction]

Introduction

Strains belonging to the genus *Azospirillum* are known as beneficial bacteria, which can positively affect plant growth and productivity (Bashan and Levanony 1990). The mode of action of *Azospirillum* on plants is unclear. Several mechanisms have been proposed; i.e., nitrogen fixation (Rennie and Thomas 1987), hormonal effects (Kucey 1988), enhanced mineral uptake (Bashan et al. 1990; Murty and Ladha 1988), bacterial nitrate reductase (Ferreira et al. 1987), and an accumulative effect

comprising several small-magnitude mechanisms operating simultaneously or in succession (Bashan and Levanony 1990).

Proton efflux through the membranes of root cells is known as a major mechanism affecting many physiological properties of living root cells (Spanswick 1981). Inoculation of wheat plants with *A. brasilense* Cd significantly enhanced proton extrusion from the roots (Bashan et al. 1989a), possibly through the release of one or more bacterial signal(s) affecting root cell membranes (Bashan 1990).

The objectives of this study were to (i) measure the effect of *A. brasilense* inoculation on the electrical membrane potential (E_m) of root cells, (ii) locate the root site producing the major membrane activity, (iii) determine whether live *Azospirillum*

¹A study dedicated to the memory of the late Mr. Avner Bashan.

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cells are necessary to affect root cell membranes, and (iv) determine whether different strains of *A. brasilense* share this ability.

Materials and methods

Bacterial growth conditions

Azospirillum brasilense Cd (ATCC 29710), *A. brasilense* Sp-245 (Baldani et al. 1986), and *A. brasilense* Somali-67 (Favilli et al. 1988) were grown in nutrient broth (Difco) in Erlenmeyer flasks (equipped with shallow grooves), agitated on a rotary shaker (250 rpm, 14–16 h, $30 \pm 2^\circ\text{C}$), and harvested in the exponential phase of growth. This procedure improved aeration of the culture and eliminated aggregation, thus producing a population of single cells in a relatively short growth period. Root-associated nonbeneficial bacteria, *Pseudomonas* sp. (OH 88004) (Bashan et al. 1989a) were cultured on nutrient broth supplemented with streptomycin sulphate (200 mg mL^{-1}). Bacteria were prepared for plant inoculation as previously described (Bashan 1986; Bashan and Levanony 1985).

Plant growth conditions

Soybean seeds (*Glycine max* (L.) Merr., cv. Pella) were surface disinfected with 3% NaOCl for 5 min and then thoroughly washed with deionized water. Seeds were transferred to 0.6% water-agar in flat trays and germinated at $25 \pm 2^\circ\text{C}$ in the dark for 48 h. Then, seedlings were inoculated and transferred into plastic and paper growth pouches (five seedlings/pouch) (Northrup King Seed Co., Minneapolis, MN) previously sterilized with ethylene oxide and wetted with 12 mL nutrient solution consisting of (mM) K_2SO_4 , 0.75; MgSO_4 , 0.65; KH_2PO_4 , 0.1; CaSO_4 , 0.5; H_3BO_3 , 1×10^{-2} ; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 1×10^{-4} ; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5×10^{-3} ; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.5×10^{-3} ; and $\text{H}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$, 0.5×10^{-6} (Marschner et al. 1982). The pH of the nutrient solution was adjusted to 5.8 with NaOH. Then, the seedlings were transferred to a fully controlled growth chamber (Conviron, model EF7H, Controlled Environments Co., Canada) at $25 \pm 1^\circ\text{C}$ and a photoperiod of 14 h light ($160 \mu\text{E m}^{-2} \text{ s}^{-1}$) and 10 h darkness. Seedlings were moistened with distilled water when required. Controls included seedlings dipped into 0.01 M potassium phosphate buffer supplemented with 0.15 M NaCl (PBS).

Bacterial inoculation and continuous perfusion of inoculated roots

Soybean seedlings were inoculated by dipping the radicle of each seedling into a freshly prepared suspension of *A. brasilense* or *Pseudomonas* sp. (10^6 colony-forming units (cfu) per millilitre) for 90–120 min (this bacterial concentration and incubation time are known to be optimal for causing marked effects on root development and bacterial adsorption to root surfaces (Bashan 1986; Bashan and Levanony 1989a)). Then the roots were perfused. Inoculation during perfusion was carried out similarly; however, the bacteria were suspended in $1 \times$ medium (described later), and perfusion of the roots was performed immediately after inoculation (<1 min). Continuous perfusion of inoculated roots was accomplished as previously described (Bashan 1990).

