Azospirillum plant growth-promoting strains are nonpathogenic on tomato, pepper, cotton, and wheat

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Abstract: Six strains of *Azospirillum* belonging to five species of plant growth-promoting bacteria (*A. brasilense, A. lipoferum, A. amazonense, A. irakense,* and *A. halopraeference*) did not cause visible disease symptoms on the roots or leaves of tomato, pepper, cotton, and wheat, failed to inhibit seed germination, and did not reduce plant dry weight when seven standard techniques for the inoculation of plant pathogens were used. Similar inoculation conditions with plant pathogens (*Pseudomonas syringae* pv. *tomato, Xanthomonas campestris* pv. *vesicatoria, Xanthomonas campestris* pv. *translucens,* and *Xanthomonas campestris* pv. *malvacearum*) induced typical disease symptoms. None of *Azospirillum* strains caused the hypersensitive reaction on eggplant, whereas all pathogens did. All *Azospirillum* strains increased phytoalexin production in all disease-resistant plant species to moderate levels, but the levels were significantly lower than those induced by the compatible pathogens. The various phytoalexins produced in plants had the capacity to inhibit growth of all *Azospirillum* strains. *Azospirillum amazonense, A. irakense,* and *A. halopraeference* had no apparent effect on plant growth, while A. *brasilense* and *A. lipoferum* increased the dry weight of all plant species. Under partial mist conditions, all *Azospirillum* strains were capable of colonizing leaf surfaces (10^3-10^7 cfu/g dry weight) regardless of the plant species. These results provide experimental evidence that *Azospirillum* sp. might be considered safe for the inoculation of several plant species.

Key words: *Azospirillum*, beneficial bacteria, environmental protection, plant inoculation, plant growth-promoting bacteria.

Résumé : Six souches d'*Azospirillum* appartenant á cinq espéces de bactéries promotrices de croissance chez les plantes soit: *A. brasilense, A. lipoferum, A. amazonense, A. irakense* et *A. halopraeference*, n'ont pas causé de symptômes de maladies sur les racines ou les feuilles de la tomate, du piment, du coton et du blé, n'ont pas réussi à inhiber la germination des semences et n'ont pas réduit le poids sec des plantes, suite à l'utilisation de sept méthodes standard d'inoculation des agents phytopathogénes. Des conditions similaires d'inoculation avec des agents phytopathogènes, dont les *Pseudomonas syringae* pv. *tomato, Xanthomonas campestris* pv. *vesicatoria, Xanthomonas campestris* pv. *translucens, Xanthomonas campestris* pv. *malvacearum*, n'ont induit aucun symptôme typique de maladie. Aucune des souches d'*Azospirillum* n'a causé des réactions d'hypersensibilité chez l'aubergine, alors que tous les agents phytopathogènes y sont parvenu. Toutes les souches d'*Azospirillum* ont favorisé la production de phytoalexine chez toutes les espèces végétales résistantes aux maladies, à des niveaux modérés mais significativement inférieurs aux niveaux induits par les agents pathogènes compatibles. Les différentes phytoalexines produites chez les plantes étaient capables d'inhiber la croissance de toutes les souches d'*Azospirillum*. Les espèces *A. amazonense, A. irakense* et *A. halopraeference*, n'ont eu aucun effet apparent sur la croissance des plantes, tandis que l'*A. brasilense* et l'*A. lipoferum* ont accrû le poids sec de toutes les espèces végétales. Dans des conditions d'humidité partielle, toutes les souches d'*Azospirillum* ont réussi á coloniser les surfaces foliaires (10^3-10^7 ufc/g de poids sec) sans égard á l'espèce végétale. Ces résultats fournissent une évidence expérimentale que les *Azospirillum* sp. peuvent être considérés comme sans danger pour l'inoculation de nombreuses espèces végétales.

Mots clés : *Azospirillum*, bactéries bénéfiques, protection environnementale, inoculation des plantes, bactéries promotrices de la croissance des plantes.

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Introduction

With the concept of plant growth-promoting bacteria gaining worldwide acceptance, a large number of bacterial strains have been isolated (Baldani et al. 1986; Chanway and Holl 1993) and evaluated primarily for promotion of plant growth (Kloepper et al. 1991). Strains failing to demonstrate a positive effect or inducing a negative plant response are routinely discarded and their negative effects are seldom reported (Nehl et al. 1997).

