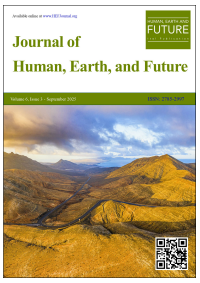
Available online at www.HEFJournal.org

ISSN: 2785-2997

Journal of
Human, Earth, and Future

Vol. 6, No. 3, September, 2025



Salt-Tolerant Phosphate-Solubilizing Bacteria from Saline Soils: Phosphate Mobilization Mechanism

Irina Smirnova [1*], Amankeldi Sadanov [1], Gul Baimakhanova [1], Zere Turlybayeva [1], Mereke Alimzhanova [2]

[1] *Research and Production Center of Microbiology and Virology LLC, Almaty 050010, Kazakhstan.*

[2] *Center of Physical-Chemical Methods of Research and Analysis, Al Farabi Kazakh National University, Almaty 050040, Kazakhstan.*

Received 18 March 2025; Revised 16 July 2025; Accepted 03 August 2025; Published 01 September 2025

Abstract

Soil salinity is a significant constraint on agricultural productivity and disturbs the availability of nutritional elements necessary for plants, such as phosphorus. Microorganisms have specialized adaptation features, such as tolerance to salt, stimulation of growth, and the ability to promote plant survival under saline conditions. The objectives of this investigation were to isolate new salt-tolerant, phosphate-solubilizing bacterial strains, assess their phosphate-solubilizing activity, and investigate the mechanisms of phosphate mobilization under salinity stress. Phosphate-solubilizing bacteria were isolated from saline pasture soils in southeastern Kazakhstan, and their salt tolerance and phosphate-solubilizing activity were identified. Three highly active strains were selected and identified by Sanger-based molecular genetic analysis: *Enterobacter cloacae* FY3, *Pseudomonas putida* FT4, and *Bacillus megaterium* F7A. Organic acid production by these strains under salt stress was analyzed using gas chromatography. The results indicated that the strains produced 12 organic acids, with differences in composition and amount between strains. The most important phosphate solubilization mechanism under salinity stress is the secretion of organic acids. Organic acid secretion, pH reduction, and increased soluble phosphorus content were positively correlated. These findings provide an effective method for phosphorus mobilization in salt soils, and the recovered strains are likely candidates for making biofertilizers applicable to salt environments. These new strains are candidates for the development of biofertilizers for saline soils.

Keywords: Phosphate-Solubilizing Bacteria; Activity; Screening; Salt Tolerance; Organic Acids.

1. Introduction

There has been an increase in the area of saline agricultural soils worldwide [1]. According to the FAO, salinity affects more than 20% of the world's irrigated areas and is increasing [2, 3]. Soil salinity is a serious stressor for crops, and significantly reduces agricultural productivity [4, 5]. Salinity deteriorates soil structure, reduces humus accumulation, decreases the biodiversity of the microbiome, and consequently lowers fertility [6, 7]. In addition, salinity negatively affects the uptake of the main nutrient-nitrogen and phosphorus by plants [8-10]. These processes render the soil unsuitable for agriculture, leading to the reduction or complete suppression of agricultural crop growth [11, 12].

Phosphorus is the second main element in plants after nitrogen. It participates in many metabolic processes in organisms and includes DNA, RNA, and enzymes [13]. Low phosphorus content in the soil inhibits the synthesis of

* Corresponding author: dr_smirnova@inbox.ru

http://dx.doi.org/10.28991/HEF-2025-06-03-09

proteins and carbohydrates, delays plant growth, and leads to the loss of resistance to stresses such as drought and salinity [14, 15]. Phosphorus deficiency also significantly affects plant metabolism and suppresses photosynthesis, respiration, protein synthesis, and lipid metabolism [16, 17].

Phosphorus in soils mainly represents hard-to-access compounds, and despite the high amount of phosphorus in soils, phosphorus deficiency exists because of low availability Owing to phosphorus deficiency, and crop productivity is severely limited [18]. The availability of phosphorus in soil depends on factors such as soil mineral composition, metal oxides, pH, organic matter, temperature, aeration, and moisture [19]. Salinity increases the unavailability of phosphorus, the deficiency of which increases in arid, semiarid, and irrigated lands [20]. Severe salinity makes 1.5 million ha of irrigated and 1-2% of arid and semi-arid lands unsuitable for cultivation each year. If salinity continues this way, 50% of the arable land will become salinized by 2050 [21]. Consequently, maintaining high levels of phosphorus in farming in saline soils may become a severe challenge for agriculture in the future.

Phosphorus deficiency caused by salinity is the primary abiotic stress in agroecosystems and negatively affects all aspects of plant development, from germination to fruiting and leaf senescence [22]. Phosphate fertilizers have been used to overcome this deficiency [23, 24]. The systematic use of large doses of phosphorus fertilizers negatively affects the soil, biodiversity of the microbiome, and water. In addition, phosphorus introduced into the soil quickly forms poorly soluble phosphates [25, 26]. Thus, phosphorus management and elimination of salt stress in agriculture in saline soils are the primary problems of the current study. Therefore, finding alternative ways to increase phosphorus content in saline soils is a highly relevant problem.

Breeding salt-tolerant plant varieties or genetic engineering can improve crop productivity in saline soils [27]. However, traditional breeding is a long process, and breeding of salt-tolerant varieties has not made much progress because salt tolerance has many traits, and salt-tolerant varieties often have low yields and low grain quality [28]. The application of genetic engineering and plant genome editing has not revolutionized the production of salt-tolerant plant varieties, because dozens of genes that determine salinity tolerance define the genetic mechanisms of salt tolerance [29]. However, these approaches are not always efficient or sustainable [30, 31].

In addition, the application of phosphate fertilizers, the traditional method of increasing phosphorus content in the soil, cannot solve the problem of phosphorus deficiency and cause significant harm to the environment. Phosphates themselves are non-renewable natural resources.

Microbiological solubilization of phosphates is an alternative to the use of phosphate fertilizers to reduce phosphorus deficiencies in saline soils [32, 33]. Microbial phosphate solubilization relies on microorganisms, most often bacteria, which can convert insoluble phosphates into soluble forms that are available to plants [34-36].

These bacteria act as biological fertilizers and can stimulate growth and soften salt stress in plants [37-39]. Phosphate-solubilizing bacteria positively affect agricultural crop productivity by improving phosphorus nutrition and stimulating growth processes [35, 40]. Bacteria also positively affect crop adaptation to stressful situations through the production of various metabolites [41-43]. However, the application of these bacteria is constrained by several factors, one of which is their inability to dissolve phosphates in saline soils. Salt-tolerant bacteria can alleviate this problem by directly increasing plant tolerance to salinity stress in the rhizosphere [44]. The support of plant growth by bacteria in saline soils is associated with difficult biochemical processes.

Phosphate-solubilizing bacteria (PSB) in the plant rhizosphere have been reported to enhance plant salinity tolerance and other abiotic stress factors, growth, and nutrient uptake [45, 46]. Several studies from different countries have established the excellent growth-promoting potential of these bacteria under saline soil conditions, and PSB is thus a promising strategy for mitigating soil salinization issues in agriculture.

However, as can be seen from the literature, most research conducted on enhancing the resistance of crops to salinity through the application of PSB has been performed by foreign scientists. There is certainly a need for additional research aimed at discovering salt-resistant, phosphate-dissolving bacteria acclimatized to the particular natural and climatic conditions of Kazakhstan. The isolation and selection of salt-resistant phosphate-solubilizing bacterial strains from Kazakh salt soils is an extremely relevant research target. These microorganisms could be useful for improving phosphorus availability and mitigating the negative impact of soil salinity on farm productivity.

The originality of this research lies in the isolation and characterization of salt-tolerant phosphate-solubilizing bacterial strains new to science from Kazakhstani saline soils, which have soil and climatic conditions that are significantly different from those in the previously investigated areas. This study eliminates a gap in the research and provides a rationale for the selection of bacterial strains adapted to local environmental conditions and ecological belts [43].

There are several mechanisms by which the bacterial conversion of insoluble phosphates, within soils to readily accessible forms, is achieved. These mechanisms include chelation, acidification, production of polymers, sequestration by organic acids, release of protons (H^+), and redox processes. Through this, bacteria create solubility of otherwise fixed phosphorus, thereby increasing the availability of nutrients for plants [47-49].

Low molecular weight organic acid production is one of the most frequent mechanisms of bacterial phosphate-solubilizing activity [50, 51]. Organic acids contribute to the solubilization of phosphate by lowering the pH as well as through chelation or cation competition by phosphate-associated cations employing their carboxyl and hydroxyl functional groups, thereby enhancing the solubility and bioavailability of mineral phosphates [52]. Moreover, organic acids participate in the detoxification of pollutants, improve the bioavailability of heavy metals, stimulate microbial activity, and influence soil microbiome structure and function [53, 54].

It is important to understand the processes through which phosphorus availability is increased by phosphate-solubilizing bacteria for efficient application in agriculture, especially in increasing soil fertility and crop yield.

Phosphate solubilization is a complex process regulated by various environmental parameters, such as temperature, pH, oxygen level, and water [40, 55]. Although numerous studies have explained the contribution of such factors to microbial phosphate solubilization, the mechanisms employed by salt-tolerant bacteria under saline conditions remain poorly explored [51, 56]. It is recognized that salt-tolerant strains of bacteria possess the capability to produce organic acids, the amount and type of which vary according to the strain; however, there is no extensive research on the qualitative and quantitative profiles of organic acids generated by salt-tolerant phosphate-solubilizing bacteria.

The objectives of this study were to screen new salt-tolerant phosphate-solubilizing bacterial strains, evaluate their phosphate-solubilizing activities, and elucidate the mechanisms of phosphate solubilization under saline conditions.

We assumed the following hypotheses:

- Hypothesis 1 (H1): Phosphate-solubilizing bacteria isolated from saline soils grow under high soil salinity.
- Hypothesis 2 (H2): Newly isolated salt-tolerant bacteria can solubilize insoluble phosphates under high salinity conditions.
- Hypothesis 3 (H3): Newly isolated phosphate-solubilizing salt-tolerant bacteria produce organic acids.
- Hypothesis 4 (H4): A possible mechanism for phosphate solubilization in new strains of salt-tolerant bacteria is the secretion of organic acids.

The importance of this study is that it will contribute to the development of new technologies for the application of salt-tolerant phosphate-solubilizing bacteria in agriculture in Kazakhstan and to understand the mechanisms of phosphate solubilization in salty soils. The ultimate objective was to create a biopreparation based on recently obtained local strains of bacteria adapted to the available soil and climate conditions in Kazakhstan, which would increase the fertility of saline pasture soils. The significance of this study is emphasized by its potential to promote livestock development in southeastern Kazakhstan, which offers economic empowerment to this region.

By increasing the fertility of low-productive pastures through phosphate-solubilizing bacteria, this research can contribute to the support of the fodder base and, therefore, improve livestock growth, as well as add to the economic well-being of rural settlements in the region.

2. Material and Methods

The research procedure of the present study includes a diligent collection of materials, experimental methods, and data analysis procedures, for example, verification of hypotheses and comparison of experimental results with theoretical predictions. The design of the research was properly thought through and decided in advance prior to conducting experiments to enable the proper choice of methods and ensure secure interpretation of data. To resolve research tasks, sophisticated techniques such as microbiology, biochemistry, molecular biology, and statistical data analysis are used. These techniques were chosen to achieve accurate, reproducible, and scientifically valid results.

