European Journal of Soil Biology xxx (2012) 1–9

Contents lists available at SciVerse ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

Evaluation of rhizobacterial isolates from Argentina, Uruguay and Chile for plant growth-promoting characteristics and antagonistic activity towards *Rhizoctonia* sp. and *Macrophomina* sp. *in vitro*

Q3 Lorena Belén Guiñazú [a,*], Javier Alberto Andrés [b], Marisa Rovera [c], Mónica Balzarini [d], Susana Beatríz Rosas [a]

[a] *Departamento de Biología Molecular, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta 36 Km 601, 5800 Río Cuarto, Argentina*
[b] *Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Físico-Químicas y Naturales y Laboratorio de Microbiología Agrícola, Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto, Ruta 36 Km 601, 5800 Río Cuarto, Argentina*
[c] *Departamento de Microbiología, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta 36 Km 601, 5800 Río Cuarto, Argentina*
[d] *Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Ciudad Universitaria s/n, 5000 Córdoba, Argentina*

ARTICLE INFO

Article history:
Received 3 October 2011
Received in revised form
10 July 2012
Accepted 17 September 2012
Available online xxx
Handling editor: Kristina Lindström

Keywords:
Alfalfa
Plant growth-promoting rhizobacteria
Phosphate solubilization
Siderophores
Rhizoctonia spp.
Macrophomina spp.

ABSTRACT

This study was conducted over 3 years in soils of Uruguay and Chile and two years in soils of Argentina. 686 bacterial isolates were phenotypically characterized by testing in relation to the presence of plant growth promoting properties: phosphate solubilization, production of siderophores, starch hydrolysis, production of exopolysaccharides and biological control of *Macrophomina phaseolina* and *Rhizoctonia* spp. In all samples analyzed, the number of Gram-positive bacteria exceeded that of Gram negative. Ten bacterial isolates were selected for their plant growth promoting properties and API Test and 16S rRNA gene (rDNA). Six of these isolates belong to the genus *Pseudomonas*, three to the genus *Bacillus* and one to *Janibacter*. This is the first report of a strain from the genus *Janibacter* with promising plant growth-promoting attributes. The results obtained allow us to improve the microbial germplasm of plant growth promoting bacteria from soils of Chile, Argentina and Uruguay with a view to their potential use in the formulation of mixed inoculants that promote the growth of alfalfa.

1. Introduction

The rhizosphere, defined as the volume of soil adjacent to and influenced by the plant root [1], is of great importance to plant health and soil fertility [2]. This space is limited and it extends to a few millimeters from the root surface [3]. Root secretions, such as amino acids and carbohydrates, constitute a rich source of energy and nutrients for microorganisms, thus, microbial populations are high in this area.

Plant growth-promoting rhizobacteria (PGPR) [4] are non pathogenic beneficial bacteria that play a fundamental role in plant health and nutrition. They can benefit plant growth through diverse mechanisms such as solubilization of mineral phosphates [5–7] and other nutrients, production of growth-regulating compounds [8,9], and prevention against pathogens attacks [10,11].

Alfalfa (*Medicago sativa* L.) participates in a high percentage of the cultivation area in the agricultural-cattle systems of Chile, Uruguay and Argentina. At the present time, it constitutes one of the most important forage resources due to its enormous adaptation to different climates and soils. In Argentina, the highest cultivated area corresponds to the provinces of Córdoba, Santa Fe, Buenos Aires and La Pampa, where water availability and soils with low pH and low levels of soluble phosphates are the main obstacles for an effective biological nitrogen fixation (BNF) [12]. In Chile, it is successfully cultivated in central and northern-central regions, where there are favorable soils and weathers. However, production of this crop is limited in southern and southern-central areas of the country because of acid soils derived from volcanic ashes (andisols), furthermore, these soils contain high concentrations of Al and Mn and a low concentration of P [13,14]. In Uruguay, alfalfa is cultivated in the core area of intensive animal production, where it is in full expansion due to its persistence.

Plagues and diseases can considerably reduce the quality, persistence and nutritious value of forage [15]. Alfalfa is affected by several diseases that attack leaves, stems, crown and roots. Pathogens that attack roots and crown, such as *Macrophomina* spp. and

* Corresponding author. Tel.: +54 0358 4676103; fax: +54 0358 4676232.
E-mail address: lguinazu@exa.unrc.edu.ar (L.B. Guiñazú).

1164-5563/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejsobi.2012.09.007

Rhizoctonia spp., directly define the longevity or the productive period of alfalfa.

It is of great interest to develop biotechnological products that combine the positive effects of different species of rhizosphere microorganisms (multiple inoculants) in order to benefit plants with a higher capacity to incorporate water and nutrients thus improving their health and yields.

In this work, we analyzed the potential plant growth-promoting traits and the biocontrol capacity of a representative bacterial isolates sample obtained from six pre and post alfalfa planting soils from Chile (Carillanca and Faja Maisan), Uruguay (Punta Espinillo I and II) and Argentina (Manfredi and Balcarce).

Our objective was to determine the proportion and diversity of plant growth-promoting characteristics and the antagonistic ability against *Rhizoctonia* spp. and *Macrophomina* spp. *in vitro* of bacteria isolated from different South American soils, and if these attributes are related to each other and/or to the isolation site.

2. Materials and methods

2.1. Soil and soil sampling

Soil samples were collected from six sites located in Chile, Uruguay and Argentina. The samples from Chile and Uruguay were conducted for three consecutive years (2004–2005–2006), while trials in Argentina corresponded to the years 2004–2005. This was due to climatic factors that influenced the phenological cycle of the crop. The samples processed following the method described by Frioni [16] included soil (preplant sampling) and rhizosphere soil (sampling post seeding). Rhizosphere soil samples were obtained from fields of alfalfa cultivation inoculated with *Sinorhizobium meliloti* B399 (strain recommended by the Instituto Nacional de Tecnología Agropecuaria – INTA for alfalfa inoculation in Argentina) with a dose of approximately of 10^3–10^4 CFU/g seeds) and uninoculated fields. Identical field assays were designed in all three countries. At least two different agro-ecological regions were selected where, during the past five years, no alfalfa or other *S. meliloti* host plant had been planted. All information on different soils is compiled in Table 1.

2.2. Isolation of bacteria and determination of colony forming units

One gram of each soil was suspended into 9 ml of sterile saline solution (9 g l^{-1} NaCl). Serial 10-fold dilutions were performed. Then, a 0.1 ml aliquot was plated onto 25% Tryptic Soy Agar (TSA; Britania Laboratories), in triplicate. Plates were incubated at 28 °C for 24–48 h. Results were expressed as colony forming units g^{-1} soil (CFU g^{-1} soil).

2.3. Selection of bacterial isolates

The first characterization consisted of direct observation of isolated colonies, taking into account color, shape, elevation, margins, diameter, surface, opacity and texture [17]. Colonies showing visible morphological differences were re-isolated on 25% TSA and yeast extract mannitol agar (YEMA) supplemented with congo red [18] to differentiate the colonies of rhizobia (not selected for further testing). The selected strains were conserved at −80 °C in Tryptic Soy Broth (TSB-Britania®, Argentina), amended with 20% glycerol.

2.4. In vitro tests

Each isolate was first subjected to Gram stain and to the complementary 3% KOH test [19]. Isolates were characterized by means of phosphate solubilization [7], siderophore production [20], starch hydrolysis and exopolysaccharide production [21] assays. Also, assays of antifungal capacity were carried out in 25% TSA medium. To this end, a mycelial disc (9 mm diameter) of an actively growing target fungus was placed on the center of a Petri plate and bacterial isolates were inoculated in the periphery. Plates were incubated for 7 days at 28 °C. Inhibition zones were recorded and compared to a growth control (target fungus alone). The strain *Pseudomonas clororaphis* subsp. *aurantiaca* SR1 [22,23] was used as positive control. The phytopathogenic fungus strains were isolated from infected plant tissue and identified by Laboratorio de Micología, Departamento de Microbiología e Inmunología, Universidad Nacional de Río Cuarto [24,25].