Bacterial cell treatments

Dead bacteria were obtained after autoclaving 10^8 cfu mL^{-1} for 20 min. Starved bacteria were obtained by incubating washed *A. brasilense* Cd cells for 10 h in PBS. Heat treatment was accomplished by transferring a bacterial culture grown at $30 \pm 2^\circ\text{C}$ to $45 \pm 2^\circ\text{C}$ for 6 h. Anaerobic stress was done by transferring aerobic cultures into jars containing helium, for 6 h. Antibiotic stress was accomplished by inserting filter-sterilized ($0.45 \mu\text{m}$) streptomycin sulphate at 500 mg L^{-1} (final concentration) into bacterial cultures, followed by incubation for 6 h. The streptomycin sulphate was washed from the bacterial cells by five successive washings with PBS ($7000 \times \text{g}$, 10 min each time) before inoculation.

Bacterial counts on roots

Root colonization measurements by *A. brasilense* strains was performed by the improved selection technique (Bashan and Levanony

1985). *Pseudomonas* sp. was counted by the dilution plate count method after homogenization of roots on nutrient agar medium, supplemented with 200 mg mL^{-1} streptomycin sulphate.

Electrophysiological measurements

Intact seedlings having a single root (63 h old) were taken from the pouch and mounted horizontally on a Plexiglas holder and washed for 3 h in an aerated solution ($1 \times$) composed of 1 mM KCl, 1 mM $\text{Ca}(\text{NO}_3)_2$, 0.25 mM MgSO_4 , and 66 mM NaH_2PO_4 , final pH 5.7 (Higinbotham et al. 1964, as modified by Érsek et al. 1986). This rinsing was essential to equilibrate the cells in the perfusion medium.

Microcapillaries with glass microfibers (WP Instruments) were pulled to microelectrodes using a horizontal electrode puller. Microelectrodes having a tip diameter of $0.6 \pm 0.2 \mu\text{m}$, tip potential of -2 to -16 mV , and tip resistance of $5\text{--}14 \text{ M}\Omega$ were used. Microelectrodes were filled with 3 M KCl, eliminating air bubbles trapped inside the microelectrode. Each microelectrode was microscopically tested for tip perfection. A reference salt bridge, a piece of tubing 4 cm long and 2 mm inner diameter, was filled with 3 M KCl in 2% agar. Both salt bridges were connected through Ag–AgCl wire with an electrometer amplifier and a chart recorder. Microelectrodes were inserted with a micromanipulator into the vacuole of a single cell at the selected root site, continuously observed with a horizontally mounted stereoscopic microscope, and illuminated with fiber optics. The reference electrode was placed in the bathing solution. The Plexiglas chamber containing the seedling and the holder (total volume of 7 mL) was perfused with $1 \times$ medium at a flow rate of 8 mL min^{-1} (Érsek et al. 1986). E_m was defined as a potential difference between the bathing solution and the cell interior. Reduction of E_m referred to E_m values that are less negative (the interior of a plant cell is negative relative to the outside and their E_m is minus voltage). After the E_m measurements, seedlings were returned to the growth pouches in the growth chamber for an additional 8 to 10 days. Then, the dry weights of the roots and shoots were determined.

Dry weight measurements

Dry weight was determined immediately after drying the roots and the shoots in a forced-air oven at 50°C for 48 h.

Experimental design and statistical analysis

Each experiment was repeated twice, each in a completely randomized design with five replicates. A replicate consisted of two seedlings. Significant differences among treatment means were determined at $P \leq 0.05$ according to either Fisher's least significant difference (LSD) or Student's *t*-test (comparison between different root parts). The number of bacteria was analyzed by standard deviation. Bars in the graph represent standard error.