Theoretical framework of the research consists of five interrelated steps:

- Problem formulation: phosphorus deficiency in saline soils;
- Definition of the subject of study: saline soils and their corresponding microbial communities;
- Research objective formulation: isolation and characterization of new salt-tolerant, phosphate-solubilizing bacterial strains, and investigation of the mechanisms of phosphate solubilization under saline conditions;
- Hypothesis formulation: formation of research hypotheses (H1–H4, as presented in the Introduction);

This structured approach ensures uniformity of the study and enables the generation of relevant and feasible results associated with the research aims. The primary goal is to discover new bacterial strains and assess whether they can improve phosphorus bioavailability in the salt soils of south-eastern Kazakhstan. The experimental process consisted of field soil collection from saline soils, isolation and screening of bacterial strains, and investigation of phosphate-solubilization processes.

Specifically, the use of microbial biofertilizers can significantly reduce or even eliminate the need for chemical phosphorus fertilizers, which are known to have harmful environmental effects. Consequently, this study also promotes the environmental safety of crop production, enhances the sustainability of cattle breeding, and strengthens the fodder base for pasture livestock breeding, especially for sheep breeding, in southern Kazakhstan.

This study identified the effectiveness of biological methods for enhancing phosphorus availability, particularly during salt stress. The implementation of salt-tolerant strains of bacteria has been proposed as a probable method to facilitate pasture crop growth by making phosphorus more accessible. The current research offers a utility-oriented contribution to the process of developing sustainable practices for raising soil fertility and addressing salinity problems in Kazakhstan and similar parts of the world.

2.1. Sampling of Saline Soils and the Characterization of the Study Sites

The soil collection for isolation of phosphate-solubilizing bacteria occurred in 2024 in Southeast Kazakhstan, 120 km from the city of Almaty (Almaty region), in the vicinity of Akshiy village, on the pastures of the farm "ZhasKanat," coordinates: 43°59′31″ N, 76°19′25″ E, 507 m above the sea level (see Figure 1).

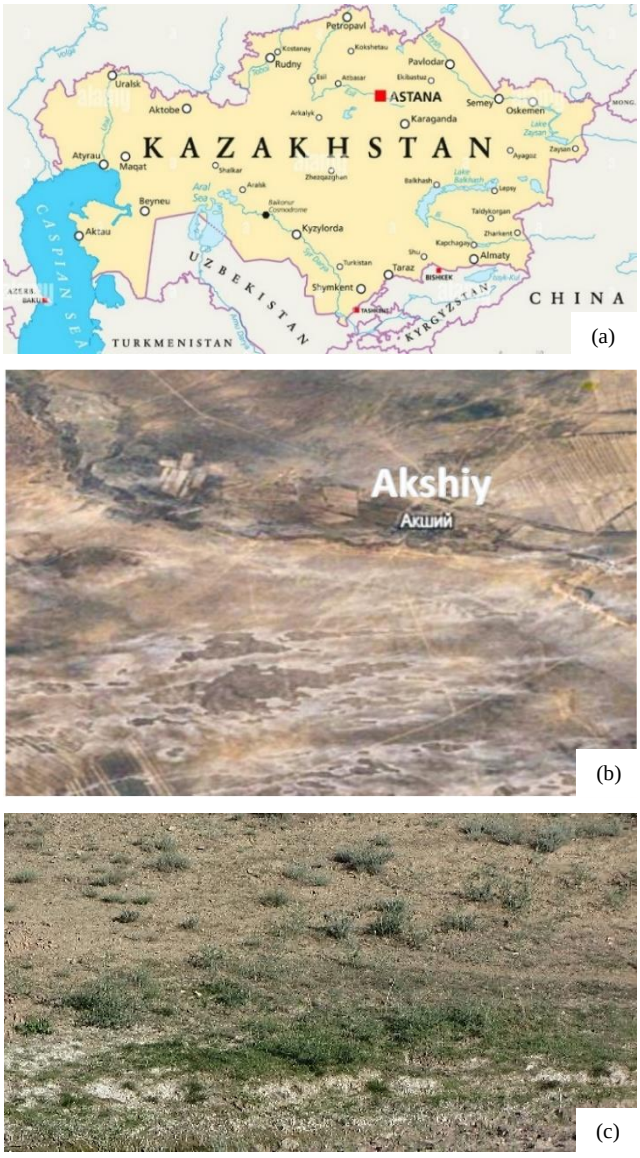


Figure 1. Location of soil sample collection. (a): Map of Kazakhstan [57]; (b): vicinity of the village Akshiy, Google Public Maps [58]; (c): pasture, photos made by the authors in summer 2024

The study area has a strongly continental climate, and the mean annual precipitation never exceeds 300 mm. Soil samples were collected from pasture grounds with varying salinity according to the relevant State Standard [59]. Sampling was performed under aseptic conditions, and the samples were stored in sterile vessels until analysis. Each pasture occupied an area of more than 20 ha. The depth of soil collection was 8-10 cm. Agrochemical and physicochemical properties of collected soils were studied in the Soil Chemical Analysis Laboratory in LLP "Kazakh Research Institute of Soil Science and Agrochemistry" (Kazakhstan, Almaty).

2.2. Isolation of Phosphate-Solubilizing Bacteria

Isolation of phosphate-solubilizing bacteria was conducted with 1 g of soil, which was split into 100 mL distilled water. Ten-fold serial dilutions ranging from 10^{-1} to 10^{-6} of each was used and the aliquots 100 µL of were cultured onto Petri dishes with normal National Botanical Research Institute's phosphate (NBRIP) medium but made supplemented with 10% NaCl. Plates were incubated at 30 °C [60]. The formation of insoluble calcium phosphate ($Ca_3(PO_4)_2$ in the medium caused uniform turbidity. Colonies of bacteria that formed a clear halo zone, indicating phosphate solubilization, were identified as phosphate-solubilizing strains. The colonies were plated on agar slants for further purification and detailed analysis.

2.3. Salt Tolerance of Phosphate-Solubilizing Bacteria

For salt tolerance estimation, bacteria were grown in NBRIP liquid medium with NaCl added at concentrations of 100, 250, and 500 mM. The Growth was carried out for 3 days at 30 °C and 180 rpm in a thermostatically controlled shaker. The growth of bacteria was determined using a spectrophotometer (PD-303, "Apel," Japan) at 540 nm with a scale in which (-) indicates no growth, (+) indicates growth, and (+) indicates the strength of growth. Five replicates were used for this study.

2.4. Phosphate-Solubilizing Activity of Bacteria Under the Conditions of Salt Stress

For phosphate-solubilizing activity analysis by bacteria, bacteria were inoculated in the center of NBRIP medium Petri plates and incubated at 30 °C for seven days. The colony diameter and halo zone (media-clearing zone) were then measured using a caliper. The solubilization index (SI) was calculated as the sum of the total diameter (colony + halo zone) divided by the colony diameter [61]:

$$\mathrm{SI} = \frac{\text{Colony diameter} + \text{Diameter of halo zone}}{\text{Colony diameter}}$$

The solubilization index (SI) of the strains was classified according to the methodology where SI<2 was low, SI>2, SI<3 was medium, and SI>3 was high [62]. Five replicates were performed for each strain.

2.5. Quantitative Estimation for Phosphate Solubilizing Ability of Bacteria

For quantitative determination of the phosphate-solubilizing ability of bacteria, 30 ml of NBRIP medium with 5% NaCl was filled in flasks and inoculated with 0.3 ml bacterial suspension at a concentration of 108 CFU/ml. Bacteria were grown in a thermostatic shaker at 150 rpm and 30 °C for seven days. After cultivation, the bacterial suspension was centrifuged at 10000 rpm for 10 min and then filtered through a 0.22 µm nylon filter. Quantitative measurement of phosphorus released from inorganic phosphates was carried out by a spectrophotometric method using a phosphorus-molybdenum complex [63]. A 1:3 volume reagent mixture of vanadate-molybdate was added to 1 mL of the supernatant and the absorbance of the solution was recorded using a D-303 spectrophotometer (Apel, Japan) at 430 nm. The dissolved phosphate concentration in the liquid medium was determined using a calibration curve. A clean medium free of bacteria served as a control. The pH of the culture medium was measured in triplicates using a digital pH meter (Consort C931, Belgium).

2.6. Identification of Bacteria by Molecular Genetic Method

The bacteria were identified by Sanger molecular genetic method by sequencing of the 16S rRNA gene. Daily bacterial cultures were isolated for genomic DNA analysis using PureLink® Genomic DNA Kits (Invitrogen, USA), and bacteria were identified by sequence analysis of the 16S rRNA gene region using universal primers [64]. Bacterial 16S rRNA gene fragments were sequenced on a 3500 DNA Analyzer (Applied Biosystems, USA) using a Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) following the manufacturer's instructions (BigDye® Terminator v3.1 Cycle Sequencing Kit, Protocol of Applied Biosystems, USA). The sequencing data were processed using the SeqA program (Applied Biosystems, USA). These identical 16S rRNA gene nucleotide sequences were obtained using BLAST computer software within the worldwide NCBI GenBank database [65]. For comparative analysis of homologous strains of bacteria, the computer software MEGA6 was used. Trees were constructed with several alignments of nucleotide sequences from ClustalW using the NJ method.

2.7. Production of Organic Acids by Bacteria

Bacteria were grown on NBRIP medium containing 5% NaCl in a thermostatic shaker at 180 rpm and 28°C for five days. Identification and measurement of organic acids were achieved through solid-phase microextraction (SPME) to analyze the organic compounds in the bacterial culture broth. Ten milliliters of culture broth were transferred to a 20 mL screw vial with a screw cap (Agilent Technologies, Santa Clara, CA, USA). After conditioning, the vial was stored at 40°C for 30 min to reach equilibrium between gas and liquid phases. The headspace organic compounds were absorbed on SPME fibers coated with 50/30 µm DVB/CAR/PDMS (Supelco, Bellefonte, PA, USA). Measurements were performed in triplicate for each sample [66].

Gas chromatography (GC) was performed on a 7890 gas chromatograph, along with a 5977 A MSD mass spectrometer (Agilent, Santa Clara, USA). For the DB-35 ms column (30 m × 0.25 mm, 0.25 µm film thickness, J&W Scientific Inc., Folsom, CA, USA), helium (>99.995%, Orenburg-Tehgas, Russia) was utilized as the mobile phase (carrier gas) at 1.0 mL/min flow rate. The temperature program of the GC oven started at 40 °C (5 min hold) and was ramped to 240 °C at 5 °C/min. injector and transfer line temperatures were 250 °C and 280 °C, respectively. Mass spectrometric detection was at 70 eV, scanning a mass range of m/z 34–850 amu. Instrument control and data processing were performed using Agilent MSD ChemStation (version 1701EA). Data processing included computation of retention times and peak areas; peak identification was performed via comparison with the Wiley 11th edition and NIST'02 libraries.

The SPME/GC-MS method was used to analyze the composition of the bacterial culture broth at a high speed. For the semi-quantitative analysis of the components of the culture fluid, an internal normalization method was used. The sum of the peak areas was taken as 100% to determine the mass percentage of one of the components of the culture fluid from the area of a single peak. This method assumes that all the chemicals present in the culture medium are detected in the chromatogram, and the proportion of each peak area to the total area is proportional to the content of the substance.

The bacterial culture broth was quantified in triplicate. Blank runs were performed under three conditions: blank for the fiber, blank for the empty vial, and blank for the culture broth without bacteria.

