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# Research Article
# Characterization of Plant Growth Promoting Rhizobacteria Isolated from an Arid Area Soil of Date Palm in Saudi Arabia

[1,2]Yassine Elmaati, [1]Fouad Msanda, [2]Abdelazize Eljiati, [2]Hammou Ouchaou, [1,3]Hassan Boubaker and [1]Mohamed Ait Hamza

[1]Laboratory of Biotechnology and Valorization of Natural Resources, Faculty of Sciences, Ibn Zohr University, Agadir, Morocco
[2]Department of R and D, YALA Laboratory, Yousef Abdul Latif and Sons Agriculture Ltd. (YALA) Company, Al-Qassim, Saudi Arabia
[3]Laboratory of Microbial Biotechnology and Plant Protection, Faculty of Sciences, Ibn Zohr University, Agadir, Morocco

## Abstract

**Background and Objective:** Many soil rhizospheric bacteria can transform insoluble forms of phosphorus and potassium to an accessible soluble form, contributing to plant nutrition as Plant Growth-Promoting Rhizobacteria (PGPR). The main purpose of this study was to isolate and identify the native population of phosphate and potassium solubilizing bacteria in the rhizospheres of date palm (*Phoenix dactylifera* L.) grown in the Al-Qassim region, Saudi Arabia. **Materials and Methods:** The rhizobacterial strains were isolated and *in vitro* screened for their phosphate and potassium solubilizing potential and ammonia production by using routine plate assay methods. The selected strains were identified based on nucleotide sequence data from the 16S ribosomal rRNA encoding gene. **Results:** The strains PSA1 and AZA2 have exhibited the highest qualitative efficiency of phosphate solubilization, while the strains AZA2 and PSA2 showed the most pronounced ability to solubilize potassium. The strains S1-3, I2 and AZS2 were the most effective for the production of ammonia. Based on the rRNA sequencing, these strains were identified as *Enterobacter ludwigii* (PSA1, PSA2 and AZA2), *Bacillus subtilis* (AZS2), *Bacillus cereus* (I2) and *Klebsiella oxytoca* (S1-3). **Conclusion:** These results suggest that some indigenous rhizobacterial strains could be an efficient and eco-friendly alternative to chemical fertilizers in the process of bio-fertilization of date palm.

Key words: Plant growth promoting rhizobacteria, date palm, Phosphate solubilizing bacteria, Potassium solubilization, Ammonia production, 16 S rRNA

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Corresponding Author: Yassine Elmaati, Laboratory of Biotechnology and Valorization of Natural Resources, Faculty of Sciences, Ibn Zohr University, Agadir, Morocco, Yousef Abdul Latif and Sons Agriculture Ltd. (YALA) Company, Al-Qassim, Saudi Arabia Tel: 0966541882741



Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The date palm, *Phoenix dactylifera* L., belonging to the Arecaceae family, is one of the most common crops cultivated in the Al-Qassim region, Saudi Arabia. This tree represents an important socio-economic and ecologic crop for the arid and semi-arid areas of many countries. However, its agricultural expansion in Saudi Arabia faces huge challenges, which are typical for dry regions characterized by water scarcity, low rainfall, high evapotranspiration, alkalinity and infertile (nutrient deficiency and/or insolubility) with salt-affected soils etc. In addition, agricultural activities in the Al-Qassim region are known to depend on the use of chemical fertilizers and pesticides to enhance soil fertility and crop yield. The excessive use of chemical fertilizers often results in adverse effects i.e., development of pathogen resistance to the applied agents and their non-target environmental impacts[1] including accumulation of nitrate in plant tissues, leaching of nitrate into groundwater and surface runoff of phosphorus and nitrogen[2]. Eco-friendly biological alternatives and the use of best management practices in order to reduce the use of chemicals are required to maintain agricultural productivity and protect the environment. Therefore, biological approaches to improve crop production became of great importance during last few decades. In this context, there is an ongoing rigorous interest worldwide in the beneficial microbes, including the so-called Plant Growth Promoting Rhizobacteria (PGPR), to promote plant growth since the term was first coined by Kloepper *et al*., in the late 1970s[3]. The PGPR generally refer to a group of soil and rhizosphere free-living bacteria colonizing roots in a competitive environment and consequently influence soil fertility and exerting a beneficial effect on plant growth[4]. The PGPR strains may have at least two of the three following criteria: aggressive colonization, plant growth stimulation and biocontrol properties[5,6]. PGPR enhance crop yield by direct mechanisms such as the improvement of nutrient availability to the crop by atmospheric nitrogen fixation, organic matter mineralization, solubilization of dicalcium phosphate, tri-calcium phosphate and other nutrients[7,8]. They also promote crop production or change many plant growth regulators concentration like indole acetic acid, gibberellic acid, cytokinins and ethylene[9] and stress regulating hormone 1-aminocyclopropane-1-carboxylate (ACC), deaminase hydrogen cyanate (HCN) and ammonia production, nitrogenase activity[10]. Indirect mechanisms include competitive exclusion of pathogens and stimulation of other beneficial organisms for the plant[11]. They could also act as antagonist against phytopathogenic microorganisms (biocontrol agents) by production of iron chelating siderophores[12], antibiotics[13] and cyanide[14], triggering induced local or systemic resistance and competition for nutrients and niches[15-17] or preventing deleterious effects of xenobiotics by degradation (rhizoremediators) by acting as rhizoremediators[18]. PGPR improve soil structure and bioremediate polluted soils by sequestering toxic heavy metal and degrading xenobiotic compounds[19]. Depending on their beneficial roles in the rhizosphere, PGPR have been classified as biofertilizers, phytostimulators, rhizoremediators and biopesticides[20].

Their application as crop microbial inoculants would be an attractive option as it would substantially reduce the use of inorganic fertilizers and pesticides, which often pollute the environment. Due to the increased availability of molecular tools, a large array of bacterial species was identified as PGPR in last few decades including species like *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligens*, *Arthobacter*, *Burkholderia*, *Bacillus* and *Serratia*, have been reported to enhance plant growth[9,21].

