Changes in proton efflux of intact wheat roots induced by *Azospirillum brasilense* Cd

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Inoculation of wheat seedings with *Azospirillum brasilense* Cd increased proton efflux from the roots. Inoculation of seeds or young seedlings using bacterial cultures at the logarithmic phase of growth caused the strongest proton extrusion. The increased effect lasted up to 20 h. No difference was detected between inoculated and noninoculated plants 20 h after inoculation. Both inoculated and noninoculated plants decreased the final pH of the nutrient solution to 3.2 and had an average proton extrusion of 4.3 μmol $H^+ \cdot$ (g fresh weight)$^{-1} \cdot h^{-1}$. *Azospirillum brasilense* Cd inoculation of wheat roots in which proton efflux was inhibited by the addition of either nitrate, dicyclohexylcarbodiimide, or orthovanadate resulted in partial recovery of proton efflux activity in these roots. Inoculation of wheat seedlings also changed the regular pattern of root proton efflux over prolonged periods of time. It is suggested that *A. brasilense* Cd inoculation influenced membrane activity and subsequent proton efflux in roots of wheat seedlings.

Key words: *Azospirillum*, plant–bacteria interaction, proton efflux, rhizosphere bacteria, *Triticum aestivum*, wheat.

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L'inoculation de plantules de blé avec de l'*Azospirillum brasilense* Cd a augmenté l'efflux de protons des racines. L'inoculation de grains ou de jeunes plantules avec des cultures bactériennes lors de la phase logarithmique de croissance a causé la plus forte extrusion de protons. Cet effet d'accroissement a eu une durée de 20 h. Vingt heures après l'inoculation, aucune différence n'a pu être notée entre les plantes inoculées et les non inoculées. Aussi bien les plantes inoculées que les non inoculées ont réduit le pH final de la solution nutritive à 3,2 et leur moyenne d'extrusion de protons s'est située à 4,3 μmol $H^+ \cdot$ (g de poids frais)$^{-1} \cdot h^{-1}$. L'inoculation avec de l'*A. brasilense* Cd de racines de blé dont l'efflux de protons avait été inhibé par addition soit de nitrate, de dicyclohexylcarbodiimide ou d'orthovanadate, a conduit à une récupération partielle de l'activité d'efflux de protons dans ces racines. L'inoculation des plantules de blé a aussi changé le profil régulier d'efflux de protons des racines durant une période de temps prolongée. La suggestion est avancée qu'une inoculation avec de l'*A. brasilense* Cd influence l'activité membranaire et l'efflux subséquent des protons dans les racines des plantules de blé.

Mots clés : *Azospirillum*, interactions plantes–bactéries, flux de protons, bactéries de la rhizosphère, *Triticum aestivum*, blé.

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Introduction

Changes in plant rhizosphere pH which result from an imbalance of ion uptake occur in the rhizosphere of several plants, mainly dicotyledons (Marschner *et al.* 1986; Riley and Barber 1971; Nye 1981). Proton efflux through membranes of root cells, which result in the acidification of the rhizosphere, was proposed to be a major mechanism in the mobilization of minerals in plants (Gardner *et al.* 1982; Riley and Barber 1971; Römheld and Marschner 1981, 1984). Proton extrusion in roots was found to be correlated with extension of root growth (Pilet *et al.* 1983), geotropism (Mulkey and Evans 1981), iron deficiency (Marschner *et al.* 1982; Römheld *et al.* 1984), or phosphorous deficiency (Hedley *et al.* 1983). Recently, we have demonstrated the presence of a proton efflux mechanism in intact wheat roots, determined its location as the basal portion of the root, and measured its capacity (Bashan and Levanony 1989).

More than a decade of intensive study on the mode of action of the beneficial rhizosphere bacteria of the genus *Azospirillum* have not yet revealed the main mechanism directly responsible for their positive effects on the growth of inoculated plants. Among the mechanisms proposed so far, increased mineral uptake by the plant as a result of inoculation has been suggested to play a major role (Lin *et al.* 1983; Morgenstern and Okon 1987; Okon and Kapulnik 1986; Sarig *et al.* 1988). However, no attempt has been made to correlate the mineral uptake activity of the plant to root membrane activity. Furthermore, the possible effect of *Azospirillum* inoculation on root membrane potential have not been evaluated.

The aim of the present study was to find out whether inoculation with *Azospirillum* affects membrane and proton efflux activities in intact wheat roots. A preliminary report of this study was presented elsewhere (Bashan and Levanony 1988).

