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Original Article

Diversity of nitrogen-fixing and phosphorus-solubilizing bacteria associated with the rhizosphere of Andean maize in Ecuador

Diversidade de bactérias fixadoras de nitrogênio e solubilizadoras de fósforo associadas à rizosfera do milho andino, no Equador

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Abstract

A great diversity of microorganisms in the soil plays an important role in the sustainability of agricultural production systems. Among these microorganisms are bacteria that have the ability to fix atmospheric nitrogen or mineralize phosphorus, thus making it easily assimilable for plants. Maize is the main crop in the highlands of Ecuador (above 2000 meters) and it is predominantly traditional, using native seeds and very little or no agrochemicals. The National Institute of Agricultural Research (INIAP) has a collection of bacteria collected from the rhizosphere of maize in the highlands of Ecuador that has not been taxonomically identified. This research aimed to carry out a biochemical and genetic characterization to establish the identity of the collected nitrogen-fixing and phosphorus-solubilizing bacteria and to understand better the diversity of microorganisms present in the root biome of Andean maize. The hypothesis consisted of determining if there is a difference in the bacteria associated with the rhizosphere of maize in the Andean region of Ecuador compared with other regions. The bacteria underwent classical biochemical characterization based on catalase, oxidase, urease, sulfates, indole, sulfate-indole motility (SIM), and lactose, among others, and genetic identification by 16S rDNA ribosomal gene sequencing, PCR, and SANGER sequencing. A great diversity of microorganisms associated with the rhizosphere of the crop was found, including the genera *Agrobacterium, Bacillus, Stenotrophomonas, Acinetobacter, Brevundimonas, Pseudomonas,* and *Pseudoxanthomonas*. INIAP conserves these bacteria in a bank of microorganisms associated with crops of economic importance. They are useful for the development of biofertilizers that could contribute to a more sustainable agriculture in the region.

Keywords: soil, 16S rDNA, plant growth promoting bacteria (PGPR), microbiome, maize.

Resumo

Existe uma grande diversidade de microrganismos no solo que desempenham um papel importante na sustentabilidade dos sistemas de produção agrícola. Um grupo deles pertence a bactérias que fixam o nitrogênio atmosférico ou mineralizam o fósforo tornando-o assimilável pelas plantas. O milho é a principal cultura por extensão nas terras altas do Equador (altitude superior a 2.000 m acima do nível do mar) e quase todo o seu cultivo é feito de forma tradicional, com sementes nativas e com muito pouco ou nenhum uso de agroquímicos. O Instituto Nacional de Pesquisa Agropecuária (INIAP) possui uma coleção de bactérias coletadas da rizosfera do milho nas terras altas do Equador que não possuem uma identificação taxonômica conclusiva. O objetivo desta pesquisa foi realizar uma caracterização bioquímica e genética para identificar a identidade das bactérias fixadoras de nitrogênio e solubilizadoras de fósforo coletadas, a fim de conhecer e entender melhor a diversidade de microrganismos presentes no bioma raiz do milho andino. A hipótese consistia em determinar se existe diferença nas bactérias associadas à rizosfera do milho na região andina do Equador, em comparação com outras regiões. As bactérias passaram por uma caracterização bioquímica clássica baseada em catalase, oxidase, urease, sulfatos, indol, motilidade indol sulfato (SIM), lactose, entre outros, e identificação genética através do sequenciamento do gene ribossomal 16S rDNA, por PCR e sequenciamento SANGER. Uma grande diversidade de microrganismos associados à rizosfera da cultura foi encontrada, incluindo os gêneros *Agrobacterium*, *Bacillus*, *Stenotrophomonas*, *Acinetobacter*, *Brevundimonas*, *Pseudomonas* e *Pseudoxanthomonas*. Estas bactérias são conservadas pelo INIAP num banco de microrganismos associados a culturas de importância económica, sendo úteis para o desenvolvimento de biofertilizantes que possam contribuir para uma agricultura mais sustentável na região.

