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REGULAR ARTICLE

Screening of plant growth-promoting traits in arsenic-resistant bacteria isolated from the rhizosphere of soybean plants from Argentinean agricultural soil

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Abstract
Aims The purpose of this study was to investigate plant-growth promoting traits in native and arsenic (As) highly-resistant bacterial strains isolated from the rhizosphere of soybean (*Glycine max*) plants grown in an Argentinean agricultural field.
Methods Determination of MICs (Minimum inhibitory concentration) was carried out on solid media supplemented with arsenite (As 3+) or arsenate (As 5+). Morphological, cultural, physiological, biochemical and molecular characterization, and in vitro determination of plant growth promoting (PGP) properties of As resistant isolates were carried out. Arsenic in soil samples was determined by ICP-OES while residual arsenic on post-removal culture medium and accumulation in cells were estimated by GF-AAS after wet acid digestion.
Results Isolated strains included γ-proteobacteria such as *Enterobacter sp.* and *Pseudomonas sp.,* and actinobacteria as *Rhodococcus sp.* All bacterial strains grew in presence of very high arsenite -over 24 *mM*- and arsenate –over 400 *mM*- concentrations. *Pseudomonas* sp. strains presented simultaneously several in vitro PGP traits, although *Rhodococcus erythropolis* AW3 did not display PGP traits. However, *R. erythropolis* AW3 was the most As resistant strain and removed and accumulated the greatest amounts of the metalloid.
Conclusion The presence of As resistant and plant-growth promoting bacterial strains in the rhizosphere of *Glycine max,* in arsenic containing agricultural soil, suggest that they could potentially play an important role in plant-growth promotion in stressed conditions. These strains were able to remove and accumulate As from liquid media, thus they could be beneficial for sustainable crop production.

Keywords Arsenic resistance · Soybean · Plant-microbe interaction · Plant growth promotion · Agricultural soil

Introduction

Arsenic is a toxic metalloid widely distributed in the environment as a result of natural geochemical processes and anthropogenic activities. It has been classified as a human carcinogen by the International Agency for Research on Cancer (IARC 1987) and the US Environmental Protection Agency (EPA 1988). The two major forms of inorganic arsenic are the reduced form, arsenite (As 3+), and the oxidized form, arsenate (As 5+) (Cullen and Reimer 1989). In the soil environment, both inorganic forms are present and can be taken up by plants, thus food chain contamination is an important risk in

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arsenic containing soils. Arsenic bioavailability in soil is mainly determined by speciation and redox state. Although As 5+ is found primarily in aerobic conditions, it typically remains bounded to minerals in the solid phase and thus is less available to be taken up by plants. On the other hand, As 3+ is most common in the aqueous phase in soils, where it is more mobile and can be taken up by plants more easily under most environmental conditions.

In Argentina, besides anthropogenic sources, As occurs naturally in soils in the central and northern portion of the country (Smedley et al. 2002, 2005; Bundschuh et al. 2000, 2004) because of weathering of arsenic-rich large scale polymetalliferous emplacements. Thus, elevated arsenic concentrations have been detected in natural groundwaters and some soils. Arsenic concentration determinations in these contaminated groundwaters have often exceed the safe drinking water limit of $1{,}3\times10^{-4}mM$ recommended by World Health Organization (WHO 1993) and by the Argentine drinking water standard (CAA 1994). In particular, in the south of Córdoba province, an important agricultural area, As groundwater concentrations higher than $2\times10^{-2}mM$ have been found (Bundschuh et al. 2008). In these areas, huge quantities of As contaminated water are usually used for irrigation purposes which increases As accumulation in soils. In addition, use of arsenical pesticides and herbicides is other possible source of As, although its application is actually illegal. This situation poses an important health risk since there is potential for inadequate levels of arsenic to enter the food chain.

In the plant rhizosphere, As bioavailability and mobility is strongly influenced by native microbial communities, through changes in pH, redox potential, organic matter proportion and mineral constitution. For example, As 5+ reducing bacteria have been found to mediate the reduction of As 5+ under highly aerobic conditions resulting in enhanced mobilization of As from limed mine tailings (Macur et al. 2001). On the other hand, soil bacterial populations frequently secrete plant growth-promoting (PGP) substances like siderophores, phytochelators and other compounds that alter metal bioavailability and complexation and thus alleviate metal toxicity (Kamaludeen and Ramasamy 2008). As a result, in recent years, research and application of PGP bacteria has been extended for treatment of contaminated soils in association with plants (Cavalca et al. 2010).