Materials and methods

Plant growth conditions

Wheat seeds (*Triticum aestivum* cv. Deganit) were surface dis-

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infected with 1% NaOCl for 5 min and then thoroughly washed with tap water. Seeds were then transferred to an hydroponic system (25 seeds per system) consisting of a deep petri dish (9 cm in diam., 7 cm deep) containing nutrient solution (replaced daily) and supported with a stainless steel stand with cheesecloth on its upper surface immersed inside the solution. All experiments were conducted in a fully controlled growth chamber (Conviron, model EF7H, Controlled Environments Co., Canada) maintained at a temperature of 22 ± 1°C with a photoperiod of 10 h light (130 $\mu E \cdot m^{-2} \cdot s^{-1}$) and 14 h darkness or, alternatively, at room temperature (22 ± 3°C) with a photosynthetic lamp (150 $\mu E \cdot m^{-2} \cdot s^{-1}$; Sylvania). Seedlings at ages ranging from 2 days (first root emergence) up to two leaves were used unless otherwise indicated.

Nutrient solution

The following nutrient solution was used (mM): K_2SO_4, 0.75; $MgSO_4$, 0.65; KH_2PO_4, 0.1; $(NH_4)_2SO_4$, 0.5; $CaSO_4$, 0.5; H_3BO_3, 1×10^{-2}; $MnCl_2 \cdot 4H_2O$, 1×10^{-4}; $ZnSO_4 \cdot 7H_2O$, 0.5×10^{-5}; $CuSO_4 \cdot 5H_2O$, 0.5×10^{-5}; $H_2MoO_4 \cdot H_2O$, 0.5×10^{-6} (Marschner *et al.* 1982). The pH of all nutrient solutions was adjusted to 5.8 with NaOH. In some experiments, the ATPase inhibitor, orthovanadate, and the oxidative phosphorylation inhibitor, dicyclohexylcarbodiimide (DCCD), were mixed separately with the nutrient solution at final concentrations of 5, 10, 50, 100, and 200 μM (orthovanadate), or 100 μM (DCCD). Experiments in the presence of nitrate were performed by replacing $(NH_4)_2SO_4$ and $CaSO_4$ with 2 mM $Ca(NO_3)_2$.

Bacterial growth conditions

Azospirillum brasilense Cd (ATCC 29710) was grown in nutrient broth (Difco) in Erlenmeyer flasks (equipped with shallow grooves) which were agitated with a rotary shaker (250 rpm, 14–16 h, 30 ± 2°C, logarithmic phase of growth). This procedure improved aeration of the culture and eliminated aggregation of *A. brasilense* Cd, thus producing a population of single cells in a relatively short growth period. When older cultures were used, the same growth conditions were used. Three root-associated bacteria, *Pseudomonas* sp. (OH 88004), *Bacillus* sp. (OH 88012), and *Azotobacter* sp. (OH 88022) were isolated from washed surface roots of young *Zea mays* seedlings grown in Crosby silt loam soil in Columbus, OH. The isolates were cultured on nutrient agar mediuim supplemented with streptomycin sulphate (200 mg/L). Bacteria were prepared for plant inoculation as previously described (Bashan 1986; Bashan and Levanony 1985).

Bacterial inoculation

Disinfected wheat seeds and wheat seedlings were inoculated by one of two methods. (*i*) The seeds were imbibed for 1 h at ambient temperature under a light vacuum created by a small water pump to facilitate penetration inside seed cavities of 10^6 cfu/mL double washed *A. brasilense* Cd culture (this bacterial concentration is known to be optimal for causing marked effects on wheat root development (Barbieri *et al.* 1988; Bashan 1986)). Then, the inoculated seeds were transferred to the hydroponic systems. (*ii*) Three days after imbibition in sterile tap water and growth in the hydroponic system, the nutrient solution was replaced by fresh nutrient solution supplemented with 10^6 cfu/mL *A. brasilense* Cd for 24 h. Then, the bacterial solution was replaced by fresh nutrient solution and proton efflux activity was measured.