Palavras-chave: chão, 16S rDNA, bactérias promotoras de crescimento de plantas (PGPR), microbioma, milho.

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1. Introduction

The rhizosphere is a complex and dynamic microbial ecosystem surrounding plant roots. It has a significant impact on plant growth and development, as well as soil health and fertility. Microorganisms are involved in the decomposition and mineralization of organic matter and are essential for long-term soil sustainability (Arruda et al., 2013; Ikeda et al., 2019).

Bacterial diversity in the rhizosphere is of great interest due to its potential to improve agricultural production of important crops such as maize (*Zea mays*). Thus in Cuba (Hernandez et al., 2003; Pérez et al., 2020), China (Gao et al., 2004; Chen et al., 2021), Brazil (Arruda et al., 2013), Mexico (Amezquita et al., 2022), Uruguay (Montañez et al., 2009; Battistoni et al., 2023), Argentina (Anzuay et al., 2021), and Peru (León and Rojas, 2015), extensive colonization by bacteria of the genus *Bacillus, Pseudomonas, Stenotrophomonas, Enterobacter, Azospirillum, Azotobacter, Xanthomonas, Serratia, Burkholderia, Klebsiella Streptomyces, Pantoea,* and *Brevundimonas* was found. However, no studies of bacterial diversity associated with maize have been conducted in the high Andean region or in the "Sierra" where environmental conditions and crop management differ from that in the coast (at sea level).

In the highlands or "Sierra" of Ecuador maize is mainly grown by subsistence farmers using indigenous seeds and very little or no agrochemicals (Zambrano et al., 2021a). This contrasts with the "Litoral" or Coastal region, where most farmers use hybrid seeds with synthetic fertilizers and greater use of pesticides. On the other hand, the high Andean ecosystems have a high soil organic carbon content due to the volcanic origin and the cold prevailing climate, as they have low mineralization rates (Rojas et al., 2018).

The altitudinal gradient and soil temperature influences the organic carbon storage capacity of high Andean soils. As the altitude increases, the soil has a greater capacity to store organic carbon (Huamán-Carrión et al., 2021). The diversity of bacteria associated with the roots of maize plants may be influenced by factors such as carbon content, climate, and management conditions of the crop.

The maize rhizosphere is not simply a passive environment but a highly dynamic ecological niche where bacteria interact with plant roots and other soil microorganisms. These interactions can influence nutrient uptake, pathogen resistance, and abiotic stress tolerance, among other aspects critical for the health and yield of maize crops (Souza et al., 2015). The rhizosphere harbors microorganisms of agricultural interest, many of which are commercialized worldwide as biofertilizers (Zambrano et al., 2021b).

The National Institute of Agricultural Research (INIAP) of Ecuador, through the Maize Program, has a set of nitrogen-fixing and phosphorus-solubilizing bacteria collected in several provinces of the highlands that still need a conclusive taxonomic identification. However, the effect of inoculation of several of these isolates in maize has been evaluated in different environments, observing a significant increase in yield compared to the control without fertilization (Sangoquiza-Caiza et al., 2022a, b). These bacteria are an invaluable source for the development of biofertilizers that contribute to a sustainable agricultural system in the Andean region. Therefore, our study aimed to carry out a biochemical and molecular characterization of the collection of bacteria to know the diversity of species associated with the cultivation of Andean maize in Ecuador.

2. Materials and Methods

2.1. Collection

Samples of the rhizosphere (part of the soil near the roots of the plant) were taken from the plant at different points in the field at a depth of 15 cm using a soil auger. Maize plants were in the vegetative stage between V10 and VT (V10 = plant with 10 collar leaves, VT = male flowers or tassels are fully visible). Sampling was carried out in a zigzag pattern in farmer plots at several representative locations of maize production in the Ecuadorian highlands (Table 1 and Figure 1). The plots were chosen randomly in the provinces and locations with more production of maize. Approximately 2 kg of soil was collected per site, and the sample was homogenized by manual stirring. All sampling tools were disinfected with 70% ethanol before each sampling. Samples were placed in sterile Ziploc" bags, labeled, and stored in a cooler at 4°C for transport. The samples were taken to the Biofertilizers Laboratory of the Maize Program (PM) of the Santa Catalina Experimental Station (EESC) of INIAP to carry out the study.

