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Survey on the characteristics and ability to promote plant growth of indigenous bacteria isolated from root nodules of mung bean *Vigna radiata* (L.) R. Wilczek grown in Thanh Duc, Tay Ninh province, Vietnam

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Abstract

This study isolated bacteria from mung bean root nodules in Thanh Duc, Tay Ninh, and evaluated their growth-promoting effects on 2-month-old plants grown in Leonard jars under garden conditions. Nodule surfaces were disinfected, with sterility confirmed via TYGA medium plating of the final rinse. Ammonium, soluble phosphate, and IAA concentration were subsequently determined through colorimetric analysis. The most promising strains were identified using MALDI-TOF MS. Finally, an in vivo assay was conducted using Leonard jars containing half-strength modified Hoagland's solution (with and without nitrogen) to measure the impact of single and dual inoculations on two-month-old mung bean plants. A total of 32 strains were isolated and all exhibited ability to fix nitrogen, solubilize phosphate, and synthesize IAA with an average concentration of 0.53 – 1.13 mg/L for NH_4^+, 109.63 – 143.17 mg/L for P_2O_5 and 0.51 – 4.03 mg/L for IAA, respectively. The three most effective strains-SX1, SX22 and SX27 were identified as *Paenibacillus barcinonensis*, *Bacillus pumilus* and *Mesobacillus jeotgali*. In vivo plant growth promoting results on mung bean plants showed that strain SX27, when inoculated individually, had the best impact on shoot biomass under growth conditions using half-strength modified Hoagland's solution without nitrogen. Meanwhile, the simultaneous inoculation of two strains SX1 and SX27 produced the highest values of shoot height, shoot biomass and root biomass under growth conditions using half strength modified Hoagland's solution

Keywords: Hoagland's Solution; Leonard Jar; MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization - Time Of Flight Mass Spectrometry); Mung Bean [*Vigna Radiata* (L.) R. Wilczek]; Tay Ninh Province; Plant Growth Promoting

1. Introduction

The use of legumes in agricultural systems to utilize biological nitrogen fixation (BNF) has long been considered an economically viable and environmentally friendly way to reduce input costs and improve soil nutrient content. BNF from symbiotic bacteria in legumes was estimated to account for about 8.0×10^{10} kg, equivalent to 20 – 200 kg N fixed ha^{-1} $year^{-1}$ [1]. The legume-rhizobia symbiotic system was considered the main source of BNF. The use of rhizobia fertilizer for lentil, soybean and mung bean reduced nitrogen input by about 12 – 15 kg/ha. It also helped increase crop productivity and soil fertility to the highest level [2].

In the context of non-symbiotic BNF, free-living bacteria capable of nitrogen fixation have been increasingly discovered and published. BNF properties along with other abilities such as phosphate solubilization, phytohormone production, activation of Induced Systemic Resistance (ISR) of these bacteria have created the concept of plant growth promotion, which has made this group of subjects a bright candidate for sustainable agricultural development. Beneficial free-living soil bacteria, commonly known as plant growth-promoting rhizobacteria (PGPR), have the ability to colonize

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rhizosphere soil, root surface (rhizoplane) or within root tissues (endophytes) [3]. In legumes, it has been observed that common PGPRs such as *Pseudomonas*, *Bacillus*, *Azotobacter* interact with rhizobia populations in the rhizosphere and helped create a zone where microorganisms benefited from additional nutrient resources. These PGPRs also interacted synergistically with plants as well as with inoculated rhizobia to influence nodule formation, nitrogen fixed capacity and performance [2, 4].

A total of 34 non-rhizobial bacteria isolates were obtained from the root nodules of three legume species (*Cicer arietinum* L., *Vicia faba* L., and *Vigna unguiculata* L. Walp), of which 31 isolates were capable of nitrogen fixation. IAA production fluctuated from 2.68 to 8.94 mg ml^{-1}, with the highest yield observed in isolate C1 *Stenotrophomonas maltophilia*. Two isolates demonstrated the ability to solubilize phosphate indicated by the formation of a halo around its colony consisting of C1 *Stenotrophomonas maltophilia* and Vf1 *Bacillus cereus* [5]. In 101 strains isolated from mung bean, in addition to rhizobium including *Bradyrhizobium*, *Rhizobium*, *Mesorhizobium*, *Ensifer*, there were also non-rhizobium genera including *Leifsonia*, *Bacillus*, *Agrobacterium*, *Mycolicibacterium*, *Kaistia*. In particular, the mung bean plants were inoculated with strain B071 *Bradyrhizobium yuanmingense* showed the best increase in shoot dry weight [6]. Nodule-assisting bacteria were thought to be either free-living PGPRs or endophytic bacteria. Endophytic bacteria resided intercellularly or intracellularly in host tissues and thus had an advantage through their ability to protect against environmental stresses and microbial competition [4]. Recent studies on interactions between legumes and rhizobacteria have been limited to soybean (*Glycine max* L.), cowpea (*Vigna unguiculata* L.), chickpea (*Cicer arietinum* L.), the common bean (*Phaseolus vulgaris* L.), and red clover (*Trifolium pretense* L.) [4].

