Bulgarian Journal of Agricultural Science, 28 (No 2) 2022, 217–228

Potential of endophytic phosphorus-solubilizing bacteria to improve soil fertility, P uptake, and yield of maize (*Zea mays* L.) cultivated in alluvial soil in dikes in Vietnam

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Abstract

Khuong, N. Q., Quang, L. T., Thuc, L. V., Huu, T. N., Thu, L. T. M., Van, T. T. B., Xuan, D. T., Hue, N. H., Le, N. T. T., Tien, P. D., Nhan, T. C., Xuan, L. N. T. & Xuan, N. T. T. (2022). Potential of endophytic phosphorus-solubilizing bacteria to improve soil fertility, P uptake, and yield of maize (*Zea mays* L.) cultivated in alluvial soil in dikes in Vietnam. *Bulg. J. Agric. Sci., 28 (2)*, 217–228

This study aimed to (i) select endophytic bacteria in maize root for its phosphorus solubilization ability and (ii) evaluate the efficacy of selected indigenous bacterial strains on soil fertility, phosphorus (P) uptake, growth, and maize yield. A total of 31 maize root samples were collected from An Phu District, An Giang Province, to isolate the bacteria. In addition, the pot experiment was conducted with nine treatments of (i) 100% P of the recommended fertilizer formula (RFF), (ii) 75% P of the RFF, (iii) 50% P of the RFF, (iv) 25% P of the RFF, (v) 75% P of the RFF plus a mixture of the 3 selected strains, (vi) 50% P of the RFF plus a mixture of the 3 selected strains, (vii) 25% P of the RFF plus a mixture of the 3 selected strains, (viii) 0% P of the RFF plus a mixture of the 3 selected strains, and (ix) 0% P of the RFF plus none of the 3 selected strains. The results identified 72 isolates from LGI medium. All isolates were screened in a broth medium at pH 5.0, but only 16 isolates obtained $OD_{660} > 0.5$. All 16 isolates were able to solubilize various insoluble P fractions. Of those, strain ASD-21 had the highest P solubilization ability for the insoluble Al-P compound. Strains ASD-08 and ASD-21 had the highest Fe-P concentration at 39.8–41.5 mg P L^{-1}. Strains ASD-08 and ASD-21 had the highest Ca-P concentration (46.6–51.3 mg P L^{-1}) for 48 h of incubation. They were identified as *Enterobacter* spp. Results also showed the application of a mixture of strains ASD-08, ASD-10, and ASD-21 increased soil fertility by increasing phosphorus content by 13.7 mg P kg^{-1} and replacing 25% of P fertilizer, but this mixture did not change maize yield.

Keywords: alluvial soil; dike; endophytic bacteria; maize yield; phosphorus solubilization

Introduction

Phosphorus (P) is very crucial for plant growth, as it is one of the key factors in energy metabolisms and plant organic compounds (Sultenfuss & Doyle, 1999). Despite its abundance in agriculture soil as mineral salts or organic compounds (Otieno et al., 2015), P is mainly present in insoluble forms, including Al-P, Fe-P, and Ca-P (Zhang et al., 2021). Nevertheless, P chemical fertilizers are inefficient due to their fixation, their harm to environment due to their cadmium content, and their cost (Seshadri et al., 2016). Therefore, the need arises for bacterial sources of available P for plants (Alori et al., 2017; Qarni et al., 2021). Many efficient P-solubilizing strains have been reported, such as *Arthrobacter*, *Bacillus*, *Burkholderia*, *Natrinema*, *Pseudomonas*, *Rhizobium* and *Serratia* (Divjot et al., 2021). This indicates there are many potential bacterial sources for altering chemical fertilizers as a P supplement for plant growth. Moreover, maize is a popular cultivar as a cereal crop in many countries (Scott et al., 2016). Based on the FAO database, in 2013, maize was the top crop in the word. Maize is mainly produced in the USA, China, Brazil, Mexico, and Argentina.

Therefore, the yield and demand of maize has been well studied. According to Barry & Miller (1989), the greatest P treatment from the first stage had the highest yield (8.2 ton ha^{-1}) at the 6-leaf stage. The highest grain yield is correlated with at least 5.0 g kg^{-1} of shoot P concentrations. Moreover, minimum P demand for maize has been also calculated to be about 6–23.4 kg ha^{-1} (Ten Berge et al., 2019). Furthermore, Mkoma (2015) suggested that for optimum maize yield, 15 kg P ha^{-1} is the agronomic efficiency of P fertilizer rate. However, alluvial soils are deposited by surface water. In-dike alluvial soils, also called unconsolidated alluvial soils, are alluvial soils bordered by dikes for flood prevention. Unconsolidated alluvial soil is commonly found on upland fields in Vietnam. The in-dike alluvial soil possesses both coarse-grained sediment and extensive fine-grained lacustrine deposits (Holzer, 1984).

The P content of 16 samples of alluvial soil were determined by Chang & Juo (1963). Levels of Al-P, Fe-P, and Ca-P were 29–77 ppm, 38–265 ppm, and 48–536 ppm, respectively. As can be seen, alluvial soils contain large amouts of Ca-P. However, Ca-P decreases while soils are kept at field moisture capacity, according to Chang & Chu (1961), which leads to the need to apply more P to the soil. Thus, this work aimed to (i) select endophytic bacteria in maize root for its phosphorus solubilization ability and (ii) evaluate the efficacy of selected indigenous bacterial strains on soil fertility, P uptake, growth, and maize yield in alluvial soil in dikes.

Materials and Methods

Place and time: The pot experiment was conducted in the greenhouse at College of Agriculture, Can Tho University from September 2020 to March 2021.

Samples for bacteria isolation: Roots of hybrid maize were sampled in the period of 40 – 45 days after planting. The total of 31 maize root samples was collected from maize fields at An Phu town and communes including Vinh Hau, Vinh Loc, Vinh Loi, Quoc Thai, Phu Huu, Khanh An and Da Phuoc, An Phu district, An Giang province in December, 2019. Moreover, 48 strains of endophytic bacteria from root maize of previous work were also used for screening steps in this research.

Maize kernels: hybrid maize used in this experiment was CP 888, whose growth cycle was 95-100 days. The length of the ear was 22 cm, small ear, orange yellow kernels, firm, drought tolerant, hard stem, green leaves and good sustainment. The yield was high and stable from 10 to 12 ton/ha.

Fertilizers: Chemical fertilizers used in this experiment consisted of urea (46% N), super phosphorus (16% P_2O_5) and potassium chloride (60% K_2O).

*Bacteria***:** The bacteria sources used in experiments were three strains of P-solubilizing endophyte *Enterobacter cloacae* ASD-21, *E. mori* ASD-08 and *E. asburiae* ASD-10 in maize hybrid, isolated from in-dyke alluvial soil in An Giang province.