2.2. Isolation of nitrogen-fixing bacteria (NFB)

Ten grams of homogenized rhizosphere soil was diluted in 90 mL of 0.1% peptone water. Then, 100 µl of each dilution (10-4) were seeded into tubes containing 9 mL of nitrogen-free semi-solid medium (NFB agar) and homogenized by shaking. The tubes were incubated at 30ºC for 8 days; those that changed the color of the medium (from green to blue and that presented a thick white veil between 0.5 and 1.5 mm below the surface of the medium) were selected; 100 µl of the white veil were

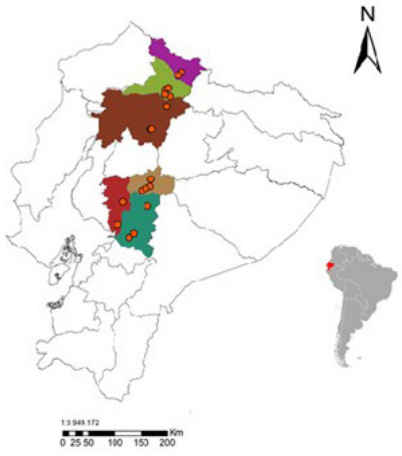

Figure 1. Map of the locations where sampling of bacteria associated with the rhizosphere of the Andean maize was carried out in the highlands of Ecuador.

Table 1. Sampling locations for the isolation of bacteria associated with the rhizosphere of the Andean maize in the highlands of Ecuador.

Province	District (Cantón)	Parish	Location	Altitude (m a.s.l.)	Average temperature (ºC)*
Carchi	Espejo	Mira	San Luis	2980	15
		San Isidro	San Joaquín	3043	13
Imbabura	Cotacahi	Quiroga	Cuicocha Pana	2393	16
		El Sagrario	Hacienda Tumibamba	2399	16
	Otavalo	San Pablo del Lago	San Pablo	2921	11
		Quinche	Pisabo	2568	16
	Antonio Ante	Atuntaqui	El Rosario	2351	17
Chimborazo	Riobamba	Quimiag	Guntuz	2911	13
	Alausí	Sibambe	Cochapamba	3008	13
		Tixan	Somisón	2933	13
Tungurahua	Mocha	Mocha	Mocha	2786	14
	Pelileo	Pelileo	El Tambo	2633	17
	Pillaro	Emilio Teran	El Capulicito	2725	16
	Quero	Quero	La Concepción	2817	15
Bolívar	Chillanes	Chillanes	Hacienda Pacay	2370	15
	Guaranda	Veintimilla	Laguacoto II	2608	13
Pichincha	Pedro Moncayo	La Esperanza	Cubinche	2712	15
	Quito	Amaguaña	Sección Oriental	2756	14
			Carapungo	2641	14

* Meteoblue, 2023.

taken and inoculated on the solid NFB agar at 30ºC for 7 days, as indicated by Espinoza (Espinoza, 2004).

2.3. Isolation of phosphorus solubilizing bacteria (PSB)

Twenty grams of soil was weighed and placed in 180 mL of sterile 0.85% NaCl saline solution. This dilution was shaken at 120 rpm for 15 minutes, and then serial dilutions to 10-6 were made in test tubes containing 9 mL of sterile distilled water. 100uL of the last dilution was seeded on KB and Pivoskaya (PVK) agar. The plates were incubated at 30ºC for 48 hours and the strains that showed fluorescence on KB agar and those that presented a transparent halo on PVK agar were isolated as indicated by Pincay (2014).