These strains can be beneficial (Glick 1995), harmful (Nehl et al. 1997), saprophytic, saprophytic becoming pathogenic under stress conditions (Soroker et al. 1984), or can be any of these by changing the host (Pimentel et al. 1991). Some pathogenic strains exhibit beneficial features such as N_2-fixation (Kanvinde and Sustry 1990), whereas others produce antibiotics that control soil-borne pathogens (Slininger et al. 1996). Some strains may exhibit beneficial effects on one plant species but harmful or variable effects on others (Gardner et al.

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1984; O'Neill et al. 1992). For environmental safety, because of the existing variability in a strain's features, potential harmful effects need to be evaluated for each strain before release into the environment.

Of the various rhizosphere-associated bacteria, *Azospirillum* species are probably the most studied and appear to have significant potential for commercial applications (Bashan and Holguin 1997). Although their beneficial effects on plants are unpredictable (Bashan and Levanony 1990), most greenhouse studies have demonstrated positive effects on plant growth (Mertens and Hess 1984). Commercial *Azospirillum* sp. inoculants are rare (Fages 1992) and it is difficult to evaluate whether some *Azospirillum* strains may exhibit harmful effects on plant growth in the absence of any significant published literature describing negative results (Harris et. al. 1989; Smith et al. 1984).

Azospirillum species have been reported to produce bacteriocin (Tapia-Hernández et al. 1990) to inhibit nitrogenase activity of other bacteria (Drozdowicz and Ferreira Santos 1987) and growth of other bacteria in mixed culture (Holguin and Bashan 1996). Furthermore, inoculation at levels of $>10^9$ cfu/mL inhibited wheat root growth (Barbieri et al. 1988; Bashan 19866). Other negative effects of *Azospirillum* and most of its commercial field research are not documented in the scientific literature (Bashan and Holguin 1997), especially that research done by commercial entities. Therefore, pathogenic effects may have escaped the knowledge of the scientific community.

The aims of this study were (*i*) to evaluate potential deleterious effects of common strains of the five known *Azospirillum* species, (*ii*) to evaluate plant phytoalexin responses to inoculation with *Azospirillum* species and several common plant pathogenic bacteria, and (*iii*) to evaluate the inhibition potential of several common phytoalexins on *Azospirillum* strains.

Material and methods

Organisms and growth conditions

The following *Azospirillum* strains were used: *A. brasilense* Cd (DSM 7030, Braunschweig, Germany), *A. brasilense* Sp-245 (donated by J. Döbereiner, Empresa Brasileira de Pesquisa Agropecuaria, Rio de Janeiro, Brazil), *A. lipoferum* 1842 (DSM 1842), *A. halopraeference* Au4 (donated by B. Reinhold-Hurek, Max Plank Institute, Marburg, Germany), *A. amazonense* 2787 (DSM 2787), and *A. irakense* KBC 1 (donated by P. Kaiser, Institute National Agronomique, Paris). The following bacterial pathogens were used: *Pseudomonas syringae pv. tomato* WT-1 (specific pathogen of tomato) (Bashan et al. 1978), *Xanthomonas campestris* pv. *vesicatoria* R-3 (specific pathogen of tomato and pepper) (Diab et al. 19826), *Xanthomonas campestris* pv. *translucens* BCS6 (specific pathogen of cereals) (isolated from infected wheat plants in Ceudad Constitucion, Mexico), and *Xanthomonas campestris* pv. *malvacearum* DSM 1220 (specific pathogen of cotton). Wheat (*Triticum aestivum* cv. Deganit), tomato (*Lycopersicon esculentum* Mill. cv. Na'ama, fresh market tomatoes susceptible to bacterial speck of tomato, and cv. Ontario 7710, resistant to the disease), pepper (*Capsicum annuum* cv. Ma'or, fresh market peppers susceptible to bacterial scab, and cv. Odem, resistant to the disease), and cotton (*Gossypium barbadense cv.* Pima S-5, susceptible to bacterial blight, and *Gossypium hirsutum cv.* Acala, resistant to the disease) were used as host plants. Resistant cultivars were used for phytoalexin induction and susceptible ones for pathogenicity tests. Eggplant (*Solanum melongena* cv. Malka Shechora) was used as the bioassay plant for hypersensitive reaction.