Isolation, selection and identification of endophytic P-solubilizing bacteria (EPSB) isolated from root maize

Samples preparation. Roots of hybrid maize were washed. Then, they were cut into small fragments and washed again with tap water. Finally, they were left dried at room temperature.

Bacterial isolation. 10 g of 1-cm long maize roots was transferred into a 250 mL Erlenmeyer flask. The flask was then added with 20 mL of alcohol 96%, and shaken on a shaking machine at the speed of 100 rpm for 10 min. Then, the alcohol was removed, and 50 mL of distilled water was put in. Finally, the flask was shaken again at the speed 100 rpm for 5 min for samples cleanliness (Repeat four times). Twenty milliliters of calcium hypochlorite 2% was applied with shaking at 100 rpm for 10 min, and samples were washed four times with distilled water as above. 150 µL of liquid at the last wash was inoculated on petri dishes with TYGA medium, incubating at 30°C. After 48 h, if there were no colonies on the medium, the samples would be considered to be qualified. After that, the samples were smashed by a sterilized mortar and pestle. The mortar was then applied with 1.5 mL of distilled water, the mixture was stirred and each of 500

µL extract for each sample was transferred into three tubes with semisolid NFb medium. All of the tubes were shaken and incubated at 30°C for 3 days. During observation, the appearance of a thin membrane on the medium surface indicated the existence of endophytic bacteria in the sample extracts. The semisolid medium containing endophytic bacteria was spread on another solid medium, and then incubated at 30°C. Dilution might be applied in case of too high bacterial density. After 48 h, colonies appearing on medium surface were inoculated to other media in order to obtain isolated and pure colonies in shape. The purity was checked by microscopes via living bacteria drop method. When the purity was maintained, the bacteria was transferred into tubes with correspondent media and stored at 4°C for the following pot experiment. We also used 48 bacterial strains isolated from maize in our previous research for this screening.

Methods to measure P content of maize root endophytic bacteria. To determine P solubilizing ability of the bacteria, NBRIP medium was adjusted by adding an amount of aluminum phosphorus (insoluble P compound), 1 g L^{-1} $AlPO_4 \bullet 2H_2O$. The 0.5 mL extract from each bacterium, whose OD_{660} value was 0.5, was put into a tube containing 5 mL of liquid NFb medium, being shaken at 120 rpm under dark condition for 2 days. 1.0 mL of bacterial extract was centrifuged for 15 min at 10,000 rpm. From the extract, P solubilized content was measured by ascorbic acid method at 880 nm wavelength by spectrophotometer (Murphy & Riley, 1962). To determine P-solubilizing capability of iron phosphorus and calcium phosphorus, $AlPO_4 \bullet 2H_2O$ was replaced by $FePO_4 \bullet 2H_2O$ or $Ca_3(PO_4)_2$, respectively, in NBRIP medium. Broth culture without bacteria was used as a negative control.

Identification of selected endophytic bacteria. It is based on the P-solubilizing screening output from above experiments. There were 3 strains of endophytic bacteria selected. The selected strains were identified, using 16S rDNA sequence analysis. For 48 h, under microaerobic light condition, the selected EPSB strains were grown in LGI broth at pH 7.0. From each culture, 2 mL was centrifuged at 10 000 rpm for 5 min in order to obtain cell pellet. From the cell pellet obtained, DNA extraction took place by using Genomic DNA Prep Kit (BioFACT™) following the manufacturer's instructions. For visualization, the genomic DNA of EPSB strains was observed by electrophoresis technique, resolving DNA samples on 1.0% w/v agarose gel and inspecting the bands under the condition of UV-trans illuminator for the concentration and purity determination. Bacterial 16S rDNA sequences were amplified by PCR technique, based on a pair of primers; 16S Forward Primer – 8F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 16S Reverse Primer – 1492R (5′- GGT TAC CTT GTT ACG ACT T-3′) (Suzuki et al., 2003) as instruction in iProof™ High-Fidelity PCR Kit – Bio-Rad (BioRad, Hercules, CA) using a T100™ thermo cycler (BioRad). The amplification reactions were carried out by the following profile: 95°C pre-denaturation for 5 min; following by 30 cycles of 95°C denaturation for 30 s, 55°C annealing for 30 s and 72°C extension for 2 min; and the final extension at 72°C for 10 min. The PCR products were detected by DNA marker based on electrophoresis technique (1.0% w/v agarose gel and 1X TAE buffer), then examined under UV-transilluminator. The products were purified by using a Purification Kit of TIANquick Midi (Tiangen Biotech Ltd., Beijing, China) following the instruction of the manufacturer.

The purified PCR products were sequenced by an automated DNA sequencer at Macrogen DNA Sequencing Service (Macrogen, Seoul, Korea) and analyzed by using BioEdit, version 7.0.5.3 for sequencing results and ChromasPro version 1.7 (http://technelysium.com.au/wp/chromaspro) for chromatograms. In Gen bank, the analyzed sequences were collated with the available sequences in database to find out the most similar sequences by Basic Local Alignment Search Tool (BLAST) of National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov). Multiple sequences were aligned by using CLUSTALW. Based on the use of MEGA software, version 6.06, the phylogenetic tree was built based on neighbor-joining method. From the neighbor-joining trees, evolutionary distance matrix was formed by Jukes–Cantor model and their topologies were checked by 1000 replicates bootstrap resampling method.

Potential of selected endophytic P-solubilizing bacteria on soil fertility, P uptake, growth and yield of maize

Experimental design. Experiment was performed in a completely randomized design (CRD) with 9 treatments, 4 replicates. Each replicate was a pot with one plant. The treatments were described as follows: (i) Control, 100% P of recommendation fertilizers formula (RFF), (ii) 75% P of RFF, (iii) 50% P of RFF, (iv) 25% P of RFF, (v) 75% P of RFF and a mixture of *Enterobacter* spp. ASD-08, ASD-10 and ASD-21, (vi) 50% P of RFF and a mixture of *Enterobacter* spp. ASD-08, ASD-10 and ASD-21, (vii) 25% P of RFF and a mixture of *Enterobacter* spp. ASD-08, ASD-10 and ASD-21, (viii) 0% P of RFF and a mixture of *Enterobacter* spp. ASD-08, ASD-10 and ASD-21, and (ix) 0% P of RFF and no endophytic bacteria.

Soil preparation. Soil used in this study was collected from Chau Phu district, An Giang province. The residue materials were removed from soil for greenhouse pot experiments during mixing and drying in air. Pots were filled with

10 kg of each soil sample. The recommended fertilizer dosage for maize was applied: 200 N-90 P_2O_5-80 K_2O kg ha^{-1}. The commercial fertilizers for N, P, and K were urea (46% N), superphosphate fertilizer (16% P_2O_5), and potassium chloride (60% K_2O), respectively.

