Received: 3 March 2024 | Accepted: 4 July 2024

DOI: 10.1002/sae2.12114

Global Initiative of Sustainable Agriculture and Environment
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RESEARCH ARTICLE

Assessing the role of field isolated *Pseudomonas* and *Bacillus* as growth-promoting rizobacteria on avocado (*Persea americana*) seedlings

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Funding information
Instituto Nacional de Innovación Agraria, Grant/Award Number: CUI 2487112; Fondo Nacional de Desarrollo Científico y Tecnológico, Grant/Award Number: No 177-2015-FONDECYT; National Council of Science, Technology and Technological Innovation of Peru, Grant/Award Number: 009-2017-FONDECYT

Abstract

Introduction: This research aims to assess the efficacy of two genera of rhizobacteria from avocado field isolated: *Pseudomonas* and *Bacillus*, as plant growth-promoting microorganisms in Hass avocado trees grafted onto Zutano rootstock.

Materials and Methods: The siderophore-producing and phosphate-solubilizing capacity of each isolated strain was determined and plant growth-promoting activity, nutrient accumulation, and nutrient use efficiency in Zutano variety avocado seedlings were evaluated. Molecular identification was carried out by amplification of the 16S rDNA gene of the isolated strains.

Results: *Pseudomonas putida*, *Lysinibacillus macroides*, *Lysinibacillus xylanilyticus*, *Lysinibacillus fusiformis*, *Bacillus subtilis* and *Pseudomonas plecoglossicida*, were identified as the PGPR of the *Bacillus* and *Pseudomonas* genera, predominant in the avocado rhizosphere. There was found 11 phosphate solubilizing strains and 2 siderophore-producing strains. The phosphate-solubilizing strains, *B. subtilis* and *P. plecoglossicida*, stimulated the growth of Zutano seedlings, increasing their root dry weight (g), stem dry weight (g), leaf dry weight (g) and leaf area (cm^2). Significant differences were found in nutrient uptake efficiency between inoculated plants and noninoculated plants. The increase in root biomass responded to greater phosphorus and potassium absorption in plants inoculated with *P. plecoglossicida*, due to this strain's high phosphate solubilization efficiency (266%).

Conclusions: The highest plant growth promotion strains were Bac F (*B. subtilis*), Bac M (*P. plecoglossicida*) and P1 (*P. putida*), which achieved the highest increase in root and leaf dry weight, as well as the highest nutrient extractions and nutrient uptake efficiency.

KEYWORDS
16S rDNA gene, avocado, nutrient uptake efficiency, PGPR, rhizosphere

1 | INTRODUCTION

The avocado (*Persea americana* Mill.) is one of the most economically significant fruit trees in tropical and subtropical regions (Apaza et al., 2019). In Peru, approximately 33,000 hectares of Hass avocado are cultivated across diverse soil and climatic conditions, spanning from inter-Andean valleys to highland jungles, with a predominant presence in the valleys and irrigated areas along the Peruvian coast (Romero, 2019; Vittery & Colchao, 2020). In recent years its growth has been significant, especially within the Chavimochic Irrigation, and throughout the Peruvian coast (Apaza et al., 2019). As a result, avocado production in Peru is poised to remain a lucrative venture, fuelled by the emergence of new markets and a growing base of consumers who value its nutritional attributes (Araújo et al., 2018).

Plantation management requires intensive agrochemical usage, geared towards safeguarding both the vegetative and reproductive growth of the plants (Apaza et al., 2019). The rise in global demand for avocados from Peru must be accompanied by quality improvement strategies, such as integrated disease management and chemical product use reduction (Apaza et al., 2019). Moreover, exploring the potential of microorganisms, particularly those with plant growth-promoting activity, as inoculants offers a promising avenue for optimizing plant growth while minimizing environmental impact (Montañez Orozco et al., 2010). Among bacterial diversity with plant growth activity, *Pseudomonas* and *Bacillus* stand out.

Pseudomonas and *Bacillus* have been identified as having growth-promoting activities through their metabolic versatility and ability to utilize root exudates released by the plant for their development. In addition, they have short generation times, high mobility, biological nitrogen fixation activity and secondary metabolites production that can regulate plant growth and rhizospheric microbial populations (Kapulnik & Okon, 2002).

Pseudomonas spp. are widely studied as biological controllers due to their ability to colonize the root, compete aggressively with other microorganisms, adapt to different environmental stresses and synthesize antibiotics, enzymes and volatile organic compounds, as well as activate systemic acquired resistance in plants (Weller, 2007). On the other hand, *Bacillus* spp. act as plant growth promoters through mechanisms that facilitate plant development, such as phosphate solubilization, plant growth-regulating hormone production and biological nitrogen fixation (Zúñiga, 2009).

Consequently, the present research aims to identify plant growth-promoting rhizobacteria (PGPR) associated with the genera Pseudomonas and Bacillus in the rhizosphere of avocado trees in La Libertad, Peru. Additionally, it seeks to determine the siderophore-producing and phosphate-solubilizing capacities of each isolated strain. Finally, the study will evaluate the plant growth-promoting activity and nutrient accumulation in avocado seedlings of the Zutano variety.

2 | MATERIALS AND METHODS

2.1 | Soil sampling

The search for bacteria of the genus *Pseudomonas* and *Bacillus* was conducted within avocado plantations situated in Viru, La Libertad, a highly significant production zone. The area is positioned at an elevation of 75 m above sea level, with coordinates of latitude 08°32′22″ and longitude 78°40′57″. These fields reported root rot disease but did not reduce avocado production yields. In each sampled field, five trees were selected. A volume of rhizospheric soil was collected from each tree, from the four cardinal points, with a total equivalent of 250 g. Each sampled tree was a subsample. Subsamples were placed in separate hermetically sealed bags and labelled (A, B, C, D, E). Samples were transported under cold conditions using Gel Pack packaging, to maintain their temperature and humidity, to the Microbial Ecology Laboratory for microbial isolation at La Molina National Agrarian University (UNALM). Finally, the subsamples were physicochemically characterised in the Soil Laboratory at UNALM.

2.2 | Rhizospheric soil physicochemical characteristics

Rhizospheric soil from which bacteria were isolated had the following characteristics: pH: 6.57, EC: 1.16 dS m^{-1}, 0.1% $CaCO_3$, 0.57% O.M., 26.7 ppm P and 24 ppm K. The soil is slightly acidic, very slightly saline, low in organic matter content, high in phosphorus and low in potassium.