Bacterial counts on roots

Verification of root colonization by *A. brasilense* Cd and bacterial counts were performed by the improved selection technique (Bashan and Levanony 1985) or by the indirect enzyme-linked immunosorbent assay (Levanony *et al.* 1987). Other rhizosphere bacteria were counted by the dilution plate count method after homogenization of the roots grown on nutrient agar medium supplemented with streptomycin sulphate (200 mg/L).

pH measurements

Ten seedlings (first or second leaf stage, separately) grown in the hydroponic system were transferred to a glass beaker (50 mL) which contained 20 mL of fresh nutrient solution. The beaker was wrapped with aluminum foil to prevent direct illumination of roots, placed in a controlled temperature water bath at 25 ± 0.5°C, and illuminated with a photosynthetic lamp. A pH electrode (Broadley James Corp.) was inserted into the solution and pH changes were monitored with a Unicorder U-228 (Pantos, Japan) with a chart speed of 10 mm/h. The pH was also measured manually with a portable pH meter.

In long-term experiments (more than 24 h of continuous measurements) the nutrient solution was replaced daily to eliminate any nutrient or oxygen deficiencies. The pH of every new solution was adjusted precisely to the pH of the nutrient solution in the beaker. Replacement of the nutrient solution was done gradually to avoid any shock to the plants. First, 20 mL of new nutrient solution was added to the used solution and half of the new mixture was pumped out. This procedure was repeated five times. This type of experiment was terminated after 7 days. At that time the volume of the roots developed inside the nutrient solution was too large for the experimental conditions.

The quantity of protons released by the roots was determined at the end of the experiment or after 10 h by titrating the nutrient solution with 0.01 M NaOH to the initial pH value. Excess water was removed from the roots by blotting with soft paper prior to fresh weight determination. The amount of protons released into the nutrient solution was expressed as $\mu mol\ H^+ \cdot (g\ fresh\ weight)^{-1} \cdot h^{-1}$.

Measurement of root surface area

Average root surface area of three seedlings per treatment was determined using the gravimetric method of Carley and Watson (1966).

Experimental design and statistical analysis

Each experiment was carried out five to eight times in a completely randomized design with five to six replicates. A replicate consisted of either a beaker containing 10 seedlings, or five hydroponic systems. Significance among treatments was determined by Duncan's Multiple Range Test ($P \leq 0.05$) and in linear regression by $P \leq 0.05$ or $P \leq 0.01$.

Results

Increase proton efflux of wheat roots by A. brasilense *Cd inoculation*

Root proton efflux in wheat seedlings inoculated either in the seed or at the seedling stage was compared to the normal proton efflux pattern of young noninoculated wheat seedlings. Inoculation with *A. brasilense* Cd increased proton efflux as indicated by pH decrease of the nutrient solution surrounding the roots. Inoculation of seeds caused the most marked effect; however, later inoculation of young seedlings also increased proton efflux of the roots (Fig. 1). These differences between inoculated and noninoculated plants were most marked 10 h after the transfer of seedlings to a fresh nutrient solution. When nutrient solution pH reached a minimal value of 3.2 ± 0.2 in any treatment, no further decrease was detected and the amount of released protons was almost similar in inoculated and noninoculated plants (Fig. 1). However, when a repeat experiment was terminated earlier after 10 h, a significant difference in proton efflux between inoculated and noninoculated plants was observed, and the highest value was recorded in seed-inoculated seedlings (underlined values in Fig. 1). Periodical counting of *A. brasilense* Cd on the roots revealed a population size ranging from 2×10^4 to 3×10^5 cfu/g fresh weight whereas the root internal population ranged from 3×10^5 to 2×10^6 cfu/g fresh weight.

Changes in nutrient solution pH during prolonged periods

Fluctuations in pH of the nutrient solution were measured continuously for 7 days. During the experimental period a bimodal proton efflux activity was observed: two periods of

proton extrusion and two periods when an alkaline compound(s) was released (Fig. 2). The release of this alkaline compound(s) was smaller than proton extrusion and the released compound(s) was not able to titrate all the released protons. This activity was not dependent on photoperiodic condition of the experiment. Inoculation of seeds with *A. brasilense* Cd eliminated the fluctuation effect. Once the solution reached a pH level of 3.1 ± 0.1 units, it remained at this low level throughout the entire period (Fig. 2).

Effect of seedling age on proton efflux from inoculated roots

Seeds were inoculated during the imbibition period. After time intervals ranging from 2 to 12 days, proton efflux was monitored after transfer of seedlings to fresh nutrient solution. Inoculation with *A. brasilense* Cd generally increased both proton efflux and pH decrease, but this effect depended upon plant age (Fig. 3). Proton efflux was greater in very young seedlings concomitantly with decreased solution pH. Response of older seedlings was smaller and after 12 days the response was minimal. Although a decrease in pH and a measurable amount of extruded protons could be detected in older seedlings, proton efflux activity was relatively small in relation to the fresh weight of roots.

