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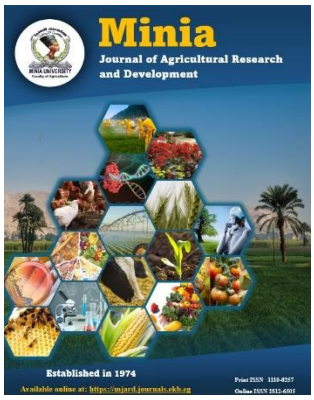

Isolation and Molecular Identification of Novel Phosphate-Solubilizing Bacteria and Their Plant Growth-Promoting Activities

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ABSTRACT

This investigation aimed to isolate and characterize novel phosphate-solubilizing bacterial (PSB) isolates using both conventional and molecular techniques. Additionally, the study evaluated their plant growth promoting activity on sugar beet (*Beta vulgaris*). Three distinct colonies that produced clear halos on Pikovskaya's agar (PKA) plates were selected and designated as YMP5, YMP6, and YMP7 for microbiological and molecular characterization. Due to the 16S rRNA gene sequence analysis and comparison with strains in the NCBI GenBank database, isolates YMP5 and YMP7 were identified as *Pseudomonas indica*, while isolate YMP6 belonged to the genus *Priestia*. Phylogenetic analysis further confirmed that YMP5 and YMP7 clustered with *Pseudomonas indica* strains IMT37 and NBRC 103045, whereas YMP6 clustered with *Priestia endophytica* strain 2DT. The 16S rDNA gene sequences of these PSB isolates were submitted to the NCBI database under accession numbers PV652948 to PV652950. In terms of plant growth promoting activities, all isolates positively influenced vegetative growth parameters, enhanced NPK nutrient content, and increased photosynthetic pigment concentrations compared to the untreated control. These findings suggest that beneficial PSB strains can be isolated from Egyptian soils and utilized as sustainable bio-inoculants in agriculture. Such bio-fertilizers have the potential to enhance plant development, reduce reliance on chemical fertilizers, and mitigate crop yield losses.

Keywords: PSB, *Pseudomonas*, *Priestia*, 16S rRNA sequence, vegetative growth

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INTRODUCTION:

Phosphate-solubilizing bacteria (PSB) have an important role in the biological processes of the soil as they can change insoluble phosphorus compounds into plant-available forms through the secretion of organic acids, enzymes, or other chelating compounds (**Pan and Cai, 2023**). This natural process positively increasing the availability of phosphorus in the rhizosphere helps to mitigate the heavy reliance on synthetic phosphorus fertilizers which are expensive and contribute to environmental damage like water eutrophication, (**Li *et al.*, 2023**).

Phosphorus solubilization serves as another subspecialty for PSB. The latter also contributes to plant growth and soil health in other ways. They can inhibit the growth of plant pathogens thereby promoting healthier crops (**Luo *et al.*, 2024**) and also produce phytohormones, i.e., indole-3-acetic acid. Furthermore, PSB application in agriculture has enhanced soil microbial diversity and resilience, which strengthens the agroecosystem's health and sustainability (**Lei *et al.*, 2025**). Considering the declining world natural phosphorus reserves, incorporation of PSB into agricultural practices is an environmentally friendly and efficient way of sustaining soil fertility and increasing crop yield (**Ibrahim *et al.*, 2022**).

The 16S ribosomal RNA (rRNA) gene is extremely significant as a molecular marker for the identification and phylogenetic study of phosphate-solubilizing bacteria (PSB). This gene contains regions that are conserved and variable which makes species delineation straightforward for researchers. For example, **Sembiring and Sumanto (2023)** performed phylogenetic analysis of the PSB isolate, RZ02, which had a high phosphorus solubilization index, and found that it was a *Pseudomonas aeruginosa*, which was closely matched in the phylogenetic analysis, and identified it using 16S rRNA gene sequencing. Also, **Wang *et al*. (2017)** reported that identifying PSB strains from the rhizosphere of Chinese cabbage using 16S rRNA sequencing, which helped to understand their taxonomic positions and value in agriculture.

The use of 16S rRNA gene sequencing in the identification of phosphorus-solubilizing bacteria (PSBs) enhances the precision of taxonomy, enlightens the ecological functions of such microorganisms, and enables their prospective application in environmentally friendly agricultural management. An investigation by **Rasul *et al.* (2024)** in India, for example, was successful in isolating PSBs from the rhizospheres of rice paddies and, through 16S rRNA gene analysis, isolated strains having close phylogenetic relationships with *Acinetobacter* and *Pseudomonas* species. This study demonstrated the strains potential as bio-fertilizers to increase the availability of phosphorus in soils (**Rasul *et al.*, 2024**). These molecular identification methods are essential for choosing PSB strains that will produce bio-fertilizer and enhance soil health and crop yield. Thus, the current study aims to isolate and characterize novel phosphate-solubilizing bacteria (PSB) molecularly using the 16s rRNA gene sequencing approach and assess their efforts to promote sugar beet plant growth.