In this work, the legume soybean was selected because it is a principal crop in the studied region in Argentina, and it is an important high-protein feed for humans and animals. Moreover, legumes are important components of biogeochemical cycles in agricultural and natural ecosystems, since atmospheric nitrogen fixation by legume-microorganism symbiosis enhances site fertility (Reichman 2007). The aim of this study was to investigate plant-growth promoting traits in native and As highly-resistant bacterial strains isolated from soybean rhizosphere for subsequent studies of plant-microbe interactions and development of strategies that minimize health risk in food production and lead to better and sustainable agriculture programs.

Materials and methods

Site description and plant material

An intensive agricultural soil (Paraje La Escondida, Alcira Gigena, Córdoba, Argentina, located at 32°40′ 83″S, 64°33′40″W) used for soybean cropping, without inoculation treatment at the time of sampling (March 2009) was studied. The non-inoculated plants were extracted and the upper 20 cm of the plant root system with undisturbed soil around the roots was manually removed, placed in plastic bags to avoid moisture loss and delivered to the laboratory on the same day. The handling of the root-soil system to obtain the two soil fractions (bulk and rhizosphere) for separate analyses is described. The bulk soil fraction was obtained by carefully shaking the plants to remove non-adhering soil. The bulk soil fractions collected from all samples were pooled together. Then the roots were vigorously shaken in water for 1 h. After that, they were removed and the suspensions were defined as the rhizosphere soil fraction. The pH of these soil suspensions was checked. A sample of non-contaminated bulk soil from a confined area of the Río Cuarto National University was also processed in the same way and used as control soil.

Chemical analyses for As and other metals concentration in soil samples

The determination of metal content in soil samples was carried out in the University of Seville (Spain)

with the collaboration of Dr. E. Pajuelo. Samples of 1 g of bulk soil were dried in an oven for 48 h at 60 °C on filter paper. The soil was triturated and homogenized using sieves from a pore size of 5 mm, in order to get rid of small stones, to a final size of 0.21 mm. The metal elements were determined by Inducted Coupled Plasma Optical Spectrophotometry (ICP-OES) after aqua regia treatment in a microwave system according to Murillo et al. (1999). A BCR Certified Reference Material was used as quality control of the analytical procedures. Final results, obtained as mean values of ICP-OES determination conducted in triplicate, were expressed as $mg\,kg^{-1}$ dry weight.

Isolation of rhizospheric arsenic-resistant bacteria

To isolate As-resistant bacteria, appropriate dilutions of rhizospheric soil suspensions were plated onto solid YEM (Yeast Extract Mannitol) agar (Vincent 1970) containing $4\,mM$ As 3+, in the form of sodium *m*-arsenite (*m*-$NaAsO_2$) (Sigma), and the plates were incubated at 25±2 °C for 10 days. After incubation, colonies with varying morphologies were selected from the plates and streaked for purity on the same medium at least five times. The strains were maintained in glycerol stocks at −80 °C.

Arsenic resistance and oxidation tests

As resistance in isolated bacterial strains was evaluated by minimum inhibitory concentration (MIC) tests onsolid mediain order to compare results with literature, Agar plates containing either YEM or LB (Luria Bertani) media, with increasing As 3+ or As 5+ concentrations were used. Arsenite was used at concentrations from 12 to $38\,mM$.Arsenate resistance was also tested in the range of 400–$700\,mM$. These concentrations may be considered extreme, however many authors have found these levels of resistance in bacterial strains isolated from diverse environments. Plates were incubated at 25±2 °C for 15 days. Prior to use, the strains were grown 24 hs in YEM broth at 25±2 °C in agitation.

The obtained arsenite-resistant bacteria were tested for their abilities to oxidize As 3+ using a qualitative $KMnO_4$ screening method modified from Salmassi et al. (2002). Each arsenite-resistant bacterium was inoculated in MM9 broth with a final concentration of $1\,mM$ $NaAsO_2$ and then shaken at 110 rpm for 3 days at 28 °C. For each isolate 1 mL culture was added to a 1.5 mL centrifuge tube containing 30 μL of $0.01\,M$ $KMnO_4$ and the color change of $KMnO_4$ was monitored. A pink color of the mixture indicated a positive arsenite oxidation reaction (formation of As 5+). Sterile MM9 medium with and without the same amount of $NaAsO_2$ were used as control.

