Rhizosphere 14 (2020) 100200

Contents lists available at ScienceDirect

Rhizosphere

journal homepage: www.elsevier.com/locate/rhisph

Inoculation of bacterial consortium increases rice yield (*Oryza sativa* L.) reducing applications of nitrogen fertilizer in San Martin region, Peru

Winston F. Ríos-Ruiz[a], Edson E. Torres-Chávez[b], Jorge Torres-Delgado[a], José C. Rojas-García[a], Eulogio J. Bedmar[c], Renzo A. Valdez-Nuñez[a,d,*]

[a] Universidad Nacional de San Martín-Tarapoto, San Martín, Perú
[b] Programa Nacional de Arroz-Instituto Nacional de Innovación Agraria (INIA), Tarapoto, Perú
[c] Department of Soil Microbiology and Symbiotic Systems, Estación Experimental Del Zaidín-CSIC, Granada, Spain
[d] Universidad Nacional de Barranca, Barranca, Lima, Perú

ARTICLE INFO

Keywords:
Biofertilizer
Plant growth promotore rhizobacteria
Rice productivity

ABSTRACT

Rice feeds more than 50% of the population worldwide, representing a great energy contribution in low-income families. The exaggerated use of synthetic chemical fertilizers to maintain high levels of yield causes alterations in the physical, chemical and biological quality of the soils. A sustainable alternative is the use of beneficial microorganisms that promote plant growth in crops. The objective of the study was to isolate and evaluate the Plant Growth Promoting (PGP) traits of rhizospheric rice bacteria in soils of the San Martin region, evaluate the effect of promoting growth in rice under pot experiments conditions and finally evaluate the effect of selected strains on the yield under different doses of nitrogen fertilizer under field conditions. Initially, 27 strains were selected for their diazotrophic characteristics and characterized by PGP traits. Through a multivariate analysis of main components, five strains were selected and evaluated in pot experiments. In this stage, the rice seeds were inoculated with the five selected strains at the rate of 10^9 CFU g^{-1} and were evaluated at 135 days. The strains showed that the parameters such as Shoot Dry Weight (SDW), tillering and grain quality were superior and even similar between inoculated treatments receiving doses of 50% Nitrogen (N) (75 kg N ha^{-1}) and treatment receiving full dose (150 kg of N ha^{-1}). For the field experiments, *Burkholderia ubonensis* la3c3, *Burkholderia vietnamiensis* la1a4 and *Citrobacter bitternis* p9a3m were selected, which were inoculated in a consortium at a rate of 10^9 CFU mL^{-1} in the nursery stage and at the time of transplantation. Grain yield was higher but not significant between 2.5 and 13.5% in inoculated treatments receiving 75% and 100% (150 kg N ha^{-1}) of the nitrogen fertilizer dose compared to the treatment without inoculation and without fertilization. Grain quality was superior in inoculated treatments versus non-inoculated treatments, reaching specific increases of 32.8% (N), 45.5% (P) and 27.9% (K) in inoculated treatments receiving low doses of N fertilizer (25%) versus treatment receive a full dose of fertilizer, also a significant increase of 2.5% in the percentage of whole grain, with respect to mill quality. The rentability (14.7–88.6%) and the utility (17.7–94.1%) were also higher in inoculated treatments. It is concluded that the use of selected native bacterial consortiums reduces the use of nitrogen fertilizer by up to 25%, increasing the productivity of rice cultivation in the San Martín region.

1. Introduction

The rice (*Oryza sativa* L.) is a priority food product for the human population, being considered as a strategic crop for food security due to its wide distribution in soils and climates worldwide (IRRIInternational Rice Research Institute, 2016), as well as in a scenario of climate change (Fahad et al., 2019). It is estimated that world rice production for the period 2018/2019 will be 487.2 million t (USDA, 2018). In Peru, rice cultivation represents the first product based on the area planted and harvested (INIA, 2018). The San Martin region is considered the first producing region of this cereal, with a harvested area of 110,442 ha and an average yield of 7.4 t ha^{-1} (MINAGRI, 2018). To maintain and/or increase productivity, the application of excess N- and P-based fertilizers has led to an unprecedented contamination of soils and waters, leading to harm to ecosystems, causing pollution, and spreading disease; nutrient depletion, soil acidification and

* Corresponding author. Departamento Académico de Ciencias Básicas, Facultad de Ingeniería, Universidad Nacional de Barranca, Lima, Peru.
E-mail addresses: wrios@unsm.edu.pe (W.F. Ríos-Ruiz), etorres@inia.gob.pe (E.E. Torres-Chávez), jotode@gmail.com (J. Torres-Delgado), jocaroga@gmail.com (J.C. Rojas-García), eulogio.bedmar@eez.csic.es (E.J. Bedmar), rvaldez@unab.edu.pe, rvaldez@unab.edu.pe (R.A. Valdez-Nuñez).

https://doi.org/10.1016/j.rhisph.2020.100200
Received 24 February 2020; Received in revised form 28 March 2020; Accepted 30 March 2020
Available online 09 April 2020

eutrophication are also common consequences of inadequate soil management (Basosi et al., 2014).

The consumption of diets with high nitrate content may produce thyroid diseases, various types of cancer and diabetes (Ahmed et al., 2017). In addition, as a result of the increase in the use of N-fertilizers, the global N-cycle has been altered, resulting in higher production of greenhouse gases, depletion of stratospheric ozone, decrease of the soil organic matter and loss of biodiversity (Hakeem et al., 2016; Singh, 2018). To mitigate the harmful effects of excessive fertilization farming practices should be identified that, combined with appropriate addition of fertilizers, maintain productivity and achieve a better fertilizer's use efficiency (Bordoloi et al., 2019). Likewise, the development of new hybrid rice cultivars and the application of mechanized planting technologies with an adequate density of plants could reduce the effects of nitrogen fertilization (Hou et al., 2019). Submerged rice cultivation, a widespread practice worldwide is responsible for the release into the atmosphere of the greenhouse gases nitrous oxide (N_2O) and methane (CH_4) thus contributing to the global climatic change (Baldocchi et al., 2016; Hong-Xing et al., 2018).

Microorganisms are essential for the maintenance and health of soil function in both natural and managed agricultural systems due to their involvement in fundamental processes such as soil structure formation, decomposition of organic matter, toxin removal, suppression of plant disease and, overall, the cycling of C, N, P and S. Within the soil microbiota, some bacterial populations are able to competitively colonize plant roots and stimulate growth, thereby reducing the incidence of plant diseases, for which the term plant growth-promoting rhizobacteria was coined, and are commonly recognized by the initials PGPR (for reviews see Olanrewaju et al., 2017 and references therein).

Potential functions of PGPR involved in plant growth promotion include: Direct, indol acetic acid production, inorganic phosphorus solubilization, the presence of 1-aminocyclopropane-1-carboxylate deaminase (ACCD) activity, and Indirect: Antibiotic production and lytic enzymes, induction of systemic resistance, competition for sites in the plant roots, production of siderophores (see Olanrewaju et al., 2017 and Zhao et al., 2018 for reviews). These mechanisms, whether direct or indirect, can be used by PGPR, and may take place simultaneously o sequentially at different plant growth stages (Keswani et al., 2016).

In recent years, there has been much interest in the application of single or combined application of PGPR to improve the development and increase the productivity of agricultural crops, including rice. The fertilization with 30 kg ha^{-1} P_2O_5 together with the inoculation of different combinations of *P. aeruginosa*, *P. putida*, *P. fluorescens*, *Azotobacter chroococcum* and *Azospirillum brasilense* was proven effective for rice production and economically cheaper than the control treatments containing 60 kg ha^{-1} P_2O_5 (Yadav et al., 2014). In addition, the combination of *P. putida*, *P. fluorescens* or *A. chroococcum* with different concentrations of inorganic nitrogen produced higher grain yield than rice plants amended with the greater conventional nitrogen doses (Ghaffari et al., 2018). In a 65-d period, under greenhouse conditions, Tan et al. (2015) showed that rice plants inoculated with the N_2-fixing species *Lysinibacillus xylanilyticus* and *Bradyrhizobium japonicum* resulted in N content of up to 63 kg ha^{-1}, which evidences the beneficial synergistic activities of the used strains. In a field experiment, Khan (2018) also demonstrated that inoculation of rice seeds with *Azospirillum* and *Trichoderma* reduced by 25% the use of inorganic nitrogen with yields similar to the plants fertilized with the recommended dose of 180 kg N ha^{-1}. Recently, Banik et al. (2019), using *Azotobacter* sp. Avi2 as a bioinoculant for rice obtained higher chlorophyll concentration in the leaves and grain yield.

Knowing the interactions between PGP bacteria and plants provides us with opportunities to generate innovative strategies that generate increasingly sustainable production alternatives, especially in rice cultivation. Despite the fact that the use of inoculants in rice to improve productivity is not new (Baldani et al., 2000; Yanni and Dazzo, 2010; Punschke and Mayans, 2011; de Souza et al., 2013; Etesami and Alikhani, 2016; Jha et al., 2020), a unique inoculant consortium has been selected and developed in terms of its composition and PGP characteristics based on native rhizospheric bacteria adapted to rice cultivation in tropical edaphoclimatic conditions. This inoculant consortium is capable of increasing nutrient adsorption and therefore reduced chemical inputs, constituting a sustainable and respectful agricultural system with the environment, improving the productivity and competitiveness of rice cultivation in Peru. Accordingly, the aim of this study was a) the isolation of rhizosphere bacteria from rice plants, b) the determination of some of their PGP characteristics, c) the molecular identification of the isolates, and d) the evaluation of their potential use as bioinoculants focused on the reduction of synthetic fertilizers for rice fields.

2. Materials and methods

2.1. Rhizospheric soil sampling

Rice roots were taken from 50 healthy pre-flowering plants of the varieties La Esperanza (30), Conquista (10) and Capirona (10) grown in the agricultural fields of Cacatachi (6°28′8.63"S 76°26′32.29"W, 284 m above sea level (m.a.s.l.)) and Picota (6°55′24.9"S 76°21′43.6"W, 196 m.a.s.l.) both located in the San Martin region of Peru and with similar field history at the beginning (primary forests) and then converted into subsistence farming and pastures, respectively. The samples were placed into polypropylene bags, kept at 4 °C and brought to the laboratory for use within 24 h after collection.

After cleaning of the bulk soil, the remaining adhering rhizosphere soil was carefully removed and pooled together. Then, 0.2 g samples were placed in Eppendorf tubes and containing 1 mL sterile saline solution, shaken in a vortex for 30 s and centrifuged at 3000 rpm for 1 min in a microfuge. For the isolation of N_2-fixing bacteria, 100 μL aliquots of the supernatant were used to inoculate 100-mL flasks containing 60 mL of either N-free MJV (Reis et al., 2004) consisting of g L^{-1}: Mannitol, 1.0; K_2HPO_4, 0.4; KH_2PO_4, 0.4; $MgSO_4$ $7H_2O$, 0.2; $CaCl_2$, 0.02; Na_2MoO_4, 0.002; $FeCl_3$, 0.01; pH 7.0; or N-free Burk (Wilson and Knight, 1952) consisting of g L^{-1}: Solution A: K_2HPO_4, 6.4; KH_2PO_4, 1.6; dH_2O, 1L.; Solution B: NaCl, 2.0; $MgSO_4$ $7H_2O$, 2.0; $CaSO_4$ $2H_2O$, 0.5; dH_2O, 1L. Solution C: $NaMoO_4$ $2H_2O$, 0.01; $FeSO_4$, 0.03; dH_2O, 1L. Final Composition: Solution A, 0.1L; Solution B, 0.1L; Solution C, 0.1L; Glucose, 5.0 g; dH_2O, 0.7 L; pH 7.2; both semisolid (0.3% agar) media. High purity products were used to prevent trace N in the media. Flasks were closed with screw caps and incubated at 30 °C until a dense cellular pellicle appeared in the subsurface of the medium (5–7 d). The top of the culture medium was removed, the bacterial film transferred to 50 mL Falcon tubes containing 5 mL sterile saline, homogenized by vortexing for 1 min and then centrifuged at 1500 rpm for 60 s. Supernatants were serially diluted and used for inoculation of Petri dishes containing either Burk or MJV solid (1.5% agar) media. Cells were incubated at 30 °C until the appearance of colony forming units (CFUs). Colonies were chosen that represented all of the colony types that could be distinguished after observation with a stereoscope and were purified by reculturing on the same medium. Tryptone Soybean Agar (TSA) or broth (TSB) media were used for routine bacterial growth.

2.2. Acetylene reduction activity (ARA)

Nitrogenase activity of the isolates was evaluated by the acetylene-dependent ethylene production assay (acetylene reduction activity, ARA) according to Talbi et al. (2010). The assay was carried out in triplicate. Briefly, cells were inoculated in liquid Burk medium ($OD_{600nm} \sim 0.05$), closed with rubber stoppers to allow injection and withdrawal of the gas samples and incubated at 30 °C until cultures reached an optical density of about 0.5. Then, 10% of the internal atmosphere of each tube was removed and replaced with the same

volume of acetylene. Gas samples (250 μL) were taken every 6 h and analyzed by ethylene production using a Hewlett Packard gas chromatograph (Model 5890, USA) equipped with a flame ionization detector and a column (180 × 3.2 mm) packed with Porapak Q (80–100 mesh). Oven, injector and detector temperatures were 60, 120 and 105 °C, respectively. Nitrogen was used as a carrier gas. Ethylene concentration was calculated by interpolation with respect to a standard curve prepared using pure ethylene.

2.3. Auxin production

Auxin production were assayed spectrophotometrically as described by Gravel et al. (2007). The bacterial cultures were grown in TSB medium to reach about 10^8 cells mL^{-1}, sedimented by centrifugation and the supernatant used to mix with Salkowsky reagent (1:2; v:v). Color intensity was measured spectrophotometrically at 535 nm after 30 min. Auxin levels were estimated using standard curves prepared with pure Indol 3-Acetic Acid. The effect of L-tryptophan on auxin production was also evaluated after addition of 600 mg L^{-1}. The assay was carried out in triplicate.

2.4. Siderophores production

Production of siderophores was assayed in triplicate following the Chromeazurol S (CAS)-shuttle assay following the methodology reported by Schwyn and Neilands (1987). Briefly, bacteria were grown at 30 °C to reach about 10^8 cells mL^{-1} in Fe-free SM minimal medium (Sayyed et al., 2005) consisting of g L^{-1}: K_2HPO_4, 6.0; KH_2PO_4, 3.0; $MgSO_4$ $7H_2O$, 0.2; $(NH_4)_2SO_4$, 1.0; and succinic acid 4.0, pH 7.0. After centrifugation as above, the supernatant was mixed (1:1; v: v) with CAS solution, allowed to stand for 20 min and then used to assay the intensity of blue color at 630 nm. A mixture of CAS solution and SM medium (1:1; v:v) was used as a reference. The production of siderophores was estimated following the formula: % of siderophore units = [(Ar–As)/Ar] x 100, where, Ar = Absorbance of reference and As = absorbance of the sample. Gram positive bacteria strains that showed poor growth in the CAS agar assay, the O-CAS method was used (Pérez-Miranda et al., 2007).