Actual acid standards were used to identify organic acids. The concentrations of organic acids were determined from the peak areas for authentic standards of acetic, butyric, propionic, gluconic (Sigma-Aldrich, USA), lactic, oxalic, malic, succinic, formic, citric, malonic, tartaric, caprylic, palmitic, pelargonic, lauric, valerianic, myristic, phthalic, and cyclopropaneacetic (Supelco, USA). The organic acid values are the mean of three replicates.

2.8. Statistical Analysis

Statistical analysis of data from the experiments was performed using STATISTICA software package (version 10.0, v. 6.0) [67]. Differences were considered significant at $p < 0.05$. Results are presented as mean values (M) ± standard error of the mean (SEM) [68].

2.9. Flowchart of Research Methodology

Figure 2 shows a flowchart detailing the experimental design and step-by-step execution of all steps of this study to visualize scientific studies and understand their requirements.

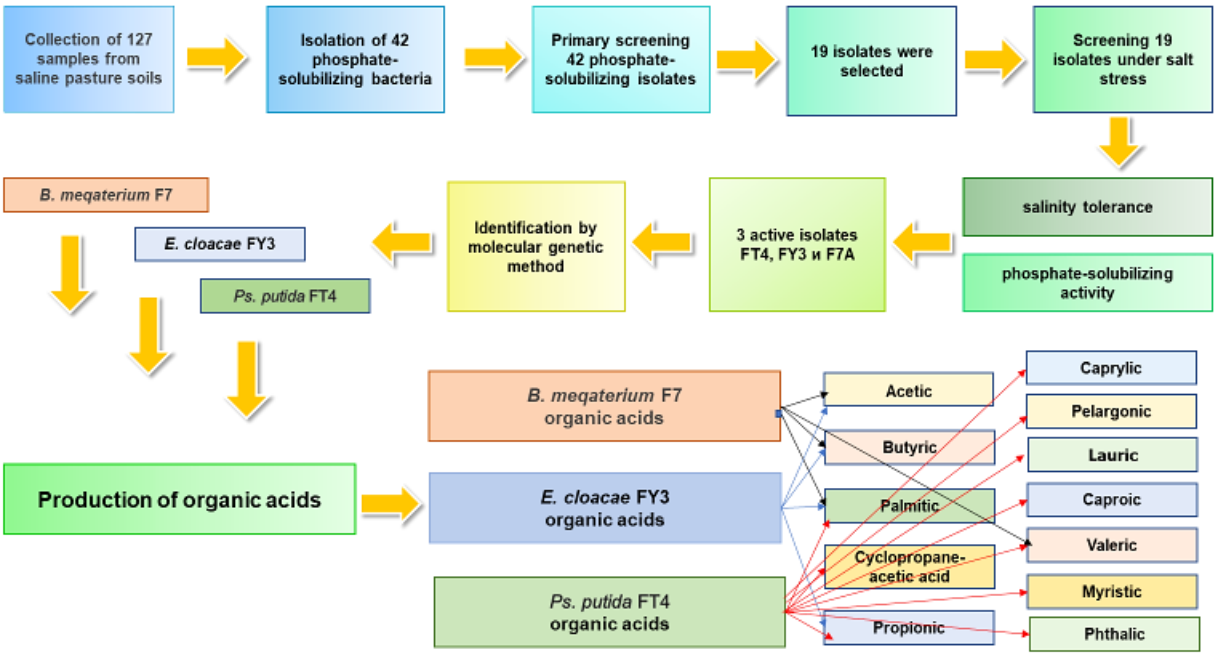


Figure 2. Flowchart design of experimentation and investigations of this research

To solve the set aim of the study, the following tasks were outlined and traced on the presented flowchart (Figure 2): isolation of local phosphate-solubilizing bacteria (1), bacterial salt tolerance study (2), study of phosphate solubilization ability under high salinity (3), selection of the most active ones (4), and organic acid secretion study (5). These results will provide prerequisites for the confirmation of a possible mechanism of phosphate solubilization in isolated bacterial strains.

3. Results

A total of 127 soil samples from saline pastures in the Almaty province of Kazakhstan were examined over a period of one year. Table 1 shows the principal agrochemical parameters of the studied saline pasture soils in terms of dynamics and their findings.

Table 1. Agrochemical indicators of water extract in (%) on absolutely dry weight of soil in dynamics (vicinity of Akshiy village, 2024)

Fields	Sum salts (%)	Alkalinity (mM (eq) / 100 g soil)	Ionic concentration (mM (eq) / 100 g soil)				
		HCO_3^-	Cl^-	SO_4^{2-}	Ca^{2+}	Mg^+	Na^+
Spring (April)							
Field 1	0.198±0.01	0.02-0.03	0.007-0.008	0.119-0.120	0,025-0,027	0,014-0,015	0,014-0,016
Field 2	0.781±0.02	0.019-0.02	0.024-0.026	0.335-0.338	0.073-0.074	0.035-0.036	0.032-0.033
Summer (July)							
Field 1	0.282±0.01	0.018-0.019	0.009-0.01	0.173-0.175	0.037-0.038	0.016-0.017	0.021-0.023
Field 2	0.829±0.02	0.015-0.017	0.034-0.035	0.515-0.517	0.098-0.099	0.047-0.049	0.062-0.063
Autumn (October)							
Field 1	0,350±0.01	0.016-0.018	0.015-0.017	0.228-0.230	0.045-0.047	0.023-0.024	0.029-0.031
Field 2	1.054±0.06	0.015-0.016	0.057-0.06	0.541-0.543	0.069-0.072	0.070-0.071	0.084-0.086

The data from Table 1 show that field 1 soil was medium saline, and the total salt content ranged seasonally from 0.198% to 0.350%. Field 2 was extremely saline, with a total salt content ranging from 0.781% to 1.054%. The soils of the two pastures were alkaline in terms of HCO3 − ion content. Soil alkalinity increases due to ion toxicity, oxidative pressure, and excessive pH, which adversely affects plant growth and causes loss of pasture productivity.

The physical and chemical properties of the soils collected from saline pastures were analyzed. The collected data are listed in Table 2.

Table 2. Physicochemical properties of pasture soils (vicinity of Akshiy village, July 2024)

Indicators	Units	Content	
		Field 1	Field 2
Total humus	%	1.1 ± 0.02	0.84 ± 0.01
Hydrolyzed nitrogen	mg/kg	58.2 ± 0.3	56.4 ± 0.2
Available phosphorus	mg/kg	23.4 ± 0.1	22.2 ± 0.1
Available potassium	mg/kg	473.4± 7.2	465.1± 5.8
Soil temperature	°C	26,9 ± 0.2	27,2 ± 0.1
Moisture content	%	35.7 ± 0.1	36.1 ± 0.1
pH		8.9±0.01	9.2±0.01

Note: Values are means ± standard error of the mean (SEM), n = 3; $p < 0.05$

The studied soils were ordinary grey soils of different types. Ordinary grey soils form in dry steppes under conditions of moisture and heat deficiency. These are poor soils with wormwood-ephemeral vegetation communities. The climate of the region is semiarid. However, the region is promising for livestock breeding, particularly sheep breeding, as it pertains to pasture lands with medium productivity. Table 2 shows that the soils had a low content of total humus (0.84 ± 0.01/1.1 ± 0.02%), easily hydrolyzed nitrogen (56.4 ± 0.3/58.2 ± 0.2 mg/kg), available mobile phosphorus (22.2 ± 0.1/23.4 ± 0.1 mg/kg), and average available mobile potassium (465.1 ± 5.8/473.4 ± 7.2 mg/kg); the pH value was highly alkaline (pH = 8.9 ± 0.01/9.2 ± 0.01, $p<0.05$), indicating a severe adverse effect on plant growth.

Phosphate-solubilizing bacteria were isolated and studied under laboratory conditions in 2024 (Almaty, Kazakhstan). On NBRIP medium plates containing 10% NaCl, 42 isolates with medium clarification zones (halo zones) were obtained, illustrating their phosphate-solubilizing ability. The population of phosphate-solubilizing bacteria was low, comprised 5-7% of the total number of bacteria.

To select active bacterial isolates, primary screening was carried out by phosphate solubilization (Figure 3).

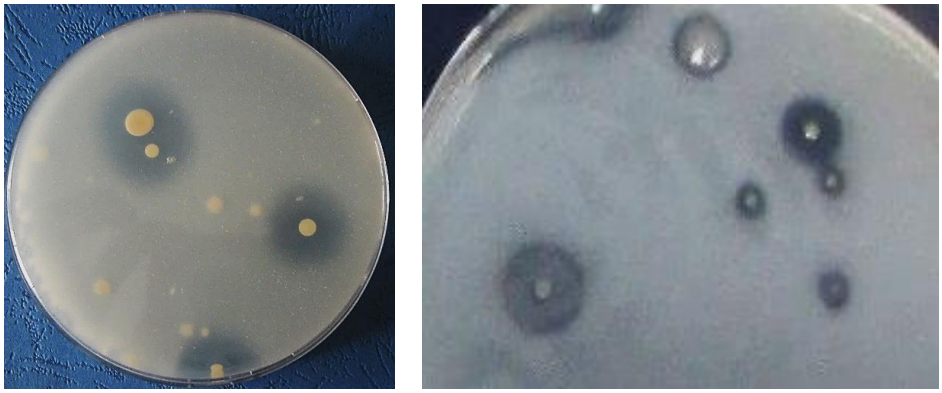


Figure 3. Colony of isolated phosphate-solubilizing bacteria and halo zones around colonies in the NBRIP medium with 10% NaCl

Primary screening of bacteria occurred on a solid medium with an insoluble phosphorus compound, which gave the medium uniform turbidity. To create a high salinity, 10% NaCl was added to the medium. For bacterial screening, colonies characterized by a large halo zone were selected (Figure 3). These isolates were considered to be active phosphate-solubilizing bacteria. Under high salt stress, out of 42 isolates, only 19 formed a large and distinct halo zone around the colonies. For a more complete study, individual colonies of these bacteria were transferred from Petri dishes to sloped agar and used in further studies.

To investigate the resistance of the selected isolates to salt stress, they were grown in NBRIP liquid medium with different concentrations of NaCl (100, 250, and 500 mM). Table 3 shows the obtained data.

Table 3. Screening for salinity tolerance of the isolates

Isolates	Concentration NaCl (mM)		
	100	250	500
FT4	++++	+++	++
FT15	+++	+	-
FT17	++	+	-
FT21	+++	-	-
FT25	+++	-	-
FT34	+++	+	-
F7A	++++	+++	++
F23A	+++	++	-
FM9/5	++++	+++	++
FM12/4	+++	+	-
FM30/5	+++	++	-
FM12	++++	+++	++
FM27	+++	+	-
FY3	++++	+++	++
FY18	+++	++	-
FC11	++++	+++	++
FC28	+++	++	-
FT34/2	+++	+	-
FT36/2	+++	-	-

Note: Qualitative analysis: (-) indicates absence of growth; (+) indicates occurrence of growth; further (+) indicates intensity of growth exhibited by the isolates.

The salt tolerance of the isolates varied significantly. Although all isolates grew optimally in a culture medium containing 100 mM NaCl, the increased salinity of the medium suppressed their growth. Thus, on a 250 mM NaCl concentration, there were three failed growth isolates and six isolates with reduced growth, while salinity was increased to 500 mM NaCl in the medium of 19 isolates; only six of the bacteria FT4, F7A, FM9/5, FM12, FY3, and FC11 grew, while the rest did not.