MATERIALS AND METHODS:

1. Soil samples

Samples of soil were taken from the vegetable experimental farm at Minia University, Faculty of Agriculture, rhizosphere of squash (*Cucurbita pepo* L.) variety Eskandrani. After being carefully

removed without affecting the soil in their rhizosphere, squash plants were placed in sterile plastic bags and transported to the lab in an icebox. After giving the plants a good shake, the dirt was removed from the roots, sieved through a 2 mm sieve, put in a sample bag, and kept in a refrigerator at 4 ^{o}C until it could be further investigated.

2. Isolation and selection of phosphate solubilizing bacterial isolates (PSB)

Nine milliliters of double-distilled water were mixed with one gram of rhizosphere soil, and the mixture was rapidly stirred for five minutes. 0.1 milliliters of each dilution were spread out on Pikovskaya agar (PKA) plates and incubated for five days at 30 degrees Celsius after a milliliter of the solution was further diluted to produce a 2–5 dilution (**Pikovskaya, 1948**). The development of clear zones surrounding the bacterial colonies is a sign of the phosphate solubilizing activity. To get pure colonies, isolates with distinct zones underwent visual screening before being purified. Following incubation, the samples yielded three isolates with exceptional phosphate solubilization activity; these bacterial colonies were then subjected to sub-culturing for identification.

3. Phosphate solubilization index (Psi) determination

In a shaking incubator, all of the chosen isolates were cultivated in Nutrient Broth (NB) for twenty-four hours at 28±2°C. The bacterial cultures were put onto PKA plates in a very small amount (0.1µl). To ensure that each culture had at least 10^6 cells per milliliter, the optical density was set between 0.5 and 1 at 550 nm. Sterile cotton buds were used for stab inoculation on PKA plates. The phosphate solubilization index was measured after the plates were incubated for seven days at 30 ^{o}C. The bacteria's degree of phosphate solubilizing activity is indicated by the phosphate solubilization index. The following formula is used for determining it (**Edi Premono *et al.*, 2007**).

Phosphate Solubilization Index (SI) = B/A

Where A is the colony's diameter and B is the total diameter (colony + clear zone)

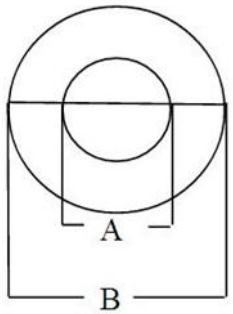


Figure1. Description of Phosphate solubilizing activity

4. Characterization of phosphate solubilizing bacterial isolates (PSB)

Based on their morphological, microscopic, and biochemical characteristics, the bacterial isolates were described.

A- Morphological examinations

Gram reaction, bacterial motility, and cell shape were examined in order to characterize the morphology of the bacteria that solubilize phosphate. According to **Aneja (2006**), the pure cultures' cell morphological features were examined under a microscope during log phase. According to **Hucker and Conn (1923),** Gram's staining was used to ascertain if the bacterial isolates that were solubilizing phosphate were gram positive or gram negative. **Elbeltagy *et al.* (2000)** investigated the ability of bacterial isolates to move independently on semi-solid nutrient agar plates (0.2% agar).

B-Antibiotic resistance

A test for antibiotic resistance was conducted on Nutrient Agar (NA) medium supplemented with one of the following

antibiotics (µg/ml): Amikacin (AK 30), Azitromycin (AZM 15); Erythromycin (E 15), Colistin (CT 100), Ampicillin (A 10), Ceftriaxone (CTR 30), and Oxacillin (O30). The zones surrounding antibiotic discs were measured to ascertain the antibiotics' resistances and sensitivity, in accordance with **Clower and Hay (1968)**.

C-Biochemical tests

Oxidase test: The capacity of bacterial isolates to produce cytochrome c oxidase was tested according to the procedure provided by **Cappuccino and Sherman (1996).**

Catalase activity: was conducted as elaborated by **Aneja (2006).**

Gelatin hydrolysis: To confirm that the gelatinase enzyme was active in hydrolyzing gelatin, gelatin liquefaction was used, as described by **Aneja (2006)**.

The capacity of the selected bacterial isolates to hydrolyze starch by generating amylase enzyme was tested in accordance with **Aneja (2006)**.

5. Molecular identification of phosphate solubilizing bacterial (PSB) isolates

A-Genomic DNA isolation

Following the procedure outlined by **Kiruthika and Padmanabha (2018)**, the whole genomic DNA of the chosen bacterial isolates was extracted using the Cornell extraction buffer, which is made up of 500 mM NaCl, 100 mM Tris-HCl (pH 8.0), 50 mM EDTA, and 0.84% SDS. Freshly made lysozyme buffer (20 mg/mL), phenol:chloroform:isoamyl alcohol (25:24:1), absolute ethanol (96–100%), and RNase (50 mg/mL) were additional reagents used in the extraction procedure. After that, the isolated DNA was dissolved in 100 µL of TE buffer and used as the PCR amplification template.

