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Plant-growth-promoting compounds produced by two agronomically important strains of *Azospirillum brasilense*, and implications for inoculant formulation

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Abstract We evaluated phytohormone and polyamine biosynthesis, siderophore production, and phosphate solubilization in two strains (Cd and Az39) of *Azospirillum brasilense* used for inoculant formulation in Argentina during the last 20 years. Siderophore production and phosphate solubilization were evaluated in a chemically defined medium, with negative results. Indole 3-acetic acid (IAA), gibberellic acid (GA_3), and abscisic acid (ABA) production were analyzed by gas chromatography-mass spectrometry. Ethylene, polyamine, and zeatin (Z) biosynthesis were determined by gas chromatography-flame ionization detector and high performance liquid chromatography (HPLC-fluorescence and -UV), respectively. Phytohormones IAA, Z, GA_3, ABA, ethylene, and growth regulators putrescine, spermine, spermidine, and cadaverine (CAD) were found in culture supernatant of both strains. IAA, Z, and GA_3 were found in all two strains; however, their levels were significantly higher ($p<0.01$) in Cd (10.8, 2.32, 0.66 μg ml^{-1}). ABA biosynthesis was significantly higher ($p<0.01$) in Az39 (0.077 μg ml^{-1}). Ethylene and polyamine CAD were found in all two strains, with highest production in Cd cultured in NFb plus L-methionine (3.94 ng ml^{-1} h^{-1}) and Az39 cultured in NFb plus L-lysine (36.55 ng ml^{-1} h^{-1}). This is the first report on the evaluation of important bioactive molecules in strains of *A. brasilense* as potentially capable of direct plant growth promotion or agronomic yield increase. Az39 and Cd showed differential capability to produce the five major phytohormones and CAD in chemically defined medium. This fact has important technological implications for inoculant formulation as different concentrations of growth regulators are produced by different strains or culture conditions.

Keywords *Azospirillum* · Abscisic acid · Gibberellic acid · Indole 3-acetic acid · Ethylene · Cadaverine · Plant-growth-promoting rhizobacteria

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Introduction

Agricultural manipulation of symbiotic and free-living plant-growth-promoting rhizobacteria has become a significant component of modern agricultural practice in many countries (Bashan and Holguín 1998). For this purpose, the most successful plant–bacteria relationships have been those involving symbiotic leguminous *Rhizobium* and free-living, nonleguminous *Azospirillum, Pseudomonas, Bacillus*, and *Azotobacter* (Döbereiner and Pedroza 1987). In many reports, *Azospirillum* is considered the most important rhizobacterial genus for improvement of plant growth or crop yield worldwide (Bashan et al. 2004). This group of bacteria initially interested researchers because of their capacity: (a) to fix atmospheric nitrogen under microaerophilic conditions; and (b) to colonize internal tissues of gramineous plants, like endophytic rhizobacteria. Based on these factors, a number of inoculant companies experimented with agronomical application of liquid or solid formulation of *Azospirillum* sp. in seeds of gramineous plants such as wheat or maize, and the technique was slowly introduced into agriculture on a small scale. The early formulations had limited success in extending viability of the bacteria long enough for use in field conditions and in increasing crop yield homogeneously (Bashan and Holguin 1997). Evaluation of 20 years of data indicated that 60–70% of field experiments were successful, with yield increases ranging from 5 to 30% (Okon and Labandera-Gonzalez 1994). Yield increases could not be explained based on nitrogen fixation alone, because the bacterial contribution to plant growth is minimal (usually <5%; Bashan and Holguin 1997). In spite of many intensive studies on physiology and molecular biology of these bacteria, the exact mode of their effect on plants is still unclear. Various authors have proposed the following direct promoting mechanisms in addition to biological nitrogen fixation: (a) production of phytohormones such as indole 3-acetic acid (IAA), gibberellic acid (GA_3) and ethylene (Bashan et al. 2004), zeatin (Z; Tien et al. 1979), and abscisic acid (ABA); (b) production of plant growth regulatory substances such as polyamines (Thuler et al. 2003), particularly cadaverine (CAD), which may be correlated with root growth promotion (Niemi et al. 2002) and osmotic stress response in plants (Aziz et al. 1997); (c) phosphate solubilization (Seshadri et al. 2000); (d) siderophore production (Saxena et al. 1986). Today, the most common explanation for the effect of rhizobacteria on plants is based on the production of phytohormones that alter plant metabolism and morphology, leading to improved mineral and water absorption. The Additive Hypothesis of Bashan and Levanony (1990) proposes that multiple mechanisms operate simultaneously or in succession to promote plant growth.