2.4.1. Phenotypic characterization

Based on the results of the preliminary characterization described above, ten promising isolates were identified to the species level by the API identification system assisted by analytical profile index (API) Plus computer software (bioMe'rieux_SA, Marcy-l'Etoile, France). Gram positive, endospore forming rods were identified to the species level using API 50 CH test strips. Gram positive, short, nonmotile (verified by the SIM (Hydrogen–Sulfide, Indole, Motility) [26], non-spore-forming, strictly aerobic, catalase positive, and oxidase negative rods were identified by using the API Coryne strip. Gram negative rod isolates with only oxidative reaction in OF basal medium [27] were identified using the API 20 NE test strip. The API strip consists of microtubes containing dehydrated media and substrates. The media microtubes containing conventional tests were inoculated with a bacterial suspension which reconstituted the media. After incubation, the metabolic end products were detected by indicator systems or the addition of reagents. The substrate microtubes contained assimilation tests and were inoculated with a minimal medium. If the isolates were capable of utilizing the corresponding substrate, they grew.

2.5. Genotypic characterization of bacterial isolates

2.5.1. DNA extraction from isolates. Partial nucleotide sequences of the 16S rRNA gene and BOX-PCR genomic fingerprints

Ten isolates showing the greatest P and Fe solubilization halos together with the highest antifungal ability were selected for

Table 1
Description of soils from the six sites. Q2

Country/location	% Organic matter	pH	P (ppm)	K	N	Ca	Mg	Na	Soil type	Cropping history
Uruguay/Punta Espinillo 1 (56° 25′ W, 34° 49′ S)	0.7	5.9	27	0.55[a]	ND	8.4[a]	3.4[a]	0.31[a]	ND	Intensive horticulture
Uruguay/Punta Espinillo 2 (56° 25′ W, 34° 49′ S)	1.8	5.5	31	0.80[a]	ND	4.7[a]	4.7[a]	0.13[a]	ND	Intensive horticulture
Chile/Carillanca (72° 40′ W, 39° 06′ S)	19.0	5.7	23	0.53[b]	25	2.99[b]	0.4[b]	0.1[b]	Typic Hapludand	Mixed prairie
Chile/Faja Maisan (72° 55′ W, 39° 05′ S)	17.0	5.4	14	1.23[b]	37	4.52[b]	1.27[b]	0.13[b]	Typic Hapludand	Mixed prairie
Argentina/INTA Manfredi (63° 44′ W, 31° 50′ S)	1.71	6.2	46	ND	0.105[d]	ND	ND	ND	Haplustol Entic	Gramineous crops
Argentina/INTA Balcarce (58° 15′ W, 37° 50′ S)	4.9	6.1	12.8	2.3[a]	0.352[d]	15.5[a]	2.9[a]	0	Petrocalcic Paleudol	Gramineous crops

ND: not determined.
Inorganic N (ppm).
[a] meq 100 g^{-1}.
[b] cmol kg^{-1}.
[d] Total N (%).

Please cite this article in press as: L.B. Guiñazú, et al., Evaluation of rhizobacterial isolates from Argentina, Uruguay and Chile for plant growth-promoting characteristics and antagonistic activity towards *Rhizoctonia* sp. and *Macrophomina* sp. *in vitro*, European Journal of Soil Biology (2012), http://dx.doi.org/10.1016/j.ejsobi.2012.09.007

further genomic characterization and identification by means of molecular techniques.

The genomic DNA was obtained following the methodology used by Guiñazú et al. [28]. The partial nucleotide sequences of the 16S rRNA gene (rDNA) were determined by direct sequencing of appropriate PCR products. A DNA region corresponding to nucleotides 20 to 338 of *Escherichia coli* 16S rDNA was amplified from each strain with the universal primers Y1 (5′-TGG CTC AGA ACG AAC GCT GGC GGC-3′) and Y2 (5′-CCC ACT GCT GCC TCC CGT AGG AGT-3′) as previously described for proteobacteria [29]. The 25 μl PCR mixtures contained: 0.5 μM of each primer, 200 μM dNTPs, 3 mM $MgCl_2$, PCR reaction buffer (50 mM KCl, 20 mM Tris HCl, pH 8.0), 1 U *Taq* DNA polymerase (Promega Corp.) and 2 μl of template DNA. Amplifications were carried out in a Thermo Cycler (I Cycler-BioRad). The cycling conditions were as follows: 94 °C for 2 min, 35 cycles at 94 °C for 20 s, 52 °C for 20 s, 72 °C for 45 s, and 72 °C for 2 min. After the reaction, 10 μl of the PCR reaction were analyzed in 1.5% agarose gels containing 1 μg ml^{-1} of ethidium bromide and photographed with a Kodak DC290 digital camera. The nucleotide sequence of the PCR products was determined for both strands with an Automatic Laser Fluorescent DNA Sequencer (Macrogen, Korea). The partial sequences of the 16S rDNA genes have been deposited in the GenBanK and compared with the complete database using the BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Table 2).

Strains were genotypically characterized by BOX-PCR, using BOX A1R (5′- CTA CGG CAA GGC GAC GCT GAC G-3′) [30] as primer. BOX-PCR was performed in 25 μl reaction mixture containing: 2.5 μl

Table 2
BLAST comparison between partial 16S ribosomal gene of selected local isolates.

Strain and origin	Description	Identity	GenBank no.[a]
FM7d (GQ 373391)[a] Faja Maisán, Chile (soil)	*Pseudomonas teessidea*, PR65T	100%	AM419154.2
	Pseudomonas sp. DK6	100%	EU158320.1
	Pseudomonadaceae bacterium FND-2	100%	EF017809.1
	Pseudomonas sp. NZ039	100%	AY014808.1
	Uncultured bacterium, clone FD03B06	100%	FM873410.1
M7c (GQ 373390)[a] Manfredi, Argentina (soil)	*Bacillus* sp. AiL3	100%	FJ860227.1
	Bacillus sp. XL62	100%	FJ465166.2
	Bacillus licheniformis strain H3	100%	FJ713021.1
	Bacillus velezensis	100%	AB244463.1
	Bacillus amyloliquefaciens strain BCRC 14193	99%	EF433408.1
Car (GQ 853341)[a] Carillanca, Chile (rhizosphere soil)	*Pseudomonas* sp. 'Disko Bay 23′	100%	FJ581931.1
	Pseudomonas sp. PSB8	100%	EU184085.1
	Pseudomonas sp. isolate 06025	100%	AM779883.1
	Pseudomonas putida isolate PhyCEm-187	100%	AM921634.1
	Pseudomonas jessenii strain B2-122	100%	AM911021.1
	Uncultured gamma proteobacterium clone NL5BD-02-E06	100%	FM252630.1
	Uncultured *Pseudomonas* sp. clone XZELH54	100%	EU703156.1
Bal1 (GQ 853342)[a] Balcarce, Argentina (rhizosphere soil)	*Bacillus* sp. PCSAS2-25	100%	GQ284490.1
	Bacillus subtilis strain NK-2	100%	GU064894.1
	Bacillus amyloliquefaciens strain dhs-28	100%	GQ903336.1
	B. subtilis strain BRZ5	100%	GQ853552.1
	Paenibacillus favisporus strain BKB30	100%	FJ864725.1
	Bacillus licheniformis strain H3	100%	FJ713021.1
Bal3 (GQ 853343)[a] Balcarce, Argentina (rhizosphere soil)	Uncultured Firmicutes bacterium clone B02	100%	GQ249508.1
	Bacillus sp. FR-W11a2 strain FR-W11a2	100%	FN395284.1
	Bacillus simplex strain B2-223	100%	FN298254.1
	Bacillus muralis strain K1-20	100%	FM992648.1
	Bacillaceae bacterium BTRH40	100%	FJ013312.1
	Bacillus macroides strain B1-42	100%	FM208190.1
Manf (GQ 853344)[a] Manfredi, Argentina (rhizosphere soil)	*Pseudomonas* sp. IMER-A2-8	100%	FJ436428.1
	P. putida isolate Tg	100%	EU275363.1
	Pseudomonas sp. strain T1P110	100%	FM211673.1
	Pseudomonas jessenii strain T2P28	100%	FM209481.1
	P. putida strain CM5002	100%	EF529517.1
UI (GQ 853345)[a] Punta Espinillo I, Uruguay (rhizosphere soil)	*Pseudomonas* sp. ATCC 43928	100%	GQ856536.1
	Pseudomonas sp. strain R-32728	100%	AM403615.1
	Pseudomonas frederiksbergensis strain SS17	100%	AB365797.1
	Pseudomonas sp. CHNCT24	100%	EF471228.1
	Uncultured proteobacterium clone DOK_CONFYM_clone433	100%	DQ828685.1
	Pseudomonas sp. ML1	100%	AB013426.1
CH2 (GQ 853346)[a] Carillanca, Chile (rhizosphere soil)	*Pseudomonas pseudoalcaligenes* strain MHF ENV 11	100%	GU055765.1
	Uncultured bacterium clone FR_B_D10	100%	GQ443095.1
	P. putida strain JD1	100%	FJ010624.1
	Pseudomonas sp. IMER-A2-21	100%	FJ434133.1
	P. putida strain 23975	100%	FJ227304.1
UII (GQ 853347)[a] Punta Espinillo II, Uruguay (rhizosphere soil)	*Janibacter* sp. 52NP14	100%	AB242707.1
	Janibacter marinus strain 095-2.2-CV-A-02	100%	EF010551.1
	Janibacter sp. B-1141	99%	DQ399749.1
	Janibacter sp. HRG7	98%	AY845398.1
Ch4 (GQ 853348)[a] Carillanca, Chile (rhizosphere soil)	*Pseudomonas chlororaphis* strain BS1393	100%	FJ652609.1
	Pseudomonas chlororaphis strain 30-84	100%	FJ652607.1
	Uncultured bacterium clone nbt19e09	100%	FJ893299.1
	Pseudomonas tolaasii strain NCPPB 741	100%	AF320992.1
	Pseudomonas sp. enrichment culture clone SY-2 16S	100%	FJ638892.1
	Pseudomonas chlororaphis subsp. *aurantiaca* strain Pa40	100%	FJ515773.1