B- Analysis and amplification of 16S rDNA gene

Using universal bacterial primers, Forward (5′-AGAGTTTGATCCTGGCTCAG-3′) and Reverse (5′-ACGGCTACCTTGTTACGACTT-3′), the fragment of 16S rDNA gene was amplified, as described by **Weisburg *et al.* (1991).** 4 µL of template DNA, 2 µL of each primer, and 17 µL of sterile double-distilled water were used in the 50 µL final reaction volume for the PCR amplifications. An additional 25 µL of 10X DreamTaqTM Green Buffer was added to finish the reaction volume. A three-minute initial denaturation at 94°C, thirty cycles of denaturation at 94°C for 30 seconds, annealing at 57°C for 30 seconds, and extension at 72°C for two minutes, and a final extension at 72°C for ten minutes were used to optimize the PCR conditions. The purified PCR products were sequenced using an ABI 3730xl DNA sequencer at GATC Biotech Ltd. (The London BioScience Innovation Centre, London, United Kingdom). The NCBI's Basic Local Alignment Search Tool (BLAST) was used to compare the sequences' similarity. Under accession numbers PV652948 to PV652950, all of the 16S rRNA gene sequences acquired for this investigation were uploaded to the National Center for Biotechnology Information (NCBI) database. Using the Neighbor-Joining (NJ) technique and the MEGA X software program (**Tamura *et al.*, 2018**), a phylogenetic tree was produced. Cluster construction was done using the un-weighted pair group.

6- Evaluation of Plant Growth Promoting Activity

A- Surface sterilization of sugar beet seeds

Sugar beet (*Beta vulgaris* L. var. BTS 185) seeds were acquired from the Ministry of Agriculture's Crop Field Research Institute, Agricultural Research Centre (ARC), in Giza, Egypt. To remove any surface microbes linked to seeds' epiphytes, sterilization was done. They underwent a one-minute surface sterilization with 70% ethanol, a three-minute washing with 3% sodium hypochlorite, and a one-minute 70% ethanol wash. After three rounds of rinsing in sterile distilled water, the seeds were patted dry.

B- Preparation of bacterial inoculum

After 48 hours at 30°C under aerobic conditions with continuous shaking at 150 rpm, the three phosphate-solubilizing bacterial (PSB) isolates, YMP5, YMP6, and YMP7, were grown in nutritional broth until they reached a density of around 10^6 CFU/mL. To pellet the cells, the bacterial cultures were centrifuged for 10 minutes at 13,000 rpm following incubation. According to **Thompson (1996),** the collected cells were then re-suspended in sterile distilled water and made ready for inoculation.

C- Seed bacterization

Ten millilitres of bacterial suspension with 10^6 cfu/ml were used to soak the seeds for two hours before they were allowed to dry in the shade. As a control, sterile distilled water-soaked seeds were retained.

D- Pot experiments

Three kg of sterilized soil/sand in a 1:1 ratio were placed in plastic pots (12 cm wide) in a greenhouse setting to examine the impact of the PSB isolates on the vegetative growth parameter of the sugar beet plants. Four grams of rock phosphate and 100 grams of sterilized vermiculite were added to the sterilized soil mixture. In a fully randomized experimental design, three replicate pots were maintained for every treatment. Each container included five sugar beet seeds that had been infected with the test isolates and given sporadic watering. After 45 days of sowing, sugar beet seedlings were collected, and several growth metrics, including fresh weight of the shoots and roots, as well as shoot and root length, were measured. Levels of carotenoid, chlorophyll a, and chlorophyll b were measured in the second, third, and fourth fresh leaves of seedlings in the apical region. Five milliliters of 100% acetone were used to soak 0.5 grams of leaves in a closed test tube at 4°C in the lab. Following five days of incubation, the colored solution from the supernatant was carefully decanted into a 25 ml volumetric flask, crushed with a blunt glass rod, and then five milliliters of new acetone were added to the test tube and allowed to sit for fifteen minutes. After that, the supernatant solution was obtained by centrifuging for five minutes at 13,000 rpm. Eventually, new acetone was added, increasing the level to 10 ml. Using a spectrophotometer, the levels of carotenoid, chlorophyll a, and chlorophyll b were measured at 663, 645, and 440.5 nm, respectively (**Lichtenthaler and Wellburn, 1983**). The procedures outlined by **Dawwam *et al.* (2013)** were used to determine the amount of nitrogen, phosphorus, and potassium.

7- Statistical analysis

A variance analysis (ANOVA) was performed using the MSTAT software package (Version 4). The data set was subjected to detailed analysis by ANOVA, followed by analysis using the least significant difference (LSD) procedure at 5% significance level (**Gomez and Gomez, 1984**).

RESULTS AND DISCUSSION:

A-Isolation of phosphate solubilizing bacteria (PSB)

Phosphate-solubilizing bacteria (PSB) were isolated from rhizospheric soil samples of squash plants using Pikovskaya agar (PKA) medium. Numerous bacterial colonies exhibiting diverse morphologies and growth characteristics were observed (**Figure 2**). Among them, three distinct colonies that formed prominent clear halos around their growth zones on PKA plates indicative of phosphate solubilization were selected for further analysis. These isolates were designated as YMP5, YMP6, and YMP7.