Results

Effect of inoculation on the membrane potential of cells in different root parts

Noninoculated soybean root parts exhibited similar E_m values, regardless of the root parts and within the range of $-146 \pm 6 \text{ mV}$ (Table 1). Inoculation of roots with *A. brasilense* Cd significantly reduced the E_m in every root part (compare each inoculated with noninoculated root part). The differences in E_m reduction significantly varied between root parts, being maximal in the root elongation zone (Table 1). Variation in root colonization levels among the root parts was minimal (Table 1).

Effect of inoculation with different *A. brasilense* strains and *Pseudomonas* sp. on the membrane potential of cells in the root elongation zone

Inoculation of soybean roots with three strains of *A. brasilense* significantly reduced the E_m of cells in the root elongation zone compared with noninoculated plants (Table 2). Inoculation with *Pseudomonas* sp. had no effect on E_m of these cells.

TABLE 1. Membrane potential differences (E_m) between interior of soybean cells and solution bathing the roots at different sites of the root 16 h after inoculation with *A. brasilense* Cd

Root part*	E_m (mV)†		No. of <i>A. brasilense</i> Cd (cfu/cm root \pm SD)‡
	Noninoculated	Inoculated	
Root tip§	-150aA	-141aB	$(3.2 \pm 0.66) \times 10^5$ ¶
Elongation zone**	-144aA	-109bB	$(4.4 \pm 1.33) \times 10^5$
Root hair zone	-147aA	-132cB	$(4.6 \pm 1.56) \times 10^4$

NOTE: Numbers in body of table followed by different lower case letters differ significantly at $P \leq 0.05$ according to LSD analysis, whereas numbers, for each root part separately, followed by different capital letters, differ significantly at $P \leq 0.05$ according to *t*-test analysis.

*By the technique described by Levanony and Bashan (1989).

†Average of two experiments each in five replicates; a total of 20 different measurements.

‡Obtained from samples taken from the same seedlings after E_m measurements and counted according to Bashan and Levanony (1985).

§Including the meristematic zone.

¶Values calculated from root segment containing only the root tip.

**Including the differentiation zone until first appearance of root hairs.

TABLE 2. Membrane potential differences (E_m) between interior of soybean root elongation zone cells and solution bathing the roots 16 h after inoculation with several *A. brasilense* strains and *Pseudomonas* sp.

	E_m (mV)*	No. of bacteria (cfu/g root fresh weight \pm SD)†
<i>A. brasilense</i>		
Cd	-110c	$(1.7 \pm 0.66) \times 10^6$
Sp-245	-118c	$(5.7 \pm 0.89) \times 10^5$
Somali-67	-127b	$(2.9 \pm 1.11) \times 10^5$
<i>Pseudomonas</i> sp. OH 88004	-144a	$(9.6 \pm 0.89) \times 10^7$
Noninoculated plants	-146a	None‡

NOTE: Numbers in body of table followed by a different letter differ significantly at $P \leq 0.05$ according to LSD analysis.

*Average of two experiments each in five replicates; a total of 20 different measurements.

†Bacteria were counted on elongation zone segments obtained from the same seedlings after E_m measurements according to Bashan and Levanony (1985).

‡Tested only for the above isolates.

Colonization ability of the root elongation zone slightly varied between *A. brasilense* strains. However, colonization by *Pseudomonas* sp. was significantly higher compared with *A. brasilense* strains (Table 2).

Effect of inoculation with treated *A. brasilense* Cd cells on membrane potential of cells in the root elongation zone

In general, dead *A. brasilense* Cd cells or cells stressed by either starvation or exposure to anaerobic conditions or to a high level of streptomycin sulphate prior to inoculation exhibited an inhibited effect on the E_m of the root cells. Heat-stressed bacteria were able to decrease the E_m of the elongation zone cells, but to a significantly lower degree compared with unstressed *A. brasilense* Cd cells (Table 3). These stresses had no apparent effect on root colonization of *A. brasilense* Cd (Table 3).

Response of soybean seedlings to inoculation with various *A. brasilense* strains and *Pseudomonas* sp. with and without stress to the bacterial cells prior to inoculation

Soybean plants obtained from E_m measurement experiments described in Tables 2 and 3 were grown further and the dry weight of roots and shoots was determined. Inoculation with three live nonstressed *A. brasilense* strains resulted in a significant increase in root dry weight over noninoculated plants, and two strains also significantly increased shoot dry weight

(Table 4). Inoculation with *Pseudomonas* sp. caused no increased growth in soybean plants. Stressed bacteria improved soybean growth to an extent similar to nonstressed bacteria, whereas streptomycin-treated cells or dead cells caused no apparent effect on soybean growth (Table 4).

