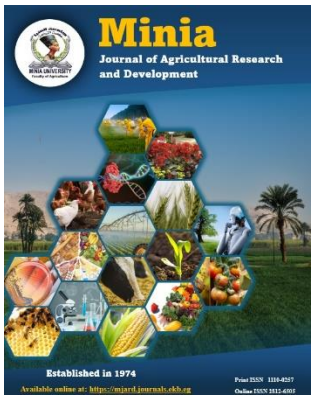
Minia J. of Agric. Res. & Develop., Minia Univ., Vol. 45 (4): 549 - 561, 2025

Minia Journal of Agricultural Research and Development

Journal homepage & Available online at:

https://mjard.journals.ekb.eg



Isolation and Identification of non-symbiotic nitrogen fixing and phosphate-solubilizing salt tolerant bacteria.

Gaber Z. Breisha[1], Omar A. O. Saad[1], Afifi M. M.I [2] and Walaa M. Nagieb[1]

[1]Department of Agricultural Microbiology, Faculty of Agriculture, Minia University, Minia, Egypt.
[2]Institute of Soil, Water and Environmental Research, Agricultural Research Center, Giza, Egypt.

Received: 20 July 2025 Accepted: 23 July 2025

ABSTRACT

Azotobacter a non-symbiotic nitrogen-fixing bacterial genus, along with some species of Bacillus genus as phosphate-solubilizing bacteria (PSB), are recognized for their significant contributions to sustainable agriculture. These microorganisms enhance soil fertility and plant productivity by facilitating key nutrient cycles most notably through the biological fixation of atmospheric nitrogen and the solubilization of insoluble phosphate compounds. However, soil salinity hinders the work of these bacteria and makes them unable to supply plants with nitrogen or phosphorus. In this work an efficient strains of non-symbiotic nitrogen fixing bacteria and phosphate dissolving bacteria were isolated from saline soil. These isolates were used for microorganisms identification based on cultural, morphological and biochemical characteristics as mentioned in Bergey's Manual of Systematic Bacteriology. These isolates were used for microorganisms identification based on cultural, morphological and biochemical characteristics as mentioned in Bergey's Manual of Systematic Bacteriology. The ability of these isolates to fix nitrogen or dissolve precipitated phosphorous were tested. Probabilistic and then confirmatory identification tests confirmed that the best strain isolated from saline soil that fixes nitrogen non-symbiotically is *Azotobacter chroococcum* while the best one for phosphorus solubilization is *Bacillus megatherium*.

Keywords: saline soil, Identification, nitrogen, Azotobacter, Phosphorous, Bacillus

* Corresponding author: Walaa M. Nagieb
E-mail address: walaagomea@gmail.com

1-INTRODUCTION

Nitrogen and phosphorus are essential macronutrients for plant growth and development, yet their availability in agricultural soils is often limited, posing a significant challenge to global food security (Zhang *et al*., 2021). Conventional agricultural practices rely heavily on chemical fertilizers to address these nutrient deficiencies; however, their excessive use led to severe environmental consequences, including soil degradation, water pollution, and greenhouse gas emissions (Savci., 2012). Sustainable substitutes like plant growth-promoting rhizobacteria (PGPR), which can increase nutrient availability and soil fertility and the quantity of phosphates dissolved by the chosen isolates, are becoming more and more popular in response. Through the action of the nitrogenase enzyme, nitrogen-fixing bacteria are essential in transforming atmospheric nitrogen into ammonia, a form that plants may easily absorb. (Bhattacharjee *et al*., 2020). Similarly, by releasing organic acids and enzymes, phosphate-solubilizing bacteria dissolve insoluble phosphates, releasing phosphorus for plant uptake. (Alori *et al.,* 2017). These bacteria, have gained significant attention for their ability to promote plant growth through biological nitrogen fixation and phosphate solubilization (Kour *et al*., 2021).

Azotobacter is a well-known genus of free-living nitrogen-fixing bacteria that live in the rhizosphere, not only enriching soil nitrogen but also producing phytohormones that stimulate plant growth. Some species of the genus Bacillus are also well known for their ability to dissolve precipitated phosphorus and convert it into a form that is readily available to the plant. (Jiménez *et al.,* 2020). Despite their potential, the practical application of these bacteria in agriculture faces several problems related to adverse environmental conditions that cause the bacteria to lose their ability to benefit plants. Among the most important adverse environmental conditions is soil salinity. One of the main issues affecting agricultural output worldwide is salinity. As mentioned by Manuel *et al* .2017) 33% of irrigated land and 20% of all farmed land worldwide are degraded and damaged by salt.