In this study, surface disinfection helped remove PGPRs in soil and rhizoplane. The plant samples collected were nodules of mung bean plants grown in Thanh Duc commune, Tay Ninh province, a locality in the Southeast region of Vietnam. The cultivated area of mung bean in the Southeast region was 9,610 hectares. Productivity reached 1,237 tons per hectare, ranking third in the country (Statistics in 2015 by the National Institute of Agricultural Planning and Projection) [7]. To date, there have been very few publications on bacteria related to mung bean nodules grown in Vietnam and this region. Therefore, we isolated bacteria in mung bean nodules and studied their ability to fix nitrogen, solubilize phosphate, and produce IAA in vitro. To evaluate the effectiveness of bacteria in promoting the growth of mung bean plants, the Leonard jar growing experiment was conducted. The Leonard jar and its improved forms were self-watering growing systems. These designs were used to study the effectiveness of rhizobia on legumes, including mung bean. Plant growing solution was a N-free liquid medium for the purpose of evaluating the nitrogen fixation efficiency of legume symbiotic bacteria [8]. A hydroponic growing solution developed and improved by Hoagland and Arnon (1938; 1950) called Hoagland nutrient solution was conveniently used in the Leonard jar model. In addition to the full strength solution, half strength Hoagland's solution and half strength Hoagland's deficient in nitrogen were also used, which could assist in evaluating the N-fixing ability of plants and microorganisms inoculated into plants [9, 10]. The P-free nutrient solution was also used in plant watering along with the addition of tricalcium phosphate to the culture section of the Leonard jar for designs that evaluate the phosphate solubilization of bacteria [11].

2. Material and methods

2.1. Samples collection and preparation

Samples were collected from 3 mung bean fields in Thanh Duc commune, Tay Ninh province, with coordinates of 11° 11 '50.6" North and 106° 12' 46.4" East. The selected plants were in a period of strong growth. Samples were collected including the root system and the soil around the roots with a diameter of 15 cm and a depth of 15 cm. The stems were cut off from a height of over 10 cm from the roots [12].

At the laboratory, the roots were washed under strong tap water. Large, healthy nodules without visible signs of infection were collected. These samples were then sterilized according to the procedure of [12]. Ethanol solution 70° was poured into the flask to cover the sample and gently shaken for 10 minutes. Samples were washed with sterile water 3 times (5 minutes/time). Hypochloride calcium 2% was added to the flask and gently shaken for 10 minutes. Samples were washed with sterile water 4 times (5 minutes/time). Water from the last wash was taken in a volume of 100 µL and spread evenly onto TGY (tryptone-glucose-yeast extract) agar plates, incubated at 30°C. After 24 – 48 hours of observation, if no bacteria growth appeared, it proved that the sample had met sterilization requirements [12].

2.2. Isolation and morphological characterization of bacterial isolates

The sterilized sample was placed in a sterile mortar and pestle, pounded, and 1 mL of sterile distilled water was added to the mortar [12]. The sample was stirred well and 100 µL of extract was pipetted, then dropped onto LB agar plates and spread evenly. Samples were incubated at 30°C. After 24 – 48 hours, different colonies growing on the agar surface

were collected and streaked until pure colonies were obtained. The purity of bacterial strains was checked by observing under a microscope. The pure strains were transferred to test tubes containing agar slants for storage [12].

Colony morphology including form, elevation, margin, surface and size were recorded after 24 hours of growth on LB agar plates at 30°C. Cell size, shape, and motility were observed by light microscopy. The Gram reaction was performed [13].

2.3. Quantification of nitrogen fixation, phosphate solubilization and IAA production

Before the quantitative survey, bacterial suspensions were prepared by culturing in liquid media: Burk's N-free medium for ammonia and IAA quantification experiments, and NBRIP medium for soluble phosphate quantification experiments. After 48 hours, the growing bacterial suspension was collected and adjusted to turbidity according to the McFarland standard of 0.5 (1.5×10^8 CFU/mL) [13].

Each 250 μL of bacterial suspension adjusted to turbidity according to McFarland 0.5 standard was added to 10 mL of free Burk's N or NBRIP broth and incubated at 120 rpm, 30°C. Suspensions were collected periodically: 2, 4, 6, 8 DAI (days after inoculation) for quantification of nitrogen fixation and IAA production; and 5, 10, 15, 20 DAI for quantification of phosphate solubilization [13].

At the time of quantification, each 1.5 mL of suspension was collected and placed in an Eppendorf tube for ultracentrifugation at 12,000 rpm, for 5 minutes. The supernatant was collected for colorimetric experiments with corresponding reagents [13].

For quantification of nitrogen fixation, reagent formula described by Solarzano (1969) were applied [14]. Sample and reagent ratio was 5:1 (v/v). The absorbance of the sample was measured by spectrophotometer with 640 nm wavelength after 30 minutes of reaction stability. Quantitative survey of phosphate-solubilizing was conducted with the reagent formula described by Murphy and Riley (1962) [15] and the sample and reagent ratio was also 5:1 (v/v). The The absorbance at 880 nm was measured after 15 minutes of reaction stability. The Fe-H_2SO_4 reagent formula was according to [16], the sample and reagent ratio was 5:1 (v/v), the wavelength was 530 nm and the reaction stabilization condition was 15 minutes in the dark [13].