2.3 | Climatic conditions where rhizospheric soil comes from

The average annual temperature fluctuates between 18°C and 26°C, with an average higher than 20°C. Precipitation is very low and is considered less than 50 mm $year^{-1}$. Despite this, humidity fluctuates between 70% and 80%. Winds are very strong, forcing agriculturists to use protective curtains for their crops (Vittery & Colchao, 2020).

2.4 | *Pseudomonas* and *Bacillus* genera rhizobacteria counting and isolation

From each sample, 1 g of soil was placed in test tubes with 9 mL of peptonized water under continuous agitation and aseptic conditions. Seven serial dilutions were made: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}. Subsequently, the last dilutions were sown in tubes with asparagine broth for *Pseudomonas* count or plates with tryptone glucose extract (TGE) medium for *Bacillus* count, and incubated at 28°C for 4 days, to determine the species richness by counting (Martínez, 2010). From the final dilution sown, pure colonies of

Pseudomonas and *Bacillus* were isolated on cetrimide agar and TGE plates, respectively.

Additionally, the number of *Pseudomonas* colonies was estimated by the most probable number (MPN) method that consists of batteries of three tubes per dilution. A 1 mL sample from the 10^{-1} dilution bottle was inoculated into a tube with 10^{-2} diluted asparagine broth. This was performed in triplicate. After 48 h, the tubes were examined under ultraviolet light in a dark room. Green fluorescent pigmentation production constituted a presumptive positive test. Tube contents were inoculated onto the surface of cetrimide agar using a Kolle loop, and the growth characteristics of the resulting colonies were evaluated. The results were expressed in MPN g^{-1} of dry soil, as shown in Table A1.

2.5 | Isolated bacteria conservation

Isolated strains were preserved in duplicate by cryopreservation at −80°C. Probable *Pseudomonas* and *Bacillus* were sown in nutrient broth and incubated for 24 h in a tube shaker at 150 rpm. Sterile glycerol (cryoprotectant) was added to the inoculum in properly labelled Eppendorf tubes at a ratio of 1:2. The mixture was homogenized with a vortex, sealed with parafilm and stored in the freezer at −80°C.

2.6 | Phosphate solubilizing activity determination

To determine the phosphate solubilization index in a solid medium, isolated and grown in nutrient broth for 48 h strains were sown in triplicate, using the superficial puncture technique on National Botanical Research Institute's phosphate growth medium NBRIP agar with 1 g L^{-1} of inorganic P, as Tricalcium phosphate. To reach the required concentrations, 5.0 g of tricalcium phosphate was added. The relative phosphate solubilization efficiency of isolates on NBRIP medium was determined by observing the formation of transparent halos and/or medium acidification around them. The solubilization index was calculated using values obtained at 120 h, following the formula described by Lara et al. (2011).

$$\text{Solubilization index} = \frac{\text{solubilization halo diameter + number of colonies}}{\text{colony diameter}} \times 100.$$

2.7 | Siderophore synthesis determination

A bacterial suspension of each studied strain was prepared in nutrient broth and incubated for 48 h at 10^8 CFU mL^{-1} concentration. Ten microliters suspension aliquots were taken and sown in triplicate on Petri dishes with chrome azurol S (CAS) agar at three microdrops rate per dish. The plates were incubated for 48 h at 28 ± 2°C, according to the methodology proposed by Patel et al. (2018). Siderophore production positive results are indicated by colour change from blue to yellow around bacterial growth (Schwyn & Neilands, 1987).

2.8 | Rhizobacteria's 16S rRNA gene molecular identification

Twenty-six isolated strains were activated in nutrient broth at 37°C for 18 h. Genomic DNA was extracted from each strain using the JetFlex™ Genomic DNA Purification Kit (Thermo Scientific) according to the manufacturer's instructions. Identification was performed through 16S rRNA gene sequencing. PCR amplification, product purification and sequencing were conducted by Macrogen.

The gene sequence data was edited using CodonCode Aligner package v. 8.02. The sequence alignment was performer using Clustal W v. 2.0 (Larkin et al., 2007). Sequence similarity searches were conducted using the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990). The obtained sequences were compared against microbial sequences in the National Center for Biotechnology Information (NCBI) database.

2.9 | Phylogenetic analysis of rhizobacteria identified in the rhizosphere of *P. americana*

The phylogenetic tree was constructed using Randomized Axelerated Maximum Likelihood (RAxML) (Stamatakis, 2006) with the GTR substitution model (Lanave et al., 1984). A bootstrap analysis with 1000 replicates was performed using the maximum likelihood method (Stamatakis et al., 2008).

As reference sequences, 16S rRNA gene sequences were randomly selected from the NCBI database (Saitou & Nei, 1987). For the construction of the *Bacillus* phylogenetic tree, the sequences used were KC540951.1, MF188191.1, KX450400.1, KJ872853.1 and LC183868.1. For the construction of the *Pseudomonas* phylogenetic tree, the sequences from *Pseudomonas putida* isolates included AY972169.1, KM435272.1, KY586134.1 and HG421014.1.

2.10 | Plant growth-promoting activity from rhizosphere-isolated strains

4 L of sterilized Premix ® N°8 substrate (pH: 5.5, EC: 0.75 dS m^{-1}, P: 25 ppm, and K: 100 ppm) were added into plastic pots. Approximately 50 g Zutano variety avocado seeds were donated by the Avo Hass Peru company. Seeds were sterilized in 10% sodium hypochlorite for 10 min before sowing and subsequently soaked for 24 h in bacterial broth from each selected strain (P1, P3, P4, P6, P10, P11, P12, BAC L, BAC M, BAC F). The

TABLE 1 Rhizobacteria molecular identification isolated from *Persea americana*.

Isolation	Identity (BLAST NCBI)	GENEBANK access number	Similarity (%)
P1	*Pseudomonas putida*	MT982622	99
P2	*P. putida*	MT982623	99
P3	*P. putida*	MT982624	99
P4	*P. putida*	MT982625	99
P6	*P. putida*	MT982626	99
P7	*P. putida*	MT982627	99
P10	*P. putida*	MT982628	99
P11	*Pseudomonas sp.*	MT982629	99
P12	*P. putida*	MT982630	99
P14	*P. putida*	MT982631	99
BAC A	*Lysinibacillus macroides*	MT982632	99
BAC B	*Lysinibacillus xylanilyticus*	MT982633	99
BAC C	*Lysinibacillus fusiformis*	MT982634	99
BAC D	*L. fusiformis*	MT982635	99
BAC E	*L. xylanilyticus*	MT982636	99
BAC F	*Bacillus subtilis*	MT982637	99
BAC G	*L. xylanilyticus*	MT982638	99
BAC H	*Bacillus sp.*	MT982639	99
BAC I	*Bacillus sp.*	MT982640	99
BAC J	*L. fusiformis*	MT982641	99
BAC K	*Lysinibacillus sp.*	MT982642	98
BAC L	*Pseudomonas sp.*	MT982643	99
BAC M	*Pseudomonas plecoglossicida*	MT982644	99
BAC N	*Pseudomonas sp.*	MT982645	99
BAC O	*Pseudomonas sp.*	MT982646	99

bacterial broth contained each strain grown for 48 h in nutrient broth (10^8 CFU mL^{-1}). Sterile bags were used to contain seeds and broth in 50 mL per seed proportion. Once germinated, the seeds were allowed to grow in a growing room with a 20°C average temperature, 60% humidity and a 12:12 light/darkness photoperiod until seedlings were obtained. At the end of 6 weeks, its growth characteristics were evaluated. The plants were watered with sterile C2-S1 class water periodically.