In Saudi Arabia, the most microbial biofertilizer agents in the market are imported from other countries. To the best of our knowledge, no local Plant Growth Promoting Rhizobacteria (PGPR) is available in the market. It has been shown that the survival of PGPR isolated from a temperate climate rapidly decreases when added to arid soil[22]. Isolation of native strains adapted to the arid environment may contribute to formulation of inoculants suitable for use with local regional crops. Native isolates may be preferred in the selection of bacteria for inoculation, as they are adapted to the environment and can be, thereby more competent than imported microbial strains[23]. Therefore, it is necessary to develop effective biofertilizers containing native PGPR strains that can adapt well to the arid environment. The rhizosphere of date palm, which is well adapted to the local environment, seems to be the best source of native microbial strains. The present investigation objective was isolation and evaluation of rhizospheric bacteria, associated with date palm in Al-Qassim region, for their phosphate and potassium solubilizing and ammonia production potential. In addition to identifying the selected bacteria based on nucleotide sequence data from the 16S ribosomal RNA (rRNA).

## MATERIALS AND METHODS

**Study area:** The isolation and characterization of plant growth-promoting Rhizobacteria lasted 25 months (from March, 2017 to April, 2019) at YALA Laboratory, Yousef Abdul Latif and Sons Agriculture Ltd. (YALA) Company, Al-Qassim province, Saudi Arabia. Al-Qassim area (Fig. 1a) has along,

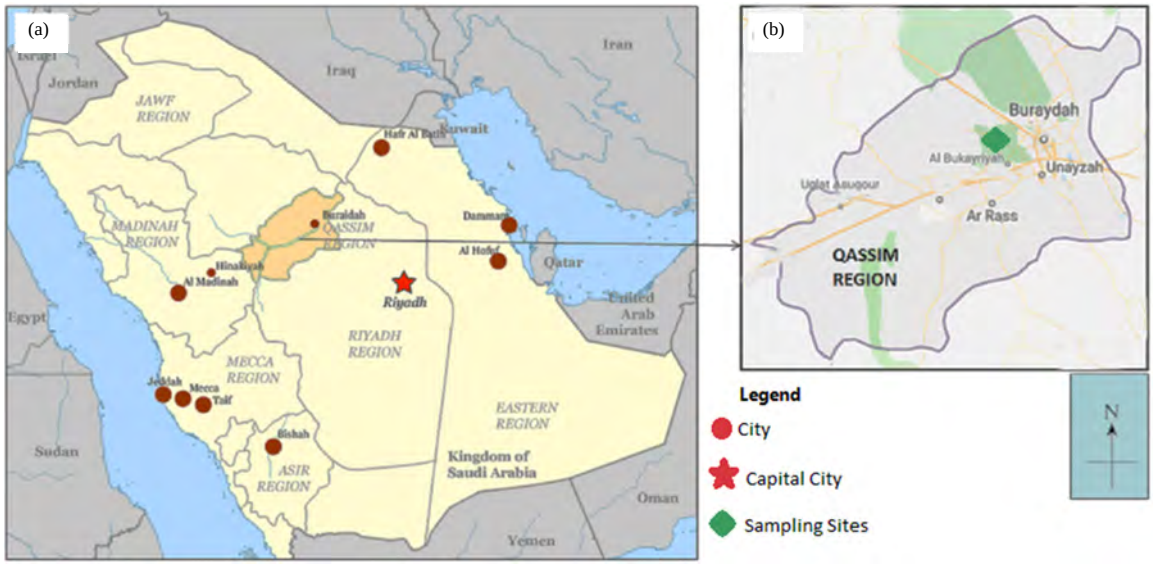


Fig. 1: (A) Map of Saudi Arabia showing Al-Qassim region in yellow, (B) Map of Al-Qassim region showing site of the study (http://en.wikipedia.org/wiki/File:Saudi_Arabia_location_map.svg)

sweltering and arid summer. Winter is cool and dry. The maximum temperature during the year when the study was conducted was 47°C in June and the minimum temperature reached-2.3°C in February. Annual total rainfall was 96.4 mm and annual average humidity was 17%, harsh conditions for most life forms.

**Collection of soil samples:** Samples of rhizospheric soil and root system from healthy plants of date palm were randomly collected in one of the farms (Nafeessa farm) of Yousef Abdul Latif and Sons Agriculture Ltd. (YALA) Company in Al-Qassim province, Saudi Arabia (Fig. 1b).

The rhizospheric soil samples of approximately 1000 g were obtained from different date palm sites. After collection, the soil samples were placed in plastic bags to avoid water loss. Each bag is provided with a reference code to identify the site of the sample and the date of sampling, then immediately transferred to the laboratory and stored in the refrigerator at 4°C in the dark for further process.

**Isolation of rhizobacteria from soil samples:** The Rhizobacteria were isolated from the samples using a dilution plate technique. Ten grams of soil was suspended in 50 mL of sterile physiological water (0.9% NaCl) (w/v). The soil suspension was vortexed for 30 min. Serial ten-fold dilutions were prepared by mixing, in Eppendorf tubes, 10 µL of the suspension made into 999 µL of sterile distilled water, until the$10^{-5}$ dilution was obtained.

In order to prevent the development of fungi and yeasts, the nutrient agar medium (R2A-F medium) was supplemented with 0.050 g L of cycloheximide by sterile filtration via syringe filters after autoclaving R2A-F medium at a temperature of 121°C and a pressure of 15 psi (1 bar) for 15 min. A volume of 50 µL of each dilution was plated onto R2A-F medium using a Drigalski spatula. The spread-plate cultures were sealed with cellophane and then incubated at 25±1°C in darkness until the appearance of bacterial colonies. Three replicates were made for each dilution. Then, the colonies differentiated morphologically based on shape, appearance and color were selected from each plate, isolated and purified by their sub-culture on fresh agar plates. Each isolate was given a specific code and sub-culture was carried out till pure colonies were obtained. Pure culture isolates were maintained on nutrient agar slants at 4°C before being used in the experiments (short term conservation) and were placed in a cryostorage box and stored at-80°C (long term conservation).

