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Research Paper

Isolation and characterization of indigenous *Streptomyces* and *Lentzea* strains from soils containing boron compounds in Argentina

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The Salta Province – in the northwest of Argentina – is the main worldwide producer of hydroboracite and leads in exports of boron mineral and its derivatives in Latin America. In addition to the natural presence of boron compounds in the soils, there are others contaminated due to the boron mining industry. Although some bacteria are known to require boron for their growth or to be capable of storing boron, no studies have been published about *Streptomyces* or *Lentzea* genera's capacity to tolerate high boron concentrations, or about their metabolic capacities in boron contaminated environments. The results of this research show the isolation and molecular characterization of eight strains belonging to the actinobacteria phylum collected from different soils contaminated with high boron concentration in Salta state. The boron tolerance assays, which show that three of the strains were able to tolerate up 60–80 mM boron, demonstrate the potential capability of this group of bacteria to grow and maybe to remove boron from the environment. They appear to be promising, considering that these microorganisms are infrequent pathogens, are metabolically versatile and many *Streptomyces* can synthesize boron containing metabolites.

Abbreviations: ADH – arginine dihydrolase; ADI – adipic acid; ARA – L-arabinose; CAP – capric acid; CIT – trisodium citrate; ESC – esculin; GEL – gelatin; GLF – D-glucose fermentation; GLU – D-glucose; GNT – potassium gluconate; MAL – D-maltose; MAN – D-mannitol; MIC – minimum inhibitory concentration; MLT – malic acid; MM – minimal medium; MNE – D-mannose; NA – nutrient agar; NAG – *N*-acetyl-glucosamine; NB – nutrient broth; NO_3^- – nitrate reduction into nitrites; PAC – phenylacetic acid; PCR – polymerase chain reaction; PNPG – 4-nitrofenil-βD-galactopiranoside; SCA – starch casein agar; SI – sequence identities; TRP – L-tryptophane; URE – urea

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Introduction

Boron (B) is a metalloid, whose importance as a nutrient for some plant growth has long been known [1]. In the last decade, its essentiality in the structure of plants and cell walls was clearly demonstrated [2]. Evidence suggests that some animals and unicellular eukaryotes including humans [3] also require boron, but the concentrations needed vary widely according to the species. Cyanide bacteria [4] and *Bacillus boroniphilus* sp. nov. [5] are the only bacteria where boron has been determined to be essential for growing. Boron has been reported to stimulate nitrogen fixation mechanisms in *Azotobacter* [6] and also intervenes in mediation mechanisms of Quorum Sensing in *Vibrio harveyi* [7].

Boron is toxic for cells when found in greater than threshold values. Due to the toxic effect on some microorganisms, boron is used in the treatment of

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vulvovaginitis caused by *Candida* and *Saccharomyces* [8], as a food preservative [9] and as a cockroach pesticide [10]. There are many metabolites containing boron synthesized by bacteria, like aplasmomycins [11], tartorlon A and B [12], boromycines [13] and borophycin [14]. Since the 19th century this element has been used on modern lifestyles. There is over 200 boron bearing minerals, but boron industrial exploitation is feasible for only five of them, which are found in very few places in the world. Borate deposits are located in the Puna in the central Andes. This region encompasses about 100,000 km^2 in the northwestern Argentine, where Salta Province is located (Fig. 1). This province is ranked as the first Latin American borate producer, the first world producer of hydroboracite (116,099 tons in 2010), and the third world producer of borates [15]. Boron recovery may be the longest-standing mining activity in Argentina. This intense industrial development has an important environmental impact, fundamentally on water and soils which are hardly recoverable. A case is Baradero, where a firm worked until 1993 producing borax and boric acid, leaving behind an important environmental burden. This area, (46,620 m^2) is surrounded by the densely inhabited neighborhoods. Studies conducted in the area (1997–2000), revealed a high content of soluble boron and salinity in soil, thus showing that the land was not suitable for human, farming, and domestic animal breeding activities [16].

Anthropogenic boron contamination in Salta has three essential sources: (1) borate extraction, (2) borate transportation, and (3) industrialization/refining process. The current treatment used to reduce the environmental contamination produced by the industrialization of boron products involve direct waste disposal *in situ*, recycling of processes streams, waste reutilization, and the re-engineering of operations and processes [17].

