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Bioprospecting Of Endophytic Bacteria With Plant Growth Promoting Activity Associated With Rice Varieties From The Colombian Caribbean

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Abstract

Endophytic bacteria have been studied for carrying out important processes within plant tissues. Among the diverse processes are plant growth-promoting activities such as nitrogen fixation, phosphate solubilisation and indole-3-acetic acid (IAA) production. The aim of this study was to evaluate in vitro the efficiency of rice tissue-associated endophytic bacteria with potential activity for nitrogen fixation, indole acetic acid production and phosphate solubilization. The population density of endophytic bacteria was shown from highest to lowest as follows: FMocarí $8.25x10^9$, F473 $7.30\ x10^9$, F2000 $9.15x10^8$, F733 $8.25x10^8$ CFU/g tissue. The root tissue in all the varieties under study was the most colonised. From the 16S rRNA gene sequences, Burkholderia cepacia, B. diazotrophica, B. tropica, Pseudomona fluorescens, Bacillus pumilis, B. thuringiensis and Herbaspirillum rubrisubalbicans were identified. All species had the ability to solubilise phosphate, fix nitrogen and produce IAA. Endophytic bacteria with plant growth promoting activity play an important role on plant growth and development. In this study, Burkholderia cepacia, B. diazotrophica, B. tropica, Pseudomona fluorescens, Bacillus pumilis, B. thuringiensis and Herbaspirillum rubrisubalbicans promoted plant growth in vitro. This makes them an excellent alternative for the application to soils with low fertility and to increase the production of crops of economic interest.

Keywords**:** indole-3-acetic acid, Rice, Endophytic bacteria, Nitrogen fixation.

Introduction

The agricultural sector in Colombia and in many countries is characterised by the trend towards monoculture where intensive cropping systems require a high demand for mineral nutrients and biomass production is mainly determined by the demand for nitrogen, on which in turn the demand for other macronutrients depends (Jethva et al., 2019; Sietz et al., 2021; Demirdogen et al., 2022). Nitrogen is considered the main nutritional constraint in crops, because its source in the soil is organic matter which mineralises only 1-3% of total nitrogen per year (Thapa et al., 2018; Gaimaro et al., 2022). Additionally, another important nutrient in development is phosphorus, which is found in the soil as a soluble form in low concentrations (5-30 mg/Kg^{-1}), where its content in the soil can vary until depletion, especially those soils that are acidic, where the need arises to correct phosphorus deficiency to obtain a response to nitrogen (Chen et al., 2020; Bai et al., 2022). Due to the nutritional requirements of plants and the nutritional exhaustion of the soil by intensive cultivation techniques, the constant use of chemical fertilisers is necessary to provide the soil with the nutrients needed for optimal crop development (Han et al., 2021; Shao et al., 2022). However, despite the fact that the use of fertilisers has yielded excellent results in terms of production yields, it is inevitable to deny the environmental problems caused by their indiscriminate use, especially in developing countries such as Colombia (Tian et al., 2022).

It is known that, in the use of nitrogen fertilisers, more than 75% of nitrogen is lost through leaching, due to the fact that they are highly soluble in water, which causes processes of eutrophication of water sources that lead to alterations in the composition, structure and dynamics due to the excess of nutrients that allow an alteration in the trophic chain of aquatic ecosystems and, by default, an environmental imbalance (Shuquin & Fang, 2018). Agriculture is held responsible for contributing 70-80% of the amount of nitrous oxide emitted into the environment, and it has been determined that 70% of nitrous oxide comes from the soil (Wu et al., 2018). Likewise, the misuse of nitrogen-based chemical fertilisers increases pollution because more than 50% of the fertiliser is lost through volatilisation (Zheng et al., 2019). In turn, phosphorus-based fertilisers react with elements such as calcium, iron or aluminium causing their precipitation and immobilisation, thus decreasing their availability to plants (Xiao et al., 2018; Yu et al., 2019; Liu et al., 2022).

One of the base crops of the agricultural sector economy in Colombia is rice, which globally ranks third after wheat and maize (Cadena et al., 2021). It is considered one of the most important foods for the basic diet

of thousands of people, it is also true that its production requires for its optimal performance the use of high amounts of chemical fertilisers (Adhikari et al., 2018), although they achieve the expected purpose, they generate a series of environmental problems (Wu et al., 2018). In response to this problem, research has recently increased on the search for more environmentally friendly mechanisms that can metabolise nitrogen and phosphorus into plant-assimilable components (Montes et al., 2022).

One of the alternatives to reduce the application of chemical fertilisers to crops is the application of endophytic bacteria, which are associated with different plant tissues without causing any type of disease (Khan et al., 2020). They are considered an important biotechnological tool to improve crop yields through plant growth promoting capacity by phosphate solubilisation, siderophore production, nitrogen fixation and ACC deaminase production (Rho et al., 2018; Krishnamoorthy et al., 2020; Matsumoto et al., 2021; Wang et al., 2021). In turn, they produce secondary metabolites that inhibit pathogen growth and induce systemic resistance to their host (Álvarez et al., 2020; Ramos et al., 2020). Under this perspective, the aim of this study was to evaluate in vitro the efficiency of endophytic bacteria associated with rice tissues with potential activity to fix nitrogen, produce indole acetic acid and solubilise phosphate.

Materials and methods

1. **Sampling área.** Plant tissue samples were collected from rice crops of four commercial varieties from the La Victoria Research Centre of Fedearroz, Montería - Córdoba, with coordinates 8º 89' north latitude and 75º 49' west longitude, with respect to the Greenwich meridian; at an altitude of 20 m above sea level, average temperature of 28 ºC, 1,200 mm of annual rainfall and an average relative humidity of 80%, characteristic of a tropical dry forest.
2. **Isolation and quantification of endophytic bacteria.** A zig-zag sampling was carried out by collecting 4 complete rice plants of the varieties F473, F2000, FMocarí and F733. The samples were labelled with their respective variety and date of collection. The samples were stored and preserved in plastic boxes at 4 °C for transport to the Microbiological Research Laboratory of the University of Sucre and processed within 24 hours after collection. For the disinfection process, the methodology proposed by Cordero et al. (2010) was followed, which consists of washing the root, stem and leaf tissues previously cut to 1 cm. After the disinfection process, each tissue was

placed in a porcelain dish and macerated until a homogeneous mixture was obtained, which was then inoculated in 9 mL of peptone and left in agitation for 24 hours at a temperature of 34 °C. From the homogenate, serial dilutions were made (10^{-1} to 10^{-8} CFU/g tissue) which were seeded by diffusion on the surface of R2A agar and incubated at 28 °C for 72 hours, after which time direct colony counting was performed on a plate. During counting, colonies distinguishable in shape, surface appearance, colour and size were observed and selected. Selected morphotypes were purified and maintained on R2A agar for further analysis.

