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ORIGINAL ARTICLE

Characterization of plant growth-promoting bacteria associated with rice cropped in iron-stressed soils

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Abstract Plant growth-promoting rhizobacteria (PGPR) are able to promote plant growth using a wide variety of mechanisms as well as provide bioprotection against biotic and abiotic stresses. The objectives of this study were to isolate and characterize putative PGPR associated with rice cultivars with a distinct tolerance to iron toxicity grown in two areas: one area with a well-established history of iron toxicity and another without iron toxicity. Bacterial strains were selectively isolated based on their growth in selective media and were identified by partial sequencing of their 16S rRNA genes. Bacterial isolates were evaluated for their ability to produce indolic compounds, siderophores, and ACC deaminase and to solubilize tricalcium phosphates. In vitro biological nitrogen fixation was evaluated for the bacterial isolates used in the inoculation experiments. A total of 329 bacterial strains were isolated. The composition of the bacterial genera and the occurrence of different plant growth-promoting (PGP) traits were significantly affected by the iron conditions and by the cultivar. Strains belonging to the *Burkholderia* and *Enterobacter* genera were the most abundant of all the Gram-negative isolates, and those belonging to the *Paenibacillus* and *Bacillus* genera were the most abundant of the Gram-positive isolates. A large number of putative PGPR belonging to different bacterial genera presented several PGP traits. Strains belonging to the *Burkholderia*, *Chryseobacterium*, and *Ochrobactrum* genera contributed to plant growth as well as to enhanced nutrient uptake of the rice plants in in vivo experiments. Growth and nutrient uptake of plants inoculated with isolate FeS53 (*Paenibacillus* sp.) in the presence of an iron excess were similar to those of plants submitted to the control iron condition, indicating that this bacterium can mitigate the effects caused by iron stress.

Keywords Plant growth-promoting rhizobacteria · PGP traits · Rice · Iron toxicity · Plant growth

Introduction

The plant rhizosphere, which is the narrow zone of soil influenced by the roots, is a multidimensional and dynamic ecological environment of diverse microbe–plant interactions. Plant-beneficial, root-colonizing bacteria, commonly referred to as plant growth-promoting rhizobacteria (PGPR), are rhizospheric bacteria that can enhance plant growth using a wide variety of direct and indirect mechanisms (Glick 2012). The favorable effects of PGPR inoculation on plant growth have been extensively reported (Sasaki et al. 2010; Ambrosini et al. 2012; Glick 2012; Arruda et al. 2013).

Increasing attention is currently being directed towards the contribution of beneficial microorganisms to the management of abiotic stresses due to the growing recognition that plant-associated microorganisms, including the rhizoplane and endophytic rhizobacteria and symbiotic fungi, play an important role in conferring tolerance to these stresses (Grover et al. 2011). Several studies addressing

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the positive effects of bacterial strains in plants grown under abiotic stress have been conducted, demonstrating, for example, the enhancement of salt tolerance in rice (Bal et al. 2013), the enhancement of tolerance to high temperatures in sorghum seedlings (Ali et al. 2009), and mitigation of the negative effects of drought in the common bean (Figueiredo et al. 2008). Moreover, the subject of PGPR eliciting tolerance to abiotic stresses has also been reviewed recently by Dimkpa et al. (2009a) and Grover et al. (2011).

Iron (Fe) is an essential micronutrient for plants. It is required in large abundance, as it is involved in various important biological processes, such as photosynthesis, respiration, chlorophyll biosynthesis (Kobayashi and Nishizawa 2012), and biological nitrogen fixation (Dixon and Kahn 2004). In aerobic conditions, the solubility of iron is very low due to the predominance of ferric (Fe^{3+}) ions usually found as oxihydroxide polymers, thereby limiting the iron supply for plant uptake, especially in calcareous soils (Andrews et al. 2003; Lemanceau et al. 2009). In anaerobic and acid soils, high concentrations of ferrous (Fe^{2+}) ions generated by the reduction of iron oxides (Fe^{3+}) in flooded soils may lead to iron toxicity due to excessive iron uptake (Stein et al. 2009). However, when absorbed in high concentrations by plants, it can generate oxidative stress (Stein et al. 2009). Iron toxicity is well-recognized as the most widely distributed nutritional disorder in lowland-rice production (Dobermann and Fairhurst 2000). Rice genotypes differ widely in their tolerance to iron toxicity. In the study by Stein et al. (2009), susceptible cultivars of rice plants grown in fields with high iron concentrations showed lower levels of the chlorophylls and soluble proteins and possessed higher levels of carbonyls, indicating oxidative damage. The excess of iron was also shown to inhibit plant growth and to cause a decrease in productivity.

As mentioned above, it has been shown that inoculation with PGPR can alleviate abiotic stress effects in different plant species. In contrast, there is an absence of studies on the rhizospheric microbial diversity associated with rice cropped in iron toxicity conditions, as well as none focusing on the contribution of PGPR in alleviating iron toxicity effects. It would therefore be interesting to determine whether an excess of iron interferes with the interaction between these microorganisms and the host plant (rice in our case) and with the PGP traits. The selection of microbial strains that exhibit the capability to stimulate plant growth under abiotic stress depends on traditional culture-based methods (Ambrosini et al. 2012). Therefore, the aims of our study were to (1) assess the diversity of putative PGPR strains associated with rhizospheric soil and roots of rice plants cropped in a field with a history of iron toxicity; (2) evaluate several plant growth promotion (PGP) activities of bacterial isolates; (3) test some of these bacteria for their ability to promote plant growth and/or alleviate the stress promoted by an excess of iron.

Materials and methods

Sampling and processing

Plants from two rice (*Oryza sativa* L.) cultivars displaying different levels of tolerance to iron toxicity [BR-IRGA 409 (susceptible) and IRGA 420 (tolerant); Bacha and Ishiy 1986] and the rhizospheric soil associated with these cultivars were collected from two regions in the state of Rio Grande do Sul, Brazil: Camaquã (30°54′07.96″S, 51°51′26.25″W), which has a well-documented history of iron toxicity, and Cachoeirinha (29°56′51.91″S, 51°06′46.36″W), which was used as a control (it does not have an excess of iron). All samples were collected in December 2010. Plants from the susceptible cultivar showed typical symptoms of iron toxicity (such as discoloration of leaves and necrosis in older leaves) only when grown at the iron-toxic site (Camaquã).

Ten samples (0.5 kg each) of fresh-weight soils were also collected from each site down to a depth of 15 cm; these were combined to obtain a representative composite sample. Three subsamples (0.5 kg fresh weight) of soil from each site were analyzed for pH, clay, phosphorus (P), potassium (K), Fe, exchangeable aluminum (Al), calcium (Ca), magnesium (Mg), and organic matter (OM) contents using standard methods (Sparks et al. 1996; Table 1).