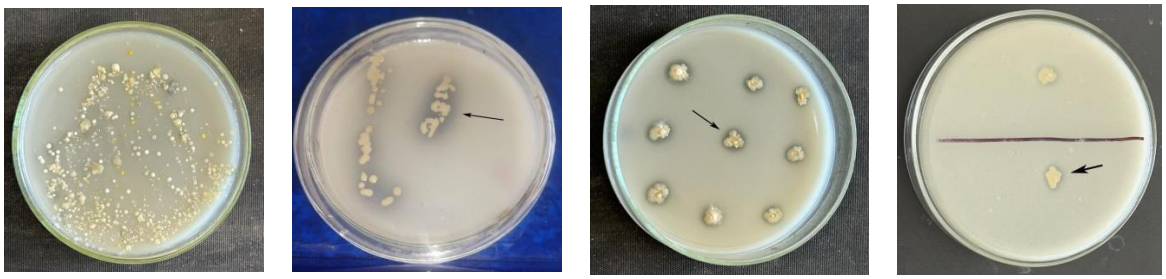


Figure 2. Variability in shape and nature of phosphate solubilizing bacteria (PSB) distinct clear zones in PKA plates.

B-Phosphate solubilization index

The potential of the three chosen isolates to solubilize tricalcium phosphate in vitro was evaluated and the findings are presented in **Figure 3** and **Table 1**. The data are presented as the mean ± standard error of three independent replicates. Of the isolates screened, YMP7 had the largest phosphate solubilizing index (2.51 cm), reflecting a greater extent of efficiency in solubilization. On the other hand, YMP5 and YMP6 isolates exhibited comparatively low but similar phosphate solubilizing indices with readings of 1.25 cm and 1.12 cm, respectively, on Pikovskaya agar plates. Bacterial solubilization of tricalcium phosphate ($Ca_3(PO_4)_2$) can be attributed to the production of organic acids by the bacteria, which decrease the pH of the ambient environment, thereby contributing to the solubilization of insoluble phosphate compounds. The cations, i.e., Ca^{2+}, bound to phosphate become complexed by other organic acids such as lactic acid, oxalic acid, gluconic acid, and citric acid. Through this activity, phosphate ions are made available in soluble form for absorption by plants (**Wang *et al.*, 2025**)

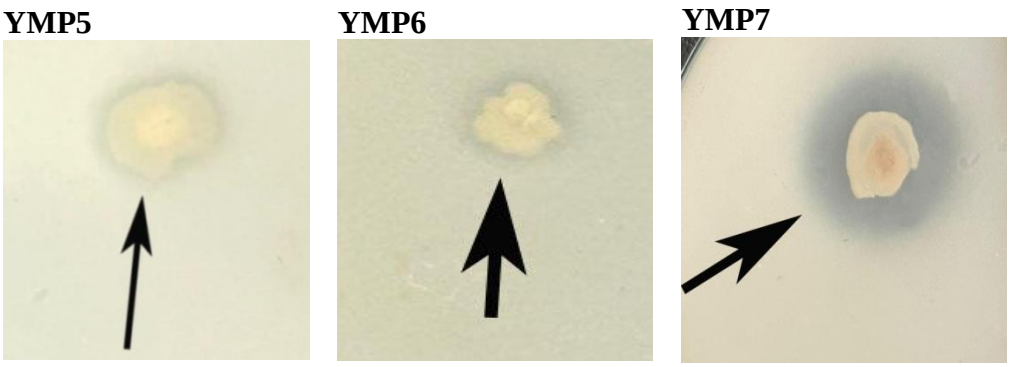


Figure 3. Clear zones generated by the three PSB isolates (YMP5, YMP6 and YMP7) after incubation at 30±2°C for 5 days on Pikovskaya agar plates

Table1. Phosphate solubilization index (PSI) of the three bacterial isolates in Pikovskaya's agar plates.

Isolates	phosphate solubilizing index (PSI) Mean ± SE
YMP5	1.25±0.015
YMP6	1.12±0.009
YMP7	2.51±0.012

C-Microbiological characterization of phosphate solubilizing bacteria (PSB)

After purification of colonies, the three chosen bacterial isolates were characterized using a variety of physiological and biochemical assays, as indicated in **Table (2).** The pure bacterial isolates all had rod-shaped, motile cells. While YMP6 was gram positive, the other two isolates, YMP5 and YMP7, were gram negative.

Table 2. Microbiological and physiological profiles of PSB isolates.

Isolates	Cell shape	Motility	Gram reaction	Catalase	Oxidase	Gelatin hydrolysis	Starch hydrolysis
YMP5	Rod	+	-	+	+	+	+
YMP6	"	+	+	+	+	+	-
YMP7	"	+	-	+	+	+	+

As revealed in **Table (2),** all the tested isolates were positive for catalase and oxidase activity. These enzymes are thought to be necessary for bacteria to neutralize cellular toxicity (**Anwar *et al.*, 2024**) and are important for shielding bacterial cells from various plant-derived reactive oxygen species (**Alfei *et al.*, 2024**).