2.4. Identifying selected isolates by their molecular fingerprints

This experiment was limited to selected strains with the best results in terms of nitrogen fixation, phosphate solubilization and IAA production. Isolates were grown on LB medium for 24 hours then identified by MALDI-TOF mass spectrometry to determine the unique protein fingerprint of an organism. The experiment was conducted by Center for Biological Science and Technology, Ho Chi Minh City University of Natural Sciences.

Species-level identification was considered reliable when score values ranged from 2.300 to 3.000. Genus-level identification and probable species identification were accepted for scores between 2.000 and 2.299. Scores between 1.700 and 1.999 indicated probable genus identification, whereas scores below 1.700 were considered unreliable [13].

2.5. Evaluation of the ability to promote plant growth in vivo on mung bean plants grown in Leonard jars

Mung beans (variety DX208, produced by Tre Viet Seeds Company, Vietnam) were surface sterilized for 30 seconds with 70% alcohol, followed by sodium hypochlorite 3% for 10 minutes and finally washed with sterile distilled water (seven times). Each 20 seeds were cultured in each agar plate (10 g agar and 12 g sucrose per liter) and allowed to germinate aseptically in the dark at room temperature [17]. Seeds that did not show signs of infection would be transferred to a 500 mL culture flask (5 plants/flask) containing agar medium (10 g agar and 12 g sucrose per liter) and placed at room temperature, with a lamp lighting regime of 12 hours/day, illuminance of 2,000 lux. The purpose of transplanting seeds twice was to control and reduce the infection rate of seeds before growing into seedlings to be put into Leonard jars.

After 2 days, when the roots grew 1 – 2 cm, the seedlings without signs of infection were collected and soaked in a bacterial suspension (SX1; SX27; SX1+SX27) with a density of 12×10^8 CFU/mL for 30 minutes before transferring to a Leonard jar. The non-bacterial control was sterile distilled water (Deionized water).

The Leonard jar was assembled as described by [18] with a substrate consisting of sand and perlite particles in a 1:1 ratio. Plant growing solutions include half strength modified Hoagland's solution (Hoagland 1/2) [19] and nitrogen-free modified Hoagland's solution (Hoagland 1/2 N-free) [20]. The non-nutritive control was sterile distilled water (Deionized water) (Figure 1)

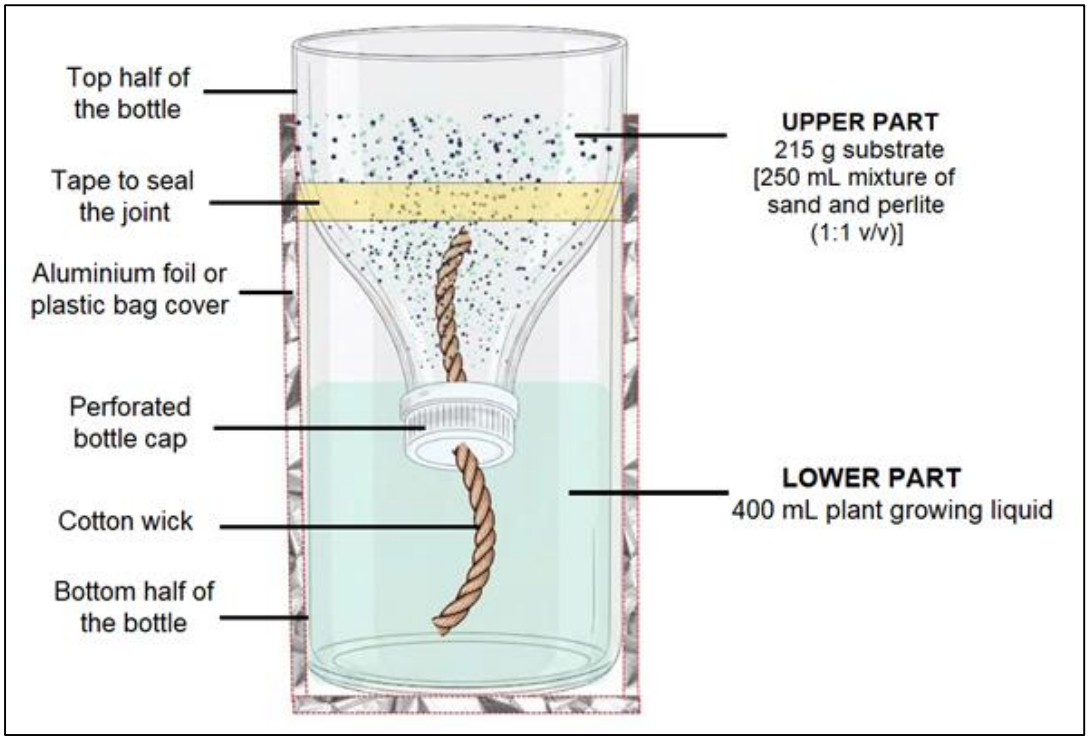

Figure 1 Schematic of the Leonard jar system

There was a total of 12 treatments repeated 5 times (5 growing jars) creating 60 experimental units (60 growing jars). These jars were arranged completely randomly under the garden's natural temperature and light conditions, with protective nets to prevent animals from destroying plants.