3. **In vitro evaluation of biological nitrogen fixation and phosphate solubilising activity of endophytic bacteria**

- **Biological nitrogen fixation.** For this, a direct surface seeding of the isolated bacteria was performed on selective Burk agar medium: 5 g $MgSO_4$, 20 g KH_2PO_4, 5 g K_2HPO_4, 3.25 g $CaSO_4$, 1.45 g $FeCl_3$, 0.253 g $NaMoO_4$, 1000 mL sterile distilled water (Park et al., 2005) lacking a nitrogen source as a nitrogen-fixing activity evaluator, which uses a combined carbon source to recover a larger number of microorganisms with possible nitrogen-fixing activity, selecting only those with the enzyme system that allows them to reduce atmospheric nitrogen and use it in their metabolism. The culture medium was incubated for 7 days at 30°C. The results were observed according to the growth of the bacteria in the medium. The morphotypes that showed qualitative nitrogen fixing activity, the amount of nitrogen fixed in the form of ammonium ion was determined using the Berthelot colorimetric method. This consisted of incubating the isolates in Burk's broth at 30°C for 72 hours at 150rpm. After this time, 2M KCl was added to the medium and incubated again under the same conditions for 1 hour. Next, 10 mL of supernatant were taken and centrifuged at 8000 rpm for 20 min. At the end of this time, an alcoholic solution of 10% phenol, 0.5% C_5N_6OFe and 1 mL of oxidant solution (20 g $Na_3C_6H_5O_7$, 1 g NaOH and 1 mL NaOH) was added to the medium, followed by an alcoholic solution of 10% phenol, 0.5% C_5N_6OFe and 1 mL of oxidant solution (20 g $Na_3C_6H_5O_7$, 1 g NaOH and 1 mL NaClO 1.5N in 100 mL H_2O), the solution plus the sample was incubated for 1 hour and then the results were analysed by absorbance reading at 632 nm in a Genesys 10S UV-Vis spectrophotometer. The standard curve used was the one standardised by the biotechnology laboratory of the University of Cordoba (Mantilla et al., 2007). Each isolate was evaluated in triplicate.
- **Phosphate solubilisation.** For preliminary assessment of phosphate solubilising activity, NBRID solid culture medium was used: 10 g

glucose, 5 g $Ca_3(PO_4)_2$, 5 g $MgCl_2$ * $6H_2O$, 0.25 g $MgSO_4$ * $7H_2O$, 0.2 g KCl, $(NH_4)SO_4$ in 1000 mL of distilled water (Dawwam et al., 2013). The appearance of clear halos around the colonies is considered as a positive indicator for phosphate solubilisation. Quantitative estimation for phosphate solubilisation was done using NBRIP culture medium enriched with tricalcium phosphate for 72 hours at 30°C at 150 rpm. After this time the samples were centrifuged at 8000rpm for 20 minutes. Then 1 mL of the supernatant was taken and resuspended in 7 mL of distilled water, 2 mL of Vaneate Molybdate reagent was added to this suspension and the samples were incubated for 30 minutes. After this time, absorbance readings were taken at 540 nm, the data were analysed using the standardised standard curve in the biotechnology laboratory of the University of Cordoba (Lara et al., 2013).

- **Indole acetic acid (IAA) production.** The indole acetic acid production capacity of the endophytic bacteria was evaluated using the liquid medium Burk Korea: 0.41 g KH_2PO_4, 0.52 g K_2HPO_4, 0.05 g Na_2SO_4, 0.2 g $CaCl_2$, 0.1 $MgSO_4$ * $7H_2O$, 0.01 g Fe SO_4 * $7H_2O$, 0.0025 g $NaMoO_4$, in 1000 mL of distilled water, and supplemented with 0.1 g tryptophan (indole acetic acid precursor). The optical density of the cultures was measured in order to have the same concentration of 10^6 CFU/g tissue, and then they were seeded in the medium described above and incubated at 150 rpm for 72 hours, after which time 1 mL of the bacterial suspension was taken and centrifuged at 12000 rpm for 5 min. Then an aliquot of the supernatant was taken and Salkowski's reagent (Dawwam et al., 2013) was added, incubated in the dark for 30 min, after which the absorbance of the sample was measured at 450nm. For the quantitative determination, the standardised curve was used using standard solutions of pure 3-indole acetic acid previously carried out by the biotechnology laboratory of the University of Cordoba (Lara et al., 2011), each isolate was evaluated in triplicate.

4. **DNA extraction from Endophytic Bacteria.** DNA extraction was performed according to the protocol described by Oliveira et al. (2013). Bacterial isolates were purified and activated in LB medium for 18 hours, after which time 1 mL of the bacterial suspension was taken and centrifuged at 12000 rpm to obtain a bacterial pellet; this pellet was resuspended in 0.5M EDTA and centrifuged at 12000 rmp for 20 minutes. The pellet was treated with lysis buffer (EDTA 0.5m and SDS 0.25%) and incubated at 60°C for 1 hour. After this time, 5M NaCl was added with incubation at 4°C for 5 minutes, and the samples were centrifuged at 12000rpm. The resulting suspension was added an

equal volume of phenol-chloroform-isoamyl alcohol 25:24:1 and centrifuged at 12000rpm. Half a volume of isopropanol was added to the suspension and incubated for 14 hours. Finally, the samples were added ethanol to dry the DNA and then resuspended in 0.5X TE buffer.

The 16S rRNA gene was amplified using 5 sets of specific oligonucleotides: FBLS342 and R1392, for the firmicutes class; F948β and R1492 for the beta-proteobacteria class; FD2 and RP1 for the gamma proteobacteria class; F243 and R1492 for the actinobacteria class; F203 and R1492 for the alpha proteobacteria class (Oliveira et al., 2013; Pandey et al., 2018). The amplified products were purified and sent for sequencing to Macrogen. The sequences obtained were compared with those stored in Genbank. Base alignment was performed in the Clustal W program, phylogenetic inferences were obtained by Neighbor Joining method based on the kimura-2-parameter model with bootstrap test 1,000 replicates with the MEGA X program.