Effect of A. brasilense *Cd culture age on proton efflux from wheat roots*

Seeds were inoculated with *A. brasilense* Cd cultures at either the logarithmic phase of growth, the stationary phase, or with 48-h-old cultures. Only inoculation with bacteria in the logarithmic phase of growth significantly increased proton efflux over noninoculated plants (Fig. 4).

Effect of A. brasilense *Cd inoculation on wheat roots treated with proton efflux inhibitors*

Application of the inhibitor DCCD to noninoculated wheat seedlings 1 h after transfer of the seedlings to fresh nutrient solution completely inhibited proton efflux 6 h later. Application of a similar DCCD concentration to inoculated plants had some inhibiting effect on proton efflux but did not totally inhibit proton extrusion (Fig. 5A; compare to Fig. 1). Addition of the ATPase inhibitor, orthovanadate, inhibited proton efflux of noninoculated plants even at relatively low concentration (50 μM). In wheat roots inoculated with *A. brasilense* Cd, orthovanadate also decreased proton efflux (Fig. 6). At 100 μM orthovanadate, inhibition of root proton efflux was even greater than the inhibition measured in noninoculated seedlings (a decrease of 2.9 compared to 1.8 μM $H^+ \cdot$ (g fresh weight)$^{-1} \cdot h^{-1}$, respectively). However, since the initial proton efflux activity of inoculated plants was higher, the plants retained some proton efflux activity. This inhibitor concentration in noninoculated plants completely inhibited proton efflux. No effect of inoculation on proton efflux was observed at the highest inhibitor concentration, which completely inhibited proton efflux activity in both inoculated and noninoculated plants (Fig. 6).

Effect of A. brasilense *Cd inoculation on proton efflux of nitrate treated plants*

Two-day-old seedlings grown in nutrient solution containing nitrate, which inhibits proton efflux in wheat roots (Bashan and Levanony 1988), were inoculated with *A. brasilense* Cd for 14 h. The nutrient solution was then replaced and 10 h later, proton efflux was initiated in these plants (Fig. 5B). However, this activity was lower than the activity in ammonium-cultured plants (compare Fig. 1, trace corresponding to seedling inoculation, with Fig. 5B).

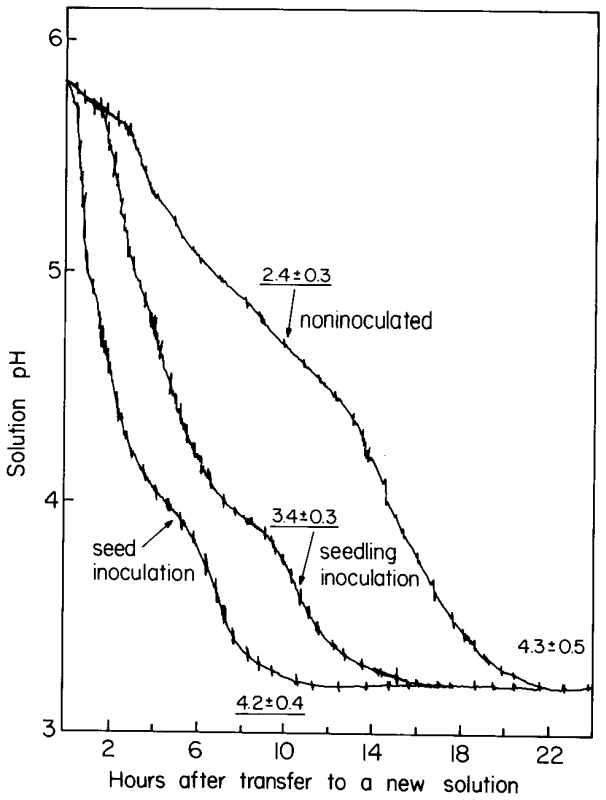

FIG. 1. Effect of inoculation with *A. brasilense* Cd on proton efflux in young intact wheat roots. All graphs were recorded automatically and drawn on a paper moving at a rate of 10 mm/h. The experiment was repeated six times and the lines are from a representative experiment. Numbers ± SE represent the average of proton efflux (μmol $H^+ \cdot$ (g fresh wt.)$^{-1} \cdot h^{-1}$) from all experiments after 10 (underlined values) or 22 h (not underlined).