Sixty days after sowing, the entire mung bean plants were harvested and the roots were washed under running water. Shoot height was measured from the root collar to the tip of the apical bud of a tree's main stem. Root length was measured from the root collar to the tip of the longest root. For fresh weight, samples were stored in nylon bags at room temperature to avoid evaporation and weighed immediately. For dry mass, the sample was dried at 70°C and weighed every 2 hours until the mass remained constant.

2.6. Processing statistics

The experiment was arranged in completely randomized design, repeated 3 times for in vitro experiments and 5 times for in vivo experiments. All quantitative data were analyzed for One-way ANOVA (Analysis of Variance) and Least Significant Difference (LSD) with $\alpha=0.05$ using IBM SPSS Statistics 26.0.

3. Results and discussion

3.1. Morphological characterization of bacterial isolates

There were 32 strains isolated from 3 pooled samples that each included all nodules obtained from 5 mung bean plants grown in a field. Characteristics of the strains were described in detail in Table 1. The main morphological characteristics of the colonies were white color (75.0%); circular shape (90.6%), entire margin (71.9%), raised elevation (84.4%) (Figure 2). Diameters of colonies were measured ranging from 0.5 to 5.0 mm. The cells were predominantly cocci (62.5%) and Gram-negative (68.8%).

Table 1 Morphological characteristics of colonies and cells of 32 isolates

Strain	Colony characteristics					Cell characteristics	
	Color	Shape	Margin	Elevation	Diameter (mm)	Cell shape	Gram
SX1	Cream	Irregular	Irregular	Raised	5.0	Bacillus	+
SX2	White	Circular	Lobate	Raised	2.3	Coccus	+
SX3	White	Circular	Entire	Raised	1.8	Coccus	-
SX4	White	Circular	Lobate	Raised	2.5	Coccus	-
SX5	White	Circular	Entire	Raised	2.2	Coccus	-
SX6	White	Circular	Entire	Raised	0.9	Coccus	-
SX7	White	Circular	Entire	Raised	2.3	Bacillus	-
SX8	White	Circular	Entire	Raised	4.0	Bacillus	-
SX9	White	Irregular	Entire	Raised	1.2	Coccus	+
SX10	White	Circular	Entire	Raised	1.0	Coccus	-
SX11	White	Circular	Entire	Raised	0.5	Coccus	-
SX12	White	Circular	Entire	Depressed	2.1	Coccus	-
SX13	White	Circular	Entire	Raised	2.2	Bacillus	+
SX14	White	Circular	Entire	Raised	1.4	Coccus	+
SX15	White	Circular	Entire	Raised	4.3	Bacillus	-
SX16	Yellow	Circular	Lobate	Raised	0.8	Bacillus	+
SX17	White	Circular	Entire	Raised	1.5	Coccus	-
SX18	White	Circular	Entire	Raised	1.2	Bacillus	-
SX19	White	Circular	Entire	Raised	3.5	Bacillus	-
SX20	Yellow	Circular	Entire	Depressed	1.3	Coccus	-
SX21	Yellow	Circular	Entire	Depressed	1.0	Bacillus	+
SX22	Cream	Circular	Irregular	Raised	2.2	Bacillus	+
SX23	Yellow	Circular	Serrated	Raised	1.5	Bacillus	-
SX24	White	Circular	Entire	Raised	2.1	Coccus	-
SX25	White	Circular	Entire	Raised	2.5	Coccus	-
SX26	White	Irregular	Entire	Raised	2.1	Coccus	+
SX27	Cream	Circular	Irregular	Raised	1.2	Bacillus	+
SX28	Yellow	Circular	Serrated	Depressed	1.6	Coccus	-
SX29	White	Circular	Entire	Raised	2.0	Coccus	-
SX30	White	Circular	Lobate	Depressed	1.7	Coccus	-
SX31	White	Circular	Entire	Raised	2.3	Coccus	-
SX32	White	Circular	Entire	Raised	1.8	Coccus	-