The obtained results only partially confirmed hypothesis 1 (H1), as out of all the isolates that were collected and tested, only six isolates—FT4, F7A, FM9/5, FM12, FY3, and FC11—were able to grow under high salinity, while the other isolates were not salt-tolerant and did not grow under high salinity. These six isolates were selected as the most salt-tolerant isolates for further study.

One of the significant indicators for application of phosphate-solubilizing bacteria in saline soil is phosphate solubilization efficiency. The ability of the isolates to solubilize phosphate under high salt stress was investigated. The isolates were grown in liquid NBRIP medium supplemented with different concentrations of NaCl.

Phosphorus solubilization activity was measured based on the solubilization index (SI). The phosphorus solubilization index is very important in determining the phosphate-solubilizing activity of bacteria and is used to explore their phosphorus solubilization capacity. A higher value of this index reflects active phosphorus dissolution by bacteria. Table 4 lists these data.

Table 4. Phosphate-solubilizing activity of isolates grown under different concentrations of NaCl in medium

Bacterial isolates	Concentration of NaCl (mM)			
	0	100	250	500
	Phosphate solubilization index (SI)			
FT4	7.45±0.03	6.67±0.01	5.50±0.02	4.78±0.01
F7A	7.12±0.03	6.08±0.01	5.44±0.01	4.39±0.01
FM9/5	5.89±0.03	4.85±0.02	4.11±0.01	3.29±0.02
FM12	6.28±0.02	5.87±0.01	4.10±0.02	3.18±0.01
FY3	7.16±0.04	6.77±0.03	5.69±0.01	4.61±0.02
FC11	6.22±0.03	5.82±0.02	4.21±0.02	3.15±0.01

Note: Values are means ± standard error of the mean (SEM), n = 5; $p<0.05$

The findings in Table 4 indicate that the selected isolates were able to solubilize phosphate under salt stress conditions. In the presence of 100 mM salt concentration in the medium, all isolates were marked by high solubilization of phosphate, with the phosphate solubilization index value (SI) ranging from 5.89 ± 0.03 to 7.45 ± 0.03 ($p<0.05$). Solubilization activity decreased with increasing salinity of the medium.

However, in the presence of high salt stress (500 mM NaCl), high phosphate solubilization activity (SI > 3.0) was observed in all isolates. The highest phosphate solubilization index (SI) value was observed in isolate FT4 (4.78 ± 0.03, $p<0.05$), followed by FY3 (4.61 ± 0.02) and F7A (4.39 ± 0.01, $p<0.05$). Therefore, three strains had a phosphate solubilization index greater than 4 and the rest had an SI greater than 3.0 (Table 4).

According to the range of solubilization index classification, all isolates under high salt stress (500 mM NaCl) had high solubilization indices (SI>3), but three isolates (FT4, FY3, and F7A) had the highest index values. These isolates were selected as the most active. These results confirmed that the selected bacteria were salt-tolerant and had high phosphate solubilization under severe salt stress.

Thus, our research confirmed hypothesis 2 (H2): the isolated salt-tolerant bacteria are capable of solubilizing phosphates under salt-stress conditions.

To determine the taxonomic status of the isolates, we used Sanger molecular genetic method based on nucleotide sequence analysis of the 16S rRNA variable gene. The Sanger method enables the determination of the sequence of 16S rRNA genes and the identification of bacteria using a database for comparing sequences. We utilized this method as it is extremely precise, allows the analysis of relatively small RNA fragments, and provides reliable results for microorganism identification.

To determine the identity of the bacteria, nucleotide sequences of the strains were compared with the 16S rRNA sequences of closely related bacterial strains in the NCBI GenBank database. Phylogenetic trees were constructed in MEGA 6.0 using the neighbor-joining cluster method to determine genetic distances (see Figure 4).

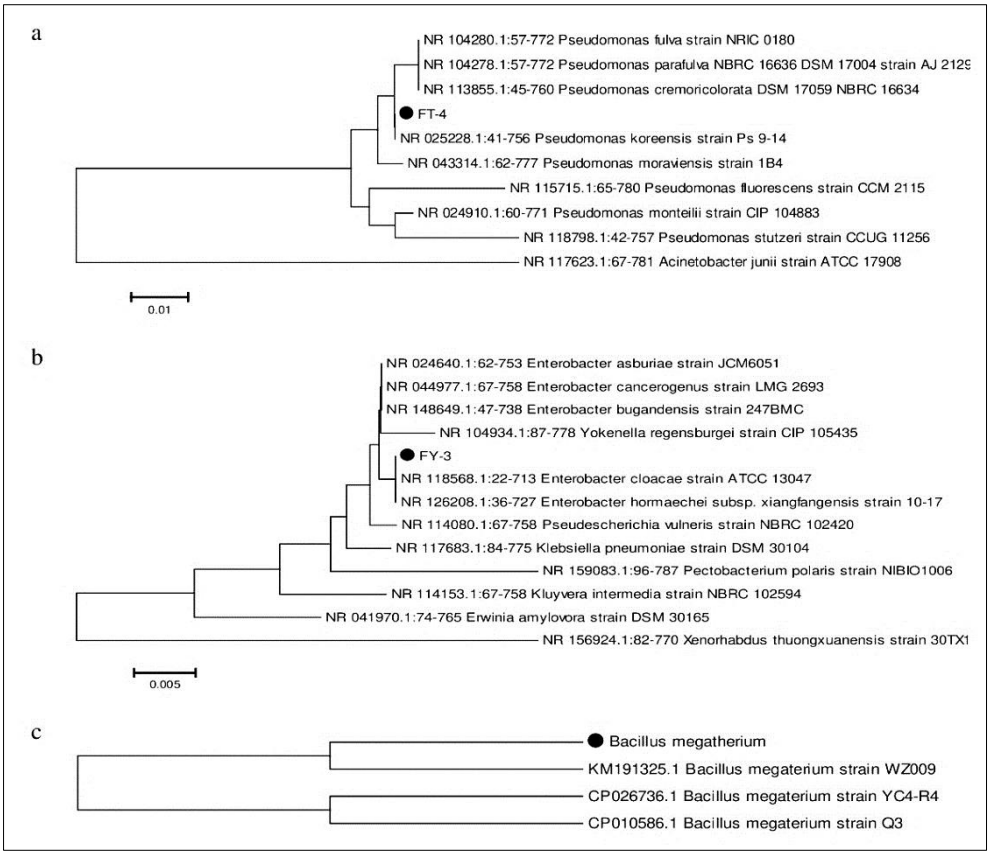


Figure 4. Neighbor-joining trees with bootstrapping 1000 replicates showing the phylogenetic relationship of three bacteria FT4 (a), FY3 (b), F7A (c) and their most closely related species based on the concatenated sequences from 16S rRNA

Figure 4 shows that strain FT4 was the most phylogenetically similar to strains of the genus *Pseudomonas*. Strain FT4's maximum similarity was 99.72 %with *Pseudomonas koreensis* strain Ps9-14's nucleotide sequence of the 16S rRNA gene from the GenBank database. Strain FY3 was closest to the genus *Enterobacter* and was found on the same branch as the GenBank database reference strains *Enterobacter cloacae* ATCC 13047 and *Enterobacter hormaechei subsp. xiangfangensis* strain 10-17. The percentage identity of these strains was 99.58%. Strain F7A was phylogenetically closest to *Bacillus* genus strains and was most similar to the nucleotide sequence of the 16S rRNA gene of *Bacillus megaterium* strain WZ009 (99.92% identity). Thus, three salt-tolerant phosphate-solubilizing bacterial strains were isolated and identified as strain FT4 (*Pseudomonas koreensis)*, strain FY-3 (*Enterobacter cloacae)*, and strain F7A (*Bacillus megaterium)*.

One of the processes involved in insoluble phosphate solubilization by bacteria is the synthesis of organic acids. However, the qualitative and quantitative composition of organic acids released by salt-tolerant bacteria under salt-stress conditions has not been well studied. In this regard, organic acid production by three salt-tolerant bacterial strains of various genera, *Pseudomonas*, *Bacillus* and *Enterobacter* has been studied.

Gas chromatography (GC) was used to analyze the bacterial secretion of organic acids. GC is an analytical procedure based on the separation and examination of volatile and semivolatile substances in a mixture. Qualitative and quantitative analyses of the organic acid composition were performed by comparison with standards. This method was used because it is precise, efficient, and reproducible.

To determine organic acids, *E. cloacae* FY3, *Ps. putida* FT4, and *B. megaterium* F7A were grown in NBRIP. NaCl (5%) was added to the medium to induce high salt stress. The collected data are listed in Table 5.

Table 5. Organic acid production by salt-tolerant phosphate-solubilizing bacteria

Strains	Organic acid (μg/ml)											
	Acetic	**Butyric**	**Caprylic**	**Palmitic**	**Propionic**	**Caproic**	**Pelargonic**	**Lauric**	**Valeric**	**Myristic**	**Phthalic**	**CPA***
*B. **megaterium*** F7A	24.33 ± 0.9	25.07 ± 0.3	ND	38.85 ± 0.4	ND	ND	ND	ND	26.55 ± 0.3	ND	ND	ND
E. cloacae FY3	68.21 ± 1.2	16.50 ± 0.4	ND	38.52± 0.6	25.07 ± 0.7	ND	ND	ND	ND	ND	ND	ND
Ps. putida FT4	ND	ND	7.93 ± 0.1	21.89 ± 0.4	3.30 ± 0.1	5.84 ± 0.2	7.25 ± 0.1	14.06 ± 0.3	44.4 ± 0.9	9.06 ± 0.2	11.13 ± 0.2	48.37 ± 0.5

Note: CPA—cyclopropaneacetic acid; ND—not detected; values are means of three replicates ± standard error of the mean (SEM); $p < 0.05$, n=3.

Table 5 shows that during the dissolution of insoluble tricalcium phosphate, the tested strains produced 12 organic acids: acetic, butyric, propionic, caprylic, palmitic, caproic, pelargonic, lauric, valerian, myristic, phthalic, and cyclopropaneacetic acids. Strain *Ps. putida* FT4 produced the highest number of organic acids (10 acids): propionic, caprylic, palmitic, caproic, pelargonic, lauric, valeric, myristic, phthalic, and cyclopropaneacetic. Strains *B. megaterium* F7A and E. cloacae FY3 produced four organic acids each, with three of the acids being the same: acetic, butyric, and palmitic acids (Table 5). Palmitic acid was the only acid that was produced by all three bacterial strains. Acetic and butyric acids were two of the acids that were produced by strains *B. megaterium* F7A and *E. cloacae* FY3. Strain *Ps. putida* FT4 did not form these acids but produced huge amounts of cyclopropaneacetic acid (48.37 ± 1.3 μg/ml), a byproduct in the acetic acid synthesis [69]. Cyclopropaneacetic acid was the principal organic acid in strain *Ps. putida* FT4 and accounted for 28% of the sum of all organic acids; in strain *E. cloacae* FY3, it was 46% of acetic acid; and in strain *B. megaterium* F7A, 34% palmitic acid. Although all strains had high phosphate-solubilizing activity, the amount and structure of organic acids in different strains varied significantly and were strain-dependent on the bacteria. Thus, different bacteria produce different organic acids, the quantitative and qualitative structure of which is strain-dependent.

The results completely validated hypothesis 3 (H3), because the isolated phosphate-solubilizing salt-tolerant bacteria were capable of producing organic acids.