Formulation of *Azospirillum brasilense* inoculants in Argentina

From 1981 to 1996, an intensive microbiological program was carried out in Argentina by the Instituto de Microbiología y Zoología Agrícola (IMYZA), in collaboration with the Instituto Nacional de Tecnología Agropecuaria (INTA) The main aims of the program were: (a) identification of strains of *Azospirillum* sp. isolated to different agroecological environment, (b) selection of the best strains for potential use in formulation of inoculants; (c) evaluation of the yield and productivity increase capacity for each isolated strain in comparison with *Azospirillum brasilense* Cd (used as "reference" strain), inoculated on wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) in various field sites near Buenos Aires, Argentina. Fifty different isolates of *Azospirillum* sp. were obtained, 25% corresponding to *A. brasilense* and 10% to *A. lipoferum*. Az39 and Cd strains of *A. brasilense* were selected based on their ability to increase yield and productivity by 13 and 33%, respectively, in wheat and maize. These results led the Servicio Nacional de Sanidad Agropecuaria (SENASA), together with Argentinean inoculants companies, to recommend *A. brasilense* Az39 for formulation of maize and wheat inoculants in Argentina.

From the physiological point of view, plant growth promotion mechanisms other than nitrogen fixation in *A. brasilense* Az39 or Cd have not been systematically evaluated. The principal aim of the present study was to evaluate phytohormone and polyamine biosynthesis, siderophore production, and phosphate solubilization in these two strains, which have been successfully used for inoculant formulation in Argentina during the past 20 years.

Materials and methods

Azospirillum strains

A. brasilense Az39, recommended for maize and wheat inoculation in Argentina, was provided by Ing. Agr. Alejandro Perticari (Agriculture Collection Laboratory; IMYZA-INTA, Argentina; see "Introduction"). *A. brasilense* Cd, recommended worldwide for inoculation of various crop plants, was obtained from American Type Culture Collection (ATCC 29710).

Culture media

Bacteria were grown in NFb (Döbereiner et al. 1976). The formulation was modified for ethylene and CAD determination by addition of (g l^{-1}): methionine (0.1; NFb*m*) or L-lysine (0.1; NFb*l*). For determination of phosphate solubilizing compounds, pentacalcium phosphate agar (TPA; g l^{-1}; Katznelson and Bose 1959) composed of soybean trypticase broth (10.0), $Ca_5(PO_4)_3OH$ (4.0), and agar (15.0) pH 6.8 was used. For siderophore determination, chrome-azurol medium (CAS; Schwyn and Neilands 1987) was used.

Culture conditions

A. brasilense Az39 and Cd were grown in individual 250 ml flasks containing 100 ml NFb medium at 30°C and 80 rpm shaking, until exponential growth phase (OD_{600} ~1), equivalent, respectively, to 0.98 and 1.02×10^8 colony-forming units (c.f.u.) ml^{-1} in NFb-agar. An aliquot was taken from each pure culture for evaluation of phosphate solubilization, siderophore production, and phytohormone identification and quantification.

Phosphate solubilization

Phosphate solubilization was measured on TPA plates by the methods of Katznelson and Bose (1959). Plates were inoculated with 1 µl NFb pure bacterial culture in halfway points of a Petri dish plate containing trypticase soya agar medium with $Ca_5(PO_4)_3OH$. Experiments were performed in triplicate. Plates were incubated at 30°C and observed daily for formation of transparent halos around each colony for up to 4 days. Positive control was made with *Pseudomonas fluorescens* strain P1 (provided by the Agriculture Collection Laboratory, IMYZA-INTA) in similar culture conditions.