Isolation of putative diazotrophic root and rhizospheric bacteria

Rhizospheric and root-associated bacteria were isolated from five independent plants with adhering (rhizospheric) soil that were spaced at least 2 m apart. Samples were randomly taken and bulked to obtain a representative composite sample. Bacteria from each sampling site were isolated according to Döbereiner (1988), using modified nitrogen-free, semi-solid NFb, LGI, and LGI-P media (Ambrosini et al. 2012; Souza et al. 2013). Bacilli and other Gram-positive bacteria were isolated according to Seldin et al. (1983) with the modifications described by Beneduzi et al. (2008), except that only rhizospheric soil was used for the bacterial isolation. After incubation, distinct colonies from each plate were randomly selected and grown in liquid LB medium (Sambrook and Russel 2001) at 28 °C with agitation (200 rpm). Different colonies were isolated according to the size, shape, and color, among other characteristics. These bacterial isolates were individually analyzed by Gram-staining and immediately stored in sterile glycerol solution (50 %) at −20 °C.

Extraction of bacterial DNA

Bacterial DNA was extracted according to Ambrosini et al. (2012) and Souza et al. (2013). Phenol–chloroform extraction and ethanol precipitation were performed as described by

Table 1 Abiotic soil characteristics of the sampling sites

Sampling sites[a]	Clay (%)	OMC (%)	pH (H_2O)	P (mg dm^{-3})	K (mg dm^{-3})	Fe (%)	Ca_{exc} ($cmol_c$ dm^{-3})	Al_{exc} ($cmol_c$ dm^{-3})	Mg_{exc} ($cmol_c$ dm^{-3})
CaR	28	3.0	4.8	9.0	43.0	0.18	2.7	0.5	1.0
CaS	28	3.0	4.7	22.2	73.0	0.15	2.7	0.4	1.0
FeR	24	1.1	5.4	0.9	54.0	0.25	2.1	0.4	1.3
FeS	25	1.1	5.5	0.9	57.0	0.35	2.4	0.4	1.6

P Phosphorus, *K* potassium, *Fe* iron, *Ca* calcium, *Al*, aluminum, *Mg* magnesium, *OMC* Organic matter content, *exc* exchangeable

[a] *CaR* Tolerant cultivar, Cachoeirinha; *CaS* susceptible cultivar, Cachoeirinha; *FeR* tolerant cultivar, Camaquã; *FeS* susceptible cultivar, Camaquã

Sambrook and Russel (2001). The quality and integrity of the DNA were determined by electrophoresis in 0.8 % agarose gels containing ethidium bromide and visualized under UV light. Fifty nanograms of bacterial DNA was used as the template for PCR procedures.

PCR amplification and sequencing of the bacterial 16S rRNA gene

Partial sequences of the 16S rRNA gene (roughly 450 bp) from each Gram-negative isolate were amplified using the conditions described by Souza et al. (2013). Bacilli and other Gram-positive bacteria were identified at the genus level by analysis of the 16S rRNA gene (approximately 1,500 bp) using the BacPaeF (AGAGTTTGATCCTGGCTCAG; Stackebrandt and Liesack 1993) and Bac1542R (AGAAAG GAGGTGATCCAGCC; Edwards et al. 1989) primers. Thermal cycling was performed according to Garbeva et al. (2003). PCR products were analyzed by electrophoresis in 1 % agarose gels containing ethidium bromide and visualized under UV light. Sequencing was determined in a Megabace 1000 automatic sequencer using a DYEnamic™ ET Dye Terminator Cycle Sequencing Kit (GE HealthCare, Madison, WI).

Sequences were trimmed to exclude low-quality sequenced nucleotides. DNA sequences were compared with sequences from the EzTaxon Server version 2.1 (http://eztaxon-e.ezbiocloud.net/) and the GenBank using BLASTN software (http://blast.ncbi.nlm.nih.gov/). The nucleotide sequences of the 329 partial 16S rRNA gene segments determined in this study have been deposited in the GenBank database (accession numbers KC550309–KC550638).

Diversity indices

Diversity indices (*H′* and *H″*; Shannon and Weaver 1949) were estimated based on the total number of individuals and the number of genera identified for each sampled cultivar. Principal component analysis (PCA) was used to verify the correlation between soil properties and rhizospheric bacterial diversity (Rico et al. 2004).

Evaluation of the characteristics that promote plant growth

The putative PGP capacity of the bacterial isolates was evaluated by in vitro tests. Bacterial suspensions (10 μl of 10^8 CFU ml^{-1}) of each isolate grown in LB medium at 28 °C with agitation (125 rpm) for 48 h were used as inocula for the PGP experiments. All bacterial isolates were analyzed for the production of indolic compounds (IC) and siderophores, ACC deaminase, and tricalcium phosphate solubilization activities. In vitro biological nitrogen fixation assays were subsequently performed on nine selected isolates.

In the in vitro IC production assay, the bacterial isolates were incubated for 72 h in Kings B medium supplemented with tryptophan (Glickmann and Dessaux 1995) at 28 °C, with agitation (200 rpm). As described by Ambrosini et al. (2012), the supernatant (500 μl) was mixed with an equal volume of Salkowski reagent (12 g l^{-1} $FeCl_3$+7.9 M H_2SO_4) in test tubes, and the mixture was kept in the dark for 30 min to allow for color development. The pink to red color produced after exposure to the Salkowski reagent is considered to be indicative of the bacterial production of indolic compounds. The samples were measured spectrophotometrically at 550 nm using a standard curve for calibration.

Siderophore production was assayed according to Schwyn and Neilands (1987) using King B medium (Glickmann and Dessaux 1995) without tryptophan. The isolates were spot inoculated onto chrome azurol S agar plates and incubated at 28 °C for 48–72 h. Development of a yellow, orange, or violet halo around the bacterial colony was considered to be positive for siderophore production.

The method described by Ambrosini et al. (2012) was used to identify isolates able to solubilize tricalcium phosphate. Bacteria were grown in glucose yeast medium. Two other solutions were prepared separately, one containing 5 g K_2HPO_4 in 50 ml of distilled water, and the other containing 10 g $CaCl_2$ in 100 ml of distilled water. These solutions were added to 1 l of GY medium just before it was poured into petri dishes, and the combination resulted in the formation of an insoluble layer of calcium phosphate that made the medium opaque. The plates were inoculated with the bacterial isolates and then incubated for 7 days at 28 °C. Those isolates that

formed visibly clear halos around their colonies were considered to be tricalcium phosphate-solubilizers.

The nitrogen-fixing ability of isolates was tested using the acetylene reduction assay (ARA), as described by Boddey (1987) and Ambrosini et al. (2012). Nitrogenase activity was measured after growth into 10-ml vials containing 4 ml of semi-solid (0.18 % agar–agar) N-free media, either NFb, LGI or LGI-P. After 72 h of incubation at 28 °C in the dark, the vials were sealed with rubber septa and 10 % (v/v) of the air phase was replaced with acetylene. The cultures were then incubated for 1 h in acetylene, after which the amount of C_2H_4 produced was measured for three vials for each isolate using a Clarus 600 gas chromatograph (PerkinElmer, Waltham, MA). Protein concentration in the resulting mixture was determined by a standard method (Bradford 1976). *Azospirillum brasilense* Sp7 and *Paenibacillus riograndensis* SBR5 were used as a positive control.