Furthermore, the ability for As 3+ oxidation of the bacterial strains was also evaluated by use of the $AgNO_3$ method (Lett et al. 2001). Agar plates were flooded with a solution of $0.1\,M$ $AgNO_3$. A brownish precipitate revealed the presence of As 5+ in the medium (colonies expressing arsenite oxidase), while the presence of As 3+ (lacking arsenite oxidase) was detected by a bright yellow precipitate.

Morphological, physiological and biochemical tests

As a first-step approach to the identification of the isolates, morphological characterization of each bacterial colony was done after growth on Petri dishes. Gram determination (Gram stain kit; Britannia Labs, Argentina) as well as catalase and oxidase tests were carried out. Biochemical analyses were done by using the standardized micromethod Api 20 NE and Api 20 E (BioMérieux SA, Lyon, France) following manufacturer instructions. Substrate assimilations were registered after 24 and 48 h. Interpretation of the results was performed after 48 h using the Api identification software version 6.0.

Identification by 16s rRNA gene sequencing

To confirm the results observed in the morphological and biochemical characterization, a fragment of the 16S rRNA gene of each isolate was amplified by PCR using universal primers and sequenced by Macrogen Company Inc. (Seoul, Korea). The 16S rRNA gene sequences were then compared to the entire GenBank nucleotide database using the Basic Local Alignment Search Tool N (BlastN) program. (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Qualitative determination of plant-growth promoting characteristics of isolates

Metalloid resistant isolates were tested for their ability to grow on 1-aminocyclopropane-1-carboxylate (ACC) as

the sole nitrogen source, to solubilize phosphate, and to produce siderophores and indole acetic acid (IAA).

Utilization of ACC as the sole nitrogen source

The ability to use ACC as nitrogen source is a consequence of enzymatic activity of ACC deaminase. Bacteria were first cultured in solid rich medium (LB) and then transferred into Erlenmeyer flasks with a minimal medium containing KH_2PO_4 0.4 gL^{-1}, K_2HPO_4 2 gL^{-1}, $MgSO_4 \cdot 7H_2O$ 0.2 gL^{-1}, $FeSO_4 \cdot 7H_2O$ 0.1 gL^{-1}, $CaCl_2$ 0.1 gL^{-1}, NaCl 0.2 gL^{-1}, $NaMoO_4 \cdot 2H_2O$ 0.005 gL^{-1}, glucose 10 gL^{-1} and ACC 0.3 gL^{-1}. Stock solution of ACC (0.15*M*) (Sigma) was filter-sterilized (0.2 μm), aliquoted and frozen at −20 °C. Prior to inoculation, the ACC solution was thaw and appropriately added to sterile minimal medium. Each strain was also inoculated into Erlenmeyers flasks containing the same medium with and without $(NH_4)_2SO_4$ 2 gL^{-1} as nitrogen source as controls. A strain of *Pseudomonas putida* capable to use ACC was used as the positive control. Following inoculation, the cultures were incubated at 25±2 °C on a rotary shaker at 110 rpm for 192 h. Bacterial growth was monitored for every 48 h by measuring optical density at 600 nm.

Qualitative screening of phosphate-solubilizing bacteria

The ability of isolates to solubilize phosphate was tested in NBRI-BPB medium containing the dye bromophenol blue (BPB) according to Mehta and Nautiyal (2001). The pH of the media was adjusted to 7.0 before autoclaving. The plates were incubated for 7 days in the dark at 25±2 °C. Clear halos around the colonies on blue agar were indicative of phosphate solubilization.

Production of siderophores

The ability of isolates to produce siderophores was checked by growing the bacterial strains on Chrome azurol S (CAS) (Sigma) agar plates as described by Schwyn and Neilands (1987). The plates were incubated for 7 days in the dark at 25±2 °C. Orange halos around the colonies on blue agar were indicative of siderophores production.