Few microorganisms have been studied for their boron tolerance: *Bacillus boroniphilus* sp. nov. [5], *Gracilibacillus boraciitolerans* sp. nov. [18], *Lysinibacillus boronitolerans* [19], *Lysinibacillus parviboronicapiens* [20], *Bacillus*, *Rhodococcus* sp., *Rhodococcus erythropolis*, and *Microbacterium foliorum* [21] as Gram-positive bacteria and *Chimaereicella boritolerans* sp. nov. [22] and *Variovorax boronicumulans* sp. nov. [23] as Gram-negative, among others *Pseudomonas*, *Shewanella*, and *Variovorax* [21] less tolerant.

Actinobacteria are the most abundant group of bacteria in soils (90%) and show primary biodegradative activity, secreting extra cellular enzymes to metabolize

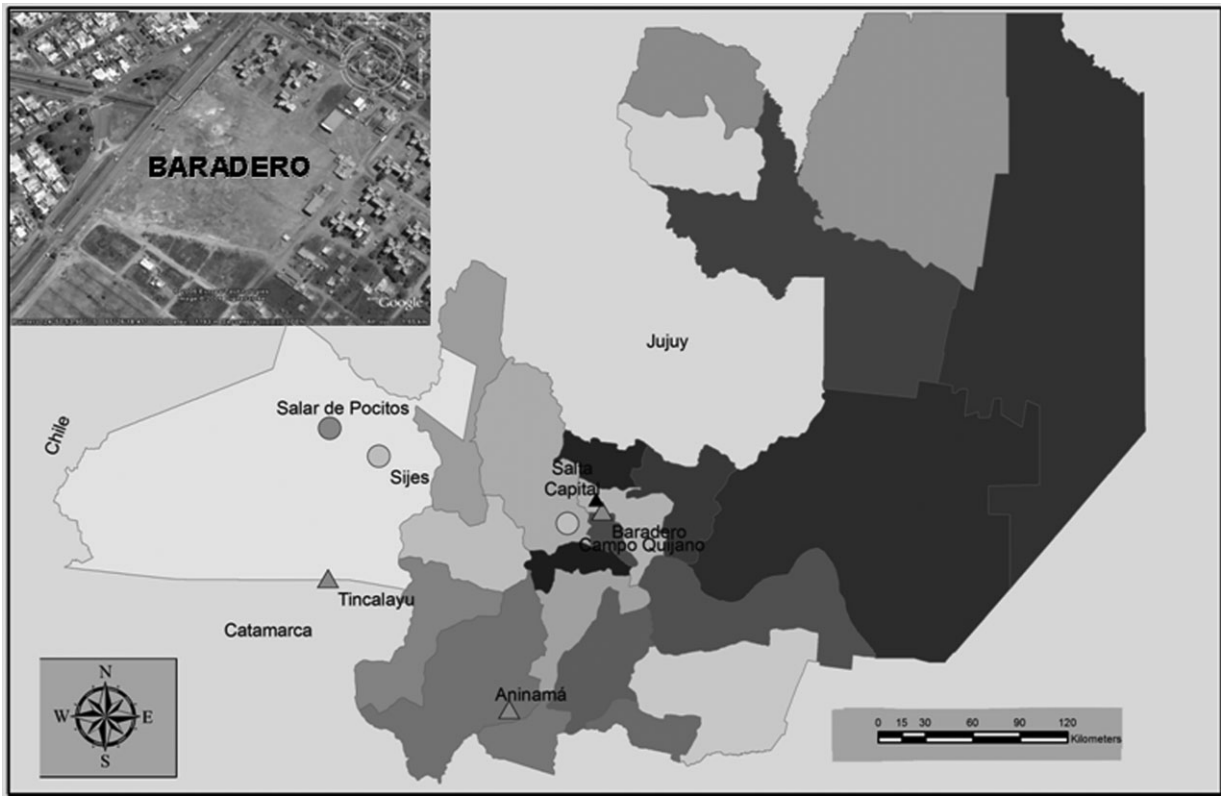

Figure 1. Boron mineral deposits and anthropogenically contaminated locations in the Province of Salta, Argentina. The boron anthropogenically contaminated area, Baradero, as a zoom.

recalcitrant molecules [24]. Amongst actinobacteria in soil, there are examples of different strategies, from cycles of rapid proliferation and sporulation to maintenance the population by prolonged slow growth and scavenging. This versatility gives them a great deal of potential for biotechnological applications [25].

The aim of this work was to characterize boron-tolerant actinobacteria from soils naturally containing high concentrations of boron and from those anthropogenically contaminated. To the best of our knowledge, there is no specific published information about *Streptomyces* or *Lentzea* tolerant to high boron concentrations.