This study aims to isolate, characterize, and identify saline tolerant free nitrogen-fixing bacteria and phosphate-solubilizing bacteria from saline soil as their native inhabit. Evaluating the efficiency of the isolates in nitrogen fixation and phosphate solubilization was also one of the objectives of this study.

2. MATERIALS AND METHODS

2. 1. Sampling

Eight saline soil samples were taken from different areas of Kafr El-Sheikh Governorate (Baltim - Sidi Salem - Al-Hamoul – Tlumpat 7)

2.2 Isolation

Ashby's Mannitol Agar or broth modified *by* **Abdel-Malek and Ishac *(*1968*)*** was used for Azotobacter isolation after incubation at 30°C for 3-5 days.

Bunt and Rovira Medium (1955) was used for isolation of phosphate dissolving bacteria. Colonies showing clear zones around them were selected as phosphate-solubilizing bacteria

2. 3. Primary Identification

Single colonies of nitrogen fixers or phosphate dissolvers were picked up, purified by successive streaking on a suitable medium (deficient nitrogen medium for nitrogen fixers and

nutrient agar medium for phosphate dissolvers). Based on the cultural, morphological, and biochemical traits listed in Bergey's Manual of Systematic Bacteriology, these isolates were used to identify microorganisms. These features include gram stain, motility, cell shape, color, and consistency. The hanging drop method was used to measure the isolates' motility. (Narendran *et al*., 2016). Slides were prepared and motility was observed under microscopic oil immersion lens. Purified isolates were maintained on at 4^0C or subsequent use.

2.4. Nitrogen fixing efficiency

The nitrogen fixing capability of the isolates was achieved using the ambient assay of N-ase activity according to Postgate(1972)

2. 5. Screening the isolates for phosphate solubilizing ability

Using the free phosphate liquid Bunt and Rovira medium, which was put in 250 mL flasks each with 100 mL of the medium and 1 g of tri-calcium phosphate added to each flask as a source of insoluble phosphate, the effectiveness of the phosphate-solubilizing bacterial isolates were assessed. Following sterilization, 1 mL of a pure culture of phosphate-solubilizing bacteria was added to the flasks, which were left to age for 48 hours. After three weeks of incubation at 28 °C in a shaking incubator (100 cycles per minute), 10 milliliters of the liquid culture were removed from the flasks and centrifuged (6000 cycles per minute). A pH meter was used to estimate the media's pH. A spectrophotometer was used to quantify the amount of dissolved phosphorus at a wavelength of 660 nanometers.

2. 6. Molecular Identification

DNA of the most efficient nitrogen fixers and phosphate dissolvers was extracted according to the method described by **Maniatis *et al* (1982).** Samples of the extracted DNA have been sent to Lab of Sigma-Aldrich Company, South Koria for identification using molecular techniques and Sequencing of 16s rRNA and Phylogenetic analysis

3. RESULTS AND DISCUSSION

3. 1. Primary identification of Free living saline tolerant nitrogen fixers

Thirty- eight colonies of what are believed to be Azotobacter bacteria were picked up and purified by successive streaking on nitrogen deficient medium. After 48 hours of growth in a nitrogen-free liquid culture, the results revealed that 15 of the 38 isolates had rounded ends and were gram negative. Initially semi-transparent and slightly viscous, the colonies grown on nitrogen-free medium eventually turned dark brown. According to the findings, the isolated compounds were oxidase negative, catalase positive, and capable of converting nitrate to nitrite. These characteristics are consistent with what was reported by **Atlas (1997)** and are considered evidence that certain bacterial isolates belong to the genus Azotobacter.

3. 2. Nitrogenase activity

The fifteen isolates, identified as a single species or species of the genus Azotobacter, were subjected to nitrogenase activity testing. The purpose of this experiment was to test the isolates' nitrogen-fixing efficiency. The results of this experiment are illustrated in Table (1) and Fig. (1). The results showed a significant variation in the ability of Azotobacter