Evaluation of the ACC deaminase activity was based on the ability of the isolates to use ACC as a nitrogen source and was determined according to Penrose and Glick (2003). Bacterial suspensions were washed three times in sterile saline solution, and the bacteria were then inoculated onto plates containing 25 ml of DF salts medium with ACC (0.5 M). Inoculated plates without ACC were used as a negative control. All plates were incubated at 28 °C for 5 days and examined every day.

Catalase activity was determined by incubating a loopful of each culture mixed with 50 μl of 3 % (v/v) H_2O_2 on a glass slide at room temperature to observe the release of oxygen, which was recorded as a positive reaction for catalase.

Each isolate was classified according to the level of IC production, with level 1 isolates producing between 0.1 and 10 μg IC ml^{-1} and level 2 isolates producing >11 μg IC ml^{-1}. For tricalcium phosphate solubilization and siderophore production, the development of a halo around the bacterial colony was considered to be indicate a positive reaction. The halo size was used as an estimate of the degree of phosphate solubilization/siderophore production, with "+" indicating halos ranging from 0.1 to 0.6 mm, and "++" indicating halos >0.6 mm.

In vivo experiments on PGP by native PGPR isolates

The growth chamber experiment was conducted with a photoperiod cycle of 14 h light at 28 °C and 10 h dark at 20 °C. The experimental units consisted of pots (15×20 cm) sterilized with 0.7 % sodium hypochlorite solution before the seeds were planted. Rice seeds from the BR-IRGA 409 cultivar (susceptible to the effects of excess iron) provided by IRGA (Instituto Rio Grandense do Arroz, Brazil) were surface disinfected as described by Souza et al. (2013). The sterilized seeds were immediately inoculated with different isolates (described below) for 30 min, and the treated seeds were planted in sterile vermiculite, 2 cm below the surface, and irrigated with nutrient solution. The plant nutrient solution used was that reported by Yoshida (1981) with some modifications: 0.1 mM KCl, 0.1 mM KH_2PO_4, 0.7 mM K_2SO_4, 1 mM $Ca(NO_3)_2.4H_2O$, 0.5 mM $MgSO_4$, and micronutrients. After germination and growth in vermiculite for 12 days, the plant roots were re-inoculated by submerging them in 10 ml bacterial culture for 5 min and then immediately transferred to hydroponic conditions (pots containing 500 ml of the same nutrient solution and covered with aluminum foil). Each pot harbored two plants, which were kept for 10 days in iron control solution. Under these same hydroponic conditions the plants were then subjected to different treatments: (1) excessive iron (500 mg l^{-1} Fe) or (2) control (6.5 mg l^{-1} Fe). In both conditions, $Fe_2(SO_4)_3.5H_2O$ was used as the iron source, and the pH of the solution was 5.0 ±0.1. Nutrient solutions were replaced every 72 h to maintain the concentration and solubility of the iron. Five repetitions per treatment were performed.

Bacterial isolates (nine) were grown in LB medium with agitation (125 rpm) for 48 h at 28 °C, and pure bacterial cultures were centrifuged and diluted to a final concentration of 10^8 CFU ml^{-1} in sterile saline solution. Seeds were inoculated with 10-ml aliquots of the cell suspensions. Two independent experiments were conducted in hydroponic conditions (anaerobic conditions). The first experiment was carried out with isolates which were iron-susceptible (FeS) at the Camaquã site, namely, FeS14, FeS42, FeS56, and FeS57, and a non-inoculated control. The second experiment was carried out with bacilli isolates that were iron-tolerant and iron-susceptible at the Cachoeirinha site (CaR114 and CaS40, respectively), as well as with FeR64, FeS24, FeS53, and a non-inoculated control. Both experiments were run for 30 days, after which time several parameters were evaluated. Plants were harvested, and the length data were recorded. Shoots and roots were dried at 65 °C to a constant weight to evaluate the dry matter. All shoot dry matter contents from each treatment were combined for elemental analysis. The shoot nutrient content was determined by the Kjeldahl method (detection limit 0.01 %) for nitrogen (N), and P and K content were determined by nitric-perchloric wet digestion/inductively coupled plasma–optical emission spectrometry (detection limit 0.01 %). The nutrient content of the plants was estimated for each treatment through the uptake per gram of plant tissue multiplied by the total yield per treatment [(yield) × (percentage nutrient per gram of plant tissue)] (Adesemoye et al. 2009).

Statistical analysis

The microbial community structure of each sample studied (cultivar × iron status) was compared using Pearson's chi-squared test. A residue analysis was used to detect differences between individual bacterial genera, which were considered to be significant for standardized adjusted residues of>|1.96|. Data from the pot trials were statistically analyzed using analysis of

variance, and the means were compared using the Scott–Knott test (p=0.05 %). Homoscedasticity and normality were verified using Levene's test and histogram analysis.

Results

Isolation and identification of putative PGP bacteria

In this work, 329 cultivable and putative nitrogen-fixing and plant growth-promoting rhizobacteria were isolated. From these 329 bacterial strains, 229 were isolated based on their growth in nitrogen-free media under aerobic conditions (Table 2), and 100 were isolated based on their growth on nitrogen-free medium under anaerobic conditions (Table 3). The 26 distinct bacterial genera to which the identified Gram-negative isolates belong and the one genus to which the identified Gram-positive isolate belongs are given in Table 2 that were identified. Table 3 shows the eight bacterial genera to which the identified putative nitrogen-fixing bacilli and other Gram-positive isolates belong. Both tables show the bacterial genus identified and the frequency of that genus in the rhizospheric soil and root samples for the two rice cultivars investigated.

As can be observed in Table 2, bacterial isolates belonging to the *Burkholderia* and *Enterobacter* genera were the most abundant among all aerobic isolates. Bacterial genera composition was significantly affected by the cultivar (p=0.001) and by the environment (p=0.01), indicating that both cultivars were present in different associated bacterial communities and that soil composition also affected the composition of the bacterial community.

The associated bacterial communities of the tolerant cultivars changed according to environmental conditions (p= 0.002): when samples from the tolerant cultivar cropped in the iron-stressed location (Camaquã) were analyzed, the most abundant strains identified belonged to the *Burkholderia* genus (20 isolates), whereas the same cultivar cropped in the Cachoeirinha locality (without an excess of iron) was represented by only ten isolates belonging to this genus, which was a significant difference. Moreover, strains belonging to the *Cronobacter* and *Pantoea* genera were found preferentially in samples collected in the control location, and strains belonging to *Leclercia* genus were only found in samples collected in the iron-stressed location. On the other hand, the associated bacterial community of the susceptible cultivar did not change according to iron stress (p=0.114).

The proportion of bacterial genera was significantly different between the susceptible and tolerant cultivars (Table 2). In the iron-stressed location, the associated bacterial community was different for the two rice cultivars (p=0.025), with the occurrence of strains belonging to the *Leclercia* genus being more associated with the tolerant cultivar than with the susceptible cultivar. In the control location, the composition of the bacterial genera was also affected by the cultivar (p= 0.001), with strains belonging to the *Burkholderia* genus being more associated with the susceptible cultivar than with the tolerant cultivar, whereas strains belonging to the *Enterobacter* and *Cronobacter* genera were more associated with the tolerant cultivar than with the susceptible cultivar.