Azospirillum strains were grown on N-free malate medium (OAB; Bashan et al. 1993) for 16 h at 30 ± 1 °C at 200 rpm. The medium for growing *A. halopraeference* Au4 was as described by Reinhold et al. (1987). All bacteria were harvested by centrifugation (7000 x *g* for 10 min), washed twice in potassium phosphate buffer (pH 7.0, 0.06 M) supplemented with 0.15 M NaCl, and were prepared for root and leaf inoculation at a concentration of 8×10^6 cfu/mL (Bashan 19866). Pathogenic bacteria were grown as previously described for *P. syringae* pathovars (Bashan et al. 1978) and for *X. campestris* pathovars (Diab et al. 1982a). To avoid atypical symptom formation (Bashan et al. 1978), pathogenic bacteria were inoculated at a concentration of 1×10^6 - 2×10^6 cfu/mL.

Plants were grown in oven-sterilized quartz in 500 mL black plastic pots as previously described by Bashan et al. (1989). All incubations were done in a greenhouse (28 ± 4°C, natural illumination) or growth chamber (28 ± 2°C for pepper, tomato, and cotton and 22 ± 2°C for wheat; 14 h of illumination at 200 µmol/($m^2 \cdot$ s); Conviron TC 16, Controlled Environments, Winnipeg). The following experiments were done: wheat, once in the greenhouse (Gr) and once in the growth chamber (Gc); tomato, once in Gr and twice in Gc; pepper, twice in Gr and twice in Gc; and cotton, once each in Gr and Gc.

Inoculation techniques

Inoculation with the bacterial suspension was done by spraying it, until runoff using an atomizer, onto the plant leaves and the root system, which was extracted carefully from the sand and rinsed to remove adhering particles. After inoculation, plants were reported with fresh sand. Seven pathogen inoculation techniques were employed: injury with carborundum (Bashan et al. 1978), exposure for 24 h to high relative humidity (misting) before inoculation, treatment of leaves with NaOH (Diab et al. 1982a), treatment with a diluted mixture of pectinases (0.005%) and cellulases (0.01%) (Takabe and Nagata 1984), injection of a bacterial suspension into the vascular system of the plants (Bashan 1984), heat shock (40°C for 3 h for tomato, pepper, and cotton, and 32°C for 3 h for wheat), low temperature treatment (6°C for 12 h.) (Diab et al. 1982a), and inhibition of seed germination (Bashan 1986a).

After each pretreatment, plants were maintained under partial mist conditions in the greenhouse as previously described (Diab et al. 19826).

Phytoalexin analyses

Rishitin (tomato), capsidiol (pepper), and terpenoid phytoalexins (cotton) in inoculated plants were quantified by TLC, GC, and HPLC methods as follows: rishitin by GC (D'Harlingue et. al. 1995), capsidiol by TLC and GC (Yoshizawa et al. 1994), and terpenoid phytoalexins by TLC and HPLC (Essenberg et al. 1990).

Phytoalexin bacterial inhibition tests

Phytoalexin bacterial inhibition tests were done as described by Platero Sanz (1981) with pathogenic bacteria grown in nutrient broth medium (Difco) and *Azospirillum* strains grown in N-free OAB medium. Phytoalexins used in these tests were extracted from resistant plants inoculated with pathogenic bacteria using the methods described by Chávez-Moctezuma and Lozoya-Gloria (1996) for capsidiol, Suleman et al. (1996) for rishitin, and Essenberg et al. (1992) for terpenoid phytoalexins.

Evaluation of disease development and disease indices

Symptom formation was scored according to indices previously described for the four pathogens (Bashan et al. 1978; Brinkerhoff et al. 1984; Diab et. al. 1982a; Watkins et al. 1986) with 0 for a healthy plant and 5 for a dying plant. This index was used also for *Azospirillum* spp. treatments. Symptoms in roots were scored as follows: 0, no visible effect (apparently healthy root system); 1, slight change of root appearance, browning, thickening of roots, lesions; 2, ibid but with a higher magnitude; 3, major deformation of the roots, growth inhibition, rotting.