Materials and methods

Soil sampling and physicochemical characterization

Three locations from the Province of Salta were selected. Baradero, located in an urban area, presents anthropogenic contamination with boron compounds, Tincalayu and Animaná, which are naturally high in boron content (Fig. 1). The samples from Tincalayu were obtained randomly from three different sectors (A, B, and C) of an exploitation mine. The third site, Animaná (Fig. 1), was also sampled randomly from three different sectors of the river side (D, E, and F). In each case 10 g of soil from each sector were taken from the 0–15 cm deep surface layer, considering it to be the depth, where most microorganisms should be concentrated.

A systematic sampling of Baradero soil was conducted on a rectangular grid [26] (zoom in Fig. 1), following the recommendations for homogeneous areas below five hectares [27]. The total area of 46,620 m^2 was divided into nine 70 m $\times$ 74 m sectors of 5180 m^2, and each of them was subdivided into five equidistant points to take the 10 g samples. Thus, 50 g of soil was obtained per sector in only three (S1, S2, S3) of the nine sectors according to Álvarez-Manilla et al. (2002) [28] for this type of analysis.

The soil samples from Baradero, Tincalayu, and Animaná were analyzed for water content, organic matter content, pH, B_2O_3, and total boron [29]. The concentrations of boron were measured and those values were used later as a reference to study the tolerance.

Microorganisms: isolation and preservation

Each soil sample was placed on a 3 cm thick plastic and all of the elements larger than 2 cm were hand picked. The sample was quartered; 10 g were taken and added to 90 ml of 1% sodium hexametaphosphate sterile solution, used as extracting agent. It was vortexed 10 min and then let stand for 30 min for the solids to settle. Three successive tenfold dilutions from the previous solution were poured in Petri dishes containing a general medium, nutrient agar (NA; Britania), and a specific for flora present in soils, starch casein agar (SCA; 1% starch, 0.1% hydrolyzed casein, 0.05% K_2HPO_4, 1.5% agar, pH 7 $\pm$ 0.5) and incubated at 30 °C for 7 d. The colonies obtained were isolated in SCA. Pure cultures were stored at 4 °C in SCA tubes until used.

Characterization of bacteria

Only 50 out of the 127 cultures initially isolated were characterized by their ability to grow at 30 °C in nutrient broth (NB) with NaCl added to final concentrations of 0.5, 1, 2.5, 5, 7.5, 10, 15, and 20% w/v; pH was adjusted to 7 $\pm$ 0.2 prior to sterilization. Bacterial growth with and without NaCl was carried out to analyze if they were halotolerant [30, 31]. In order to know their ability to grow at different temperatures, 0.1% v/v inocula of fresh cultures in NB of the 50 isolates were cultured in NB at 4, 15, and 30 °C, for 24 and 48 h.

Fresh cultures (NB, 24 h) were Gram stained. *Salmonella* sp. and *Staphylococcus aureus* were used as controls.

The identification of the Gram negative isolates (33) was carried out using the commercial kit API® 20 NE (BioMérieux, France). The other 17 isolated strains were also tested to elucidate and/or confirm some information related to their capacity of assimilating and degradating of some compounds. This standardized system combines eight conventional assays and 12 assimilation tests of different carbohydrates.

Boron tolerance studies

Eight actinobacteria isolates were selected in order to further study boron-tolerance by different methods. A qualitative screening assay was carried out in Petri dishes containing SCA. Rectangular troughs were cut in the center of plates and filled with 600 μl of sterile boric acid solutions. Five different concentrations were tested: 80, 150, 340, 390, and 440 mM. Isolates were inoculated by streaking 3 cm with a sterile stick perpendicularly to the troughs. Microbial growth was used as the qualitative parameter of boron tolerance. Three categories depending on the length of growth were defined: limited (1 cm), partial (2 cm), and total (3 cm). The inoculated plates were incubated at 30 °C for 7 d [32].

For semi-quantitative screening, 50 μl of seven H_3BO_3 solutions (80, 150, 210, 270, 340, 390, and 440 mM) were used to fill the wells of Petri dishes containing SCA medium previously inoculated with 200 μl of a spore suspension (4×10^6 CFU ml^{-1}) of the strain to be tested. Sterile water was used as a control. After 24 h incubation

at 30 °C the growth inhibition diameter was measured. The strains were considered tolerant to that boron concentration when the diameter of inhibition was less than 7 mm, and non-tolerant when it was higher, according to an arbitrary criteria [32].

The minimum inhibitory concentration (MIC) was also determined as an indication of boron tolerance. Different concentrations of boric acid solutions (5, 10, 15, 20, 30, 40, 50, 60, 70, and 80 mM) in NB were inoculated with a standardized number of cells (from a spore suspension) grown in minimal medium (MM, composition in g/L: glucose, 10.0; L-asparagine, 0.5; K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.20; $FeSO_4.7H_2O$, 0.01; pH 7) at 30 °C, 250 rpm, for 6 h. The tubes were then incubated for 72 h at 30 °C. The MIC was determined as the lowest concentration of boric acid preventing growth.