Production of indole acetic acid (IAA)

IAA production was tested using the protocol described by Glickman and Dessaux (1995). Bacteria were first cultured for 24 h in Erlenmeyers flasks containing 10 mL LB medium. Then the isolates were re-inoculated in Erlenmeyers flasks containing 10 mL LB medium with and without 0.5 mg mL^{-1} tryptophan. After incubation in the dark during 72 h at 110 rpm, 1.5 mL of the cell suspension was transferred into a tube and centrifuged for 10 min at 10,000 rpm. Then 1 mL of the supernatant was mixed vigorously with 2 mL Salkowski's reagent (37.50 mL H_2SO_4, 1.88 mL 0.5 M $FeCl_3 \cdot 6H_2O$ and 60.62 mL distilled H_2O) allowed to stand at room temperature for 25 min, after which absorbance at 530 nm was determined. Final concentrations expressed as $\mu g mL^{-1}$ were calculated from an adjusted calibration curve. A strain of *Azospirillum sp.* that produces IAA was used as a positive control (Kaushik et al. 2000) and non-inoculated LB medium was the negative control.

Determination of arsenite removal and accumulation in bacterial biomass

For arsenite removal experiments, bacterial strains were grown at 25±2 °C, in the dark with continuous shaking at 110 rpm, in 20 mL YEM broth containing 4 *mM* sodium arsenite. Cultures were incubated 96 h, then they were centrifuged (10 000 rpm for 10 min) and the supernatants were used for As 3+ removal estimations. Then, the bacterial pellets were washed once with 1 mL of 20 mM EDTA to eliminate As adsorbed to the cells. After that, the pellets were dried till constant weight to measure total biomass and total As accumulation. A control medium without inoculum was included to evaluate possible arsenite losses by flask adsorption or evaporation (control 100 %). Total residual arsenic concentration and As accumulation in all samples was measured by a graphite furnace atomic absorption spectrophotometer (GF-AAS) technique after a wet acid digestion with nitric acid. Briefly, 100 mL of sample were heated and evaporated with 5 mL of HNO_3. Then, distilled water and acid were added till the solution cleared. The solution was cooled and filtered if necessary and taken to a final volume of 100 mL.

Results

Chemical analyses for As and other metals concentration in soil samples

Soil samples were collected in situ from an intensive agricultural field used for soybean cropping, without inoculation treatment at the time of sampling. The pH of the rhizospheric soil fraction from La Escondida sample was between 5.0 and 5.5. The concentrations of As were very similar between the studied soil and the control one, while the greatest differences were detected for Cr and Zn concentration (Table 1).

Isolation and biochemical characterization of rhizospheric arsenite-resistant bacteria

After several transfers on solid media, a total of six arsenite-resistant bacterial strains were isolated from soybean rhizosphere, and were named as AW1 to AW6. The isolation of native bacterial strains was possible on the basis of their ability to grow in the presence of As 3+ added at a concentration of 4 *mM*, selecting for highly arsenic-resistant bacterial strains, and according to the criteria of different colony morphology. Strain AW3 was Gram-positive, while all the others were Gram-negative (Table 1). All strains isolated were positive for catalase AW1 and AW3 were negative for oxidase while the others were positive. All As 3+ resistant bacteria were rods in the microscopy.

Minimum inhibitory concentrations

All isolates were investigated for their capability to grow at high concentrations of As 3+ or As 5+. The MIC, defined as the lowest concentration of arsenite/arsenate that inhibited growth in YEM solid media, was performed. Microbial resistance to arsenic species is widespread in nature but resistance to concentration of As 3+ higher than 20 *mM* is considered high (Cai et al. 2009). All isolated strains were highly resistant to As 3+ and As 5+, with MICs>20 *mM* and >400 *mM*, respectively (Table 2). Strain AW3 proved to be the most As resistant strain, still growing in the presence of 38 *mM* of As 3+ and 700 *mM* of As 5+.

Identification of rhizospheric arsenite-resistant bacteria

The identification of the isolates was confirmed by partially (1,400–1,500 bp) sequencing the 16S rRNA gene. The phylogenetic analyses indicated that the strains were allocated to 3 genera distributed among: *Gammaproteobacteria* (*Enterobacter* and *Pseudomonas)* and *Actinobacteria* (*Rhodococcus*).

Arsenite oxidation by isolates

All As 3+ resistant strains were tested for their ability to oxidize As 3+. None isolate was able to oxidize As 3+ even after a prolonged incubation period neither with $KMnO_4$ method or $AgNO_3$ method.