2.5. Biocontrol activity

The antifungal activity of the isolates against *Rhizoctonia solani* R1 and *R. oryzae* R2 was evaluated according to Castellano-Hinojosa et al. (2015). These phytopathogens are the main cause of sheath blight and sheath rot diseases in rice, respectively. Both strains were isolated from Tumbes, preserved and donated by the Department of Plant Pathology of UNSM-Perú. For antagonism assays, the fungi were first grown in Potato Dextrose Agar (PDA) medium and used to take 1 cm-diameter agar plugs that were placed in the middle of plates containing PDA medium previously inoculated independently with 100 μL of each of the isolates cultured in TSB. Cells were incubated for 8 days at 25 °C and the diameter of the inhibition zones recorded every 2 days for 8 days. The percentage of inhibition was calculated according to the formula: % Inhibition = $[(R_1\text{-}R_2/R_1)$ x 100, where R_1 is the diameter of the fungal mycelium in plates not inoculated with the bacterial culture and R_2 is the diameter of the fungal mycelium in plates inoculated with the bacterial culture.

For the antibiosis test, the agar plugs containing the fungus were placed near the border of a plate containing PDA medium and incubated at 25 °C for 48 h. Then, the strain to be tested was streaked in straight line on the opposite side of the plate. The inhibitory effect on fungal growth was evaluated every 2 days for 8 days at 25 °C, and the percentage of inhibition relative to the control (without bacteria) was evaluated as indicated above. Both assays was carried out in triplicate.

2.6. DNA extraction, PCR amplification and sequencing of the 16S rRNA gene

For DNA extraction and PCR amplifications, genomic DNA was isolated from bacterial cells using the RealPure Genomic DNA Extraction Kit (Durviz, Spain), according to the manufacturer's instructions. The quantity of DNA was determined by using a NanoDrop spectrophotometer (NanoDrop ND1000) (Thermo Fisher Scientific, USA). Amplification of the 16S rRNA gene fragment was carried out by the polymerase chain reaction (PCR) using primers fD1 (5′-CCGAATT CGTCGACAACAGAGTTTGATCCTGGCTCAG-3′) and rD1 (5′-CCCGGGA TCCAAGCTTAAGGAGGTGATCCAGCC-3′) and conditions described by Weisburg et al. (1991). The amplification products were purified using the Qiagen PCR product purification system, and they were subjected to cycle sequencing using the same primers as for PCR amplifications, with ABI PRISM Dye Chemistry, and analyzed with a 3130xl automatic sequencer at the sequencing facilities of Estación Experimental del Zaidín, Granada (Spain). All the obtained sequences were compared with those deposited in the EzBioCloud.net (Yoon et al., 2017). MEGA7.0 was used for all the phylogenetic analyses (Kumar et al., 2016). ClustalW (Thompson et al., 1994) was used for the alignments, the 2-parameter model of Kimura (1980) for the calculation of distances and the neighbor-joining grouping algorithm (Saitou and Nei, 1987) for the development of the phylogenetic trees.

2.7. Germination percentage and pathogenicity test

Strains were grown in TSB broth at 30 °C and upon reaching the exponential phase, they were inoculated in “La Esperanza” rice seed disinfected on the surface according to the methodology suggested by Mia et al. (2012). The seeds were soaked in each inoculum of PGP bacteria, and TSB broth without inoculation was used as a control. Twenty-five air-dried seeds were placed in Petri dishes containing 1% Agar water and incubated at 30 °C for 120 h. Assay were carried out in triplicate. The germination percentage was calculated with the following formule: (Number of germinated seeds/Number of seeds sown) x 100. The pathogenicity test was performed with 21-day-old rice seedlings “La Esperanza” grown under controlled conditions (Cottyn et al., 2001). For each PGP strain, ten seedlings were inoculated by injecting 0.1 mL of the inoculum (3×10^8 CFU mL^{-1}), 2 cm above the soil. TSB broth without inoculation was used as a negative control and the bacterium *Burkholderia glumae* THT was used as a positive control (Valdez-Nuñez et al., 2020). At 7 days post-inoculation, the appearance of a necrotic area in the wound is considered positive, on the contrary, it will be negative.

2.8. Pot experiments

For the pot experiment, soil was obtained from the 20-cm layer of the arable topsoil from El Porvenir Experimental Station (06°36′15″ S; 76°25′15″ W, 330 masl) of Instituto Nacional de Innovación Agraria (INIA) (Juan Guerra, San Martín, Perú). According to the standard textural triangle the soil was a clay soil texture (Bouyoucos Hydrometer) with the following physicochemical properties: pH in water, 7.7; electrical conductivity, 4.097 dS m^{-1}; organic matter, 3.12%; total N, 0.20%, available P, 10.00 mg kg^{-1}; available K, 223 mg kg^{-1}; Ca, 21.10 meq 100 g^{-1}; Mg, 2.34 meq 100 g^{-1} and Na, 1.00 meq 100 g^{-1}. The cationic exchange capacity of the soil was 25 cmol(c) kg^{-1}. Soil was fertilized with phosphorus (P) (46 kg P_2O_5 ha^{-1} as super phosphate) and potassium (K) (120 kg KCl ha^{-1}) at the beginning of the experiment and used to fill 5-kg PVC pots (4 L volume). Seeds of rice var. La Esperanza were surface-disinfected with 70% ethanol for 1 min, washed with 5% sodium hypochlorite for 5 min, rinsed thoroughly with sterile water, left in sterile water for 1 h and then allowed to germinate at 30 °C in the dark. Seeds were sowed in the pots (6 pot^{-1}) and inoculated. For inoculation, the bacterial

suspensions were prepared by cell growth until reaching the stationary phase in TSB broth at 170 rpm at 30 °C, and then diluted to an OD_{600nm} of 1.0 (10^9 cells mL^{-1}). Then, the bacterial suspensions were independently added to sterile peat (1:1; v/v) in polypropylene bags and kept at 30 °C for 24 h. As recommended by Ferreira et al. (2010), the obtained mixture was then blended with the surface-sterilized seeds (10 g bacteria + peat mix per 1 kg seeds) and finally using the proportion 0.6 g solid inoculant pot^{-1}. The treatments (n = 5) consisted of *S. hominis* p7b1m, *B. vietnamiensis* la1a4, *M. yunnanensis* la2b2, *C. bitternis* p9a3m, *B. ubonensis* la3c3 and non-inoculated control (TSB Broth), all receiving 3 doses of urea as nitrogen fertilizer at 0 (0%), 37.5 (25%) y 75 (50%), and a treatment receiving the dose of 150 kg ha^{-1} (100%). 40% of the urea was applied 15 days after planting and the remaining 60% 25 days later. After emergency, plants were trimmed to 3 pot^{-1}. Pots were maintained under greenhouse conditions for 135 days with a temperature range of 28 ± 2 °C, humidity of 80 ± 5%, natural light and 12:12 photoperiod (light: dark). During the experiment, the pots were covered by a water sheet of 2–3 cm, which was removed previous to fertilization. Non-treated urea soil was used as a control. The parameters evaluated were: shoot dry weight (SDW), tillers per plant (TP), panicles per plant (PP), grain per panicle (GP), weight of 1000 grain (W1000G) and grain protein (GProt). The protein content of the grain was calculated: Total N in grain x factor (5.95). Total N in grain sample was estimated after digestion in H_2SO_4 in digestion Block using the standard methods described by AOAC (1970). This factor is based on the N content (16.8%) of the main protein of rice, glutelin (Araújo et al., 2013).

2.9. Field trials

The experiments were carried out using the facilities of El Porvenir Experimental Station. First, after the shaking and leveling, the soil was fertilized with P and K as above for the pots and then 8 nurseries (8 m^2 $subplot^{-1}$ in a 70 m^2 total area) were prepared and designed installed on August 18, 2017. All subplots were covered by a 3 cm water sheet for 2 days prior to seed sowing. Seeds (60 kg ha^{-1}) were sown scattering on the plots and inoculated independently with the different bacterial suspensions prepared as indicated above and added to the irrigation water (1.2 L bacterial cells 60 kg $seeds^{-1}$). Following recommendation by Govindarajan et al. (2008) and Islam et al. (2012), a second inoculation was carried out at the garbas stage (see below).

Twenty-seven d-old seedlings were gently uprooted to form garbas (300–500 seedlings $garbas^{-1}$) that were manually transplanted every 20 cm to the adjacent experimental field where a split-plot design was used with N-fertilization rates as the main-plot treatments and inoculation or non-inoculation as the sub-plot treatments. Each subplot presented a size of 3.5 m length × 3.0 m width, 1 m between rows, with 4 replicates/treatment. A total area of 1736 m^2, was planted with 2.5 × 10^5 seedlings ha^{-1}.

As for the nursery, at soil preparation, all subplots received P and K. N-fertilization was applied as urea at 37.5 (25%), 75.0 (50%), 112.5 (75%) and 150 kg N ha^{-1} (100%) at 15 (40%) and 25 days (60%) after the transplant from the nursery, the latter coinciding with the evaluation of cotton point. Seeds were inoculated independently with the different bacterial suspensions prepared as above and added to the irrigation water. The strains used were *Citrobacter bitternis* p9a3m, *B. vietnamiensis* la1a4 and *B. ubonensis* la3c3. The strains were confronted with each other to evaluate their growth inhibition capacity according to Lima et al. (2017). The treatments applied were 12: Uninoculated control soil, 4 urea concentrations and 7 single and combined bacterial suspensions. During the field trials, soil was flooded according to the height of the plants, from 5-cm during the first 25 days up to 13–15 cm at the beginning of plant maturation. Plants were harvested 135 days after field installation. The parameters evaluated were: Root length (RL), plant height (H_1), dry weight of root (RDW) and SDW of the plants were estimated 27 days after the beginning of the nursery stage. After plant transplant the chlorophyll content at the start of the panicle development was determined (90 d) using a chlorophyll meter SPAD-502Plus (Konica Minolta). At the harvest, the straw yield (SY), plant height (H_2), number of tillers $hill^{-1}$ (NT), weight of 1000 grain (W1000G), panicle length (PL), number of panicles $hill^{-1}$ (NPH), number grains $panicle^{-1}$ (NGP), N, P and K content, milling quality expressed as broken grain and whole grain were also determined (Martínez-Racines et al., 1989; Yadav et al., 2014). Finally, production costs and rentability analysis between the inoculated and non-inoculated treatments was performed (Aon et al., 2015).

2.10. Statistical analysis

Measured variables in this study were first explored using the Shapiro–Wilk test to check whether they meet the normality assumptions. In the case of parametric data, we used the LSD-Fisher, Duncan, Scott and Knott. For non-parametric data, the Kruskal–Wallis and Conover–Iman combined tests were used for comparisons among treatments. A covariance-based (N-1) principal component analysis (PCA) was run to analyze relationships among ARA, siderophore production, auxin production and biocontrol activity. Analysis of variance ANOVA-1 was used to determine if there were differences between the means of the treatments. The LSD-Fisher mean comparison test was used to establish which treatments were significantly different in pot experiments, as well as the nursery stage at 27 days and grain yield under field conditions, similarly was used Scott & Knott mean comparison test for data of SY, H, NT, W1000G, PL, NPH, NGP and grain quality. All the statistical analyses were run using INFOSTAT version 2012.1 software (Di Rienzo et al., 2012).

3. Results

3.1. Isolation of N_2-fixing bacteria from rhizospheric soil of rice

A total of 27 bacterial strains were isolated from the rhizosphere of rice plants (Table 1) that were capable of growing in N-free medium. Most of the strains (62.5%) were isolated from the rhizosphere of plants grown in Cacatachi's agricultural fields of rice and the remaining 37.5% from those of Picota. ARA activity was detected only in 53.13% (17) of the 27 bacterial strains isolated from N-free media, with values varying from 92.59 to 2240.97 nmoles of ethylene mg^{-1} protein h^{-1} (Table 1).

3.2. Auxin production

In the absence of L-tryptophan in the growth medium, 27 strains (84.4%) produced auxin. Production of auxin increased with addition of L-tryptophan to the medium until a threshold concentration (600 mg mL^{-1}) was reached from which further increase in auxin concentration was not observed. Under all conditions examined, with and without L-tryptophan in the culture medium, strain la5c5 was the largest producer of auxin (Table 1).

3.3. Siderophore production

Most of the bacteria used in this study (93.75%) grew well in liquid Fe-free SM medium (Table 1). The strain la6c1 had maximum efficiency (1212.39%) in siderophore production followed by strains la6c5 and la3c3; in contrast, evaluation of unit siderophore production in liquid medium showed that greatest values corresponded to strains la3c3 and la4c2.

3.4. Biocontrol activity

About 84.4% and 68.8% of the rhizospheric bacteria from rice plants showed in vitro biocontrol activity against *R. solani* R1 and *R. oryzae* R2. When the fungi and the bacteria were in contact, strain la3c3

Table 1
Auxin production, nitrogen fixation and siderophore production by rhizospherical bacterial isolates.