5. **Statistical análisis.** Data were statistically analysed by one-way analysis of variance (ANOVA). Duncan's test was also applied to establish differences ($p<0.05$) between the population density of endophytic bacteria in relation to variety and colonised tissue. Likewise, with the plant growth promoting activity. The statistical programme Infostat student version was used and for the graphic edition the programme R 3.4.1.

Results

Endophytic bacteria population density

According to the results of the analysis of variance showed that there are significant statistical differences ($p<0.05$) between the population density of endophytic bacteria among the colonised varieties and tissue. The population density of endophytic bacteria ranged from 7. $9x10^8$ to $8.7x10^9$CFU/g tissue. The rice variety with the highest population density of endophytic bacteria was FMocarí $8.25x10^9$ CFU/g tissue, followed by F473 7.30 $x10^9$ CFU/g tissue, then followed by F2000 $9.15x10^8$ CFU/g tissue and finally F733 $8.25x10^8$ CFU/g tissue (figure 1).

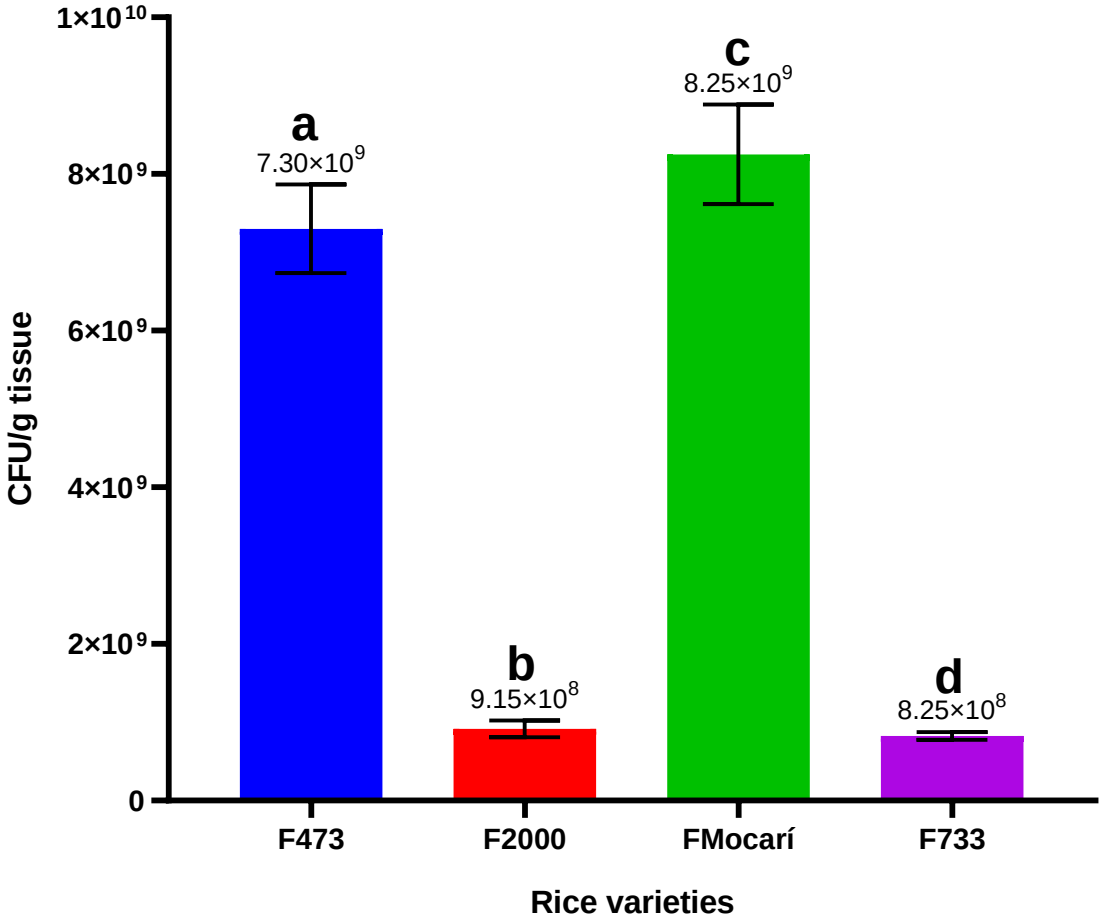


Figure 1. Population density of endophytic bacteria (CFU/g tissue) in the different rice varieties planted in the Colombian Caribbean. Means with a common letter are not significantly different ($p > 0.05$).

The Duncan multiple range test to establish differences in the population density of endophytic bacteria present in the tissues of the rice varieties showed that there are significant statistical differences ($p<0.05$). The highest population density per tissue was $9.05x10^7$ CFU for the root of variety FMocarí, followed by variety F473 with a density of $8.55 x10^7$ CFU (figure 2). The variety that presented the highest population density in the stem tissue was F473 $3.45 x10^7$ CFU, followed by the variety FMocarí $3.10 x10^7$ CFU. While for the leaf tissue the variety F733 presented the highest population of bacteria with $3.95x10^6$ CFU, followed by the variety F2000 with 3.65 x106 CFU.