Table 3 shows that the strains belonging to the *Paenibacillus* and *Bacillus* genera were the most abundant groups among the putative nitrogen-fixing bacilli and the other Gram-positive bacterial isolates. The associated bacterial community was significantly different in the susceptible cultivar under both environmental conditions (p<0.001), with the occurrence of strains belonging to the *Bacillus* genus being more associated with the control location than with the iron-stressed location. Strains belonging to the *Leifsonia* genus were only found in samples collected in the iron-stressed location. On the other hand, the associated bacterial community was similar in terms of the tolerant cultivar at the same environmental conditions (p=0.408). Moreover, the composition of the bacterial genera was similar between the cultivars at both the control location (p=0.095) and the iron-stressed location (p=0.446) (Table 3).

Diversity indices for each sampling site are also presented in Tables 2 and 3. According to Table 2, the bacterial community isolated from samples obtained from the susceptible cultivar cropped in the iron-stressed location presented the highest genetic diversity (H'=2.23), whereas that isolated from samples from the tolerant cultivar cropped in the same region presented the lowest diversity index (H'=1.65). On the other hand, in terms of the diversity of the Gram-positive bacteria (H''; Table 3), the bacterial community isolated from samples collected from the susceptible cultivar cropped in the iron-stressed location presented the highest diversity index (H''=1.27), whereas much lower indices were obtained from those isolated from the other samples (Table 3).

The chemical analysis of soils (Table 1) indicated low pH values in both sampling areas. However, soil from the iron-stressed site showed higher values of pH and Fe, as well as lower levels of OMC and P, than samples from the control site. PCA was used to investigate the relationships between bacterial diversity (H' and H'') and abiotic soil parameters (Fig. 1). The first two dimensions of PCA (PCA1 and PCA2) explained 86.12 % of the total variation, with component 1 accounting for 61.35 % and clearly separating the control site (Cachoeirinha) from the iron-stressed site (Camaquã). Component 2 accounts for 24.77 % of the variance and separates the susceptible from the tolerant cultivars. This analysis showed that of the soil factors assessed, Al, clay, Ca, and OM contents were the most closely related to H', and K and P contents seemed to be the major soil parameters affecting the diversity of the Gram-positive isolates (H'').

Table 2 Bacterial genera found in association with the roots and rhizospheric soil of rice cultivars cropped in areas with different levels of iron and the Shannon diversity indices (*H'*) for each sampling site

Bacterial genus	Cachoeirinha				Camaquã				
	CaR		CaS		FeR		FeS		Total
	1[a]	2[a]	1	2	1	2	1	2	
Achromobacter	1	–	1	–	–	–	–	–	2
Bosea	–	–	1	–	–	–	–	–	1
Burkholderia	4	6	15	8	13	7	12	3	68
Cedecea	–	–	–	1	–	–	2	–	3
Chryseobacterium	–	–	–	–	–	–	–	1	1
Cronobacter	4	3	–	–	1	–	–	–	8
Citrobacter	1	1	1	1	1	6	2	1	14
Dyella	–	–	1	1	–	–	1	–	3
Enterobacter	12	8	1	4	5	14	2	11	57
Escherichia	–	1	–	–	–	–	–	1	2
Gluconacetobacter	–	–	–	–	1	–	–	–	1
Herbaspirillum	–	3	–	3	1	–	1	–	8
Klebsiella	–	–	–	–	1	–	–	–	1
Leclercia	–	–	–	–	4	–	–	–	4
Lysinibacillus	–	–	–	–	–	–	–	1	1
Luteibacter	–	–	–	1	–	–	–	–	1
Microvirga	–	–	1	–	–	–	–	–	1
Ochrobactrum	–	–	–	–	–	–	–	1	1
Pandoraea	1	–	2	–	–	–	2	–	5
Pantoea	1	6	1	8	–	1	1	3	21
Pseudomonas	3	–	3	–	1	1	2	4	14
Rhizobium	–	–	–	–	–	–	1	–	1
Rahnella	–	1	–	–	–	–	–	–	1
Salmonella	1	1	–	–	–	–	–	2	4
Serratia	1	–	1	2	–	–	–	–	4
Spirillum	–	–	–	–	–	–	1	–	1
Yokenella	1	–	–	–	–	–	–	–	1
H'	2.12	2.04	1.65	2.23	229				

–, Not identified

[a] Bacterial genera isolated from: 1, Rhizospheric soil; 2, roots

Screening of PGP traits of bacterial isolates

All 229 strains selectively isolated based on their growth on nitrogen-free media under aerobic conditions were analyzed for the presence of four PGP properties (Table 4). According to Table 4, one of the most evident characteristic among these isolates was their ability to produce IC, which ranged from 0.11 to 50 μg IC ml^{-1}, with 22 isolates producing >10 μg IC ml^{-1} after 72 h of incubation. Of these 22 isolates, 16 were isolated from the susceptible cultivar cropped in the iron-stressed site (Camaquã). The level of IC production was also different according to the environment for the susceptible cultivar ($p=0.001$). There were more level-1 IC producers in the control site than in the iron-stressed site ($p<0.05$), while there were more level-2 IC producers in the iron-stressed site ($p<0.05$). For the tolerant cultivar, the IC production ability of the isolates presented very similar levels ($p>0.05$) for both sites. Strains identified as good IC producers were those belonging to the *Burkholderia*, *Enterobacter*, and *Pantoea* genera.

Another ability displayed by most of the aerobic isolates was siderophore production (216/229; Table 4), with 41 strains isolated from samples from the tolerant cultivar cropped in the iron-stressed site presenting larger halos (++) compared to 28 strains isolated from the same cultivar cropped in the control site ($p=0.002$). Isolates from the

Table 3 Gram-positive bacterial genera found associated with rhizospheric soil of rice cultivars cropped in areas with different levels of iron and the Shannon diversity indices (*H″*) for each sampling site

Bacterial genus	Cachoeirinha		Camaquã		
	CaR	CaS	FeR	FeS	Total
Arthrobacter sp.	–	–	–	2	2
Bacillus sp.	1	10	3	1	15
Clostridium sp.	–	1	–	–	1
Leifsonia sp.	–	–	3	5	8
Micrococcus sp.	–	–	1	–	1
Paenibacillus sp.	16	23	18	13	70
Sinomonas sp.	–	–	–	1	1
Staphylococcus sp.	–	–	1	1	2
H″	0.22	0.73	1.00	1.27	100

–, Not identified

susceptible cultivar from both regions did not differ in the ability to solubilize iron (p=0.076). Isolates identified as belonging to the *Burkholderia*, *Enterobacter*, and *Pantoea* genera were identified as good siderophore producers.

In addition to the above characteristics, 102 of 229 isolates were able to solubilize tricalcium phosphates (Table 4). The isolates of the tolerant cultivar differed in their tricalcium phosphate solubilization ability between the environments (p=0.009), a higher number of the best tricalcium phosphate solubilizers found in the control site (16 isolates with halo ++) than at the iron-stressed site (3 isolates with halo ++) (p<0.05). For the susceptible cultivar, however, there was no difference in the tricalcium phosphate solubilization ability displayed by the isolates for both environments (p=0.387). Isolates identified as belonging to the *Burkholderia* genus were identified as good phosphate-solubilizing strains.