The plant height (cm), number of leaves, leaves fresh weight (g), stem fresh weight stem (g), root fresh weight (g), root dry weight (g), stem dry weight (g) leaf dry weight (g), chlorophyll content (measured with SPAD device) and leaf area (cm^2) were evaluated. N, P, K, Ca, Mg and S macronutrient concentrations were also evaluated. In all cases, significant statistical differences were found between treatments ($p < 0.05$) through variance analysis and Duncan's test.

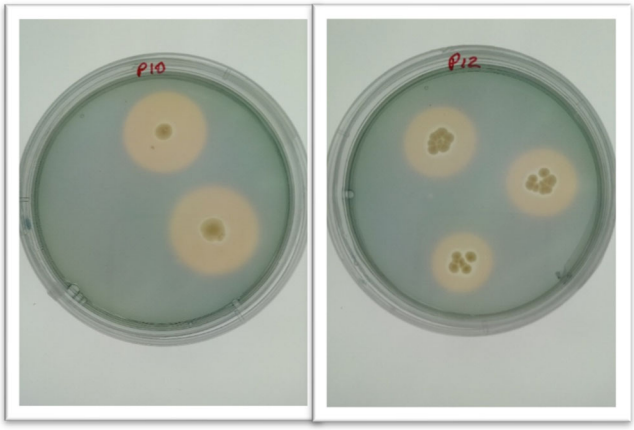

FIGURE 1 Siderophores production around bacterial growth for: P10 strain; P12 strain.

2.11 | Nutrient absorption efficiency (NAE) and nutrient efficiency index (NEI)

Determination of NUE is useful to differentiate plant species genotypes and agronomics practices for their influence to absorb (uptake) and utilize (assimilation) nutrients for maximum production of dry mater and yields (Baligar et al., 2001). The NUE is based on (a) NAE (acquire from soil, influx rate into roots, influx kinetics, radial transport in roots are based on root parameters per weight or length and uptake is also related to the amounts of the particular nutrient applied or present in soil), and (b) utilization efficiency (based on remobilization, whole plant, i.e., root and shoot parameters) (Weih et al., 2018).

The NEI was calculated following the formula suggested by Gerloff and Gabelman (1983), considering the total dry weight in g per unit of nutrient in mg. The NAE was calculated following the formula suggested by Gourley et al. (1994), considering the total nutrient extraction in mg per unit of root dry weight in g.

$$\text{Nutrient efficiency index (NEI)} = \frac{\text{Total dry weight (g)}}{\text{Total nutrient extraction (mg)}},$$

$$\text{Nutrient Absorption Efficiency (NAE)} = \frac{\text{Total nutrient extraction (mg)}}{\text{Root dry weight (g)}}.$$

TABLE 2 Agronomic characteristics evaluated in *Persea americana* var. Zutano seedlings treated with rhizobacteria.

Treatment	Height (cm)		Number of leaves		Root dry weight (g)		Stem dry weight (g) (g)		Leaves dry weight (g)		Aerial dry biomass (g)	Chlorophyll content (SPAD)		leaf área (cm^2)	
Control	33.6	ab	7.1	abc	1.9	ab	1.4	a	2.5	de	3.9 a	45.5	e	287.4	de
P1	39.0	abc	8.7	cd	2.6	cd	2.7	c	2.7	de	5.4 c	42.9	de	311.8	de
P3	39.3	abc	8.0	bcd	1.6	a	1.9	ab	2.2	bcd	4.1 b	35.7	bc	256.5	bcd
P4	42.0	c	6.8	ab	1.4	a	1.7	a	1.6	a	3.3 a	37.8	bcd	191.8	a
P6	40.5	bc	8.0	bcd	2.4	bc	2.0	ab	2.8	e	4.8 b	35.1	b	322.5	e
P10	37.7	abc	6.0	a	1.7	ab	1.8	a	1.8	ab	3.6 a	28.7	a	207.1	ab
P11	34.3	ab	6.0	a	1.9	ab	1.8	a	2.4	cde	4.2 b	35.4	b	281.2	cde
P12	32.5	a	7.0	abc	1.9	ab	1.5	a	2.0	abc	3.5 a	39.9	bcd	231.2	abc
BacF	39.6	bc	9.0	d	3.3	d	3.2	c	4.3	g	7.5 e	41.9	de	507.6	g
BacL	36.2	abc	6.5	ab	2.8	cd	1.9	ab	2.6	de	4.5 b	40.8	cde	308.5	de
BacM	35.0	ab	8.0	bcd	2.8	cd	2.6	bc	3.8	f	6.4 d	35.4	b	444.1	f

Note: Different letters in a column are statistically different (Duncan, $p \leq 0.05$).

FIGURE 2 *Persea americana* var. Zutano seedlings inoculated with plant growth-promoting rhizobacteria.

2.12 | Statistical analysis

The Statistic Package for Social Sciences (SPSS) programme from IBM, version 24, was used for statistical analysis. Obtained data in each experiment were subjected to variance analysis by using the *F* test and treatments were compared in case of significance by using Duncan's test, to determine statistically significant differences between strains. Less than 5% probability alpha error was considered significant.

3 | RESULTS

3.1 | *Pseudomonas* and *Bacillus* genera rhizobacteria counting and isolation

In general, greater amounts of the isolated *Pseudomonas* strains were observed rather than *Bacillus* in avocado trees rhizosphere, cultivated on the northern coast of Peru as shown in Table A1.

3.2 | *Bacillus* and *Pseudomonas* isolates molecular identification

16S rDNA gene amplification of the identified strains presented bands between 1197 and 1863 bp. The similarity between obtained nucleotide sequences and Gen Bank database sequences using BLAST analysis showed that the isolates belong to *Pseudomonaceae* and *Bacillaceae* families, and are related to *Pseudomonas*, *Bacillus* and *Lysinibacillus* genera, respectively (Table 1, Figures A1 and A2).