isolates to exhibit nitrogenase enzyme activity and thus there is a great difference in the ability of these isolates to fix nitrogen. Five azotobacter isolates exhibit high nitrogenase activity comparing to the other isolates. The highest nitrogenase activity given by the isolate number 9 was 870 nmole C_2H_4/L/hr followed by the isolates numbers 3, 13, 10 and 7, which gave nitrogenase activity of 287, 150, 144 and 138 nmole C_2H_4/L/hr respectively. The nitrogenase activity of the other isolates ranged from only 21 to 60 nmole C_2H_4/L/hr. Because Azotobacter species can synthesize one or more alternative nitrogenases in situations where molybdenum is scarce, in addition to using the traditional Mo-containing enzyme for BNF, they can be regarded as evolving bacteria, which explains this variation in nitrogenase activity. Three distinct nitrogenase enzymes with distinct structural subunits were discovered to be encoded by A. vinelandii, for instance: (1) the conventional Mo-nitrogenase; (2) an enzyme that contains vanadium (nitrogenase-2, encoded by the vnf-H, vnf-D, G, and K genes); and (3) an iron-containing nitrogenase (nitrogenase-3, encoded by the H, D, G, and K gene cluster). (Robson *et al*., 1986; Leigh, 2002).

Table (1) Nitrogenase activity (nanomole C2H4 ml-1 hr-1) of Azotobacter isolates:

No.	Samples code	N_2 – ase activity nmole C_2H_4/L/hr
1	T_1	40.0
2	T_2	30.0
3	T_3	287.0
4	T_4	44.0
5	T_5	64.0
6	T_6	21.0
7	T_7	138.0
8	T_8	60.0
9	T_9	870.0
10	T_{10}	144.0
11	T_{11}	21.0
12	T_{12}	70.0
13	T_{13}	150.0
14	T_{14}	40.0
15	T_{15}	60.0

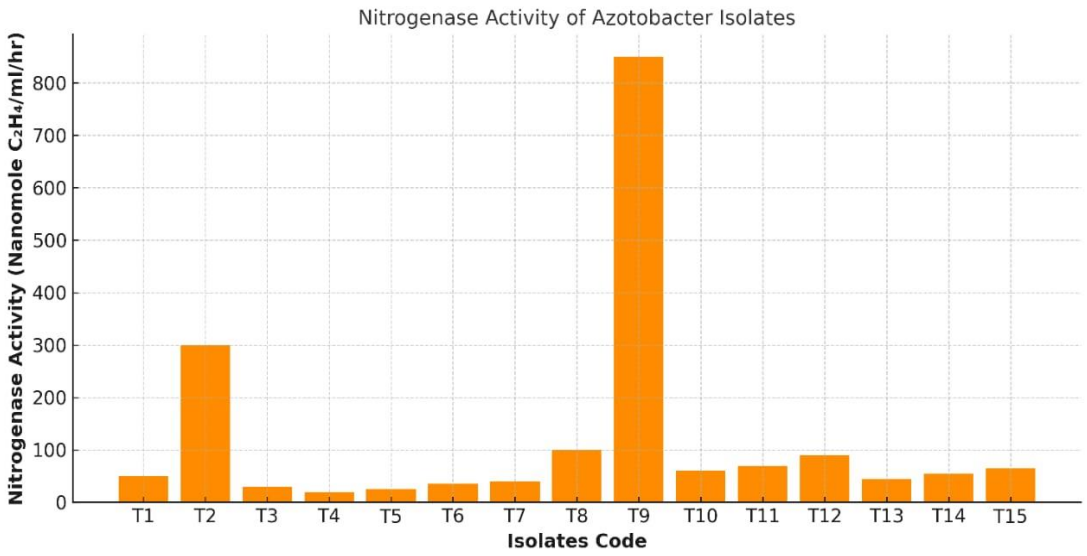


Fig (1): Nitrogenase activity (nanomole C_2H_4 ml-1 hr-1) of either Azotobacter isolates.

3. 3. Saline tolerant phosphate dissolvers.

The formation of clear zones around colonies of bacteria grown in Bunt and Rovera medium was taken as evidence of the ability of these bacteria to dissolve precipitated phosphate. The results illustrated in Table (2) and Fig. 2 show colonies diameter (mm), clear zones diameter (mm) and solubilizing index (SI) of thirty-two colonies of bacteria grown on Bunt and Rovera medium. The SI of each colony was obtained by dividing the diameter of the clear zone by the diameter of the colony. The efficiency rating of phosphate-dissolving bacteria published by Pikovskaya, (1948) and Nautiyal (1999) shows that bacteria with efficiency rating ≥2.5, 1.5- 2.5 or < 1.5 are considered highly, moderately, or lowly active in dissolving phosphate, respectively. Accordingly, twenty colonies were highly active in phosphate solubilizing activity, eight colonies were moderately active and four colonies had low activity. Wang *et al* (2021) isolated a few species of salt-tolerant bacteria from saline–alkali soil and their phosphate solubilization characteristics were evaluated under different levels of salt stress. However, there is little information about bacterial strains able to solubilize inorganic phosphates in saline soil. Khan *et al* (2009) reported that strains with SI > 2 are potential candidates as biofertilizers (Khan *et al*., 2009).