[a] GenBank accession numbers of the sequences analyzed in this paper.

Please cite this article in press as: L.B. Guiñazú, et al., Evaluation of rhizobacterial isolates from Argentina, Uruguay and Chile for plant growth-promoting characteristics and antagonistic activity towards *Rhizoctonia* sp. and *Macrophomina* sp. *in vitro*, European Journal of Soil Biology (2012), http://dx.doi.org/10.1016/j.ejsobi.2012.09.007

buffer (10x), 0.5 μl of BOX A1R, 2.5 μl $MgCl_2$, 2.5 μl of each dNTP, 0.2 μl of *Taq* DNA polymerase, 14.5 μl of sterile bidistilled water and 2.3 μl of template DNA. PCR-amplifications were performed in a Thermo Cycler (I Cycler-BioRad) and the temperature profile was as follows: denaturation at 95 °C for 7 min, 35 cycles at 94 °C for 1 min, annealing at 52 °C for 1 min, extension at 65 °C for 8 min, and a final extension step at 65 °C for 16 min. The BOX amplification products were separated by horizontal electrophoresis on 1.5% agarose gels containing 12 μl ethidium bromide every 100 ml; gels were photographed using a Polaroid 667 type of film.

2.6. Statistical analyses

Results obtained from the PGPR characteristics tests were statistically analyzed by means of a principal co-ordinate analysis (PCO) of a 686 isolates × 6 variables (PGPR tests) matrix. The PCO was carried out with the Dice similarity metric, transformed in distance through the complement's square root function from one, to arrange the PGPR characteristics (columns of the entrance matrix). The average number of PGPR characteristics in the isolates as well as the proportion of isolates with different desirable promoting characteristics according to the soil of origin and the sampling time was estimated. All data were subjected to statistical analysis using the Info-gene [31] software.

3. Results

The total pre and post-planting bacterial population was in the order of 10^6–10^7 CFU g^{-1} soil or rhizosphere soil. The mean count was 1.8×10^6 for soil and 1.5×10^7 for rhizosphere soil (for the 3 countries, respectively). A total of 2370 bacterial isolates were obtained from soils of Chile, Uruguay and Argentina. 686 bacterial isolates were phenotypically characterized by testing in relation to the presence of plant growth promoting properties: phosphate solubilization, production of siderophores, starch hydrolysis, production of exopolysaccharides and biological control of *Macrophomina phaseolina* and *Rhizoctonia* spp.

It was observed that in all samples (pre and post-planting) the number of Gram-positive bacteria was higher than that of Gram negative (approximately 80% of the isolates were Gram positive). Actinomycetes were detected in most of the samples.

The PCO analysis allowed us to infer that the characteristics of the isolates were similar in all three countries. Rhizobacteria capable of solubilizing Fe and P (Fe S and P. S.) are opposed in the classification plane to those that produce exopolysaccharides (Ex. P.), hydrolyze starch (S. H.) or antagonize *M. phaseolina* (M.p.). In addition, the ability to control the phytopathogenic fungus *Rhizoctonia* spp. appeared to be shared by the isolates that solubilize Fe and P and those that produce exopolysaccharides and lytic enzymes, since it was not opposed to none of these groups in the coordinate axis obtained from Chile and Uruguay. On the other hand, the coordinate axis from Argentina allowed us to observe that the ability to control this phytopathogen is shared by those isolates that produce exopolysaccharides and hydrolyze starch, and it would not be present in those rhizobacteria with nutrient solubilizing capacities (Fig. 1).

The number of plant growth promoting characteristics was higher in pre-planting samplings (Manfredi (Argentina), Faja Maisan (Chile) and Punta Espinillo I and II (Uruguay)) than in post-planting samplings (Fig. 2a, c, e and f, respectively). On the other hand, in the pre-planting samplings carried out in Balcarce (Argentina) and Carillanca (Chile) the rhizobacteria were positive in a lesser number of tests (Fig. 2b and d, respectively).

The number of desirable plant growth promoting characteristics was lower in rhizobacteria from inoculation assays than in those without inoculation in the post-planting samplings from Argentina. Also, the number of desirable characteristics decreased during the second sampling (Pp2) in both assays (Fig. 2a and b).

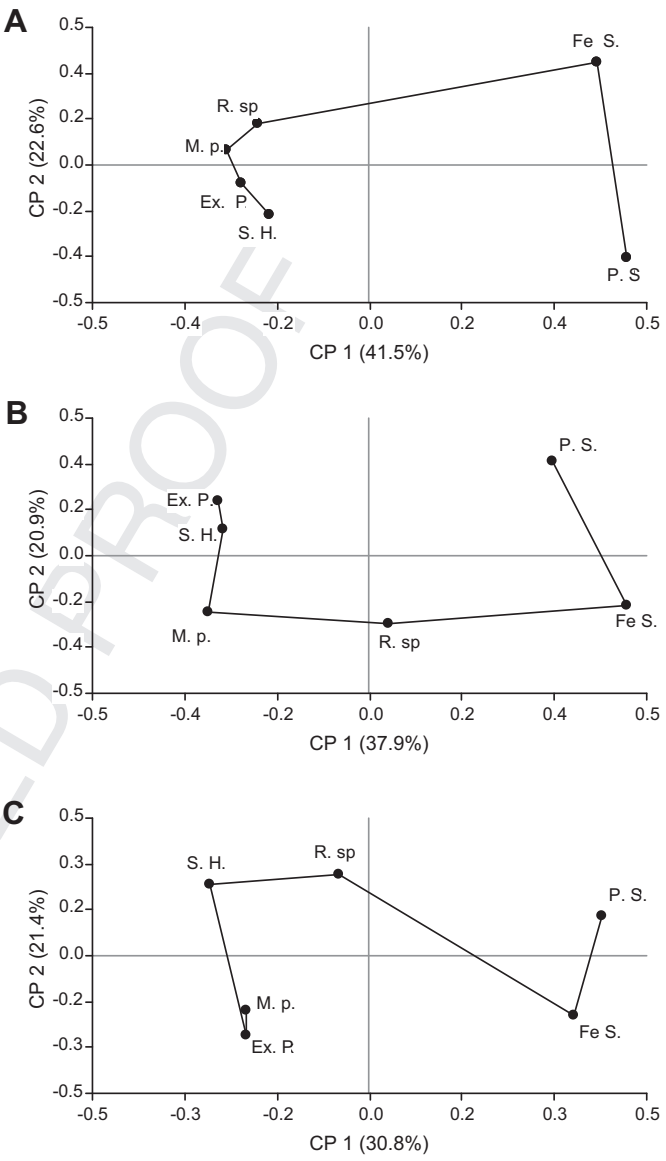

Fig. 1. PCO Argentina–Balcarce and Manfredi- (top); Chile–Carillanca and Faja Faisán- (center) and Uruguay–Espinillo 1 and 2- (bottom). References: S. H: starch hydrolysis; Ex. P.: Exopolysaccharide production; P. S.: Phosphate solubilization; Fe S.: Siderophore production; M. p.: *Macrophomina phaseolina*; R. sp: *Rhizoctonia* sp.