3.3 | Phosphate solubilizing capacity and siderophore production determination of 26 evaluated strains

3.3.1 | Phosphate solubilization

Fourteen strains were negative to the phosphate solubilization test out of 26 evaluated strains. The other 12 strains, Bac I, P10, P12, P3, Bac N, Bac O, P11, P2, P1, P4, Bac M and Bac L, turned out to have the capacity to solubilize precipitated tricalcium phosphates with an efficiency of between 100% and 300% (Table A2). The Bac L strain showed the highest tricalcium phosphate solubilizing efficiency

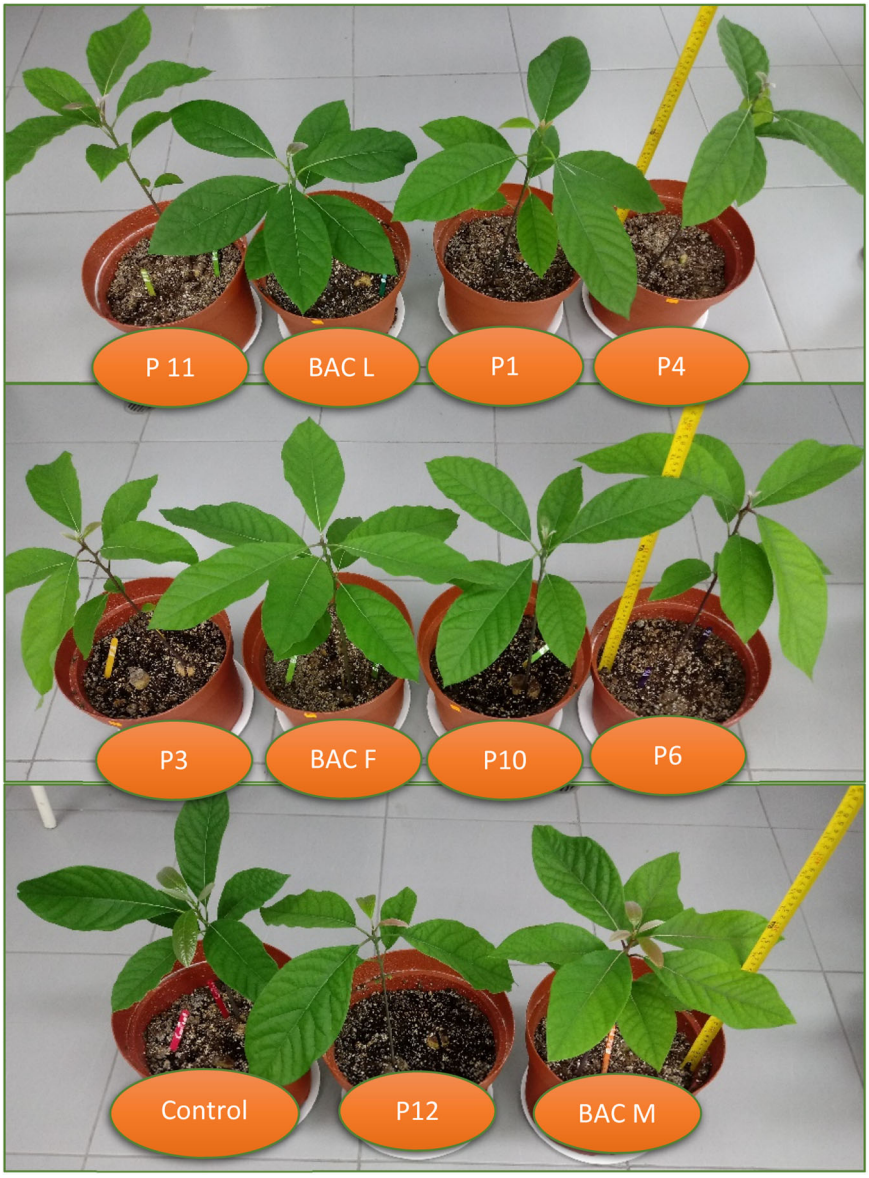

FIGURE 3 *Persea americana* var. Zutano seedlings inoculated with plant growth-promoting rhizobacteria.

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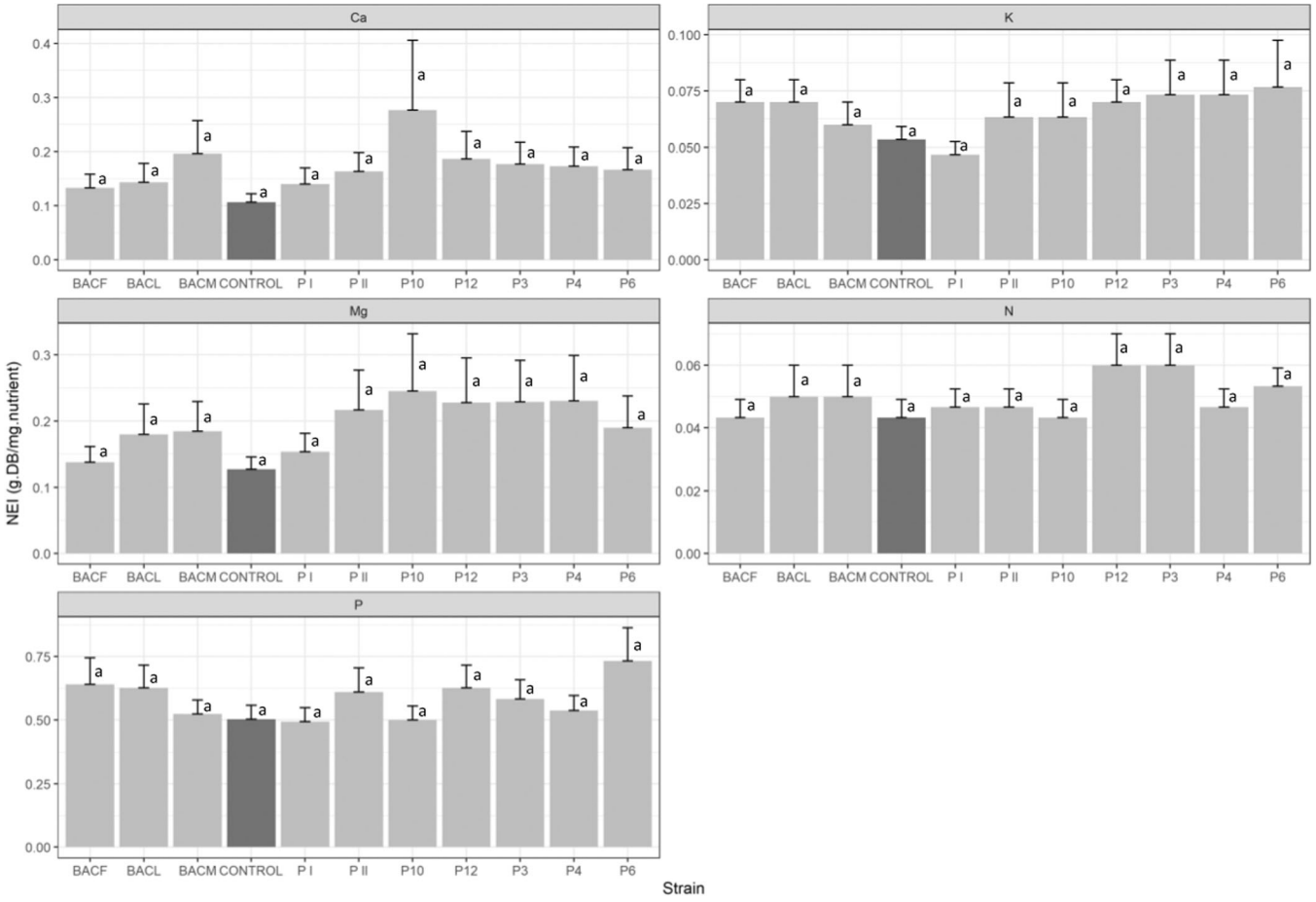