Effect of inoculation with nonbeneficial, root-associated bacteria on proton efflux

Inoculation of wheat seeds with nonbeneficial, root-associated bacteria affected neither the proton efflux activity in roots of germinating wheat seedlings nor their root surface area, although the bacteria were capable of root colonization. On the other hand, inoculation with *A. brasilense* Cd caused increased proton efflux and root surface area of the seedlings (Table 1).

Discussion

The genus *Azospirillum* is known for its potential to affect plant growth, mainly cereals (Okon 1985; Patriquin *et al.* 1983), as well as vegetables (Bashan *et al.* 1989; Kolb and Martin 1985). The mode of beneficial action of *Azospirillum* is still an open question. Although several explanations have been suggested, none is commonly accepted. One of the proposed explanations is improved mineral uptake of plants caused by the bacteria (Kapulnik *et al.* 1985; Lin *et al.* 1983; Sarig *et al.* 1988). However, it is not clear how bacterial colonization of roots affects cell membranes. This is of importance since mineral uptake is usually closely related to membrane activity.

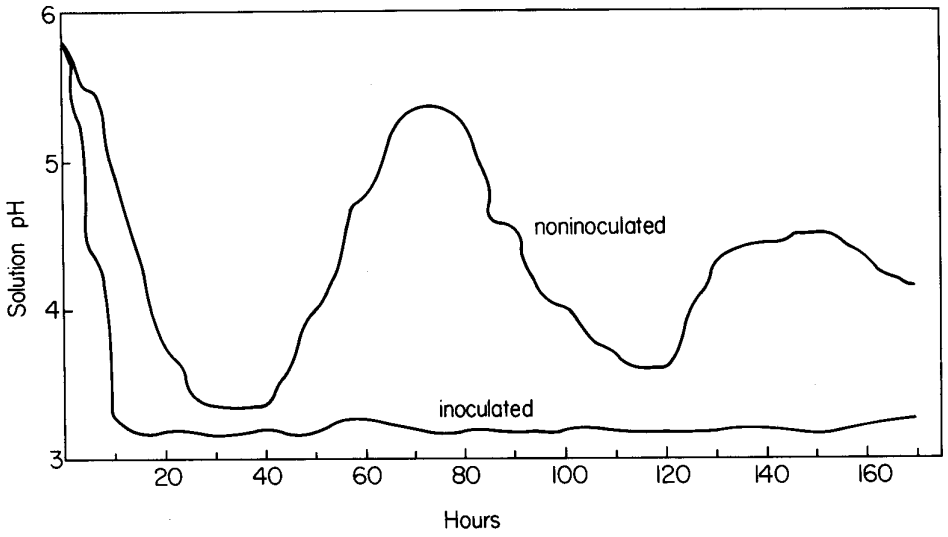

FIG. 2. Changes in nutrient solution pH caused by *A. brasilense* inoculated and noninoculated wheat seedlings. Details as in Fig. 1. The experiment was repeated four times.

