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IDENTIFICATION OF MICROORGANISMS FOUND IN THE ROOTS OF PLANTS AND IN THE RHIZOSPHERIC SOIL OF THE DRIED BED OF THE ARAL SEA

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Abstract: In the context of global climate change and increasing environmental degradation, extreme ecosystems such as the dried bottom of the Aral Sea have become important natural laboratories for studying plant–microbe interactions under severe abiotic stress conditions. These environments, characterized by high salinity, aridity, and complex geochemical factors, significantly influence the structure and function of rhizosphere microbial communities. However, the diversity and ecological role of cultivable microorganisms associated with halophytic plants in such environments remain insufficiently explored.

The **aim** of the study was to identify cultivable microorganisms associated with the roots and rhizosphere of halophytic plants, particularly Salsola richteri, growing in the Aralkum region, and to evaluate their ecological and biotechnological potential.

Methods. The study employed classical microbiological and analytical approaches, including soil and root sampling from the Aral Sea dried seabed, serial dilution and streak plate techniques for isolation of pure bacterial cultures, and morphological, physiological, and Gram staining analyses. Precise taxonomic identification of isolates was carried out using MALDI-TOF mass spectrometry. Additionally, salt tolerance assays were performed to assess the adaptive capacity of isolates under hypersaline conditions.

Results. A total of 13 bacterial isolates were obtained, including 40% Gram-positive and 60% Gram-negative bacteria, predominantly rod-shaped forms. Taxonomic analysis revealed that the isolates mainly belonged to the genera Bacillus, Enterobacter, Providencia, Pseudomonas, and Rhodococcus. Among them, Rhodococcus ruber and Enterobacter cancerogenus were the most frequently detected species. Several isolates demonstrated high salinity tolerance, with the ability to grow at NaCl concentrations up to 12–14%, indicating strong physiological adaptation to extreme environmental conditions.

The predominance of halotolerant and stress-adapted microorganisms in the rhizosphere suggests their active involvement in plant survival under harsh conditions. These microorganisms likely contribute to plant adaptation through mechanisms such as osmotic regulation, nutrient mobilization, and stimulation of plant growth.

Conclusion. The obtained results highlight the ecological significance of rhizosphere microbiota in the Aral Sea region and demonstrate their potential application in phytomelioration, soil restoration, and the development of biofertilizers. The identified strains represent promising candidates for future biotechnological applications aimed at improving plant resilience in saline and arid environments.

Keywords: Aral Sea, *Salsola richteri*, rhizosphere microorganisms, saline soil, extreme environment, MALDI-TOF MS, *Bacillus*, *Enterobacter*, *Rhodococcus*, phytomelioration.

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Hyper-extreme ecosystems, such as salt lakes and the areas formed as a result of their desiccation, are distributed worldwide; however, they differ in salinity levels and chemical composition, which are largely determined by local geological characteristics (de la Haba et al., 2011). In addition to their hypersaline nature, these ecosystems are often characterized by other environmental extremes, including high alkalinity, low oxygen availability, and intense ultraviolet (UV) radiation (Edwardson & Hollibaugh, 2018).

A prominent example of such environments is the Aral Sea and its desiccated seabed. The Aral Sea is an endorheic saline lake located between Kazakhstan in the north and Uzbekistan in the south. It began to shrink in the 1960s and had largely transformed into a desert by the 2010s (Wikipedia contributors, n.d.).

As a consequence of the Aral Sea's desiccation, sandy landscapes have formed within its former basin (Song et al., 2024), initiating processes of soil formation (Jabbarov et al., 2024). Currently, these newly formed areas cover approximately 5.2 million hectares, of which 3.2 million hectares are located within the territory of Uzbekistan.

The distribution of vegetation across the dried seabed of the Aral Sea varies by region—southern, eastern, western, northern, and central zones. In the southern region alone, 216 plant species have been identified (UzDaily, 2024), whereas in the western region, 27 plant species belonging to four genera have been recorded (Shomurodov et al., 2021).

Over the years, changes in the composition of vegetation in the dried bed of the Aral Sea have been observed. In particular, a significant transformation has occurred in mesophytic plant communities as a result of the decline in groundwater levels. In addition, the movement and redistribution of salts have led to the emergence of new plant species, while others have disappeared (Adilov et al., 2021). The primary factor influencing the gradual transformation of vegetation is soil salinization (Duan et al., 2024). Furthermore, as a result of desertification processes, approximately 68% of the dried Aral Sea bed has undergone degradation (Song et al., 2021).

In the northern part of the dried Aral Sea basin, the main processes of soil degradation include anthropogenic salinization, wind erosion, and soil alkalization (Issanova et al., 2022). Initially, the water of the Aral Sea was not highly saline; however, over time, it became increasingly saline, which contributed to severe soil salinization and the formation of solonchaks (Panin et al., 2024). At present, the dried bed of the Aral Sea is predominantly covered by halophytic and psammophytic plant species, whose projective cover changes over time (Issanova et al., 2023). Periodic climatic changes further influence the distribution patterns of vegetation (He et al., 2022). The intensification of soil salinity has a negative impact on plant growth and development (Micklin et al., 2020).