**Screening of pure bacterial isolates:** The screening of the bacterial isolates for various plant growth promoting activities like phosphate solubilization, potassium solubilization and NH3 production were performed according to the standard methods given by Mehta and Nautiyal[24], Hu *et al.*[25] and Duman and Soylu[26], respectively. The descriptions of these methods are as follows.

**Screening for phosphate solubilization activity:** Phosphate-solubilizing bacterial screening technique is based on the formation of clear halos around the colonies able to solubilize calcium phosphate[24]. Three aliquots of 5 µL from each bacterial isolate were plated on Pikovskaya's solid medium[27] and onto NBRIP agar medium[28] with the following ingredients (g $L^{-1}$): Pikovskaya Medium ((PVK)): Glucose, 10.0, $Ca_3(PO_4)_2$, 5.0, Yeast extract, 0.5, $(NH_4)_2SO_4$, 0.5, $MgSO_4 \times 7\ H_2O$, 0.1, KCl, 0.2, $FeSO_4 \times 7\ H_2O$, 10 mL (0,0136 g in 250 mL), Micro Agar, 12.0, the pH of medium is adjusted with 1 N HCl and NaOH solutions for 6.8-7.0. NBRIP-Medium: Glucose, 10.0, $Ca_3(PO_4)_2$, 5.0, $(NH_4)_2SO_4$, 0.1, NaCl, 0.2, $MgCl_2 \times 6\ H_2O$, 5.0, $MgSO_4 \times 7\ H_2O$, 0.25, KCl, 0.2, Micro Agar, 12.0, the pH of medium is adjusted with 1 N HCl and NaOH solutions for 7.0. Three replicates were made for each bacterial isolate using the two media. The plates were then sealed with cellophane and incubated at 25±2°C in darkness for 3-5 days.

They are observed for P-solubilization: the clear zones (halo zone) formed around colonies were measured with a ruler then the data were recorded. P-Solubility Index (PSI) was calculated using the following formula[29]:

$$\text{PSI (\%)} = \frac{\text{H-B}}{\text{B}} \times 100$$

Where:

H = Diameter of halo zone (cm)

B = Diameter of bacterial colony (cm)

**Screening for potassium solubilization activity:** The bacterial isolates were subjected to a Potassium solubilizing test in Aleksandrov medium[25]. Plates of modified Aleksandrov medium having $K_2HPO_4$ as a source of potassium were prepared. The medium contains 5.0 of Glucose, 2.0 g of $Ca_3(PO_4)_2$, 0.5 g of $MgSO_4 \times 7H_2O$, 0.1 g of $CaCO_3$, 0.005 g of $FeCl_3$ and 12.0 g of Agar in 1 l of deionized sterile water. The pH of medium is adjusted with 1 N HCl and 1N NaOH solutions for 7.2±0.2.

For the optimization of the assay and to improve the visualization of halo zone formation around the colonies on agar plates, dyes were used[30] by adding an acid-base indicator dyes ((bromothymol blue) (BTB)) to Aleksandrov modified medium from stock solutions (5 g $L^{-1}$) prepared in 70% (weight/volume) ethanol. An amount of 2 mL of stock dye solution were mixed in 100 mL of Aleksandrov agar medium to achieve final concentrations of 100.0 mg $L^{-1}$. After adding the measured amounts of dye solution, the medium was autoclaved for 15 min at 121°C then poured into petri plates. After spotting the rhizobacterial strains on the plates and incubation at 25±2°C for 72 hrs, the colonies exhibiting clear zones are selected and the K-Solubility Index (KSI) was calculated using the same above formula (PSI).

**Qualitative estimation of ammonia production:** Ammonia production of the bacterial isolates was tested using the qualitative method described by Duman and Soylu[26]. Peptone water broth (Peptone-10 g, NaCl 5 g, Dist. Water 1 L, pH-7) and Nessler's reagent were prepared: 20 mL of sterile peptone water broth medium was added to each test tube and inoculated with freshly grown culture of each rhizobacterial isolate. Two test tubes were prepared as a control. The tubes were incubated for 5-7 days at 37°C in darkness. Nessler's reagent was added in each tube. Test tubes with a brown to yellow color were considered positive for ammonia production and test tubes with no color change indicates negative test.

**Molecular identification of potent PGPR:** The six most efficient rhizobacterial strains selected based on one or more of the above mentioned PGP activities were genetically characterized. The 16S rRNA sequence analysis was used.

**Genomic DNA extraction:** Genomic DNA of rhizobacterial isolates was extracted using Insta Gene Matrix genomic DNA extraction kit (Bio-Rad, USA), according to the protocol recommended by the manufacturer. The isolated rhizobacterial colonies are picked up with a sterilized toothpick and suspended in 100 µL of sterilized saline water in a microfuge tube. The tube is then centrifuged at 10,000 rpm for 1 min. After removal of supernatant, the pellet is suspended in 200 µL of Insta Gene Matrix and incubated at 56°C for 30 min. The tube was vortexed at high speed for 10 sec and placed in a 100°C in boiling water bath for 8 min. The tube was vortexed again at high speed for 10 sec and spun at 10,000 rpm for 2-3 min. Finally, 20 µL of the resulting supernatant was used per 50 µL PCR reaction and the remainder of the supernatant was stored at-20°C.

**PCR amplification of 16s rRNA genes:** The 16S rRNA gene amplification was performed using the universal bacterial-specific primer sets, 27F 5' (AGA GTT TGA TCM TGG CTC AG) 3' and 1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'[31].

The PCR amplification of 16S rRNA genes was carried out using a program that starts with an initial denaturation cycle at 95°C for 5 min, followed by 30 cycles of 0.5 min denaturation at 95°C, annealing at 55°C for 2 min and 1.5 min extension at 68°C and ending with final extension cycle at 68°C, for 1.5 min.