Siderophore production

Siderophore production was determined by the method of Schwyn and Neilands (1987). Plates were seeded with 1 µl NFb pure bacterial culture in halfway points of a Petri dish plate containing agar CAS medium. Experiments were performed in triplicate. Plates were incubated at 30°C and observed daily for orange color formation around each colony for up to 4 days. Positive control was made with *P. fluorescens* strain P1 (provided by the Agriculture Collection Laboratory, IMYZA-INTA) in similar culture conditions.

Identification and quantification of IAA, ABA, GA3, and Z

Bacterial cultures (NFb) in exponential growth phase as defined in "Culture conditions" were separated into several 20 ml fractions for determination of IAA, ABA, GA_3, and Z. Fractions were centrifuged at 8,000 rpm for 20 min at 4°C, and supernatants were acidified at pH 2.5 with acetic acid solution (1% v/v). Individual samples were then added with 100 ng of corresponding $^2H_6$-ABA (kindly supplied by Prof. Richard P. Pharis, Univ. of Calgary, Canada), $^2H_5$-IAA, or $^2H_2$-GA_3 (OlChemIm, Czech Republic) deuterated internal standard and kept at 4°C for 2 h. No deuterated internal standard was used for Z. Each sample was partitioned four times with the same volume of acetic-acid-saturated ethyl acetate (1%, v/v). After the last partition, acidic ethyl acetate was evaporated to dryness at 36°C. Dried samples were diluted in 100 µl acetic acid/methanol/water (1:30:70) for ABA determination, acetic acid/acetonitrile/water (1:15:85) for IAA determination, and methanol/water (30:70) for GA_3 and Z determination. They were injected into a reverse phase C_{18} HPLC column (µBondapack, 300×3.9 mm, Waters Associates, Milford, MA) in a Konik 500 (Konic Instruments) system coupled to a UV-Vis Konik 3000 diode-array spectrometer. For each sample, elution was performed at 1 ml min^{-1} flow rate, and fractions eluting at the retention time corresponding to each pure standard were collected.

Z was identified and quantified by HPLC-UV at 254 nm (Tien et al. 1979). IAA, ABA, and GA_3 were identified and quantified by gas chromatography-mass spectrometry with selective ion monitoring (GC-MS-SIM). UV-absorbing fractions at 254, 262, and 220 nm were grouped for IAA, ABA, and GA_3 determination, respectively, then methylated with ethereal diazomethane and silylated with 1:1 pyridine/BSTFA [bis (trimethylsilyl) trifluoroacetamide] plus 1% trimethyl-chlorosilane (Fluka Chemika, Switzerland) to obtain methyl-trimethylsilyl derivatives of IAA, ABA, and GA_3. Aliquots of each sample were injected directly into a DB1-15N (15 m×0.25 mm, 0.25 µM methyl silicone) capillary column (J&W Scientific) fitted in a Hewlett-Packard 5890 Series II GC with a capillary direct interface to a 5970B Mass Selective Detector. The GC temperature program was 60 to 195°C at 20°C min^{-1}, then 4°C min^{-1} to 260°C. Carrier gas (He) flow rate was 1 ml min^{-1}, interface temperature was 280°C, and data acquisition was controlled by a HP 300 Series computer. The amount of free ABA was calculated by comparison of peak areas of the ion at a mass/charge (m/z) 196 (molecular ion for [$^2H_6$]ABAMeTMSi) and the ion at m/z 190 (molecular ion for [^{1}H]ABAMeTMSi) at the corresponding time (Kovats 1958). Similarly, we calculated the amount of free IAA by comparison of peak areas for the parent ion (m/z) 194 and (m/z) 189 and amount of free GA_3 by comparison of peak areas for parent ion (m/z) 506 and (m/z) 504. Data from three experiments were analyzed by analysis of variance (ANOVA) followed by post hoc Tuckey test.