FIGURE 4 Nutrient use efficiency index (NEI) of nitrogen, phosphorus, potassium, calcium and magnesium in plants of *Persea Americana* var. Zutano treated with different rhizobacteria of the genera *Bacillus* and *Pseudomonas*.

(300%) followed by the Bac M strain. *P. putida* strains, showed low phosphate solubilizing activity (less than 200%), except for P1 and P4 strains.

3.3.2 | Siderophore production

Only strains P10 and P12 of *P. putida*, among the 26 strains evaluated, were positive in the siderophore production assay as shown in Figure 1.

3.4 | Plant growth-promoting activity from rhizosphere-isolated strains

As shown in Table 2, P4 treatment with *P. putida* reached a 42 cm plant height, higher than the control with 33.6 cm. The control, P1 and BAC F treatments presented the highest chlorophyll contents with 45.5, 42.9 and 41.9 SPAD units, respectively. Chlorophyll content decreased in all treatments compared to the control, which may indicate that the bacterial effects of promoting chlorophyll production are not clearly understood.

Regarding other experimental variables, *B. subtilis*, BAC F strain had greater dry matter accumulation than the control treatment without inoculation (Figure 2, Table 2). This strain increased the total dry biomass by 92.31%, 73.68% root dry weight, 72% leaf dry weight and 128.57% stem dry weight. Likewise, it managed to increase the number of leaves by 26.76% and the leaf area by 76.62%, that is, it has a greater photoassimilate production source (Figure 3, Table 2).

B. subtilis (Bac F strain) inoculated plants presented the highest total nitrogen extraction values due to greater dry matter production. However, Bac F treated plants showed nonsignificant differences nitrogen efficiency index (Figure 4). This characteristic indicates that a greater nitrogen concentration and accumulation is not related to a greater total biomass accumulation. The advantage of Bac F lies in increasing nitrogen absorption efficiency, that is, greater nitrogen absorption due to root dry matter accumulation (Figure 5). In this sense, Bac F is a root biostimulant that allows greater nitrogen absorption.

Table 3 shows the percentages of N, P, K, Ca, Mg and S macronutrient concentrations. Inoculated plants with *P. putida* (P10 strain) presented the highest nitrogen concentration. P10 strain inoculated plants have the same behaviour than *B. subtilis*, Bac F strain. They achieved a high nitrogen absorption efficiency, but a

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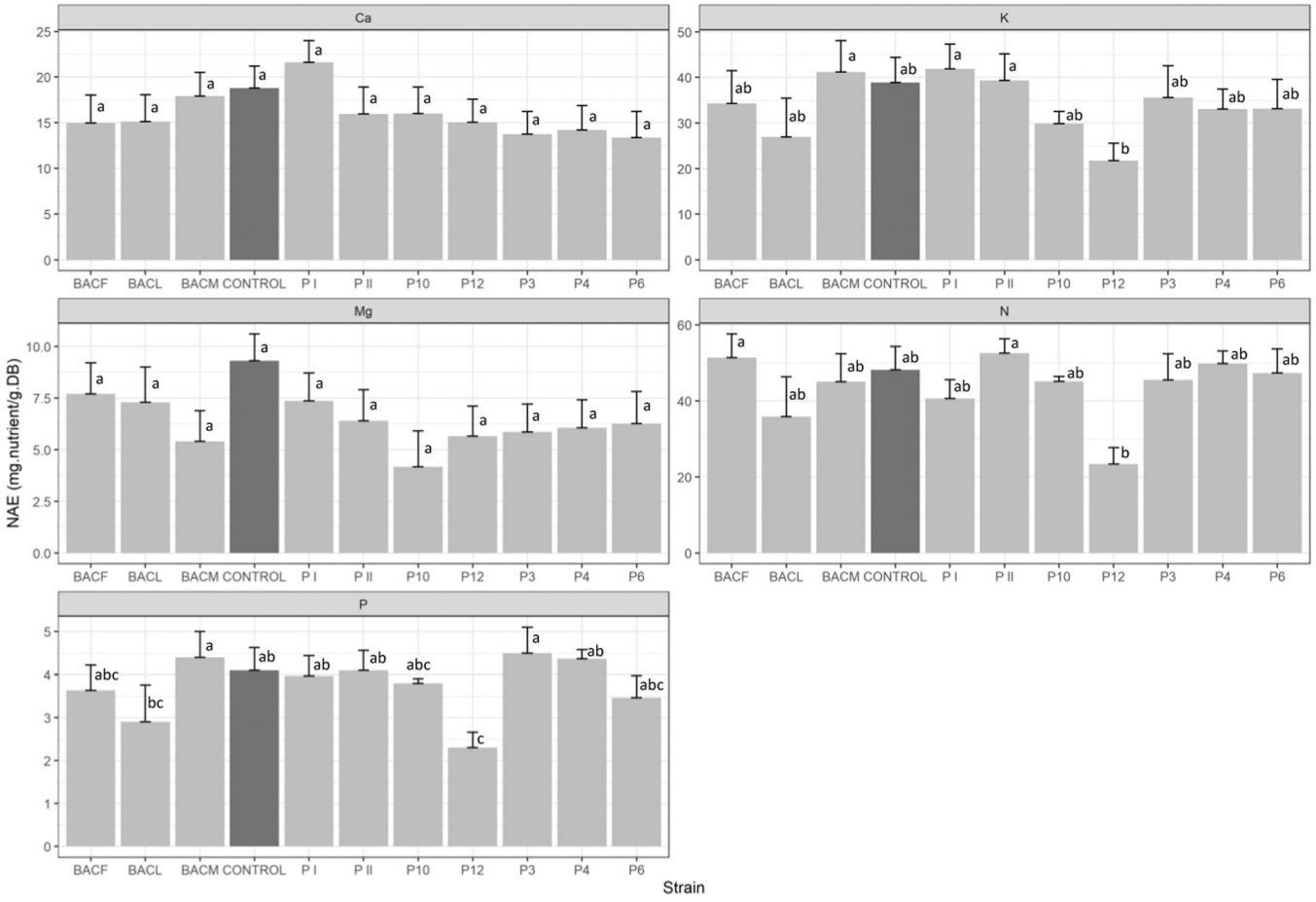