The Pp1 sampling, carried out in Faja Maisan (Chile), allowed us to observe that isolates from the rhizosphere of inoculated alfalfa plants showed a lower number of beneficial characteristics compared to those from the rhizosphere of uninoculated plants. The opposite happened with isolates from Pp2 sampling. The Pp3 sampling reflected that, although somewhat lower, the number of plant growth promoting characteristics was similar among isolates from the rhizosphere of inoculated and uninoculated alfalfa plants. In the assay established in Carillanca, the isolated rhizobacteria from the Pp1 sampling, the rhizosphere of inoculated alfalfa from Pp2 sampling, and the rhizosphere of uninoculated alfalfa from Pp3 sampling were positive for more than 2 tests (Fig. 2c and d, respectively).

The number of plant growth promoting properties had increased in the Pp1, Pp2 and Pp3 samplings from Punta Espinillo I.

Please cite this article in press as: L.B. Guiñazú, et al., Evaluation of rhizobacterial isolates from Argentina, Uruguay and Chile for plant growth-promoting characteristics and antagonistic activity towards *Rhizoctonia* sp. and *Macrophomina* sp. *in vitro*, European Journal of Soil Biology (2012), http://dx.doi.org/10.1016/j.ejsobi.2012.09.007

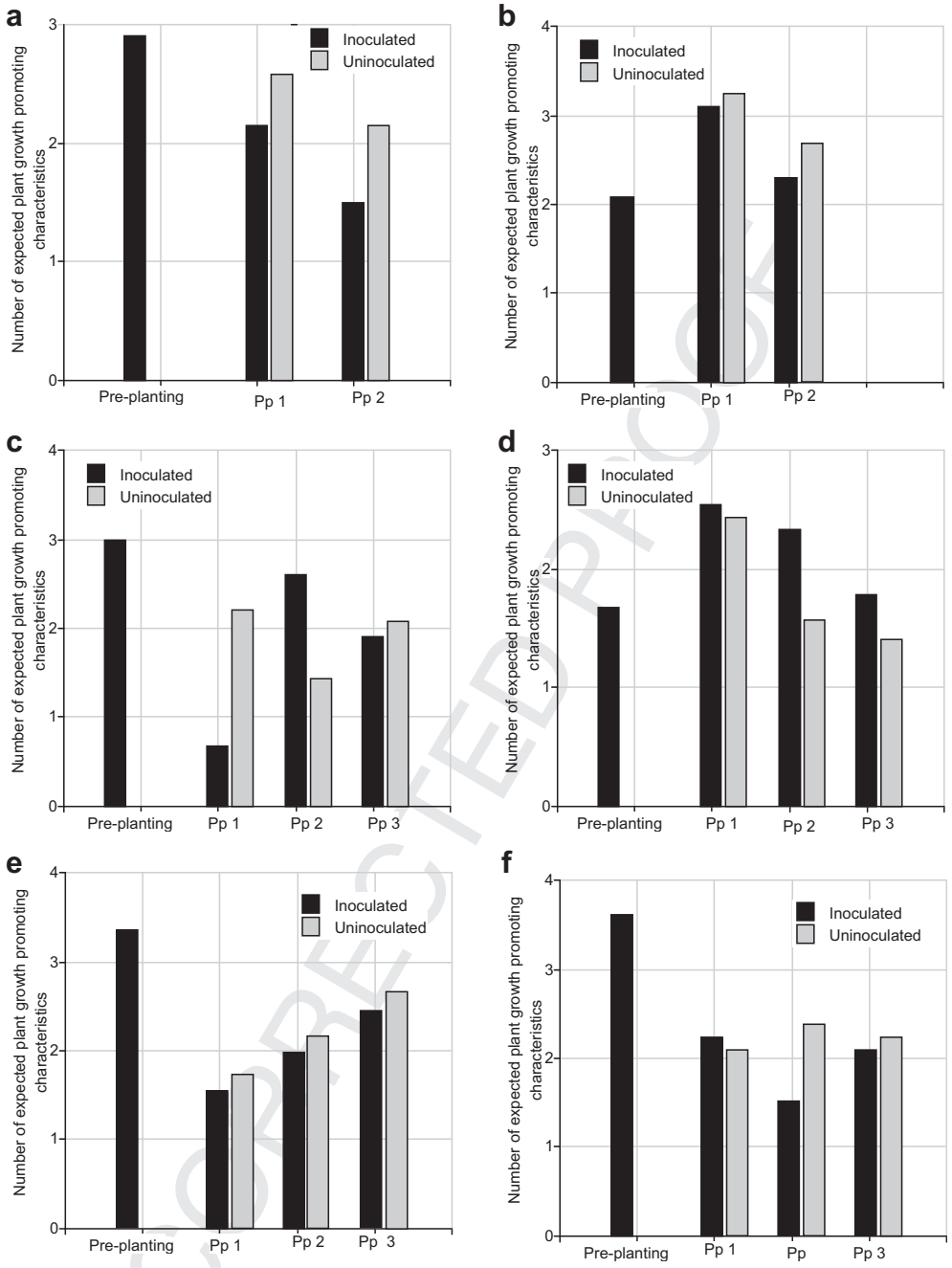

Fig. 2. Number of expected PGPR characteristics in isolates according to country, assay and sampling time. (a) Manfredi – (b) Balcarce – (c) Faja Maisan – (d) Carillanca – (e) Punta Espinillo I – (f) Punta Espinillo II. References: Pp: post-planting.

Isolates from Punta Espinillo II showed more than 2 positive characteristics, except for Pp2 samplings from rhizosphere of uninoculated alfalfa (Fig. 2e and f, respectively).

In the assay established in Manfredi, the proportion of isolates with capacity of producing exopolysaccharides (Ex. P.) and hydrolyzing starch (S. H.) was higher than that of the solubilizers of Fe and P and the antagonists of phytopathogenic fungi (Fig. 3a). In Balcarce, the proportion of isolates with capacity of antagonizing phytopathogenic fungi, producing exopolysaccharides (Ex. P.) and hydrolyzing starch (S. H.) was similar; nevertheless, it was higher when compared to isolates with capacity of solubilizing nutrients (Fig. 3b). In the Faja Maisan assay, the majority of the isolates showed capacity to produce exopolysaccharides as well as to hydrolyze starch (approximately 40%), being reduced the quantity of rhizobacteria, in some of the samplings, with solubilizing properties (Fig. 3c). A similar behavior can be observed in isolates from Carillanca, but the proportion of solubilizers was higher in the different samplings (Fig. 3d).

In the assays established in Uruguay, the proportion of solubilizing isolates was higher (near 20%) that in the other countries; in fact, it reached 40% in some of the post-planting samplings. The proportion of biocontrolling and exopolysaccharides and lytic enzymes producing rhizobacteria varied with the localization of the assay and the sampling time (Fig. 3e and f, respectively).