Identification and quantification of ethylene

Ethylene production was measured by gas chromatography-flame ionization detection (Strzelczyk et al. 1994). NFb bacterial culture (1 ml) in exponential growth phase (OD_{600} ~1) was transferred to 250 ml Erlenmeyer flasks containing 100 ml NFb or NFb*m*. Flasks were fitted with rubber plugs tightened with metal cowls and incubated 48 h at 30°C and 80 rpm, corresponding to 1.25 and 1.28×10^8 c.f.u. ml^{-1} in NFb and 1.34 and 1.31×10^8 c.f.u. ml^{-1} in NFb*m*, for Cd and Az39, respectively. To test ethylene production, a 500 µl air sample was taken from the flasks after 7d and analyzed by a Konik KNK 3000 gas chromatograph equipped with Kromapek column (2 m length) packed with chromosorb W-AW-DCMS, operated isothermally at 110°C, with nitrogen as gas carrier and a flame ionization detector (170°C). Pure ethylene (Air Liquid Group, Argentina) was used as standard. Experiments were performed in triplicate.

Identification and quantification of CAD and other polyamines

These substances were evaluated by the method of Tiburcio et al. (1997). Az 39 and Cd bacterial cultures were collected from logarithmic growth phase (8 h) by centrifugation at 15,000 g, 4°C, 10 min. An aliquot of (mmol l^{-1}) heptadiamine (HTD; 0.1) was added and supernatants were treated with pure perchloric acid to obtain a 5% (v/v) solution and centrifuged at 8,000 g. Supernatants were neutralized with sodium bicarbonate, added with 400μl (g 1^{-1}) dansyl chloride acetone solution (20.0), and kept in darkness for 16 h. Each sample was then added with 100 μl (g 1^{-1}) proline acuose solution (100.0) and kept in darkness for 30 min. Dansyl-amides were extracted in 500 μl toluene. The organic phase was evaporated, resuspended in 100 μl acenonitrile, and injected in a HPLC-fluorescence (ISCO) system for identification at 510 nm. Quantification was performed by injection of individual standard solutions of (mmol l^{-1}) putrescine (0.1), spermine (0.1), spermidine (0.1), and CAD (0.1). Relative abundance of each polyamine was compared with HTD at the same retention time.

Results

Our results indicate that *A. brasilense* strains Az39 and Cd show differential patterns of plant growth regulator (e.g., CAD) and phytohormone biosynthesis (e.g., IAA, Z, ABA, GA_3, and ethylene). Production and release of regulators and phytohormones in defined media was the only direct putative plant growth promoter mechanism detected in these strains, and quantitative differences were found among them. Two strains grew satisfactorily in these experimental conditions; however, no production of siderophores or phosphate solubilizers was observed.

IAA production was significantly (almost four times) higher for Cd, which produced 10.8 μg ml^{-1} l in chemically defined NFb medium, compared to Az39, which produced 2.9 μg ml^{-1} l in exponential phase (Fig. 1a).

Z production was also significantly (over three times) higher for Cd (2.37 μg ml^{-1} l) in Nfb medium compared to Az39 (0.75 μg ml^{-1} l) in exponential phase (Fig. 1b).

GA_3 production was also higher for Cd (0.66 μg ml^{-1} l) than for Az39 (0.30 μg ml^{-1} l; Fig. 1c).