According to Table 4, ACC deaminase activity was the rarest ability among the isolates (51/229). The occurrence of ACC deaminase activity was dependent on both the environment and the cultivar (p=0.012). In the control site, the susceptible cultivar possessed a greater number of ACC deaminase producers (p<0.05). On the other hand, in the iron-stressed site, the highest number of ACC deaminase producers was found in the tolerant cultivar (p<0.05). Isolates belonging to the *Burkholderia* genus were identified as good ACC deaminase producers.

The PGP traits of putative diazotrophic bacilli and other Gram-positive bacteria are shown in Table 5. The most evident characteristic among those Gram-positive strains was the production of IC, as 94 isolates were able to produce IC in vitro, of which seven produced >10 μg IC ml^{-1} after 72 h of incubation. Only two strains were able to produce siderophores, 11 were able to solubilize tricalcium phosphate, and four isolates presented ACC deaminase activity under our in vitro conditions. There were no strains among the bacterial communities isolated from the tolerant cultivar from the control region displaying any other ability than IC production. The rhizosphere soils from the samples collected with both cultivars cropped in the iron-stressed site were found to have the higher numbers of phosphate-solubilizing strains compared to those from the other site.

The biological nitrogen fixation and catalase activity in vitro assays were performed for nine bacterial isolates that were selected based on their taxonomic identification and PGP attributes in the in vivo experiment in the growth chamber. Table 6 shows the results of the PGP activities evaluated for these isolates. Four bacterial isolates were used to inoculate the rice plant seeds in the first experiment: FeS14 (*Burkholderia* sp.), FeS42 (*Chryseobacterium* sp.), FeS56 (*Ochrobactrum* sp.), and FeS57 (*Lysinibacillus* sp.). The

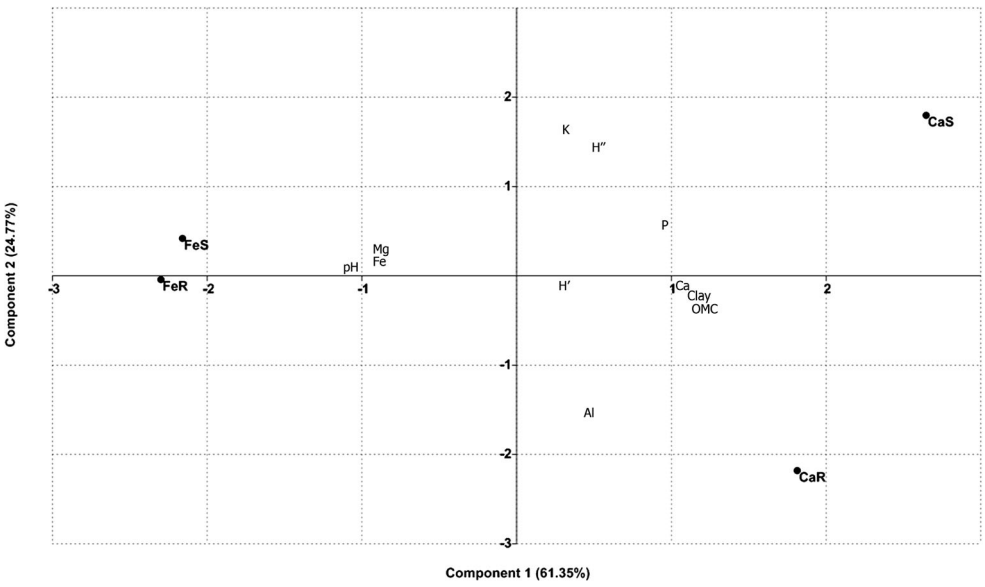

Fig. 1 Principal component (PC) analysis of the diversity indices (*H′* and *H″*) of the sampling sites (*CaR* tolerant cultivar, Cachoeirinha; *CaS* susceptible cultivar, Cachoeirinha; *FeR* tolerant cultivar, Camaquã, *FeS* susceptible cultivar, Camaquã) in relation to different soil properties [clay, organic matter content (*OMC*), pH, phosphorus (*P*), potassium (*K*), iron (*Fe*), calcium (*Ca*), and aluminum (*Al*)]. PC 1 and PC 2 accounted for 61.35 and 24.77 % of the total variation, respectively

Table 4 Number of isolates, siderophore, ACC deaminase, and indolic compound producers, and tricalcium phosphate solubilizers among the bacterial isolates at each sampling site

Site	Source	Number of isolates	Siderophore producers[a]		Phosphate solubilizers[b]		ACC deaminase producers	IC producers (μg ml^{-1})[c]	
			+	++	+	++		1	2
CaR	Rhizosphere	30	15	14	9	7	5	29	1
	Root	30	16	14	8	9	3	29	1
CaS	Rhizosphere	28	5	21	5	8	7	26	2
	Root	29	13	15	7	5	8	28	1
FeR	Rhizosphere	28	3	23	15	2	13	24	1
	Root	29	10	18	5	1	7	28	0
FeS	Rhizosphere	27	6	15	8	4	6	21	6
	Root	28	19	9	5	4	2	18	10
Total number		229	216	102	51	203	22		

IC Indolic compound

[a] Development of a yellow, orange, or violet halo around a bacterial colony was considered to indicate positivity for siderophore production. The halo size was used as an estimate of the degree of siderophore production: +, Halos 0.1–0.6 cm; ++, halos >0.6 cm

[b] Development of a halo around a bacterial colony was considered to indicate positivity for tricalcium phosphate solubilization. The halo's size was used as an estimate of the degree of phosphate solubilization: +, Halos 0.1–0.6 cm; ++, halos >0.6 cm

[c] Each isolate was classified according to the level of IC production: level 1 isolates produced between 0.1 and 10 μg IC ml^{-1} ; level 2 isolates produced >11 μg IC ml^{-1}

Gram-positive isolates CaR114 (*Paenibacillus* sp.), CaS40 (*Paenibacillus* sp.), FeR64 (*Bacillus* sp.), FeS24 (*Paenibacillus* sp.), and FeS53 (*Paenibacillus* sp.) were used in the second experiment.

First plant assay: growth-promoting effect of rice plants with bacterial treatments

To test the interaction between Gram-negative PGPR and rice plants, an in vivo experiment was conducted in a growth chamber with the four previously identified selected isolates (Table 6). The plants were submitted to different treatments under hydroponic conditions, including an excess of iron (500 mg l^{-1} iron) and the control treatment (6.5 mg l^{-1} iron). Individual plants submitted to the iron stress condition + inoculation did not present any significant differences (data not shown).

Under the growth chamber conditions, most of the PGPR used for the inoculation of rice plants resulted in positive effects on plant growth in the iron control treatment (Table 7). Rice plants inoculated with the FeS42 and FeS56 strains had significantly higher dry shoot biomass, length, and dry biomass of roots than the non-inoculated plants.