FIGURE 5 Nutrient uptake efficiency (NAE) of nitrogen, phosphorus, potassium, calcium and magnesium in plants of *Persea Americana* var. Zutano treated with different rhizobacteria of the genera *Bacillus* and *Pseudomonas*.

TABLE 3 Macronutrients concentration (%) in total dry matter of *Persea americana* var. Zutano seedlings treated with rhizobacteria.

Strain	N (%)		P		K		Ca		Mg		S	
CONTROL	2.32	cd	0.20	bc	1.88	bc	0.93	c	0.80	c	0.16	bcd
P 1	2.10	abcd	0.20	c	2.16	c	0.74	bc	0.66	abc	0.18	bcd
P3	1.76	ab	0.17	abc	1.38	ab	0.59	ab	0.46	a	0.11	ab
P4	2.13	bcd	0.19	bc	1.42	ab	0.61	ab	0.46	a	0.17	bcd
P6	1.90	abc	0.14	a	1.34	a	0.63	ab	0.55	abc	0.09	a
P10	2.41	d	0.20	bc	1.60	ab	0.42	a	0.44	a	0.12	abc
P 11	2.13	bcd	0.17	abc	1.60	ab	0.64	ab	0.49	ab	0.15	abcd
P12	1.62	a	0.16	abc	1.50	ab	0.57	ab	0.47	a	0.17	bcd
BACF	2.24	bcd	0.16	ab	1.50	ab	0.77	bc	0.74	bc	0.19	cd
BACL	2.02	abcd	0.16	abc	1.51	ab	0.73	bc	0.58	abc	0.20	d
BACM	1.96	abcd	0.19	bc	1.79	abc	0.54	ab	0.56	abc	0.15	abcd

Note: Different letters in a column are statistically different (Duncan, $p \leq 0.05$).

low efficiency index value, and this is due to a very low dry matter accumulation.

Regarding phosphorus, plants inoculated with the Bac M, P1 and Bac F strains presented a higher total phosphorus extraction of 12.24, 10.99 and 11.91 mg, respectively. This response is due to a greater accumulation of dry matter of plants inoculated with Bac M, P1, and Bac F of 6.4, 5.4 and 7.5 g, respectively (Table 2). However, the accumulation of dry matter did not respond to greater accumulation of phosphorus, as no significant statistical differences were found in the phosphorus use efficiency index of the inoculated plants concerning the control (Figure 4). However, root dry matter accumulation responded significantly to increased phosphorus absorption, attributed to a higher phosphorus absorption efficiency observed in plants inoculated with the Bac M and P3 strains compared to those inoculated with other strains (Figure 5). The high accumulation of root dry matter due to greater phosphorus absorption is due to the high phosphate solubilization efficiency of the Bac M and P3 strains 266% and 100%, respectively.

Concerning potassium, *P. putida* (P1 strain) inoculated plants resulted the highest potassium concentration value (Table 3). Inoculated plants with P1, Bac M and Bac F strains achieved a high total potassium extraction, due to a high dry matter accumulation (Figure 3). Additionally, the influence of Bac M and P1 treatments on plants resulted in the highest absorption efficiency values (Figure 5). Specifically, a greater root biomass influenced higher potassium concentration; however, this increased potassium concentration did not lead to greater biomass accumulation.

Regarding calcium and magnesium, the inoculated plants with the Bacillus subtilis and Pseudomonas putida strains a noninoculated plants did not present significant differences in nutrient use efficiency (NEI) and NAE. Therefore, the calcium and magnesium concentration in the plant does not explain a greater total biomass and root biomass accumulation.

4 | DISCUSION

In this study, it was discovered that the *Pseudomonas* genus was the most abundant microbes of 26 evaluated strains in the rhizosphere of avocado plants of the Zutano variety in La Libertad, with counts ranging from $24{\cdot}10^4$ to $11{\cdot}10^5$ MPN g^{-1} of dry soil. These results coincide with Mamani and Aragón (2018), who reported *Pseudomonas* spp. populations in avocado of $2.3{\cdot}10^6$ MPN g^{-1} in Lima, $4.3{\cdot}10^5$ MPN g^{-1} in Huaral and $>1.1{\cdot}10^8$ MPN g^{-1} in Casma. That is, there is a population variation of *Pseudomonas* in the avocado rhizosphere depending on the locality and management practices. Furthermore, Yang et al. (2001) and Pliego et al. (2008) reported that *Pseudomonas* are the most abundant rhizobacteria in avocado tree roots.

It was identified that *P. putida* (P1), *P.plecoglossicida* (Bac M) and *Pseudomonas* sp (Bac L.), reached the highest percentages of phosphate solubilization (Table A2); and some *P. putida* strains (P10 and P12), achieved a high phosphate solubilization percentage of and siderophore production (Table A2, Figure 1). Inoculation with phosphate-solubilizing strains P1, Bac M and Bac L resulted in increased root, leaf, and total biomass, as well as leaf area, in avocado plants. Additionally, inoculation with P1 and Bac M strains led to higher extraction of total nitrogen, phosphorus and potassium from the plant, along with an improvement in the nutrient uptake efficiency (Figure 5). This indicates that the phosphate-solubilizing capacity facilitated greater absorption of phosphorus, nitrogen and potassium, resulting in increased biomass production. Similar results were reported by Mamani and Aragón (2018), who observed biostimulation of root and leaf growth by inoculating avocado trees with *Pseudomonas* strains from the rhizosphere in Casma. Likewise, Sharafzadeh (2012) noted that inoculation with *Pseudomonas*, *Azotobacter* and *Azospirillum* increased nitrogen, phosphorus and potassium absorption in tomato plants, accompanied by the greatest increase in both root and shoot dry weight. Lavakush et al. (2014) determined that rice plants inoculated with a *P. aeruginosa*, *P. putida*, *P. fluorescens*, *Azotobacter chroococum*, and *Azospirillum brasilense* based-microbial consortium, and 30 kg ha^{-1} of P_2O_5, save 50% of chemical fertilizer, increasing the plant nutrient content and grain yield, that is, the plants were more efficient.