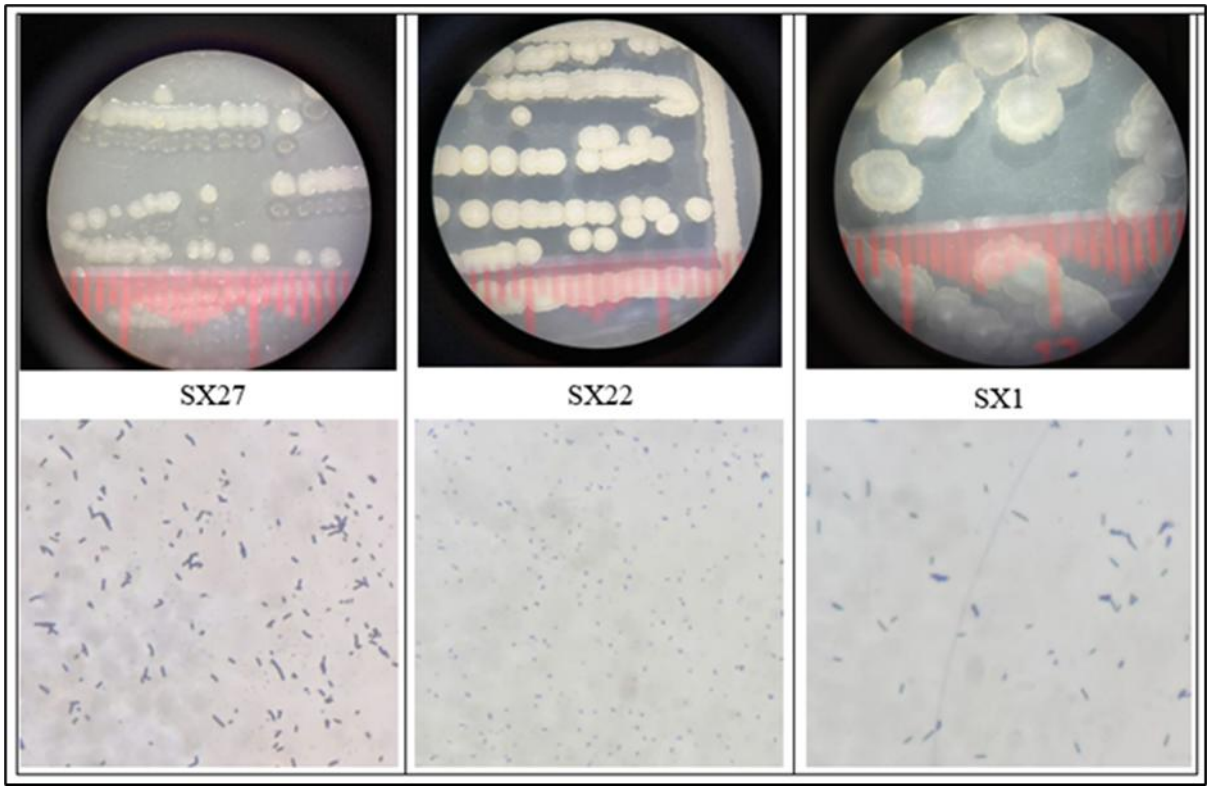

Figure 2 Colony and cell morphology of the 3 strains SX27, SX22, SX1

PGPR that were widely exploited as microbial inoculants in agricultural production were the genera *Pseudomonas* and *Bacillus* [21]. However, Gram-positive bacteria capable of forming endospores such as *Bacillus* and *Paenibacillus* also have the advantage of surviving in harsh environmental conditions and were favorable for the development of products used in agricultural production, including biofertilizers and biological pesticides based on their properties of improving crop productivity as well as soil health [22-24].

3.2. Quantitative results of nitrogen fixation, phosphate solubilization and IAA production

Quantitative results of in vitro PGP (Plant-Growth-Promoting) ability including nitrogen fixation, phosphate solubilization and IAA production of 32 strains were presented in Table 2. The average values of the four sampling periods of 32 treatments corresponding to 32 isolates ranged from 0.53 – 1.13 mg/L for NH_4^+, 109.63 – 143.17 mg/L for P_2O_5, and 0.51 – 4.03 mg/L for IAA.

Table 2 Quantitative results of NH_4^+, P_2O_5 (converted), IAA of 32 isolated strains

Treatment	NH_4^+ (mg/L)	P_2O_5 (mg/L)	IAA (mg/L)
SX1	**$1.123^{a} \pm 0.601$**	$129.113^{a-e} \pm 4.995$	$0.798^{d} \pm 0.156$
SX2	$1.014^{ab} \pm 0.240$	$129.886^{a-e} \pm 3.885$	$0.674^{d} \pm 0.304$
SX3	$0.595^{bc} \pm 0.110$	$128.016^{a-e} \pm 7.068$	$0.707^{d} \pm 0.114$
SX4	$0.623^{bc} \pm 0.022$	$127.059^{a-e} \pm 13.153$	$0.640^{d} \pm 0.191$
SX5	$0.857^{a-c} \pm 0.425$	$122.900^{a-e} \pm 9.921$	$0.929^{d} \pm 0.090$
SX6	$0.681^{bc} \pm 0.127$	$141.451^{ab} \pm 13.773$	$0.526^{de} \pm 0.233$
SX7	$0.874^{a-c} \pm 0.195$	$136.755^{a-d} \pm 4.394$	$0.789^{d} \pm 0.157$
SX8	$0.789^{a-c} \pm 0.163$	$128.966^{a-e} \pm 4.895$	$0.681^{d} \pm 0.165$
SX9	$0.605^{bc} \pm 0.066$	$131.609^{a-e} \pm 6.648$	$0.787^{d} \pm 0.257$