Liquid endophytic biofertilizers in the greenhouse. Maize kernels were cultivated at a density of one kernel per pot. This provided 4.2×10^3 cells g^{-1} DSW (6.3×10^6 cells $kernel^{-1}$, dry soil 10 kg pot^{-1}). Each liquid EPSB biofertilizer was applied at 5.0 mL (initial cell density 10^8 cells mL^{-1}, dry soil 10 kg pot^{-1}) to provide roughly 0.33 x 10^5 cells g^{-1} DSW for each stage at days 10, 20 and 45 of cultivation. This resulted in a total EPSB population of approximately 1.0×10^5 cells g^{-1} DSW (5.0000 log cells) for three stages (one crop). Therefore, each soil pot was inoculated with 1.042×10^5 cells g^{-1} DSW (5.0179 log cells), or roughly 1 x 10^5 cells g^{-1} DSW from liquid EPSB biofertilizer plus cells inoculated in the maize kernels.

Maize kernels were partially sterilized by submersion in 70% ethanol and 1% sodium hypochlorite solution for 3 and 10 min, respectively. Then they were washed with sterile distilled water and incubated for 24 h in darkness condition to germinate. Approximately 1000 maize kernels germinated, which were separately soaked in 63 mL EPSB suspension, in sterile beakers containing either single or a mixed EPSB (roughly 10^8 cell mL^{-1}), while sterile deionized water without EPSB served as a control. The sterile beakers were covered with aluminum foil, shaken in a reciprocating shaker at 60 rpm for 1 h and then dried in a laminar airflow cabinet for 1 h. Finally, approximately 6.3×10^6 EPSB cells per kernel and control kernels without EPSB were planted.

Solid biofertilizers. Solid biofertilizers were prepared following the method of Kantha et al. (2015) with a slight modification from ash and leaves maize at ratio of 1:4. In brief, each bacteria strain was separately cultured at pH 4.5 for 48 h. A cell suspension was then prepared in distilled water to obtain a cell density of 10^9 cells mL^{-1} for use as an inoculant. To prepare the solid formulae, 30 mL were added into 120 g of carrier to produce a final cell density of roughly 10^8 cell g^{-1}. The mixed biofertilizer was packed in plastic bags for 1 month in darkness at room temperature prior to use. The cell density of biofertilizer was counted before inoculation. One pot contained one seed of maize, which gave out a density of 4.2×10^3 cells g^{-1} dry soil weigh (DSW) (6.3×10^6 cells $seed^{-1}$, dry soil 10 kg pot^{-1}). Solid biofertilizer was used at the amount of 5.0 g (initial cell density 10^8 cells mL^{-1}, dry soil 10 kg pot^{-1}) so as to maintain roughly 0.33 x 10^5 cells g^{-1} DSW for each stage at 10, 20 and 45 days after cultivation, which led to a bacterial density of 1.0×10^5 cells g^{-1} DSW (5.0000 log cells) for three stages in one season. Therefore, each pot contained with 1.042×10^5 cells g^{-1} DSW (5.0179 log cells), or roughly 1 x 10^5 cells g^{-1} DSW from solid biofertilizer and cells inoculated in the maize seeds.

Parameters of survey

Growth and yield parameters were collected at the stage of physiological maturity R6 (115 days after planting, DAS). Four pots of each treatment were all measured. Plant height (cm): the measurement occurred from the ground to the peak of a plant in each pot. Plant height was determined at 115 DAS. Height of appeared first ear formation (cm): it was measured by the height from the ground to the first ear formation. Stem diameter (cm): it was determined from the average diameter values of top, middle and bottom stem. Number of leaves (leaves/plant): Total of leaves was counted in each plant of each treatment.

Yield components were measured as following description. Ear length (cm): it was measured by the length from both ends. Ear diameter (cm): it was measured by the diameter of ear body. Number of row/ear (rows): the number of row in an ear was counted. Number of kernels/row (kernels): the number of kernels was counted in each row of an ear. 100-kernel weight (g): 100 kernels were randomly collected in each treatment and then weighed by an electronic scale with three digits.

Maize yield (g pot^{-1}). All maize ears were collected from plants. The fresh weight of ears was measured. Then, the ears were naturally dried; kernels were derived and collected into separate paper bags labeled with codes for each treatment. After that, bags with kernels were dried out at 70°C in 72 h, until humidity reached 15.5%, then, dry kernel weight was checked.

Biomass of grain, stem, leaves and root. Grain, stem, leaves and root were weighed at the stage of R6 of each pot. They were separately then dried out at 70°C for 72 h to obtain entire dried biomass for measurement.

Soil analysis. Analysis of soil samples followed the standard methods described by Sparks et al. (1996). Briefly, to determine pH, soil pH_{KCl} and pH_{H2O} were extracted with 1 M KCl and deionized water at a soil: solvent ratio of 1:5. The extracted solution was also used for electrical conductivity (EC) determination. Total P was digested by mixture of perchloric acid and nitric acid and detected by ascorbic acid method via spectrometer at wavelength of 880 nm; Available P (P_{avail}) was measured by the Bray II method. Quantification of total nitrogen (N_{tot}) was by the regular Kjeldahl method after converting organic N to inorganic N. Salicylate was used to determine NH_4^+-N concentration from soil extracted with 2 M KCl. Total carbon (C_{tot}) was determined using dichromate oxidation by a thermal conductivity technique in

sulfuric acid using titration with ferrous sulfate heptahydrate after converting organic C to inorganic C.

Plant analysis: Stover straw and kernel samples were collected at the physiologically mature stage and dried at 65 – 70°C for 72 h before they were cut, ground, and passed through a 0.5 mm sieve for the analysis of N_{tot} and P_{tot} in above ground components. Specifically, N_{tot} content was determined by Kjeldahl distillation and P_{tot} concentration was analyzed by the UV-VIS method (Walinga et al., 1989). Elemental accumulations of N and P in the kernel and the stover straw were calculated from these concentrations.

Data analysis

The data shown are means of four replications, unless otherwise stated. The data were subjected to one-way analysis of variance (ANOVA) using SPSS software version 13.0. Means were separated by ANOVA and the significance of differences was assessed by Duncan's post-hoc test at $P < 0.05$.

Results

Selection and identification of endophytic P-solubilizing bacteria isolated from maize root

Among 72 strains of isolated endophytic bacteria from hybrid maize roots, there were 13 strains possessing an OD_{660} value higher than 0.5 in an acidic medium. Three strains, including ASD-02, ASD-13, and ASD-21, reached the highest OD_{660}, with the values of 0.884–0.986 (Data not shown) under an incubating condition with a pH of 5.0.