Characterization of potential PGP As-resistant rhizobacteria

In stressed environments, rhizosphere bacteria with PGP characteristics could play an important role in plant growth. To identify potential PGP rhizobacteria, the isolates were qualitatively screened for their ability to utilize ACC as the sole nitrogen source, and to solubilize phosphate, to secrete siderophores into the growth medium and to produce IAA. Table 3 shows that, with the exception of *R. erythropolis* AW3, all isolates possessed at least one PGP trait tested. On the contrary, strain *Pseudomonas putida* AW4 had three traits. In accordance with the literature, all *Pseudomonas* strains (*P. fluorescens* AW2, *P. putida* AW4, *P. poae* AW5 and *P. poae* AW6) were able to produce siderophores and to solubilize phosphate.

Determination of arsenic removal in liquid culture media and As bacterial accumulation

R. erythropolis AW3 was able to remove from the culture solution about 37 % sodium arsenite, followed

Table 1 Heavy metals and metalloids concentration in soil samples (mg kg^{-1})

Soil sample	As	Cd	Co	Cu	Cr	Hg	Ni	Pb	Zn
Control	9.50	< 0.20	11.90	21.10	33.80	< 0.05	12.80	17.10	68.00
La escondida	9.80	< 0.20	9.6	20.80	23.20	< 0.05	8.20	15.20	61.10

Table 2 Phenotypic, morphological and physiological characteristics of As-resistant bacterial isolates

	AW1	AW2	AW3	AW4	AW5	AW6
Colony features in YEM Agar after 48 h incubation	Medium, circular, white, mucous	Small, circular, white, mucous	Medium, circular, salmon, mucous	Small, punctiform, circular, white	Small, irregular, white	Small, irregular, white
Gram	−	−	+	−	−	−
Catalase	+	+	+	+	+	+
Oxidase	−	+	−	+	+	+
As (III) resistance (MIC)	24 mM	26 mM	>38 mM	26 mM	28 mM	28 mM
As (V) resistance (MIC)	600 mM	600 mM	>700 mM	700 mM	500 mM	500 mM
16S rRNA Closest Genbank relative	*Enterobacter cloacae*	*Pseudomonas fluorescens*	*Rhodococcus erythropolis*	*Pseudomonas putida*	*Pseudomonas poae.*	*Pseudomonas poae*

by *P. fluorescens* AW2 and *P. poae* AW5 (Fig. 1). In accordance with this result, *R. erythropolis* AW3 was also the strain that accumulated more As. Other possible bacterial mechanisms involved in removal process are currently under study. No losses by flask adsorption or evaporation were detected.

Discussion

The presence of moderate to high levels of As in soil, and/or groundwater used for irrigation, is an increasing concern, in Argentina as well as in other countries around the world. This is due to the toxic effects of this metalloid in plants, and most importantly because its noxious accumulation through the food chain. Also, this situation obstructs sustainable socio-economic growth of many affected rural areas. Therefore, development of efficient technologies and strategies that promote safe and sustainable agriculture in affected regions is needed. Each plant species in interaction with the surrounding microbial community affect toxic metals bioavailability, as part of the adaptation process to survive in a specific environment. Thus, it is important to study each plant species with its associated bacterial community in its own context.

In the studied agricultural soil sample from Córdoba, Argentina, arsenic concentration wasaround 10 $mg kg^{-1}$ and there were not significant differences compared with the control soil.Although worldwide natural soil concentrations are around 5 $mg kg^{-1}$, this can vary depending on the origin of the soil and there are indications that 5–10 $mg kg^{-1}$ is the background level (Heikens 2006).These similarities may be due to the proximity of the sampling areas (100 km approx.) and to the common origin of the loessic sediments in the south of Córdoba province in Argentina (Bundschuh et al. 2008). Even when soil samples appeared to be not contaminated with As, there is a great problem of arsenic contamination in groundwater, which are usually used for irrigation purposes. If inputs of As into soils from irrigation waters are not equivalent to outputs, there will be a buildup of As in these soils with a rate depending on the amount and quality of irrigation water used, and therefore the amounts of As that are taken up by crops will increase.