Effect of continuous perfusion of *A. brasilense* Cd inoculated roots on the membrane potential of cells in the root elongation zone

Continuous perfusion of the root, immediately after inoculation, eliminated E_m reduction in the elongation zone cells. The measured E_m resembled the E_m of noninoculated plants (Fig. 1). However, the number of *A. brasilense* Cd cells colonizing the roots under permanent perfusion was similar to the colonization level of roots that were perfused only after initial adsorption of *A. brasilense* Cd (compare Fig. 1, Δ data, with Table 2).

Discussion

The *Azospirillum* mode of promoting plant growth has been under continuous debate for over a decade. One of the mechanisms suggested thus far (Murty and Ladha 1988), but not unequivocally confirmed (Bashan et al. 1990), is the enhancement of the mineral uptake of plants induced by the inoculated bacteria. Changes in mineral uptake may be directly linked to root membrane activities and to their direct consequence, proton

TABLE 3. Membrane potential differences (E_m) between interior of soybean root elongation zone cells and solution bathing the roots 16 h after inoculation with treated *A. brasilense* Cd cells

No. of inoculated bacteria (cfu/mL)	Stress	E_m (mV)*	No. of <i>A. brasilense</i> Cd (cfu/g root fresh weight \pm SD)†
None	None	-147a	None‡
1×10^6	None	-111c	$(5.4 \pm 0.46) \times 10^5$
1×10^6	Starved	-148a	$(4.6 \pm 0.89) \times 10^5$
1×10^6	45°C	-135b	$(5.6 \pm 0.66) \times 10^5$
1×10^6	Dead	-145a	None‡
1×10^6	Anaerobic	-138a	$(5.1 \pm 0.89) \times 10^5$
1×10^6	Streptomycin	-141a	$(4.7 \pm 0.66) \times 10^4$

NOTE: Numbers in body of table followed by a different letter differ significantly at $P \leq 0.05$ according to LSD analysis.

*Average of two experiments each in five replicates; a total of 20 different measurements.

†Bacteria were counted on elongation zone segments obtained from samples taken from the same seedlings after E_m measurements according to Bashan and Levanony (1985).

‡Tested only for *A. brasilense* Cd.

TABLE 4. Growth responses of soybean (cv. Pella) seedlings to inoculation with various *A. brasilense* strains and *Pseudomonas* with and without stress to bacterial cells prior to inoculation

	Stress	Dry weight (mg/plant)	
		Root	Shoot
<i>A. brasilense</i> *			
Cd	None	3360a	1450a
Sp-245	None	3390a	1490a
Somali-67	None	3010a	1310b
<i>Pseudomonas</i> sp. OH 88004*			
<i>A. brasilense</i> Cd†	Starved	3280a	1430a
	Anaerobic	3330a	1410a
	45°C	3170a	1430a
	Streptomycin	2820b	1230b
	Dead	2680b	1190b
Noninoculated plants	None	2670b	1210b

NOTE: Numbers in body of table, in each column separately, followed by a different letter differ significantly at $P \leq 0.05$ according to LSD analysis.

*Plants obtained from E_m experiments described in Table 2.

†Plants obtained from E_m experiments described in Table 3.

efflux from the roots. Significant changes in proton efflux activity of inoculated roots were recently demonstrated (Bashan 1990; Bashan et al. 1989a). This study was aimed to add evidence to the previous studies and to show that concomitantly with proton efflux changes induced in the root by *Azospirillum* inoculation, membrane potential is also changed. This was accomplished by using an electrophysiological technique. Changes in E_m are a very sensitive indicator of the root cell membrane's status and can be executed with negligible interruption in the essential cellular functions (Novacky et al. 1976, 1978). This technique allows examination of cell membranes *in situ* (Novacky 1983; Pavlovkin et al. 1986) and the seedlings can later continue growing for further examination of plant responses towards inoculation at more advanced stages of growth.