Table (2): Phosphate Solubilization and Phosphate Solubilizing index (SI) of bacteria from saline soil

Isolates No.	Colony diameter (mm)	Clear halo zone (mm)	(SI)	Interpretation (Efficiency)	Isolates No.	Colony diameter (mm)	Clear halo zone (mm)	(SI)	Interpretation (Efficiency)
B1	3	14	4.66	High	B17	3.2	12.5	3.91	High
B2	2.5	18	7.2	High	B18	2.7	4.45	1.64	Moderate to High
B3	3.6	11	3.05	High	B19	2.9	7	2.41	Moderate to High
B4	3	15	5.0	High	B20	3.2	10	3.13	High
B5	3.7	13	3.51	High	B21	2.7	8	2.96	High
B6	4.2	8	1.90	Moderate to High	B22	3	18	6.0	High
B7	0.9	0.5	0.55	Low	B23	3	15	5.0	High
B8	3.9	14	3.58	High	B24	2.9	2.5	0.86	low
B9	4.5	8	1.78	Moderate to High	B25	2.6	6	2.30	Moderate to High
B10	3	16	5.33	High	B26	3	12	4.00	High
B11	3.4	13	3.82	High	B27	3.2	11	3.43	High
B12	3.9	7	1.79	Moderate to High	B28	2.6	5	1.92	Moderate to High
B13	3.2	9	2.81	High	B29	3	14	4.66	High
B14	3.9	4	1.03	Low	B30	2.6	13	5.00	High
B15	3	0.5	0.1	low	B31	3	10.5	3.50	High
B16	1.6	2.5	1.56	Moderate to High	B32	2.5	9.5	3.8	High

Efficiency Rating: (Pikovskaya, 1948; Nautiyal, 1999):

- High: SI ≥2.5 or P ≥50 µg/mL.
- Moderate: SI = 1.5–2.5 or P = 20–49 µg/mL.
- Low: SI < 1.5 or P < 20 µg/mL.
- Solubilization Index (SI) = $\frac{\text{halozone diameter (mm)}}{(\textit{colony diameter (mm)})}$

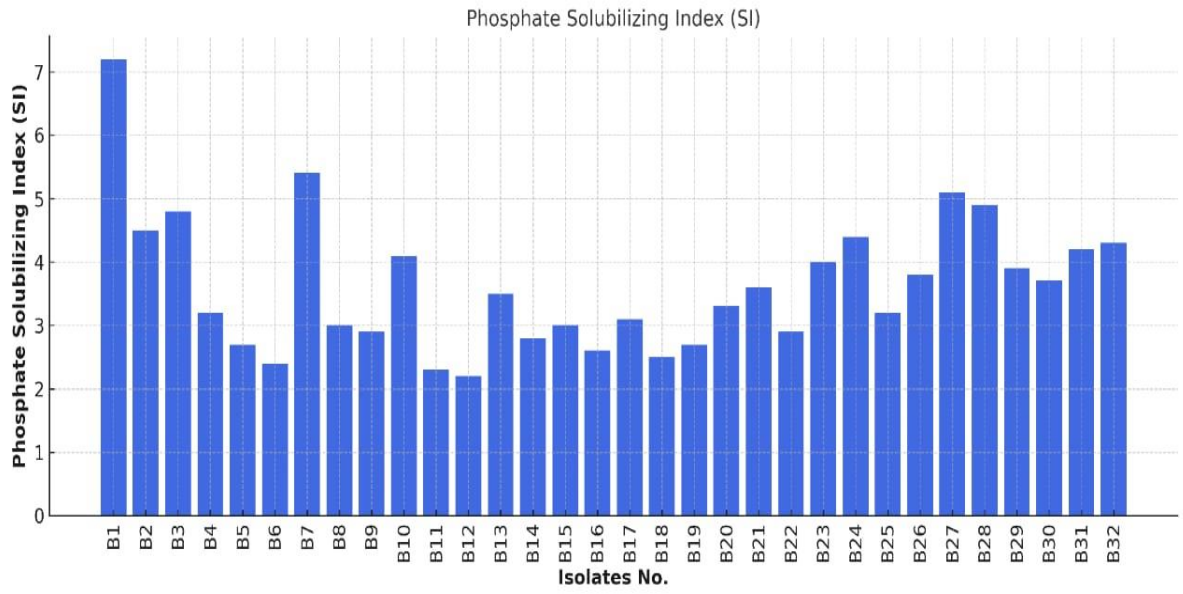


Fig (2): Phosphate Solubilizing index (SI)