Ten strains selected for their potential PGPR properties were identified by API test and partial 16S RNA sequencing. Three Gram

Please cite this article in press as: L.B. Guiñazú, et al., Evaluation of rhizobacterial isolates from Argentina, Uruguay and Chile for plant growth-promoting characteristics and antagonistic activity towards *Rhizoctonia* sp. and *Macrophomina* sp. *in vitro*, European Journal of Soil Biology (2012), http://dx.doi.org/10.1016/j.ejsobi.2012.09.007

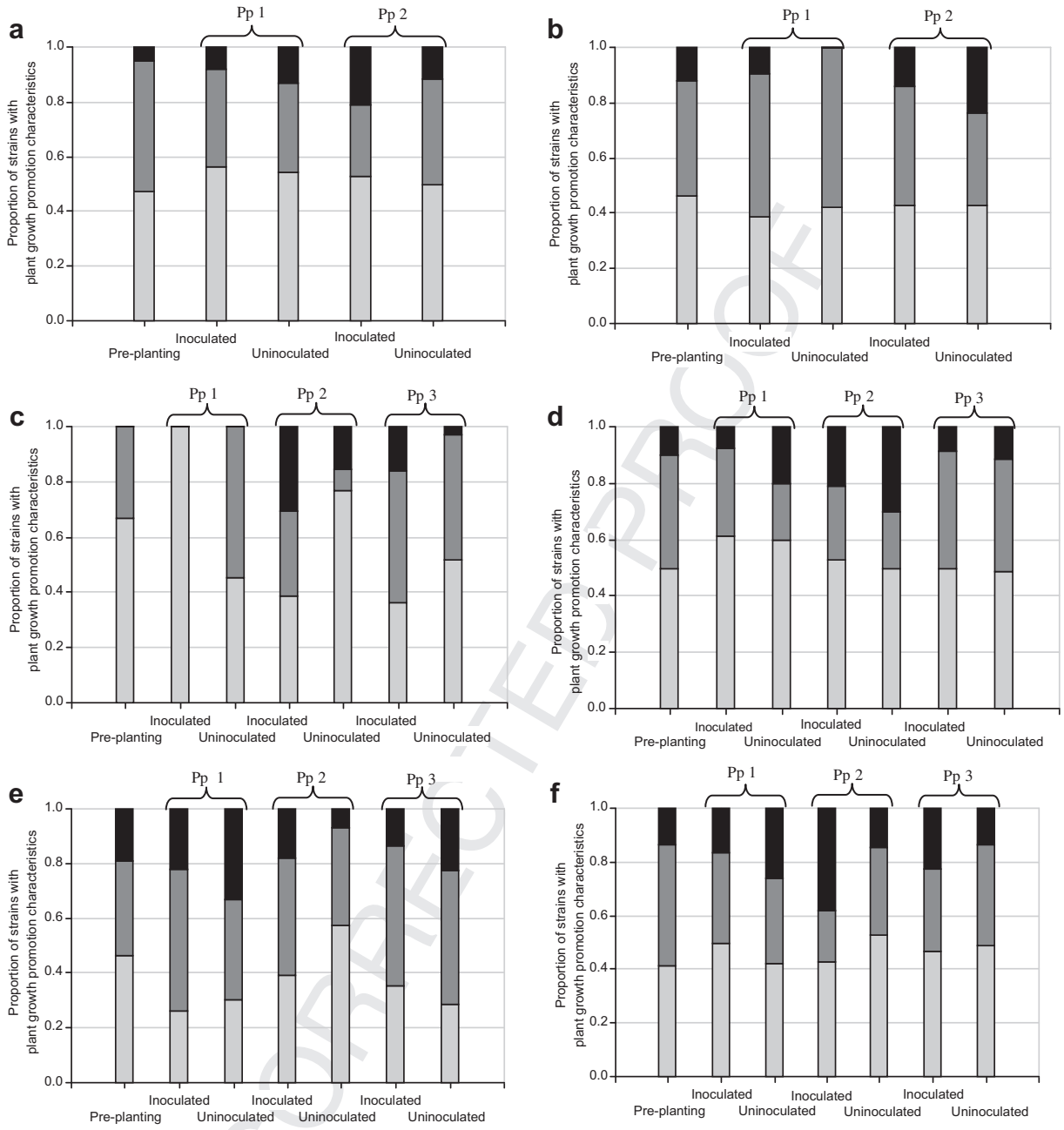

Fig. 3. Proportion of isolates with different desirable PGPR characteristics according to country, origin assay and sampling time. (a) INTA Manfredi, (b) INTA Balcarce, (c) Faja Maisan, (d) Carillanca, (e) Punta Espinillo I and (f) Punta Espinillo II. References: Pp: Post-planting; P and Fe solubilizers; biological control; starch hydrolysis and exopolysaccharides production.

positive strains were identified with The API 50 CH as members of the genus *Bacillus* (two corresponded to *Bacillus liqueniformis* and one to *Bacillus* spp.) One strain was identified as *Arthrobacter* spp. (API Coryne strip) and the 6 remaining isolates corresponded to the genus *Pseudomonas* (API 20NE tests). Of these, three where identified as *Pseudomonas putida* and the rest were identified as *Pseudomonas* spp.

PCR amplifications of the corresponding 16S rDNA gene were performed obtaining a 297 bp fragment from M7c, a 288 bp fragment from FM7d, a 287 bp fragment from Car, a 299 bp fragment from Bal1, a 299 bp fragment from Bal3, a 287 bp fragment from Manf, a 289 bp fragment from UI, a 287 bp fragment from Ch2, a 293 bp fragment from UII and a 287 bp fragment from Ch4. Pairwise comparisons were made between homologous 16S gene segments and showed that most of the 16S rDNA sequences from the isolates had maximum levels of identity (100–99%) with *Bacillus* sp. or *Pseudomonas* sp. The isolate UII showed 100–98% similarity to strains of the genus *Janibacter* (Table 2). This strain was isolated in Punta Espinillo, Uruguay, and showed ability to solubilize P and Fe *in vitro*.

Diversity of amplification patterns was observed in the band profiles obtained during genetic analyses. Consequently, we suppose that the PGPR effects would not be associated to a single genotype of wide distribution, but to a pronounced bacterial genetic diversity (Fig. 4).

Please cite this article in press as: L.B. Guiñazú, et al., Evaluation of rhizobacterial isolates from Argentina, Uruguay and Chile for plant growth-promoting characteristics and antagonistic activity towards *Rhizoctonia* sp. and *Macrophomina* sp. *in vitro*, European Journal of Soil Biology (2012), http://dx.doi.org/10.1016/j.ejsobi.2012.09.007

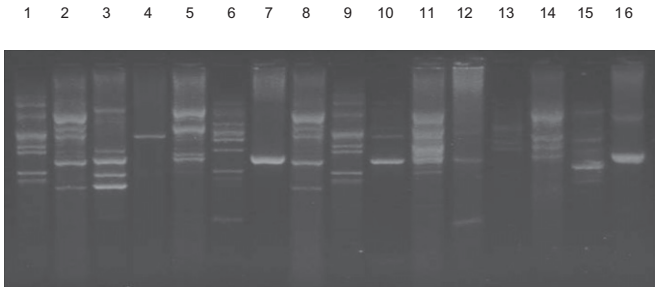

Fig. 4. Fingerprint obtained from genomic DNA of isolates from alfalfa rhizosphere and reference strains, using the BOX A1R primer. References: 1) Standard, 2) *P. putida* SP 21; 3) FM7d, 4) FM 10a, 5) Car, 6) Bal1, 7) *P. aurantiaca* SR1; 8) *P. putida* SP 22; 9) Standard, 10) Bal3, 11) M7c, 12) UI, 13) Manf, 14) Ch2, 15) UII, 16) Ch4.

4. Discussion

The method used to isolate bacteria, by means of serial dilutions and later inoculation on enriched media, is a broadly used procedure in studies of soil microbiology [32,33].

The cell extraction techniques require a balance between cell removal and cell death and the proportion of bacteria recovered may be small, influencing diversity. In addition, it is generally accepted that culturing imposes considerable bias on bacterial communities [34]. Nevertheless, no culture medium is sufficiently complex for the growth of all of the edaphic species, since their nutritional requirements are unknown and because of the complexity and variability of the root environment. Therefore, reports usually refer to a fraction of the total when informing bacterial colony counts. As described by McCaig et al. [34], we used a relatively non-selective medium (TSA) for isolating bacteria, which may have favored the growth of fast-growing aerobic bacteria. Also, the selection of isolates on the basis of colony morphotype was chosen to obtain a wide range of bacteria. Nevertheless, this technique may also have imposed bias since a single bacterial species may possess several distinguishable colony morphologies [35].