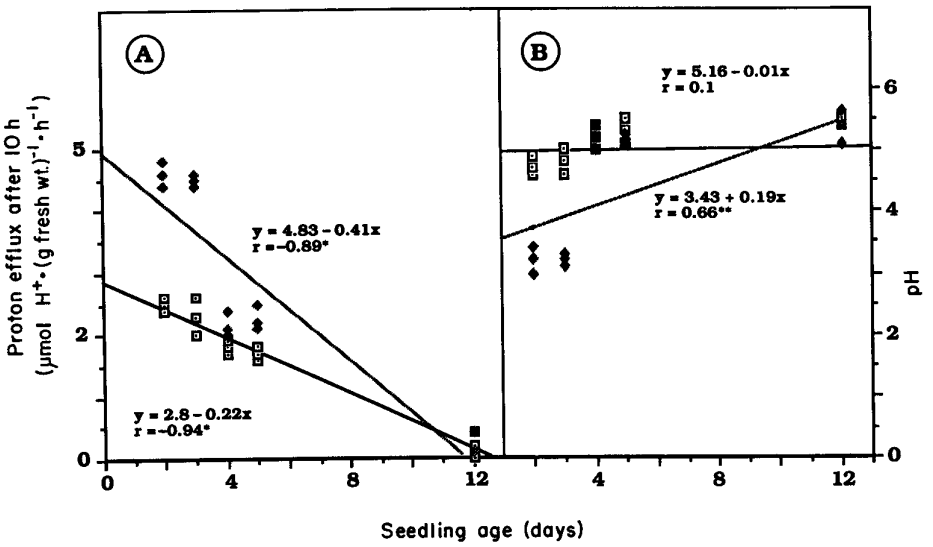

FIG. 3. Correlations between wheat seedling age and (A) proton efflux of roots and (B) the pH of the nutrient solution around the roots of *A. brasilense* inoculated (♦) and noninoculated (⊡) seedlings. When superimposed, ♦ and ⊡ symbols appear as solid squares. Inoculation was performed on seeds and the initial pH of the nutrient solution was 5.8. The significance of each correlation is given at either $P \leq 0.01$ (*) or $P \leq 0.05$ (**). The experiment was repeated three times, with three replicates for each time point. Each symbol represents the mean of three replicates.

The capacity of an intact plant to lower the pH of its rhizosphere is exhibited by several plant species. Exchange of extruded protons for potassium and ammonium ions that are taken up is a well-known phenomenon (Marschner *et al.* 1986). This activity is carried out mainly by electrogenic proton pumps localized in various membranes (Churchill and Sze 1983). These pumps may create chemical and electrical gradients across the membranes (Spanswick 1981). Thus, proton efflux may act as a driving force for energy-dependent transmembrane uptake of essential ions. In addition, proton efflux induces plant cell enlargement (O'Neill and Scott 1983).

Species of *Azospirillum* are known to attach to the cell wall of their host (Berg *et al.* 1979; Gafni *et al.* 1986; Jain and Patriquin 1984; Umali-Garcia *et al.* 1980) and to produce fibrillar material connecting the bacteria to the plant cell wall (Bashan *et al.* 1986). However, there is no evidence that the presence of *Azospirillum* near or in close proximity to plant cell walls affects plant membrane and proton efflux activities. To the best of our knowledge, this is the first report on the effect of any associative beneficial bacteria on the proton efflux of plant roots.

The capacity of the proton efflux is of particular importance. Rates of proton extrusion of 1 up to 6 μmol H^+ · (g fresh

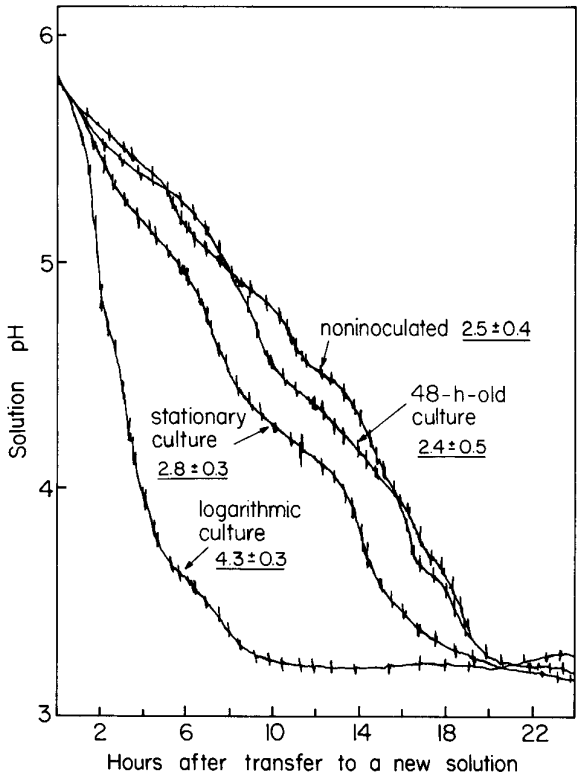

FIG. 4. Effect of *A. brasilense* Cd culture age at inoculation on proton efflux in young wheat seedlings. Details as in Fig. 1. The experiment was repeated twice. Two additional experiments were terminated after 10 h to quantify proton efflux (underlined numbers ± SE).