Isolate code	Auxin production (μg/ml)		ARA (nmol ethylene/h/mg protein/h)*	Siderophore production	
	-Tryp	+Tryp		**(%)	*** US
****Control	4.54(± 1.09)^{MN}	9.63(± 1.50)^{JKL}	ND	NE	NE
la1a1m	0.20(± 0.82)^{N}	19.27(± 10.47)^{FGHIJ}	ND	361.11(± 14.72)^{NO}	0.94(± 0.41)^{I}
la3c3	63.62(± 3.52)^{C}	10.71(± 1.76)^{JKL}	300.96(± 13.24)^{G}	966.67(± 1472)^{B}	51.88(± 0.21)^{A}
p3a3m	4.22(± 0.32)^{MN}	11.15(± 1.45)^{JKL}	1227.65(± 10.21)^{CD}	728.57(± 8.26)^{GHI}	6.09(± 0.35)^{F}
la2b2	5.76(± 1.72)^{MN}	16.23(± 2.75)^{HIJK}	456.83(± 4.52)^{F}	771.43(± 8.26)^{EF}	1.33(± 0.08)^{I}
la5b5m	5.71(± 1.16)^{MN}	8.07(± 1.61)^{KL}	327.38(2.46)^{FG}	771.43(± 8.26)^{EF}	9.95(± 2.12)^{E}
1a3c5	20.24(± 2.06)^{HIJ}	18.08(± 4.05)^{GHIJK}	ND	611.11(± 6.42)^{J}	5.01(± 0.87)^{FGH}
1a1a6	43.08(± 9.66)^{EFG}	83.65(± 6.95)^{A}	ND	442.22(± 8.81)^{M}	5.94(± 0.46)^{F}
p7d1m	13.99(± 1.30)^{JKLM}	74.22(± 5.53)^{A}	705.13(± 24.21)^{EF}	757.14(± 8.26)^{EFG}	ND
1a2b3	9.79(± 1.92)^{KLMN}	13.02(± 1.68)^{IJKL}	1821.49(± 12.68)^{B}	909.52(± 12.61)^{C}	1.84(± 0.80)^{HI}
p9a3m	0.38(± 1.40)^{N}	28.46(± 7.20)^{EFGHI}	1211.11(± 21.23)^{CD}	490.47(± 4.77)^{L}	10.00(± 0.48)^{E}
1a6c5	22.32(± 4.32)^{HIJ}	ND	ND	971.43(± 21.85)^{B}	45.39(± 0.26)^{B}
1a5cl	50.47(± 7.03)^{DEF}	ND	92.59(± 11.01)^{I}	235.71(± 10.92)^{Q}	1.74(± 0.88)^{HI}
p1a2	28.75(± 3.38)^{H}	46.87(± 2.62)^{BC}	ND	616.67(± 4.17)^{J}	1.65(± 0.15)^{HI}
1a2b4	28.05(± 1.15)^{H}	30.05(± 1.29)^{DEFGH}	893.25(± 21.04)^{E}	380.48(± 5.37)^{N}	3.73(± 0.48)^{FGHI}
1a5c5	147.71(± 1.97)^{A}	58.49(± 2.15)^{B}	ND	767.89(± 13.85)^{EF}	ND
1a6c1	52.09(± 0.56)^{DE}	25.05(± 0.85)^{FGHIJ}	146.82(± 2.90)^{GH}	1212.39(± 35.79)^{A}	2.00(± 0.29)^{HI}
p9f3m	12.89(± 2.11)^{JKLM}	32.00(± 4.96)^{DEFG}	ND	871.43(± 8.26)^{D}	ND
1a3c6	17.74(± 6.70)^{IJKL}	ND	133.13(± 16.90)^{H}	766.67(± 9.63)^{EF}	32.54(± 4.04)^{C}
p4c3m	1.31(± 1.12)^{N}	11.88(± 2.43)^{JKL}	ND	737.5(± 7.23)^{FGH}	ND
1a4c2	0.51(± 1.60)^{N}	ND	1540.94(± 21.56)^{C}	ND	49.00(± 1.81)^{A}
1a5c4	18.44(± 0.94)^{IJK}	10.47(± 1.36)^{JKL}	767.00(± 21.67)^{E}	272.22(± 3.46)^{P}	ND
1a5c2	8.39(± 0.43)^{LMN}	15.71(± 3.37)^{HIJKL}	ND	700(± 7.23)^{I}	13.31(± 0.90)^{E}
p5b2m	24.07(± 1.52)^{HIL}	18.20(± 4.14)^{GHIJK}	2240.97(± 24.98)^{A}	ND	ND
1a1a4	102.4(± 1.19)^{B}	24.33(± 2.06)^{FGHIJ}	430.56(± 21.84)^{F}	220.83(± 4.17)^{Q}	2.15(± 1.33)^{GHI}
p9e3m	39.82(± 5.88)^{G}	36.26(± 8.48)^{CDEF}	ND	571.43(± 8.26)^{K}	ND
p7b1m	58.75(± 2.97)^{CD}	42.50(± 8.48)^{CDE}	1181.25(± 17.24)^{D}	347.62(± 9.54)^{NO}	13.15(± 0.89)^{E}
1a6c4	42.48(± 1.18)^{FG}	38.05(± 5.15)^{CDE}	299.14(± 19.22)^{G}	717.38(± 23.78)^{HI}	5.06(± 0.38)^{FGH}
CV(%)	**21.05**	**36.34**	**13.45**	**3.16**	**16.96**

Values are mean (n = 3) and values followed by the same letter in each column are not significant from each other as detected by Duncan ($p < 0.05$). (± Standard error). +Tryp: TSB medium supplemented with 600 mg L^{-1} Tryptophan; *ARA(Acetylene Reduction Activity); **(%) Efficiency in the qualitative production of siderophore; ***(US) Efficiency in the quantitative production of siderophore (US mg^{-1}) **** *Ensifer americanum* 5750 R.

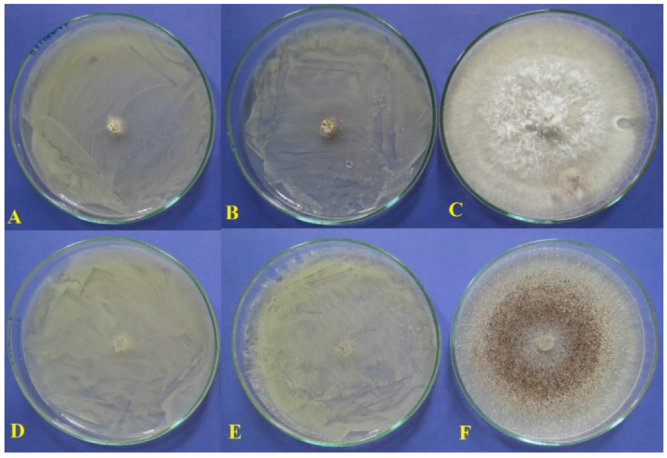

Fig. 1. Antagonism activity of selected PGPR strains: *B. vietnamiensis* la1a4 (A), *M. yunnanensis* la2b2 (B and D), *B. ubonensis* la3c3 (E) against *R. oryzae* R2 (C) and *R. solani* R1 (F).

inhibited significantly the mycelial growth of *R. solani* R1 with percentage of inhibition of 88.59% (Fig. 1E) and 69.85% (Fig. 1D) with strain la2b2, after incubation for 5 days (Table 2). Strain p7b1m (Fig. 2E) inhibited significantly *R. solani* R1 growth (90.09%) and the strain la2b2 (Fig. 2A) to *R. oryzae* R2 growth (91.67%) when bacteria and fungi were not in contact.

Most bacteria showed biocontrol activity against both *R. solani* and *R. oryzae*. Biocontrol activity, whether antagonism or antibiosis against *R. solani* R1 and *R. oryzae* R2, was found in 43.75% of the strains and only 15.6% showed no antifungal activity against them.

3.5. Multivariate analysis

Our results showed that all strains had at least one PGP characteristic, that 25 were able to produce siderophores and expressed biocontrol activity. Altogether, only 11 strains had all the characteristics analyzed in this study.

A PCA analysis including the variables ARA, siderophore production, auxin production, antibiosis and antagonism resulted in two new factors (Fig. 3). Factor 1 was responsible for 39.1% of the total variation and is mainly described by antagonism and antibiosis against *R. oryzae sativae*. Factor 2 accounted for 19.5% of the total variation and is supported mainly by production of auxin supplemented with tryptophan caused greater variability.

A Pearson correlation analysis was performed among the 4 PGP characteristics evaluated, evidencing a positive relationship between basal auxin production and antibiotic activity against *R. oryzae* ($r^2 = 0.42$). On the contrary, a negative correlation between the quantitative production of siderophores and the nitrogenase activity ($r^2 = -0.41$) was evidenced.

3.6. Identification of bacteria

The five strains selected from the multivariate analysis were identified by the amplification and sequencing of the 16S rRNA gene. The comparative analysis with type strains of species deposited in the EZ-BioCloud program, allows to affirm that the p9a3m strain shows a low affiliation with *C. bitternis* SKKUI-TP7^{T} (98.17%), so it could be a new species in the gender. Likewise, strain p7b1m presented affiliation with *Staphylococcus hominis* subsp. *novobiosepticus* GTC 1228^{T} in 98.96%. Strains la1a4 and la3c3 presented affiliation with *B. vietnamiensis* LMG 10929^{T} and *B. ubonensis* CIP 107078^{T}, with a percentage of similarity of 99.79 and 99.24%, respectively. With respect to strain la2b2, it has a

Table 2
Antagonistic and antibiotic activity of rhizospherical bacterial isolates.

Isolate code	Antagonistic activity		Antibiotic activity	
	RS	RO	RS	RO
la1a1m	52.74(± 3.62)^{G}	55.78(± 232)^{E}	52.45(± 0.42)^{F}	31.45(± 0.11)^{I}
la3c3	88.59(± 1.80)^{A}	66.22(± 2.96)^{ABC}	91.01(± 0.05)^{AB}	89.89(± 0.60)^{AB}
p3a3m	ND	ND	31.04(± 5.89)^{H}	ND
la2b2	70.22(± 4.38)^{CD}	69.85(± 1.61)^{ABC}	91.25(± 0.27)^{AB}	91.67(± 0.10)^{A}
la5b5m	ND	ND	65.40(± 0.55)^{E}	39.80(± 2.65)^{H}
1a3c5	ND	ND	77.11(± 1.73)^{D}	49.70(± 2.27)^{F}
1a1a6	ND	ND	44.85(± 0.23)^{G}	44.18(± 0.48)^{G}
p7d1m	ND	ND	68.68(± 0.12)^{E}	57.80(± 0.73)^{E}
1a2b3	68.44(± 2.96)^{CD}	72.37(± 3.48)^{A}	90.31(± 0.81)^{AB}	87.63(± 1.25)^{AB}
p9a3m	60.67(± 2.57)^{EF}	58.52(± 3.17)^{DE}	88.96(± 0.15)^{AB}	90.84(± 0.25)^{A}
1a6c5	58.00(± 2.45)^{FG}	58.52(± 3.17)^{DE}	92.72(± 0.03)^{A}	88.80(± 0.56)^{AB}
1a5cl	ND	ND	75.66(± 0.20)^{D}	50.08(± 0.97)^{F}
p1a2	ND	ND	76.03(± 1.22)^{D}	61.40(± 0.79)^{DE}
1a2b4	68.15(± 3.92)^{CD}	65.41^{BC}	87.54(± 0.74)^{B}	88.37(± 3.86)^{AB}
1a5c5	66.52(± 4.87)^{DE}	63.56(± 3.57)^{CD}	90.43(± 0.70)^{AB}	87.88(± 0.52)^{AB}
1a6c1	ND	ND	81.28(± 1.35)^{C}	62.18(± 0.29)^{D}
p9f3m	ND	ND	ND	ND
1a3c6	74.3(± 2.86)^{BC}	69.33(± 2.19)^{ABC}	91.51(± 0.19)^{AB}	89.13(± 0.14)^{AB}
p4c3m	60.3(± 0.30)^{EF}	ND	1.26(± 1.26)^{J}	ND
1a4c2	57.56(± 3.33)^{FG}	29.46(± 1.83)^{F}	75.43(± 0.39)^{D}	46.50(± 1.21)^{FG}
1a5c4	65.78(± 3.36)^{DE}	64.37(± 1.87)^{CD}	91.11(± 0.12)^{AB}	90.31(± 0.38)^{AB}
1a5c2	ND	ND	53.44(± 1.83)^{F}	29.46(± 5.43)^{I}
p5b2m	ND	ND	ND	ND
1a1a4	79.78(± 1.89)^{E}	70.81(± 2.39)^{AB}	89.52(± 0.16)^{AB}	87.68(± 1.14)^{AB}
p9e3m	ND	ND	24.81(± 0.74)^{I}	ND
p7b1m	64.52(± 1.83)^{DE}	67.33(± 2.95)^{ABC}	90.09(± 0.42)^{AB}	80.35(± 1.55)^{C}
1a6c4	63.15(± 1.84)^{DE}	66.44(± 4.29)^{ABC}	90.70(± 0.75)^{AB}	85.80(± 1.74)^{B}
CV(%)	**10.94**	**12.27**	**4.28**	**5.09**

Values are mean (n = 3) and values followed by the same letter in each column are not significant from each other as detected by Duncan ($p < 0.05$). (± Standard error). RS: *Rhizoctonia solani* R1; RO: *Rhizoctonia oryzae* R2; ND: not detected; NF: no evaluate.

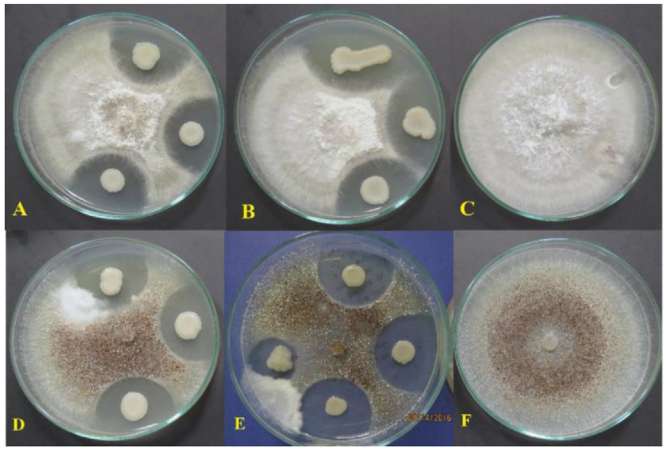

Fig. 2. Antibiosis activity of selected PGPR strains: *M. yunnanensis* la2b2 (A and D), *B. ubonensis* la3c3 (B), *S. hominis* subsp. *novobiosepticus* p7b1m (E) against *R. oryzae* R2 (C) and *R. solani* R1 (F).

closer affiliation with *M. yunnanensis* YIM 65004^{T}, with a similarity percentage of 99.79% (Table 3). For a better visualization of the phylogenetic approaches, phylogenetic trees were constructed according to the phylum Proteobacteria (Fig. 4) and Firmicutes, actinobacteria (Fig. 5).

3.7. Germination percentage and pathogenicity test

Treatment of the seeds with 5 strains of selected PGP bacteria increased the germination percentage significantly compared to the control treatment (Table 4), resulting negative to the pathogenicity test. In contrast, the treatment inoculated with *B. glumae* THT showed necrosis in the puncture site.

3.8. Pot experiments

The agronomic parameters evaluated in the treatments inoculated with PGP strains were superior to the treatments that did not receive inoculation, independently of the dose of nitrogenous fertilization. The treatments inoculated with *C. bitternis* p9a3m plus 50% nitrogen fertilizer increased the SDW by 2.9%, with respect to the treatment receiving the 100% fertilizer dose. Likewise, the treatment inoculated with *B. vietnamiensis* la1a4 plus 50% of nitrogen fertilizer, managed to increase the number of tillers and panicles per plant, as well as the number of grains per panicle, by 4.7%, 10.5% and 4.1%, respectively, with respect to the 100% fertilized treatment. Regarding the grain, the weight of 1000 seeds was higher in the treatment inoculated with *B. ubonensis* la3c3 in 3.0% compared to the 100% fertilized treatment; finally, the accumulation of proteins in the grain, unlike the other parameters, was higher in treatments with low fertilization (25%) (4.1%), especially in the treatment inoculated with *B. ubonensis* la3c3 (Table 5). *S. hominis* subsp. *novobiosepticus* p7b1m was excluded from the experiments due to be considered human pathogen.