To prepare the spore suspension, 10 ml of sterile water were added to three Petri dishes with fresh cultures grown in SCA at 30 °C for 5 d. The agar was carefully scraped with a loop to release the spores. The spore suspension obtained was filtered through a compacted cotton bed and the filtered was then centrifuged at 5400 rpm. The supernatant was discarded and the pellet resuspended in 1 ml of sterile water. The spore concentration was determined by colony count.

Isolation of chromosomal DNA

DNA from the eight selected strains was isolated using a commercial kit (DNeasy Blood & Tissue Kit from QIAGEN), with some variations in the conditions described by the manufacturer's protocol. Briefly, biomass as spore suspension was collected from 5-d pure cultures from Petri dishes of SCA. Proteinase K (Promega) was added to achieve a final 70 $\mu g\ ml^{-1}$ concentration and the mixture was incubated at 55 °C for 1 h. Then, 560 μl of Buffer AL was added and the resulting solution was incubated at 85 °C for 15 min. DNA was precipitated by adding 560 μl of ethanol and mixing 5 s. Finally, to avoid the inhibition of enzymatic reactions, 8 μl of RNase H was added and the mixture was incubated at 37 °C for 20 min.

PCR amplification of 16S rDNA

Total genomic DNA from the eight strains was used as the template for polymerase chain reaction (PCR) amplifications. The amplifications were performed in 25 μl reaction volume using universal oligonucleotide primers (Sigma): 63 Forward (5′-CAGGCCTAACACATGCAAGTC-3′) and 1389 Reverse (5′-ACGGGCGGTGTGTACAAG-3′) [33]. PCR reactions of 16S rDNA were performed in an automated thermal cycler GeneAmp® PCR 9600 (Applied Biosystems). PCR products were detected by electrophoresis in a 2% w/v agarose gel, stained with ethidium bromide, and then visualized using the program Kodak-EDAS. The 100 base pair DNA Ladder (Promega) was used as a molecular weight marker. The amplified fragments were shipped to Korea to be purified and sequenced.

Sequencing of 16S rDNA and phylogenetic analyses

DNA sequencing on both strands was performed by Macrogen® Korea (Macrogen, Seoul, Korea) on an ABI 3730 XL automatic DNA sequencer, using the same primers as used for PCR amplification.

Sequences belonging to the same species or closely related species were selected by comparing 16S rDNA sequences of isolated strains with the database of nucleotides sequences deposited at the NCBI web server (www.ncbi.nlm.nih.gov), through Basic Local Alignment Tool (BLAST) program (http://www.ncbi.nlm.nih.gov/blast/) [34]. With this procedure the percentages of sequence identities (SI) among the strains were obtained.

The sequences of 16S rDNA were aligned using CLUSTALW software [35]. The alignments were used to calculate phylogenetic distances using the Kimura two-parameter model [36]. The phylogenetic tree for the data sets was inferred from the neighbor-joining method [37] using the software MEGA version 5 [38]. In the phylogenetic tree, defined clusters for phylogenetic similarity coefficients were compared to the classifications proposed by Williams [39] for the *Streptomyces* group in the Bergey's Manual of Determinative Bacteriology (1994).

Results

Soil physicochemical characterization

The physicochemical characterization of soils obtained at S1, S2, S3 from Baradero, Tincalayu (A, B, and C), and Animaná (D, E, and F) were determined (Table 1). The soil in the central sector of Baradero (S2) was very compact, sandy, and dry but not as much so as that in sector S1. Sector S3 differed from both of them as it was more humid and sandy with incipient vegetation. The sample obtained in this latter sector was used as control of low boron contamination from Baradero. As expected, samples from the exploitation mine in Tincalayu, showed the greater boron content values, especially in sectors A and C. Animaná samples seemed rather uniform.

Microorganisms: isolation and characterization

From the 127 strains isolated initially, 50 isolates were selected so as to leave behind unicellular and filamentous fungi. From the isolates, 33 were identified

Table 1. Physicochemical characterization of soils from Baradero (sectors S1, S2, and S3), Tincalayu (A, B, and C), and Animaná (sectors D, E, and F).