Rice plants inoculated with the FeS14, FeS42, and FeS56 isolates showed enhanced N uptake compared to that of the non-inoculated plants. Moreover, plants inoculated with the FeS14, FeS42 and FeS56 strains showed a significant increase in the amount of K accumulated in the shoots compared to that

Table 5 Number of isolates, siderophore, ACC deaminase, and indolic compound producers, and tricalcium phosphate solubilizers among Gram-positive bacterial isolates from the rhizospheric soil of the rice cultivars

Site	Source	Number of isolates	Siderophore producers	Phosphate solubilizers	ACC deaminase producers	IC producers (μg ml^{-1})[a]	
						1	2
CaR	Rhizosphere	17	0	0	0	13	4
CaS	Rhizosphere	34	1	1	1	33	1
FeR	Rhizosphere	26	0	4	0	25	1
FeS	Rhizosphere	23	1	6	3	16	1
Total		100	2	11	4	87	7

[a] Each isolate was classified according to the level of IC production: level 1 isolates produced between 0.1 and 10 μg IC ml^{-1} ; level 2 isolates produced >11 μg IC ml^{-1}

Table 6 Plant growth-promoting abilities of selected bacterial isolates

Isolate[a]	Phosphate solubilization	Siderophore production	IC production (μg ml^{-1})	ARA (nmol C_2H_4 mg protein h^{-1})	ACC deaminase activity	Catalase activity
FeS14	–[b]	+	3.33	29.60	+	+
FeS42	−	+	6.81	0.38	−	+
FeS56	−	+	3.91	0.39	−	+
FeS57	+	+	14.04	0.56	−	+
CaR114	−	−	5.85	259.53	−	+
CaS40	−	+	2.95	0.16	−	+
FeR64	−	−	3.91	1.89	−	+
FeS24	+	−	0.51	4.14	+	+
FeS53	−	−	Nd	1.45	+	+
Control Sp7	nd	nd	Nd	112.59	Nd	Nd
Control SBR5	nd	nd	Nd	501.95	Nd	Nd

Nd Not determined, *ARA* acetylene reduction assay

[a] FeS14 (*Burkholderia* sp.), FeS42 (*Chryseobacterium* sp.), FeS56 (*Ochrobactrum* sp.), FeS57 (*Lysinibacillus* sp.), CaR114 (*Paenibacillus* sp.), CaS40 (*Paenibacillus* sp.), FeR64 (*Bacillus* sp.), FeS24 (*Paenibacillus* sp.), FeS53 (*Paenibacillus* sp.). Controls: Sp7 (*Azospirillum brasilense*), SBR5 (*Paenibacillus riograndensis*)

[b] +, Positive for siderophore production, tricalcium phosphate solubilization, ACC deaminase, and catalase activity; −, negative for siderophore production, tricalcium phosphate solubilization, ACC deaminase, and catalase activity

in the non-inoculated control plants [Electronic Supplementary Material (ESM) Table S1].

Second plant assay: contribution of inoculation with Gram-positive isolates in rice plants submitted to iron stress

After 9 days of exposure to iron stress, plants from the susceptible cultivar, BR-IRGA 409, developed typical symptoms of iron toxicity, with the appearance of bronzing and necrotic lesions on the leaves, and the roots acquiring a dark brown and orange color. These symptoms appeared concomitantly with a decrease in shoot number and nutrient uptake.

Figure 2 shows that in the treatment with an excess of iron, plants inoculated with the FeS53 isolate differed statistically from un-inoculated plants in terms of dry matter of shoot. Moreover, plants subjected to the excess iron condition had reduced dry shoot biomass compared to those in the iron control treatment, with the exception of plants inoculated with the FeS53 isolate, which had statistically the same values under both conditions.

The potential role of bacilli isolates to alleviate the iron stress was also evaluated by determining the concentration of nutrients accumulated in shoot tissue (Table 8). For most of the treatments, rice plants subjected to an excess of iron reduced their uptake of nutrients, such as Ca, Mg, Cu, and Zn (data not shown), and of N, P, and K (Table 8) compared to those subjected to the iron control treatment. With respect to the plant N content, in the iron control treatment, rice plants inoculated with the CaR114 strain showed enhanced N uptake compared with the non-inoculated control plants. In the treatment with excess iron, plants inoculated with the FeS53 isolate were statistically equivalent to plants which had not

Table 7 Results of in vivo experiments with selected bacterial isolates in the growth promotion of rice plants under growth chamber conditions

Isolates[a]	Shoot growth	Root growth		
	Length (cm)	Dry matter (mg)	Length (cm)	Dry matter (mg)
FeS14	57.71±0.91 a	238±20.21 a	15.87±0.46 b	75±6.20 b
FeS42	57.37±0.97 a	243±10.12 a	17.30±1.18 a	85±5.50 a
FeS56	57.51±1.91 a	228±10.19 a	17.75±1.03 a	84±12.5 a
FeS57	56.85±0.70 a	206±14.30 b	16.24±0.78 b	68±3.91 b
NI	57.75±1.92 a	220±19.80 b	16.29±1.16 b	76±5.82 b

Data are presented as the mean ± standard deviation (SD) of 5 replicates of plants grown in vermiculite in a controlled environmental chamber. Values in the same column followed by the same lowercase letter did not differ significantly at $p>0.05$ (Scott–Knott test).

[a] FeS14 (*Burkholderia* sp.), FeS42 (*Chryseobacterium* sp.), FeS56 (*Ochrobactrum* sp.), FeS57 (*Lysinibacillus* sp.); NI, Not inoculated

been inoculated in terms of N, P, and K uptake, although the K content in the shoots was higher in plants inoculated with FeS53. On the other hand, plants inoculated with the FeS53 strain showed similar results in terms of N and K uptake compared with plants in the iron control and iron toxicity treatments (Table 8).

The concentration of iron accumulated in rice shoot tissue was also evaluated (ESM Table S2). The shoot tissue of rice plants in the excess iron condition had higher iron concentrations than shoot tissue of rice plants in the control iron condition. We also observed that the inoculation of rice seeds with the bacilli isolates, including the isolate FeS53, resulted in a reduction in the concentration of iron in the shoot tissue compared with that in the non-inoculated control plants.

Discussion

Isolation and identification of putative PGP bacteria

To investigate how an excess of iron interferes with the diversity of putative PGPR associated with rice, we isolated specific bacterial communities from two rice cultivation areas with different concentrations of iron. The toxic character of these experimental sites had been determined prior to our study by Stein et al. (2009), who observed a very high concentration of ferrous (Fe^{2+}) ions (>280 mg l^{-1}) in soil samples from the experimental site, Camaquã, and a much lower concentration in those from the control site, Cachoeirinha (29 mg l^{-1}). Our results suggest that both soil abiotic factors and the genotype of the rice cultivar have significant effects on the composition of the Gram-negative bacterial communities isolated from the roots and rhizospheric soils (Table 2), thus corroborating the hypothesis that plants can actively select their bacterial community under different nutrient conditions (Costa et al. 2013). Briones et al. (2002) showed that different cultivars of the same plant species can affect the populations of ammonia-oxidizing bacteria. Inceoglu et al. (2012) studied the bacterial community in the potato rhizosphere using PCR followed by denaturing gradient gel electrophoresis and observed the effects of soil type and cultivar on the microbial community structures. These authors concluded that soil type was the most determinative parameter shaping the functional communities, whereas cultivar type also exerted some influence.