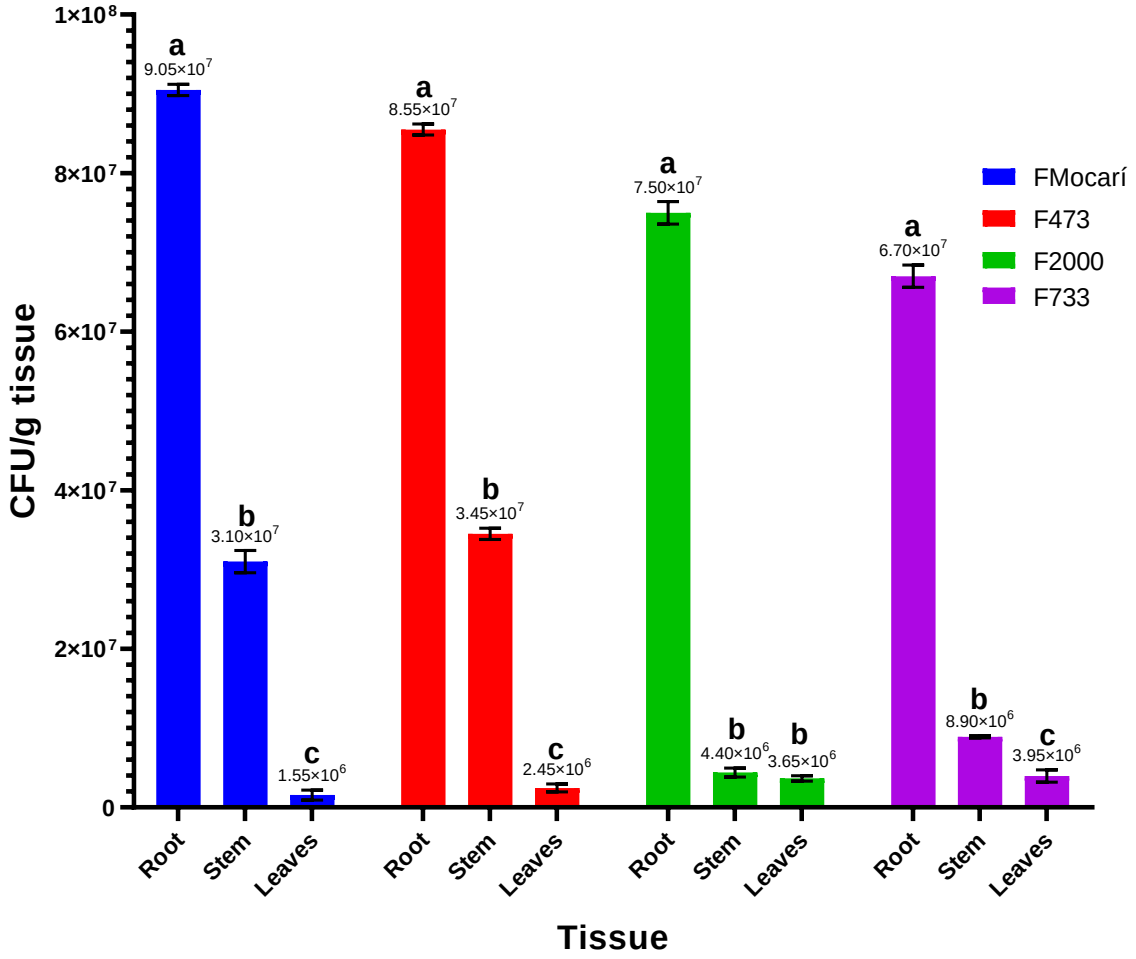


Figure 2. Population density of endophytic bacteria (CFU/g tissue) in the tissues of the different rice varieties. Means with a common letter are not significantly different ($p > 0.05$).

From the total population density of endophytic bacteria isolated from the different tissues of the rice varieties, colonies were selected according to shape and colour. For variety F473, 3 stem morphotypes (F4T1, F4T2 and F4T3), 2 leaf morphotypes (F4H2 and F4H4) and 1 root morphotype (F4R1) were isolated; for variety F2000, 2 root morphotypes (F2R1 and F2R2), 2 stem morphotypes (F2T1 and F2T2) and 1 leaf morphotype (F2H1) were isolated; variety 733 isolated 1 root morphotype (F7R1), 3 stem morphotypes (F7T1, F7T2 and F7T3) and 1 leaf morphotype (F7H1); finally, 1 isolated root morphotype (MOCR1), 1 isolated stem morphotype (MOCT2) and 2 leaf morphotypes (MOCH2, MOCH3, MOCH4) for variety FMocarí (figure 3).

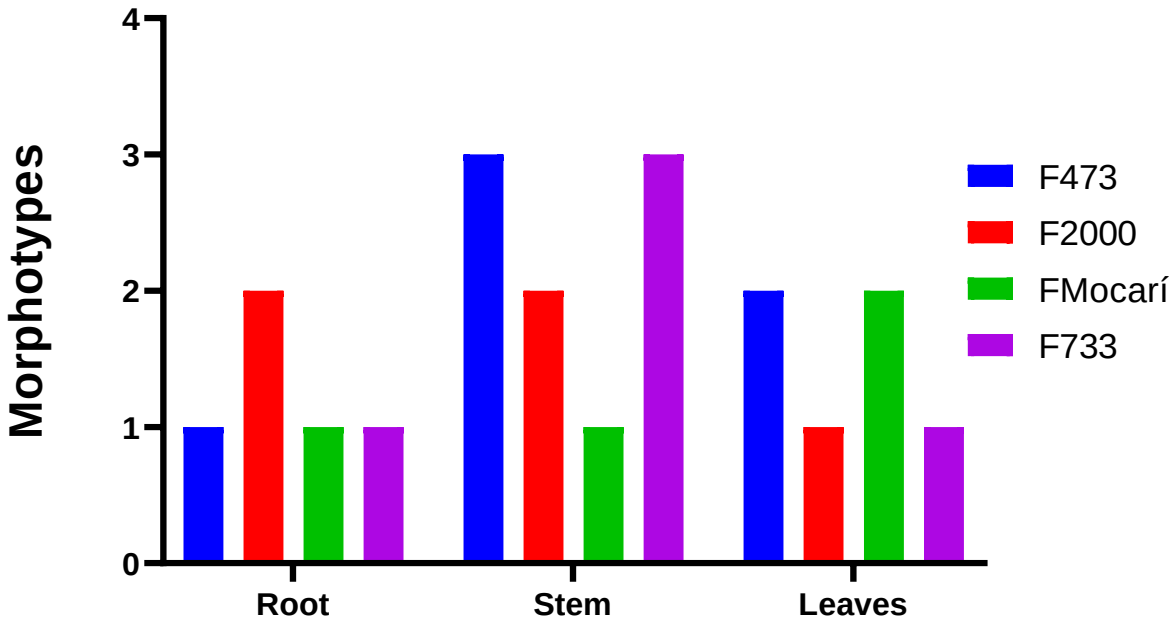


Figure 3. Number of assorted morphotypes of tissues of rice varieties

In vitro plant growth promotion of endophytic bacteria

Nitrogen fixation. The morphotypes isolated from the tissues of the rice varieties all had the ability to grow on Burk medium, indicating that this is a positive test for nitrogen fixation. In turn, quantitative nitrogen determination from ammonium ion indicated that all isolates belonging to different varieties showed ammonium ion production at concentrations higher than 0.2 mg/L. The root morphotype MOCR1 isolated from the FMocarí variety showed the best average ammonium ion production (2.2 mg/L), followed by the leaf morphotype MOCH3 from the same rice variety, with an average ammonium ion reduction of (1.9 mg/L) respectively (figure 4).