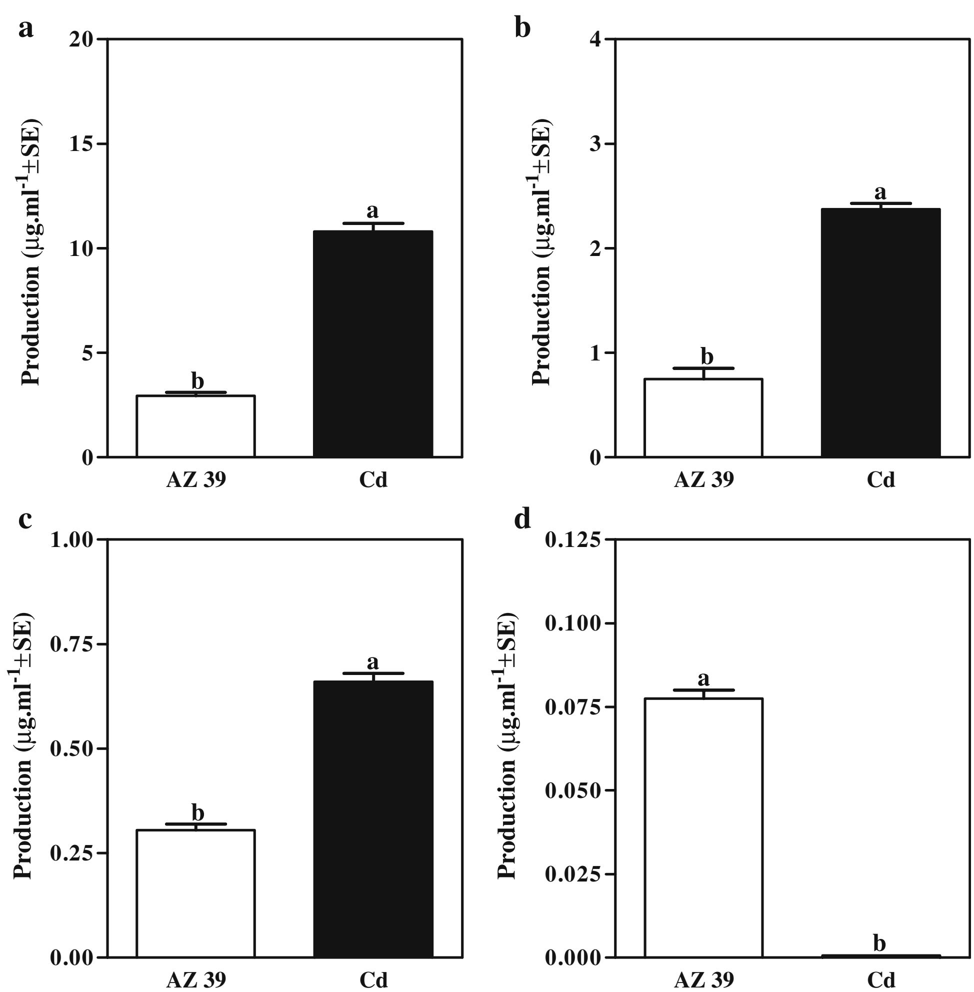

Fig. 1 Identification and quantification of phytohormones by HPLC-UV-GC-MS-SIM, obtained from chemically defined medium of *A. brasilense* Az39 (*white bars*) and Cd (*black bars*): **a** IAA, **b** Z, **c** GA_3, and **d** ABA obtained from 48 h NFb cultures at 30°C and 80 rpm. Data were analyzed by ANOVA and post hoc Tuckey test

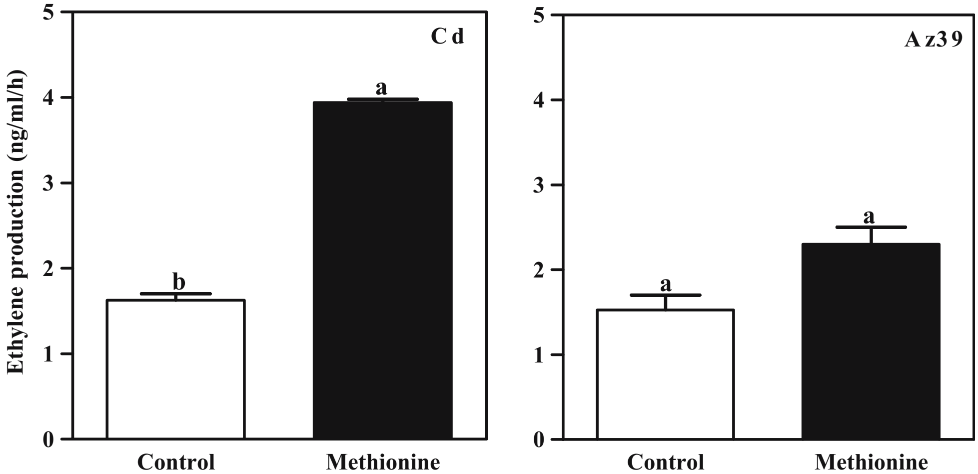

Fig. 2 Identification and quantification of ethylene by FID-HPLC obtained from 0.1 g l^{-1} NFb (*white bars*) and NFb plus methionine (*black bars*) cultures of *A. brasilense* Az39 and Cd at 30°C and 80 rpm for 48 h. Data were analyzed by ANOVA and post hoc Tuckey test. Values with the same letter are not significantly different at $p<0.01$

In contrast, ABA production was almost 12 times higher for Az39 (7.70 ng ml^{-1} l) in chemically defined Nfb medium than for Cd (0.65 μg ml^{-1} l) in exponential phase (Fig. 1d).