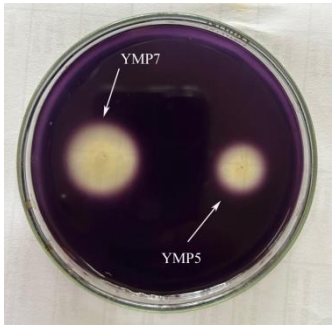
One essential mechanism for energy storage is the breakdown of gelatin and starch. Gelatinase and amylase were examined in connection with the enzymatic

activity of phosphate-solubilizing bacterial isolates, as indicated in **Table 2**. Every bacterial isolate has the capacity to manufacture gelatinase into deep tubes of nutritional gelatin. Microorganisms manufacture gelatinase enzymes, which hydrolyze gelatin into its constituent sub-compounds (polypeptides, peptides, and amino acids) that the organism may utilize after passing through the cell membrane. Various gelatinase enzymes were found to be produced by a number of bacterial species, especially *Bacillus* spp. and *Pseudomonas* spp. (**Pathade *et al.*, 2024**). In contrast, amylase activity was only exhibited by the two bacterial isolates YMP5 and YMP7, as shown through clear zones in starch agar media (**Figure 4**).

D-Antibiotic resistance test

Using NA medium treated with one of eight antibiotics, the three PSB isolates' true antibiotic resistance was confirmed **Figure 5** and **Table 3.**

A



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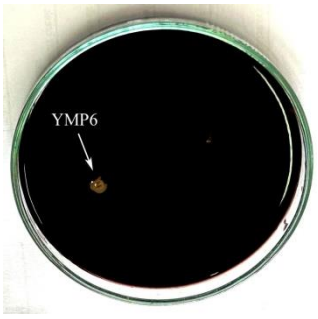

Figure 4. Amylase activity of PSB isolates. A: Clear zones generated by the two phosphate solubilizing bacterial isolates (YMP5 and YMP7) and B: No amylase activity of YMP6 isolate after incubation at 30±2°C for 5 days in Pikovskaya agar plates.

A **B** **C**

Figure 5. Effect of antibiotics on growth of phosphate solubilizing bacterial (PSB) isolates. (A) Amikacin (AK 30), Azitromycin (AZM 15), Erythromycin (E 15) and Colistin (CT 100) on YMP5, (B) Amikacin (AK 30), Azitromycin (AZM 15), Erythromycin (E 15) and Colistin (CT 100) on YMP6, (C) Amikacin (AK 30), Azitromycin (AZM 15), Erythromycin (E 15) and Colistin (CT 100) on YMP7

Table 3. Growth of PSB isolates at the presence of different antibiotics on NA plates.

Isolates	Amikacin (AK 30)		Azitromycin (AZM 15)		Erythromycin (E 15)		Colistin (CT 100)		Ampicillin (A 10)		Ceftriaxone (CTR 30)		Oxacillin (O30)	
	D/Z	R/S	D/Z	R/S	D/Z	R/S	D/Z	R/S	D/Z	R/S	D/Z	R/S	D/Z	R/S
YMP5	16	S^{+}	8	S	10	S^{+}	2	S	8	S	10	S^{+}	20	S^{++}
YMP6	18	S^{+}	10	S^{+}	10	S^{+}	0	R	0	R	2	S	14	S^{+}
YMP7	26	S^{++}	0	R	0	R	8	S	2	S	14	S^{+}	2	S

Abbreviations: Resistant (R); Weakly Sensitive (S); Sensitive (S^{+}); Highly Sensitive (S++); Diameter (mm) of Zone around Antibiotic (D/Z).

The results indicated that YMP5 isolate had different levels of sensitivity to all tested antibiotics. The YMP6 isolate was resistant to the two antibiotics (Colistin CT 100 and Ampicillin A 10) on the other side, it exhibited different levels of sensitivity to other tested antibiotics. Finally the isolate YMP7 was able to growth in presence of Azitromycin (AZM 15) and Erythromycin (E 15) while it was sensitive to the other tested antibiotics. Microbes use antibiotic resistance as a defensive strategy to shield themselves from adverse environments, especially when antimicrobial drugs are present. The existence of many resistance genes is reflected in the variation in antibiotic resistance characteristics across microbial isolates. These genes produce proteins that either prevent the absorption of antibiotics or encourage their efflux, which lowers their intracellular concentrations and efficacy, as well as enzymes that can break down or alter antibiotics, including β-lactamases (**Blair *et al.*, 2015**).