Table 6. pH value, soluble phosphate content and total organic acids

Strains	pH		Soluble (released) P(μg/ml)	Total organic acid (μg/ml) *
	Starting	Final		
*B. **megaterium*** F7A	6.9±0.03	4.05±0.02	318.7±1.5	114.8±1.3
E. cloacae FY3	6.8±0.01	3.52±0.01	778.7±3.2	148.3±2.0
Ps. putida FT4	7.0±0.01	3.43±0.01	805.1±2.4	173.23±2.2

Note: Values are the mean of three replicates ± standard error of the mean; $p<0.01$; $*p<0.05$; n = 3.

The results of the data (Tables 5 and 6) reveal that the general mechanism of solubilization of phosphates in the strains under investigation is the secretion of organic acids, since it follows from a decrease in pH values from 7.0 to 4.0-3.5 and an increase in the quantitative content of soluble phosphorus. The acid ratio in the pH value was higher, and the soluble phosphorus content was higher. The strain *Ps. putida* produced the greatest yield of organic acids (173.23 ± 2.2 μg/mL, $p<0.05$), the greatest decrease in pH of the medium (3.43 ± 0.01, $p<0.01$), and the greatest content of soluble phosphorus (805.1 ± 2.4 μg/mL, $p<0.01$). A little lower production of organic acids was observed in the strain *E. cloacae* FY3 (148.3 ± 2.0 μg/mL, $p<0.05$). The lowest production of organic acids was in the strain *B. megaterium* F7A (114.8 ± 1.3, $p<0.05$), which also had the lowest soluble phosphorus content (318.7 ± 1.5 μg/mL, $p<0.01$).

Our tests indicated that the organic acid content in the medium is equivalent to the phosphate-solubilizing activity of bacteria and that there is a positive and direct correlation between organic acid release, medium acidification (pH decrease), and phosphorus solubilizing capacity. These results confirmed that the major mechanism of phosphate solubilization by the studied bacterial strains was the release of organic acids. Thus, the results fully corroborated our hypothesis 4 (H4) that the basic phosphate-solubilizing mechanism in novel salt-tolerant bacterial strains is organic acid secretion.

4. Discussion

Soil salinity is one of the greatest crop yield-enhancing factors, as it inhibits or even curbs plant development. Soil salinity drastically decreases the fitness of the soil to host nutrients destined for plants, particularly phosphorus. Phosphorus applied via fertilizer has a very limited effect as it is immediately fixed in plant-unavailable forms. To this extent, phosphorus inhibition is one of the major causes of crop yield reduction in saline soils.

Microbial solubilization of phosphates is a potential solution to this problem because it increases the plant availability of insoluble phosphates. Therefore, the use of phosphate-solubilizing bacteria is a promising approach. However, not all bacteria possess the ability to solubilize phosphate in saline soil. Therefore, it is necessary to screen, isolate, and select salt-tolerant phosphate-solubilizing bacteria capable of effectively solubilizing insoluble phosphorus in saline soils.

In this study, 127 soil samples from Southeast Kazakhstan (Almaty Province) saline pastures and 42 phosphate-soluble bacteria were isolated. In saline soils, their number is not high, but only 5-7% of the total number. This corresponds to data showing that the number of phosphate-solubilizing salt-tolerant bacteria in salt soils is small,

accounts for 3-12% on average [70, 71]. Other researchers believe that this effect occurs because osmotic pressure and increased salt concentration in soil change the protein properties and structure of bacteria and adversely affect the enzymatic activity of microbes, which reduces their numbers in soils [72, 73].

The salinity effect experiment on bacteria indicated that increased salinity inhibited their growth, and at 100 mM NaCl, all the isolates grew, while at 500 mM NaCl, six isolates (FT4, F7A, FM9/5, FM12, FY3, and FC11) grew, and these isolates were salt-tolerant. Higher salinity has also been found to inhibit the growth of phosphate-solubilizing bacteria in other studies [74-76]. We found that very few phosphate-mobilizing bacteria were salt-tolerant, as only six out of all bacteria isolated and tested were salt-tolerant and could grow under high-salt stress conditions. Hence, hypothesis 1 (H1), that is, that phosphate-solubilizing bacteria from salinized soils can grow in high salinity soil, was only partially confirmed. One of the resistance mechanisms of bacteria to salinity is their ability to store relatively high and harmful amounts of sodium ions within their cells to equalize osmotic pressure [77].

Simultaneously, in saline soil microbiomes, we identified phosphate-solubilizing bacteria that were not tolerant to salt. Most likely, they are physiologically inactive, but under variable conditions, such as reduced salinity, they become active. No similar studies have been performed, but indirect evidence has been described [76].

One of the most crucial traits in the selection of phosphate-solubilizing bacteria in saline soils is their ability to solubilize phosphate under high salt stress conditions. Salt-tolerant phosphate-solubilizing bacteria were screened on solid nutrient medium under active conditions. Isolates were selected based on the diameter of the halo zone. The findings of other studies have also been shown. Other studies have shown that only a few bacteria can function at high salinity [78, 79]. We obtained bacteria with a high ability to solubilize phosphate under high salt stress that formed clear and stable halo zones on solid media when grown under high salinity (10% NaCl), thereby demonstrating a high ability to solubilize phosphates under high salinity. By that, the phosphate solubilization index (SI) under high salt stress in bacteria was greater than three ($SI>3$, $p<0.05$), and in three of the isolates, FT4, FY3, and F7A, it was greater than four, equalling 4.4-4.8 ($p<0.05$). In other studies, the value of this index was significantly lower [75, 76], and several studies have indicated that high salinity completely inhibits phosphate solubilization [80, 81]. This indicates that the isolated bacteria were more resistant to salinity and had higher phosphate solubilization than bacteria isolated in other studies. Thus, the results of the current study support hypothesis 2 (H2) and demonstrate that pure salt-tolerant bacteria can efficiently solubilize insoluble phosphates under salt-stress conditions. There are very few analogous studies, but some research suggests that the application of salt-tolerant phosphate-solubilizing bacteria could be an effective and inexpensive method for increasing phosphorus availability in salt soils [21, 82].

Three salt-tolerant and highly active isolates, FT4, FY3, and F7A, were the most promising. Identification of these bacteria by Sanger molecular genetic method made it possible to define them as *Pseudomonas putida* FT4, *Enterobacter cloacae* FY3, and *Bacillus megaterium* F7A strains.

There are several studies on soil phosphate-solubilizing bacterial diversity, but there are very few studies on the identification of salt-tolerant phosphate-solubilizing bacteria. Studies by different authors have confirmed that the prevalent genera of salt-tolerant phosphate-solubilizing bacteria are *Pseudomonas* and *Bacillus*; other genera such as *Pantoea, Alcaligenes*, and *Flavobacterium* possess much less ability to dissolve phosphates in soils [79]. These studies are in agreement with our results. Simultaneously, there are few studies in which *Enterobacter* bacteria can solubilize phosphate under saline stress, although these bacteria are widespread in soil [83].

Phosphate-solubilizing bacteria can solubilize phosphates through different mechanisms [49, 77, 84]. The major ones are (1) production of metabolites that are involved in solubilizing phosphate in the soil (hydroxyl ions, siderophores, and organic acids), (2) extracellular enzyme production (phytases and acid phosphatases), and (3) phosphorus release after the breakdown of insoluble phosphate-rich substrates. However, organic acid secretion is generally accepted to be a process of insoluble phosphate solubilization. Phosphate-solubilizing bacteria release organic acids that degrade mineral phosphates and render them more accessible to plants, thereby enhancing their growth [85, 86]. The results of our gas chromatography analysis indicated that the bacterial strains isolated under salt stress conditions have the ability to release organic acids, degrade insoluble phosphate forms, and increase phosphorus availability to plants.

Hence, our work supported hypothesis 3 (H3) and determined that the novel phosphate-solubilizing salt-tolerant bacteria isolated were proficient in producing organic acids. The production of organic acids by bacteria under salt stress does not occur or is scarce. Moreover, no data on the composition of organic acids produced by salt-tolerant phosphate-solubilizing bacteria in qualitative and quantitative terms are available. We found that salt-tolerant bacterial strains produced different compositions and amounts of organic acids when they dissolved the same source of phosphorus. Experiments illustrating that different phosphate-solubilizing bacterial strains produce an array of organic acids validate these results [56]. The studied strains produced 12 organic acids: acetic, butyric, propionic, caprylic, palmitic, caproic, pelargonic, lauric, valeric, myristic, phthalic, and cyclopropaneacetic acids.

The basic mechanism of solubilization of insoluble phosphates in the studied bacteria was the production of organic acids, which was proven by a positive correlation between organic acid excretion, decrease in pH value in the medium, and growth of the quantitative content of soluble phosphorus (i.e., acidification) being the primary factor in phosphate solubilization. The study findings fully verified hypothesis 4 (H4) and showed that the elementary mechanism of phosphate solubilization in novel strains of salt-tolerant bacteria is organic acid excretion. Furthermore, there was a clear relationship between the medium pH reduction and phosphate solubilization activity. Similar studies involving salt-tolerant bacteria are absent, but indirect confirmation is provided in [40, 87].

Therefore, we conducted studies in this direction. We isolated and studied salt-tolerant phosphate-solubilizing bacteria to support the growth of soybean cultures in saline soils [88]. In this study, bacterial strains isolated from the soybean rhizosphere showed growth-stimulating activity and the ability to increase soybean yield in saline soils. However, species specificity of bacteria exists in their host plants, which is dependent on the host plant [89]. Therefore, bacteria from the soybean rhizosphere will not be efficient when applied to saline pasture soils, and will not have a high positive effect on the growth of pasture grasses. In addition, there is a relationship between plants and particular species of phosphate-mobilizing bacteria, meaning that plants in the rhizosphere select certain species of bacteria through their secretions [90]. The new contribution of this study is that it aims to improve the productivity of saline pastures.

The isolated new strains of bacteria will be adapted to pasture grasses, soil, and climatic conditions of saline soils of pastures of south-eastern Kazakhstan, and will be effective in their practical application. In addition, new local strains will reduce the doses of phosphorus fertilizers, which are expensive for farmers and will favorably affect the environment and increase the productivity of saline pastures in south-eastern Kazakhstan. This will lead to more efficient development of the fodder base for livestock breeding, because sheep breeding is of great economic importance and determines the employment and income of the rural population in this region.

Thus, new active phosphate-solubilizing salt-tolerant bacterial strains were isolated, which have broad prospects for application in saline soils because they are characterized, in addition to high salt tolerance, by the ability to solubilize phosphates under high salt stress; they are also indigenous and adapted to the soil and climatic conditions of Kazakhstan.

It was established that the basic mechanism of solubilization of insoluble phosphate in the studied bacteria was the secretion of organic acids, as demonstrated by the positive correlation between the secretion of organic acids and the increase in soluble phosphorus content. However, the mobilization of phosphates by the secretion of organic acids is not the only mechanism; therefore, it is planned to study other mechanisms of phosphate dissolution by new strains, such as the production of siderophores and the synthesis of enzymes that break down phosphorus compounds (phosphatases and phytases).

Our results have broad prospects for farming in saline soils because new bacterial strains can replace or partially reduce the use of phosphorus fertilizers, and their application can be an effective method to increase crop productivity in saline soils.