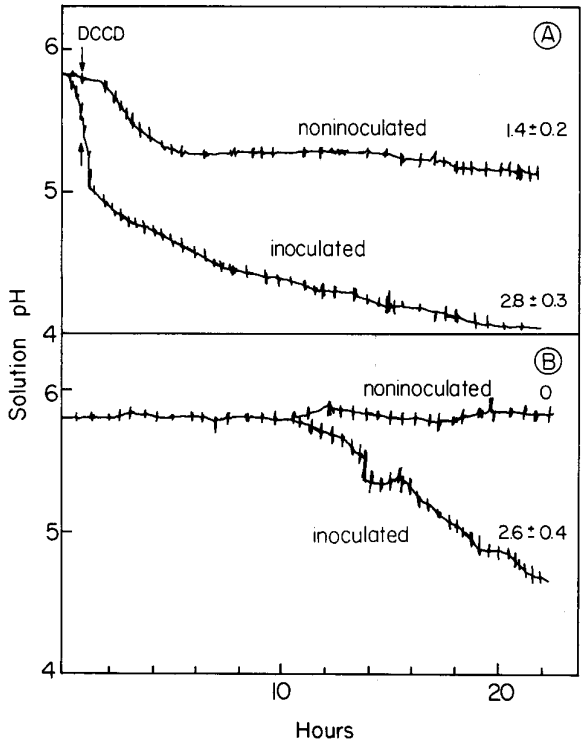

FIG. 5. (A) Effect of applying the inhibitor DCCD (arrows) on proton efflux of inoculated and noninoculated wheat seedlings. (B) Effect of inoculating with *A. brasilense* Cd on proton efflux in wheat seedlings submerged in a nutrient solution containing NO_3^-, which inhibits proton efflux. Details as for Fig. 1. The experiments were repeated three and five times, respectively.

weight)$^{-1}$ · h^{-1} were reported (Glass and Siddiqi 1981; Mengel and Malissiovas 1982; Pitman *et al.* 1975). Our previous results showed that, under optimal conditions, the amount of proton extrusion ranged from 2.6 to 5.7 μmol H^+ · (g fresh weight)$^{-1}$ · h^{-1} with an average of 4.2. Simultaneously, the plants decreased the pH level of their surroundings from 5.8 to a minimum of 2.9, with an average pH value of 3.2 (Bashan and Levanony 1989). The presence of *A. brasilense* Cd in the roots increased proton efflux and the surrounding nutrient solution reached its minimal pH value in about half of the time required by noninoculated wheat plants. On the other hand, inoculation with *A. brasilense* Cd did not increase the total capacity of root proton efflux. However, it did alter proton efflux as demonstrated by the elimination of the bimodal pattern of proton efflux found in noninoculated roots (Fig. 2). Attempts to increase proton efflux in wheat roots by inoculating with nonbeneficial, root-associated bacteria other than *Azospirillum*, such as *Pseudomonas, Bacillus*, and *Azotobacter*, were not successful. These bacteria did not induce an increase in either proton efflux or root surface area, a parameter known to be affected by beneficial bacteria (Bashan 1986; Fallik *et al.* 1988). However, this study does not claim that this ability is unique and (or) specific to the genus *Azospirillum*. Other associative beneficial bacteria may induce this phenomenon as well. The effect of rhizobia inoculation on membrane potentials in soybean was recently demonstrated (Ersek *et al.* 1986). The biological meaning of these changes in root activity needs further study.

A unique feature of the increased proton efflux phenomenon of wheat roots is the requirement for high metabolic activity in both bacteria and plants. Only a logarithmic phase culture of *A. brasilense* Cd was able to induce proton efflux and the strongest activity was found in very young seedlings immediately after germination. At this seedling age, marked effects of *A. brasilense* Cd inoculation on cell division of root tips and enlargement of the elongation zone were recently found (Levanony and Bashan 1989). These findings give further physiological evidence to observations made by many workers indicating that young seedlings respond better to inoculation than older plants (for review, see Patriquin *et al.* 1983).

Replacement of NH_4^+ ion with NO_3^- resulted in the elimination of proton efflux of wheat seedlings. When NO_3^--supplemented nutrient solution was given to the plants, only inoculated plants were able to create proton efflux activity in the roots. *Azospirillum* species are known to be able to transform various nitrogenous compounds (Okon 1985); therefore, the bacteria probably converted nitrate into ammonium and the later induced the proton efflux.