3.9. Field experiment

The promotion of the crop growth at 27 days after sowing (nurserie) was evidenced in all inoculated treatments unlike the non-inoculated control. The strains are compatible with each other, there is no inhibition between them, so the formulation of the consortium is safe. The DWS and DWR were statistically significant, especially in the treatments inoculated with *B. ubonensis* la3c3 (Table 6) (see Table 7).

At 90 days of culture, chlorophyll levels were higher in the inoculated treatments, it is worth noting that the treatment inoculated with the consortium receiving 75% of the nitrogen dose was similar to the treatment received 100% of the dose of nitrogen fertilizer (Data not showed).

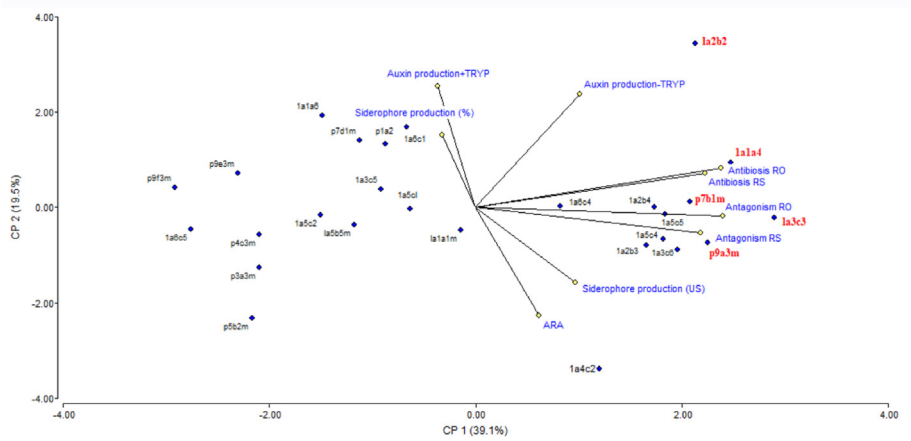

Fig. 3. Biplot analysis of major components (CP1 and CP2) obtained from the study of 9 PGPR characteristics in 27 strains of rice rhizospheric bacteria.

Table 3
Percentages of sequence similarity of the 16S rRNA gene with type strains of different genres using the EZ-Biocloud program.

Strain	Species related type closest. Type strain (T)	Similarity %	Base pairs
p9a3m	*Citrobacter bitternis* SKKUI-TP7 (T)	98.17	1474
la1a4	*Burkholderia vietnamiensis* LMG 10929 (T)	99.79	1463
p7b1m	*Staphylococcus hominis* subsp. *novobiosepticus* GTC 1228 (T)	98.96	1486
la3c3	*Burkholderia ubonensis* CIP 107078 (T)	99.24	1482
la2b2	*Micrococcus yunnanensis* YIM 65004 (T)	99.79	1425

Through the Pearson correlation analysis, it was shown that the agronomic parameters evaluated after harvesting as straw yield (r^2 = 0.81), height (r^2 = 0.81), panicle length (r^2 = 0.83), number panicle per Hill (r^2 = 0.60), number grain per panicle (r^2 = 0.67), weight thousand grains (r^2 = 0.43) are correlated with grain yield (Tables 8 and 9).

Regarding grain yield (t ha^{-1}), significant differences are reported in relation to nitrogen fertilizer doses (p = 0.0024), but without significant statistical differences in relation to inoculant treatments.

Although there was no interaction between inoculants and nitrogen fertilization doses, significant differences were observed between experimental treatments. The highest yield in grain was obtained in the treatment inoculated with the bacterial consortium plus 100% of the nitrogen fertilization (8.15 t ha^{-1}), increasing by 13.51% with respect to the treatment without inoculation plus 100% fertilization.

Likewise, the yield obtained in the treatment receiving 75% of the dose of nitrogen fertilizer plus the inoculation of the bacterial consortium (7.36 t ha^{-1}) was comparable and 2.5% higher than the yield of the treatment receiving 100% of the fertilizing dose (7.18 t ha^{-1}). In general, the use of a bacterial consortium with 75% nitrogen fertilizer gave best result with respect to grain yield (Table 9).

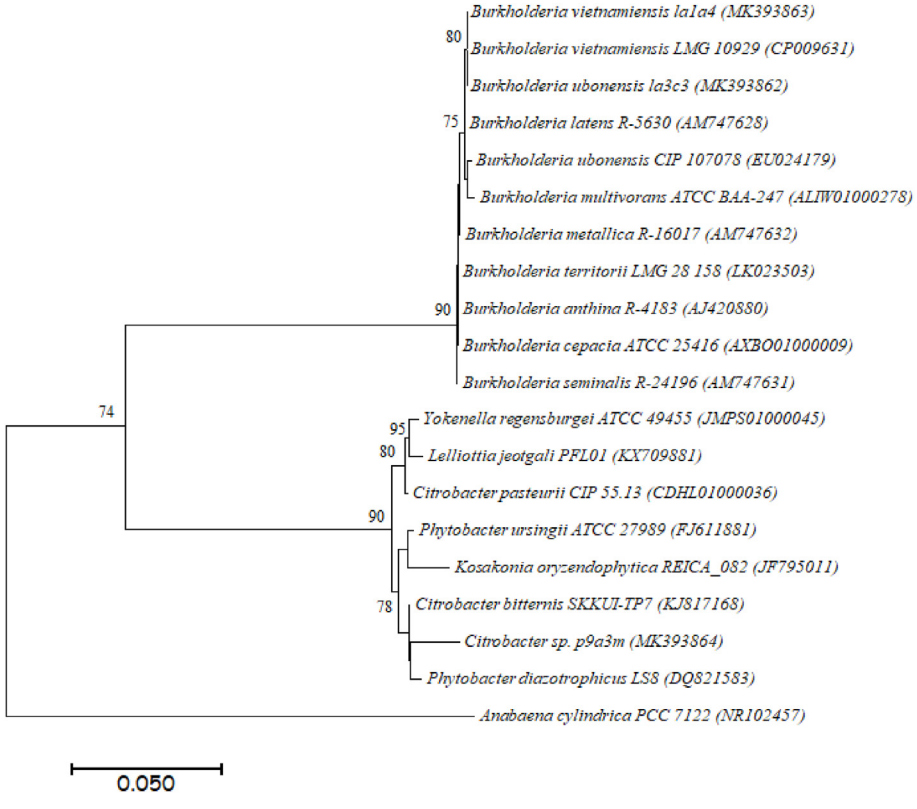

Fig. 4. NJ-phylogenetic tree based on partial 16S rRNA gene sequences of the PGPR strains Gram negative and phylogenetically related type species. The significance of each branch is indicated by a bootstrap value calculated for 1000 subsets; values lower than 70% are not indicated. Bar, 5 substitutions per 100 nucleotide position. The tree is rooted on *Anabaena cylindrica* PCC 7122^T.

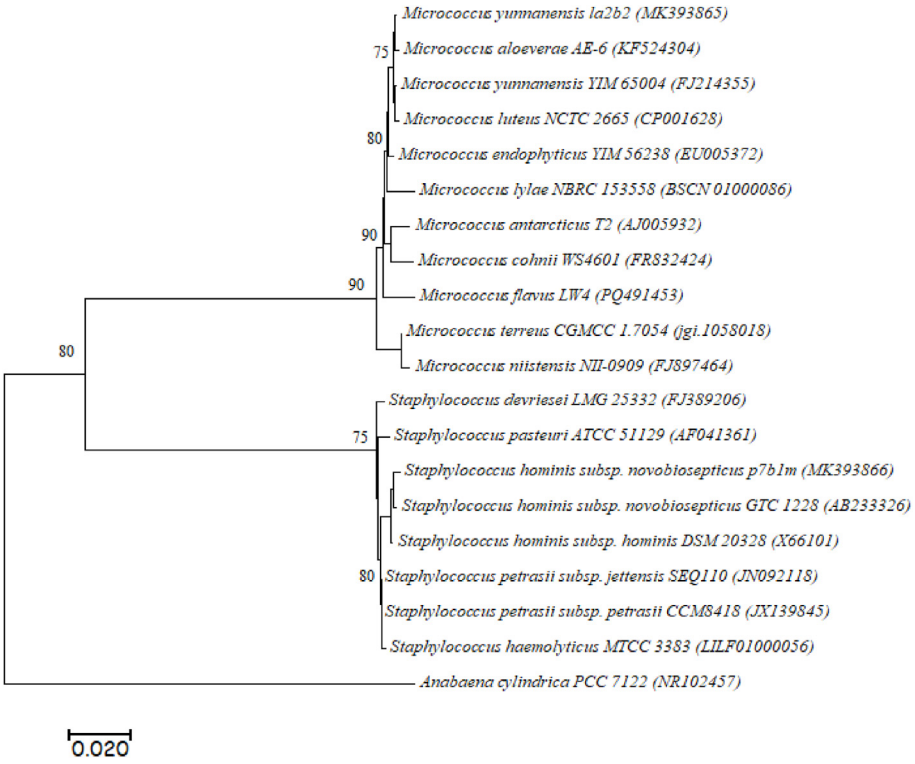

Fig. 5. NJ-phylogenetic tree based on partial 16S rRNA gene sequences of the PGPR strains Gram positive and phylogenetically related type species. The significance of each branch is indicated by a bootstrap value calculated for 1000 subsets; values lower than 70% are not indicated. Bar, 2 substitutions per 100 nucleotide position. The tree is rooted on *Anabaena cylindrica* PCC 7122^{T}.

Table 4
Effect of 5 PGP bacteria on germination percentage in rice seeds "La esperanza".

Strain	Germination (%)
Control	79.67(± 4.03)^{C}
C. bitternis p9a3m	83.00(± 2.08)^{B}
M. yunnanensis la2b2	91.67(± 2.19)^{A}
B. ubonensis la3c3	90.33(± 0.67)^{AB}
B. vietnamiensis la1a4	90.00(± 2.00)^{AB}
S. hominis p7b1m	82.33(± 2.33)^{B}
C.V. (%)	4.87

Values are mean (n = 3) and values followed by the same letter in each column are not significant from each other as detected by Duncan ($p < 0.05$). (± Standard error).

The accumulation of N, P and K at the grain level was higher and statistically significant ($p < 0.0001$) in the inoculated treatments than in the non-inoculated treatments, independently of the fertilization dose (Table A1). In relation to the N content, the plants that received 25% fertilizer plus inoculation of *C. bitternis* p9a3m and *B. vietnamiensis* la1a4 were statistically significant and superior to the treatment receiving the full dose of fertilizer. On the contrary, although the N content was higher in the treatment inoculated with the consortium received 75% dose of fertilizer (1.34%) compared to the treatment received 100% of the dose (1.22%), it was not significant. The content of P was higher in the treatments inoculated with the bacterial consortium receiving 25% and 75% of the fertilizer with respect to the 100% fertilized treatment, in 45.46% and 36.36%, respectively. The treatment inoculated with the bacterial consortium received 50% of the fertilizer showed statistically significant enhancement in the accumulation of K (0.88%) in the grain, unlike the treatment fertilized with the full dose (0.61%).

Another parameter of commercial importance in the cultivation of rice and that defines the price of it, is the milling quality of the grain. The treatments inoculated with the bacterial consortium were statistically significant in obtaining whole grain (%) after the pile, with respect to the other treatments. The grain from the treatment inoculated with the bacterial consortium receiving 75% of the fertilizer had a higher percentage of whole grain (4.99%) compared to the treatment received the full dose of fertilizer. There was also a statistically significant improvement ($p = 0.0092$) in obtaining total grain (whole grain plus broken grain), where the treatment inoculated with the bacterial consortium receiving 75% of fertilizer reached 71.30% grain, unlike the treatment received 100% of the dose that reached 69.53% (Table A2). Regarding the economic analysis, the rentability of rice cultivation increased from 14.7% to 26.6% and the utility from 17.7% to 27.0%, both being marketed as paddy rice and piled grain, respectively, in the reduction scenario of 25% of nitrogen fertilizer (Table 10).

4. Discussion

Rice is the most important source of food energy in the world. Practices such as monoculture and fertilizer abuse make it a hostile crop for the environment, because they contribute 46% of N_2O emissions in agriculture. When nitrogen levels exceed the needs of the plant, different mechanisms such as denitrification result in the emission of N_2O, leading to an inefficient use of nitrogen fertilizers (Hussain et al., 2015). The challenge of modern agriculture is to reduce the use of nitrogen fertilizers without losing performance (Bordoloi et al., 2019). One of the strategies, is the use of microorganisms with promotion activities as a complement to chemical fertilizers (Sahu et al., 2018), especially in crops with high nitrogen requirements such as rice cultivation (Othman and Panhwar, 2014). Several antecedents have been reported related to the selection of PGP bacteria to improve productivity in rice cultivation (Sharma et al., 2014; Etesami and Alikhani, 2017; Braga et al., 2018; Bisht and Chauhan, 2020).

In this research, 27 isolates with different microscopic and morphocolonial characteristics were obtained. Only 63.0% of the isolated bacteria were confirmed by ARA activity as true diazotrophs, a situation previously reported by other researchers at 18.52% by Park et al. (2005) and 18.33% by Sarathambal et al. (2015). The non-diazotrophic bacteria recovered in the semi-solid media are probably oligotrophic bacteria, which use the nitrogen fixed and released by the true

Table 5
Agronomic parameters of response to inoculation with selected five strains of PGPR bacteria in *Oryza sativa* var. La Esperanza under 3 levels of nitrogen fertilization under pot experiments conditions.