5. Conclusion

This study enabled the isolation of salt-tolerant phosphate-solubilizing bacteria from the saline soils of Kazakhstan and the identification of the most efficient strains with good phosphate solubilization under high salinity. This study included the identification of isolates, establishment of their taxonomic affiliation, and investigation of phosphate solubilization processes. The bacterial strains were able to secrete organic acids, and the qualitative and quantitative compositions of the secreted acids were investigated. We found that the acid composition depended on the bacterial strain.

5.1. Scientific Novelty

For the first time, new local strains of salt-tolerant phosphate-solubilizing bacteria have been isolated from Kazakhstani saline soils. It was established that their numbers were low and represented 5-7% of all bacteria. Using the Sanger method of 16S rRNA gene sequencing, new bacterial strains were detected and found to belong to *Pseudomonas putida* FT4, *Enterobacter cloacae* FY3, and *Bacillus megaterium* F7A.

The solubilization mechanism of insoluble phosphates in new bacterial strains involves secretion of organic acids and acidification of the medium.

5.2. Theoretical Significance of the Study Results

The results of this study are of immense theoretical significance, as they complement and add to the information on phosphate-solubilizing bacteria. The findings of this study allow us to better understand the mechanisms of the bacterial dissolution of insoluble phosphates. The study of the mechanisms of phosphate dissolution in saline soils is of

utmost importance for the elaboration of methods for mobilization of phosphorus from poorly soluble compounds and their transfer into readily accessible forms for plants, and investigation into the mechanisms of microbial phosphate solubilization of insoluble phosphates will allow the development of efficient methods for solving the problem of soil salinization not only in Kazakhstan but also in other countries with similar climatic and soil conditions for restoration of the fertility of saline soils and the productivity of pastures.

5.3. Practical Significance of the Study Results

Kazakhstan is a developing region of grassland agriculture that brings significant national income in the form of dairy and meat products. The country obtains more than 45% of its gross agricultural output from the sector. Breeding animals on pastures reduces food product prices and increases the profitability of animal breeding on farms. The well-being and nutrition of most of the population of Kazakhstan depend significantly on the condition and productivity of natural pastures. Therefore, the use of new local strains of salt-tolerant phosphate-solubilizing bacteria to stimulate phosphorus availability and alleviate salt stress is of crucial practical importance. Efficient biopreparation based on these bacteria will reduce the use of expensive phosphate fertilizers and limit the negative impact of these fertilizers on the environment. In addition, these biopreparations may be promising for maintaining productive pastures in saline soils and re-establishing them as productive lands.

5.4. Limitations and Future Research

This was a complete logical study. However, laboratory approaches have some limitations because they estimate phosphate solubility in microbial solubilization to be slightly higher than that in field tests. In this regard, laboratory studies require validation through field testing of strains.

The final goal of our study was to create biofertilizers to increase the productivity of pasture grasses in saline soils. Therefore, in future, we plan to conduct studies on the influence of bacteria on the physiological functions of plants and study the ability of bacteria to produce metabolites that support plant growth under salt stress in the field. Such biofertilizers are promising because local bacterial strains can acclimatize to the natural conditions of saline soils and synergize the interaction between plants and microbes.

6. Declarations

6.1. Author Contributions

Conceptualization, I.S. and A.S.; methodology, I.S.; software, A.S.; validation, A.M., G.B., and T.Z.; formal analysis, A.M.; investigation, G.B.; resources, T.Z.; data curation, A.S.; writing—original draft preparation, I.S.; writing—review and editing, T.Z.; visualization, A.M.; supervision, A.S.; project administration, I.S.; funding acquisition, G.B. All authors have read and agreed to the published version of the manuscript.

6.2. Data Availability Statement

The data presented in this study are available on request from the corresponding author.

6.3. Funding

The work was carried out within the framework of the grant project of the program “Development of rational technology to increase the fertility of degraded saline soils of pastures in the South-East Kazakhstan”, funded by the Ministry of Science and Higher Education of the Republic of Kazakhstan, AP 23487733.

6.4. Institutional Review Board Statement

Not applicable.

6.5. Informed Consent Statement

Not applicable.

6.6. Declaration of Competing Interest

The authors declare that there are no conflicts of interest concerning the publication of this manuscript. Furthermore, all ethical considerations, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

7. References

[1] Negacz, K., Malek, Ž., de Vos, A., & Vellinga, P. (2022). Saline soils worldwide: Identifying the most promising areas for saline agriculture. Journal of Arid Environments, 203, 104775. doi:10.1016/j.jaridenv.2022.104775.

[2] FAO. (2021). Global Map of Salt Affected Soils Version 1.0. Available online: https://www.fao.org/soils-portal/data-hub/soil-maps-and-databases/global-map-of-salt-affected-soils/en/ (accessed on August 2025).

[3] Tarolli, P., Luo, J., Park, E., Barcaccia, G., & Masin, R. (2024). Soil salinization in agriculture: Mitigation and adaptation strategies combining nature-based solutions and bioengineering. iScience, 27(2), 108830. doi:10.1016/j.isci.2024.108830.

[4] Atta, K., Mondal, S., Gorai, S., Singh, A. P., Kumari, A., Ghosh, T., Roy, A., Hembram, S., Gaikwad, D. J., Mondal, S., Bhattacharya, S., Jha, U. C., & Jespersen, D. (2023). Impacts of salinity stress on crop plants: improving salt tolerance through genetic and molecular dissection. Frontiers in Plant Science, 14. doi:10.3389/fpls.2023.1241736.

[5] Palmgren, M., & Shabala, S. (2024). Adapting crops for climate change: regaining lost abiotic stress tolerance in crops. Frontiers in Science, 2. doi:10.3389/fsci.2024.1416023.

[6] Nainwal, R. C., Chaurasiya, P., Kumar, A., Singh, M., Singh, D., & Tewari, S. K. (2024). Phytoremediation: A Sustainable Approach to Combat Soil Salinity. Advances in Environmental and Engineering Research, 5(2), 1–16. doi:10.21926/aeer.2402015.

[7] El-Ramady, H., Prokisch, J., Mansour, H., Bayoumi, Y. A., Shalaby, T. A., Veres, S., & Brevik, E. C. (2024). Review of Crop Response to Soil Salinity Stress: Possible Approaches from Leaching to Nano-Management. Soil Systems, 8(1), 11. doi:10.3390/soilsystems8010011.

[8] Sarker, A., Ansary, M. W. R., Hossain, M. N., & Islam, T. (2021). Prospect and Challenges for Sustainable Management of Climate Change-Associated Stresses to Soil and Plant Health by Beneficial Rhizobacteria. Stresses, 1(4), 200–222. doi:10.3390/stresses1040015.

[9] Loi, L. T., Minh, V. Q., & Duy, N. N. (2022). Changing of Soil Properties under Drought and Saline Water Intrusion Conditions in Different Land-Use Patterns – A Case Study in the Ben Tre Province, Vietnam. Journal of Ecological Engineering, 23(8), 112–118. doi:10.12911/22998993/150692.

[10] Muhammad, M., Waheed, A., Wahab, A., Majeed, M., Nazim, M., Liu, Y. H., Li, L., & Li, W. J. (2024). Soil salinity and drought tolerance: An evaluation of plant growth, productivity, microbial diversity, and amelioration strategies. Plant Stress, 11, 100319. doi:10.1016/j.stress.2023.100319.

[11] Zhu, H. Y., Gan, B., Xu, J. B., Liang, F., Zhou, B. B., Sun, Y., Duan, M. L., & Bai, Y. H. (2023). Effect of Soil Salinity on Nitrogen Transformation in Soil with Nitrogen Fertilizer Application. Applied Ecology and Environmental Research, 21(6), 5625–5642. doi:10.15666/aeer/2106_56255642.

[12] Xie, W., Yang, J., Gao, S., Yao, R., & Wang, X. (2022). The Effect and Influence Mechanism of Soil Salinity on Phosphorus Availability in Coastal Salt-Affected Soils. Water (Switzerland), 14(18), 2804. doi:10.3390/w14182804.

[13] Lovio-Fragoso, J. P., de Jesús-Campos, D., López-Elías, J. A., Medina-Juárez, L. Á., Fimbres-Olivarría, D., & Hayano-Kanashiro, C. (2021). Biochemical and molecular aspects of phosphorus limitation in diatoms and their relationship with biomolecule accumulation. Biology, 10(7), 565. doi:10.3390/biology10070565.

[14] Meng, X., Chen, W. W., Wang, Y. Y., Huang, Z. R., Ye, X., Chen, L. S., & Yang, L. T. (2021). Effects of phosphorus deficiency on the absorption of mineral nutrients, photosynthetic system performance and antioxidant metabolism in Citrus grandis. PLoS ONE, 16(2 February), 246944. doi:10.1371/journal.pone.0246944.

[15] Khan, F., Siddique, A. B., Shabala, S., Zhou, M., & Zhao, C. (2023). Phosphorus Plays Key Roles in Regulating Plants' Physiological Responses to Abiotic Stresses. Plants, 12(15), 2861. doi:10.3390/plants12152861.

[16] Mishra, S., Levengood, H., Fan, J., & Zhang, C. (2024). Plants under Stress: Exploring Physiological and Molecular Responses to Nitrogen and Phosphorus Deficiency. Plants, 13(22), 3144. doi:10.3390/plants13223144.

[17] Ualiyeva, R. M., Zhangazin, S. B., & Altayeva, I. B. (2022). Structural Organization of Vitelline Cells of Trematode with Undifferentiated Body of Azygia Lucii (Muller, 1776). OnLine Journal of Biological Sciences, 22(1), 10–17. doi:10.3844/ojbsci.2022.10.17.

[18] Wang, J., Qi, Z., & Wang, C. (2023). Phosphorus loss management and crop yields: A global meta-analysis. Agriculture, Ecosystems and Environment, 357, 108683. doi:10.1016/j.agee.2023.108683.

[19] Solangi, F., Zhu, X., Khan, S., Rais, N., Majeed, A., Sabir, M. A., Iqbal, R., Ali, S., Hafeez, A., Ali, B., Ercisli, S., & Kayabasi, E. T. (2023). The Global Dilemma of Soil Legacy Phosphorus and Its Improvement Strategies under Recent Changes in Agro-Ecosystem Sustainability. ACS Omega, 8(26), 23271–23282. doi:10.1021/acsomega.3c00823.

[20] Bouras, H., Mamassi, A., Devkota, K. P., Choukr-Allah, R., & Bouazzama, B. (2023). Integrated effect of saline water irrigation and phosphorus fertilization practices on wheat (Triticum aestivum) growth, productivity, nutrient content and soil proprieties under dryland farming. Plant Stress, 10, 100295. doi:10.1016/j.stress.2023.100295.

[21] Dey, G., Banerjee, P., Sharma, R. K., Maity, J. P., Etesami, H., Shaw, A. K., Huang, Y. H., Huang, H. Bin, & Chen, C. Y. (2021). Management of phosphorus in salinity-stressed agriculture for sustainable crop production by salt-tolerant phosphate-solubilizing bacteria—a review. Agronomy, 11(8), 1552. doi:10.3390/agronomy11081552.

[22] Kaya, C., Uğurlar, F., Ashraf, M., Alyemeni, M. N., Dewil, R., & Ahmad, P. (2024). Mitigating salt toxicity and overcoming phosphate deficiency alone and in combination in pepper (Capsicum annuum L.) plants through supplementation of hydrogen sulfide. Journal of Environmental Management, 351, 119759. doi:10.1016/j.jenvman.2023.119759.

[23] Zheng, S., Yin, K., & Yu, L. (2022). Factors influencing the farmer's chemical fertilizer reduction behavior from the perspective of farmer differentiation. Heliyon, 8(12), 11918. doi:10.1016/j.heliyon.2022.e11918.