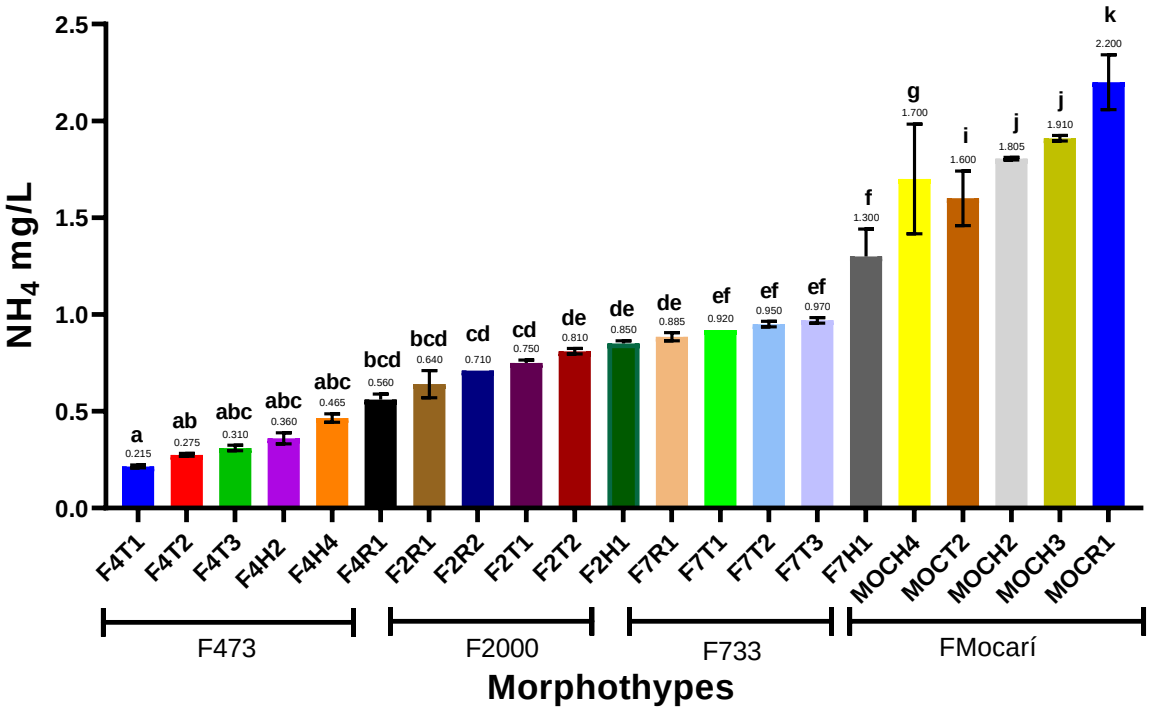


Figure 4. Concentration in mg/L of ammonium ion produced by endophytic bacteria isolated from rice varieties. Means with a common letter are not significantly different ($p > 0.05$).

Phosphate solubilisation Phosphate solubilisation results showed that all 21 morphotypes formed a transparent halo in NBRID culture medium, which is a positive result in solubilising phosphorus in vitro. All morphotypes showed significant statistical differences ($p<0.05$) in phosphorus solubilisation in NBRIP liquid medium. Morphotypes MOCR1 and MOCH3 of the FMocarí variety showed the best phosphate solubilisation averages of 1203 mg/L, pH 3.4 and 967 mg/L, pH 3.7 respectively (figure 5).

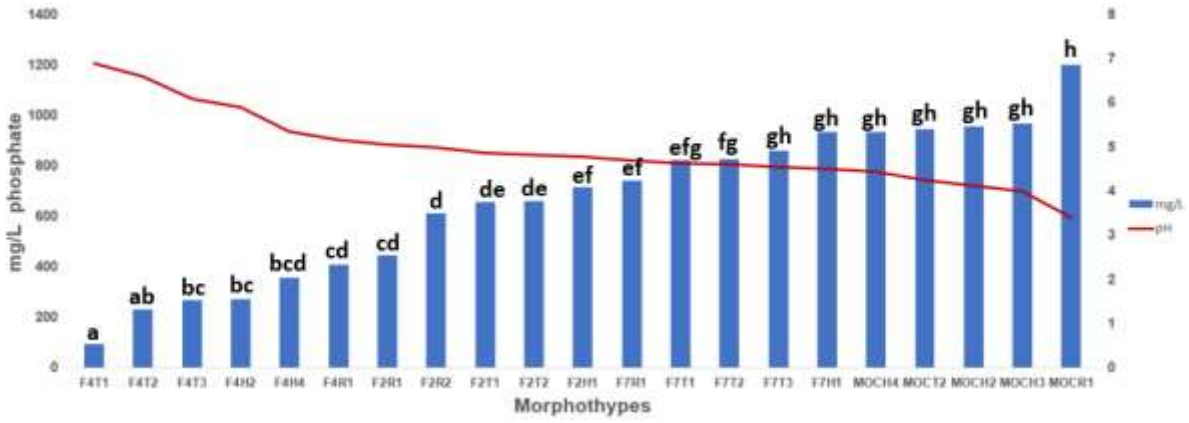


Figure 5. Concentration in mg/L of phosphorus solubilised by endophytic bacteria isolated from rice varieties. Means with a common letter are not significantly different ($p > 0.05$).

Indole acetic acid production. All 21 morphotypes isolated from the different rice varieties showed the capacity to produce indole acetic acid (IAA) and showed significant statistical differences ($p<0.05$) in terms of IAA production. Morphotypes F4T1 and F4H4 of variety F473 showed the highest average IAA production with 10.60 mg/L and 9.0 mg/L respectively (figure 6).