In addition to being tolerant of acid, the 13 strains were also capable of solubilizing the insoluble P compounds. The amount of Al-P dissolved was 3.0–55.3 mg P L^{-1}. Among them, there were 7 strains which had the highest content of solubilized Al-P, including ASD-02, ASD-13, ASD-15, ASD-18, ASD-21, ASD-40, and ASD-48 (Figure 1a). For Ca-P, strain ASD-B21 had the highest solubilizing ability (51.3 mg L^{-1}) and was significantly different from other strains at 5%, except for strain ASD-10 (46.6 mg P L^{-1}). The second highest one for Ca-P was ASD-25, with 43.7 mg P L^{-1} (Figure 1b). Strains ASD-08 and ASD-21 had the highest amount of dissolved Fe-P at 39.8–41.5 mg P L^{-1}. The others had the smaller amounts, from 6.2 to 32.2 mg P L^{-1}. Similarly, strain ASD-13 had the second highest Fe-P content (Figure 1c). In short, in the application of alluvial soil in dikes, bacteria are able to solubilize all types of insoluble forms, so strains ASD-08, ASD-10, and ASD-21 were selected for cultivating maize in the pot experiments. They were identified as *Enterobacter cloacae* ASD-21, *E. mori* ASD-08, and *E. asburiae* ASD-10 (Figure 2).

Potential of selected endophytic P-solubilizing bacteria on the improvement of soil fertility, P uptake, growth, and yield of maize

Effects of selected endophytic P-solubilizing bacteria on soil alluvial fertility in dike-cultivated maize. The reduction of P fertilizer levels and supplementation with bacteria from maize root changed several in-dike alluvial soil properties (Table 1).

Starting with the least significant difference, the pH_{KCl} value and the total N content changed unremarkably during the experiment. Meanwhile, pH_{H2O} value and the total P content were significantly different among treatments. However, their trends were unclear. For the other properties, including available N and P, the differences revealed the effect of P fertilizer levels and bacteria supplementation. With P fertilizer, following the decrease in the amount of P supplement from control treatment with 100% N and 100% P to only 25% P only, the amount of available P dropped as well from 62.8

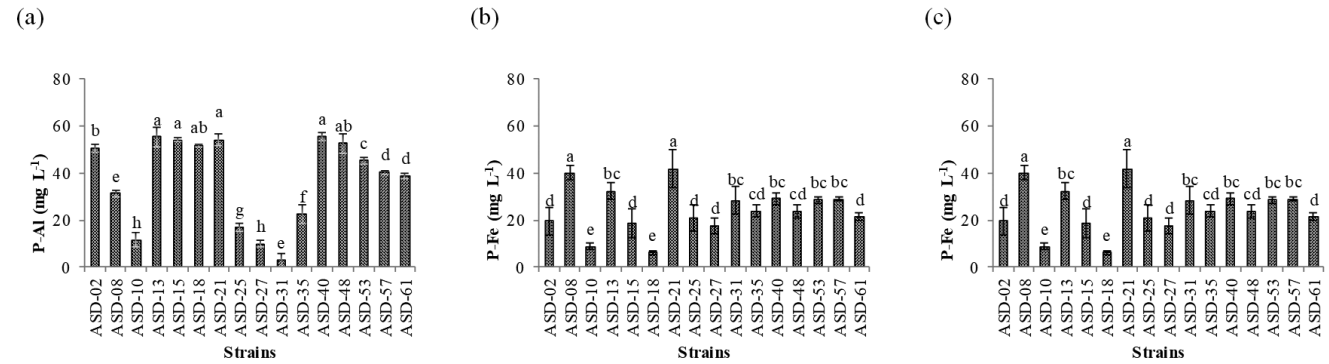

Fig. 1. Selection of acid resistant endophytic bacteria to solubilize insoluble P from (*a*) $AlPO_4$, (*b*) $Ca_3(PO_4)_2$ and (*c*) $FePO_4$

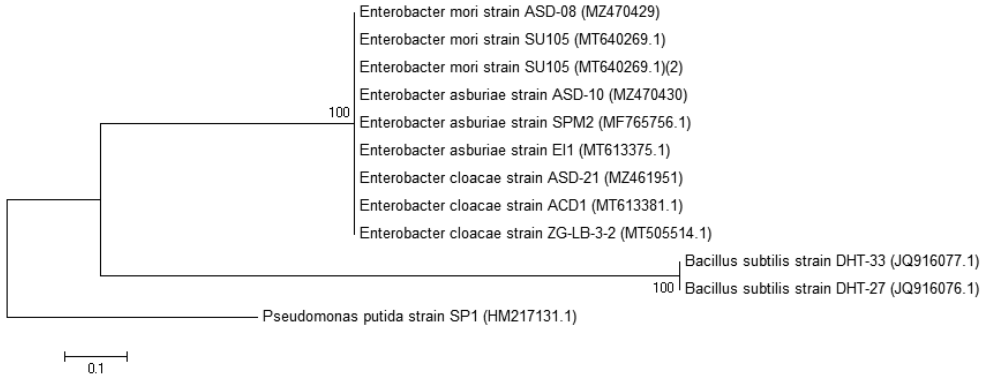

Fig. 2. Neighbor-joining phylogenetic trees based on 16S rDNA sequences of three selected endophytic bacterial strains compared to the closely related strains in the GenBank database. The percentage levels of bootstrap analysis of 1000 replicates are indicated at each node. Bar, 0.1 substitutions per nucleotide position. *Pseudomonas putida* strain PS1 was used as the outgroup strain. Access numbers of GenBank sequences are implied in brackets

Table 1. Effects of addition of endophytic P-solubilizing bacteria on soil alluvial fertility in dyke cultivated maize

Treatment	pH (1:2.5~ Soil:H_2O)	pH (1:2.5~ Soil:KCl)	N_{total} (%)	$N_{available}$ (mg NH_4^+ kg^{-1})	P_{total} (%)	$P_{available}$ (mg P kg^1)
100% P	6.15^{abc}	5.20	0.27	113.2^{a}	0.143^{bc}	73.3^{b}
75% P	6.22^{abc}	5.24	0.27	101.5^{b}	0.133^{d}	62.8^{c}
50% P	6.30^{a}	5.22	0.27	97.9^{bc}	0.158^{a}	58.5^{d}
25% P	6.28^{ab}	5.29	0.24	87.7^{d}	0.140^{cd}	55.3^{e}
75% P + EPSB	6.16^{abc}	5.23	0.27	114.4^{a}	0.155^{a}	76.5^{a}
50% P + EPSB	6.18^{abc}	5.22	0.23	98.5^{bc}	0.140^{bcd}	62.6^{c}
25% P + EPSB	6.08^{bc}	5.26	0.23	94.2^{c}	0.150^{a}	56.8^{de}
0% P + EPSB	6.04^{c}	5.26	0.23	84.9^{d}	0.158^{a}	51.7^{f}
0% P	6.08^{bc}	5.22	0.23	67.4^{e}	0.158^{a}	45.7^{g}
F	*	*ns*	*ns*	*	*	*
CV (%)	2.28	1.36	16.1	14.8	7.00	15.8