Cells within the root cortex are electrically coupled by plasmodesmata (Spanswick 1972). Therefore, the microsites of the microelectrode measurements are not important. Each cortical cell may be directly affected by the inoculated bacteria or may get the signal from nearby affected cells (Érsek et al. 1986). However, the electrophysiological technique employed

in this study cannot differentiate between the E_m 's of the plasmalemma and the tonoplast (Findlay and Hope 1976; Higinbotham 1973). Furthermore, Bertl and Felle (1985) using a pH microelectrode suggested that the microelectrode tip is located within the cytoplasm. Although the maximal contribution of the tonoplast is restricted to several millivolts (Novacky 1983), the data presented in this study reflect the contribution of both cell membranes.

In general, an increase in proton efflux in plant tissues was found to be related to membrane hyperpolarization such as in fusicoccin application (Marré 1979). Most observations, however, were performed in leaves or in the plant's storage roots (Reinhold and Kaplan 1984). In this study the effect of *A. brasilense* in regular soybean roots differed in this respect. The bacteria depolarized the membrane and concomitantly increased proton efflux from the roots. Other root-beneficial bacteria, such as various species of rhizobia, were previously shown to have the same effect on soybean root membranes as well (Érsek et al. 1986). The observed membrane depolarization may be related to an increase in the solute uptake via the secondary active transport, the H^+ -solute cotransport after the

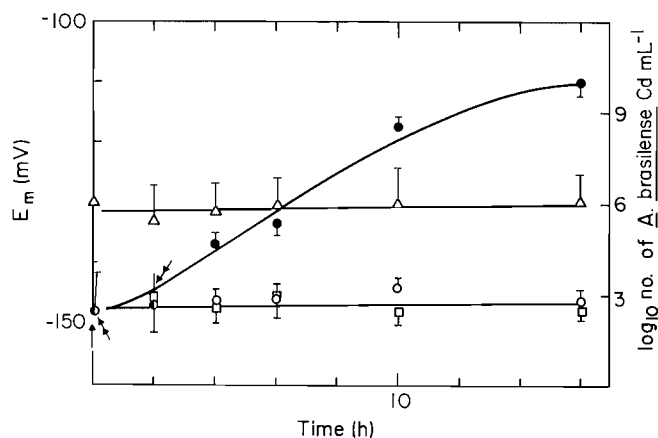


FIG. 1. Membrane potential differences between interior of soybean root elongation zone cells and solution bathing the roots during continuous perfusion of the roots from inoculation time with *A. brasilense* Cd. ●, E_m values for inoculated roots perfused 120 min after inoculation; ○, E_m values for noninoculated roots; □, E_m values for inoculated roots perfused immediately after inoculation; △, numbers of *A. brasilense* Cd cells in the perfusion cell. Arrow indicates time of inoculation. Double arrows indicate beginning of perfusion. The values are means from 20 separate determinations in the elongation zone, four determinations per seedling. Bars represent standard error.

A. brasilense "signal molecules" affect the proton pump. In this respect, an accumulation of amino acids or sugars, or both, will be evident in the plant tissue. Increase in nitrogenous compounds in general as a result of *Azospirillum* inoculation is a well-documented phenomenon (Bashan and Levanony 1990).

The data collected to date for transport of amino acids in higher plants suggest a common carrier capable of transporting all amino acids (Poole 1978; Reinhold and Kaplan 1984). If this is the case for *A. brasilense*, it will also support the notion that the beneficial effects of the bacteria are nonspecific (Bashan and Levanony 1990; Bashan et al. 1989b). Thus, a concomitant analysis of amino acids and sugars in the plant tissue should follow future E_m studies in order to determine if this physiological mechanism is being triggered by *A. brasilense* in inoculated plants.

The unidentified signal(s), which was previously proposed (Bashan 1990) to induce changes in proton efflux, also affects membrane activity. The existence of such a bacterial signal(s) is indirectly proven by these studies. Since there is no direct contact between *Azospirillum* cells in the cortical intercellular spaces and the membranes of these cells (Levanony et al. 1989), the bacterium cell should produce a diffusible signal that has the ability to cross plant cell walls to affect membranes. Thus, it is further suggested that inoculation with *A. brasilense* triggers changes in the membrane potential of cortical cells via a bacterial signal(s), which in turn results in increased proton efflux from the roots. How the signal(s) is transmitted from the bacterium cell is not known, since thorough understanding of the *Azospirillum* cell wall and membranes is still lacking (Bachhawat and Ghosh 1987a; Murray and Moyles 1987).