[24] Krasilnikov, P., Taboada, M. A., & Amanullah. (2022). Fertilizer Use, Soil Health and Agricultural Sustainability. Agriculture (Switzerland), 12(4), 462. doi:10.3390/agriculture12040462.

[25] Johan, P. D., Ahmed, O. H., Omar, L., & Hasbullah, N. A. (2021). Phosphorus transformation in soils following co-application of charcoal and wood ash. Agronomy, 11(10), 10. doi:10.3390/agronomy11102010.

[26] Liu, L., Zheng, X., Wei, X., Kai, Z., & Xu, Y. (2021). Excessive application of chemical fertilizer and organophosphorus pesticides induced total phosphorus loss from planting causing surface water eutrophication. Scientific Reports, 11(1), 23015. doi:10.1038/s41598-021-02521-7.

[27] Alexander, A., Singh, V. K., & Mishra, A. (2020). Halotolerant PGPR Stenotrophomonas maltophilia BJ01 Induces Salt Tolerance by Modulating Physiology and Biochemical Activities of Arachis hypogaea. Frontiers in Microbiology, 11. doi:10.3389/fmicb.2020.568289.

[28] Egea, I., Estrada, Y., Faura, C., Egea-Fernández, J. M., Bolarin, M. C., & Flores, F. B. (2023). Salt-tolerant alternative crops as sources of quality food to mitigate the negative impact of salinity on agricultural production. Frontiers in Plant Science, 14. doi:10.3389/fpls.2023.1092885.

[29] Jeon, D., Kim, J. B., Kang, B. C., & Kim, C. (2023). Deciphering the Genetic Mechanisms of Salt Tolerance in Sorghum bicolor L.: Key Genes and SNP Associations from Comparative Transcriptomic Analyses. Plants, 12(14), 2639. doi:10.3390/plants12142639.

[30] Wijitkosum, S., Sriburi, T., & Krutnoi, L. (2024). Taking Advantage of Disposal Bamboo Chopsticks to Produce Biochar for Greenhouse Crop Cultivation. Emerging Science Journal, 8(3), 917–932. doi:10.28991/ESJ-2024-08-03-07.

[31] Huang, K., Li, M., Li, R., Rasul, F., Shahzad, S., Wu, C., Shao, J., Huang, G., Li, R., Almari, S., Hashem, M., & Aamer, M. (2023). Soil acidification and salinity: the importance of biochar application to agricultural soils. Frontiers in Plant Science, 14. doi:10.3389/fpls.2023.1206820.

[32] Elhaissoufi, W., Ghoulam, C., Barakat, A., Zeroual, Y., & Bargaz, A. (2022). Phosphate bacterial solubilization: A key rhizosphere driving force enabling higher P use efficiency and crop productivity. Journal of Advanced Research, 38, 13–28. doi:10.1016/j.jare.2021.08.014.

[33] García-Berumen, J. A., Flores de la Torre, J. A., de los Santos-Villalobos, S., Espinoza-Canales, A., Echavarría-Cháirez, F. G., & Gutiérrez-Bañuelos, H. (2025). Phosphorus dynamics and sustainable agriculture: The role of microbial solubilization and innovations in nutrient management. Current Research in Microbial Sciences, 8, 100326. doi:10.1016/j.crmicr.2024.100326.

[34] Silva, L. I. da, Pereira, M. C., Carvalho, A. M. X. de, Buttrós, V. H., Pasqual, M., & Dória, J. (2023). Phosphorus-Solubilizing Microorganisms: A Key to Sustainable Agriculture. Agriculture (Switzerland), 13(2), 462. doi:10.3390/agriculture13020462.

[35] Cheng, Y., Narayanan, M., Shi, X., Chen, X., Li, Z., & Ma, Y. (2023). Phosphate-solubilizing bacteria: Their agroecological function and optimistic application for enhancing agro-productivity. Science of the Total Environment, 901, 166468. doi:10.1016/j.scitotenv.2023.166468.

[36] Zhu, Y., Xing, Y., Li, Y., Jia, J., Ying, Y., & Shi, W. (2024). The Role of Phosphate-Solubilizing Microbial Interactions in Phosphorus Activation and Utilization in Plant–Soil Systems: A Review. Plants, 13(19), 2686. doi:10.3390/plants13192686.

[37] Sunita, K., Mishra, I., Mishra, J., Prakash, J., & Arora, N. K. (2020). Secondary Metabolites from Halotolerant Plant Growth Promoting Rhizobacteria for Ameliorating Salinity Stress in Plants. Frontiers in Microbiology, 11. doi:10.3389/fmicb.2020.567768.

[38] Zulfikar, T., Siregar, T. N., Rozaliyani, A., & Sutriana, A. (2024). Antimicrobial Potential of Calotropis gigantea Leaf against Klebsiella pneumoniae in Ventilator-Associated Pneumonia. Journal of Human, Earth, and Future, 5(3), 456–470. doi:10.28991/HEF-2024-05-03-010.

[39] Ahmad, M., Naqve, M., Lihong, W., Zia, M. A., Mahmood, A., Javaid, M. M., Ameen, M., Rashed, A. A., Rasheed, A., Hassan, M. U., &Qari, S. H. (2024). Mitigating negative impact of salinity on berseem (Trifoliumalexandrinum) by foliar application of salicylic acid. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 52(1), 13467. doi:10.15835/nbha52113467.

[40] Pan, L., & Cai, B. (2023). Phosphate-Solubilizing Bacteria: Advances in Their Physiology, Molecular Mechanisms and Microbial Community Effects. Microorganisms, 11(12), 2904. doi:10.3390/microorganisms11122904.

[41] Liu, Y., Xun, W., Chen, L., Xu, Z., Zhang, N., Feng, H., Zhang, Q., & Zhang, R. (2022). Rhizosphere microbes enhance plant salt tolerance: Toward crop production in saline soil. Computational and Structural Biotechnology Journal, 20, 6543–6551. doi:10.1016/j.csbj.2022.11.046.

[42] Ondrasek, G., Rathod, S., Manohara, K. K., Gireesh, C., Anantha, M. S., Sakhare, A. S., Parmar, B., Yadav, B. K., Bandumula, N., Raihan, F., Zielińska-Chmielewska, A., Meriño-Gergichevich, C., Reyes-Díaz, M., Khan, A., Panfilova, O., Fuentealba, A. S., Romero, S. M., Nabil, B., Wan, C., … Horvatinec, J. (2022). Salt Stress in Plants and Mitigation Approaches. Plants, 11(6), 717. doi:10.3390/plants11060717.

[43] Iqbal, B., Li, G., Alabbosh, K. F., Hussain, H., Khan, I., Tariq, M., Javed, Q., Naeem, M., & Ahmad, N. (2023). Advancing environmental sustainability through microbial reprogramming in growth improvement, stress alleviation, and phytoremediation. Plant Stress, 10, 100283. doi:10.1016/j.stress.2023.100283.

[44] Khoso, M. A., Wagan, S., Alam, I., Hussain, A., Ali, Q., Saha, S., Poudel, T. R., Manghwar, H., & Liu, F. (2024). Impact of plant growth-promoting rhizobacteria (PGPR) on plant nutrition and root characteristics: Current perspective. Plant Stress, 11, 100341. doi:10.1016/j.stress.2023.100341.

[45] Fu, M., Liu, L., Fu, B., Hou, M., Xiao, Y., Liu, Y., Sa, D., & Lu, Q. (2024). Effects of salt stress on plant and rhizosphere bacterial communities, interaction patterns, and functions. Frontiers in Plant Science, 15. doi:10.3389/fpls.2024.1516336.

[46] Benitez-Alfonso, Y., Soanes, B. K., Zimba, S., Sinanaj, B., German, L., Sharma, V., Bohra, A., Kolesnikova, A., Dunn, J. A., Martin, A. C., Khashi u Rahman, M., Saati-Santamaría, Z., García-Fraile, P., Ferreira, E. A., Frazão, L. A., Cowling, W. A., Siddique, K. H. M., Pandey, M. K., Farooq, M., … Foyer, C. H. (2023). Enhancing climate change resilience in agricultural crops. Current Biology, 33(23), R1246–R1261. doi:10.1016/j.cub.2023.10.028.

[47] Wang, C., Pan, G., Lu, X., & Qi, W. (2023). Phosphorus solubilizing microorganisms: potential promoters of agricultural and environmental engineering. Frontiers in Bioengineering and Biotechnology, 11. doi:10.3389/fbioe.2023.1181078.

[48] Rababah, S., Alawneh, A., Albiss, B. A., Aldeeky, H. H., Bani Ismaeel, E. J., & Mutlaq, S. (2024). Enhancing Soil Stability through Innovative Microbial-Induced Calcium Carbonate Techniques with Sustainable Ingredient. Civil Engineering Journal, 10(8), 2536–2553. doi:10.28991/CEJ-2024-010-08-08.

[49] Faller, L., Leite, M. F. A., & Kuramae, E. E. (2024). Enhancing phosphate-solubilising microbial communities through artificial selection. Nature Communications, 15(1), 1649. doi:10.1038/s41467-024-46060-x.

[50] Yue, L., Jiao, L., Tao, M., Xu, L., Cao, X., Chen, F., Wang, C., Cheng, B., & Wang, Z. (2023). Dynamics of organic acid exudation and rhizobacteria in maize rhizosphere respond to N-CDs. Science of the Total Environment, 901, 166500. doi:10.1016/j.scitotenv.2023.166500.

[51] Zuluaga, M. Y. A., de Oliveira, A. L. M., Valentinuzzi, F., Jayme, N. S., Monterisi, S., Fattorini, R., Cesco, S., & Pii, Y. (2023). An insight into the role of the organic acids produced by Enterobacter sp. strain 15S in solubilizing tricalcium phosphate: in situ study on cucumber. BMC Microbiology, 23(1), 184. doi:10.1186/s12866-023-02918-6.

[52] Li, H. P., Han, Q. Q., Liu, Q. M., Gan, Y. N., Rensing, C., Rivera, W. L., Zhao, Q., & Zhang, J. L. (2023). Roles of phosphate-solubilizing bacteria in mediating soil legacy phosphorus availability. Microbiological Research, 272, 127375. doi:10.1016/j.micres.2023.127375.

[53] Seregin, I. V., & Kozhevnikova, A. D. (2024). The Role of Low-Molecular-Weight Organic Acids in Metal Homeostasis in Plants. International Journal of Molecular Sciences, 25(17), 9542. doi:10.3390/ijms25179542.

[54] Wiesenbauer, J., Gorka, S., Jenab, K., Schuster, R., Kumar, N., Rottensteiner, C., König, A., Kraemer, S., Inselsbacher, E., & Kaiser, C. (2025). Preferential use of organic acids over sugars by soil microbes in simulated root exudation. Soil Biology and Biochemistry, 203. doi:10.1016/j.soilbio.2025.109738.

[55] Janati, W., Bouabid, R., Mikou, K., Ghadraoui, L. El, & Errachidi, F. (2023). Phosphate solubilizing bacteria from soils with varying environmental conditions: Occurrence and function. PLoS ONE, 18(12), 289127. doi:10.1371/journal.pone.0289127.