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Table 1. The effect of inoculation with five species of *Azospirillum* and four plant pathogenic bacteria (*X. campestris* pv. *translucens*, *P. syringae* pv. *tomato*, *X. campestris* pv. *vesicatoria*, *X. campestris* pv. *malvacearum*) on wheat, tomato, pepper, and cotton plants.

Bacterial species	Host plant	Method of inoculation*	Bacterial concentration (cfu/mL)	Change in dw over noninoculated (%)†	Root colonization (cfu/g dw)‡	Leaf colonization (cfu/g dw)‡	Seed germination§	Observed deformations in plants‖	No. of repetitions
A. brasilense Cd	Wheat	1,2,4,6,7	8×10^6	112 *bc*	$5.2\times10^5\pm0.6\times10^5$	$2.8\times10^6\pm0.7\times10^6$	94±2 *a*	Increased root hairs (22%) and number of side roots (44%)	2
A. brasilense Sp-245	Wheat	1,2,4,6,7	8×10^6	126 *c*	$6.7\times10^5\pm0.4\times10^5$	$4.1\times10^7\pm0.1\times10^7$	93±3 *a*	Increased root hairs (18%) and number of side roots (33%)	2
A. lipoferum 1842	Wheat	1,2,4,6,7	8×10^6	118 *c*	$3.2\times10^5\pm0.7\times10^5$	$3.4\times10^6\pm0.3\times10^6$	91±3 *a*	Increased root hairs (28%) and number of side roots (37%)	2
A. amazonense	Wheat	1,2,4,6,7	8×10^6	106 *b*	$7.4\times10^4\pm0.3\times10^4$	$7.1\times10^6\pm0.6\times10^6$	92±1 *a*	None	2
A. irakense	Wheat	1,2,4,6,7	8×10^6	109 *bc*	$6.6\times10^4\pm0.9\times10^4$	$6.8\times10^5\pm0.4\times10^5$	90±2 *a*	None	2
A. halopraeference	Wheat	1,2,4,6,7	8×10^6	98 *b*	$3.9\times10^4\pm0.3\times10^4$	$1.7\times10^4\pm0.4\times10^4$	88±2 *a*	None	2
X. campestris pv. *translucens*	Wheat	1	1×10^6	58 *a*	$8.1\times10^4\pm0.7\times10^4$	$7.4\times10^6\pm0.2\times10^6$	87±3 *a*	Black chaff (15%)	2
Noninoculated	Wheat	1,2,4,6,7	None	100 *b*	None	None	92±2 *a*	None	2
A. brasilense Cd	Tomato	1–7	8×10^6	133 *d*	$9.3\times10^6\pm0.3\times10^6$	$6.6\times10^5\pm0.3\times10^5$	89±2 *b*	Increased root hairs (36%) and number of side roots (41%)	3
A. brasilense Sp-245	Tomato	1–7	8×10^6	127 *c*	$2.4\times10^7\pm0.5\times10^7$	$4.9\times10^5\pm0.2\times10^5$	86±3 *b*	Increased root hairs (26%) and number of side roots (17%)	3
A. lipoferum 1842	Tomato	1–7	8×10^6	144 *d*	$4.8\times10^6\pm0.7\times10^6$	$5.3\times10^4\pm0.7\times10^4$	84±4 *b*	Increased root hairs (32%) and number of side roots (19%)	3
A. amazonense	Tomato	1–7	8×10^6	106 *b*	$7.8\times10^5\pm0.1\times10^5$	$5.5\times10^4\pm0.6\times10^4$	87±2 *b*	None	3
A. irakense	Tomato	1–7	8×10^6	101 *b*	$6.9\times10^4\pm0.8\times10^4$	$6.2\times10^4\pm0.4\times10^4$	86±3 *b*	None	3
A. halopraeference	Tomato	1–7	8×10^6	97 *b*	$7.1\times10^4\pm0.9\times10^4$	$9.8\times10^3\pm0.2\times10^3$	84±4 *b*	None	3
P. syringae pv. *tomato* WT-1	Tomato	1	1×10^6	68 *a*	$8.2\times10^5\pm0.4\times10^5$	$4.7\times10^6\pm0.3\times10^6$	72±5 *a*	Falling leaves (33%) and yellow leaves (22.3%)	3
Noninoculated	Tomato	1–7	None	100 *b*	None	None	88±3 *b*	None	3
A. brasilense Cd	Pepper	1–7	8×10^6	128 *d*	$3.8\times10^7\pm0.4\times10^7$	$2.1\times10^6\pm0.4\times10^6$	90±2 *a*	Increased root hairs (23%) and number of side roots (11%)	4
A. brasilense Sp-245	Pepper	1–7	8×10^6	141 *d*	$4.4\times10^7\pm0.6\times10^7$	$3.7\times10^5\pm0.7\times10^5$	88±3 *a*	Increased root hairs (27%) and number of side roots (13%)	4
A. lipoferum 1842	Pepper	1–7	8×10^6	118 *c*	$4.6\times10^6\pm0.9\times10^6$	$4.8\times10^5\pm0.6\times10^5$	89±3 *a*	Increased root hairs (30%) and number of side roots (15%)	4