**Sequencing and analysis of 16s rRNA genes:** For the purification of PCR products, the unincorporated PCR primers and dNTPs were removed using Montage PCR Clean up kit (Millipore). The purified PCR were directly sequenced using 2 primers: 785F 5′ (GGA TTA GAT ACC CTG GTA) 3′ and 907R 5′ (CCG TCA ATT CMT TTR AGT TT) 3′. Sequencing was performed using Big Dye Terminator Cycle sequencing kit v.3.1 (Applied Biosystems, USA) following the manufacturer's instructions. Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied Biosystems, USA). The 16S rRNA gene sequences of the bacterial isolates were compared to known sequences listed in NCBI's GenBank (National Center for Biotechnology Information (NCBI), National Library of Medicine, USA (http://www.ncbi.nlm.nih.gov/)), using BLAST search program[32]. Proposed taxonomic assignment of culturable bacteria was based on BLAST annotation using sequence identity and query cover as main criteria.

**Statistical analysis:** All data were subject to statistical analyses conducted in R 3.2.0 and multivariate analyses were performed using R language (R Development Core Team)[33].

## RESULTS

**Isolation of rhizospheric bacteria:** Morphologically distinct rhizobacteria isolates were isolated from date palm rhizosphere samples and purified by successive subculturing. A total of 25 isolates of rhizospheric bacteria were successfully isolated from the rhizospheric soil of date palm in Al-Qassim region.

### Rhizobacterial isolates screened for plant growth promoting traits

**Phosphate solubilization by bacterial isolates:** Phosphate-solubilizing potential of the isolates was screened by routine plate assay method using Pikovskaya agar medium and NBRIP agar medium. The P-solubilization potential was detected qualitatively by observing the formed halo zones around the bacterial colonies indicating the phosphorus solubilized from tri-calcium phosphate added to the two-agar media and PSI was calculated. Phosphate Solubilizing Bacteria (PSB) showing clear zone were selected. The result indicated that seven isolates (ATS1, AZA1, AZA2, AZS1, PSA1, PSA2 and PSS2) were able to form a clear transparent halo zone around the colony on Pikovskaya (PVK) medium (Fig. 2a) and on National Botanical Research Institute Phosphate (NBRIP) medium (Fig. 2b).

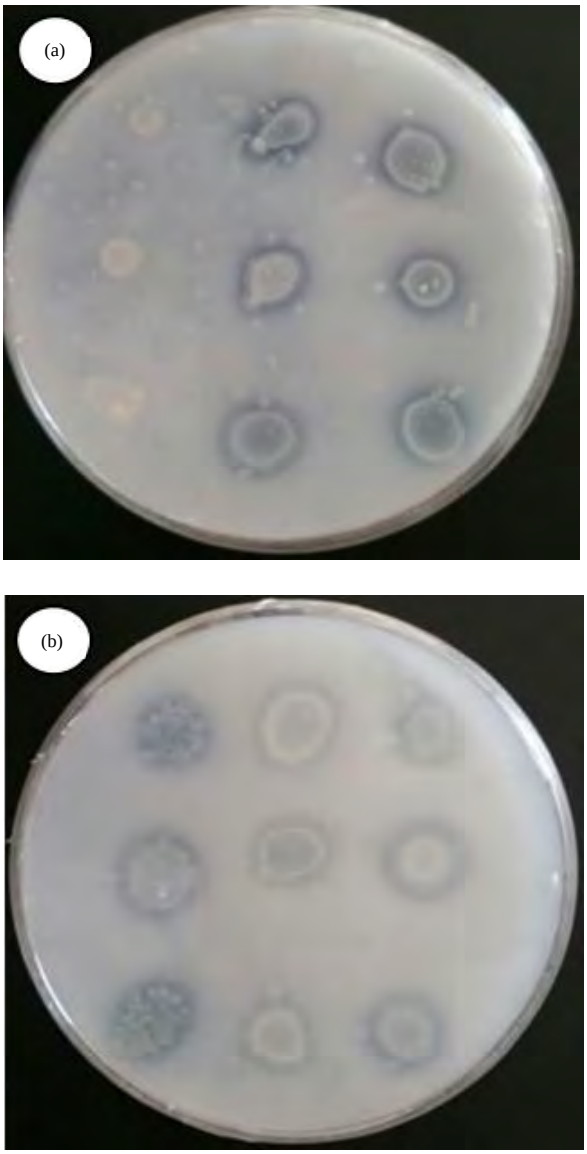


Fig. 2(a-b): (a) Growth of PSB on the Pikovskaya (PVK) medium and (b) Growth of PSB on National Botanical Research Institute Phosphate (NBRIP) medium containing insoluble phosphate characterized by the clear zone around the colony

Screening data of Pikovskaya medium is shown in Fig. 3a, while for NBRIP medium it is shown in Fig. 3b. Results indicated that all isolates possess phosphate solubilization activity, on both media but not to the same extent. Qualitative efficiency of phosphate solubilization by bacterial isolates showed significant differences. Results of Phosphate solubilization index (PSI) on both media clearly showed that two strains PSA1 and AZA2exhibited the highest qualitative efficiency. The efficiency of the two strains PSA1