[56] de Almeida Leite, R., Martins da Costa, E., Cabral Michel, D., do Amaral Leite, A., de Oliveira-Longatti, S. M., de Lima, W., Konstantinidis, K. T., & de Souza Moreira, F. M. (2024). Genomic insights into organic acid production and plant growth promotion by different species of phosphate-solubilizing bacteria. World Journal of Microbiology and Biotechnology, 40(10), 311. doi:10.1007/s11274-024-04119-3.

[57] ElDala.kz. (2025). Available online: https://eldala.kz/novosti/kazahstan/12539-opublikovana-administrativnaya-karta-kazahstana-s-uchetom-novyh-oblastey (accessed on August 2025).

[58] Google Public Maps. (2025). Available online: https://www.google.kz/maps/ (accessed on August 2025).

[59] GOST 17.4.4.02-84. (2017). Nature Protection: State Standard. Soils. Methods of Sampling and Preparation of Material for Chemical, Bacteriological, and Helminthological Analysis. Standartinform, Moscow, Russia. (in Russian).

[60] Qingwei, Z., Lushi, T., Yu, Z., Yu, S., Wanting, W., Jiangchuan, W., Xiaolei, D., Xuejiao, H., & Bilal, M. (2023). Isolation and characterization of phosphate-solubilizing bacteria from rhizosphere of poplar on road verge and their antagonistic potential against various phytopathogens. BMC Microbiology, 23(1), 221. doi:10.1186/s12866-023-02953-3.

[61] Reis, M. N. O., Bessa, L. A., de Jesus, A. P., Guimarães Silva, F., Moreira, M. A., & Vitorino, L. C. (2021). Efficiency of the Hydroponic System as an Approach to Confirm the Solubilization of CaHPO4 by Microbial Strains Using Glycine max as a Model. Frontiers in Plant Science, 12. doi:10.3389/fpls.2021.759463.

[62] Amy, C., Avice, J. C., Laval, K., & Bressan, M. (2022). Are native phosphate solubilizing bacteria a relevant alternative to mineral fertilizations for crops? Part I. when rhizobacteria meet plant P requirements. Rhizosphere, 21, 100476. doi:10.1016/j.rhisph.2022.100476.

[63] Wieczorek, D., Żyszka-Haberecht, B., Kafka, A., & Lipok, J. (2022). Determination of phosphorus compounds in plant tissues: from colourimetry to advanced instrumental analytical chemistry. Plant Methods, 18(1), 22. doi:10.1186/s13007-022-00854-6.

[64] Winand, R., Bogaerts, B., Hoffman, S., Lefevre, L., Delvoye, M., Van Braekel, J., Fu, Q., Roosens, N. H. C., De Keersmaecker, S. C. J., & Vanneste, K. (2020). Targeting the 16s rRNA gene for bacterial identification in complex mixed samples: Comparative evaluation of second (illumina) and third (oxford nanopore technologies) generation sequencing technologies. International Journal of Molecular Sciences, 21(1), 298. doi:10.3390/ijms21010298.

[65] NCBI. Available online: http://www.ncbi.nlm.nih.gov/ (accessed on August 2025).

[66] Sadanov, A., Alimzhanova, M., Ismailova, E., Shemshura, O., Ashimuly, K., Molzhigitova, A., & Daugaliyeva, S. (2023). Antagonistic and protective activity of Lactobacillus plantarum strain 17 M against E. amylovora. World Journal of Microbiology and Biotechnology, 39(11), 314. doi:10.1007/s11274-023-03765-3.

[67] Borovikov, V. P. (2018). A popular introduction to modern data analysis in STATISTICA-10. Publishing House "Hot Line - Telecom", Moscow, Russia. (in Russian).

[68] Gómez-de-Mariscal, E., Guerrero, V., Sneider, A., Jayatilaka, H., Phillip, J. M., Wirtz, D., & Muñoz-Barrutia, A. (2021). Use of the p-values as a size-dependent function to address practical differences when analyzing large datasets. Scientific Reports, 11(1), 20942. doi:10.1038/s41598-021-00199-5.

[69] Merli, G., Becci, A., Amato, A., & Beolchini, F. (2021). Acetic acid bioproduction: The technological innovation change. Science of the Total Environment, 798, 149292. doi:10.1016/j.scitotenv.2021.149292.

[70] Tarigan, D. M., Barus, W. A., Munar, A., & Lestami, A. (2023). Exploration and Morphological Characterization of Phosphate-Solubilizing and Nitrogen-Fixing Bacteria in Saline Soil. Sabrao Journal of Breeding and Genetics, 55(2), 550–563. doi:10.54910/sabrao2023.55.2.27.

[71] Koczorski, P., Furtado, B. U., Gołębiewski, M., Hulisz, P., Thiem, D., Baum, C., Weih, M., & Hrynkiewicz, K. (2022). Mixed growth of Salix species can promote phosphate-solubilizing bacteria in the roots and rhizosphere. Frontiers in Microbiology, 13. doi:10.3389/fmicb.2022.1006722.

[72] Zhang, G., Bai, J., Zhai, Y., Jia, J., Zhao, Q., Wang, W., & Hu, X. (2024). Microbial diversity and functions in saline soils: A review from a biogeochemical perspective. Journal of Advanced Research, 59, 129–140. doi:10.1016/j.jare.2023.06.015.

[73] Zhang, H., Chang, D., Zhu, Z., Meng, C., & Wang, K. (2024). Soil priming effects and involved microbial community along salt gradients. Biogeosciences, 21(1), 1–11. doi:10.5194/bg-21-1-2024.

[74] Saranya, K., Sundaramanickam, A., Manupoori, S., & Kanth, S. V. (2022). Screening of multi-faceted phosphate-solubilising bacterium from seagrass meadow and their plant growth promotion under saline stress condition. Microbiological Research, 261, 127080. doi:10.1016/j.micres.2022.127080.

[75] Zhang, C., Chen, H., Dai, Y., Chen, Y., Tian, Y., & Huo, Z. (2023). Isolation and screening of phosphorus solubilizing bacteria from saline alkali soil and their potential for Pb pollution remediation. Frontiers in Bioengineering and Biotechnology, 11. doi:10.3389/fbioe.2023.1134310.

[76] Teles, E. A. P., Xavier, J. F., Arcênio, F. S., Amaya, R. L., Gonçalves, J. V. S., Rouws, L. F. M., Zonta, E., & Coelho, I. S. (2023). Characterization and evaluation of potential halotolerant phosphate solubilizing bacteria from Salicornia fruticosa rhizosphere. Frontiers in Plant Science, 14. doi:10.3389/fpls.2023.1324056.

[77] Kumawat, C., Kumar, A., Parshad, J., Sharma, S. S., Patra, A., Dogra, P., Yadav, G. K., Dadhich, S. K., Verma, R., & Kumawat, G. L. (2022). Microbial Diversity and Adaptation under Salt-Affected Soils: A Review. Sustainability (Switzerland), 14(15), 9280. doi:10.3390/su14159280.

[78] Aliyat, F. Z., Maldani, M., El Guilli, M., Nassiri, L., & Ibijbijen, J. (2022). Phosphate-Solubilizing Bacteria Isolated from Phosphate Solid Sludge and Their Ability to Solubilize Three Inorganic Phosphate Forms: Calcium, Iron, and Aluminum Phosphates. Microorganisms, 10(5), 980. doi:10.3390/microorganisms10050980.

[79] Kouas, S., Djedidi, S., Ben Slimene Debez, I., Sbissi, I., Alyami, N. M., & Hirsch, A. M. (2024). Halotolerant phosphate solubilizing bacteria isolated from arid area in Tunisia improve P status and photosynthetic activity of cultivated barley under P shortage. Heliyon, 10(19), 38653. doi:10.1016/j.heliyon.2024.e38653.

[80] Wang, W., Sun, X., Huang, W., Men, X., Yi, S., Zheng, F., Zhang, Z., & Wang, Z. (2023). Soil P solubilization and plant growth promotion by a saline-alkali-tolerant P-solubilizing bacterium, Bacillus sp. DYS211. Journal of Plant Ecology, 16(6), 1-13. doi:10.1093/jpe/rtad028.

[81] Fuskhah, E., & Purbajanti, E. D. (2023). Exploration and selection of salt tolerant phosphate solubilizing bacteria from the Indonesian saline soils. IOP Conference Series: Earth and Environmental Science, 1246(1), 12026. doi:10.1088/1755-1315/1246/1/012026.

[82] Belkebla, N., Bessai, S. A., Melo, J., Caeiro, M. F., Cruz, C., & Nabti, E. H. (2022). Restoration of Triticum aestivum Growth under Salt Stress by Phosphate-Solubilizing Bacterium Isolated from Southern Algeria. Agronomy, 12(9), 2050. doi:10.3390/agronomy12092050.

[83] Kumar, M., & Prasad, V. (2025). Growth and functional evaluation of Enterobacter cloacae under salinity stress. Biocatalysis and Agricultural Biotechnology, 64, 103495. doi:10.1016/j.bcab.2025.103495.

[84] Rawat, P., Das, S., Shankhdhar, D., & Shankhdhar, S. C. (2021). Phosphate-Solubilizing Microorganisms: Mechanism and Their Role in Phosphate Solubilization and Uptake. Journal of Soil Science and Plant Nutrition, 21(1), 49–68. doi:10.1007/s42729-020-00342-7.

[85] Guan, N., & Liu, L. (2020). Microbial response to acid stress: mechanisms and applications. Applied Microbiology and Biotechnology, 104(1), 51–65. doi:10.1007/s00253-019-10226-1.

[86] Suriani, N. L., Suprapta, D. N., Suarsana, I. N., Reddy, M. S., Gunawan, S., Herlambang, S., Resiani, N. M. D., Pratiwi, E., Sabullah, M. K., Alfarraj, S., & Ansari, M. J. (2022). Piper caninum extract and Brevibacillus agri mixture suppresses rice leaf spot pathogen; Nigrospora oryzae and improves the production of red rice (Oryza sativa L). Frontiers in Sustainable Food Systems, 6. doi:10.3389/fsufs.2022.1080481.

[87] Setiawati, T. C., Erwin, D., Mandala, M., & Hidayatulah, A. (2022). Use of Bacillus as a Plant Growth-Promoting Rhizobacteria to Improve Phosphate and Potassium Availability in Acidic and Saline Soils. KnE Life Sciences, 7(3), 11160. doi:10.18502/kls.v7i3.11160.

[88] Smirnova, I., Sadanov, A., Baimakhanova, G., Faizulina, E., & Tatarkina, L. (2024). Phosphate-Solubilizing Salt-Tolerant Bacteria to Support Soybean Growth (Glycine max (L.) Merr.) On Saline Soils. OnLine Journal of Biological Sciences, 24(1), 9–18. doi:10.3844/ojbsci.2024.9.18.

[89] Wang, Z., Lajoie, G., Jiang, Y., Zhang, M., Chu, C., Chen, Y., Fang, S., Jin, G., Jiang, M., Lian, J., Li, Y., Liu, Y., Ma, K., Mi, X., Qiao, X., Wang, X., Wang, X., Xu, H., Ye, W., … Kembel, S. W. (2024). Host specificity of plant-associated bacteria is negatively associated with genome size and host abundance along a latitudinal gradient. Ecology Letters, 27(6), 14447. doi:10.1111/ele.14447.

[90] Gómez-Godínez, L. J., Aguirre-Noyola, J. L., Martínez-Romero, E., Arteaga-Garibay, R. I., Ireta-Moreno, J., & Ruvalcaba-Gómez, J. M. (2023). A Look at Plant-Growth-Promoting Bacteria. Plants, 12(8), 1668. doi:10.3390/plants12081668.