Arsenic tolerance levels in the isolated bacterial strains, were greater than expected for a non-contaminated

Table 3 PGP characteristics of As-resistant bacterial isolates

	As-resistant isolates					
PGP characteristic	AW1	AW2	AW3	AW4	AW5	AW6
Siderophores	−	+	ND	++	+	+++
Phosphate solubilization	++	+	−	+	++	+++
IAA production	>200 $\mu g mL^{-1}$	−	−	10 $\mu g mL^{-1}$	−	−
ACC utilization	−	−	−	−	−	−

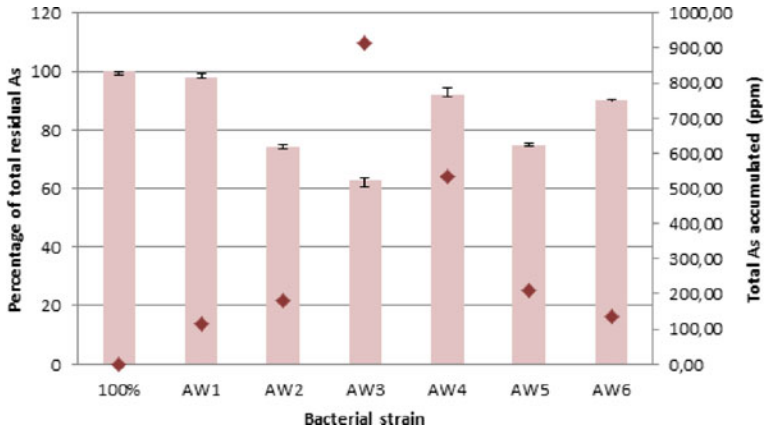

Fig. 1 Residual arsenite percentage in culture medium after 96 h. of incubation in studied strains (AW1-AW6) with an initial concentration of 4 *mM* (*bars*). Total As accumulation (ppm) in bacterial biomass (*diamonds*). *Data are represented as means ± stantard error, n= 4*

soil. In most cases, arsenic resistant microorganisms have been obtained from arsenic-contaminated soils such as mine/smelter waste or from geothermal areas (Pennanen et al. 1996; Macur et al. 2004; Gihring et al. 2003; Anderson and Cook 2004; Drewniak et al. 2008). Cai et al. (2009) also found highly arsenite-resistant bacteria only in highly contaminated soils. However, there are other reports that showed resistance to significant levels of As in laboratory strains of commonly studied bacteria such as *Escherichia coli* (Carlin et al., 1995), *Staphylococcus aureus* (Ji and Silver 1992), and *Pseudomonas aeruginosa* (Cervantes and Chavez 1992). Our results support the idea that As resistance might not be confined to organisms inhabiting arsenic-laden environments, and that even arsenic-free environments might harbor As resistant bacteria (Jackson et al. 2005). Other phylogenetic study on *arsC* gene distribution suggested that the genetic and cellular mechanisms of As resistance evolved millions of years ago when higher levels of arsenic were present in the natural environment and thus the trait may be evolutionarily widespread (Jackson and Dugas 2003). Such microorganisms could be important in transforming As that might become available in such environments through pollution or unexpected redox changes, and represent a background reservoir of arsenic resistant organisms (Jackson et al. 2005). Finally, arsenite-tolerant phenotype should also be demonstrated on liquid media because the resistance level may be overestimated on solid media due to colonial growth.

The isolates were identified as *E. cloacae* AW1 and *Pseudomonas sp.*AW2, AW4, AW5 and AW6 *(Gammaproteobacteria)* and *R. erythropolis* AW3 *(Actinobacteria)*. Members of the genus *Rhodococcus* have been isolated from contaminated and uncontaminated soil, groundwater and wastewater treatment plants, and other biological samples. In 49 actinobacteria genomes, including *Rhodococcus* altogether 99 putative metal sequestrating metallothioneins/metallohistins were identified (Schmidt and Kothe, unpublished). Concerning the genus *Enterobacter*, it has been reported the ACC deaminase-containing plant growth-promoting strain *Enterobacter cloacae* CAL2, which improve the growth of canola in the presence of arsenate (Nie et al. 2002). *Pseudomonas* was the most represented genera, and the strains were highly resistant to As 3+ considering that they were isolated from a lowly contaminated agricultural site compared with other *Pseudomonas* isolated from highly contaminated effluents. For example, the MICs for As 3+ of other isolates were 0.5 *mM* for *Pseudomonas* sp. (Valenzuela et al. 2009), 16.6 *mM* for *Pseudomonas sp ORAs5* (Pepi et al. 2007), 26 *mM* for *Pseudomonas putida* (Chang et al. 2007) and 40 *mM* for *Pseudomonas lubricans* (Rehman et al. 2010). This result is in agreement with the literature, where the genus *Pseudomonas* is broadly represented among the arsenic-resistant strains isolated from environmental samples (Pepi et al. 2007), because they are ubiquitous bacteria endowed with a remarkable adaptability to diverse environments (de Vincente et al. 1990).