Note: 100% P: 100% P of recommendation fertilization formula (RFF); 75% P: 75% P of RFF; 50% P: 50% of RFF; 25% P: 25% P of RFF; 75% P + EPSB: 75% P plus a mixture of *E. mori* ASD-08, *E. asburiae* ASD-10 and *E. cloacae* ASD-21; 50% P + EPSB: 50% P of RFF plus a mixture of *E. mori* ASD-08, *E. asburiae* ASD-10 and *E. cloacae* ASD-21; 25%P + EPSB: 25%P of RFF plus a mixture of *E. mori* ASD-08, *E. asburiae* ASD-10 and *E. cloacae* ASD-21; 0%P+ EPSB: 0%P of plus a mixture of *E. mori* ASD-08, *E. asburiae* ASD-10 and *E. cloacae* ASD-21; 0%P: 0% P of RFF (*): significant difference at 5% following Duncan test, (ns): insignificant difference; EPSB: endophytic phosphorus solubilizing bacteria

to 55.3 mg P kg^{-1}. With the addition of the bacterial mixture of *Enterobacter* spp. ASD-21, ASD-08, and ASD-10, the available P content rose and peaked at treatment of 75% P plus bacterial mixture (76.5 mg P kg^{-1}) and was significantly higher than the control treatment with 100% P fertilizer of the recommended fertilizer formula (73.3 mg P kg^{-1}). Moreover, the decrease of P in treatments reducing P fertilizer levels with adding endophytic bacteria also obtained higher concentration in the same P level without adding endophytic bacteria. This means that the bacterial mixture could be solubilized to produce 25% P fertilizer. For N nutrition, reducing P fertilizer level from 100% P to 25% P caused the amount of N to fall from 113.2 mg N kg^{-1} to 87.7 mg N kg^{-1}. In order to confirm the impact of the bacteria on the soil, treatments with mixture of bacteria produced more available nitrogen and phosphorus than those without bacterial application. As a result, the mixture of the selected endophytic bacteria significantly dominated in the amount of both available P and N compared to no added bacteria in the case of no application of chemical P fertilizer.

Effects of endophytic P-solubilizing bacteria on P content, biomass and P uptake in grain and stover of maize. Modifications of fertilizers led to changes in the components of the maize stover. The biomass dropped as P fertilizer levels fell. Specifically, grain biomass fell from 64.9 g pot^{-1} when treated with 100% P of the RFF to 54.7 g pot^{-1} when

treated with 25% P of the RFF; for treatments with 75%, 50%, or 25% P of the RFF plus a mixture of EPSB, the value of treatment 75% P of RFF was higher than no added EPSB at the same P level. In case of those treatment 0% P plus mixture of ASD-08, ASD-10 and ASD-21 was significantly higher than no both P fertilizer and bacteria supplement, which all together showed the enhancing influence of the bacterial mixture on the growth of maize biomass and its capability to reduce 25% of the chemical fertilizer.

In other parts of the maize, including the stem, leaf, and root, the weight of its biomass had the same pattern as in grain (Table 2). In grain and leaves, for both P concentration and P uptake, treatment of 75% P of the RFF plus a mixture of ASD-08, ASD-10, and ASD-21 was remarkably higher than treatment with 100% P of the RFF. The numbers were, respectively, 1.07% compared to 0.86% and 0.72 g pot^{-1} compared to 0.56 g pot^{-1} in grain, and 0.98% compared to 0.59% and 0.045 g pot^{-1} compared to 0.011 g pot^{-1} in grain. In the stem and root, 75% P of the RFF plus a mixture of ASD-08, ASD-10, and ASD-21 was insignificantly different from a treatment of 100% P of the RFF. This proved that the addition of the selected bacteria improved P uptake in maize. Additionally, there was a significant down trend from 100% P of the RFF to 25% P of the RFF (0.62 to 0.51 g pot^{-1}) and from 100% P of the RFF plus a mixture of ASD-08, ASD-10, and ASD-21 to 25% P of the RFF plus a mixture of ASD-08, ASD-10, and ASD-21 (0.81 to 0.58 g pot^{-1}). Moreover, treatment with 0% P of the RFF plus a mixture of ASD-08, ASD-10, and ASD-21 was noticeably higher than that of the same P level and no endophytic bacteria, with 0.21 compared to 0.12 g N pot^{-1} in uptake (Table 2). This also showed the influence of bacterial supplement on N uptake as well.

Effects of endophytic P-solubilizing bacteria on maize growth. According to Table 3, except for number of leaves, for other parameters, including plant height, height of the first ear, and stem diameter, there were significant differences among treatments and their trends were similar. The trend was a reduction in the value at 2 sets of treatments, from 100% P of the RFF to 25% P of the RFF and from 75% P of the RFF plus a mixture of endophytic bacteria ASD-08, ASD-10, and ASD-21 to 25% P of the RFF plus a mixture of endophytic bacteria ASD-08, ASD-10, and ASD-21, along with the decrease of P fertilizer level.

There were also unremarkable differences between 100% P of the RFF and 75% P of the RFF plus a mixture of bacteria ASD-08, ASD-10, and ASD-21; 75% P of the RFF and 50% P of the RFF plus a mixture of bacteria ASD-08, ASD-10, and ASD-21; 50% P of the RFF and 25% P of the RFF plus a mixture of bacteria ASD-08, ASD-10, and ASD-21, which showed the potential for bacteria to replace 25% of chemical

Table 2. Effects of addition of endophytic P-solubilizing bacteria on P content, biomass and P uptake in grain and stover of maize