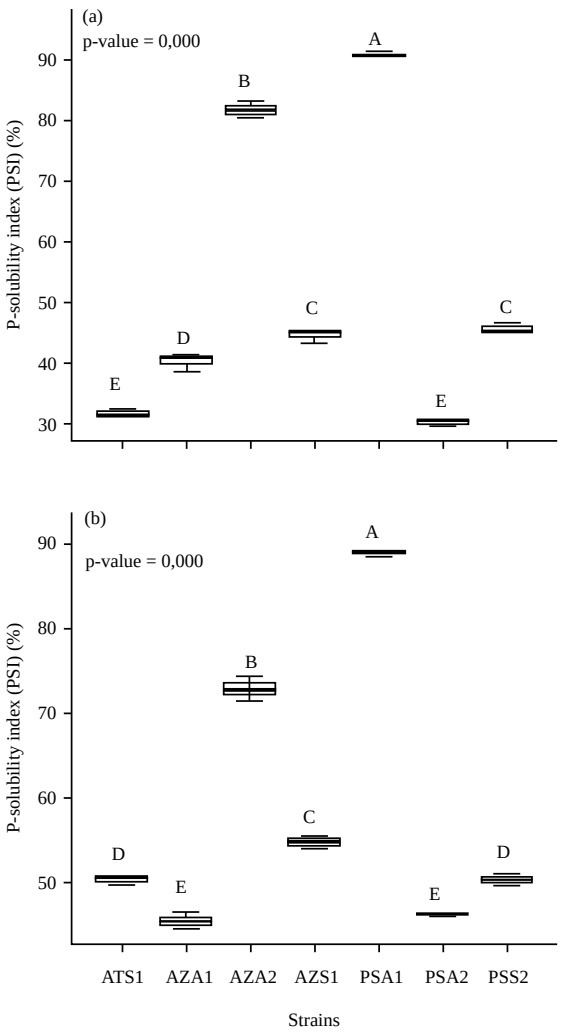


Fig. 3(a-b): (a) Index of phosphate solubilization (PSI) % on (a)Pikovskaya (PVK) medium after 7 days of incubation and (b) Index of phosphate solubilization (PSI) % on National Botanical Research Institute Phosphate (NBRIP) medium after 7 days of incubation

Means that do not share a letter are significantly different according to T-test analysis

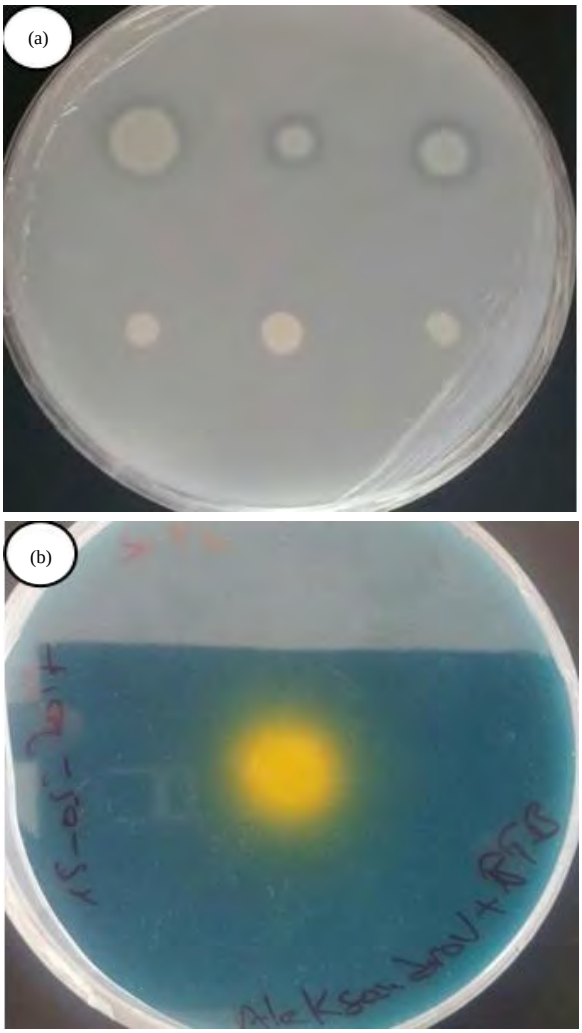


Fig. 4(a-b): (a) Growth of KSB on Aleksandrov medium and (b) Growth of KSB on Aleksandrov supplemented with bromothymol blue (BTB) medium characterized by the clear zone around the colony

and AZA2 in solubilizing phosphate was clear in both media: 90.93 and 81.8% in Pikovskaya's agar medium, respectively (Fig. 3a) and 88.9 and 72.83% in NBRIP agar medium (Fig. 3b), respectively for 3-5 days at 25±2°C. They were followed by AZS1 strain who exhibited a solubilization index of 45.5% in Pikovskaya's medium and 50.36% in NBRIP medium. PSS2 and ATS1 strains also showed good solubilization activity of 45.66 and 31.53%, respectively, in Pikovskaya's medium and 50.36 and 50.36% in NBRIP medium. Finally, PSA2 and AZA1 strains recorded the lowest phosphate solubilization efficiency of 30.2 and 40.26%, respectively, in Pikovskaya's medium and 46.2 and 45.5% in NBRIP medium, respectively (Fig. 3a and 3b).

**Potassium solubilization by bacterial isolates:** Aleksandrov agar medium (Fig. 4a) and the modified Aleksandrov agar medium using BTB (Fig. 4b) were used in the qualitative measurement of the K solubilization zone ability of the strains.

Among the bacterial isolates analyzed qualitatively for K solubilization, the best seven isolates with the highest scores of potassium-solubilizing index were selected (AZA2, S1-4, KSS1, PSA2, S1-3, I2 and S2-3). On Aleksandrov agar medium,