Treatment	DWS (g pot^{-1})	TP^{-1}	PP^{-1}	GP^{-1}	W1000G	GProt (%)
0 kg N ha^{-1} (0% N)						
S. hominis p7b1m	35.5 (± 2.8)^{BCD}	4.0 (± 0.4)^{ABC}	4.2 (± 0.4)^{ABC}	42.6 (± 2.8)^{DEFGH}	25.9 (± 0.6)^{AB}	9.09 (± 0.03)^{EFG}
*B. vietnamiensis*la1a4	29.6 (± 4.0)^{CDE}	3.2 (± 0.4)^{CD}	2.8 (± 0.2)^{E}	39.9 (± 2.9)^{EFGH}	24.4 (± 1.7)^{B}	9.16 (± 0.03)^{DEF}
M. yunnanensis la2b2	37.9 (± 2.5)^{AB}	4.5 (± 0.2)^{A}	4.8 (± 0.2)^{A}	41.5 (± 2.6)^{DEFGH}	25.4 (± 0.7)^{AB}	9.66 (± 0.03)^{AB}
C. bitternis p9a3m	28.6 (± 2.4)^{DE}	3.2 (± 0.3)^{CD}	3.6 (± 0.4)^{BCDE}	38.1 (± 3.6)^{H}	26.2 (± 0.3)^{AB}	8.61 (± 0.03)^{H}
B. ubonensis la3c3	34.2 (± 1.9)^{BCD}	2.7 (± 0.4)^{D}	3.0 (± 0.3)^{DE}	43.6 (± 2.9)^{CDEFGH}	27.2 (± 0.6)^{A}	8.62 (± 0.03)^{H}
Non inoculated	34.7 (± 2.4)^{BCD}	4.0 (± 0.3)^{ABC}	3.4 (± 0.4)^{CDE}	29.1 (± 2.1)^{I}	26.8 (± 0.5)^{A}	9.04 (± 0.03)^{FG}
37.5 kg N ha^{-1} (25% N)						
S. hominis p7b1m	37.5 (± 4.1)^{ABC}	3.2 (± 0.3)^{CD}	3.8 (± 0.4)^{ABCDE}	47.4 (± 2.2)^{BCDE}	27.1 (± 0.5)^{A}	9.31 (± 0.03)^{CDE}
B. vietnamiensis la1a4	41.5 (± 0.9)^{AB}	3.5 (± 0.4)^{ABCD}	3.8 (± 0.4)^{ABCDE}	47.4 (± 2.3)^{BCDE}	26.2 (± 0.5)^{AB}	8.01 (± 0.03) ^{J}
M. yunnanensis la2b2	35.1 (± 1.3)^{BCD}	4.2 (± 0.5)^{ABC}	4.6 (± 0.2)^{AB}	48.4 (± 3.1)^{ABCD}	26.8 (± 0.3)^{A}	9.62 (± 0.03)^{B}
C. bitternis p9a3m	38.3 (± 1.6)^{AB}	3.7 (± 0.3)^{ABC}	3.8 (± 0.4)^{ABCDE}	53.3 (± 2.0)^{AB}	25.5 (± 0.3)^{AB}	8.93 (± 0.03)^{G}
B. ubonensis la3c3	35.0 (± 1.6)^{BCD}	3.8 (± 0.5)^{ABC}	4.0 (± 0.4)^{ABCD}	39.1 (± 2.9)^{FGH}	26.0 (± 0.3)^{AB}	9.84 (± 0.03)^{A}
Non inoculated	35.2 (± 3.6)^{BCD}	3.2 (± 0.5)^{CD}	3.2 (± 0.4)^{CDE}	31.6 (± 4.1)^{I}	26.4 (± 0.1)^{A}	8.97 (± 0.03)^{FG}
75 kg N ha^{-1} (50% N)						
S. hominis p7b1m	45.9 (± 6.9)^{A}	3.7 (± 0.5)^{ABC}	3.2 (± 0.4)^{CDE}	39.3 (± 3.6)^{GH}	26.1 (± 0.8)^{AB}	8.63 (± 0.03)^{H}
B. vietnamiensis la1a4	23.8 (± 1.2)^{E}	4.5 (± 0.4)^{A}	4.2 (± 0.4)^{ABC}	53.9 (± 1.9)^{AB}	26.2 (± 0.4)^{A}	9.32 (± 0.03)^{CD}
M. yunnanensis la2b2	35.5 (± 0.5)^{BCD}	3.3 (± 0.3)^{BCD}	3.4 (± 0.4)^{CDE}	45.9 (± 1.8)^{BCDEFG}	26.3 (± 0.6)^{A}	8.24 (± 0.03)^{I}
C. bitternis p9a3m	42.1 (± 4.3)^{AB}	4.2 (± 0.5)^{ABC}	4.0 (± 0.3)^{ABC}	47.1 (± 2.5)^{BCDEF}	27.2 (± 1.2)^{A}	9.00 (± 0.03)^{FG}
B. ubonensis la3c3	41.8 (± 2.6)^{AB}	3.5 (± 0.2)^{ABCD}	3.4 (± 0.2)^{CDE}	46.7 (± 3.1)^{BCDEFG}	27.4 (± 0.2)^{A}	9.33 (± 0.03)^{CD}
Non inoculated	40.6 (± 0.9)^{AB}	4.2 (± 0.3)^{ABC}	3.6 (± 0.7)^{BCDE}	43.6 (± 2.8)^{CDEFGH}	25.5 (± 0.3)^{AB}	9.15 (± 0.03)^{DEF}
150 kg N ha^{-1} (100% N)	40.9 (± 2.4)^{AB}	4.3 (± 0.3)^{AB}	3.8 (± 0.2)^{ABCDE}	51.8 (± 3.6)^{ABC}	26.6 (± 0.3)^{A}	9.45 (± 0.03)^{BC}
CV (%)	**14.2**	**11.0**	**9.2**	**12.8**	**2.2**	**1.5**

Averages followed by the same letter in the column do not show significant differences (LSD Fisher, p ≤ 0.05). (± Standard error). **DWS**: Dry Weight Shoot; **TP:** Tillers per plant; **PP:** Panicles per plant; **GP:** Grains per panicle; **W1000G**: Weight 1000 grains; **GProt**: Grain protein.

diazotrophs (Beneduzi et al., 2013).

The production of auxin is the most used mechanism to explain the benefits of PGP on the regulation of plant growth (Prasad et al., 2019), because it is involved in the initiation, division and radical cell elongation. Tryptophan is the biologically active precursor of auxins (Mustafa et al., 2018), therefore 48.2% of the strains in our study increased the production of auxin when the TSB medium was supplemented with 600 mg L^{-1} of Tryptophan. In contrast, 51.9% of strains produced lower levels of auxin, according to Spaepen and Vanderleyden (2011), auxins levels may vary according to the strain and/or PGP species, as well as the carbon and pH limitation in the culture medium tested.

Under stress conditions, plants are supplied with nutrients such as Fe, and prevent the uptake of heavy metals, improving plant growth through of siderophores production (Etesami and Maheshwari, 2018). The frequency of siderophores production in liquid medium in our study (74.10%) was similar to that reported by Castellano-Hinojosa et al. (2015). Some strains showed the production of siderophores in solid medium and not in liquid medium, Schwyn and Neilands (1987) reported that the sensitivity of the test depends on the type of siderophore produced by the bacteria.

The biocontrol activity of PGP is usually associated with the control of plant pathogens (Choi et al., 2018). The *Rhizoctonia* complex causes losses between 21 and 50% of world rice production (Yu et al., 2017). In general, the study group showed greater antibiotic activity than antagonistic, a similar situation reported by Castellano-Hinojosa et al. (2015), where only the C17 strain significantly inhibited the growth of *Fusarium oxysporum*, when the bacteria and the fungus were not in contact. The inhibition of growth by antibiosis can be explained by the formation of metabolites diffusible to the medium such as antibiotics (cyclic lipopeptides, fengycin and surfactin) (Chandler et al., 2015) or antifungals (2,4-diacetylphloroglucinol). The antagonism could be explained by the formation of biofilms on the fungus and the consequent production of extracellular enzymes (Zhao et al., 2018).

The multivariate analysis of PGP characteristics allowed to determine that the antagonism and antibiosis as well as the production of AIA supplemented with tryptophan are the characteristics by which a bacterial consortium can be selected that can be used as an inoculant to promote the health and the growth of rice cultivation. Similarly, Castellano-Hinojosa et al. (2015) reported that the strains of *Bacillus aryabhattai* C4, *Burkholderia multivorans* C16 and *Staphylococcus aureus* subsp *aureus* C17, had the greatest contribution to the total variance, selected according to the production of siderophores and activity ACC deaminase.

Table 6
Effect of the inoculation of selected three strains of PGPR bacteria on vegetative parameters in *Oryza sativa* var. La Esperanza during the nursery stage at 27 days after inoculation under field conditions.

Treatments	RL (cm)	H_1 (cm)	DWR (mg)	DWS (mg)
B. ubonensis la3c3	13.85 (± 0.89)^{AB}	28.70 (± 0.22)^{A}	465.70 (± 6.10)^{A}	1469.57 (± 2.59)^{A}
C. bitternis p9a3m	14.70 (± 1.07)^{A}	22.55 (± 1.17)^{CD}	181.40 (± 0.64)^{E}	848.87 (± 2.22)^{G}
B. vietnamiensis la1a4	12.38 (± 0.55)^{ABC}	24.08 (± 1.67)^{BC}	184.90 (± 0.17)^{E}	847.90 (± 2.40)^{G}
la3c3 + p9a3m	11.63 (± 0.38)^{BC}	27.00 (± 0.46)^{AB}	178.20 (± 0.03)^{E}	1002.10 (± 2.18)^{F}
p9a3m + la1a4	11.88 (± 1.39)^{BC}	25.50 (± 0.79)^{ABC}	215.40 (± 1.32)^{D}	1068.43 (± 0.26)^{D}
la3c3 + la1a4	11.13 (± 0.55)^{C}	29.08 (± 1.20)^{A}	313.10 (± 0.64)^{B}	1195.80 (± 0.45)^{B}
la3c3 + p9a3m + la1a4	12.00 (± 1.02)^{BC}	22.80 (± 1.87)^{CD}	215.27 (± 1.07)^{D}	1054.13 (± 2.11)^{E}
Non-inoculated	10.95 (± 0.32)^{C}	20.45 (± 1.30)^{D}	280.07 (± 0.32)^{D}	1119.97 (± 0.03)^{C}
C.V (%)	**6.19**	**4.79**	**1.54**	**0.28**

Averages followed by the same letter in the column do not show significant differences (LSD Fisher, p ≤ 0.05). (± Standard error). **RL:** Root length; **H_1:** Plant height; **DWR:** Dry Weight Root; **DWS:** Dry Weight Shoot.

Table 7
Agronomic parameters of a rice cultivar "La Esperanza" inoculated with PGP bacteria in the presence of different levels of nitrogen fertilization under field conditions.

N	la3c3	p9a3m	la1a4	la3c3 + p9a3m	la3c3 + la1a4	p9a3m + la1a4	la3c3 + p9a3m + la1a4	Non-inoculated	Mean	CV
kg ha^{-1}	**SY (t ha^{-1})**									%
0% (0)	11.44	14.52	14.23	15.31	16.08	13.52	12.25	17.55	14.88 B	
25% (37.5)	18.47	16.63	17.52	17.11	18.25	16.16	18.00	16.83	17.37 B	
50% (75)	15.77	15.34	15.84	17.37	16.9	15.67	15.42	16.52	16.10 B	16.90
75% (112.5)	21.70	19.53	17.46	22.36	19.81	20.34	18.67	20.17	20.26 A	
100% (150)	21.73	18.83	19.78	21.08	20.77	22.17	24.02	21.84	21.28 A	
Mean	**17.82 a**	**16.97 a**	**16.97 a**	**18.65 a**	**18.36 a**	**17.57 a**	**17.67 a**	**18.58 a**		
H_2 (cm)										
0% (0)	90.00	87.33	89.33	86.67	89.33	89.67	89.33	87.33	88.63 B	
25% (37.5)	92.00	93.67	93.67	90.00	94.00	91.67	92.00	92.00	92.38 A	
50% (75)	91.00	92.00	94.00	91.67	91.67	90.00	90.33	88.33	91.13 B	3.61
75% (112.5)	96.00	94.00	94.33	89.67	95.33	93.00	94.33	92.00	93.58 A	
100% (150)	94.33	97.33	93.67	92.67	95.33	95.33	95.33	94.33	94.79 A	
Mean	**92.67 a**	**92.87 a**	**93.00 a**	**90.13 a**	**93.13 a**	**91.93 a**	**92.27 a**	**90.80 a**		
NT										
0% (0)	14.00	13.33	14.33	15.00	12.67	15.00	17.00	15.00	14.54 A	
25% (37.5)	15.67	14.00	18.33	14.33	13.00	14.67	12.67	15.00	14.71 A	
50% (75)	14.33	15.67	14.33	14.67	14.33	13.00	15.33	14.00	14.46 A	11.91
75% (112.5)	14.93	13.80	15.40	14.17	14.73	15.43	14.93	14.63	14.75 A	
100% (150)	15.33	15.67	15.00	15.67	14.00	16.33	14.33	13.33	14.96 A	
Mean	**14.85 a**	**14.49 a**	**15.48 a**	**14.77 a**	**13.75 a**	**14.89 a**	**14.85 a**	**14.39 a**		

Averages followed by the same lowercase or capital letter in the column do not present significant differences (Scott & Knott, $p \leq 0.05$). (± Standard error). La3a3: *Burkholderia ubonensis*; p9a3m: *Citrobacter bitternis*; la1a4: *Burkholderia vietnamiensis*. SY: Straw yield, H_2: Plant height and NT: Number of tillers per hill.

Table 8
Agronomic parameters of a rice cultivar "La Esperanza" inoculated with PGP bacteria in the presence of different levels of nitrogen fertilization under field conditions.