Strains belonging to the *Burkholderia* and *Enterobacter* genera were more abundant in the samples analyzed in our study. Beneficial plant–microbial interactions can be established by strains belonging to *Burkholderia* genus when associated with plants and can promote plant growth through different mechanisms. For this reason, Gyaneshwar et al. (2011) suggested that the nitrogen-fixing species of the *Burkholderia* group should be placed into a new genus to separate them from the pathogenic species. Strains belonging to the *Enterobacteria* genus have been found in association with a large number of plant species of agronomic interest and are also known to improve plant growth (Deepa et al. 2010).

Our results also suggest that in addition to the environment and the genotype of the rice cultivar having an impact on Gram-negative bacterial communities, they also affect Gram-positive bacterial communities (Table 3). Yadav et al. (2011) conducted diversity studies under stress conditions in which they analyzed the biodiversity of PGP bacilli isolated from moderately acidic soil. Their results revealed several *Bacillus* strains which were positive for multiple PGP attributes. Upadhyay et al. (2009) analyzed the genetic diversity of PGPR isolated from the rhizosphere of wheat under saline conditions and concluded that *Bacillus* and *Bacillus*-derived genera were the most abundant type of bacteria in their samples.

The results of ourt study show a correlation between abiotic soil parameters and bacterial diversity (Fig. 1). Although several soil parameters were measured and analyzed in our study, it is still possible that other unevaluated variables, such as plant genotype, root exudates, plant age, and plant community composition, can influence the diversity of microbial communities. Moreover, the differences in diversity among the bacterial communities can be the result of the combined effects of the weather and edaphic factors. Palmer and Young (2000) observed that pH and the contents of clay and OM may affect bacterial diversity, and Arruda et al. (2013) showed that the bacterial diversity of bacterial communities isolated from maize was associated with the soil clay content.

Screening of PGP traits of bacterial isolates

Rhizospheric bacteria can enhance plant growth through a wide variety of mechanisms. The production of IC by PGPR is an important attribute for the improvement of plant growth (Upadhyay et al. 2009) as it can directly influence root development, thus allowing the uptake of essential nutrients. The data obtained in our study indicate that the level of IC production differed according to the environment. The susceptible cultivar was able to select the best IC producers under conditions of iron stress, whereas the microbiota of the tolerant cultivar was unresponsive in terms of IC production (Table 4). Moreover, >90 % of all isolates were able to produce IC (Tables 4, 5). These results are in agreement with those reported by other authors (Ambrosini et al. 2012; Costa et al. 2013; Souza et al. 2013) who found that IC production was the most prevalent PGP characteristic in the majority of isolates. Dimkpa et al. (2009b) observed that the presence of metals such as Al, cadmium, and nickel, including iron, inhibited auxin production in *Streptomyces* sp. but that auxin

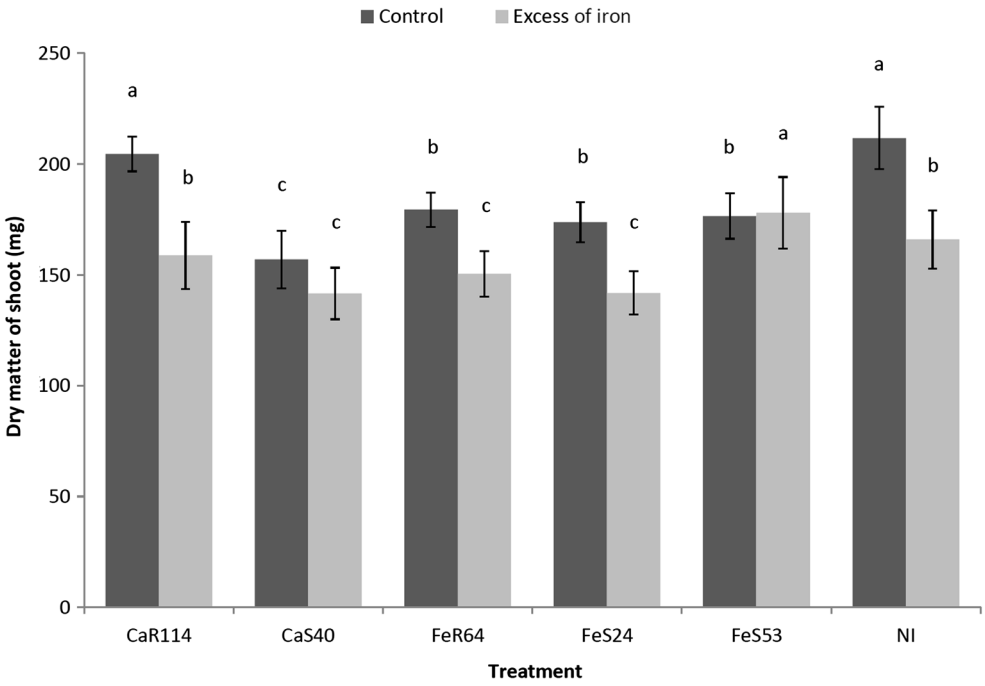

Fig. 2 Growth response of rice plants inoculated with selected native bacilli (*CaR114 Paenibacillus* sp., *CaS40 Paenibacillus* sp., *FeR64 Bacillus* sp., *FeS24 Paenibacillus* sp., *FeS53 Paenibacillus* sp.) under growth chamber conditions. *NI* Non-inoculated. Values represent means (*bars*) ± standard deviation (*whiskers*) of 5 replicates of plants. All bacterial treatments are compared only to the respective non-inoculated treatment. *Dark bars* (control) are compared only to each other, as are *gray bars* (excess iron treatment). *Bars with the same lowercase letter* did not differ significantly at $p>0.05$ (Scott–Knott test)

production was enhanced under siderophore-producing conditions. Siderophores promote auxin synthesis by chelating these metals, thereby protecting microbial auxins from degradation and enabling them to enhance plant growth.

Despite the fact that iron (Fe^{2+}) is typically available in flooded soil conditions and that paddy rice soils are subjected to periodic changes between the oxic and anoxic conditions (Becker and Asch 2005), another ability displayed by most of rhizospheric isolates in our study was siderophore production (Table 4). Many microorganisms can produce siderophores involved in the sequestration of Fe^{3+}. In flooded soil conditions of rice cultivation, Fe^{3+} can act as an electron acceptor for microbial respiration and subsequently become available to plants (Becker and Asch 2005). Moreover, the rhizosphere of rice cultivars is a potential site of Fe^{2+} oxidation, leading to a considerable Fe^{3+} accumulation and the formation of an iron plaque around rice roots (Ando et al. 1983). Our results suggest that under the iron stress condition the best siderophore producers were found in the tolerant cultivar.

The power of the rice root to oxidize iron has also been described as a possible mechanism used by tolerant rice cultivars to exclude high amounts of iron in the soil solution from the plant (Ando et al. 1983). In experiments with *Azotobacter vinelandii*, Kraepiel et al. (2009) showed that all of the nitrogenase metal cofactors (Fe, Mo, and V) were bound to siderophores and that even in iron-replete cultures, siderophore production, although reduced, was not suppressed at high iron concentrations.