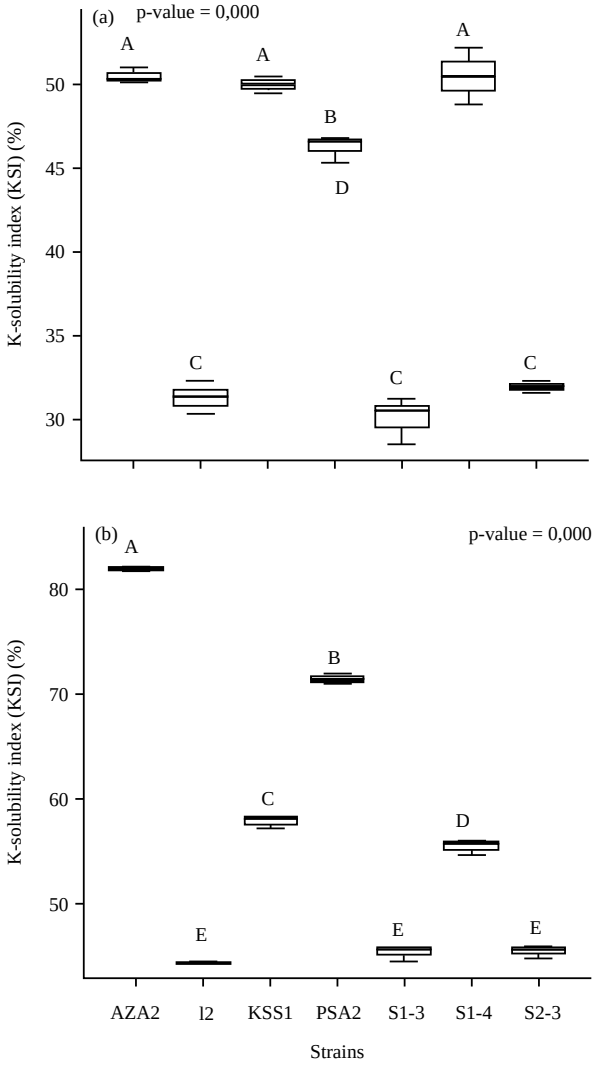


Fig. 5(a-b): (A) Index of potassium solubilization (KSI) (%) on Aleksandrov agar medium after 72 hrs of incubation in 25±2°C and (b) Index of potassium solubilization (KSI) (%) modified Aleksandrov agar using BTB medium after 72 hrs of incubation in 25±2°C

Means that do not share a letter are significantly different according to T-test analysis

strains AZA2, S1-4 and KSS1 showed the most pronounced ability to solubilize K, demonstrated by their highest solubilization index (SI) (50.5, 50.46 and 50%, respectively) followed by PSA2 strain (46.26%). However, S1-3, I2 and S2-3 solubilized the least amount of K as observed by weak Solubilization Index (SI) (30.06, 31.3 and 31.93%, respectively) compared to other strains (Fig. 5a). Data recorded of K-Solubilization Index (KSI) on the modified Aleksandrov medium using BTB revealed that the significant higher results

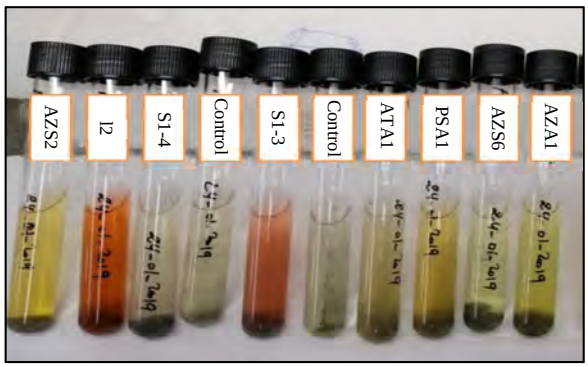


Fig. 6: Test for ammonia production

Table 1: Ammonia production by the selected strains

| Strain code | Coloration | Ammonia production |
|---|---|---|
| I-2 | Brownish yellow | +++ |
| AZS2 | Brownish yellow | +++ |
| S1-3 | Brownish yellow | +++ |
| PSA1 | light yellow | ++ |
| ATA1 | light yellow | ++ |
| AZA1 | yellow | + |

+: Low, ++: Moderate, +++: Strong

have been obtained by AZA2 and PSA2 strains (81.9 and 71.4%, respectively) as compared to other KSB strains, followed by KSS1 and S1-4 strains, which showed values of 57.83 and 55.46%, respectively. While strains I2, S1-3 and S2-3 showed the lowest values (44.43, 45.36 and 45.5%, respectively) as shown in Fig. 5b. Interestingly, strains on the modified Aleksandrov medium, using BTB, revealed a much greater zone of solubilization when compared with the strains on Aleksandrov agar medium. On the basis of K-solubilizing capacity, two efficient KSB strains, AZA2 and PSA2, were selected for further study.

**Qualitative estimation of ammonia production:** Ammonia production of the bacterial strains was studied from the 5-7th days of incubation at 37°C in darkness. According to the results obtained (Table 1), the strains I2, AZS2 and S1-3 were the most efficient in the production of ammonia compared to other strains, followed by PSA1 and ATA1, while the isolate AZA1 was low producer of ammonia (Fig. 6).

**Molecular identification of potent PGPR:** Six isolates which showed maximum PGPR activity were further characterized (AZS2, AZA2, PSA1, PSA2, I2 and S1-3) genetically.