In this work, the average of the counts obtained from the different samplings (10^6–10^7 CFU/g soil or rhizosphere soil) was similar to that obtained by Miethling et al. [36], who studied the structural differences of leguminous rhizosphere communities and determined that the alfalfa rhizosphere extract contained 2.6×10^6 CFU/ml; also, there were no statistically significant differences in colony counts from different growth stages of the crop.

Several authors have reported that Gram negative bacteria are the most abundant rhizobacteria [37–39]. On the other hand, Gram positive sporulated and non sporulated bacilli as well as Gram positive non sporulated cocci are less abundant in this area [40,41]. However, González Vázquez [42] determined that cultivation rotation methods together with organic matter and solarization, changed the rhizosphere bacterial populations of pumpkin plants to a predominant Gram positive population. In our study, the number of Gram positive bacteria was higher than that of Gram negative microbes.

Ten isolates were characterized based on biochemical and genotypic features. These isolates were selected for their potential PGPR characteristics (all of the strains were positive for P solubilization and for production of siderophores and showed antifungal activity). Six were Gram negative, all belonging to the genus *Pseudomonas*. Four strains were Gram positive, three of which belonged to the genus *Bacillus* and one *Janibacter*. These ten isolates do not represent the bacterial diversity of the soil. However, the genera found as predominant (*Pseudomonas* and *Bacillus*) have been reported by several authors. Bowen & Foster [43] observed that *Pseudomonas* sp. from the root–soil interface have a higher generation time than *Bacillus*; therefore, when nutritional, humidity and temperature conditions are appropriate; *Pseudomonas* presents a faster growth rate. According to Frederickson & Elliott [44] and Bolton et al. [45], the genus *Pseudomonas* is very competitive in the rhizosphere environment due to their metabolic versatility. The inability of most of the microorganisms to use these compounds confers *Pseudomonas* a considerable competitive advantage [46]. Moreover, other authors reported that the genus *Bacillus* prevails in the non rhizosphere environment [47–49].

Isolate UII was initially identified as belonging to the genus *Arthrobacter*. However, the 16S rDNA partial sequence analysis revealed that it showed high similarity to strains of the genus *Janibacter*. This group of microorganisms encompasses aerobically growing, asporogenous, irregularly shaped, non-partially acidfast, Gram-positive rods, generally termed coryneforms. The isolation of *Janibacter* spp. strains was reported from environmentally polluted [50,51] and clinical samples [52]. No study on the isolation of these bacteria from the rhizosphere of agronomically important crops has been published so far. Although it was not outlined as an objective, this work represents the first report on the isolation of a strain belonging to the genus *Janibacter* with promising plant growth-promoting traits.

We observed certain differences in the characteristics of the 686 PGPR isolates from different geographical regions before and after planting (Fig. 3), which could be due to multiple factors affecting microbial diversity in soil. The number of microorganisms in the rhizosphere is always substantially higher because of the plant influence. There are also changes in the biodiversity of microorganisms caused by this "rhizosphere effect", which was defined by Badalucco and Kuikman [53] as any physical, chemical or biological change occurring within the root sphere or indirectly events mediated by excretions and organic debris. An important role is played by plants in selecting and enriching the types of bacteria by the constituents of their root exudates. Thus, the bacterial community in the rhizosphere develops depending on the nature and concentrations of organic constituents of exudates, and the corresponding ability of the bacteria to utilize these as sources of energy. Soil physical and chemical properties (pH, humidity and water availability, temperature, redox, salinity, texture, stability of aggregates, fertility, organic matter content), the presence or absence of pesticides and other xenobiotic substances are examples of well known abiotic factors that can directly or indirectly affect plant growth and their interaction with soil microbiota. Abiotic factors can also directly influence PGPR activity and probably their effect on plant growth and the dynamics of root microbial communities.

We observed that an important percentage of the isolated bacterial strains was positive for 2 or more of the PGPR tests. Cattelan et al. [54] observed a similar behavior when characterizing rhizobacteria isolated from soybean (*Glycine max* L. Merr) rhizosphere *in vitro* by means of nutrients solubilization tests, production of lytic enzymes and capacity to inhibit phytopathogenic fungi.

Fe and P solubilizing bacteria were isolated in most of the samples, independently of their previous record. The presence of bacterial groups capable of solubilizing Fe (property associated to the production of antibiotics and siderophores), has a decisive influence in competition phenomenons [55]; thus, they are important in suppressing plant pathogens. Legumes like alfalfa and clover show a high positive response to P supplementation [56], but most of the supplemented P becomes unavailable when it reacts with soil components. Many soil microorganisms are able to solubilize this unavailable P through their metabolic activities exudating organic acids, which directly dissolve the rock phosphate, or chelating calcium ions that release P to the solution.

Please cite this article in press as: L.B. Guiñazú, et al., Evaluation of rhizobacterial isolates from Argentina, Uruguay and Chile for plant growth-promoting characteristics and antagonistic activity towards *Rhizoctonia* sp. and *Macrophomina* sp. *in vitro*, European Journal of Soil Biology (2012), http://dx.doi.org/10.1016/j.ejsobi.2012.09.007

In other studies, we observed that strains *Bacillus* sp. M7C and *Pseudomonas* sp FM7d showed higher solubilization capacity than the control strain *P. putida* SP22, *in vitro*. In addition, the coinoculation of alfalfa with FM7d or M7C and *S. meliloti* B399 significantly increased most parameters measured, probably related to P uptake in coinoculated plants [28].

In general, bacteria with nutrients solubilizing capacities did not show ability to synthesize exopolysaccharides. Exopolysaccharides are associated with biofilm-forming ability and motility [57].

We did not observe a direct relationship between the antagonistic capacity and the production of enzymes and siderophores. However, several antagonistic bacteria were able to produce lytic enzymes and to synthesize siderophores.

Bacteria with antagonistic activity against *M. phaseolina* and *Rhizoctonia* spp. were isolated from all soils. This is consistent with studies carried out by Weller et al. [58] and Mazzola [59], who sustained that both natural and agricultural soils possess some capacity to decrease the incidence of plant pathogens. This is due to the presence and activity of the microorganisms inhabiting those soils.

One of the 10 isolates selected and genetically characterized in this study (*Pseudomonas* sp. Ch2) showed antifungal activity against the alfalfa pathogen *M. phaseolina* in the *in vitro* as well as in the *in vivo* assays [25]. Other authors have reported on the ability of strains of *Bacillus* and *Pseudomonas*, to inhibit different pathogens.

For example, *Bacillus subtilis* BN1 exhibited strong antagonistic activity against *M. phaseolina*, and other phytopathogens including *Fusarium oxysporum* and *Rhizoctonia solani* [60]. *Bacillus pumilus* strain SG2 secretes two chitinases with antifungal activity [61]. Species of *Pseudomonas* have been known to excrete chitinases and β-1,3-glucanases to digest the fungal cell wall chitin and glucan respectively and use these as a carbon and energy source [62] and are also reported to produce wide range of antifungal metabolites [63,64].

In summary, this study reports associations between PGPR characteristics for each set of strains, defined by country and soil status of source (pre and post seeding of alfalfa). We expect the results of this work contributes to improving the microbial germplasm collection of plant growth promoting bacteria from soils of Chile, Argentina and Uruguay and their potential use for the formulation of mixed inoculants to promote the growth of alfalfa.

Acknowledgments

This research was supported by FONTAGRO (ONU), Secretaría de Ciencia y Técnica of Universidad Nacional de Río Cuarto (SECyT-UNRC), PICTO-Agencia Nacional de Promoción Científica y Tecnológica y Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina). M. Balzarini and S. B. Rosas are members of the Research Career of CONICET. L. B. Guiñazú is recipient of a postdoctoral fellowship from CONICET.

References

[1] J. Sørensen, The rhizosphere as a habitat for soil microorganisms, in: J.D. Van Elsas, J.T. Trevors, E.M.H. Wellington (Eds.), Modern Soil Microbiology, Marcel Dekker, Inc., New York, N.Y, 1997, pp. 21–45.