E-Molecular identification of phosphate solubilizing bacterial (PSB) isolates

Nucleic acid sequencing methods have recently become highly effective tools for species-level bacterial identification and classification. The most popular genetic technique for finding bacterial strains that have not yet been identified, are infrequently isolated, or exhibit aberrant phenotypes is the study of the 16S rRNA gene sequence. The 16S rRNA gene's hypervariable sections and high degree of conservation enable accurate taxonomic resolution and phylogenetic placement of bacterial species (**Chun *et al.*, 2018**).

The amplified regions of the three bacterial phosphate-solubilizing (PSB) isolates were analyzed through nucleotide sequencing with the same primers. The nucleotide sequence data have been submitted to the NCBI GenBank and can be accessed with the accession numbers listed in **Table (4).**

Table 4. Molecular identification of phosphate-solubilizing bacteria based on 16S rDNA sequence.

Isolates	accession No.	% Similarity	Closest NCBI strain and accession No.	Phylum
YMP5	PV652949	97.32	*Pseudomonas indica* strain IMT37	Gammaproteobacteria
YMP6	PV652948	97.13	*Priestia endophytica* strain 2DT	Bacilli
YMP7	PV652950	99.58	*Pseudomonas indica* strain NBRC 103045	Gammaproteobacteria

Based on the 16S rDNA sequencing analysis's identity percentages with the nearest NCBI strain or strains listed in the Genbank database, it was found that the two PSB isolates (YMP5 and YMP7) were related to the same genus and species (*Pseudomonas indica*) while the third one, was belonged to genus *Priestia* (**Table 4**). The isolate YMP5 gave 97.32% of homology with *Pseudomonas indica* strain IMT37 NR_028801while YMP6 isolate exhibited 97.13% homology with *Priestia endophytica* strain 2DT NR_025122. Finally, YMP7 showed 99.58 % homology with *Pseudomonas indica* strain NBRC 103045 NR_114196.

Pseudomonas species are well known for their function in phosphate solubilization, which improves plant availability of phosphorus and makes a substantial contribution to sustainable agriculture. By secreting organic acids like gluconic acid, which chelate calcium ions and decrease the pH of the surrounding soil, these bacteria mainly solubilize inorganic phosphates like tricalcium phosphate and hydroxyapatite, increasing the bioavailability of phosphorus (**Silva *et al.*, 2023**). The importance of *Pseudomonas* in integrated nutrient management is still being highlighted by recent research, particularly in phosphorus-deficient soils where its use has enhanced crop output and soil health (**Mehmood *et al.*, 2023**).

Promising PGPR with phosphate-solubilizing properties are found in the genus *Priestia*, which was recently reclassified from several members of the *Bacillus* genus. Through the secretion of low-molecular-weight organic acids like citric and gluconic acids, which chelate metal ions and acidify the rhizosphere, species like *Priestia megaterium* (formerly *Bacillus megaterium*) are known to solubilize inorganic phosphate and release it in a form that plants can use (**Bakki *et al.*, 2024**).

The neighbor joining (NJ) approach was used to create a phylogenetic tree based on the 16S rRNA gene sequences from the three isolates of phosphate solubilizing bacteria (PSB), as seen in **Figure (6)**. Generally, the phylogenetic tree exhibited that the two isolates YMP5 and YMP7 sharing the same cluster with two strain of *Pseudomonas indica* (*Pseudomonas indica* strain IMT37 and *Pseudomonas indica* strain NBRC 103045). While, the bacterial isolate YMP6 shared the same cluster with *Priestia endophytica* strain 2DT.

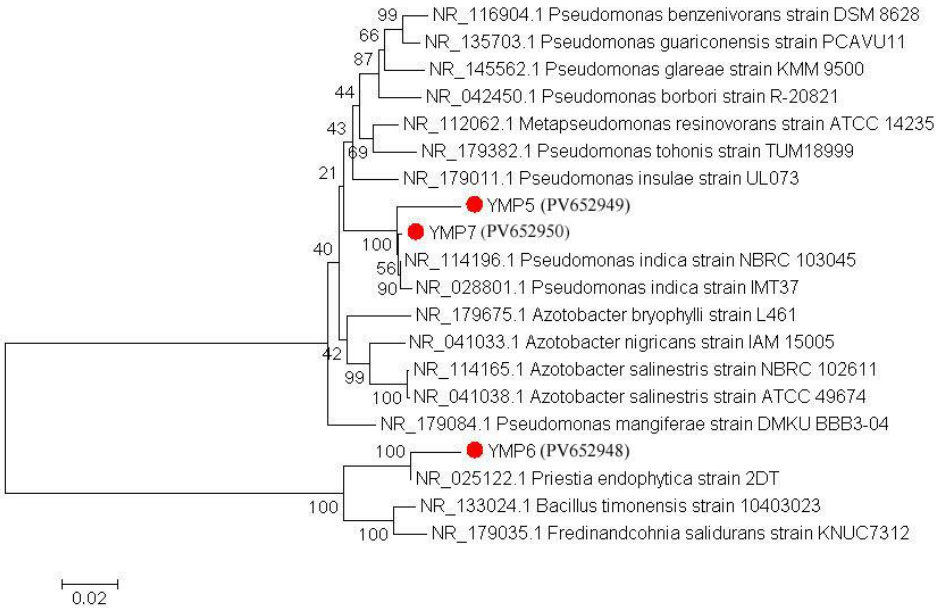

Figure 6. Phylogenetic tree of the 16S rRNA gene sequences showing the relationships of three phosphate-solubilizing bacteria. The tree was constructed using neighbor-joining. Sequence from the three PSB isolates (YMP5, YMP6 and YMP7) with accession numbers are marked by red ring.