Ethylene biosynthesis in NFb or NFb*m* culture medium was observed in both Az39 and Cd, but there were significant differences in their capacity to produce and release the gas in the presence or absence of L-methionine. Ethylene production in NFb*m* medium was higher for Cd (3.94 ng ml h^{-1}) than for Az39 (2.32 ng ml h^{-1}; Fig. 2).

Polyamine biosynthesis in NFb or NFb-*l* (0.1) culture medium, for both Az39 and Cd, was altered by the presence of CAD precursor L-lysine (Fig. 3). In NFb medium, the most abundant polyamine was spermidine, which reached a concentration of 155.0 nmoles ml^{-1}in Az39 and 107.0 nmoles ml^{-1} in Cd. Putrescine, spermine, and CAD concentrations were 18.37, 17.7, and 10.04 nmoles ml^{-1} for Az39 and 11.50, 16.95, and 6.10 nmoles ml^{-1} for Cd, respectively, under the same physiological growth conditions. In NFb-*l* medium, the most abundant polyamine was

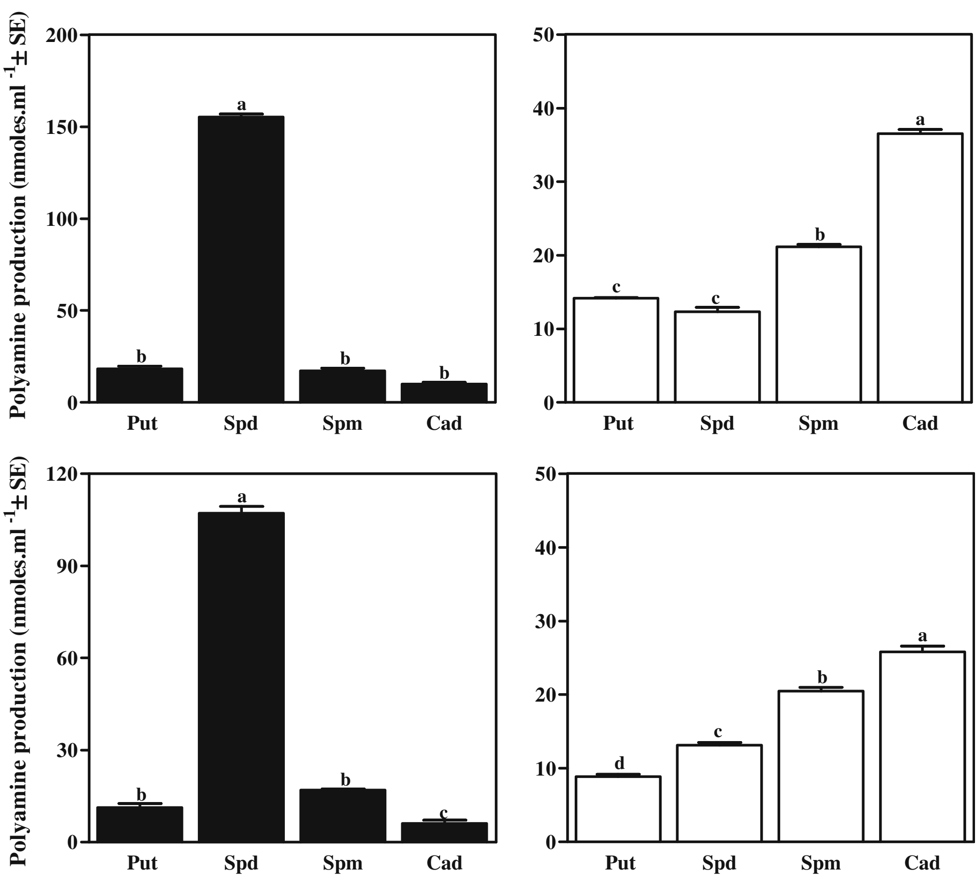

Fig. 3 Identification and quantification of polyamines by *HPLC-fluorescence*, obtained from 0.1 g^{-1} l NFb (*black bars*) or NFb-*l* lysine (*white bars*) cultures of A. *brasilense* Az39 and Cd at 30°C and 80 rpm for 48 h. Data were analyzed by ANOVA and post hoc Tuckey test. Values with the same letter are not significantly different at $p<0.01$