N	la3c3	p9a3m	la1a4	la3c3 + p9a3m	la3c3 + la1a4	p9a3m + la1a4	la3c3 + p9a3m + la1a4	Non-inoculated	Mean	CV
W1000G (g)										
0% (0)	27.86	28.08	27.98	28.33	27.89	27.67	27.70	27.92	27.93 A	
25% (37.5)	29.33	28.59	28.85	28.69	28.21	28.48	28.86	28.30	28.67 A	
50% (75)	28.69	29.19	28.87	28.21	27.58	28.31	28.81	28.18	28.47 A	1.94
75% (112.5)	28.90	28.15	28.40	29.23	29.33	28.62	28.52	28.54	28.71 A	
100% (150)	28.82	28.32	28.54	28.66	27.12	29.09	28.74	28.39	28.46 A	
Mean	**28.72 a**	**28.47 a**	**28.53 a**	**28.63 a**	**28.02 b**	**28.43 a**	**28.53 a**	**28.27 b**		
PL (cm)										
0% (0)	23.40	23.37	23.75	23.43	23.56	22.83	23.28	21.95	22.82 C	
25% (37.5)	25.31	25.08	25.33	25.10	25.45	24.99	25.51	23.41	24.44 B	
50% (75)	25.61	25.64	25.84	25.67	25.62	25.73	25.29	24.12	24.84 B	1.96
75% (112.5)	26.72	25.76	26.13	26.27	26.46	25.94	26.12	24.11	25.36 A	
100% (150)	26.32	26.32	26.82	26.26	26.75	26.64	26.58	25.36	25.82 A	
Mean	**24.75 a**	**24.58 a**	**24.81 a**	**24.63 a**	**24.84 a**	**24.38 a**	**24.61 a**	**24.60 a**		
NPH										
0% (0)	14.00	13.67	15.00	13.33	15.00	13.33	13.67	13.33	13.92 B	
25% (37.5)	13.00	15.00	15.00	13.33	14.67	14.00	13.00	13.00	13.88 B	
50% (75)	16.00	16.00	14.00	14.00	13.67	13.33	16.00	14.33	14.67 B	10.32
75% (112.5)	14.67	15.00	14.67	13.00	14.67	14.33	14.67	13.67	14.33 B	
100% (150)	16.67	15.33	15.67	15.33	16.33	16.33	18.00	18.00	16.46 A	
Mean	**14.87 a**	**15.00 a**	**14.87 a**	**13.80 a**	**14.87 a**	**14.27 a**	**15.07 a**	**14.47 a**		
NGP										
0% (0)	136.80	123.95	136.20	132.40	134.40	120.90	126.60	97.35	117.84 B	
25% (37.5)	135.80	135.35	147.85	143.20	144.40	141.05	148.95	118.20	128.90 A	
50% (75)	132.50	154.90	145.25	140.20	160.25	149.00	133.70	122.90	133.03 A	7.53
75% (112.5)	155.35	147.35	155.50	160.15	156.90	159.85	154.35	126.70	139.75 A	
100% (150)	145.85	149.50	151.95	155.05	149.55	150.70	164.00	131.40	139.02 A	
Mean	**128.17 a**	**128.53 a**	**133.38 a**	**133.36 a**	**135.17 a**	**131.19 a**	**133.24 a**	**130.62 a**		

Averages followed by the same lowercase or capital letter in the column do not present significant differences (Scott & Knott, $p \leq 0.05$). (± Standard error). La3c3: *Burkholderia ubonensis*; p9a3m: *Citrobacter bitternis*; la1a4: *Burkholderia vietnamiensis*. W1000G: Weight of a thousand grains, PL: Panicle length, NPH: Number of panicles per hill and NGP: Number of grains per panicle.

According to the phylogenetic analysis, the p9a3m strain has a low affiliation with the genus *Citrobacter*, which has been reported as a rhizospheric inhabitant of cereals such as maize (Arruda et al., 2013), sugar cane (Beneduzi et al., 2013) and rice (Hongrittipun et al., 2014). With respect to the genus *Burkholderia*, we selected two species, *B. ubonensis* la3c3 and *B. vietnamiensis* la1a4. Both species belong to the *Burkholderia* cepacia complex, that although their pathogenicity is strain dependent and independent of species, they are recognized as opportunistic human pathogens BSL level - 2 (Center for Disease Control and PreventionCDC, 2019). To date the virulent capacity of *B. ubonensis* has not been demonstrated (Price et al., 2017) and *B. vietnamiensis* is frequently isolated as diazotrophic bacterial in different cultures (Govindarajan et al., 2008; Tang et al., 2010; Shinjo et al., 2018).

Table 9
Effect of seven inoculation treatments with PGPR strains under five different doses of nitrogen fertilization on the yield (t ha^{-1}) of the variety rice cultivation "La Esperanza" in field conditions.

Dose of Nitrogen fertilization (kg N ha^{-1})	Combined treatments						
	la3c3	p9a3m	la1a4	la3c3 + p9a3m	la3c3 + la1a4	p9a3m + la1a4	la3c3 + p9a3m + la1a4	Non-inoculated
0% (0)	4.65 (± 0.42)^{C}a	4.87 (± 0.11)^{C}a	4.4 (± 0.19)^{B}ab	4.49 (± 0.26)^{C}ab	4.43 (± 0.32)^{C}ab	4.49 (± 0.18)^{B}ab	4.56 (± 0.15)^{C}ab	3.86 (± 0.10)^{C}b
25% (37.5)	5.76 (± 0.39)^{ABC}a	6.27 (± 0.26)^{AB}a	6.18 (± 0.10)^{A}a	5.68 (± 0.21)^{B}a	5.82 (± 0.43)^{B}a	5.69 (± 0.15)^{AB}a	5.80 (± 0.28)^{BC}a	5.89 (± 0.15)^{AB}a
50% (75)	5.38 (± 0.23)^{BC}a	5.71 (± 0.43)^{BC}a	5.95 (± 0.84)^{A}a	5.74 (± 0.37)^{AB}a	5.43 (± 0.54)^{BC}a	5.41 (± 1.16)^{AB}a	6.09 (± 0.17)^{BC}a	5.28 (± 0.35)^{B}a
75% (112.5)	6.33 (± 0.37)^{AB}a	6.74 (± 0.34)^{AB}a	6.66 (± 0.42)^{A}a	6.80 (± 0.39)^{A}a	6.68 (± 0.23)^{AB}a	6.57 (± 0.56)^{A}a	7.36 (± 0.95)^{AB}a	6.08 (± 0.84)^{AB}a
100% (150)	7.11 (± 0.66)^{A}a	7.10 (± 0.59)^{A}a	7.31 (± 0.36)^{A}a	6.72 (± 0.40)^{AB}a	7.27 (± 0.50)^{A}a	7.37 (± 0.52)^{A}a	8.15 (± 0.64)^{A}a	7.18 (± 0.18)^{A}a
C.V (%)	12.99	10.77	13.00	9.88	12.30	18.49	14.54	12.95

Average and standard error (n = 3) followed by the same letter (Capital letter in superscript for nitrogen fertilization dose and lowercase letters for inoculation procedure) are not significant at 5% probability by the LSD-Fisher test. (± Standard error). *Burkholderia ubonensis*,la3c3; *Citrobacter bitternis*, p9a3m; *Burkholderia vietnamiensis*,la1a4.

Table 10
Production costs and rentability analysis of the use of inoculants in the rice cultivation variety "La Esperanza" in the San Martín region.

Fertilization Dose N (%)	WITH INOCULANT		WITHOUT INOCULANT	
	75%	100%	75%	100%
Marketing of the grain as paddy rice				
Yield obtained (t ha^{-1})	6084.01	7182.01	7362.00	8154.08
Cost kg seed to the market ($ USD)	0.31	0.31	0.31	0.31
Cost of production per kg of seed ($ USD)	0.31	0.26	0.26	0.23
Utility margin per kg of seed ($ USD)	0.00	0.05	0.05	0.08
Total Gain ($ USD)	1886.04	2226.42	2282.22	2527.76
Total cost of rice production (Campaign 2017)* ($ USD)	1895.44	1895.44	1901.61	1901.61
Net ($ USD)	−9.40	330.98	380.61	626.15
Rentability	−0.50	17.46	20.02	32.93
Utility	0.00	0.17	0.20	0.33
Marketing as piled grain				
Whole Grain (%)	65.27	64.80	68.03	66.07
Whole Grain yield (kg ha^{-1})	3971.03	4653.94	5008.37	5387.40
Price rice piled (kg)	0.56	0.56	0.56	0.56
Total Gain per piled grain ($ USD)	2223.78	2606.21	2804.69	3016.94
Total Gain ($ USD)	328.34	710.77	903.08	1115.33
Rentability	17.32	37.50	47.49	58.65
Utility	0.17	0.37	0.47	0.59

*Total cost: Direct cost + Indirect cost; ** Rentability: Net x 100/Total gain.

Authors such as Baldani et al. (2000), Prasertsincharoen et al. (2015) and Sandanakirouchenane et al. (2017), reported the promotion of rice growth through the inoculation of rhizospheric diazotrophic strains of *B. vietnamiensis*. Although diazotrophy is intrinsic in *B. vietnamiensis* (Eberl and Vandamme, 2016), *B. ubonensis* la3c3 was able to reduce ethylene in acetylene, its diazotrophic capacity being reported for the first time.

The genus *Micrococcus* belongs to the edge of the actinobacteria, which have been described as common inhabitants in the rhizosphere of plants (Sekhar and Thomas, 2015). On the other hand, the presence of the genus *Staphylococcus* has been reported by several authors (Sekhar and Thomas, 2015; Tchakounté et al., 2018), probably due to the interaction and relationship between the fertilizer-soil-plant-human systems since the beginning of agriculture (Kennedy et al., 2004). Strain p7b1m was excluded from the following studies in the pot and field experiments.

The five strains selected for their PGP characteristics, when individually inoculated in rice variety "La Esperanza" under laboratory and pot experiments, increased the germination percentage and the agronomic and grain parameters associated with the improvement of crop productivity, with respect to non-inoculated treatments. The ability of bacteria to express PGP characteristics under "in plant" conditions is one of the bottlenecks faced by microbiologists to select an ideal consortium in a specific plant model. The conditions for obtaining an ideal PGP were provided by Chauhan et al. (2015), however not always a strain covers all the requirements, so the use of PGP consortium, instead of simple strains is increasingly studied (Etesami and Alikhani, 2016; Nguyen et al., 2017).

Several authors maintain that SDW, number of tillers, panicles per plant, number of grains per panicle and weight of 1000 seeds, is mainly due to the effect of production of auxins and gibberellins (Guimarães and Baldani, 2013), which is reflected in a higher crop yield. Hahn et al. (2016) reported a 19.2% increase in the number of tillers in a treatment inoculated with *Mesorhizobium* UFRGS Lc336 receiving 40 kg N ha^{-1}, compared to a fertilized treatment with 80 kg N ha^{-1}. Rodrigues et al. (2008) reported that diverse strains of *Azospirillum amazonense* were able to increase the number of panicles under greenhouse conditions.

The major protein in rice is glutelin (Oszvald et al., 2008). The protein values reported in this study (up to 9.84%) were increased in the treatments inoculated by receiving low doses of nitrogen fertilization (0 and 25%). This pattern in the protein increase was reported by Amoli et al. (2014), when the Tarom variety was inoculated with *Azospirillum* receiving 50% fertilizer (10.40%) compared to the treatment receiving 100% of the nitrogen fertilization (9.15%). Under conditions of nitrogen deprivation, depending on the genotype of the plant and the efficient expression of the nitrogenase enzyme in the inoculating strain, the Biological Nitrogen Fixation would increase the accumulation of N in the grain.

In the treatments inoculated individually and supplemented with 25 and 50% of the nitrogen fertilizer dose, the vegetative and grain parameters were comparable and even higher than the treatment receiving 100% of doses in the pot experiments. Similar results were obtained by Jetiyanon et al. (2017) when evaluating the biofertilizer "Rhizoproduct" formulation based on *Bacillus cereus* RS87 under greenhouse conditions, where the parameters were higher in the treatment 100% fertilized in a range of 11.8–15.6%. In an integrated nutrient management program, reducing the use of fertilizers supplemented with the use of biofertilizers, results in an attractive strategy for the sustainable management of rice, in other words, beginning to cut our dependence on chemicals and to target environmental technologies economically viable (Basak et al., 2017).

Under field conditions, an efficient use of nitrogen fertilizer was observed, especially when doses of 75% nitrogen fertilizer were used in treatments inoculated with the inoculant consortium when compared to the treatment with 100% nitrogen fertilizer. In a similar way that reported to those published by other authors such as Govindarajan et al. (2008), Araújo et al. (2013) and Jetiyanon et al. (2017). In the present study, a response to positive inoculation and consistent with doses of nitrogen fertilization is reported. Yanni and Dazzo (2010), in a study during several crop campaigns, was able to estimate that rhizobial inoculants were able to provide increases in yield in a range of 3.5–47.0% in all cases, so a response positive to the inoculation. Other authors have reported different values of increase in grain yield when using biofertilizers, Win et al. (2019) under greenhouse conditions, and Govindarajan et al. (2008) (9.5–23.6%), de Souza et al. (2013) (1.0–11.68%), Araújo et al. (2013) (5.71%), Sarathambal et al. (2015) (24.17%), Take-Tsaba et al. (2018) (16.74%), under field conditions.

The benefits of the use of the inoculating consortium can be discussed in two different scenarios: 1) Fertilizer input reduction scenario: The treatment inoculated with the bacterial consortium and fertilized with 75% of the nitrogen dose, increases the grain yield up to a 21.5% (1.28 t ha^{-1}) with respect to the treatment receiving only 75% of the total fertilizer dose. Even an increase of 2.5% (180 kg) was achieved with respect to the treatment receiving the full dose of fertilizer. 2) Common scenario of use of fertilizer inputs: The treatment receiving the full dose of nitrogen fertilizer to be inoculated with the bacterial consortium is achieved an increase in grain yield of up to 13.5% (972 kg of grain) with respect to treatment receiving full fertilizer dose without inoculating. The yields obtained in a definitive way confirm the beneficial effect of the use of the bacterial consortium in rice cultivation. Taking into consideration that the variety "La Esperanza" is one of the most cultivated in the San Martín region (MINAGRI, 2018), it currently reaches an average yield of 6 t ha^{-1}.

In the first scenario, the reduction in the use of nitrogen fertilizers would generate environmental benefits, Khalil et al. (2009) reported that for every 100 kg of urea are generated up to 54 kg N_2O–N ha^{-1}, reducing 25% of the nitrogenous dose would stop emitting a quarter of N_2O emission to the environment, contributing to the mitigation of climate change. In relation to the economic benefits, leaving to use 37.5 kg of N ha^{-1}, it is possible a reduction in the costs of application of fertilizers and labor in approximately 2.8% of the total costs of production in the rice crop (INIA, 2018).

In the second scenario, outstanding economic benefits are identified. The yield grain, was expressed in greater magnitude when the inoculation was accompanied by the maximum dose of nitrogen fertilization (150 kg N ha^{-1}), Punschke and Mayans (2011) and Jetiyanon et al. (2017) have reported this situation. This increase in yield is associated with a higher adsorption of nutrients (Punschke and Mayans, 2011), due to the PGP characteristics for which they were selected, in both cases, this characteristics of the bacteria allowed a greater efficiency in nutrient adsorption.

The economic analysis of rentability and utility is important to estimate crop productivity (Aon et al., 2015). The rentability and utility data were analyzed under two common scenarios in the farmers of the San Martin region. The first, when the farmer commercializes the grain directly to the miller intermediary, and a second scenario, when the farmer processes the piled grain (Table 10). The highest values of rentability and utility were reached in the inoculated treatments, confirming the economic benefits of the inoculation in rice cultivation.

5. Conclusions

The results of this study allow to conclude the following: 1) It is possible to select bacteria with PGP characteristics, which under pot experiments conditions increased the agronomic parameters. 2) The inoculation of the bacterial consortium (p9a3m, la1a4 and la3c3) turns out to be a low-cost and environmentally sustainable technology that increases the efficient use of nitrogen fertilizer and the rentability/utility of rice cultivation in the San Martin region. For future studies, the aim is to study the environmental toxicity of the members of the consortium and thus ensure the biosecurity of its use. It is important to study the patterns of colonization of the strains in consortium, and thus improve the effectiveness of the same through the monitoring of the strains during the field evaluations using different rice genotypes. It will also be important to study the consortium's nitrogen fixation potential through the ^{15}N isotopic technique.