Treatment	P concentration (%)				Biomass (g pot^{-1})				P uptake (g pot^{-1})				Total P uptake (g pot^{-1})
	Grain	Stem	Leaf	Root	Grain	Stem	Leaf	Root	Grain	Stem	Leaf	Root	
100% P	0.86^{cde}	0.11^{a}	0.59^{c}	0.63^{abc}	64.9^{a}	10.0^{ab}	4.45^{b}	3.97^{ab}	0.56^{b}	0.011^{a}	0.026^{d}	0.025^{a}	0.62^{b}
75% P	0.92^{bc}	0.09^{bc}	0.65^{c}	0.66^{ab}	61.1^{b}	9.79^{ab}	4.05^{d}	3.95^{ab}	0.56^{b}	0.009^{e}	0.027^{d}	0.026^{a}	0.63^{b}
50% P	0.81^{e}	0.11^{a}	0.60^{c}	0.68^{ab}	54.4^{d}	9.45^{bc}	4.07^{cd}	3.88^{ab}	0.44^{c}	0.010^{abc}	0.025^{d}	0.027^{a}	0.50^{c}
25% P	0.83^{e}	0.11^{a}	0.70^{bc}	0.60^{bc}	54.7^{cd}	9.04^{c}	3.95^{d}	3.54^{c}	0.45^{c}	0.010^{bc}	0.028^{d}	0.021^{b}	0.51^{c}
75% P + EPSB	1.07^{a}	0.11^{ab}	0.98^{a}	0.68^{a}	67.3^{a}	10.4^{a}	4.66^{a}	4.09^{a}	0.72^{a}	0.011^{ab}	0.045^{a}	0.028^{a}	0.81^{a}
50% P + EPSB	0.94^{b}	0.09^{bc}	1.06^{a}	0.56^{cd}	57.5^{cd}	10.1^{ab}	4.40^{b}	3.84^{b}	0.54^{b}	0.010^{abc}	0.047^{a}	0.022^{b}	0.62^{b}
25% P + EPSB	0.90^{bcd}	0.09^{c}	0.82^{b}	0.49^{d}	57.8^{c}	9.66^{bc}	4.21^{c}	3.86^{b}	0.52^{b}	0.010^{bc}	0.034^{c}	0.019^{b}	0.58^{b}
0% P + EPSB	0.81^{e}	0.10^{bc}	1.07^{a}	0.39^{e}	18.2^{e}	7.75^{d}	3.62^{e}	2.62^{d}	0.15^{d}	0.007^{d}	0.039^{b}	0.010^{c}	0.21^{d}
0% P	0.86^{de}	0.09^{c}	0.70^{bc}	0.35^{e}	9.8^{f}	7.54^{d}	2.65^{f}	1.57^{e}	0.08^{e}	0.007^{d}	0.019^{e}	0.005^{d}	0.12^{e}
F	*	*	*	*	*	*	*	*	*	*	*	*	*

Note: 100% P: 100% P of recommendation fertilization formula (RFF); 75% P: 75% P of RFF; 50% P: 50% of RFF; 25% P: 25% P of RFF; 75% P + EPSB: 75% P plus a mixture of *E. mori* ASD-08, *E. asburiae* ASD-10 and *E. cloacae* ASD-21; 50% P + EPSB: 50% P of RFF plus a mixture of *E. mori* ASD-08, *E. asburiae* ASD-10 and *E. cloacae* ASD-21; 25%P + EPSB: 25%P of RFF plus a mixture of *E. mori* ASD-08, *E. asburiae* ASD-10 and *E. cloacae* ASD-21; 0%P+ EPSB: 0%P of plus a mixture of *E. mori* ASD-08, *E. asburiae* ASD-10 and *E. cloacae* ASD-21; 0%P: 0% P of RFF (*): significant difference at 5% following Duncan test, (ns): insignificant difference; EPSB: endophytic phosphorus solubilizing bacteria

Table 3. Effects of addition of endophytic P-solubilizing bacteria on maize growth

Treatment	Plant height, cm	Height of appeared ear, cm	Number of leaves	Stem diameter, cm
100% P	183.0^{ab}	71.8^{a}	11.8	1.08^{ab}
75% P	178.0^{c}	66.3^{b}	11.8	1.00^{bc}
50% P	174.8^{cd}	65.8^{b}	11.5	1.01^{bc}
25% P	171.3^{d}	63.3^{b}	11.3	0.90^{c}
75% P + EPSB	187.3^{a}	74.3^{a}	11.3	1.16^{a}
50% P + EPSB	184.8^{a}	74.0^{a}	10.8	0.99^{bc}
25% P + EPSB	179.3^{bc}	65.3^{b}	11.8	1.06^{ab}
0% P + EPSB	163.8^{e}	59.0^{c}	10.8	0.90^{c}
0% P	151.3^{f}	53.3^{d}	11.3	0.74^{d}
F	*	*	*ns*	*
CV (%)	6.5	10.6	6.3	14.0

Note: 100% P: 100% P of recommendation fertilization formula (RFF); 75% P: 75% P of RFF; 50% P: 50% of RFF; 25% P: 25% P of RFF; 75% P + EPSB: 75% P plus a mixture of *E. mori* ASD-08, *E. asburiae* ASD-10 and *E. cloacae* ASD-21; 50% P + EPSB: 50% P of RFF plus a mixture of *E. mori* ASD-08, *E. asburiae* ASD-10 and *E. cloacae* ASD-21; 25%P + EPSB: 25%P of RFF plus a mixture of *E. mori* ASD-08, *E. asburiae* ASD-10 and *E. cloacae* ASD-21; 0%P+ EPSB: 0%P of plus a mixture of *E. mori* ASD-08, *E. asburiae* ASD-10 and *E. cloacae* ASD-21; 0%P: 0% P of RFF (*): significant difference at 5% following Duncan test, (ns): insignificant difference; EPSB: endophytic phosphorus solubilizing bacteria

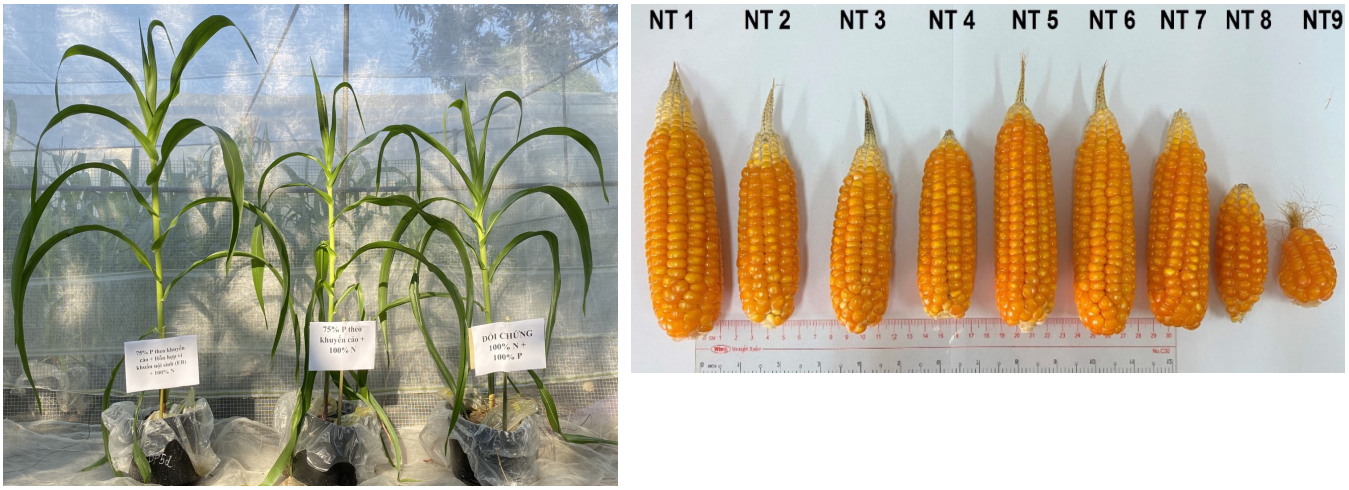

Fig. 3. (a) Growth of maize after 55 days planting in treatments 75% P plus mixture of endophytic bacteria of phosphorus solubilization, 75% P as RFF and 100% P as RFF; (b) Effects of endophytic bacteria of phosphorus solubilization on Cob maize in alluvial soil in dyke