	Baradero			Tincalayu			Animaná		
Parameters	**S1**	**S2**	**S3**	**A**	**B**	**C**	**D**	**E**	**F**
Organic matter content (%)	2.36	3.44	2.78	3.58	3.22	3.71	0.25	1.18	1.06
Humidity at 110 °C (%)	5.49	5.83	6.06	12.53	2.2	7.61	0.20	0.68	0.78
pH	8.22	8.72	7.28	ND	ND	ND	8.06	8.23	8.13
pH in KCl	8.25	8.80	7.03	9.13	9.03	9.00	7.90	7.85	7.83
$[B_2O_3]$ (g per 100 g soil)	1.08	2.72	0.56	10.38	0.84	5.24	2.21	2.26	2.37
B_{total} (g per 100 g soil)	0.34	0.84	0.17	3.20	0.26	1.62	0.69	0.70	0.74

ND, not determined.

as Gram-negative rods and most of them were later classified as *Pseudomonas*. The 17 remaining isolates were Gram positive and eight of them seemed to belong to the actinobacteria phylum, based upon macro and microscopic morphology, mycelium color, spore formation, colony consistency, and the distinctive "earthy" odor because of the geosmin present [40].

Subsequent studies were only performed with the eight aforementioned strains isolated: 048 and 050 from sector S2, 002 from S1, 053 from S3, 128 and 130 from A, and 132 and 133 from sectors E and F, respectively, in Animaná. Another strain, 129, was also isolated and even sequenced from sector C in Tincalayu soil. However, it was not considered for further studies as it was determined to be *Sphingomonas* sp. (HQ538728), which does not belong to the actinobacteria phylum.

Even though the microorganisms were able to grow properly in NB, later cultures were carried on only with SCA because distinctive features (Table 2) were observed for each colony facilitating their identification and purity control. All of the strains produced extensively branching, primary mycelium transformed during the life cycle into aerial mycelium bearing and to typical spore. Four strains (002, 048, 128, and 133) showed diffusible pigments in SCA.

Growth in NB was assessed at three different temperatures (4, 15, and 30 °C) and eight different NaCl concentrations at 30 °C (Table 3). No growth was detected in any culture before 48 h of incubation at 4 °C. Conversely, all the strains grew in 48 h at 30 °C, even with 5% w/v NaCl. Strain 128 was able to grow until 7.5% of NaCl at 72 h, while strain 053 could grow until 10% NaCl at 48 h.

Even though the kit API® 20 NE system (BioMérieux) could not be used to identify the Gram positive microorganisms, the results provided important physiological and biochemical information for the eight selected strains (Table 4). Conventional tests for NO_3^- (nitrate reduction into nitrites), TRP (indol formation from L-tryptophan), GLF (D-glucose fermentation), and ADH (presence of enzymes such as arginine dihydrolase) were negative, as expected for actinobacteria. All the strains were ESC (hydrolysis of esculin) positive. Regarding the assimilation tests, all the strains used D-glucose (GLU) and conversely none of them used capric acid (CAP). Strains 002 and 128 were the only ones that did not use trisodium citrate (CIT). Also strain 128 was the only strain that did not use D-mannose (MNE).

Boron tolerance screening

The tolerance to boron (20–440 mM) was analyzed for the eight actinobacteria. Initially, the qualitative screening showed that the microorganisms were able to grow in all of the boron concentrations tested, although not always closer to the trough. The semi-quantitative assay was then carried out in SCA. An

Table 2. Macroscopic characteristics observed for the isolated strains when grown in starch casein agar at 30 °C.

	Strains							
Property	**002**	**048**	**050**	**053**	**128**	**130**	**132**	**133**
Mycelium color	R	Y	W	Y	R	W	W	R
Spore color	W	G	G	W	W	G	G	G
Colony appearance	RU	RU	RU	SW	RU	RU	RU	RU
Pigmentation	+	+	−	−	+	−	−	+

R, red; Y, yellow; W, white; G, gray; +, positive; −, negative; RU, Rugose; SW, smooth warty.

Table 3. Culture of the isolated strains in nutrient broth with and without NaCl at different temperatures after 24 h.

	Without NaCl			With NaCl in different % w/v, at 30°C							
Strain	4 °C[a]	15 °C	30 °C	0.5%	1%	2.5%	5%	7.5%	10%	15%	20%
002	−	− (−)[b]	+	+	+	+	+	−	−	−	−
048	+	− (+)[b]	+	+	+	− (+)[b]	− (+)[b]	−	−	−	−
050	+	+	+	+	+	+	+	−	−	−	−
053	+	+	+	+	+	+	+	− (+)[b]	− (+)[b]	−	−
128	−	+	+	+	+	+	+	− (+)[c]	−	−	−
130	+	+	+	+	+	+	+	−	−	−	−
132	−	− (−)[b]	− (−)[b]	+	+	+	+	−	−	−	−
133	+	− (+)[b]	+	+	+	+	+	−	−	−	−

+, positive; −, negative.
[a]Result correspond to 4 days.
[b]Results in parenthesis correspond to 48 h.
[c]Results in parenthesis correspond to 72 h.