The ability of rhizospheric bacteria to convert insoluble forms of phosphorus to an accessible form is an important trait in PGPR. In this work, the best tricalcium phosphate solubilizers were selected from the tolerant cultivar cropped in the control site and not from that cropped in the iron-stressed site (Table 4). Among the bacilli and other Gram-positive bacteria, only a few strains were identified as tricalcium phosphate-solubilizing bacteria (Table 5). Similarly low numbers of phosphate-solubilizing bacteria have been documented by other studies. Ambrosini et al. (2012) evaluated the diversity of PGP bacteria isolated from sunflower and found 59 phosphate-solubilizing strains from among 299 bacterial isolates analyzed. Beneduzi et al. (2008), isolated 296 bacilli from rice, but of these, only 22 isolates had phosphate-solubilizing ability.

In our investigation, only a few bacterial strains (Tables 4, 5) exhibited ACC deaminase activity, showing the competency of the isolates to use ACC as a nitrogen source. Bal et al. (2013) recently demonstrated the effectiveness of rhizobacteria containing ACC deaminase to enhance salt tolerance and consequently improve the growth of rice plants under salt-stress conditions.

Contribution to plant growth and alleviation of iron stress effects with bacterial treatments

Rhizospheric strains selected by their PGP traits were tested in an in vivo experiment in an environmental chamber (Table 6). Our results suggest that FeS14 (*Burkholderia* sp.), FeS42 (*Chryseobacterium* sp.), and FeS56 (*Ochrobactrum* sp.) contribute to plant growth as well as to enhanced nutrient uptake of the rice plants in growth chamber conditions (Table 7; ESM Table S1). The FeS14, FeS42, and FeS56 isolates were not able to solubilize tricalcium phosphate in our in vitro assay

Table 8 Influence of inoculation with native bacilli on nutrient uptake in rice plants subjected to different iron treatments under growth chamber conditions

Treatment[a]	Nitrogen (N) (mg g^{-1})		Phosphorus (P) (mg g^{-1})		Potassium (K) (mg g^{-1})	
	Control iron condition	Iron excess condition	Control iron condition	Iron excess condition	Control iron condition	Iron excess condition
CaR114	8.59±0.32 a,A	6.03±0.58 b,B	1.18±0.04 a,A	0.58±0.06 a,B	11.04±0.41 b,A	8.57±0.82 a,B
CaS40	6.27±0.52 c,A	5.09±0.42 c,B	0.97±0.08 c,A	0.44±0.04 b,B	8.77±0.73 d,A	7.22±0.60 b,B
FeR64	6.99±0.30 c,A	5.87±0.40 b,B	1.13±0.05 b,A	0.48±0.03 b,B	10.21±0.44 c,A	6.92±0.47 b,B
FeS24	6.95±0.39 c,A	5.67±0.36 b,B	0.97±0.05 c,A	0.48±0.03 b,B	8.86±0.46 d,A	7.51±0.52 b,B
FeS53	6.71±0.39 c,A	6.94±0.63 a,A	0.95±0.06 c,A	0.58±0.05 a,B	9.35±0.55 d,A	9.25±0.84 a,A
NI	7.84±0.53 b,A	6.47±0.51 a,B	1.21±0.08 a,A	0.63±0.05 a,B	11.87±0.80 a,A	8.63±0.69 a,B

Data are presented as the mean ± SD of 5 replicates of plants grown in vermiculite in a controlled environmental chamber. Values in the same column followed by the same lowercase letter did not differ significantly at $p>0.05$ (Scott–Knott test). Values in the same line followed by the same uppercase letter did not differ significantly at $p>0.05$ (Scott–Knott test)

[a] CaR114 (*Paenibacillus* sp.), CaS40 (*Paenibacillus* sp.), FeR64 (*Bacillus* sp.), FeS24 (*Paenibacillus* sp.), FeS53 (*Paenibacillus* sp.)

and showed only a low capacity to reduce acetylene (Table 6); these observations could indicate that other growth promotion mechanisms, such as IC production or other mechanisms not evaluated in our study, improved nutrient uptake. Large numbers of PGPR of different bacterial genera with multifunctional traits have, therefore, been described for their potent application in boosting plant growth in modern agriculture (Bhattacharyya and Jha 2012). Strains of *Chryseobacterium* and *Burkholderia* are well known for their abilities to promote plant growth (Ambrosini et al. 2012) through their ability to synthesize an array of metabolites.

Based on our in vivo experimental data (Table 8), rice plants inoculated with the CaR114 (*Paenibacillus* sp.) strain had an improved potential for nitrogen uptake. CaR114 showed the capacity to reduce acetylene and IC production (Table 6), which may be related to the uptake of N. Beneduzi et al. (2008) demonstrated the effectiveness of PGP bacilli in promoting the plant growth of rice. Moreover, bacilli are also known for their biocontrol abilities against phytopathogens, which can occur through antibiosis, production of siderophores, as well as by processes of induced systemic resistance (Lugtenberg and Kamilova 2009).

Our analysis of plant nutrients under the condition of iron stress showed that most of the plants inoculated with different bacterial treatments had lower rates of N, P and K uptake compared to those subjected to the control iron condition, with the exception those plants inoculated with the FeS53 isolate (Table 8). Mehraban et al. (2008) reported that high concentrations of iron in the soil decreases the uptake of Ca^{2+}, Mg^{2+}, and especially N, P, and K by rice. In our study, plants inoculated with FeS53 (*Paenibacillus* sp.) strain, which showed ACC deaminase activity, in the presence of iron excess had a dry shoot biomass and nutrient uptake similar to those subjected to the control iron condition, indicating that this bacterium can mitigate the effects caused by iron stress. Bal et al. (2013) demonstrated the effectiveness of rhizobacteria that contain ACC deaminase, such as *Alcaligenes* sp., *Bacillus* sp., and *Ochrobactrum* sp., in inducing salt tolerance and consequently improving the growth of rice plants under salt-stress conditions; positive results on different growth parameters, such as germination percentage, shoot and root growth, and chlorophyll content, were similar to those of the un-inoculated control. Other studies have also reported that bacteria with ACC deaminase activity can reduce the level of stress caused by ethylene, thus resulting in a better growth of the plants under various stresses (Glick 2012). Similar results were obtained by Arshad et al. (2008) who observed that a strain of *Pseudomonas* spp. displaying ACC deaminase activity partially eliminated the effect of drought stress on the growth of peas (*Pisum sativum* L.).

In summary, the results of our study demonstrate the presence of a diverse population of PGP bacteria interacting with rice cropped in sites with different iron concentrations. The

composition of the bacterial genera (community) and the occurrence of different PGP traits were significantly affected by the iron condition of the soil sample and by the cultivar. We therefore conclude that the bacterial isolates studied here interacted positively with rice plants in the test conditions. Our results clearly indicate that further research aimed at studying the mechanism of action involved in the alleviation of iron stress against rice plants by the rhizobacteria is necessary.

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