CAD, which reached a concentration of 36.55 nmoles ml^{-1} for Az39 and 25.8 nmoles ml^{-1} for Cd. Putrescine, spermidine, and spermine concentrations were 14.13, 12.35, and 21.13 nmoles ml^{-1} for Az39 and 8.75, 13.55, and 20.47 nmoles ml^{-1} for Cd, under the same physiological growth conditions.

Discussion

A. brasilense Az39 and Cd are commonly used, free-living plant-growth-promoting rhizobacteria capable of affecting the yield of numerous plant species of agronomic interest. This promotion capacity has been studied mainly in terms of biological nitrogen fixation. Inoculation assays have demonstrated variability in growth promotion and yield increases. Differential capacity of bacterial strains to produce and release plant-growth-regulating compounds in chemically defined media, as well as during inoculant formulation, could lead to differential plant growth response.

An inoculant is a complex biological formulation that combines two elements: cultured microorganisms, and compounds secreted into their growth medium under controlled conditions. A commercially available inoculant should not be considered as a carrier of microorganisms, but as a complex resulting from the biotransformation, by the bacteria, of components added to the growth medium into different metabolites. Biological activity of phytohormones may greatly influence processes such as early germination, early seedling growth, plant colonization, and bacterial establishment. Results of the present study show that two strains of *A. brasilense*, Az39 (most widely used for inoculant formulation in Argentina) and Cd (most commonly used for research worldwide), possess the capacity to produce and release plant-growth-promoting substances such as IAA, Z, GA_3, ABA, ethylene, and polyamines.

IAA production for *A. brasilense* Az39 (Fig. 1a) was comparable to that reported by other authors for *A. lipoferum* (4.1 μg ml^{-1}) and other *A. brasilense* strains (4.5 μg ml^{-1}) in chemically defined media (Crozier et al. 1988). In contrast, Cd showed higher (10.8 μg ml^{-1}) production than Az39. Cd may therefore be a superior inoculant, because IAA production has been correlated with various physiological processes that improve root growth and development. Kolb and Martin (1985) showed that inoculation of *Beta vulgari*s with *A. brasilense* led to increased number of lateral roots, which was correlated with high concentration of IAA in pure bacterial cultures. Exogenous application of the hormone caused an effect similar to that seen in inoculated plants. Dobbelaere et al. (1999) determined that inoculation of wheat cv. Soisson with 10^8 c.f.u. ml^{-1}*A. brasilense* sp. 245 was comparable to exogenous application of IAA.

Z production was determined for strains Cd and Az39 as 2.37 and 0.75 μg ml^{-1}, respectively (Fig. 1b). Horemans et al. (1986) detected production of Z and other cytokines in *A. brasilense* in chemically defined medium by radioimmune analysis (RIA). Tien et al. (1979) used HPLC to demonstrate that *A. brasilense* produces cytokine-like compounds with biological activity equivalent to that of K (kinetin) in chemically defined medium. Tien et al. also showed that inoculated pearl millet (*Penisetum americanum* L.) had an increased number of lateral and hair roots compared to noninoculated plants and attributed this response in part to *Azospirillum* cytokine production and release. Pan et al. (1999) reported that exogenous application of 0.2 μg ml^{-1} K in maize seeds, with *Serratia liquefaciens* inoculation, increased root size and weight.

GA_3 production was significantly higher in Cd (0.66 μg ml^{-1}) than in Az39 (0.30 μg ml^{-1}; Fig. 1c). Bottini et al. (1989) evaluated the capacity of *A. lipoferum* to produce GA_1 and GA_3 in chemically defined NFb medium. Supernatant culture concentration was estimated by biological test, and it showed an equivalent GA_3 production of 20 and 40 pg ml^{-1}, respectively, for 10^9 c.f.u. ml^{-1}. Janzen et al. (1992) measured production of GA_1 and GA_3 in *A. brasilense* Cd in chemically defined medium. In this case, GA_3 production reached 5.0 μg ml^{-1} for 10^7 c.f.u. ml^{-1}.