2.4. Phenotypic (biochemical) characterization

The strains were sown on Congo red agar and King B agar. They were incubated for 24 hours at a temperature of 30ºC. For Gram staining, each bacterial sample was placed on a slide, followed by fire fixation and staining according to the procedure described by Pincay (2014).

Nitrogen-fixing bacteria (NFB) underwent biochemical characterization based on catalase, oxidase, urease, sulfates, indole, Sulfate-Indole Motility (SIM), lactose, nitrate reduction, β-polyhydroxybutyrate staining, citrate tests, and glucose as carbon source, according to the procedures described by Gamazo et al. (2005). Phosphorus solubilizing bacteria (PSB) underwent biochemical tests: oxidase, TSI (Triple Sugar Iron), citrate, gelatin hydrolysis, nitrate, urease and nitrate reduction as indicated by Jean & Faddin (Jean and Faddin, 2003).

2.5. Molecular characterization

The strains (monocultures) were sent to the University of Guelph (Canada) to extract genetic material and sequence of the ribosomal DNA16S gene, commonly used as a molecular marker for the identification of microbial species. The bacteria were lyophilized at -17 C and maintained in the laboratory of the PM of the EESC. Two independent samples of each isolate were sent as replicates to ensure the integrity or purity of the isolates. The samples were prepared as follows:

1) A small aliquot of each lyophilized culture was suspended in 20 ul of molecular-grade water and pipetted into wells of a storage plate.
2) Plates were dried prior to shipment. Drying included: speedvac centrifuge, overnight at 37C. A visible sticky film developed at the bottom of each well; a pipet tip was used to verify that no liquid was left.
3) The plates were sealed with aluminum foil.

Primers 341F (CCTACGGGNGGCWGCAG) and 785R (GACTACHVGGGTATCTAATCC) were used to amplify the 16S rDNA ribosomal gene by PCR. The sequencing results (Sanger sequencing) of the amplified fragment were sent to the BOLD SYSTEM platform (Ratnasingham and Hebert, 2007). The DNA sequences obtained were edited using the Bioedit 7.0 program, and the bacteria were identified using

BLAST (NCBI, www.ncbi.nlm.nih.gov). The phylogenetic relationship between the isolates was generated with the MEGA version 7.0 software (Kumar et al., 2016) using the neighbor-joining method, Tamura 3-parameter model. *Nitrospira marina* JQ073799 was used as an external sequence (Aviles et al., 2022).

2.6. Conservation of isolates

The bacteria are lyophilized and stored at -17 ºC in the Biofertilizers Laboratory of the PM of the EESC of INIAP. The sequences are available on the BOLDSYSTEM platform (Ratnasingham and Hebert, 2007) of the University of Guelph, Canada, in the ECORN project.

3. Results

3.1. Isolation of NFB and PSB

From the 53 rhizosphere samples, 19 NFB isolates were obtained: 2 from Bolívar province, 5 from Tungurahua, 2 from Chimborazo, 2 from Pichincha, 3 from Carchi, and 5 from Imbabura (Table 2). The isolation and purification of NFB bacteria in Congo red agar tube medium showed morphologically identical colonies of circular shape, except for C3 with an irregular shape. The elevation of the colonies varied between pulvinate, umbonate, elevated and convex, red, orange-red, fuchsia and white. In PVK medium, the growth of a wide variety of bacterial colonies was observed, but very few showed halos of phosphorus solubilization. Twelve bacteria from the maize rhizosphere that can solubilize phosphorus *in vitro* were isolated; 4 from the province of Imbabura, 3 from Bolivar, 2 from Pichincha and 3 from Chimborazo (Table 3). The percentage of PSB was found in a range from 2 to 16% of the colonies. These colonies grown in King B media were small, convex, with regular edges, gelatinous consistency, and diffusible fluorescent with pigments. There was no correlation between the sampling location, temperature, and altitude with the genera found (data not shown).