SX10	0.665^{bc} ± 0.046	119.654^{a-e} ± 6.189	0.514^{de} ± 0.227
SX11	0.677^{bc} ± 0.059	129.504^{a-e} ± 4.150	0.810^{d} ± 0.252
SX12	0.869^{a-c} ± 0.217	121.391^{a-e} ± 20.488	0.546^{d} ± 0.010
SX13	0.655^{bc} ± 0.042	129.658^{a-e} ± 10.526	0.700^{d} ± 0.303
SX14	0.809^{a-c} ± 0.145	115.369^{de} ± 13.914	0.622^{d} ± 0.042
SX15	0.804^{a-c} ± 0.149	132.662^{a-e} ± 2.521	0.716^{d} ± 0.173
SX16	0.873^{a-c} ± 0.351	121.899^{a-e} ± 11.428	0.610^{d} ± 0.101
SX17	0.905^{a-c} ± 0.320	123.938^{a-e} ± 5.743	0.876^{d} ± 0.167
SX18	0.815^{a-c} ± 0.225	131.683^{a-e} ± 4.915	0.853^{d} ± 0.164
SX19	0.894^{a-c} ± 0.079	113.529^{de} ± 14.559	0.800^{d} ± 0.278
SX20	0.897^{a-c} ± 0.162	118.608^{b-e} ± 4.948	0.864^{d} ± 0.031
SX21	0.814^{a-c} ± 0.048	119.359^{a-e} ± 6.892	0.816^{d} ± 0.100
SX22	0.558^{c} ± 0.022	**143.167^{a} ± 40.088**	0.690^{d} ± 0.173
SX23	0.798^{a-c} ± 0.148	117.210^{c-e} ± 10.534	2.918^{c} ± 0.123
SX24	0.712^{a-c} ± 0.152	127.840^{a-e} ± 6.357	3.662^{ab} ± 0.863
SX25	0.725^{a-c} ± 0.032	126.588^{a-e} ± 9.615	3.120^{bc} ± 0.411
SX26	0.704^{a-c} ± 0.066	139.898^{a-c} ± 5.488	3.120^{bc} ± 0.135
SX27	**1.125^{a} ± 0.384**	119.992^{a-e} ± 3.043	3.664^{ab} ± 0.337
SX28	0.786^{a-c} ± 0.096	109.627^{e} ± 25.362	3.548^{ab} ± 0.664
SX29	0.749^{a-c} ± 0.047	123.577^{a-e} ± 7.903	2.859^{c} ± 0.144
SX30	0.528^{c} ± 0.020	124.873^{a-e} ± 7.964	3.525^{ab} ± 0.411
SX31	0.830^{a-c} ± 0.439	132.831^{a-e} ± 5.557	3.804^{a} ± 0.141
SX32	0.549^{c} ± 0.008	132.750^{a-e} ± 6.056	4.033^{a} ± 0.811
Control	**0.000^{d}** ± 0.000	**0.000^{f}** ± 0.000	**0.000^{e}** ± 0.000

Means within a column followed by the same letters were not significantly different at α =0.05 using Duncan test.

Using the same quantitative method, the results showed that bacteria isolated from inside mung bean nodules in this study had a superior ability to dissolve tricalcium phosphate compared to bacteria isolated from soil in the rhizosphere of pepper plants grown in Binh Phuoc province, Vietnam [13] while the ability to fix nitrogen and synthesize IAA was lower. However, when compared to the results of quantifying the PGP ability of soil bacteria in the rhizosphere of sugarcane grown in Tay Ninh [25], the results obtained in this study were similar. Soil in Binh Phuoc province is fertile basaltic soil while soil in Tay Ninh province is poor acrisol soil. Soil types are thought to be a key factor in the diversity of plant root-associated bacterial communities. In which, a nutrient-poor environment stimulates bacteria to have stronger PGP activity. Root exudates of host plants and microbial communities in the soil are also factors that influence the colonization and PGP activity of plant-associated bacteria. Inoculation of a native bacterium into a specific crop also gives better results than foreign bacteria and facultative crops [26-29].

Based on the quantitative results, 3 strains SX1, SX22 and SX27 were identified. However, in the experiment to study PGP ability in vivo, we limited to two strains with the best nitrogen fixation results and good phosphate solubilization results as well as IAA production, namely SX1 and SX27. Further studies should investigate strains with strong phosphate-solubilizing activity, such as SX22 and other strains.

3.3. Results of identification of isolated strains

Identification results obtained using the Bruker Daltonik MALDI-TOF method of 3 strains SX1, SX22, and SX27 were *Paenibacillus barcinonensis*, *Bacillus pumilus*, and *Mesobacillus jeotgali* with score values 1.81, 1.79, 1.89, respectively.

The results confirmed the correct genus and possible species name. In 2005, a xylanase-producing bacterium isolated from a rice field in the Ebro Delta, Spain was reported as a new species: *Paenibacillus barcinonensis* sp. nov. [30]. Meanwhile, *Bacillus pumilus* was a PGPB bacterium widely published in many reports [31].