F-Plant growth promoting activity of PSB isolates on sugar beet plants

Effect of PSB isolates on some vegetative growth traits

After being grown in greenhouse conditions for 45 days in pots, sugar beet seedlings' vegetative growth characteristics were assessed. These included plant height, number of leaves, and the fresh and dry weights of shoots and roots (**Figure 7** and **Table 5**). The obtained results indicated that all tested PSB isolates increased plant height of sugar beet plants particularly YMP7 isolate that increased significantly this trait (10.34 cm) as compared to control (5.81cm). Data in **Table 5** showed that, all of the PSB isolates, except YMP6 significantly increased the number of leaves of seedling. The highest values of leaves number (6.50) were found in plants treated with YMP5 and YMP7 isolates as compared with control (6.00). The highest shoot fresh weight (1.05 and 0.83 g) was recorded in plant treated with the two isolates YMP7 and YFV05, respectively as compared to control (0.74 g). On the other hand, plants treated with YMP7 isolates exhibited the maximum shoot dry weight (0.18 g) as compared to control and all other tested bacterial isolates. Concerning root fresh weight, it was observed that all tested isolates improved this trait particullary YMP7 isolates which increased significantly root fresh weight (0.06 g) as compared to control (0.02 g). On the other side, all tested isolates effect positively on root dry weight trait particulary YMP7 which gave the maximium root dry weight (0.005 g) as compared to control and all other tested isolates (**Table 5**). Generally , the data in **Table (5)** show that as compared

to control, the all tested PSB isolates had positive effect in all studied vegetative parameters (Plant height, root fresh and dry weight, and shoot fresh and dry weight) particularly the two isolates YMP5 and YMP7.

Figure 7. Effect of three PSB isolates (YMP5, YMP6 and YMP7) on different growth parameter of sugar beet seedling seeds after 45 days of sowing.

Table 5. The vegetative growth parameters of sugar beet seedlings recorded after seed fortification with three PSB isolates (YMP5, YMP6 and YMP7) by pre-sowing soaking inoculation after 45 days.

Isolates	Plant height (cm)	Leaves no.	Shoot fresh weight (g)	Shoot Dry weight (g)	Root fresh weight (g)	Root Dry weight (g)
Control	5.81	6.00	0.74	0.04	0.02	0.001
YMP5	7.75	6.50	0.83	0.06	0.03	0.003
YMP6	7.33	6.25	0.79	0.05	0.03	0.004
YMP7	10.34	6.50	1.05	0.18	0.06	0.005
$LSD_{0.05}$	2.44	0.27	0.21	0.03	0.02	0.002

PSB inoculation has been shown to significantly enhance plant height, leaf number, and biomass accumulation in various crops under both greenhouse and field conditions. For instance, *Pseudomonas fluorescens* inoculation in wheat and maize has led to increased root length, shoot biomass, and photosynthesis pigments content due to improved phosphorus uptake and hormonal modulation (**Elhaissoufi *et al.*, 2020**). In addition to solubilizing phosphorus, *Pseudomonas* spp. often produce phytohormones such as indole-3-acetic acid (IAA), which further stimulates root elongation and overall vegetative growth (**Bakki *et al.*, 2024**). Similarly, *Bacillus* species, particularly *Bacillus megaterium* and its recently reclassified counterpart *Priestia megaterium*, are potent phosphate solubilizers that contribute to the enhancement of vegetative traits across many crops like sugar beet, tomato, and chickpea, *Bacillus* inoculation has resulted in significant increases in shoot and root biomass, leaf area, and plant height compared to control treatment (**Mani *et al.*, 2024**). Moreover, co-inoculation strategies using both *Pseudomonas* and *Bacillus* strains have demonstrated synergistic effects on plant growth, likely due to the complementary modes of action in phosphorus solubilization and growth promotion (**Bakki *et al.*, 2024**).

Effect of three PSB isolates on N, P and K content in leaves

Plant nutrient uptake findings are shown in **Table (6)**. Generally, it was observed that NPK contents in leaves of whole treated sugar beet seedlings were considerably higher than that of the untreated seedlings. The highest percentages of N contents were found in leaves of plants inoculated with YMP7 isolate followed by that treated with YMP5 (1.19 and 1.12%, respectively) with a significant increase than control (1.02%).