3.2. Phenotypic (biochemical) characterization

The biochemical characterization of the isolates showed great metabolic diversity. The NFB C2, C4, C5, C6, C16, C18 and C20 were identified as Gram-positive bacilli, and

Table 2. Biochemical characterization of 19 isolates of nitrogen-fixing bacteria (BFN) isolated from the rhizosphere of the Andean maize.

Id sample	BOLD SYS Code	Gram Stain	Movility	Catalase	Oxidase	Urea	Sulfates	Indol	Lactose	Nitrate reduction	Poly hydroxybutyrate stain	Citrate	Glucose
C1	ECORN001	-	+	+	+	+	-	-	+	-	-	+	+
C2	ECORN002	+	+	+	+	+	-	-	-	+	-	+	+
C3	ECORN003	-	+	+	+	-	-	-	-	-	-	+	+
C4	ECORN004	+	+	+	+	+	-	-	-	+	-	+	+
C5	ECORN005	+	+	+	+	+	-	-	-	+	-	+	+
C6	ECORN006	+	+	+	+	+	-	-	-	+	-	+	+
C7	ECORN007	-	+	+	+	-	-	-	-	-	-	-	-
C8	ECORN008	-	+	+	+	-	-	-	-	-	-	-	-
C9	ECORN009	-	+	+	+	-	-	-	-	+	-	-	+
C11	ECORN011	-	+	+	+	-	-	-	-	-	-	-	+
C12	ECORN012	-	+	+	+	-	-	-	-	+	-	-	+
C13	ECORN013	-	+	+	+	+	-	-	-	-	-	+	+
C14	ECORN014	-	+	+	+	-	-	-	-	-	-	-	+
C15	ECORN015	-	+	+	+	-	-	-	-	-	-	-	-
C16	ECORN016	+	+	+	+	+	-	-	-	+	-	+	+
C17	ECORN017	-	+	+	+	-	-	-	-	-	-	-	+
C18	ECORN018	+	+	+	+	+	-	-	-	+	-	+	+
C19	ECORN019	-	+	+	+	-	-	-	-	-	-	-	+
C20	ECORN020	+	+	+	+	+	-	-	-	+	-	+	+

Table 3. Biochemical characterization of 12 isolates of phosphorus solubilizing bacteria (PSB) isolated from the rhizosphere of Andean maize.

Id sample	BOLD SYS Code	Gram Stain	Mobility	Catalase	Oxidase	TSI	Citrate	gelatin hydrolysis	Urea	Nitrate reduction
aI2	ECORN038	-	+	+	+	K/K	+	+	+	-
aI3	ECORN039	-	+	+	+	K/K	+	+	+	+
aI5	ECORN053	-	+	+	+	K/K	+	+	+	-
aI6	ECORN040	-	+	+	+	K/K	+	+	+	+
aC1	ECORN047	-	+	+	+	K/K	+	+	+	+
aC2	ECORN044	-	+	+	-	K/K	+	-	-	-
aC4	ECORN048	-	-	+	-	K/K	+	-	-	-
aP1	ECORN045	-	+	+	+	K/K	+	+	+	+
aP2	ECORN046	-	+	+	+	K/K	+	+	+	+
aB1	ECORN055	-	+	+	+	K/K	+	+	+	+
aB4	ECORN056	-	+	+	+	K/K	+	+	+	+
aB9	ECORN054	-	+	+	+	K/K	+	+	+	-

the rest of the strains, especially the PSB, were Gram-negative with active motility, positive reaction to catalase, oxidase tests, urea and gelatin hydrolysis (Tables 2 and 3). Similar results were obtained by Hernandez et al. (2003) in different Cuban soils associated with maize cultivation in tropical and warm areas.