**Sequencing and analysis of 16s rRNA genes:** Molecular characterization of the six isolates that showed interesting

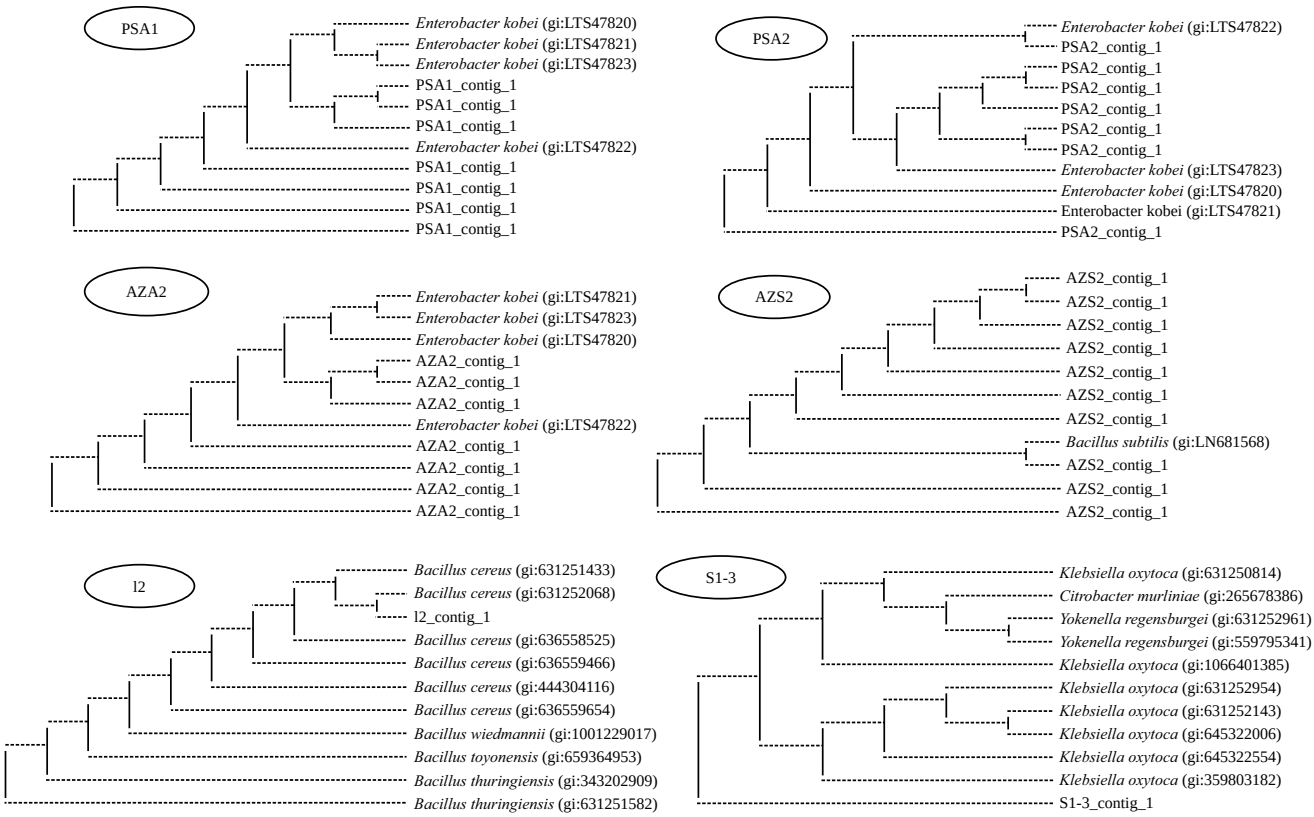


Fig. 7: Phylogenetic trees based on 16S rRNA sequence analysis and the relation between the six most efficient PGPRs isolates and the most closely related bacterial species

Table 2: BLAST search for the DNA sequence in NCBI (GeneBank)

| Isolates | GeneBank accession number | Description (Specie) | Genus | Family | Identity (%) |
|---|---|---|---|---|---|
| PSA1 | CP017279.1 | *Enterobacter ludwigii* | *Enterobacter* | Enterobacteriaceae | 99 |
| PSA2 | NR_042349.1 | *Enterobacter ludwigii* | *Enterobacter* | Enterobacteriaceae | 99 |
| AZA2 | NR_042349.1 | *Enterobacter ludwigii* | *Enterobacter* | Enterobacteriaceae | 99 |
| AZS2 | CP002905.1 | *Bacillus subtilis* | *Bacillus* | Bacillaceae | 99 |
| I2 | NR_074540.1 | *Bacillus cereus* | *Bacillus* | Bacillaceae | 99 |
| S1-3 | NR_118853.1 | *Klebsiella oxytoca* | *Klebsiella* | Enterobacteriaceae | 99 |

Samples of rhizospheric soil and root system from healthy plants of date palm were randomly collected in some squares (Square A, I and S) in Nafeessa farm of Yousef Abdul Latif and Sons Agriculture Ltd. (YALA) Company. Each isolate was given a specific codes as not to confuse us. PSA-1: PS = Phosphate Solubilizing, A = Square A, 1 = Strain 1. PSA2: PS = Phosphate solubilizing, A = Square A, 2 = Strain 2. ATA1: AT = Tested for growth in Azotobacter medium, A = Square A, 1 = Strain 1. AZA1: AZ = Tested for growth in Azospirillum medium, A = Square A, 1 = Strain 1. AZA2: AZ = Tested for growth in Azospirillum medium, A = Square A, 2 = Strain 2. AZS2: AZ = Tested for growth in Azospirillum medium, S = Square S, 2 = Strain 2. I-2: I = Square I, 2 = Strain 2. S1-3: S = Square S, 2 = Strain 2, 3 = Subculture 3

PGPR characteristics revealed that they are basically categorized into 4 different species of PGPR, based on 16S rRNA sequence analysis. Comparing the 16S rRNA sequences of the six PGPRs strains with the NCBI database using BLAST, the results indicate that the highest similarity was obtained to members of the Enterobacteriaceae family. The isolates PSA1, PSA2 and AZA2 showed 99% similarity with *Enterobacter ludwigii*, AZS2 exhibited 99% similarity with *Bacillus subtilis*. I2 showed 99% similarity with *Bacillus cereus* and S1-3 revealed 99% similarity with *Klebsiella oxytoca* (Table 2). Phylogenetic trees (Fig. 7) based on 16S rRNA sequence analysis enable us to group our six strains into 3 main genera: *Enterobacter*, *Bacillus* and *Klebsiella*. The strains PSA1, PSA2 and AZA2 were affiliated to *Enterobacter* cluster, while AZS2 and I2 belong to *Bacillus* and S1-3 formed a cluster with *Klebsiella* genus.

## DISCUSSION

Al-Qassim region, Saudi Arabia, is located in an arid zone characterized by various harsh natural environmental conditions mainly, low rainfall, extreme temperatures, nutrient

deficiency and insolubility in soils and high salinity. Rhizosphere is a suitable survival niche for soil microorganisms due to the accumulation of a variety of plant exudates, such as amino acids and sugars, providing a rich source of energy and nutrients for bacteria[34,35]. The PGPR colonize plant root surface and rhizospheric zone to exhibit beneficial effects on plant growth and development through a variety of mechanisms[36].