B. subtilis (Bac F) was not identified as a phosphate solubilizer or a siderophore producer. However, it was the rhizobacteria with the greatest effect on the increase in root and total biomass, as well as in leaf area. Furthermore, inoculation with the Bac F strain resulted in one of the highest nitrogen, phosphorus and potassium extractions, as well as the greatest increase nitrogen uptake efficiency (Figure 5). Bac F inoculated plants reached, by far, the highest root dry weight, which may indicate that *Bacillus* biostimulation mechanism is associated with auxin production, a hormone responsible for root branching. *B. subtilis* main effect on plants is root branching intensification, stimulated by N-Acyl-L-homoserine lactones (AHLS), cyclodipeptides (CPDS) and volatile organic compounds (Ortiz-Castro et al., 2017).

Avocado crops are highly susceptible to abiotic stresses, including soil salinity and oxygen deprivation. Therefore, soil microorganism's incorporation into comprehensive crop nutrition plans is essential for root and foliar biostimulation, particularly in the context of climate change (Ramírez-Gil & Morales-Osorio, 2020).

5 | CONCLUSIONS

The predominant PGPRs of the avocado Zutano variety plant rhizosphere was *Pseudomonas* with between $24{\cdot}10^4$ and $11{\cdot}10^5$ MPN g^{-1}, compared to *Bacillus*, which were found between $1{\cdot}10^5$ and $3{\cdot}10^5$ MPN g^{-1}. Twenty-six rhizosphere isolated strains were molecularly identified as *P. putida*, *L. macroides*, *L. xylanilyticus*, *L. fusiformis*, *B. subtilis* and *P. plecoglossicida*. Of these, 11 had phosphate solubilizing activity, with *Bacillus sp*. (Bac I strain), *P. putida* (P10, P12, P3, P2, P1, P4 strains) and *P. plecoglossicida* (Bac M strain), *Pseudomonas sp*. (Bac N, Bac O, P11 Bac L strains) being the

main ones. In addition, only two strains of the *P. putida* (P10 and P12 strains) species showed siderophore production capacity.

This study showed that avocado plants inoculated with *P. putida* (P3 strain), *P. plecoglossicida* (Bac M strain) and *B. subtilis* (Bac F strain) reached the highest values of total extraction of nitrogen, phosphorus and potassium. Likewise, inoculation with phosphate-solubilizing strains of *P. plecoglossicida* (P1 and Bac M strains) resulted in increased root, leaf, and total biomass, as well as leaf area, in avocado plants. The Bac M strain of *P. plecoglossicida* also increased the potassium and phosphorus uptake efficiency index. Biostimulation in the absorption and assimilation of nutrients increased the leaf area, root, leaf and total biomass of avocado plants inoculated with *B. subtilis* and *P. plecoglossicida*. These results suggest that the plant growth-promoting activity of *Pseudomonas* and *Bacillus* is due to greater stimulation of plant nutrition.

AUTHOR CONTRIBUTIONS

Richard A. Solórzano: Methodology; validation; writing—original draft; visualization; investigation. **Kenyi R. Quispe**: Conceptualization; validation; writing—review & editing.

ACKNOWLEDGEMENTS

We would like to thank to Dra. Cinthia Quispe Apaza that provided support for the realization of the phylogenetic trees. This research was funded by the CUI 2487112 INIA project Research and technology transfer services improvement in the management and recovery of degraded agricultural soils and irrigation water for small and medium-scale agriculture in the departments of Lima, Ancash, San Martín, Cajamarca, Lambayeque, Junín, Ayacucho, Arequipa, Puno and Ucayali. Richard Andi Solórzano Acosta was funded by scholarship grant No 177-2015-FONDECYT from the National Council of Science, Technology and Technological Innovation of Peru. Project No 009-2017-FONDECYT from the National Council of Science, Technology and Technological Innovation of Peru.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

ETHICS STATEMENT

The authors confirm that they have adhered to the ethical policies of the journal.

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How to cite this article: Solórzano-Acosta RA, Quispe KR. Assessing the role of field isolated *Pseudomonas* and *Bacillus* as growth-promoting rizobacteria on avocado (*Persea americana*) seedlings. J Sustain Agric Environ. 2024;3:e12114. https://doi.org/10.1002/sae2.12114