3.3. Molecular characterization

Sequencing determined that the isolates were pure due to the high quality of the sequence and the congruence between the two replicates. Sequences between 327 and 428 nucleotides long were obtained. The BLAST analysis showed the diversity in families and genera of the bacteria found (Table 4). Figure 2 shows in percentage the diversity of families and genera of the isolates in the maize rhizosphere, which indicates that the highest proportion corresponds to *Pseudomonadaceae* family with 31%, followed by the *Bacillaceae* with 27% and finally, 24% of *Xanthomonadaceae*.

The dendrogram resulting from the phylogenetic analysis showed six main clades (I, II, III, IV, V and VI). Representatives of the genus Bacillus occupied the first of them,; the second clade presented strains of the genera *Stenotropgomonas* and *Pseudoxanthomonas*; the third corresponded to bacteria of the genus Acinetobacter; the fourth clade included bacteria of the genus *Pseudomonas*; the fifth clade to *Agrobacterium* and the sixth clade to *Brevundimonas* (Figure 3).

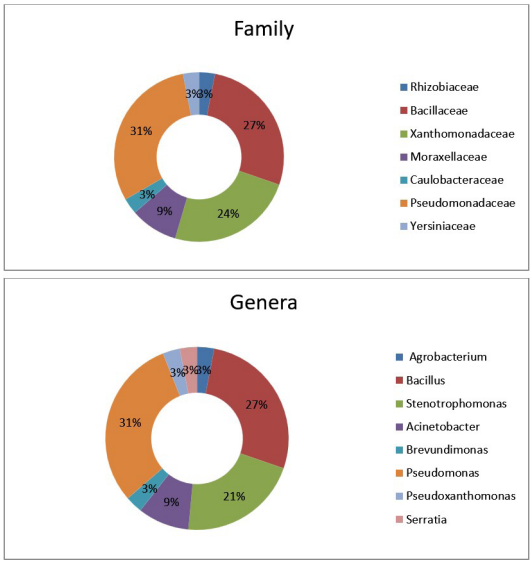

Figure 2. Diversity of families and genera of bacteria isolated from the rhizosphere of Andean maize in the highlands of Ecuador.

4. Discussion

Samples C2, C4, C5, C6, C16, C18, and C20 were identified as *Bacillus subtilis*. These bacteria are Gram-positive, rod-shaped, catalase positive, with the ability to form endospores, which allows them to resist heat, UV light, and different pH in the soil (Corrales-Ramírez et al., 2017; Rojas et al., 2016). They are considered growth promoters (PGPR) and biological control agents (BCAs), due to the production of organic compounds, antibiotics, phosphate solubilization and biological nitrogen fixation (Meena et al., 2016); they are very abundant in the rhizosphere (Saharan and Nehra, 2011; Zahid et al., 2015).

The samples aI2, aI5, aI6, aB1, aB4, aB9, and aP1 were identified as *Pseudomonas* sp., due to the sequences similarity with species of this genus. They are free-living saprophytes in the soil or water, capable of using a wide variety of organic compounds as a substrate to grow (Hernandez et al., 2003). They are Gram-negative bacilli, and their importance lies in their ability to produce a beneficial effect on plants, either as PGPR or BCAs (Fgaier and Eberl, 2010; Olanrewaju and Babalola, 2019) due to the synthesis of phytohormones, vitamins, siderophores,

Table 4. Results of local similarity analysis between sequences (BLAST) of the 16S rDNA gene of bacteria isolated from the rhizosphere of Andean maize in the highlands of Ecuador.