In the last few years, the number of worldwide identified PGPB has greatly increased. Several bacterial genera belonging to *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Burkholderia*, *Bacillus*, *Azospirillum* and *Serratia*, have already been isolated from various soils and reported as enhancing the growth of various plants [37,38]. In this study, Rhizobacterial strains were isolated from the rhizospheric soil of date palm and screened *in vitro* for different plant growth promoting activities. Several studies have been performed to isolate and screen effective local Plant Growth Promoting Rhizobacteria (PGPR) in Al-Qassim region, Saudi Arabia[39,40]. The six most efficient PGPRs isolates were characterized morphologically and genetically based on 16S rRNA gene sequence analysis. Their identification showed that they belong to the following genera: *Enterobacter*, *Bacillus* and *Klebsiella*.

In the soils of some agroecosystems, such as some arid and semi-arid regions, the total soil P concentration is adequate but 95-99% of phosphorus present is in insoluble, non-mobilizable or in precipitated forms. Therefore, plant-absorbable P (monobasic ($H_2PO_4^-$) and dibasic ($HPO_4^{-2}$) ions) is deficient[27]. Solubilization of mineral phosphorus is beneficial to enhance plant growth. In this aspect, an important function played by phosphate solubilizing bacteria (rhizosphere colonizing bacteria and endophytic) is to make mineral phosphorus more readily available for plant uptake through a variety of solubilization reactions such as acidification, chelation, exchange reactions and production of gluconicacid[41,42]. The ability of various *Enterobacter* strains to solubilize phosphate has been previously shown. Moreover, members of this genus have been found as endophytic plant growth promoters in date palm under saline conditions or in association with other diverse plants in arid lands[43]. Recently, a complete genome sequence analysis of an *Enterobacter* species, isolated from the Jizan region in Saudi Arabia, with multi-stress tolerance promoting activities has been published[44]. Shoebitz *et al.*[45] have reported that two strains of *Enterobacter ludwigii* have been qualified as PGPB because of their ability to solubilize inorganic phosphate. Many species of *Bacillus* including *Bacillus subtilis* and *Bacillus cereus* have shown their role in plant growth promotion by production of phytohormones, solubilization of phosphate and releasing ammonia from nitrogenous organic matter, etc[46]. *Bacillus* species are known for their wide distribution in many soil types and were suggested for their possible role in the adaptation of desert plants by supporting their growth[47]. Because of their spore-forming ability, they can survive in diverse biotic and abiotic environments[48].

Potassium is an essential macronutrient and most abundantly absorbed cation that play an important role in the growth of plants. The concentrations of soluble potassium in the soil are usually very low and more than 90% of potassium in the soil exists in the form of insoluble rocks and silicate minerals[49]. The capability of rhizobacteria to solubilize potassium rock has been of interest to agricultural microbiologist as it can maintain potassium status and plant uptake in soils for sustaining growth and yield[50]. Potassium Solubilizing Bacteria (KSB) are able to solubilize potassium rock through production and secretion of organic acids[51]. In this study, *Enterobacter ludwigii*, *Klebsiella oxytoca* and *Bacillus cereus* PGPR isolates identified from date palm rhizosphere were efficient in potassium solubilization. The results were supported by Wang *et al.*[52], who reported that five *Klebsilla*, one *Enterobacter* and one *Bacillus* strains isolated from the rhizosphere and roots of wheat have the potential to solubilize potassium.

The production of ammonia is an important trait of PGPR that indirectly influences the plant growth. Current study has shown that the majority of tested strains were able to produce ammonia. Mukhtar *et al.*[53] reported that the ACC-deaminase produced by *Bacillus cereus* helped to alleviate ethylene production which cleaved the ACC to α-ketobutyrate and ammonia and decreased the adverse effects of ethylene on plant growth under heat stress. Joseph *et al.*[54] reported an ammonia production in 95% of isolates of *Bacillus* followed by *Pseudomonas* (94.2%), *Rhizobium* (74.2%) and *Azotobacter* (45%).

In the present research investigation, numerous rhizobacterial strains isolated from date palm rhizosphere exhibited significant plant growth-promoting attributes *in vitro* tests. Therefore, it is crucial to test their PGPR potential in pot and field experiments to make this bio-fertilizer potential a practical option for farmers' use.

## CONCLUSION

The PGPR are involved in various biotic activities of the soil ecosystem to make it dynamic for turnover and sustainability of crop production. They are found in plant roots or in the adjacent soil and enhance plant growth through multiple direct and indirect mechanisms, including phosphate solubilization, potassium solubilization and ammonia

production. PGPR have been investigated in search of alternative methods to reduce the application of chemical fertilizers, which in turn reduces the pollution of the environment and the cost of production and increases the yield in terms of quality and quantity. Screening and evaluating of native strains of plant growth promoting rhizobacteria (PGPR) in Al-Qassim region in Saudi Arabia were conducted in this study. Numerous rhizobacterial strains were isolated from the date palm rhizosphere. Isolates, which showed interesting PGPR characteristics, were further identified via 16S rRNA sequencing. The isolated rhizobacteria were grouped into tree distinct genera according to their molecular identification (*Enterobacter*, *Bacillus* and *Klebsiella*). The isolated strains showed different growth promoting activities since they can solubilize phosphate, potassium and to produce ammonia. Based on these results, the isolated PGPRs in this study are further able to provide the plant with essential nutrients such as phosphate, potassium and ammonia and could be an efficient and eco-friendly alternative to chemical fertilizers.

## SIGNIFICANCE STATEMENT

In the present study, it was presented one of the promising techniques in the practice of sustainable agriculture: the use of plant growth promoting rhizobacteria (PGPR) to improve the quality and quantity of crops. More specifically, it was discussed the suitability of some bacteria genera(*Enterobacter*, *Bacillus* and *Klebsiella*) isolated from the rhizospheric soil of date palm in Al-Qassim region in Saudi Arabia as phosphorus and potassium solubilizers and ammonia producers. Such biological products therefore represent a sustainable alternative to the excessive use of chemical fertilizers.

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