Table 4. a. Assimilation tests of different carbohydrates with API® 20 NE (BioMérieux; GLU, D-glucose; ARA, L-arabinose; MNE, D-mannose; MAN, manitol; NAG, *N*-acetil-glucosamine; MAL, D-Maltose; GNT, potassium gluconate; CAP, capric acid; ADI, adipic acid; MLT, malic acid; CIT, trisodium citrate; PAC, phenil acetic acid) and b. Conventional tests with API® 20 NE (BioMérieux; NO3, nitrate reduction; TRP, indol formation from L-triptophane; GLF, D-glucose fermentation; ADH, presence of arginine dihydrolase enzyme; URE, presence of urease enzyme; ESC, esculine hydrolysis ferric citrate; GEL, gelatine hydrolysis; PNPG, 4-nitrofenil-βD-galactopiranoside hydrolysis).

	a. Carbohydrates assimilation[a]										b. Conventional tests[b]		
Strain	ARA	MNE	MAN	NAG	MAL	GNT	ADI	MLT	CIT	PAC	URE	GEL	PNPG
002	+	+	−	−	(+)	+	(+)	(+)	−	−	−	−	+
048	(+)	+	+	(+)	+	+	−	(+)	+	(+)	+	−	+
050	+	+	+	+	+	(+)	−	+	+	(+)	−	+	−
053	+	+	+	+	+	+	−	+	+	+	−	+	+
128	−	−	−	−	−	+	−	−	−	−	−	−	(+)
130	−	+	−	−	−	−	−	−	+	−	−	+	(+)
132	−	+	(+)	−	+	+	(+)	+	+	−	−	+	−
133	(+)	+	+	+	−	−	−	+	+	−	(+)	−	+

+, positive; −, negative; (+), weakly positive.
[a]All the strains used GLU and none of them used CAP.
[b]Tests for NO3, TRP, GLF, and ADH were all negative, as expected for actinobacteria. All the strains were ESC positive.

inhibition zone of 7 mm in diameter was arbitrarily designated as the criterion to determine boron resistance of the tested strains. All isolated strains were able to grow at 80 mM, while only 50 and 13% of them grew at 210 and 440 mM, respectively. According to the inhibition criteria adopted, all the strains were B-tolerant up to 270 mM (Fig. 2). Interestingly, strain 048 was able to grow without any inhibition over the entire range of boric acid concentrations used, while strain 128 showed tolerance even to 440 mM of B. Higher concentrations of boric acid were not used due to solubility limitations.

The MICs determined after 72 h were 20 mM for 002 and 132, 30 mM for 130, 40 mM for 133, 50 mM for 050, 60 mM for 053, 70 mM for 128, and 80 mM for 048.

Phylogenetic analyses

Only one product of amplification was obtained by PCR, as verified by electrophoresis in agarose gel. The amplified fragments were sequenced at Macrogen® and the results obtained were used to elucidate the taxonomic position of the eight isolated strains as explained before. The 16S rDNA sequences obtained for isolated strains were compared to 49 sequences belonging to the same or closely related species, available through the public databases. The sequences were uploaded to NCBI GenBank and their accession numbers are given in parentheses (Fig. 3).

The generated phylogenetic tree was divided into six major clusters on the basis of their evolutionary distances calculated through the neighbor-joining method. The first cluster was further divided into two

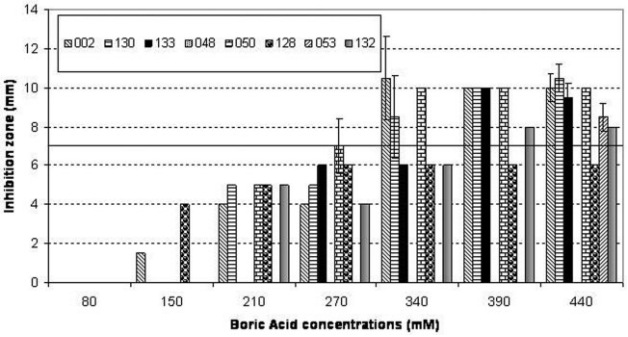

Figure 2. Semi-quantitative assay of boron tolerance measured as the diameter of the inhibition zone. The horizontal line indicates the arbitrary limit used to define boron tolerant (below) and non-tolerant (above) strains.