Id sample	BOLD SYS Code	Identification 16S DNA		
		Genetic Identity	Identity (%)	Coverage (%)
C1	ECORN001	*Agrobacterium sp*	100	100
C2	ECORN002	*Bacillus subtilis*	100	100
C3	ECORN003	*Stenotrophomonas sp*	100	100
C4	ECORN004	*Bacillus subtilis*	100	100
C5	ECORN005	*Bacillus subtilis*	100	100
C6	ECORN006	*Bacillus subtilis*	100	100
C7	ECORN007	*Acinetobacter lwoffii*	100	100
C8	ECORN008	*Brevundimonas sp*	100	100
C9	ECORN009	*Stenotrophomonas maltophilia*	100	100
C11	ECORN011	*Stenotrophomonas rhizophila*	100	100
C12	ECORN012	*Stenotrophomonas maltophilia*	100	100
C13	ECORN013	*Pseudomonas baetica*	100	100
C14	ECORN014	*Stenotrophomonas maltophilia*	100	100
C15	ECORN015	*Pseudoxanthomonas sp.*	100	100
C16	ECORN016	*Bacillus subtilis*	100	100
C17	ECORN017	*Stenotrophomonas maltophilia*	100	100
C18	ECORN018	*Bacillus subtilis*	100	100
C19	ECORN019	*Stenotrophomonas sp*	100	100
C20	ECORN020	*Bacillus subtilis*	100	100
aI2	ECORN038	*Pseudomonas sp.*	100	100
aI3	ECORN039	*Pseudomonas palleroniana*	100	99.5
aI5	ECORN053	*Pseudomonas sp.*	99	100
aI6	ECORN040	*Pseudomonas sp*	100	100
aB1	ECORN055	*Pseudomonas sp.*	100	100
aB4	ECORN056	*Pseudomonas sp.*	100	100
aB9	ECORN054	*Pseudomonas sp.*	100	100
aP1	ECORN045	*Pseudomonas sp.*	100	99.8
aP2	ECORN046	*Serratia sp*	100	100
aC1	ECORN047	*Pseudomonas fluorescens*	100	100
aC2	ECORN044	*Acinetobacter calcoaceticus*	100	100
aC4	ECORN048	*Acinetobacter calcoaceticus*	100	100
21C1	ECORN028	*Bacillus sp*	100	100
21C2	ECORN030	*Bacillus sp*	100	100

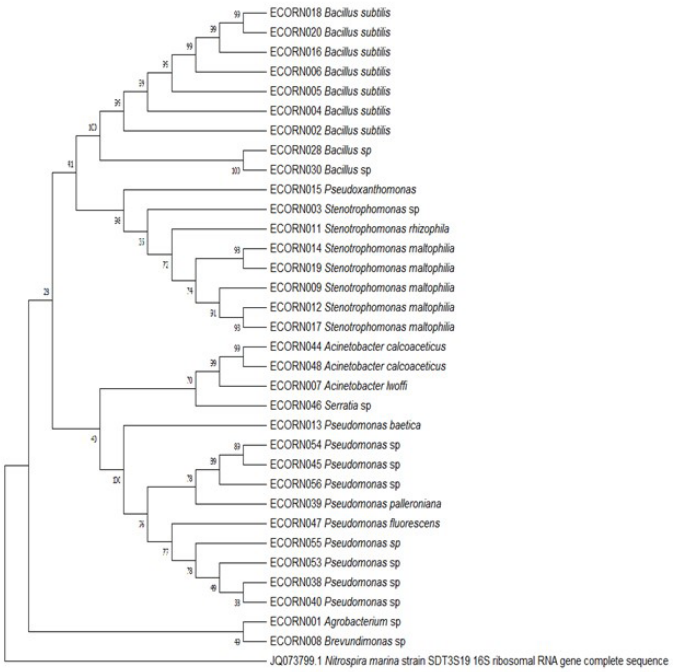

Figure 3. Phylogenetic tree inferred using the Neighbor-Joining method. The numbers in the nodes indicate bootstrap support values based on an analysis of 1000 resampled data sets. *Nitrospira marina* (JQ073799.1) was used as external taxon sequence.

and phosphorus solubilization enzymes and antibiotics (Rosas et al., 2009).