Primary identification of highly active and highly saline tolerant phosphate solubilizing bacteria

The twenty isolates showed high activity in phosphate solubilization as mentioned above were microscopically examined. The cells were small to medium-sized and convex colonies all of them were gram positive, rod shaped and spore formers. The cells were single, diploid or found in chain. Young cells (24 hr age) were motile. These characteristics are consistent with what was reported by **Atlas (1997)** and are considered evidence that these isolates belong to the genus Bacillus.

Selection of the most efficient phosphate dissolving strain

Out of the twenty isolates which gave the highest efficiency rating in phosphate solubilization, six isolates reduced the pH of Bunt and Rovera liquid medium from 7.2 to less than four (Table 3). The pHs were reduced as a result of acids. However, While the majority of research has determined that one of the primary mechanisms of PSB is the production of organic acids, certain studies continue to hold differing opinions. In their research, Zhao *et al*. (2003) discovered no correlation between the medium's organic acid content and PSB's ability to solubilize phosphorus. So that it was important to determine the amounts of soluble phosphorus produced by these twenty isolates. The results in Table (4) showed that the most efficient six isolates in pH reduction were the most efficient isolates regarding the amount of produced soluble phosphorus (ppm). Low-molecular-weight organic acids, including butyric acid, lactic acid, 2-ketogluconic acid, fumaric acid, oxalic acid, glutamic acid, propionic acid, fumaric acid, acetic acid, tartaric acid, malonic acid, glutamic acid, and propionic acid, can be secreted by phosphate-solubilizing bacteria as they grow. Patel *et al* .,2021. Under low pH conditions, these low-molecular-weight organic acids can chelate with metal ions in the soil (Fe3+, Al3+, Ca2+) through

hydroxyl and carboxyl groups. They compete with phosphates for phosphorus adsorption sites in the soil, which increases the soil's uptake of phosphate and increases the solubilizing capacity of inorganic phosphorus. This increases the solubility and availability of mineral phosphates. Their most important function is Ca2+ chelation. (Dash *et al*., 2019).

Table(4) Amount of phosphates solubilized pH values.

Blank =7.2

Isolation no.	pH-day1	pH-day2	pH-day3
B1	6.81	5.92	3.88
B2	6.78	5.86	3.74
B3	6.91	6.04	4.92
B4	6.88	5.96	4.89
B5	6.84	5.94	4.88
B6	6.95	6.06	4.93
B7	6.77	5.86	3.77
B8	6.72	5.81	4.70
B9	6.83	5.94	4.81
B10	6.96	6.08	4.93
B11	6.76	5.87	3.74
B12	6.74	5.82	4.70
B13	6.86	5.97	4.87
B14	6.90	6.00	4.89
B15	6.86	5.94	4.89
B16	6.77	5.86	4.74
B17	6.82	5.90	4.83
B18	6.89	5.97	4.88
B19	6.86	5.92	4.87
B20	6.94	6.06	4.92
B21	6.82	5.90	4.81
B22	6.78	5.86	3.78
B23	6.92	6.02	4.90
B24	6.77	5.86	3.77
B25	6.85	5.92	3.81
B26	6.93	6.03	4.91
B27	6.86	5.92	3.86
B28	6.77	5.81	4.71
B29	6.92	6.01	4.90
B30	6.87	5.91	3.89
B31	6.89	5.94	4.86
B32	6.79	5.82	4.74

Molecular Identification

According to the above results DNA of the most efficient Azotobacter isolate and the most efficient Bacillus isolate have been sent to Lab of Sigma-Aldrich Company, South Koria for identification using molecular techniques and Sequencing of 16s rRNA and Phylogenetic analysis. The company kindly sent the results which indicate the Azotobacter isolate was *Azotobacter chroococcum* and the Bacillus isolate was *Bacillus megatherium*. So, it can be concluded that this study resulted in the isolation and complete identification of a strain of nitrogen-fixing bacteria and a strain of phosphorus-dissolving bacteria with high activity and efficiency. However, the most important and remarkable thing, and what we consider a scientific achievement, is that these strains can tolerate high salinty.