APPENDIX

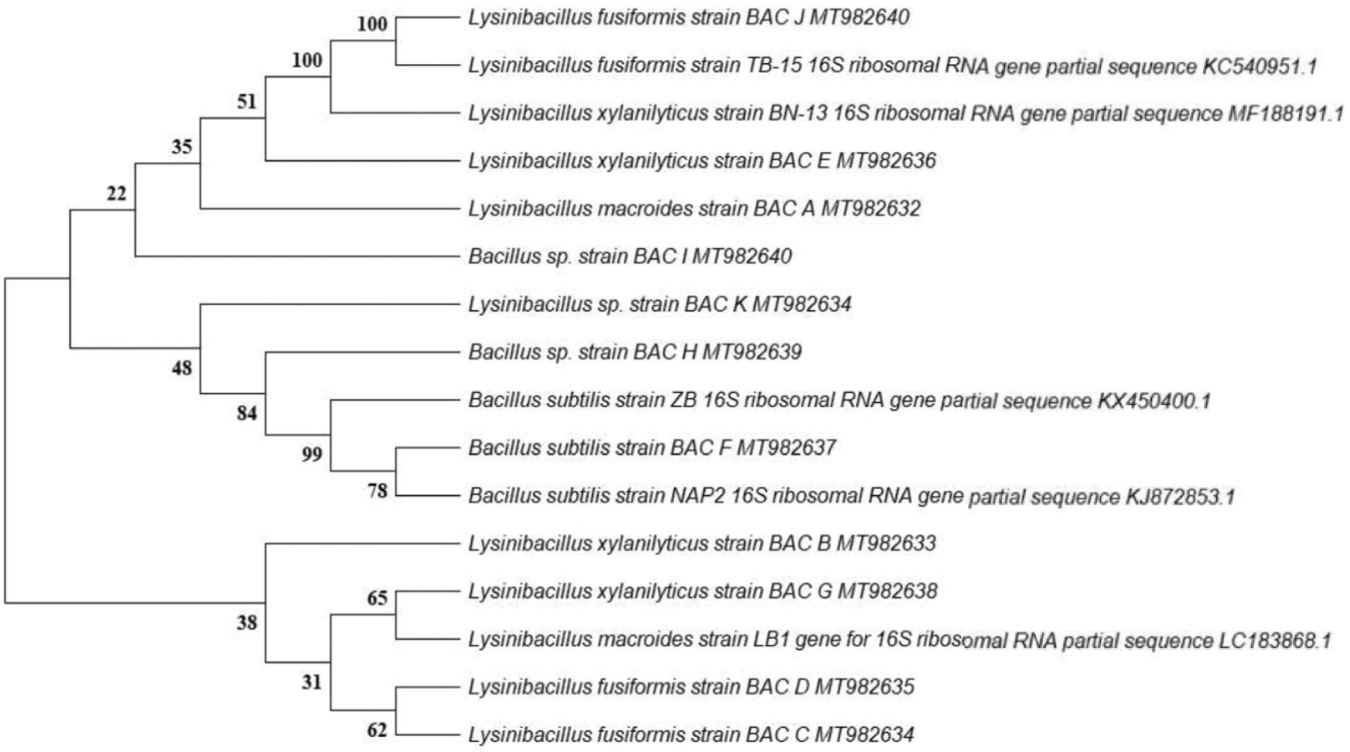

FIGURE A1 Phylogenetic tree constructed according to the Maximum Likelihood Method based on the relationship between *Bacillus* strains 16S rRNA gene sequences isolated from *Persea americana* rhizosphere and related *Bacillus* species. *Bacillus subtilis* strain 16S rRNA gene sequences were arbitrarily chosen as an external sequence to the group.

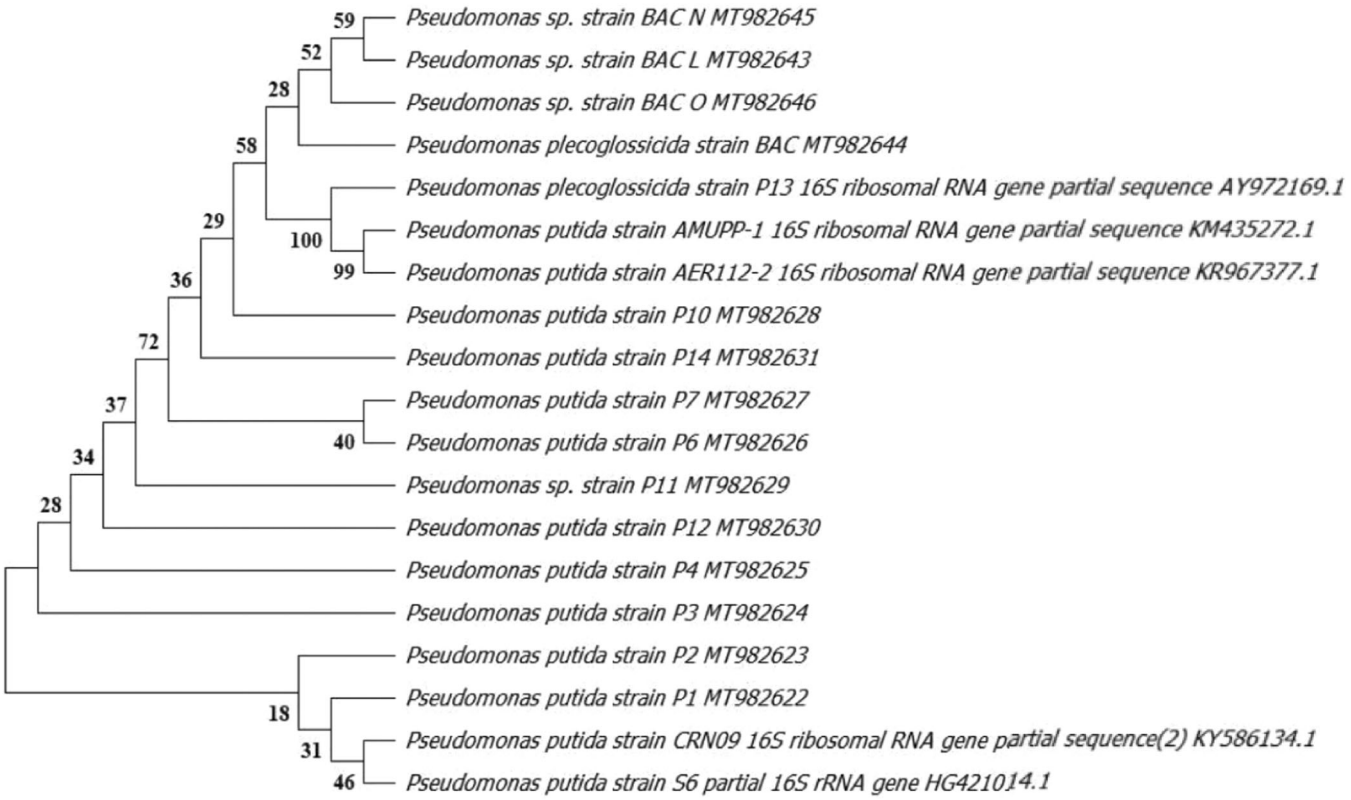

FIGURE A2 Phylogenetic tree constructed according to the Maximum Likelihood Method based on the relationship between *Pseudomonas* strains 16S rRNA gene sequences isolated from *Persea americana* rhizosphere and related *Pseudomonas* species. *Pseudomonas putida* strain 16S rRNA gene sequences were arbitrarily chosen as an external sequence to the group.

TABLE A1 Avocado rhizosphere bacterial count (MPN g^{-1}).

Tree	*Pseudomonas*	*Bacillus*
A	11×10^5	1×10^5
B	11×10^5	2×10^5
C	24×10^4	3×10^5
D	24×10^4	1×10^5
E	11×10^5	1×10^5

TABLE A2 Tricalcium phosphate solubilizing efficiency of 12 strains isolated from *Persea americana* rhizosphere.

Strain	Solubilizing efficiency (%)
BAC I	100 a
P10	100 a
P12	100 a
P3	100 a
BAC N	117 a
BAC O	117 a
P11	183 b
P2	183 b
P1	200 bc
P4	233 cd
BAC M	266 de
BAC L	300 e

Note: Average of five repetitions. Means with different letters in a column are statistically different (Duncan, $p \leq 0.05$).