[2] K. Smalla, G. Wieland, A. Buchner, A. Zock, J. Parzy, S. Kaiser, N. Roskot, H. Heuer, G. Berg, Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed, Appl. Environ. Microbiol. 67 (2001) 4742–4751.

[3] R.M. Bringhurst, Z.G. Cardon, D.J. Gage, Galactosides in the rhizosphere: utilization by *Sinorhizobium meliloti* and development of a biosensor, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 4540–4545.

[4] J.W. Kloepper, M.N. Scrhoth, T.D. Miller, Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield, Phytopathology 70 (1980) 1078–1082.

[5] J.R. De Freitas, M.R. Banerjee, J.J. Germida, Phosphate solubilizing rhizobacteria enhance the growth and yield but not of early soybean growth in nonsterile soil, our results phosphorus uptake of canola (*Brassica napus* L.), Biol. Fertil. Soils 4 (1997) 358–364.

[6] Y.P. Chen, P.D. Rekha, A.B. Arun, F.T. Shen, W.A. Lai, C.C. Young, Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities, Appl. Soil Ecol. 34 (2006) 33–41.

[7] S.B. Rosas, J.A. Andrés, M. Rovera, N.S. Correa, Phosphate-solubilizing *Pseudomonas putida* can influence the rhizobia–legume symbiosis, Soil Biol. Biochem. 38 (2006) 3502–3505.

[8] F.J. Gutierrez Mañero, N. Acero, J.A. Lucas, A. Probanza, The influence of native rhizobacteria on European alder [*Alnus glutinosa* (L.) Gaertn.] growth. II. Characterization of growth promoting and growth inhibiting strains, Plant Soil 182 (1996) 67–74.

[9] E. Dell'Amico, L. Cavalca, V. Andreoni, Improvement of *Brassica napus* growth under cadmium stress by cadmium-resistant rhizobacteria, Soil Biol. Biochem. 40 (2008) 74–84.

[10] Z.A. Siddiqui, G. Baghel, M.S. Akhtar, Biocontrol of *Meloidogyne javanica* by *Rhizobium* and plant growth-promoting rhizobacteria on lentil, World J. Microbiol. Biotechnol. 23 (2007) 435–441.

[11] H. Ahmed Idris, N. Labuschagne, L. Korsten, Screening rhizobacteria for biological control of *Fusarium* root and crown rot of sorghum in Ethiopia, Biol. Control 40 (2007) 97–106.

[12] E.H. Hijano, D.H. Basigalup, El cultivo de la alfalfa en la Argentina, in: E.H. Hijano, A. Navarro (Eds.), La alfalfa en la Argentina, INTA, 1995, pp. 12–18.

[13] A. Zurita, H. Urzúa, L. Longeri, A. Herrera, Factores de suelo limitantes del crecimiento de la alfalfa en cuatro suelos de la Xa Región. I. Disponibilidad de nutrientes, Ciencia e Investigación Agraria 21 (1994) 137–144.

[14] H. Urzúa, A. Zurita, L. Longeri, A. Herrera, Factores de suelo limitantes del crecimiento de la alfalfa en cuatro suelos de la X Región. II Acidez del suelo, Ciencia e Investigación Agraria 22 (1995) 49–56.

[15] R.L. Reed, M.A. Sanderson, J.C. Read, Harvest management of switchgrass grown for biomass, in: Forage Res, vol. 5252, TAES CPR, TX, 1994, 53–57.

[16] L. Frioni, Anexo práctico, in: Procesos Microbianos,, Fundación Universidad Nacional de Río Cuarto, Argentina, 1999, pp. 298–332.

[17] D.K. Zinniel, P. Lambrecht, N.B. Harris, Z. Feng, D. Kuczmarski, P. Higley, C.A. Ishimaru, A. Arunakumari, R. Barletta, A. Vidaver, Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants, Appl. Environ. Microbiol. 68 (2002) 2198–2208.

[18] J.M. Vincent, A Manual for the Practical Study of Root Nodule Bacteria, in: International Biology Programme Handbook No. 15, Blackwell Scientific Publications, Oxford, U.K., 1970.

[19] T.V. Suslow, M.N. Schroth, M. Isaka, Application of a rapid method for Gram differentiation of plant pathogenic and saprophytic bacteria without staining, Phytopathology 72 (1982) 917–918.

[20] D. Alexander, D. Zuberer, Siderophore producing bacteria of iron efficient and inefficient grass, in: D.L. Keister, P.B. Criang (Eds.), The Rhizosphere and Plant Growth, Kluwer Academic Publiser, The Netherlands, 1991, p. 308.

[21] M. Del Gallo, M. Negi, C. Neyra, Calcofluor- and lectin-binding exocellular polysaccharides of *Azospirillum brasilense* and *Azospirillum lipoferum*, J. Bacteriol. 171 (1989) 3504–3510.

[22] S.B. Rosas, F. Altamirano, E. Schröder, N. Correa, *In vitro* biocontrol activity of *Pseudomonas aurantiaca*, Phyton Int. J. Exp. Bot. (2001) 203–209.

[23] E. Carlier, M. Rovera, A. Rossi Jaume, S. Rosas, Improvement of growth, under field conditions, of wheat inoculated with *Pseudomonas Chlororaphis* subsp *aurantiaca* SR1, World J. Microbiol. Biotechnol. 24 (2008) 2653–2658.

[24] S.B. Rosas, N.A. Pastor, L.B. Guiñazú, J.A. Andrés, E. Carlier, M.V. Vogt, J. Bergesse, M. Rovera, Efficacy of *Pseudomonas chlororaphis* subsp. *aurantiaca* SR1 for improving productivity of several crops, in: Peeyush Sharma (Ed.), Crop Production Technologies, InTech, 2011, ISBN 978953307787-1, pp. 199–210.

[25] L.B. Guiñazú, J.A. Andrés, M. Rovera, S. Rosas, Isolation and characterization of *Macrophomina phaseolina* (Tassi) Goid antagonists rhizobacteria the cause of damping off in alfalfa (Chapter 10), in: A. Malik, E. Grohmann (Eds.), Environmental Protection Strategies for Sustainable Development, Springer Publisher, The Netherlands, 2012, pp. 329–339.

[26] J.F. Mac Faddin, Biochemical Tests for Identification of Medical Bacteria, third ed., Lippincott Williams & Wilkins, Baltimore, 2000, p. 912.

[27] R. Hugh, E. Leifson, The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria, J. Bacteriol. 66 (1953) 24–26.

[28] L.B. Guiñazú, J.A. Andrés, M.F. Del Papa, M. Pistorio, S.B. Rosas, Response of alfalfa (*Medicago sativa* L.) to single and mixed inoculation with phosphate-solubilizing bacteria and *Sinorhizobium meliloti*, Biol. Fertil. Soil 46 (2010) 185–190.

[29] J.P.W. Young, H.L. Downer, B.D. Eardly, Phylogeny of the phototrophic *Rhizobium* strain BTAi1 by polymerase chain reaction-based sequencing of a 16S rRNA gene segment, J. Microbiol. 173 (1991) 2271–2277.

[30] J. Versalovic, M. Schneider, F.J. de Bruijn, J.R. Lupski, Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction, Methods Mol. Cell. Biol. 5 (1994) 25–40.

[31] M. Balzarini, J. Di Rienzo, Info-Gen software para analisis estadístico de datos genéticos, Universidad Nacional de Córdoba, Córdoba, Argentina, 2004.