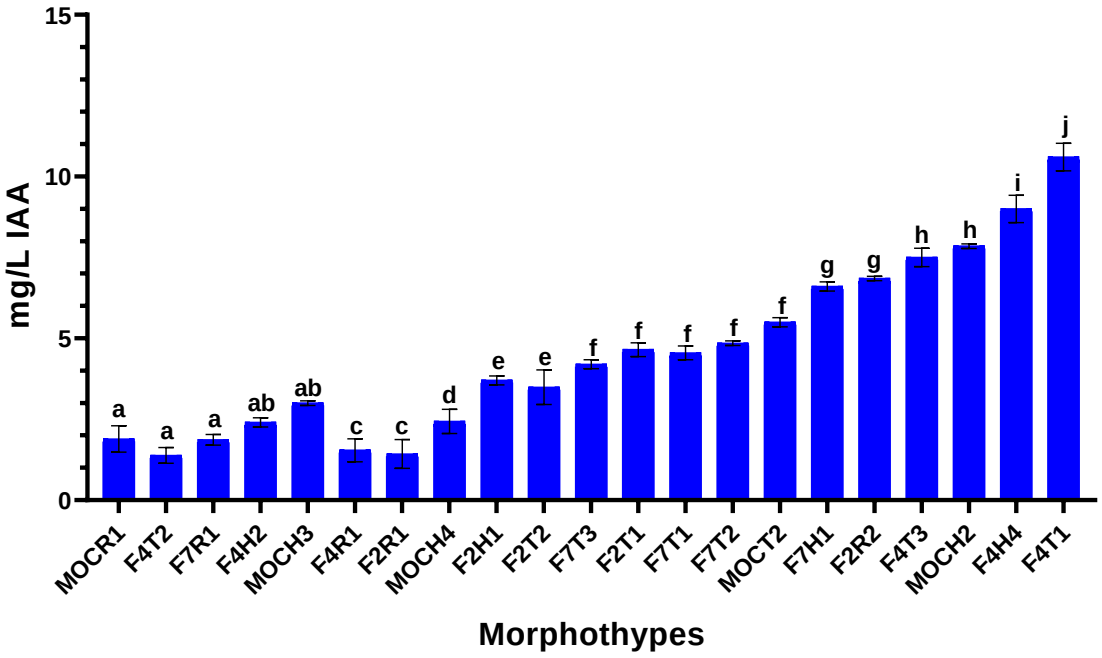
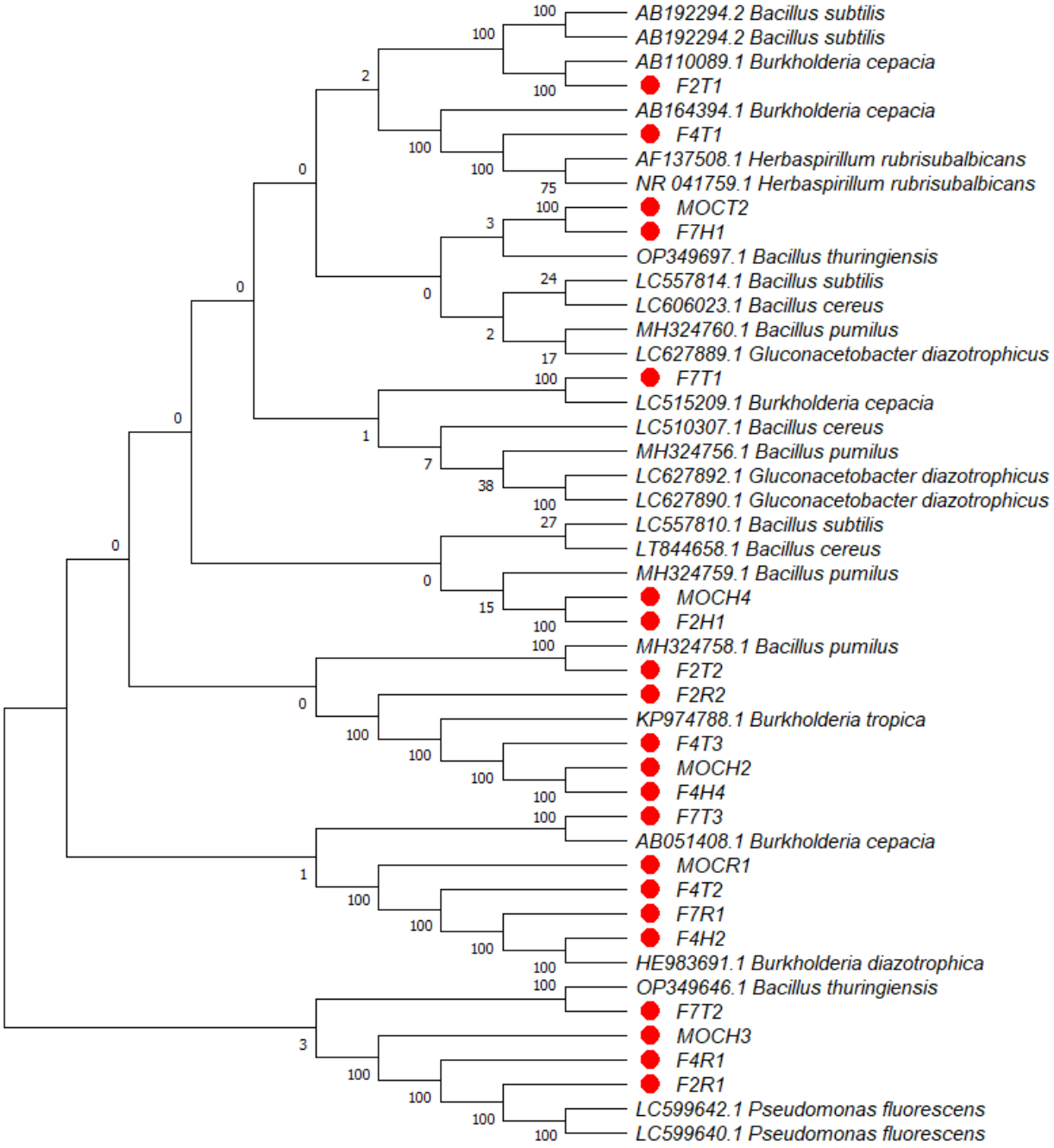


Figure 6. Concentration in mg/L of IAA produced by endophytic bacteria isolated from rice varieties. Means with a common letter are not significantly different ($p > 0.05$).