Bacillus megaterium (Isolate) [95%]

├─ *Bacillus subtilis (92%)*
├─ *Bacillus licheniformis (89%)*
├─ *Bacillus cereus (86%)*
├─ *Bacillus pumilus (90%)*
├─ *Bacillus amyloliquefaciens (88%)*
├─ *Bacillus firmus (87%)*
├─ *Bacillus thuringiensis (91%)*
├─ *Bacillus safensis (85%)*
├─ *Bacillus mojavensis (84%)*
└─ *Bacillus megaterium* (Isolate) [95%]

Scale bar: 0.02 substitutions/site

Azotobacter chroococcum (Isolate) [96%]

├─ *Azotobacter vinelandii (90%)*
├─ *Azotobacter salinestris (86%)*
├─ *Azotobacter armeniacus (88%)*
├─ *Azotobacter nigricans (84%)*
├─ *Azotobacter tropicalis (87%)*
├─ *Azotobacter beijerinckii (85%)*
├─ *Azotobacter paspali (82%)*
├─ *Azotobacter insuavis (89%)*
├─ *Azotobacter indicus (83%)*
└─ *Azotobacter chroococcum* (Isolate) [96%]

Scale bar: 0.02 substitutions/site

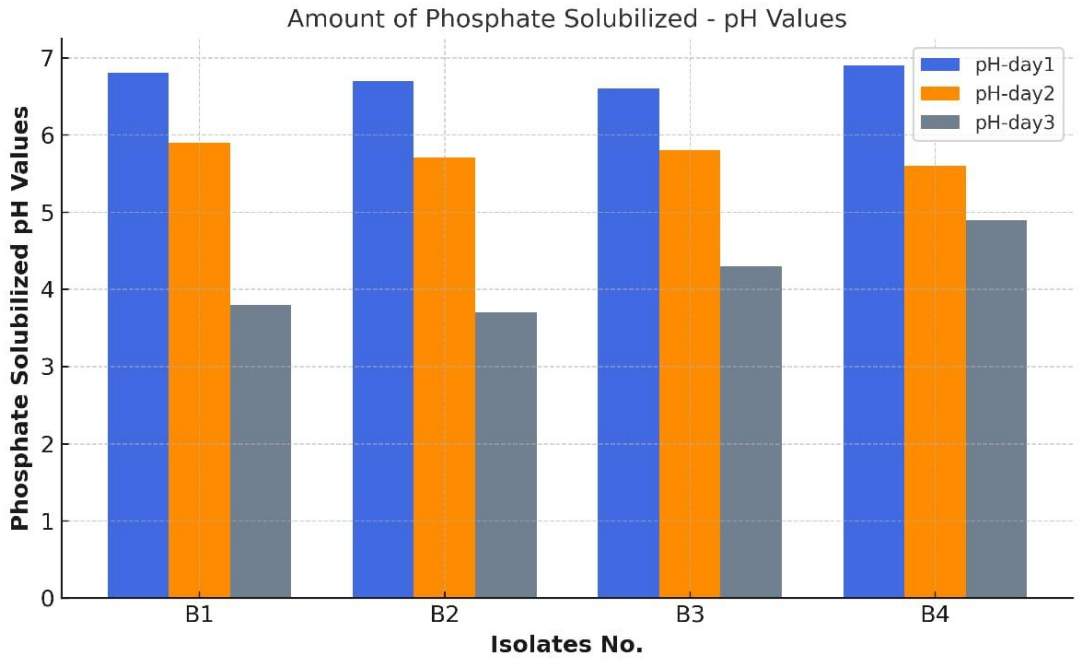


Fig (4): Amount of phosphate solubilized pH values

Table(5) Amount of phosphates soluilized by the selected isolates

• No. of isolates (PSB)	• Soluble-p(ppm)
• B1	• 273.25
• B2	• 377.00
• B3	• 248.50
• B4	• 270.22
• B5	• 146.22
• B6	• 393.11
• B7	• 550.00
• B8	• 342.11
• B9	• 592.00
• B10	• 251.23
• B11	• 370.11
• B12	• 450.22
• B13	• 470.23
• B14	• 432.11
• B15	• 460.22
• B16	• 166.75
• B17	• 186.11
• B18	• 144.22
• B19	• 191.06
• B20	• 553.00
• B21	• 463.00
• B22	• 296.00
• B23	• 378.16
• B24	• 448.32
• B25	• 532.09
• B26	• 364.52
• B27	• 258.33
• B28	• 166.37
• B29	• 231.53
• B30	• 341.80
• B31	• 421.71
• B32	• 132.82