Strains C9, C12, C14, C17 were identified as *Stenotrophomonas maltophilia*, and strains C3 and C19 matched *Stenotrophomonas* sp. *S. maltophilia* is a species commonly associated with plant growth promotion that also shows antagonistic activity against certain plant pathogens, such as cucumber green mottle mosaic virus (Li et al., 2016; Pérez et al., 2020).

Strains aC2 and aC4 were identified as *Acinetobacter calcoaceticus*; strain C7 as *Acinetobacter lwoffii*, strain C1 as *Agrobacterium* sp., strain C8 as *Brevundimonas sp*., strain C13 as *Pseudomonas baetica*, strain aC1 as *Pseudomonas fluorescens*, strain aI3 as *Pseudomonas palleroniana*, strain C15 as *Pseudoxanthomonas*, strain aP2 as *Serratia sp.,* and the C11 strain as *Stenotrophomonas rhizophila*. These results agree with those reported by other authors in diverse maize-grown conditions, on the presence of *Pseudomonas*, *Stenotrophomona*, *Serratia*, and *Bacillus* (Pereira et al., 2011; Zahid et al., 2015; Gao et al., 2004; Arruda et al., 2013). *Agrobacterium* was another microorganism found in maize rhizosphere in France and Mexico (Montañez et al., 2009).

All bacterial genera identified from the highlands in this study have been reported as PGPR in other regions (Ahemad and Kibret, 2014; Ikeda et al., 2019, Gao et al., 2004). For instance, the inoculation of *Bacillus subtilis* in maize increased grain yield by 29.1%, when *B. subtilis* was inoculated without P2O5 doses (Pereira et al., 2020). *Acinetobacter calcoaceticus* produces indole acetic acid (IAA), siderophores, and solubilizes phosphorus and zinc oxide (Rokhbakhsh et al., 2011). *Stenotrophomonas maltophilia* has nitrogenase activity (Ahemad and Kibret, 2014). *Brevundimonas* and *Serratia* can solubilize phosphorus (Breedt et al., 2017; Bhattacharyya and Jha, 2012; López et al., 2015).

Since the isolation of these microorganisms, the Maize Program of the Santa Catalina Experimental Station has carried out several studies about the beneficial effect that the inoculation of these microorganisms has had, especially strains C2 and aI5 (Molina, 2006; Sangoquiza-Caiza, 2011; Genial, 2010; Ortíz, 2010; Pallo, 2013; Rivadeneira, 2012; Changoluisa, 2013; Sangoquiza-Caiza et al., 2019, 2022a, b). However, the C2 strain was for many years called *Azospirillum* sp. based on phenotypic characteristics; but in this study, we confirmed that this bacterium corresponds to *Bacillus subtilis*.

This study showed that the diversity of rhizobacterial species associated with maize cultivation is similar in various regions of the planet, regardless of altitude or environmental conditions. In the future, it will be possible to deepen this analysis to determine the frequency and quantity of each microorganism with potential for being used as biofertilizers. Additionally, only those bacteria capable of growing in culture media could be identified. Other types of studies, such as quantitative and metagenomic analyses, are necessary to understand the magnitude of species diversity in the soils cultivated with maize in the Andean region.

5. Conclusions

There was an extensive diversity of cultivable nitrogen-fixing and phosphorus-solubilizing bacteria in the maize rhizosphere grown in the highlands of Ecuador. All the isolated bacteria have been reported as plant-growth promoters, either by nitrogen fixation, phosphorus solubilization, siderophore production, or growth hormones. *Bacillus*, *Pseudomonas,* and *Stenotrophomonas* were the most frequently found genera. The presence of *Agrobacterium*, *Acinetobacter*, *Brevundimonas, Serratia,* and *Pseudoxanthomonas* was also reported, indicating these bacteria's importance in maize production systems in the highlands of Ecuador. These bacteria are stored in the biofertilizer laboratory of the Maize Program of the INIAP Santa Catalina Experimental Station, and the 16S rDNA gene sequences are available at BOLDSYSTEM.

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