NT1: Control, 100% P of recommendation fertilizers formula (RFF), NT2: 75% P of RFF, NT3 50% P of RFF, NT4: 25% P of RFF, NT5: 75% P of RFF and mixture of Enterobacter spp. ASD-08, ASD-10 and ASD-21, NT6: 50% P of RFF and mixture of Enterobacter spp. ASD-08, ASD-10 and ASD-21, NT7: 25% P of RFF and mixture of Enterobacter spp. ASD-08, ASD-10 and ASD-21, NT8: 0% P of RFF and mixture of Enterobacter spp. ASD-08, ASD-10 and ASD-21, NT9: 0% P of RFF and no endophytic bacteria

P fertilizer for maize. Moreover, the significant difference between treatment with 0% P of the RFF plus bacterial supplement and 0% P of the RFF plus no added bacteria also support this proposal. For example, for plant height, from 100% P of the RFF to 25% P of the RFF, the number dropped from 183.0 cm to 171.3 cm, and from 75% P of the RFF plus a mixture of bacteria ASD-08, ASD-10, and ASD-21 to 25% P of the RFF plus a mixture of bacteria ASD-08, ASD-10, and ASD-21, the values were from 187.3 to 179.3 cm (Figure 3a); and treatment with 0% P of the RFF plus bacterial supplement was significantly higher than 0% P of the RFF plus no added endophytic bacteria, with 163.8 cm compared to 151.3 cm, respectively.

Effects of endophytic P-solubilizing bacteria on yield components and maize grain yield. In addition to supporting the growth of maize, bacterial application enhanced the

yield components and grain yield as well. An increase was recorded in the size of the ear and the number of kernels. Although kernel weight and the number of rows did not measurably change, the number of kernels was higher. Additionally, along with the decrease of P fertilizer level, the size of ears got smaller from 10.8 cm in length with a treatment of 100% P of the RFF to 8.0 cm with the treatment of 25% P of the RFF and from 3.8 cm to 3.4 cm in width, respectively. The number of kernels followed the same trend as the size of the ears. The 100-kernel weight ranged from 27.0–31.3 g. However, grain yield was influenced by bacterial application. Just like other parameters, the down trend appeared along with the reduction of P fertilizer levels, and the dominance of treatment with 0% P of the RFF plus bacterial supplement against 0% P of the RFF plus no added bacteria also remained. However, in this parameter, P fertilization with the soil bacteria supplement was significantly higher than that of the control treatment, 100% P of the RFF, with 55.5 g pot^{-1} compared to 52.2 g pot^{-1}, respectively (Table 4). This gave evidence that the addition of soil bacteria from maize root in fertilizers could be used to reduce more than 25% of the chemical P fertilizer.

Discussion

Soil fertility for maize cultivation in Vietnam is limited by dikes. Thus, supplementation with various P sources is vital. For sustainable agriculture, strategies focus on biological methods for providing P (Jat et al., 2021). In alluvial soil, P is present for plants; however, it fails to meet the requirements of maize due to shortages from flooding and insoluble forms, such as $AlPO_4 \cdot 2H_2O$, $FePO_4 \cdot H_2O$, and $Ca_3(PO_4)_2$ (Zhang et al., 2021). In this research, endophytic bacteria were adapted under different conditions, such as alkaline and acidic environments, and were able to solubilize the insoluble P compounds. Sixteen out of the 62 isolates possessed the ability solubilizes various insoluble P compounds (Figure 1). Specifically, strain ASD-21 produced as much available P for plant as strains ASD-02, ASD-13, ASD-15, ASD-18, ASD-21, ASD-40, and ASD-48, but it also had a higher ability to produce available P from Fe-P and Ca-P sources. Strains ASD-08 and ASD-21 released the highest concentration, with 39.8–41.5 mg P L^{-1} from Fe-P form, while ASD-08 and ASD-21 had the highest content, with 46.6–51.3 mg P L^{-1} from Ca-P compound after 48-h incubation. As a result, strains ASD-08, ASD-10, and ASD-21 were identified as *Enterobacter* spp. for application in alluvial soil in dikes. Although many endophytic bacteria have been selected to produce available P for various plants (Chen et al., 2021; Rana et al., 2021), this research selected potential strains that could contribute to sustainable maize cultivation under low P conditions in Vietnam.

For maize growth, besides N, P plays a crucial role in the sustainable development of maize and its yield. Moreover, P is the main yield-limiting nutrient in many sites worldwide (El-Batran et al., 2020). Therefore, with a decrease of P, maize quality reduces in many parameters, and this study was not an exception. According to Table 2, biomass in parts of the plant declined along with the downward trend of P level from 100% to 25%. Grain yield dropped from 64.9 > 61.1 > 54.4 ~ 54.7 g pot^{-1}, corresponding to P levels of 100%, 75%, 50%, and 25%, respectively (Figure 3b). The same re-

Table 4. Effects of addition of endophytic P-solubilizing bacteria on yield components and maize grain yield

Treatment	Ear length, cm	Ear diameter, cm	Number of row/ ear, row	Number of kernels/ row, kernels	100-kernel weight, g	Grain yield, g pot^{-1}
100% P	10.8^{a}	3.8^{b}	10.0^{ab}	21.0^{a}	31.2^{a}	52.2^{b}
75% P	9.2^{bc}	3.6^{c}	9.8^{b}	17.5^{b}	31.1^{a}	42.6^{c}
50% P	8.4^{cd}	3.5^{c}	10.0^{ab}	15.0^{b}	29.7^{ab}	37.2^{d}
25% P	8.0^{d}	3.4^{c}	9.8^{b}	16.3^{b}	29.7^{ab}	35.0^{d}
75% P + EPSB	11.1^{a}	3.9^{ab}	11.0^{a}	22.5^{a}	30.9^{a}	55.5^{a}
50% P + EPSB	10.4^{a}	4.0^{a}	10.5^{ab}	20.5^{a}	31.4^{a}	53.0^{b}
25% P + EPSB	9.4^{b}	3.8^{ab}	9.5^{b}	17.0^{b}	31.3^{a}	42.8^{c}
0% P + EPSB	5.5^{e}	3.0^{d}	7.7^{c}	9.6^{c}	28.9^{ab}	20.2^{e}
0% P	4.4^{f}	2.9^{d}	7.5^{c}	9.5^{c}	27.0^{b}	16.1^{f}
F	*	*	*	*	*	*
CV (%)	26.4	11.0	13.6	28.9	8.0	34.2