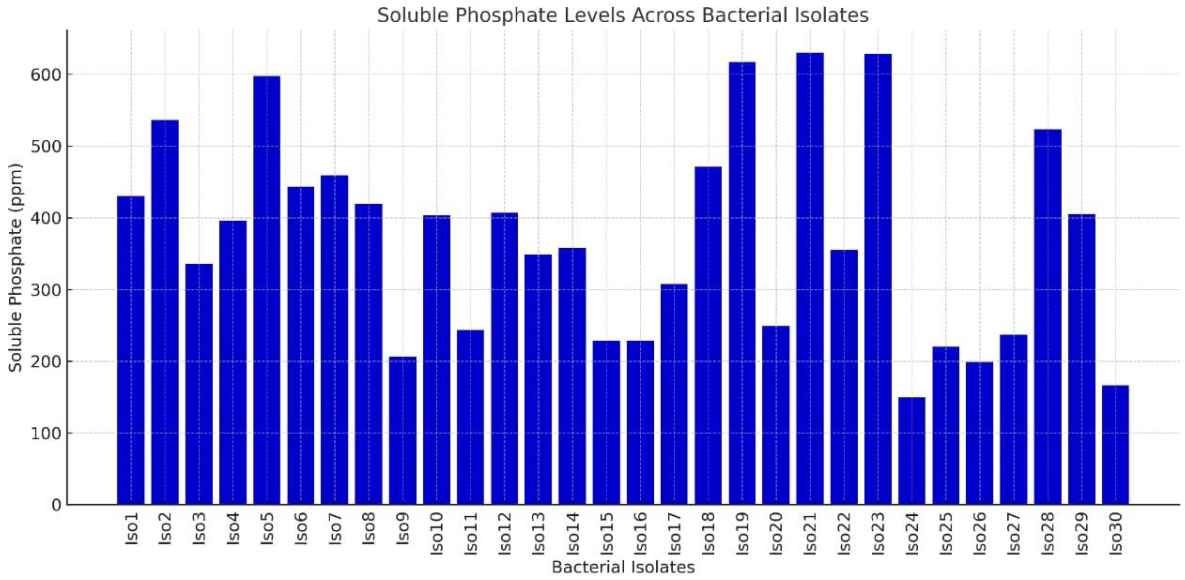


Fig (5): phosphates solubilized

REFERENCES

Abdel-Malek Y. and Ishac Y.Z. (1968). Evaluation of methods used in counting Azotobacter. Journal of Applied Bacteriology, 31(3): 269–275.

Alori, E. T., Glick, B. R. and Babalola, O. O. (2017). Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Frontiers in Microbiology, 8, 971. https://doi.org/10.3389/fmicb.2017.00971

Atlas, M. Ronald (1997). Handbook of Microbiological Media Second Edition. pp. 126. CRC Press. University of Louisville, Kentucky, USA.

Bhattacharjee, R. B., Singh, A. and Mukhopadhyay, S. N. (2020). Use of nitrogen-fixing bacteria as biofertilizer for non-legumes: prospects and challenges. Applied Microbiology and Biotechnology, 97, 1305–1314.

Bunt J. S. and Rovira ,A. D (1955). Microbiological studies of some sub-Antarctic soils. Journal of Soil Science, 6, 119–128.

Dash, S., Borah S.S .and Kalamdhad, A.S. (2019). Study of the limnology of wetlands through a one-dimensional model for assessing the eutrophication levels induced by various pollution sources. Ecol. Model. 2019;416:108907.

Jiménez, D. J., Dini-Andreote, F. and van Elsas, J. D. (2020). Metataxonomic profiling and microbial functions of rhizosphere soil and root-endosphere microbiomes in barley. Plant and Soil, 417, 139–153.

Khan, M. S., Zaidi, A. and Wani, P. A. (2009). Role of phosphate-solubilizing microorganisms in sustainable agriculture–a review.

Agronomy for Sustainable Development, 29(1), 43–54.

Kour, D., Rana, K. L., Kaur, T., Yadav, N., Yadav, A. N., Kumar, M. and Saxena, A. K. (2021). Microbial consortia and plant growth-promoting microbes for sustainable agriculture. Applied Microbiology and Biotechnology, 105(3), 1105–1127.