Bacterial siderophores, which help bacteria scavenging iron from the environment, are formed by nearly all aerobic bacteria (Neilands and Leong 1986; Loper and Buyer 1991), including *A. brasilense* (Bachhawat and Ghosh 1987b). Neilands and Leong (1986) proposed three ways by which bacterial siderophores can affect plant life: (i) solubilizing and transport of Fe^{3+} to the plant in cases of iron deficiency, (ii) facilitation of plant disease, and (iii) biocontrol of plant pathogens (Kloepper et al.

1980). However, all *Azospirillum* strains proved to be nonpathogenic and thus far there is no single report of *Azospirillum* as a biocontrol agent (Bashan and Levanony 1990). Although inoculated plants with *Azospirillum* take up more iron (Barton et al. 1986; Bashan et al. 1990) and acidification of the rhizosphere proved to be a major strategy by which plants assimilate iron (Loper and Buyer 1991), the physiological mechanism operating by plant growth promoting rhizobacteria (PGPR) lacking biocontrol activity such as *Azospirillum*, as yet has not been defined. Therefore, it is not yet clear whether the "signal molecules" of *Azospirillum* have a siderophore nature.

Azospirillum brasilense is known to have preference for colonizing the elongation zone of several plant species (Bashan et al. 1986, 1991) and to affect morphologically the growth of this zone in wheat (Levanony and Bashan 1989), despite its ability to colonize every part of the root system in cereals and other plant families (Bashan and Levanony 1989a, 1989b; Bashan et al. 1986, 1989b; Murty and Ladha 1987). This study provides evidence that the preference of *A. brasilense* to colonize the elongation zone also affects the membrane activity in that zone. Although changes in E_m were recorded in every part of the inoculated root, the greatest change was detected in the elongation zone of soybean root. Whether the elongation zone of soybean root also extruded more protons is not known. However, the elongation zone of wheat, for example, even without inoculation, is known to extrude more protons than the other root parts (Bashan and Levanony 1989c).

Previous proton efflux studies of inoculated roots revealed that metabolically active bacterial cells are essential to induce a pronounced proton efflux in roots. Dead, stressed, or old bacteria were inefficient (Bashan 1990; Bashan et al. 1989a). Similarly, this study shows that active bacterial cells are required to affect the E_m of membranes in the cortical cells. Probably stressed cells are incapable of producing the required signal, although they retain their colonization ability intact. Thus, bacterial metabolic activity, and not simply bacterial population size, is responsible for affecting root membranes. Furthermore, efficient root colonization, generally assumed to be crucial to the effect of beneficial bacteria on plants (Bashan and Levanony 1990; Suslow 1982), is not fully correlated with effects on root membranes. It is still unclear how these data are related to several previous studies (e.g., Bashan 1986) that showed that a threshold colonization number of *Azospirillum* is essential to induce plant response towards inoculation. This relationship definitely requires further study.

Enhanced proton efflux in wheat roots was originally attributed to a single strain of *A. brasilense*, strain Cd. Other root-associated, nonbeneficial bacteria of the genera *Pseudomonas*, *Bacillus*, and *Azotobacter* failed to enhance proton efflux in wheat (Bashan 1990; Bashan et al. 1989a). This study, by using two additional *A. brasilense* strains that were also capable of affecting membrane activity, may be a clue that probably more strains of *A. brasilense* are capable of inducing this phenomenon. However, this proposal needs more evidence to be conclusive. On the other hand, effect on membranes is not a general phenomenon induced by all root-associated bacteria. *Pseudomonas* sp., an excellent colonizer of soybean roots, failed to induce any detectable effect on membrane potential.

In conclusion, this study demonstrates that several strains of *A. brasilense* have the ability to reduce the membrane potential of inoculated soybean roots, and in particular, cell membranes in the root elongation zone. Active metabolism of the bacterial cells is required. Bacteria probably affect cell membranes through a release of bacterial signal(s).

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