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Table 1. (*Concluded*).

Bacterial species	Host plant	Method of inoculation*	Bacterial concentration (cfu/mL)	Change in dw over noninoculated (%)†	Root colonization (cfu/g dw)‡	Leaf colonization (cfu/g dw)‡	Seed germination§	Observed deformations in plants‖	No. of repetitions
A. amazonense	Pepper	1–7	8×10^6	101 *b*	$9.2\times10^6\pm0.1\times10^6$	$6.3\times10^6\pm0.3\times10^6$	91±2 *a*	None	4
A. irakense	Pepper	1–7	8×10^6	108 *b*	$5.7\times10^6\pm0.2\times10^6$	$6.7\times10^4\pm0.2\times10^4$	87±3 *a*	None	4
A. halopraeference	Pepper	1–7	8×10^6	106 *b*	$5.1\times10^5\pm0.4\times10^5$	$9.7\times10^4\pm0.2\times10^4$	85±4 *a*	None	4
X. campestris pv. *vesicatoria*	Pepper	1	2×10^6	86 *a*	$4.2\times10^5\pm0.3\times10^5$	$2.1\times10^8\pm0.2\times10^8$	69±4 *b*	Yellow leaves (26.5%)	4
Noninoculated	Pepper	1–7	None	100 *b*	None	None	91±2 *a*	None	4
A. brasilense Cd	Cotton	1,2,3,5,6,7	8×10^6	138 *c*	$7.4\times10^6\pm0.4\times10^6$	$1.4\times10^4\pm0.5\times10^4$	86±2 *b*	Increased root hairs (19%) and number of side roots (9%)	2
A. brasilense Sp-245	Cotton	1,2,3,5,6,7	8×10^6	123 *c*	$6.3\times10^6\pm0.6\times10^6$	$1.8\times10^4\pm0.3\times10^4$	85±3 *b*	Increased root hairs (29%) and number of side roots (17%)	2
A. lipoferum 1842	Cotton	1,2,3,5,6,7	8×10^6	100 *b*	$5.5\times10^5\pm0.7\times10^5$	$4.1\times10^3\pm0.2\times10^3$	80±2 *ab*	15% smaller leaves (in size); some plants had yellowing leaf tips (6% of leaves); thickening of 8% of roots	2
A. amazonense	Cotton	1,2,3,5,6,7	8×10^6	92 *b*	$4.9\times10^5\pm0.3\times10^5$	$3.6\times10^3\pm0.1\times10^3$	76±3 *a*	None	2
A. irakense	Cotton	1,2,3,5,6,7	8×10^6	96 *b*	$3.6\times10^3\pm0.2\times10^3$	$3.9\times10^3\pm0.4\times10^3$	84±2 *b*	None	2
A. halopraeference	Cotton	1,2,3,5,6,7	8×10^6	94 *b*	$2.7\times10^3\pm0.4\times10^3$	$4.7\times10^3\pm0.3\times10^3$	85±3 *b*	Thickening of 5% of roots	2
X. campestris pv. *malvacearum*	Cotton	1	2×10^6	78 *a*	$4.6\times10^4\pm0.8\times10^4$	$7.6\times10^8\pm0.4\times10^8$	84±3 *b*	Rot of the entire plant (12% of all plants)	2
Noninoculated	Cotton	1,2,3,5,6,7	None	100 *b*	None	None	87±2 b	None	2