Although isolated from nodule endothelium, none of the species belonged to rhizobia. However, this result was similar to results obtained in some studies. The two best isolates, C1 and Vf1, were identified by 16S rRNA gene sequencing as *Stenotrophomonas maltophilia* (KY515467.1) and *Bacillus cereus* (MG515188.1) was isolated from chickpea (*Cicer arietinum* L.) and faba beans (*Vicia faba*) [5]. From cowpea (*Vigna unguiculata* L. Walp), non-rhizobial communities were also identified including *Enterobacter*, *Chryseobacterium*, *Sphingobacterium*, and unclassified Enterobacteriaceae, made up approximately 60%. There were also other genera such as *Chitinophaga*, *Roseateles*, *Cronobacter*, *Bacillus*, *Halomonas*, and actinobacteria such as *Streptomyces*, *Nocardioides* and *Microbacteria*. The study also showed that the bacterial community inside the root nodules depends on the growing soil and not on the plant variety [32]. A list of up to 22 non-rhizobial genera was published, including the genus *Bacillus* and some other Gram-positive bacteria such as *Brevibacillus*, *Brevibacterium*, *Microbacterium*. They were isolated from common bean (*Phaseolus vulgaris* L.) and wild legumes in Mexico [33].

3.4. Ability to promote plant growth in vivo on mung bean plants grown in Leonard jars

The effectiveness of two bacterial strains SX1 (*Paenibacillus barcinonensis*) and SX27 (*Mesobacillus jeotgali*) inoculated singly or in combination on 2-month-old mung bean plants was presented in Table 3.

Table 3 Effects of single inoculation and co-inoculation of strains SX1 and SX27 on two-month-old mung bean plants

Parameters	**Growth medium**	**Treatments**			
		Control (deionized water)	**SX1**	**SX27**	**SX1+SX27**
Length of shoot (cm)	Control (deionized water)	$16.34^{b} \pm 1.96$	$17.40^{ab} \pm 1.13$	$\mathbf{21.04^{a}} \pm 3.05$	$18.44^{ab} \pm 2.39$
	Hoagland 1/2 N - free	$16.68^{b} \pm 5.98$	$19.06^{ab} \pm 2.06$	$18.78^{ab} \pm 2.21$	$17.62^{ab} \pm 2.16$
	Hoagland 1/2	$16.94^{b} \pm 3.11$	$17.68^{ab} \pm 1.01$	$19.48^{ab} \pm 2.07$	$18.28^{ab} \pm 2.24$
Length of root (cm)	Control (deionized water)	$\mathbf{13.80^{a}} \pm 1.45$	$7.42^{b} \pm 2.44$	$9.92^{b} \pm 3.95$	$10.62^{b} \pm 1.91$
	Hoagland 1/2 N - free	$8.90^{b} \pm 1.91$	$8.18^{b} \pm 2.76$	$9.18^{b} \pm 2.23$	$7.46^{b} \pm 2.24$
	Hoagland 1/2	$9.42^{b} \pm 1.95$	$8.62^{b} \pm 1.09$	$7.90^{b} \pm 1.49$	$9.18^{b} \pm 2.29$
Fresh weight of shoot (g)	Control (deionized water)	$0.7040^{de} \pm 0.0514$	$0.4700^{f} \pm 0.0414$	$0.8080^{d} \pm 0.2157$	$0.5400^{ef} \pm 0.1206$
	Hoagland 1/2 N - free	$0.5700^{ef} \pm 0.0652$	$0.7120^{de} \pm 0.0929$	$1.4380^{c} \pm 0.1796$	$0.8460^{d} \pm 0.1399$
	Hoagland 1/2	$1.6200^{bc} \pm 0.1461$	$1.4920^{bc} \pm 0.2041$	$1.6780^{b} \pm 0.1977$	$\mathbf{2.4260^{a}} \pm 0.3351$
Fresh weight of root (g)	Control (deionized water)	$0.0260^{g} \pm 0.0045$	$0.0280^{g} \pm 0.0049$	$0.0700^{fg} \pm 0.0354$	$0.1040^{ef} \pm 0.0956$
	Hoagland 1/2 N - free	$0.0960^{e-g} \pm 0.0126$	$0.0980^{e-g} \pm 0.0112$	$0.1580^{de} \pm 0.0356$	$0.1820^{cd} \pm 0.0536$
	Hoagland 1/2	$0.2140^{b-d} \pm 0.0344$	$0.2640^{ab} \pm 0.0472$	$0.2480^{bc} \pm 0.0517$	$\mathbf{0.3300^{a}} \pm 0.1127$

Dry mass of shoot (g)	Control (deionized water)	$0.0983^{d} \pm 0.0127$	$0.0743^{d} \pm 0.0152$	$0.1212^{d} \pm 0.0241$	$0.0744^{d} \pm 0.0179$
	Hoagland 1/2 N-free	$0.0869^{d} \pm 0.0208$	$0.1090^{d} \pm 0.0119$	$0.1876^{c} \pm 0.0340$	$0.1245^{d} \pm 0.0255$
	Hoagland 1/2	$0.2351^{bc} \pm 0.0446$	$0.2393^{bc} \pm 0.0230$	$0.2688^{b} \pm 0.0578$	$\mathbf{0.4002^{a}} \pm 0.0999$
Dry mass of root (g)	Control (deionized water)	$0.0034^{g} \pm 0.0009$	$0.0036^{g} \pm 0.0007$	$0.0092^{fg} \pm 0.0057$	$0.0131^{ef} \pm 0.0116$
	Hoagland 1/2 N-free	$0.0132^{ef} \pm 0.0019$	$0.0133^{ef} \pm 0.0020$	$0.019^{de} \pm 0.0042$	$0.0219^{cd} \pm 0.0064$
	Hoagland 1/2	$0.0297^{bc} \pm 0.0049$	$0.0321^{b} \pm 0.0065$	$0.0329^{b} \pm 0.0067$	$\mathbf{0.0418^{a}} \pm 0.0105$