Acknowledgments

This study was financed by the National University of San Martín with the annual financial support 2015, 2016 and 2017, with resolutions N° 138-2015; 239-2016 and 248-2017, respectively. Renzo A. Valdez-Nuñez made a stay at the Estación Experimental del Zaidín (CSIC State Agency, Granada, Spain), through fellowship N° 021-2016-FONDECYT (National Fund for Scientific, Technological and Technological Innovation). We would also like to thank Professor Eybis Flores García, for giving us the strains of *Rhizoctonia* used in this study, as well as the Rony Castro Tuanama for their support in greenhouse and field trials.

Appendix. ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rhisph.2020.100200.

References

Ahmed, M., Rauf, M., Mukhtar, Z., Saeed, N.A., 2017. Excessive use of nitrogenous fertilizers: an unawareness causing serious threats to environment and human health. Environ. Sci. Pollut. Res. 24, 26983–26987. https://doi.org/10.1007/s11356-017-0589-7.

Amoli, H.F., Niknejhad, Y., Mahmoudi, M., 2014. Effect of nitrogen fertilizer, Azospirillum and Pseudomonas bacteria on qualitative characteristics in rice (Local cv). I.J.F.A.S., vol. 3. pp. 1283–1287. http://ijfas.com/wp-content/uploads/2015/01/1283-1287.pdf.

AOAC, 1970. Official Methods Analysis, tenth ed. Association of Official Agricultural Chemistry, Washington, DC.

Aon, M., Khalid, M., Hussain, S., Naveed, M., Akhtar, M.J., 2015. Diazotrophic inoculation supplemented nitrogen demand of flooded rice under field conditions. Pakistan J. Agric. Sci. 52, 145–150. Available in: http://www.pakjas.com.pk/papers/2400.pdf.

Araújo, A.D.S., Baldani, V.L.D., Galisa, P.S., Pereira, J.A., Baldani, J.I., 2013. Response of

traditional upland rice varieties to inoculation with selected diazotrophic bacteria isolated from rice cropped at the northeast region of Brazil. Appl. Soil Ecol. 64, 49–55. https://doi.org/10.1016/j.apsoil.2012.10.004.

Arruda, L., Beneduzi, A., Martins, A., Lisboa, B., Lopes, C., Bertolo, F., Vargas, L.K., 2013. Screening of rhizobacteria isolated from maize (*Zea mays* L.) in Rio Grande do Sul State (South Brazil) and analysis of their potential to improve plant growth. Appl. Soil Ecol. 63, 15–22. https://doi.org/10.1016/j.apsoil.2012.09.001.

Baldani, V.L.D., Baldani, J.I., Döbereiner, J., 2000. Inoculation of rice plants with the endophytic diazotrophs *Herbaspirillum seropedicae* and *Burkholderia* spp. Biol. Fertil. Soils 30, 485–491. https://doi.org/10.1007/s003740050027.

Baldocchi, D., Knox, S., Dronova, I., Verfaillie, J., Oikawa, P., Sturtevant, C., Matthes, J.H., Detto, M., 2016. The impact of expanding flooded land area on the annual evapotranspiration of rice. Agric. For. Meteorol. 223, 181–193. https://doi.org/10.1016/j.agrformet.2016.04.001.

Banik, A., Dash, G.K., Swain, P., Kumar, U., Mukhopadhyay, S.K., Dangar, T.K., 2019. Application of rice (*Oryza sativa* L.) root endophytic diazotrophic *Azotobacter* sp. strain Avi2 (MCC 3432) can increase rice yield under green house and field condition. Microbiol. Res. 219, 56–65. https://doi.org/10.1016/j.micres.2018.11.004.

Basak, N., Mandal, B., Datta, A., Mitran, T., Biswas, S., Dhar, D., Badole, S., Saha, B., Hazra, G.C., 2017. Impact of long-term application of organics, biological, and inorganic fertilizers on microbial activities in rice-based cropping system. Commun. Soil Sci. Plant Anal. 48, 2390–2401. https://doi.org/10.1080/00103624.2017.1411502.

Basosi, R., Spinelli, D., Fierro, A., Jez, S., 2014. Mineral nitrogen fertilizers: environmental impact of production and use. In: López-Valdez, F., Fernández-Luqueños, F. (Eds.), Fertilizers: Components, Uses in Agriculture and Environmental Impacts. Nova science publishers, New York, pp. 3–4.

Beneduzi, A., Moreira, F., Costa, P.B., Vargas, L.K., Lisboa, B.B., Favreto, R., Baldani, J.I., Pasaglia, L.M.P., 2013. Diversity and plant growth promoting evaluation abilities of bacteria isolated from sugarcane cultivated in the south of Brazil. Appl. Soil Ecol. 63, 94–104. https://doi.org/10.1016/j.apsoil.2012.08.010.

Bisht, N., Chauhan, P.S., 2020. Comparing the growth-promoting potential of *Paenibacillus lentimorbus* and *Bacillus amyloliquefaciens* in *Oryza sativa* L. var. Sarju-52 under suboptimal nutrient conditions. Plant Physiol. Biochem. 146, 187–197. https://doi.org/10.1016/j.plaphy.2019.11.023.

Bordoloi, N., Baruah, K.K., Bhattacharyya, P., Gupta, P.K., 2019. Impact of nitrogen fertilization and tillage practices on nitrous oxide emission from a summer rice ecosystem. Arch. Agron Soil Sci. 14, 340–365. https://doi.org/10.1080/03650340.2019.1566716.

Braga, L.F., de Oliveira, F.A., do Couto, E.A.P., Santos, K.F.d.E.N., de Brito Ferreira, E.P., Martin-Didonet, C.C.G., 2018. Polyphasic characterization of bacteria obtained from upland rice cultivated in Cerrado soil. Braz. J. Microbiol. 49, 20–28. https://doi.org/10.1016/j.bjm.2017.04.004.

Castellano-Hinojosa, A., Correa-Galeote, D., Palau, J., Bedmar, E.J., 2015. Isolation of N_2-fixing rhizobacteria from *Lolium perenne* and evaluating their plant growth promoting traits. J. Basic Microbiol. 55, 1–7. https://doi.org/10.1002/jobm.201500247.

Center for Disease Control and Prevention, CDC, 2019. *Burkholderia Cepacia* in Healthcare Settings. https://www.cdc.gov/HAI/organisms/bCepacia.html, Accessed date: 1 March 2019.

Chandler, S., Van Hese, N., Coutte, F., Jacques, P., Höfte, M., De Vleesschauwer, D., 2015. Role of cyclic lipopeptides produced by *Bacillus subtilis* in mounting induced immunity in rice (*Oryza sativa* L.). Physiol. Mol. Plant Pathol. 91, 20–30. https://doi.org/10.1016/j.pmpp.2015.05.010.

Chauhan, H., Bagyaraj, D.J., Selvakumar, G., Sundaram, S.P., 2015. Novel plant growth promoting rhizobacteria—prospects and potential. Appl. Soil Ecol. 95, 38–53. https://doi.org/10.1016/j.apsoil.2015.05.011.

Choi, J.E., Nguyen, C.M., Lee, B., Park, J.H., Oh, J.Y., Choi, J.S., Song, J.K., 2018. Isolation and characterization of a novel metagenomic enzyme capable of degrading bacterial phytotoxin toxoflavin. PloS One 13, e0183893. https://doi.org/10.1371/journal.pone.0183893.

Cottyn, B., Regalado, E., Lanoot, B., De Cleene, M., Mew, T.W., Swings, J., 2001. Bacterial populations associated with rice seed in the tropical environment. Phytopathology 91, 282–292. https://doi.org/10.1094/PHYTO.2001.91.3.282.

de Souza, R., Beneduzi, A., Ambrosini, A., Da Costa, P.B., Meyer, J., Vargas, L.K., Schoenfeld, R., Passaglia, L.M., 2013. The effect of plant growth-promoting rhizobacteria on the growth of rice (*Oryza sativa* L.) cropped in southern Brazilian fields. Plant Soil 366, 585–603. https://doi.org/10.1007/s11104-012-1430-1.

Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W., 2012. InfoStat, Versión 2012.1, Grupo InfoStat, FCA. Universidad Nacional de Córdoba, Argentina. https://www.infostat.com.ar/, Accessed date: 20 December 2019.

Eberl, L., Vandamme, P., 2016. Members of the genus *Burkholderia*: good and bad guys. F1000Res. http://doi:10.12688/f1000research.8221.1 5.

Etesami, H., Alikhani, H.A., 2016. Co-inoculation with endophytic and rhizosphere bacteria allows reduced application rates of N-fertilizer for rice plant. Rhizosphere 2, 5–12. https://doi.org/10.1016/j.rhisph.2016.09.003.

Etesami, H., Alikhani, H.A., 2017. Evaluation of Gram-positive rhizosphere and endophytic bacteria for biological control of fungal rice (*Oryza sativa* L.) pathogens. Eur. J. Plant Pathol. 147, 7–14. https://doi.org/10.1007/s10658-016-0981-z.

Etesami, H., Maheshwari, D.K., 2018. Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: action mechanisms and future prospects. Ecotoxicol. Environ. Saf. 156, 225–246. https://doi.org/10.1016/j.ecoenv.2018.03.013.

Fahad, S., Adnan, M., Noor, M., Arif, M., Alam, M., Khan, I.A., Basir, A., 2019. Major constraints for global rice production. In: Hassanuzzaman, M., Fujita, M., Biswas, J.K. (Eds.), Advances in Rice Research for Abiotic Stress Tolerance. Woodhead Publishing, pp. 1–22. https://doi.org/10.1016/B978-0-12-814332-2.00001-0.

Ferreira, J.S., Baldani, J.I., Baldani, V.L.D., 2010. Seleção de bactérias diazotróficas em duas variedades de arroz. Acta Sci. Agron. 32, 179–185. https://doi.org/10.4025/actasciagron.v32i1.73210.4025/actasciagron.v32i1.732.

Ghaffari, H., Gholizadeh, A., Biabani, A., Fallah, A., Mohammadian, M., 2018. Plant Growth Promoting Rhizobacteria (PGPR) application with different nitrogen fertilizer levels in rice (*Oryza sativa* L.). Pertanika J. Trop. Agric. Sci. 41, 715–728. http://www.pertanika.upm.edu.my/Pertanika%20PAPERS/JTAS%20Vol.%2041%20(2)%20May.%202018/14%20JTAS%20Vol%2041%20(2)%20May%202018_JTAS-1208-2017_pg715-728.pdf.

Govindarajan, M., Balandreau, J., Kwon, S.W., Weon, H.Y., Lakshminarasimhan, C., 2008. Effects of the inoculation of *Burkholderia vietnamiensis* and related endophytic diazotrophic bacteria on grain yield of rice. Microb. Ecol. 55, 21–37. https://doi.org/10.1007/s00248-007-9247-9.

Gravel, V., Antoun, H., Tweddell, R.J., 2007. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). Soil Biol. Biochem. 39, 1968–1977. https://doi.org/10.1016/j.soilbio.2007.02.015.

Guimarães, S.L., Baldani, V.L.D., 2013. Produção de arroz inoculado com bactérias diazotróficas marcadas com resistência induzida ao antibiótico estreptomicina. Rev. Ciencias Agrar. 56, 125–132. https://doi.org/10.4322/rca.2013.020.

Hahn, L., Sá, E.L.S.D., Osório Filho, B.D., Machado, R.G., Damasceno, R.G., Giongo, A., 2016. Rhizobial inoculation, alone or coinoculated with *Azospirillum brasilense*, promotes growth of wetland rice. Rev. Bras. Ciênc. Solo 40. https://doi.org/10.1590/18069657rbcs20160006.

Hakeem, K.R., Sabir, M., Ozturk, M., Akhtar, M.S., Ibrahim, F.H., 2016. Nitrate and nitrogen oxides: sources, health effects and their remediation. In: In: de Voogt, P. (Ed.), Reviews of Environmental Contamination and Toxicology ume 242. Springer Charm, pp. 183–217. https://doi.org/10.1007/398_2016_11.

Hong-Xing, G., Hong-Sheng, Z., He, Z., Xu-Hui, C., Yu, S., Ling, K., 2018. The characteristics of methane flux from an irrigated rice farm in East China measured using the eddy covariance method. Agric. For. Meteorol. 249, 228–238. https://doi.org/10.1016/j.agrformet.2017.11.010.

Hongrittipun, P., Youpensuk, S., Rerkasem, B., 2014. Screening of nitrogen fixing endophytic bacteria in *Oryza sativa* L. J. Agric. Sci. 6, 66–74. https://doi.org/10.5539/jas.v6n6p66.

Hou, W., Khan, M.R., Zhang, J., Lu, J., Ren, T., Cong, R., Li, X., 2019. Nitrogen rate and plant density interaction enhances radiation interception, yield and nitrogen use efficiency of mechanically transplanted rice. Agric. Ecosyst. Environ. 269, 183–192. https://doi.org/10.1016/j.agee.2018.10.001.

Hussain, S., Peng, S., Fahad, S., Khaliq, A., Huang, J., Cui, K., Nie, L., 2015. Rice management interventions to mitigate greenhouse gas emissions: a review. Environ. Sci. Pollut. Res. 22, 3342–3360. https://doi.org/10.1007/s11356-014-3760-4.

INIA, 2018. Instituto nacional de Innovación agraria. PNIA en arroz. http://www.inia.gob.pe/programas/arroz, Accessed date: 1 March 2019.

IRRI, 2016. International rice research institute. http://irri.org/our-work/research/rice-and-the-environment, Accessed date: 1 January 2019.

Islam, M.Z., Sattar, M.A., Ashrafuzzaman, M., Saud, H.M., Uddin, M.K., 2012. Improvement of yield potential of rice through combined application of biofertilizer and chemical nitrogen. Afr. J. Microbiol. Res. 6, 745–750. http://doi: 10.5897/AJMR11.859.

Jetiyanon, K., Wittaya-Areekul, S., Plianbangchang, P., Lohitnavy, O., 2017. "Rhizoproduct", a biofertilizer containing spores of *Bacillus cereus* strain RS87 for early rice seedling enhancement and with potential for partial fertilizer substitution Songklanakarin. J. Sci. Technol. 39, 109–116. http://doi:10.14456/sjst-psu.2017.12.

Jha, P.N., Gomaa, A.B., Yanni, Y.G., El-Saadany, A.E.Y., Stedtfeld, T.M., Stedtfeld, R.D., Gantner, S., Chai, B., Cole, J., Hashsham, S.A., Dazzo, F.B., 2020. Alterations in the endophyte-enriched root-associated microbiome of rice receiving growth-promoting treatments of urea fertilizer and *Rhizobium* biofertilizer. Microb. Ecol. 79, 367–382. https://doi.org/10.1007/s00248-019-01406-7.