Molecular identification of endophytic bacteria. Phylogenetic analysis from the 16S rRNA gene for endophytic bacteria with plant growth promoting activity (figure 7) shows that the 21 morphotypes isolated from the tissues of the different rice varieties showed high similarity with the sequences stored in the GenBank database and identified as follows: Burkholderia cepacia (F7T3, F2T1, F7T1); B. diazotrophica (MOCR1, F4T2, F7R1, F4H2); B. tropica (F2R2, F4T3, MOCH2, F4H4); Pseudomona fluorescens (MOCH3, F4R1, F2R1); Bacillus pumilis (MOCH4, F2H1, F2T2);

B. thuringiensis (F7T2, MOCT2, F7H1); Herbaspirillum rubrisubalbicans



(F4T1).

Figure 7. Dendogram using the Neighbour-Joining model from 16S rRNA sequences of plant growth-promoting endophytic bacteria isolated from rice cultivars

Discussion

Density of endophytic bacteria in rice varieties. Plant-associated bacterial populations can be found both on the surface and inside plants. Most of these populations can colonise both internal and external plant tissue, making it difficult to strictly understand when a bacterium is an endophyte (Zhou et al., 2020; Haider et al., 2022). The population density of endophytic bacteria is highly variable, depending on the species of the bacteria and the genotype of the host plant, as well as the developmental stage of the plant, inoculum density and environmental conditions. Likewise, the population density of endophytic bacteria present in the different rice tissues is relatively low compared to the abundance of bacteria present in the rhizosphere (Hallman, et al., 1997), which shows that the most colonised tissue was the root in each of the varieties under study. In addition, the presence of endophytes is described for all plant tissues, with the highest number in the root, decreasing as it moves up the stem until it reaches the leaves and finally the fruit or inflorescences (Bacon & Hinton, 2007).

The main reason why there is a higher colonisation in the root as opposed to other plant organs may be due to the fact that roots are in intimate contact with the environment which harbours a myriad of microorganisms (Senthilkumar et al., 2011). Studies by Bacilio et al. (2001) indicate that their density is higher in the intercellular junction zone of the root epidermis, probably due to the space and the possibility of mobility offered by these regions, since the mucilaginous layer covering the epidermis has less tension in this area (Bowen, 1979). At the same time, the concentration of carbon as a source of energy is higher in this area, which favours the growth of these microorganisms (Bennett & Lynch, 1981). For example, studies conducted by Feng et al. (2017) showed that the most colonised tissue in the rice plant was the root with a population density of 6.80×10^4 CFU g^{-1}, which demonstrates the interaction between the root and the microbiota present in the soil (Sahoo et al., 2017). In relation to the results of this research, the F2000 variety presented a higher population density of endophytic bacteria ($9.15x10^8$ CFU/g tissue) compared to that reported by Barboza-García et al. (2022) where the same variety presented a population density of $1.4x10^4$ CFU/g tissue. In addition, the same study reports that the variety F473 presented a population density of $1.2x10^4$ CFU/g tissue, a low density compared to that found in this study of $7.30x10^8$ CFU/g tissue, respectively. This suggests that the density of endophytic bacteria can change in relation to environmental factors and the physiological development of the host (Ali et al., 2022; Pandey et al., 2022; Taheri et al., 2022).

It has been shown that the composition of the endophytic bacterial community varies according to the developmental stage of the plant and differs in different organs, indicating different modes of colonisation. However, most flowers and fruits have endophytes at low densities, ranging from 102 to 103 CFU/g fresh weight (Berg et al., 2005). In addition, studies by (Naik et al., 2009), in which populations of endophytic bacteria were isolated from 2400 rice segments collected from Southeast India at two times of the year. The rate of colonisation of disinfested tissues by bacteria varied with respect to time of year with 40.3% on roots and 25.83% on leaves during winter and 20.15% on roots and 8.66% on leaves during summer.

The density of endophytic bacteria established on the plant is prone to influences caused by the change in plant physiology. Therefore, it can be inferred that physiological changes in the plant, e.g. growth stage, soil type, influence bacterial density in a significant way (Hallmann & Berg, 2006; Singh & Dubey, 2018). For most endophytic bacteria, their ecological function within the host plant is unknown. However, endophytes can influence host physiology such as phytohormone production, modulation of ethylene levels, N_2 fixation, solubilisation of inorganic phosphate and siderophore production (Compant et al., 2010; Krishnamoorthy et al., 2020; Bahmani et al., 2021).

Molecular identification of endophytic bacteria with plant growth-promoting activity. Plant growth-promoting bacteria provide diverse benefits in agriculture due to their ability to improve crop production and protect their host against pathogens (Numan et al., 2018; Ramakrishna et al., 2019). Among the plant growth-promoting bacteria identified using the 16S rRNA gene is the genus Burkholderia. It represents one of the most common genera of endophytic bacteria in plants. They are considered a very versatile biotechnological tool due to their ability to produce volatile secondary metabolites that have the function of inhibiting pathogen growth. In addition, they induce the production of ACC deaminase, solubilise phosphates by releasing organic acids and enzymes such as phosphatases and release siderophores which prevent pathogen growth (Mendes et al., 2007; White et al., 2019; Pal at al., 2022). Pandey et al. (2005) isolated Burkholderia spp. from the root nodule Mimosa indica and determined that the bacterium has the ability to fix nitrogen producing IAA and siderophore production. Therefore, it was considered as a potent microorganism to promote plant growth. de Andrade et al. (2019) reported on the increase of strawberry biomass after inoculation of 3 bacterial strains Azospirillum brasilense, Burkholderia cepacia and Enterobacter cloacae. The authors report the

increase in biomass to IAA production and nitrogen fixation produced by the bacterial strains. Similarly, Bernabeu et al. (2015) demonstrated that B. tropica and B. diazotrophica had the ability to colonise tomato plants and induce growth effects in their host by releasing siderophores and phosphate solubilisation, which influenced the increase in biomass and fruit size. In turn, Reis et al. (2004) stated that B. tropica isolated from maize and sugarcane plants is able to fix nitrogen, solubilise phosphate and control the growth of pathogenic microorganisms by secondary metabolites. Schlemper et al. (2018) observed that the application of B. tropica together with inoculated Herbaspirillum rubrisubalbicans on sorghum plants increases the growth of the plant's root system, allowing it to absorb nutrients efficiently and in greater proportion for optimal development. The above mentioned studies support the results found in this research, since B. cepacia, B. tropica and B. diazotrophica showed good results in phosphate solubilisation, IAA production and nitrogen fixation, which demonstrates the great potential of these microorganisms in agriculture to increase the production of crops of economic interest and as an alternative to replace chemical fertilisers.