Notes: Numbers presented are the average data from all the methods used. Statistics for the change in dry weight and seed germination were calculated for each plant part separately. Numbers followed by different letters differ significantly at $P \leq 0.05$ using one-way ANOVA. None, not including saprophytic bacteria.

*1, under mist for 24 h prior to inoculation; 2, carborundum injuries prior to inoculation; 3, heat treatment (40°C for 3 h for tomato, pepper, cotton and 32°C for 3 h for wheat) of the entire plant prior to inoculation; 4, low temperature treatment (6°C for 12 h) of the entire plant prior to inoculation; 5, slow injection of bacterial suspension into the stem or leaf; 6, spraying with 1mM NaOH prior to inoculation ; 7, pectinase–cellulase treatment of roots prior to inoculation.

†Time after germination (days): wheat, 48; tomato, 62; pepper, 72; cotton, 43.

‡Time after colonization (days): wheat, 23; tomato, 18; pepper, 17; cotton, 10.

§Time after seed germination (days): wheat, 10; tomato, 15; pepper, 25; cotton, 15.

‖Deformations are atypical disease symptoms or different growth patterns compared with noninoculated plants.

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Table 2. Effect of the phytoalexins capsidiol, rishitin, and terpenoid phytoalexin on survival of *Azospirillum* and plant pathogenic bacteria cells.

Bacteria	Nontreated	Growth after 24 h: Capsidiol* No. of bacteria (cfu/mL)	Capsidiol* % survival	Rishitin* No. of bacteria (cfu/mL)	Rishitin* % survival	Terpenoid phytolexins* No. of bacteria (cfu/mL)	Terpenoid phytolexins* % survival
P. *syringae* pv. *tomato*	$6x10^{10}$	$8.4x10^{9}$	14.0	$7.1x10^{8}$	1.183	$9.4x\ 10^{9}$	15.66
X. *campestris* pv. *vesicatoria*	$8x\ 10^{9}$	$4.1x10^{8}$	5.125	$8.9x10^{8}$	11.125	$7.1x10^{8}$	8.875
X. *campestris* pv. *malvacearum*	$6x10^{9}$	$8.6x10^{8}$	14.4	$4.7x10^{8}$	7.83	$2.0x\ l\ 0^{8}$	3.33
A. brasilense Cd	$5x10^{9}$	$4.0x10^{5}$	$8x10^{-3}$	$3.2x10^{6}$	$6.4x10^{-2}$	$8.4x10^{6}$	0.168
A. brasilense Sp-245	$4x\ 10^{9}$	$3.8x10^{4}$	$9.5x10^{-4}$	$6.4x10^{5}$	$1.6x10^{-2}$	$4.2x10^{5}$	$1.05x10^{-2}$
A. lipoferum	$2x10^{9}$	$2.7x10^{4}$	$1.35x10^{-3}$	$7.3x10^{5}$	$3.65x\ 10^{-2}$	$5.7x10^{5}$	$2.85x10^{-2}$
A. amazonense	$3x10^{9}$	$4.3x10^{4}$	$1.43x10^{-4}$	$8.2x10^{5}$	$2.73x\ 10^{-2}$	$6.8x10^{5}$	$2.26x\ 10^{-2}$
A. halopraeference	$8x10^{8}$	$6.2x\ 10^{4}$	$7.75x10^{-3}$	$4.6x10^{5}$	$5.75x10^{-2}$	$3.2x10^{5}$	$4x10^{-2}$

* 100 µg/mL phytoalexin was added to the bacterial growth medium.

Leaf colonization measurements

The leaf colonization study was done as previously described for leaf pathogens (Bashan and Okon 1986) using five replicates. Samples were taken after 23 days (wheat), 18 days (tomato), 17 days (pepper), and 10 days (cotton).