In stressed environments, rhizosphere bacteria could play an important role in the promotion of plant growth, thus PGP characteristics detection are very important criteria to select promising bacterial strains for increasing crop production.

Therefore, the rhizospheric isolates were tested for their ability to produce IAA and siderophores, to solubilize phosphate and to utilize ACC as the sole nitrogen source. Four siderophores producers, two IAA producers and five phosphate solubilizing strains were

identified. None of the analyzed strains produced ACC deaminase. Similarly, siderophores and IAA production have been detected more frequently than ACC deaminase activity in rhizosphere isolates of metallicolous *Graminaceae (*Dell'Amico et al. 2005), of the Ni hyperaccumulator *Thlaspi goesingense* (Idris et al. 2004) and of the heavy metal accumulating *Salix caprea* (Kuffner et al. 2008). In the case of AW3, the bacterium did not grow in CAS culture media. This was probably because AW3 is a Gram positive bacterium. Schwyn and Neilands (1987) observed that the detergent hexadecyltrimethylammonium bromide (HDTMA) used in preparation of the CAS medium may be toxic to some microorganisms, including Gram positive bacteria and fungi. Thus, a modified version of CAS media (Milagres et al. 1999) should be used to determine siderophores production in *Rhodococcus sp*. AW3 strain.

As a first attempt to explain the high arsenic resistance of the strain, removal experiments were performed. The highest removal percentage and As accumulation was reached with *Rhodococcus erythropolis* AW3, in correlation with its highest arsenite resistance. As 3+ removal is reported to be rare and to occur by different mechanism than As 5+ removal. Our results showed that accumulation is one of the mechanisms involved in the removal process. Tentative removal mechanisms suggested include As 3+ complexation with sulfhydryl groups of surface proteins in the bluegreen algae *Scytonema* (Prasad et al. 2006) and complexation with iron in biomass from tea fermentation (Murugesan et al. 2006) and fungus (Thirunavukkarasu et al. 2003; Pokhrel and Viraraghavan 2006). Using dry biomass of *Aspergillus flavus*, the Freundlich model fits a little better than Langmuir model the As 3+ adsorption data (Maheswari and Murugesan 2011). In this work, further studies are required to establish the mechanisms involved in accumulation.

Finally, the results show that As resistant PGP rhizobacteria are present in the root zone of soybean grown in agricultural soils containing As. The isolated strains belonging to the genus *Pseudomonas* are of particular interest because they have a great potential in novel crop production strategies due to their resistance to As and the presence of several PGP traits. Further experiments will be required to assess whether these novel native bacterial strains isolated from soybean rhizosphere are adequate for increasing crop production in agricultural soil. Moreover, it is important to determine if some soybean-bacteria interaction increases plant yield and nitrogen content, and decreases plant metal accumulation, thus preventing the impact of metals in the food chain.

Conclusions

Most papers published so far have considered the ability of rhizobacteria, with or without PGP characteristics, to mobilize As in soil in association with hyperaccumulating plants. However, there are very few studies on the characterization of the interaction between rhizobacteria and legumes n in agricultural soils containing As. In the present study we isolate and identify bacteria that inhabit the rhizosphere of *Glycine max* growing in a soil with a loessic origin, as an initial step towards identifying those organisms highly resistant to As and potentially PGP in soybean. Various microbial strains present in the root–soil system were able to withstand high As concentrations and presented PGP traits. Furthermore, these strains were able to accumulate As and then remove it from liquid media. Selecting bacterial strains that are both As-resistant and able to produce PGP compounds could prove useful as inoculants in affected areas. Some of the novel As resistant bacteria with PGP characteristics obtained in this study could be adequate for future development of better strategies for sustainable agronomic production programs.

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