DCCD and orthovanadate are known as inhibitors of oxidative phosphorylation and ATPase activity, respectively. The inhibition effects of DCCD on proton efflux in sunflower seedlings were previously demonstrated (Römheld *et al.* 1984). Proton efflux inhibition by orthovanadate in radish seedlings was also reported (Cocucci *et al.* 1980). In a previous study (Bashan and Levanony 1989), the inhibitor orthovanadate decreased proton efflux in wheat seedlings. These effects in

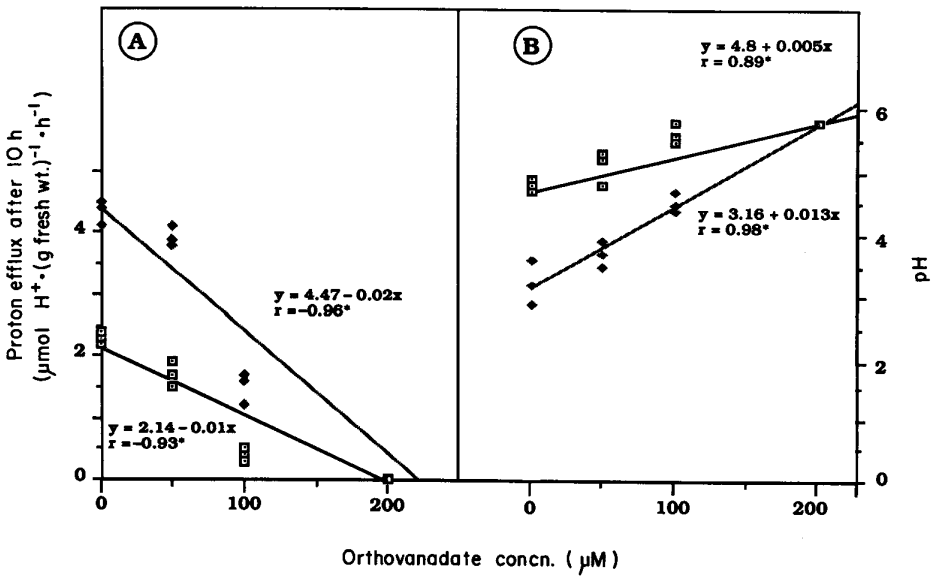

FIG. 6. Correlation between the ATPase inhibitor, orthovanadate, and (A) proton efflux and (B) the pH of the nutrient solution around the roots of *A. brasilense* inoculated (♦) and noninoculated (⊡) seedlings. The initial pH of the nutrient solution was 5.8. The significance of each correlation is given at $P \leq 0.01$ (*). The experiment was repeated three times, with two replicates for each concentration point. Each symbol represents the mean of two replicates.

TABLE 1. Effect of seed inoculation with nonbeneficial, root-associated bacteria on proton efflux of roots and on root surface area of wheat seedlings

Bacterial species	Proton efflux after 10 h, μmol $H^+ \cdot$ (g fresh wt.)$^{-1} \cdot h^{-1}$	pH after 10 h[a]	Root surface area, mg $Ca(NO_3)_2$/plant[b]	No. of bacteria/ g fresh wt.[c]
None	2.2*b*	4.6*b*	98*a*	12±3
A. brasilense Cd (ATCC 29710)	4.1*a*	3.3*a*	177*b*	$(5.1\pm0.3)\times10^5$
Pseudomonas sp. (OH 88004)	2.4*b*	4.8*b*	104*a*	$(6.7\pm0.7)\times10^6$
Bacillus sp. (OH 88012)	2.6*b*	4.5*b*	89*a*	$(3.6\pm0.4)\times10^4$
Azotobacter sp. (OH 88022)	2.4*b*	4.2*b*	93*a*	$(2.7\pm0.6)\times10^2$

NOTE: Numbers followed by a different letter differ significantly at $P \leq 0.05$. The experiment was repeated twice. Results are means of the two repetitions.

[a]From initial pH of 5.8.

[b]According to Carley and Watson (1966).

[c]*Azospirillum brasilense* Cd was counted on BL semiselective medium (Bashan and Levanony 1985); the other rhizosphere bacteria were counted on nutrient agar supplemented with streptomycin sulphate.

intact plants were interpreted in the mentioned studies as an indication of inhibition of proton efflux in roots corresponding to a membrane-bound ATPase(s). Inoculation of inhibitor-treated seedlings with *A. brasilense* Cd partially decreased proton efflux inhibition. The plants were able to retain part of this essential activity at inhibitor concentrations which normally inhibited proton efflux. Thus, it is suggested that *A. brasilense* Cd has an effect on the ATPase activity of wheat root membranes.

In conclusion, it is suggested that *A. brasilense* Cd inoculation can affect membrane activity and proton efflux of inoculated wheat roots. This activity requires high metabolic activity of both participants in the plant–bacteria association and may participate in increasing mineral uptake of *Azospirillum*-inoculated plants. The mode of action of this novel finding is currently under study.

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