Experimental design and statistical analysis

Inoculation experiments were done two to four times each (see Table 1 for details) and phytoalexin tests twice. A replicate in each experiment consisted of three pots containing one plant each. Results of single experiments showed consistent statistical differences among treatments, therefore, the results were pooled for the statistical analysis presented here.

Phytoalexin effects were determined for five replicates similar to those described above. Results of all repetitions were combined and analyzed together in a one-way analysis of variance (ANOVA) at $P < 0.05$. Results in percentages were arcsine transformed before analysis.

Results and discussion

Azospirillum species are perhaps one of the best-known rhizosphere bacteria. Because they are environmentally competent (for reviews see Bashan and Holguin 1997; Bashan and Levanony 1990), one should be cautious prior to massive field inoculation to ensure that this versatile bacteria will not cause harm upon inoculation. This study was aimed to give evidence for the harmless nature of six strains of *Azospirillum* belonging to its five characterized species.

Using seven different standard inoculation techniques for plant pathogens, no visible disease symptoms were induced by any of the six strains of *Azospirillum* used on leaves or roots of tomato, pepper, wheat, and cotton. In two cases (*A. lipoferum* on cotton and *A. halofraeference* on cotton), some minor thickening of roots was observed. Strains of *A. brasilense* and *A. lipoferum* 1842 increased plant dry weight (dw) by up to 40% (Table 1), which is consistent with previously reported responses for these species (Bashan et. al. 1989; Berge et al. 1990). *Azospirillum amazonense*, *A. irakense*, and *A. halopraeference* failed to produce any increase in plant growth. The plant pathogens caused typical symptoms on host plants and always reduced plant dry weight (14-42%). All species of *Azospirillum* were capable of colonizing the roots of the tested plant species with the colonization level varying with each isolate-plant combination. Generally, the *A. brasilense* strains and *A.* lipoferum 1842 were the best root colonizers(10^{6}-10^{7} cfu/g dw in tomato and pepper). All leaf pathogens colonized roots in relatively small numbers (10^{4}-10^{5} cfu/g dw) and leaves as expected (Sharon et al. 1982; Essenberg et al. 1992). Leaf colonization (10^{3}-10^{7} cfu/g dw leaf) by *Azospirillum* was documented for the first time in this study. Although *Azospirillum* has been isolated from leaf tissues (Chaudhury and Sengupta 1991), colonization levels (>10^{7} cfu/g dw leaf, in some cases) following artificial inoculation was an unexpected result. Colonization may have been enhanced by the partial misting conditions in which the plants were maintained. *Azospirillum* strains had no effect on seed germination apart from inhibition of germination of cotton seeds by *A.. amazonense* (24%). *Pseudomonas syringae* pv. tomato (28%) and X. *campestris* pv. *vesicatoria* (31 %) inhibited the germination of tomato and pepper seeds (Table 1) as previously reported by Bashan (1986a). None of the *Azospirillum* strains caused a hypersensitive reaction on eggplant leaves, but all pathogens did. *Azospirillum brasilense* strains and *A. lipoferum* 1842 always increased root hair formation and produced more lateral roots as documented by Morgenstern and Okon (1987). *Azospirillum amazonense*, *A. irakense*, or *A. halopraeference* did not induce such changes. Only one combination (*A. lipoferum* on cotton) showed minor negative effects on plant growth (smaller leaves and yellow tips on several leaves), although these symptoms did not affect plant dry weight. *Azospirillum* species produced no disease symptoms on leaves of wheat, tomato, pepper, and cotton. The disease index (DI; ranging from 0 to 5) of the respective pathogens were 2.7 for X. *campestris* pv. *translucens* (wheat), 4.5 for P. *syringae* pv. tomato (tomato), 3.8 for X. *campestris* pv. *vesicatoria* (pepper), and 4.1 for X. *campestris* pv. *malvacearum* (cotton). On wheat roots, none of the bacteria, including the pathogens, caused any symptoms. On tomato roots, only the pathogen *P. syringae* pv. tomato had a DI of 1.2. Similarly, on pepper roots, the pathogen X. *campestris* pv. *vesicatoria* had a DI of 0.8. On cotton, apart from A. lipoferum (DI = 0.3) and A. *halopraeference* (DI =0.2), no other *Azospirillum* inoculation induced any visible effect on the roots.