Note: 100% P: 100% P of recommendation fertilization formula (RFF); 75% P: 75% P of RFF; 50% P: 50% of RFF; 25% P: 25% P of RFF; 75% P + EPSB: 75% P plus a mixture of *E. mori* ASD-08, *E. asburiae* ASD-10 and *E. cloacae* ASD-21; 50% P + EPSB: 50% P of RFF plus a mixture of *E. mori* ASD-08, *E. asburiae* ASD-10 and *E. cloacae* ASD-21; 25%P + EPSB: 25%P of RFF plus a mixture of *E. mori* ASD-08, *E. asburiae* ASD-10 and *E. cloacae* ASD-21; 0%P+ EPSB: 0%P of plus a mixture of *E. mori* ASD-08, *E. asburiae* ASD-10 and *E. cloacae* ASD-21; 0%P: 0% P of RFF (*): significant difference at 5% following Duncan test, (ns): insignificant difference; EPSB: endophytic phosphorus solubilizing bacteria

duction applied to stem, leaves, and roots. This meant that the application of P fertilizer contributed to an increase in maize grain yield. The drawbacks of decreased P appeared not only in the inner qualities, as shown in Table 3 and Table 4, but also in the height and width of plant, the size of ear, and the number of kernels per row. Altogether, even though kernel weight decreased insignificantly, overall grain yield decreased with values of 52.2 > 42.6 > 37.2 ~ 35.0 g pot^{-1}, following a decline from 100%, 75%, 50%, and 25% of P applied to the plant. This is similar to the results of Masood et al. (2011). When the P levels applied to maize were reduced from 100 to 50 to 0 kg ha^{-1}, the grain yield dropped from 2415 > 1816 ~ 1305 kg ha^{-1}, respectively. However, with the applied P content higher at 150 and 200 kg ha^{-1}, the yield changed insignificantly. Furthermore, the changes of P level in both studies did not significantly affect the weight of grain. Although in this study, the bacterial mixture applied treatments did raise the values in yield parameters, the same descending trend also appeared along with the decrease in P levels at these parameters. Ultimately, the evidence above indicated the undeniable influence of P level on the yield of maize, i.e., the reduction in P levels led to a decline in maize yield.

Unlike N, plants cannot obtain P from the atmosphere via bacteria (Ezawa et al., 2002). P usually comes in the form of free anions or attached to metals cations like Fe, Ca, and Al (Halajnia et al., 2009). Therefore, P uptake is limited in plants. However, many studies have stated the possibility of microbes, especially bacterial species, for enhancing P uptake in plants via P solubilization. In accordance with Qarni et al. (2021), *Penicillium* sp. contributed to reaching maximum P-uptake in maize. Furthermore, the ability to provide P for maize from microbes increasing P uptake in plants is reported by the combination of inorganic phosphorus with P-solubilizing bacteria and poultry manure (Zafar et al., 2011), *Streptomyces* sp. (Battini et al., 2017), *Bacillus* sp. (Wahid et al., 2020), and for PSB only (Adnan et al., 2020). This result was consistent with those studies. In Table 2, the treatment with only the bacterial mixture of ASD-08, ASD-10, and ASD-21 had a P-uptake value of 0.15 g P pot^{-1} in grain, 0.039 g P pot^{-1} in leaves, and 0.01 g P pot^{-1} in root, which was significantly higher than those of treatments without the bacterial mixture, which were 0.08, 0.019, and 0.005 g P pot^{-1}, respectively. Nevertheless, P uptake in the stem changed insignificantly between treatments with and without bacteria addition.

Moreover, further study has pointed out that P provided by bacteria improved soil properties and maize yield. To be more specific, in Table 1, with the addition of bacterial mixture to fertilizers, the P available in the soil rose, i.e., the treatment with 75% P of the RFF plus the bacterial mixture had a higher amount of available P than that of treatment with 100% P of the RFF plus no bacteria: 76.5 mg P kg^{-1} compared to 73.3 mg P kg^{-1}, respectively. According to Qarni et al. (2021), the application of phosphorus-solubilizing microbes increased soluble P at the post-harvest stage. Moreover, the use of phosphorus-releasing bacteria *Kluyvera intermedia* or *Azospirillium brasilense* improved the availability of P in the soil for maize (Hafez et al., 2021; Liu et al., 2021) which is considered as a main factor for increased maize yield (Arif et al., 2021).

Moreover, the bacteria were inoculated into kernels, so they were able to produce available P for maize in the early stage, which has a positive correlation with optimum nutrient uptake to increase yield (Pedersen et al., 2021). Following the order of descending in P levels, available P concentration of treatments with 50 (Table 1). Moreover, the application of PSB remarkably raises the yield of maize as well (Iqbal et al., 2019; Khan, 2015). Sharing the same trend as available P content, grain yield improved with P produced by bacteria (Table 4). However, in this parameter, treatments with 75%, 50%, and 25% P of the RFF plus the bacteria mixture were remarkably higher than treatments with 100%, 75%, and 50% P of the RFF. Ultimately, it can be inferred that a mixture of bacteria strains ASD-08, ASD-10, and ASD-21 is able to replace 25% of chemical P fertilizer without reducing maize P uptake, soluble P content in soil, and grain yield. However, a mixture of PSM and plant-growth-promoting rhizobacteria is capable of replacing 50% of P fertilizers without significantly lowering the grain yield of maize (Yazdani et al., 2009). Overall, the addition of selected endophytic bacterial can effectively replace 25% of the mineral P fertilizer in alluvial soil in dikes.% P of the RFF plus bacteria and 25% P of the RFF plus bacteria were correspondingly and significantly different from treatments with 75% P of the RFF and 50% P of the RFF without the addition of bacteria

Conclusion

Seventy-two strains of endophytic bacteria in hybrid maize root were obtained. Among them, 16 strains were able to live under a low-pH condition, and strains with potential to solubilize insoluble P fractions were identified as *Enterobacter* spp. ASD-21, ASD-08, and ASD-10.

A mixture of the selected three endophytic bacteria strains helped to increase soluble P content in the soil and altered 25% of the phosphorus fertilizer but still maintained hybrid maize growth, yield, and P uptake in greenhouse conditions.

Acknowledgements

This research is funded by Vietnam National University HoChiMinh City (VNU-HCM) under grant number C2021-16-05.

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Received: May 5, 2021; *Accepted:* October 20, 2021; *Published:* April, 2022