sub-clusters. In sub-cluster Ia, the isolated strain 048 (HQ538723) was closely associated to *S. achromogenes* (99.4%) and to *S. nogalater* while strain 133 (HQ538729) was associated to *S. griseosporeus* (98.5%). In sub-cluster Ib, the isolated strain 053 (HQ538724) was closely associated to *S. albogriseolus* (100%) and to *S. thermoluteus* and the isolated strain 002 (HQ538731) was closely associated to *S. iakyrus* (100%). Most of the species located in cluster I were incorporated in the largest cluster-group A, and widely distributed in different clusters (19, 23, 12, 18, 15, 16, etc.) according to the proposed classification. In cluster II, the isolated strain 050 (HQ538725) was closely associated to *S. fradiae* (100%). The species located in cluster II were incorporated in the cluster-group G, and located only in the cluster 68. In cluster III, the isolated strain 130 (HQ538727) was closely associated to *S. polychromogenes* (100%), the species located in cluster III were incorporated in the cluster-group F and only in the cluster 61. In cluster IV, the isolated strain 128 (HQ538726) was closely associated to *S. lincolnensis* (99.8%), the species located in the cluster IV are incorporated in the cluster-group A and located in clusters 18 and 19. In cluster V, the isolated strain 132 (HQ538730) was associated to species of the genus *Lentzea* (97.6 - 97.8%). In cluster VI, the isolated strain 129 (HQ538728) was closely associated to with *Sphingomonas melonis* (99.1%).

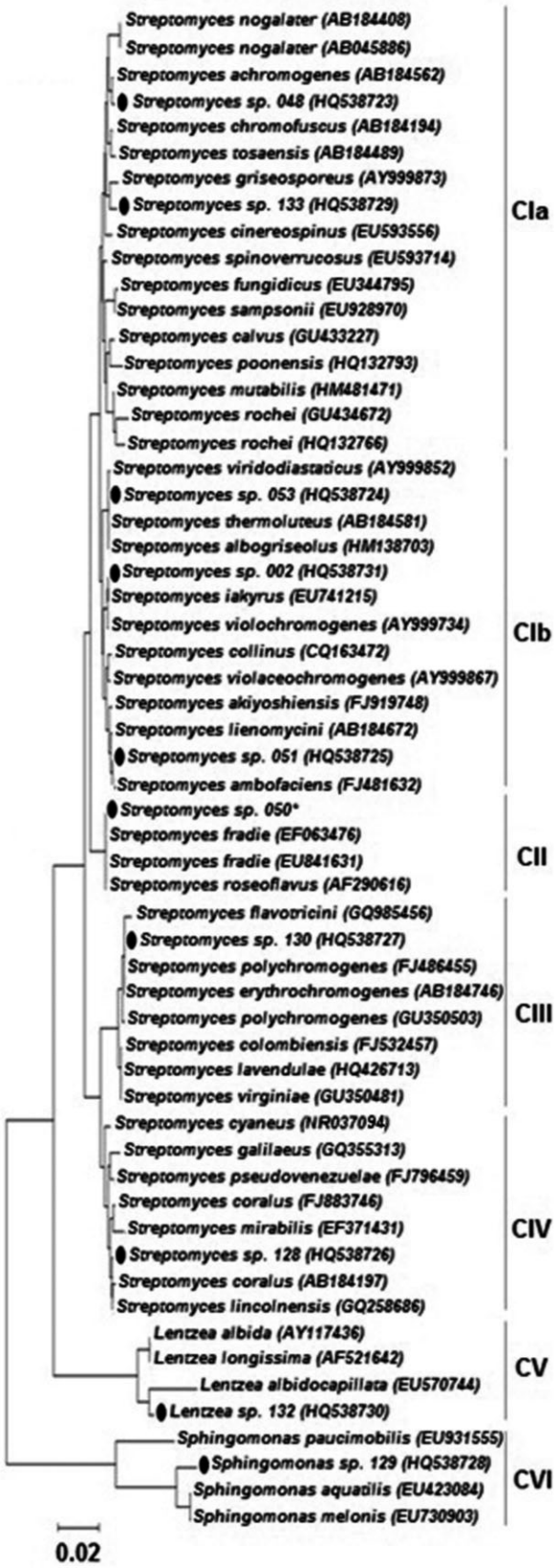

Figure 3.

Figure 3. Phylogenetic tree of actinobacteria species inferred by the neighbor-joining method [37], using Kimura's evolutionary distance [36] and based on the comparison of nearly complete 16S rDNA sequence of 49 organisms. Accession numbers of 16S rDNA sequences are given in parentheses. The label • shows the isolated strains in this study.