Mean values within a parameter followed by the same letters were not significantly different at the $\alpha = 0.05$ level using the Duncan test.

It was found that plant growth was successfully observed in terms of height, fresh weight and dry mass of shoot when seeds were inoculated singly with SX1, SX27 and also SX1+SX27 mixture compared to uninoculated control under the same nutritional regime. Among them, strain SX27 increased shoot height the best, up to 30.97% compared to the control without bacterial strain when plants were grown in sterile distilled water (the control without bacteria, without nutrients). The mixture SX1+SX27 increased the fresh weight of shoots by up to 54.21% compared to the control without bacteria when the plants were grown in half strength modified Hoagland's solution (Hoagland 1/2) (Figure 3). Similarly, this mixture also increased the dry weight of shoot by up to 70.23% compared to the above control.

Control: non-bacterial treatment; SX1+SX27: mixture of 2 bacterial strains.

Figure 3 Mung bean plants grown in half strength modified Hoagland's solution after 60 days of sowing

Regarding root growth, the greatest length measured was in the treatment without bacterial inoculation and growing plants in a non-nutrient environment, reaching 13.8 cm; while all remaining treatments reached 7.42 - 10.62 cm with no statistically significant difference. It is possible that in a non-nutrient environment, the growth of the root system in depth acts as a form of chemotaxis to find nutrients. However, SX1+SX27 co-inoculation was best on root mass increase. In Hoagland 1/2 medium, this mixture increased the fresh weight and dry mass of roots compared to the control without bacteria by 54.2% and 40.74%, respectively.

When comparing individual inoculation efficiency, out of a total of 18 indicators considering different growing environments (total number of rows in the Table minus the title row; comparing columns 4 and 5), the SX27 strain outperformed SX1 in 10 out of 18 evaluated parameters, showed similar performance in 7 parameters, and was inferior in only one parameter. In nitrogen-free plant culture solutions, single inoculation of SX27 strain also had effective effects on shoot development. It increased fresh weight and dry weight by up to 152.3% and 115.9%, respectively, compared to the uninoculated control.

In summary, strain SX27 (*Mesobacillus jeotgali*) appeared to be superior to strain SX1 (*Paenibacillus barcinonensis*), but co-cultivation of these two strains gave the best effect on 2-month-old mung bean plants grown in Leonard jars. Co-inoculation has brought good results perhaps because the interaction between the two subjects benefits each other and the host plant. Strain SX1 had statistically equal nitrogen fixation and phosphate solubilization results to strain SX27 but had better IAA production results (Table 2). Co-inoculation of *Pseudomonas putida* with *Trichoderma viride* showed maximum increase in root length (up to 86.57%), shoot length (up to 56.91%), root dry weight (up to 94.42%) and shoot dry weight (up to 56.09%) compared to uninoculated control in potted mung bean seedling experiment [34].

It has been reported that several *Paenibacillus* spp., notably *P. polymyxa*, *P. azotofixans*, *P. macerans* and *P. graminis*, are highly effective plant growth-promoting rhizobacteria (PGPR) that have been extensively studied for their potential as biofertilizers in agriculture [35]. Five strains of *Paenibacillus* sp. including JS-4, SZ-10, SZ-14, BJ-4 and SZ-15 significantly promoted plant growth and increased plant dry weight in wheat, cucumber and tomato [36]. *Peanibacillus* spp. also had good plant growth promoting effects on white clover, tomato and maize [22]. *Mesobacillus jeotgali* and *Peribacillus simplex* doubled the growth rate of wheat when grown with selenium supplementation [37]. *Bacillus pumilus* was considered one of the most interesting PGPB strains among the spore-forming bacteria of the phylum Firmicutes. This species could live in a variety of environments, tolerated abiotic stresses, and produced a variety of phytohormones and other plant growth stimulants. In particular, *B. pumilus* WP8 could stimulate plant growth when the natural microflora in the soil was altered. Therefore, this species was also a potential candidate for multifunctional biofertilizer production [31].

4. Conclusion

This study had isolated 32 bacterial strains with the ability to fix nitrogen, solubilize phosphate and synthesize IAA. The three best strains including SX1, SX22 and SX 27 were identified as *Paenibacillus barcinonensis*, *Bacillus pumilus* and *Mesobacillus jeotgali*. The combination of two strains SX1 and SX27 to co-inoculate mung bean plants brought the best PGP effect on shoot and root development within 60 days after sowing.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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