Regarding phosphorus and potassium contents in leaves, data revealed that all treated plants gave higher level of P content compare to control particularly isolate YMP7 (0.23%) and YMP5 (0.21%) as compared to untreated plant (0.16%). Inculcation with YMP7 and YMP5 isolates gave the highest percentages of K (1.27 and 1.25%, respectively) with a significant increase as compared with control (1.14%). These results revealed that inoculation with all tested PSB bacterial isolates increased the uptake of leaves N, P and K contents which were considerably higher than untreated control (**Table 6**).

The bioavailability and uptake of essential macronutrients, such as nitrogen (N), phosphorus (P), and potassium (K), are enhanced by phosphate-solubilizing bacteria (PSB) such as Pseudomonas and Bacillus species, which contribute to plant nutritional health (**Bakki *et al.*, 2024**).

Effect of three PSB isolates on photosynthetic pigments in leaves

Data presented in **Table (7),** revealed that inoculation with all tested PSB isolates increased significantly chlorophyll a, b and carotinoides pigments in leaves of sugar beet plants as compared to untreated plants. The two isolates YMP7 and YMP5 gave the highest percentages of photosynthetic pigments as compared with YMP6 and untreated control treatments.

Table 6. Effect of the three PSB isolates (YMP5, YMP6 and YMP7) isolates inoculation on N, P and K contents in sugar beet shoot after 45 days of sowing.

Isolates	N%	P%	K%
Control	1.02	0.16	1.14
YMP5	1.12	0.21	1.25
YMP6	1.09	0.19	1.22
YMP7	1.19	0.23	1.27
$LSD_{0.05}$	0.07	0.04	0.08

Table 7. Effect of the three PSB isolates inoculation on chlorophyll a, b and carotenoids in sugar beet leaves after 45 days of sowing.

Treatments	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Carotenoids (mg/g FW)
Control	0.298	0.195	0.211
YMP5	0.311	0.223	0.225
YMP6	0.307	0.217	0.223
YMP7	0.315	0.227	0.227
$LSD_{0.05}$	0.009	0.020	0.010

PSB release organic acids and phosphatases that improve phosphorus uptake, essential for chlorophyll synthesis (**Elhaissoufi, *et al.*, 2022**). Inoculation with *Pseudomonas fluorescens* has been shown to increase chlorophyll and carotenoid levels in crops such as wheat and tomato (**Noureldeen *et al.*, 2021** and **Mahmud *et al.*, 2021**). Similarly, *Bacillus spp*. improves pigment levels by enhancing nutrient absorption and producing growth-promoting hormones (**Abdelrahman *et al.*, 2023**).

Finally, these findings suggest that beneficial PSB strains can be isolated from Egyptian soils and utilized as sustainable bio-inoculants in agriculture. Such bio-fertilizers have the potential to enhance plant development, reduce reliance on chemical fertilizers, and mitigate crop yield losses.

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الملخص العربي

العزل والتوصيف الجزيئي لبكتيريا جديدة مذيبة للفوسفات (PSB) ونشاطها المعزز لنمو النباتات

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هدفت هذه الدراسة إلى عزل وتوصيف عزلات بكتيرية جديدة مذيبة للفوسفات (PSB) باستخدام التقنيات التقليدية والجزيئية. بالاضافة إلى ذلك، قيّمت الدراسة نشاطها المعزز لنمو النبات على نبات بنجر السكر (*Beta vulgais*). حيث تم إختيار ثلاث مستعمرات مميزة أنتجت هالات واضحة على أطباق بيئة أجار بيكوفسكايا (PKA) وتم تسميتها على أنها YMP5وYMP6وYMP7 واختيارها للتوصيف الميكروبيولوجي والجزيئي. بعد إجراء تحليل تسلسلى لقواعد جين الـ 16s rRNA ومقارنته بالسلالات الموجودة في قاعدة بيانات GenBank NCBI، تم توصيف العزلتين YMP5وYMP7 على أنهما *Pseudomonas indica*، بينما تنتمي العزلة YMP6 إلى جنس *Priestia* . وأكد التحليل التطوري أيضا أن YMP5 وYMP7 تجتمعان مع سلالتى *Pseudomonas indic*a IMT37 وNBRC 103045، بينما تشترك YMP6 مع سلالة *Pristia endophytica* DT2 . تم تسجيل تسلسل القواعد لجين 16s rRNA لعزلات PSB على قاعدة بيانات المركز الوطني لمعلومات التكنولوجيا الحيوية (NCBI) بأرقام الوصول (.accession no) من PV652948 الى PV652950. وفيما يتعلق بأنشطة تعزيز نمو النبات، أثرت جميع العزلات إيجابيًا على صفات النمو الخضري، ورفعت محتوى العناصر الكبرى NPK ، وزادت تركيزات الصبغة الضوئية مقارنةً بالكنترول (الغير معامل). تشير هذه النتائج إلى إمكانية عزل سلالات PSB مفيدة من التربة المصرية واستخدامها كملقحات حيوية في الزراعة المستدامة حيث تتمتع هذه الاسمدة الحيوية بالقدرة على تعزيز نمو النبات، وتقليل الاعتماد على الاسمدة الكيماوية، والحد من خسائر المحاصيل.