Analysis of phytoalexin accumulation in inoculated resistant cultivars showed all *Azospirillum* strains may function as elicitors. Induced levels were low (<0.3 µg/g dw) compared with the amounts of phytoalexins produced in experiments with the respective pathogens (approx. >1 µg/g dw; Fig 1).

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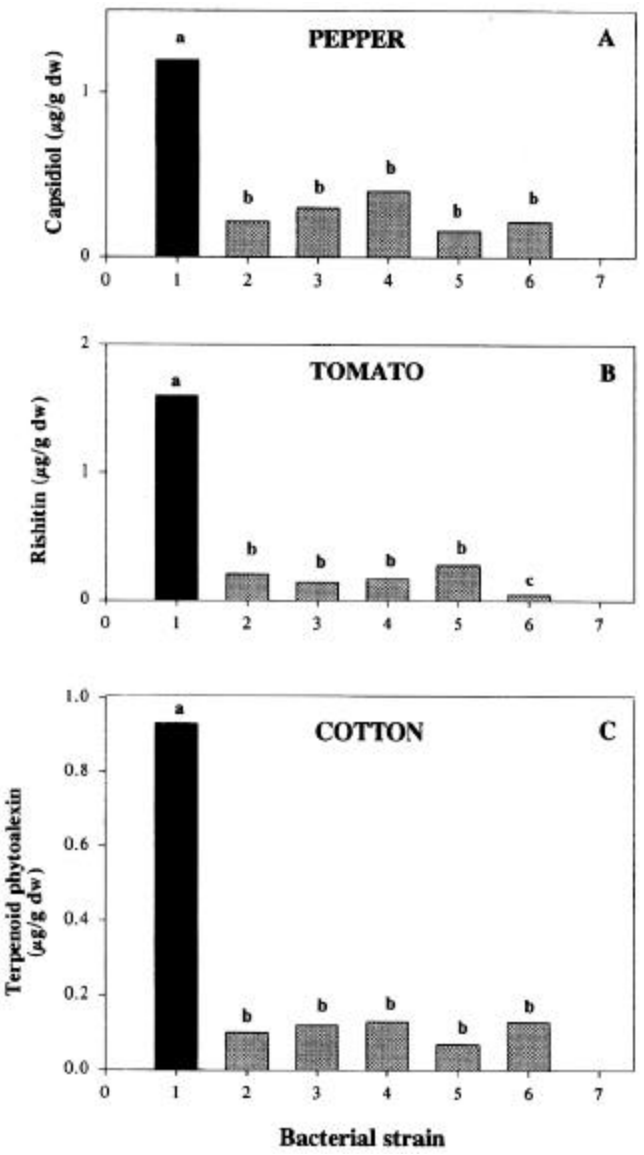
Fig. 1. Phytoalexin accumulation in inoculated resistant cultivars by *Azospirillum* strains and phytopathogenic bacteria. (A) 1, *Xanthomonas campestris* pv. *vesicatoria*; (B) 1, *P. syringae* pv. tomato, and (C) 1, X. *campestris* pv. *malvacearum*. For all parts, the *Azospirillum* strains were as follows: 2, *A. brasilense* Cd; 3, *A. brasilense* Sp-245; 4, *A. lipoferum* 1842; 5, *A. halopraeference* Au4; 6, *A. amazonense* 2787; 7, *A. irakense* KBC1 and noninoculated controls. Columns denoted by a different letter, within each subfigure, differ significantly at $P \leq 0.05$ in the one-way ANOVA.



Furthermore, the various phytoalexins produced in plants had the capacity to inhibit growth of all *Azospirillum* strains (Table 2).

The failure to induce any pathogenic reaction by inoculation points to the safety of *Azospirillum* inoculation. However, one has to consider the small size of this study (24 plantbacteria combinations) compared to the large number of known strains of this species. Before concluding definitively that *Azospirillum* is safe for agriculture, more strains and plant species combinations need to be screened.

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