Kennedy, I.R., Choudhury, A.T.M.A., Kecskés, M.L., 2004. Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited? Soil Biol. Biochem. 36, 1229–1244. https://doi.org/10.1016/j.soilbio.2004.04.006.

Keswani, C., Sarma, B.K., Singh, H.B., 2016. Synthesis of policy support, quality control, and regulatory management of biopesticides in sustainable agriculture. In: singh, H., Sarma, B., Keswani, C. (Eds.), Agriculturally Important Microorganisms. Springer, Singapore, pp. 3–12. https://doi.org/10.1007/978-981-10-2576-1_1.

Khalil, M.I., Buegger, F., Schraml, M., Gutser, R., Richards, K.G., Schmidhalter, U., 2009. Gaseous nitrogen losses from a Cambisol cropped to spring wheat with urea sizes and placement depths. Soil Sci. Soc. Am. J. 73, 1335–1344. http://doi:10.2136/sssaj2008.0153.

Khan, H.I., 2018. Appraisal of biofertilizers in rice: to supplement inorganic chemical fertilizer. Rice Sci. 25, 357–362. https://doi.org/10.1016/j.rsci.2018.10.006.

Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120. https://doi.org/10.1007/BF01731581.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874. https://doi.org/10.1093/molbev/msw054.

Lima, J.V.L., Martins, S.C.S., de Siqueira, K.A., Soares, M.A., Martins, C.M., 2017. Characterization of actinobacteria from the semiarid region, and their antagonistic effect on strains of rhizobia. Afr. J. Biotechnol. 16, 499–507. https://DOI: 10.5897/AJB2016.15724.

Martínez-Racines, C.P., Cuevas-Pérez, F.E., Medina, L.M., 1989. Evaluación de la calidad culinaria y molinera del arroz. CIAT. Cali-Colombia, pp. 73.

Mia, M.B., Shamsuddin, Z.H., Mahmood, M., 2012. Effects of rhizobia and plant growth promoting bacteria inoculation on germination and seedling vigor of lowland rice. Afr. J. Biotechnol. 11, 3758–3765. https://doi.org/10.5897/AJB09.1337.

MINAGRI - SIEA, 2018. Sistema Integrado de Estadísticas Agrarias. http://siea.minagri.gob.pe/siea/sites/default/files/nota-coyuntura-arroz-280818_2.pdf, Accessed date: 1 December 2018.

Mustafa, A., Imran, M., Ashraf, M., Mahmood, K., 2018. Perspectives of using L-tryptophan for improving productivity of agricultural crops: a review. Pedosphere 28, 16–34. https://doi.org/10.1016/S1002-0160(18)60002-5.

Nguyen, T.H., Phan, T.C., Choudhury, A.T., Rose, M.T., Deaker, R.J., Kennedy, I.R., 2017. BioGro: a Plant Growth-Promoting Biofertilizer validated by 15 Years' research from laboratory selection to rice farmer's fields of the Mekong delta. In: Singh, J., Seneviratne, G. (Eds.), Agro-Environmental Sustainability. Springer, Cham, pp. 237–254. https://doi.org/10.1007/978-3-319-49724-2_11.

Olanrewaju, O.S., Glick, B.R., Babalola, O.O., 2017. Mechanisms of action of plant growth promoting bacteria. World J. Microbiol. Biotechnol. 33, 197. https://doi:10.1007/s11274-017-2364-9.

Oszvald, M., Tömösközi, S., Larroque, O., Keresztenyi, E., Tamás, L., Békés, F., 2008. Characterization of rice storage proteins by SE-HPLC and micro z-arm mixer. J. Cereal. Sci. 48, 68–76. https://doi.org/10.1016/j.jcs.2007.08.002.

Othman, R., Panhwar, Q.A., 2014. Phosphate-Solubilizing Bacteria improves nutrient uptake in aerobic rice. In: Khan, M., Zaidi, A., Musarrat, J. (Eds.), Phosphate Solubilizing Microorganisms. Springer, Cham, pp. 207–224. https://doi.org/10.1007/978-3-319-08216-5_9.

Park, M., Kim, C., Yang, J., Lee, H., Shin, W., Kim, S., Sa, T., 2005. Isolation and characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of Korea. Microbiol. Res. 160, 127–133. https://doi.org/10.1016/j.micres.2004.10.003.

Pérez-Miranda, S., Cabirol, N., George-Téllez, R., Zamudio-Rivera, L.S., Fernández, F.J., 2007. O-CAS, a fast and universal method for siderophore detection. J. Microbiol. Methods 70, 127–131. https://doi.org/10.1016/j.mimet.2007.03.023.

Prasad, M., Srinivasan, R., Chaudhary, M., Choudhary, M., Jat, L.K., 2019. Plant growth promoting rhizobacteria (PGPR) for sustainable agriculture: perspectives and challenges. In: Singh, A.K., Kumar, A., Sing, P.K. (Eds.), PGPR Amelioration in Sustainable Agriculture. Woodhead Publishing, pp. 129–157. https://doi.org/10.1016/B978-0-12-815879-1.00007-0.

Prasertsincharoen, N., Constantinoiu, C., Gardiner, C., Warner, J., Elliman, J., 2015. Effect of root colonization of domestic rice (*Oryza sativa* L. cv Amaroo) by *Burkholderia pseudomallei*. Appl. Environ. Microbiol AEM-00317. https://doi:10.1128/AEM.00317-15.

Price, E.P., Sarovich, D.S., Webb, J.R., Hall, C.M., Jaramillo, S.A., Sahl, J.W., Kaestly, M., Mayo, M., Harrington, G., Baker, A.L., Sidak-Loftis, L.C., Settlis, E.W., Lummis, M., Shupp, J.M., Gillece, J.D., Tuanyok, A., Warner, J., Busch, J.D., Keim, P., Currie, B.J., Wagner, D.M., 2017. Phylogeographic, genomic, and meropenem susceptibility analysis of *Burkholderia ubonensis*. PLoS Neglected Trop. Dis. 11, 1–18. https://doi.org/10.1371/journal.pntd.0005928.

Punschke, K., Mayans, M., 2011. Selección de cepas de *Herbaspirillum* spp. promotoras del crecimiento de arroz. Agroc. Urug. 15, 19–26. Available in: http://www.scielo.edu.uy/pdf/agro/v15n1/v15n1a03.pdf.

Reis, V.M., Estrada-de-Los-Santos, P., Tenório-Salgado, S., Vogel, J., Stoffels, M., Guyon, S., Mavingui, P., Baldani, V.L.D., Schmid, M., Baldani, J.I., Balandreau, J., Hartmann, A., Caballero-Mellado, J., 2004. *Burkholderia tropica* sp. nov., a novel nitrogen-fixing, plant associated bacterium. Int. J. Syst. Evol. Microbiol. 54, 2155–2162. https://doi.org/10.1099/ijs.0.02879-0.

Rodrigues, E.P., Rodrigues, L.S., de Oliveira, A.L.M., Baldani, V.L.D., dos Santos Teixeira, K.R., Urquiaga, S., Reis, V.M., 2008. *Azospirillum amazonense* inoculation: effects on growth, yield and N_2 fixation of rice (*Oryza sativa* L.). Plant Soil 302, 249–261. https://doi.org/10.1007/s11104-007-9476-1.

Sahu, A., Bhattacharjya, S., Mandal, A., Thakur, J.K., Atoliya, N., Sahu, N., Manna, M.C., Patra, A.K., 2018. Microbes: a sustainable approach for enhancing nutrient availability in agricultural soils. In: Meena, V. (Ed.), Role of Rhizospheric Microbes in Soil. Springer, Singapore, pp. 47–75. https://doi.org/10.1007/978-981-13-0044-8_2.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetics trees. Mol. Biol. Evol. 4, 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454.

Sandanakirouchenane, A., Haque, E., Geetha, T., 2017. Recent studies on N_2 fixing *Burkholderia* isolates as a biofertilizer for the sustainable agriculture. Int. J. Curr. Microbiol. App. Sci. 6, 2780–2796. https://doi.org/10.20546/ijcmas.2017.611.329.

Sarathambal, C., Ilamurugu, K., Balachandar, D., Chinnadurai, C., Gharde, Y., 2015. Characterization and crop production efficiency of diazotrophic isolates from the rhizosphere of semi-arid tropical grasses of India. Appl. Soil Ecol. 87, 1–10. https://doi.org/10.1016/j.apsoil.2014.11.004.

Sayyed, R.Z., Badgujar, M.D., Sonawane, H.M., Mhaske, M.M., Chincholkar, S.B., 2005. Production of microbial iron chelators (siderophores) by fluorescent Pseudomonads. Indian J. Biotechnol. 4, 486–490. http://nopr.niscair.res.in/handle/123456789/5776.

Schwyn, B., Neilands, J.B., 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 160, 47–56. https://doi.org/10.1016/0003-2697(87)90612-9.

Sekhar, A.C., Thomas, P., 2015. Isolation and identification of shoot-tip associated endophytic bacteria from banana cv. Grand Naine and testing for antagonistic activity against *Fusarium oxysporum* f. sp. cubense. Am. J. Plant Sci. 6, 943. http://doi: 0.4236/ajps.2015.67101.

Sharma, A., Shankhdhar, D., Sharma, A., Shankhdhar, S.C., 2014. Growth promotion of the rice genotypes by PGPRs isolated from rice rhizosphere. J. Soil Sci. Plant Nutr. 14, 505–517. https://doi.org/10.4067/S0718-95162014005000040.

Shinjo, R., Uesaka, K., Ihara, K., Sakazaki, S., Yano, K., Kondo, M., Tanaka, A., 2018. Draft genome sequence of *Burkholderia vietnamiensis* strain RS1, a nitrogen-fixing endophyte isolated from sweet potato. Microbiol. Resour. Announc. 7 00820-18. http://doi: 10.1128/MRA.00820-18.

Singh, B., 2018. Are nitrogen fertilizers deleterious to soil health? Agronomy 8, 48. https://doi.org/10.3390/agronomy8040048.

Spaepen, S., Vanderleyden, J., 2011. Auxin and plant-microbe interactions. CSH Perspect. Biol. 3, 4. http://doi:10.1101/cshperspect.a001438.

Take-Tsaba, A.L., Juraimi, A.S., Yusop, M.R., Othman, R., Singh, A., 2018. Influence of biofertilizer on growth improvement of aerobic rice genotypes. J. Anim. Plant Sci. 28, 1774–1786. Available in: http://www.thejaps.org.pk/docs/v-28-06/30.pdf.

Talbi, C., Delgado, M.J., Girard, L., Ramírez-Trujillo, A., Caballero-Mellado, J., Bedmar, E.J., 2010. *Burkholderia phymatum* strains capable of nodulating *Phaseolus vulgaris* are present in Moroccan soils. Appl. Environ. Microbiol. 76, 4587–4591. https://doi.org/10.1128/AEM.02886-09.

Tan, K.Z., Radziah, O., Halimi, M.S., Khairuddin, A.R., Shamsuddin, Z.H., 2015. Assessment of plant growth-promoting rhizobacteria (PGPR) and rhizobia as multi-strain biofertilizer on growth and N_2 fixation of rice plant. Aust. J. Crop. Sci. 9, 1257–1264. 30 Dec 19. https://search.informit.com.au/documentSummary;dn=769564475884988;res=IELHSS>ISSN: 1835-2693.

Tang, S.Y., Hara, S., Melling, L., Goh, K.J., Hashidoko, Y., 2010. *Burkholderia vietnamiensis* isolated from root tissues of nipa palm (*Nypa fruticans*) in Sarawak, Malaysia, proved to be its major endophytic nitrogen-fixing bacterium. Biosci. Biotechnol. Biochem. 74, 1972–1975. https://doi.org/10.1271/bbb.100397.

Tchakounté, G.V.T., Berger, B., Patz, S., Fankem, H., Ruppel, S., 2018. Community structure and plant growth-promoting potential of cultivable bacteria isolated from Cameroon soil. Microbiol. Res. 214, 47–59. https://doi.org/10.1016/j.micres.2018.05.008.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

USDA, 2018. United States department agrary. http://www.ers.usda.gov/publications/rcs-rice-outlook.aspx, Accessed date: 1 December 2019.

Valdez-Nuñez, R.A., Ríos-Ruiz, W.F., Ormeño-Orrillo, E., Torres-Chávez, E.E., Torres-Delgado, J., 2020. Caracterización genética de bacterias endofíticas de arroz (*Oryza sativa* L.) con actividad antimicrobiana contra *Burkholderia glumae*. Revista Argentina de Microbiologia (in press). https://doi.org/10.1016/j.ram.2019.12.002.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., Lane, D.J., 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173, 697–703. https://doi.org/10.1128/jb.173.2.697-703.1991.

Wilson, P.W., Knight, S.G., 1952. Experiments in Bacterial Physiology. Burgess Publishing Co., Minneapolis, Minnesota. USA, pp. 49p.

Win, K.T., Okazaki, K., Ookawa, T., Yokoyama, T., Ohwaki, Y., 2019. Influence of rice-husk biochar and *Bacillus pumilus* strain TUAT-1 on yield, biomass production, and nutrient uptake in two forage rice genotypes. PloS One 14, e0220236. https://doi.org/10.1371/journal.pone.0220236.

Yadav, J., Verma, J.P., Jaiswal, D.K., Kumar, A., 2014. Evaluation of PGPR and different concentration of phosphorus level on plant growth, yield and nutrient content of rice (*Oryza sativa*). Ecol. Eng. 62, 123–128. https://doi.org/10.1016/j.ecoleng.2013.10.013.

Yanni, Y.G., Dazzo, F.B., 2010. Enhancement of rice production using endophytic strains of *Rhizobium leguminosarum* bv. trifolii in extensive field inoculation trials within the Egypt Nile delta. Plant Soil 336, 129–142. https://doi.org/10.1007/s11104-010-0454-7.

Yoon, S.H., Há, S.M., Kwon, S., Lim, J., Kim, Y., Seo, H., Chun, J., 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int. J. Syst. Evol. Microbiol. 67, 1613–1617. https://doi.org/10.1099/ijsem.0.001755.

Yu, Y.Y., Jiang, C.H., Wang, C., Chen, L.J., Li, H.Y., Xu, Q., Guo, J.H., 2017. An improved strategy for stable biocontrol agents selecting to control rice sheath blight caused by *Rhizoctonia solani*. Microbiol. Res. 203, 1–9. https://doi.org/10.1016/j.micres.2017.05.006.

Zhao, L., Xu, Y., Lai, X., 2018. Antagonistic endophytic bacteria associated with nodules of soybean (*Glycine max* L.) and plant growth-promoting properties. Braz. J. Microbiol. 49, 269–278. https://doi.org/10.1016/j.bjm.2017.06.007.