[32] A. Probanza, J.A. Lucas, N. Acero, F.J. Gutierrez Mañero, The influence of native rhizobacteria on European alder [*Alnus glutinosa* (L) Gaertn] growth I. Char-

Please cite this article in press as: L.B. Guiñazú, et al., Evaluation of rhizobacterial isolates from Argentina, Uruguay and Chile for plant growth-promoting characteristics and antagonistic activity towards *Rhizoctonia* sp. and *Macrophomina* sp. *in vitro*, European Journal of Soil Biology (2012), http://dx.doi.org/10.1016/j.ejsobi.2012.09.007

acterization of growth promoting and growth inhibiting strain, Plant Soil 182 (1996) 59–66.
[33] F.J. Gutierrez Mañero, B. Ramos Solano, A. Probanza, J. Mehouachi, F.R. Tadeo, M. Talon, The plant growth-promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins, Physiol. Plant 111 (2001) 1–7.
[34] A.E. McCaig, S.J. Grayston, J.I. Prosser, L.A. Glover, Impact of cultivation on characterization of species composition of soil bacterial communities, FEMS Microb. Ecol. 35 (2001) 37–48.
[35] M.W. Hahn, M.G. Höffle, Flagellate predation on a bacterial model community: interplay of size-selective grazing, specific bacterial cell size, and bacterial community composition, Appl. Environ. Microbiol. 65 (1999) 4863–4872.
[36] R. Miethling, K. Ahrends, C. Tebbe, Structural differences in the rhizosphere communities of legumes are not equally reflected in community-levels physiological profiles, Soil Biol. Biochem. 35 (2003) 1405–1410.
[37] J.W. Kloepper, C.J. Beauchamp, A review of issues related to measuring of plant roots by bacteria, Can. J. Microbiol. 38 (1992) 1219–1232.
[38] G.S. Gilbert, J.L. Parke, M.K. Clayton, J. Handelsman, Effects of an introduced bacterium on bacterial communities on roots, Ecology 74 (1993) 840–854.
[39] J.W. Kloepper, Plant growth-promoting rhizobacteria as biological control agents, in: F.B.J. Metting (Ed.), Soil Microbial Ecology, Marcel Dekker, New York, 1993, pp. 255–274.
[40] A.D. Rovira, E.I. Newman, H.J. Bowen, R. Campbell, Quantitative assessment of the rhizoplane bacteria microflora by direct microscopy, Soil Biol. Biochem. 6 (1974) 211–216.
[41] R. Campbell, Microbiology of root, in: R. Campbell (Ed.), Plant Microbiology, Edward Arnold Ltd, Londres, 1985, pp. 106–154.
[42] M. González Vázquez, Poblaciones de microorganismos asociados a la rizósfera del cultivo de calabaza (*Cucurbita moschata* Dutch.) durante un programa de agricultura sustentable, Tesis de Maestro en Ciencias. Universidad de Puerto Rico. (2005).
[43] G.D. Bowen, R.C. Foster, Dynamics of microbial colonization of plant roots, in: W.J. Broughton, C.K. John (Eds.), Proc. Symp. Soil Microbiol. Plant Nutrition, Univ. Malaysia Press, 1978, pp. 98–101.
[44] J.K. Fredrickson, L.F. Elliott, Effects on winter wheat seedling growth by toxing-producing rhizobacteria, Plant Soil 83 (1985) 399–409.
[45] B. Bolton, J.K. Fredrickson, F.E. Elliot, Microbial ecology of the rizosphere, in: F.B. Metting Jr. (Ed.), Soil Microbial Ecology: Applications in Agricultural and Environmental Management, Marcel Dekker, New York, 1993, pp. 27–63.
[46] P.J. Murphy, W. Wexler, W. Grzemski, J.P. Rao, D. Gordon, Rhizopines-their role in symbiosys and competition, Soil Biol. Biochem. 27 (1995) 525–529.
[47] E.C.S. Chan, H. Katznelson, J.W. Rovatt, The influence of soil and root extraction the associative growth of selected soil bacteria, Can. J. Microbiol. 9 (1963) 187–197.
[48] M.A. Gómez, M.A. Sagardoy, Efecto del glifosato sobre bacterias aerobias que colonizan un suelo arenoso, An. Edafol. Agrobiol. 44 (1985) 119.
[49] M. Salerno, M.A. Sagardoy, Presencia y caracterización de *Bacillus* spp. aislados de suelos cultivables de la zona semiárida de la provincia de Buenos Aires, Argentina, Agrochimica 34 (1990) 164–171.
[50] Y. Imamura, M. Ikeda, S.-I. Yoshida, H. Kuraishi, *Janibacter brevis* sp. nov., a new trichloroethylene-degrading bacterium isolated from polluted environments, Int. J. Syst. Evol. Microbiol. 50 (2000) 1899–1904.
[51] J.H. Yoon, H.B. Lee, S.H. Yeo, J.E. Choi, *Janibacter melonis* sp. nov., isolated from abnormally spoiled oriental melon in Korea, Int. J. Syst. Evol. Microbiol. 54 (2004) 1975–1980.
[52] J. Loubinoux, B. Rio, L. Mihaila, E. Foïs, A. Le Fleche, P.A.D. Grimont, J.P. Marie, A. Bouvet, Bacteremia caused by an undescribed species of *Janibacter*, J. Clin. Microbiol. (2005) 3564–3566.
[53] L. Badalucco, P.J. Kuikman, Mineralization and immobilization in the rhizosphere, in: R. Pinton, Z. Varanini, P. Nannipieri (Eds.), The Rhizosphere. Biochemistry and Organic Substances at the Soil-plant Interface, Marcel Dekker, New York, 2001, pp. 141–196.
[54] A.J. Cattelan, P.G. Hartel, J.J. Fuhrmann, Screening for plant growth-promoting rhizobacteria to promote early soybean growth, Soil Sci. Soc. Am. J. 63 (1999) 1670–1680.
[55] P. Marschner, D.E. Crowley, Iron stress and pioverdin production by a fluorescent pseudomonad in the rhizosphere of white Lupine (*Lupinus albus* L.) and Barley (*Hordeum vulgare* L.), Appl. Environ. Microbiol. 63 (1997) 277–281.
[56] P. Gyaneshwar, G.N. Kumar, L.J. Parekh, P.S. Poole, Role of soil microorganisms in improving P nutrition of plants, Plant Soil 245 (2002) 83–93.
[57] Y.W. Lee, S.Y. Jeong, Y.H. In, Lack of *O*-polysaccharide enhances biofilm formation by *Bradyrhizobium japonicum*, Lett. Appl. Microbiol. 50 (2010) 452–456.
[58] D.M. Weller, J.M. Raaijmakers, B.B. Mc Spadden Gardener, L.S. Thomashow, Microbial population responsible for specific soil suppressiveness to plant pathogens, Annu. Rev. Phytopathol. 40 (2002) 309–348.
[59] M. Mazzola, Mechanisms of natural soil suppressiveness to soilborne diseases, Antonie Van Leeuwenhoek 81 (2004) 557–564.
[60] N. Singh, P. Pandey, R.C. Dubey, D.K. Maheshwari, Biological control of root rot fungus *Macrophomina phaseolina* and growth enhancement of *Pinus roxburghii* (Sarg.) by rhizosphere competent *Bacillus subtilis* BN1, World J. Microbiol. Biotechnol. 24 (2008) 1669–1679.
[61] S. Ghasemi, G. Ahmadian, N.B. Jelodar, H. Rahimian, S. Ghandili, A. Dehestani, P. Shariati, Antifungal chitinases from *Bacillus pumilus* SG2: preliminary report, World J. Microbiol. Biotechnol. 26 (2010) 1437–1443.
[62] R.H. Leah, S. Tommerup, I. Svendsen, J. Murphy, Biochemical molecular characterization of three barley seed proteins with antifungal properties, J. Biol. Chem. 266 (1991) 1564–1573.
[63] D. Haas, G. Defago, Biological control of soil-borne pathogens by fluorescent pseudomonads, Nat. Rev. Microbiol. 3 (2005) 307–319.
[64] N.S. Ajit, R. Verma, V. Shanmugam, Extracellular chitinases of fluorescent pseudomonads antifungal to *Fusarium oxysporum* f. sp. dianthi causing carnation wilt, Curr. Microbiol. 52 (2006) 310–316.

Please cite this article in press as: L.B. Guiñazú, et al., Evaluation of rhizobacterial isolates from Argentina, Uruguay and Chile for plant growth-promoting characteristics and antagonistic activity towards *Rhizoctonia* sp. and *Macrophomina* sp. *in vitro*, European Journal of Soil Biology (2012), http://dx.doi.org/10.1016/j.ejsobi.2012.09.007