ABA production was significantly higher for Az39 (7.70 ng ml^{-1}) than for Cd (0.65 μg ml^{-1}; Fig. 1d). There are very few reports on identification of ABA in chemically defined media, or in plants inoculated with *Azospirillum* sp. Kolb and Martin (1985) reported ABA production by *A. brasilense* strain Ft326, but the method used (radioimmunological assay; RIA) may be less sensitive. The physiological role of bacterial ABA supply in plant–microbe interaction is dubious, and there is no direct evidence that this phytohormone promotes or regulates plant growth. However, in restrictive (e.g., saline) soils, microbial ABA may conceivably contribute, along with other bacterial molecules such as CAD (Aziz et al. 1997), to the plant's "stress response." This is a recently emerging line of research, particularly in free-living microorganisms that can be classified as a third group of beneficial bacteria in plants, termed "Plant Stress Homeo-regulating Rhizobacteria (PSHR)" by Cassán et al. (2005).

Ethylene biosynthesis in NFb or NFb*m* culture medium was showed in both Az39 and Cd (Fig. 2) in the presence or absence of L-methionine. Ethylene production was higher in NFb*m* medium for Cd (3.94 ng ml h^{-1}). In contrast to our results, Strzelczyk et al. (1994) found that ethylene production in chemically defined medium was completely dependent on the presence of L-methionine. The promoting effect of *Azospirillum* sp. on root growth may depend in part on plant ethylene production mediated by microbial auxin biosynthesis, or on bacterial ethylene production. Ribaudo et al. (2006) evaluated plant growth in rice seedlings (*Oryza sativa* L. var. Yeruá) inoculated with *A. brasilense* strain FT326 (IAA super producer) and showed a correlation between length and number of lateral and hair roots and plant ethylene biosynthesis (tenfold compared to control), mediated by bacterial IAA. Peck and Kende (1995) found that the limiting step for ethylene biosynthesis is the conversion of *S*-adenosylmethionine to 1-aminocyclopropane-1-carboxylic acid mediated by the ACC synthase, which is increased by exogenous application of IAA.

Polyamine biosynthesis was observed in both Cd and Az39, and the production pattern was affected by presence of L-lysine in culture medium (Fig. 3). The most abundant polyamine in *Azospirillum* sp. was spermidine, which reached 155.0 nmoles ml^{-1} in Az39 and 107.0 nmoles ml^{-1} in Cd. Similar results were obtained by Hamana et al. (1988), who reported spermidine and homospermidine as the most abundant polyamines in *Azospirillum, Rhodospirillum*, and other members of the α-group of *Proteobacteria*. Thuler et al. (2003) reported predominant production of putrescine and spermine in various *Azospirillum* strains isolated from manioc roots (*Manihot* sp.).

In the present study, CAD production was observed with or without addition of L-lysine to culture medium; This is the first report of such result in *Azospirillum* sp. L-lysine is a natural precursor of diamine CAD in a one-step reaction mediated by lysine decarboxylase enzyme. The physiological role of bacterial CAD in plant–microbe interactions is not yet clear. However, two published experiments suggest possible growth regulation by CAD in plants, in terms of: (a) increased lateral root growth (Niemi et al. 2002), (b) improved osmotic stress response (Aziz et al. 1997). One of the authors (Cassán F.) observed previously that *A. brasilense* inoculation in rice seedlings (*O. sativa* L.) cv. El Paso promoted root growth, at least in part through bacterial CAD production (unpublished data).

Our results indicate that *Azospirillum* strains Az39 and Cd have the potential capacity to promote plant growth through direct physiological mechanisms such as phytohormone and CAD production, in addition to biological nitrogen fixation. Both strains showed an intrinsic capacity to produce and release various growth-promoting compounds in chemically defined media. Evaluation of all growth-promoting components in inoculant bacterial strains is very important for accurate quality control and strain selection criteria establishment according to the species and soil characteristics where the inoculant will be used.

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