Leigh, G. J. (2002). "Front matter," in *Nitrogen Fixation at the Millennium* (Amsterdam: Elsevier Science;), iii. 10.1016/B978-0-444-50965-9.50017-3

Maniatis, T.; Fritsch, E.F. and Sambrook, J.K. (1982). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor.

Manuel,D.; Bissett A.; Eldridge D.J.;Maestre F.T.; He J.-Z.; Wang J.-T.; Hamonts K.; Liu Y.-R.; Singh B.K.; and Fierer N. (2017). Palaeoclimate explains a unique proportion of the global variation in soil bacterial communities. Nature Ecology & Evolution volume 1, pages1339–1347

Narendran, R., Rathinam, M. and Manoharan, S. (2016). Isolation and characterization of Azospirillum species from different regions of Tamil Nadu. International Journal of Current Microbiology and Applied Sciences, 5(4), 617–625.

Nautiyal, C.S. (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiology Letters, 170(1): 265–270.

Patel, D.; Murawala, P.; Archana, G.; Naresh Kumar, G. (2021). Repression of mineral phosphate solubilizing phenotype in the presence of weak organic acids in plant growth promoting fluorescent pseudomonads. Bioresour. Technol. 102:3055–3061.

Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. Microbiologiya, 17, 362–370.

Postgate, J. R. (1972). The Fundamentals of Nitrogen Fixation. Cambridge University Press.

Robson, R. L.; Eady, R. R.; Richardson, T. H.; Miller, R. W.; Hawkins, M. and Postgate J. R. (1986). The alternative nitrogenase of *Azotobacter chroococcum* is a vanadium enzyme. *Nature* 322 388–390.

Savci, S. (2012). Investigation of effect of chemical fertilizers on environment. APCBEE Procedia, 1, 287–292.

Wang, Z.; Chen, Z.; Kowalchuk, G.A.; Xu, Z.; Fu , X. and Kuramae, E.E. (2021). Succession of the Resident Soil Microbial Community in Response to Periodic Inoculations. Applied and Environmental Microbiology, 87(9), e00046‑21.

Zhang, Q.; Chen, Q.; Lu, X.; Zhao, Y.; and Wu, L. (2021). Impact of long-term fertilization on soil nutrients and microbial communities. Agriculture, Ecosystems & Environment, 319, 107582.

Zhao, X.R.; Lin, Q.M. and Li B.G. (2003).The Relationship Between Rock Phosphate Solubilization and pH and Organic Acid Production of Microorganisms. J. Microbiol.;23:5–7.

الملخص العربي

عزل وتعريف البكتيريا المثبتة للنيتروجين لا تكافليا والمذيبة للفوسفات المتحملة للملوحة

جابر زايد بريشة[1] - عمر عبد اللطيف عمر سعد[1] - محمد محمود إبراهيم عفيفي [2] - ولاء محمد نجيب جمعة[1]

[1]- قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة المنيا – مصر

2- معهد بحوث الأراضي والمياه والبيئة – مركز البحوث الزراعية بالجيزة – مصر

تعرف بكتريا الأزوتوباكتر وهي جنس بكتيري يثبت النيتروجين لا تكافليا إلي جانب بعض أنواع جنس الباسيليس كبكتريا مذيبة للفوسفات (PSB) بمساهماتها الكبيرة في الزراعة المستدامة، حيث تعزز هذه الكائنات الدقيقة خصوبة التربة وإنتاجية النباتات من خلال تسهيل دورات المغذيات الرئيسية، ولاسيما التثبيت البيولوجي للنيتروجين وإذابة مركبات الفوسفات غير القابلة للذوبان ومع ذلك تعيق ملوحة التربة عمل هذه البكتريا وتجعلها غير قادرة علي إمداد النباتات بالنيتروجين أو الفوسفور، في هذا العمل تم عزل سلالات فعالة من البكتريا المثبتة للنيتروجين لا تكافليا والبكتريا المذيبة للفوسفات من تربة ملحيه، وقد تم اختبار قدرة هذه العزلات علي تثبيت النيتروجين أو إذابة الفوسفات وقد أكدت اختبارات التعريف الاحتمالية ثم التأكيدية أن أفضل سلالة معزولة من التربة الملحية والمثبتة للنيتروجين لا تكافليا هي *Azotobacter* chroococcum في حين أن أفضل سلالة لإذابة الفوسفور هي *Bacillus megatherium* .