Pseudomona fluorescens is a rhizobacterium recognised for its beneficial effect on plant growth and against pathogens (Chin et al., 2022). The variety of mechanisms studied for the genus Pseudomona is very broad and ranges from interspecies competition for space or nutrients, nitrogen fixation, phosphate solubilisation and the production of secondary metabolites (Gouda et al., 2018). In a study by Mekureyaw et al. (2022) aimed at characterising the beneficial ability of P. fluorescens to improve tomato growth parameters and increase tolerance to drought stress. The authors report that inoculation of P. fluorescens increased plant biomass and these drought-stressed plants showed higher chlorophyll and abscisic acid content in leaves compared to non-inoculated controls. Root inoculation also increased the activity of different carbohydrate metabolism enzymes, which are important for root and leaf growth and development in drought-stressed plants. Similarly, Chavez et al, (2022), stated that P. fluorescens was able to increase the dry weight of maize seedlings by 38% to 66%; it increased germination from 20% to 51% in the number of germinated seeds, from 16% to 38% in the length of the aerial part of seedlings, from 30% to 140% in the length of seedling roots, from 15% to 32% in the fresh weight of seedlings and 21%-33% in the dry weight of seedlings and reduced the damage generated by Fusarium spp. by 75%-96%. the aetiological agent of Fusarium blight in maize. The combination of P. fluorescens, B. amyloliquefaciens and B. pumilus favoured an increase of 49.5%, 46% and 40% in the average root length of

banana compared to the control. The roots of the inoculated plants showed greater thickness, greater length and volume, abundant secondary roots. In particular, plants treated with P. fluorescens showed a larger root complex than non-inoculated plants, with more branches and secondary roots, and abundant growth in leaf area (Gamez et al., 2019).

On the other hand, many of the Bacillus spp. are known for their ability to biocontrol pathogens through the production of antibiotics and release of enzymes with the function of releasing phosphatases to solubilise phosphorus (Saxena et al., 2020). For example Bacillus pumilis, can control 81.50% of the stem rot disease caused by Sclerotinia sclerotiorum in cauliflower plants Kaushal et al. (2017). In addition, Iturin A release as an antifungal compound suppressed disease severity by 93% in greenhouse. It also exhibited the best plant growth promotion averages. Therefore, B. pumilis, is considered a potent strain for biological control application in agriculture. Pandey et al. (2019) carried out an isolation process of endophytic bacteria from chickpea. According to the results obtained P. aeruginosa and B. pumilis presented the maximum phosphate solubilization, which was in the range of 78-87.64 mg Soluble P/L. The microbial consortium improved the growth patterns like germination index, plant height, leaf area index, stem diameter and chlorophyll content by 50%, 100%, 63%, 185% and 63%, respectively, as compared to uninoculated chickpea plants. B. pumilis inoculated on rice has the ability to generate root enlargement and enhance carbohydrate metabolism and phenylpropanoid biosynthesis (Liu et al., 2020). in rice, which allows understanding that the consortium of bacterial strains generate a positive impact on plant growth.

B. thuringiensis has been reported as an endophytic bacterium of rice with the ability to fix nitrogen, produce siderophores, solubilise phosphate and produce IAA, which when applied can improve production in the agricultural sector (Dawwam et al., 2013; Djenane et al., 2017; Figueredo et al., 2022). Armada et al. (2016) evaluated the response of Lavandula dentata under drought conditions and inoculated in combination with mycorrhizae and B. thuringiensis. The mixture of mycorrhizae and endophytic bacteria increased plant biomass by producing IAA, ACC deaminase and solubilising phosphorus; they compensated for drought stress and reduced oxidative lipid damage. The authors claim that indigenous mycorrhizal species and in particular their mixture with B. thuringiensis demonstrated their potential to protect plants against drought and help them thrive in semi-arid ecosystems. Importantly, B. thuringiensis has the versatility to produce secondary

metabolites that can control soil-borne pathogens. In addition, it is characterised by Cry proteins, which are the virulence factor of the bacterium and are capable of controlling insect pests (De Bock et al., 2021; Rendon et al., 2021; Aynalem et al., 2022; Choe et al., 2022).

Bacteria belonging to the genus Herbaspirillum have been widely studied as nitrogen-fixing endophytes in economically important grass crops such as sugarcane, sorghum, maize and rice and have also been found to produce growth regulators (Senthilkumar et al., 2011). Herbaspirillum rubrisbalbicans is a beta-proteobacterium which in this study evidenced nitrogen fixation, phosphate solubilisation and IAA synthesis activities in the rice variety F473 isolated from the stem. This same bacterial species has been referenced in sugarcane stems as a nitrogen fixer (Serrato et al., 2010; Dos Santos et al., 2017). Currently, the species H. seropedicae, H. rubrisubalbicans and H. frisingense have been considered as N_2-fixing endophytes. These species are being used as bacterial inoculants in agriculture mainly in soils with low fertility, which have shown excellent results in several crops when applied directly to the soil. For this reason, the genus Herbaspirillum constitutes an important resource for designing ecological technologies for modern agricultural systems (Matteoli et al., 2020).

Conclusion

Endophytic bacteria with plant growth promoting activity play an important role on plant growth and development. In this study, Burkholderia cepacia, B. diazotrophica, B. tropica, Pseudomona fluorescens, Bacillus pumilis, B. thuringiensis and Herbaspirillum rubrisubalbicans showed activity to fix nitrogen, solubilise phosphate and produce IAA. This makes them an excellent alternative for the application to soils with low fertility. In addition, the combination of these can induce an increase in the yield and production of crops of economic interest. At the same time, they can replace the application of agrochemicals that to date are causing changes in the microbial diversity present in the soil and making pathogenic strains increasingly resistant.

Abbreviations

IAA: indole-3-acetic acid; **CFU:** Colony Forming Units; **RNA:** ribonucleic acid; **ANOVA:** Analysis of variance

5. Authorship contributions

All authors have jointly and equally contributed to the argumentation and writing of the manuscript.

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