Discussion

The eight strains selected belong to the actinobacteria phylum, a physiologically diverse bacterial group that has the soil as their natural habitat. Previous studies have demonstrated that Gram-positive bacteria with high G + C content, i.e., actinomycetes, dominated the community in non-contaminated soils as well as contaminated soils [41].

Actinobacteria are known for their ability to produce antibiotics and metabolites, and to remove heavy metals. From the eight isolates obtained in this work, strain 132 from Animaná was identified as *Lentzea* sp., while the other seven strains were *Streptomyces* sp. (Fig. 3) according to the phylogenetic tree based on 16S rDNA sequences. Some of the strains can be associated with contaminated soils, i.e., *Lentzea* with heavy-metal contaminated soils [42]. Others were reported for specific abilities to degrade some compounds, such as *S. iakyrus* and parathion [43] and *S. albogriseolus* and latex [44], to produce antibiotics such as *S. achromogenes* and streptozotocin, rubradirin [45], and tomaymycin [46], *S. ambofaciens* and spiramycin [47], *S. lincolnensis* and lyncomicine [48], and to produce enzymes, such as *S. fradie* with keratolytic activity [49]. However to the best of our knowledge there are no reports about their capacity to grow in soils or media with high boron concentrations.

Only few microorganisms have been reported previously as having boron tolerance. Three of them were actinobacteria. For *Microbacterium foliorum* intracellular boron concentration was less than 0.4 nmol per gram of dry weight and less than 0.7 nmol per gram of dry weight for both *Rhodococcus* sp. and *Rhodococcus erythropolis* [21]. Among others, Gram-positive bacteria *Lysinibacillus parviboronicapiens*, can tolerate up to 6% w/v NaCl, but only 50 mM boron [20]. *Lysinibacillus boronitolerans* can tolerate up to 150 mM boron [19], while *Bacillus boroniphilus* sp. nov. [5] and *Gracilibacillus boraciitolerans* sp. nov. [18] can tolerate over 450 mM of boric acid. *G. boraciitoloerans* actually requires boron to grow.

Gram-negative bacteria are also able to tolerate high boron concentration such as *Chimaereicella boritolerans* sp. nov. up to 300 mM B [22] and *Variovorax boronicumulans* sp. nov. could accumulate boron intracellular [23].

The results obtained in this work from the qualitative screening about boron tolerance were neither clear nor consistent. The growth was subjected to a great uncertainty since the true boron concentration at different points in the agar gel was unknown. The semi-quantitative results instead showed that all the isolates were able to grow in concentrations up to 270 mM. They also evidenced that the strains 048 and 128, both isolated from heavily contaminated soils, had even higher B-tolerance, up to concentrations of 440 mM boron. The level of B-tolerance is similar to that reported by Ahmed *et al.* [5, 18] for other Gram-positive bacteria. Regarding the MIC, the strains 048, 053, and 128 showed the highest B-tolerance (80, 60, and 70 mM, respectively). Miwa and Fujiwara [21] reported some microorganisms, including three actinobacteria, as high boron tolerant after they showed ability to grow at concentrations of 0.1 mM of boric acid in liquid media. Taking that, the strains isolated look very promising, since they are able to grow in the presence of at least 600 times higher concentrations than the reported ones.

The sensibility of the studied strains was considerably higher in liquid medium. As Majzlik *et al.* [50] suggest the differences in values of MIC on agar and liquid medium are likely to be caused by completely different environments. These authors propose that in liquid medium, bacteria are able to create a suitable environment around the cells (synthesis of peptides, proteins that are released into their immediate surroundings) and internal defense mechanisms are altered even at low concentrations. Same authors pose another feasible hypothesis based on the nature of agar as a complex organic molecule that contains binding sites that can react or form complex with boric acid in this case, thereby reducing its effective concentration. We also think that diffusive effects that are involved in the solid medium may cause this difference. In liquid medium cells are in intimate contact with boric acid while in agar not.

Finally, strains 048 and 128 look promising to deal with environmental contamination with boron. Whether the bacteria are using the boron for the production of some metabolite or just capturing and accumulating it internally will need to be elucidated through further studies evaluating the boron mass balance during growth.

Conclusions

Eight strains of actinobacteria isolated from soils obtained in the Province of Salta in Argentina showed the ability to grow in the presence of boron compounds. Three of them, showed the greatest tolerance to the highest concentrations analyzed. This is the first study about *Streptomyces* and *Lentzea* genera that are tolerant to, or able to grow in, high boron concentrations.

Further studies will be conducted to determine the feasibility of using these boron-tolerant strains for biotechnological applications. In that case the fate of the boron will have to be studied and elucidated.

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