Among the plants tolerant to salinity and drought in the Aral Sea region, *Glycyrrhiza glabra* (licorice) is recognized as a species highly resistant to various ecological stresses (Jabborova et al., 2023). Another stress-tolerant plant found in different parts of the dried Aral Sea basin is *Chenopodium quinoa* (Reimov & Fayzieva, 2014).

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Figure 1. The plant *Salsola richteri*, which occurs on the dried bottom of the Aral Sea

The study of *Salicornia* L., which is widely distributed throughout the Aral Sea surroundings, and the isolation of bacteria from its rhizosphere for application to plants in other regions have demonstrated positive results under stress conditions (Zhang et al., 2019).

In addition, *Salsola richteri* (Fig. 1), *Haloxylon aphyllum*, species belonging to the genus *Calligonum*, and other plants are present in this region as dominant species characterized by high biomass production.

The analysis of the above-mentioned literature indicates that studying the plants distributed on the dried bottom of the Aral Sea, particularly *Salsola richteri*, as well as the microorganisms inhabiting its rhizosphere zone, is of significant scientific relevance. In particular, the composition of these microorganisms and their precise identification have not been sufficiently elucidated, making research in this area highly important from both ecological and biotechnological perspectives.

Materials and Methods

Soil and root samples were collected from the rhizosphere zone (at a depth of 25–30 cm) of selected plant species growing on the dried bottom of the Aral Sea. The sampling was conducted in November 2025. The map of the Aral Sea and the geographical location of the sampling sites are presented in Figure 2.

During the study period, meteorological and weather conditions remained stable. Throughout the observation period, sunny weather prevailed, with an average daytime air temperature of approximately 22 °C (Figure 2).

Soil and root samples were collected under aseptic conditions, placed into sterile plastic containers, and transported to the laboratory within 1–2 days.

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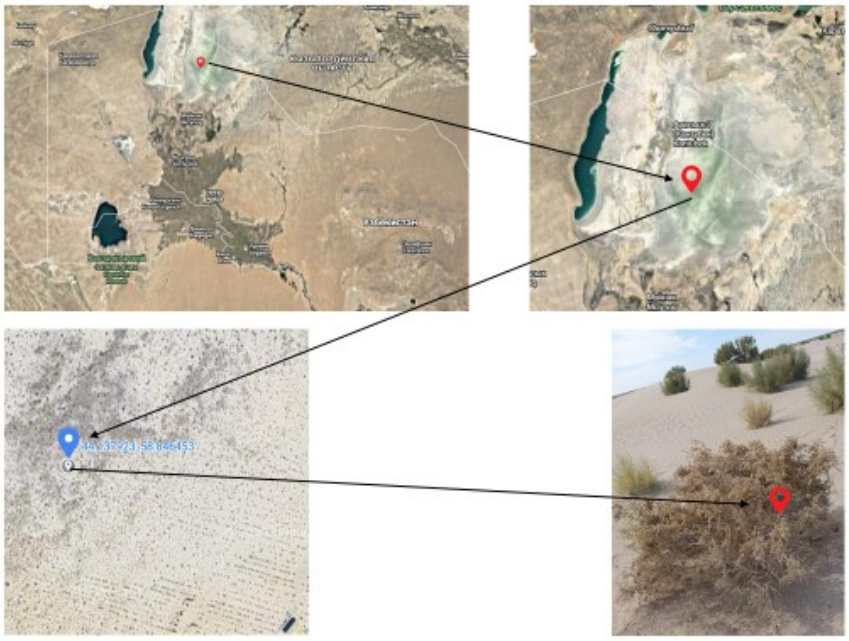


Figure 2. Sampling site of soil samples

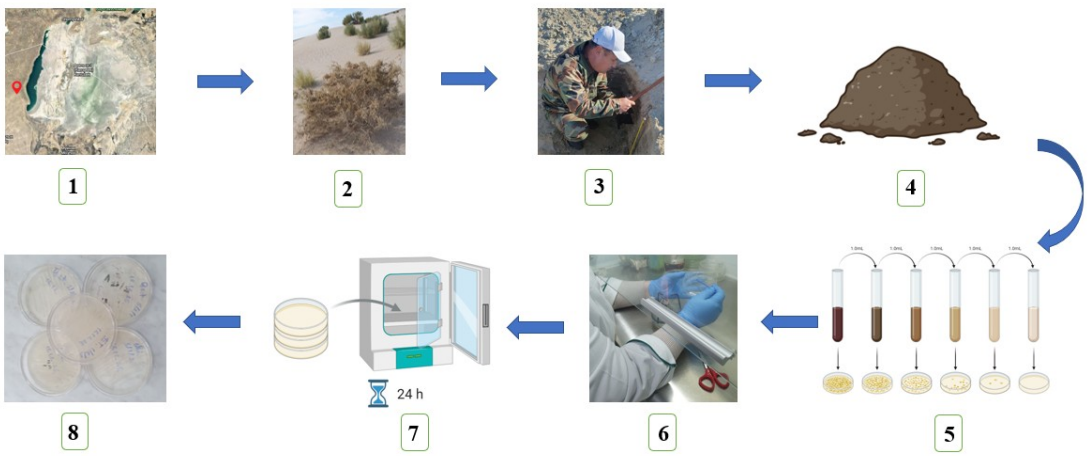
The map was created using Google Maps (https://www.google.com/maps)

A nutrient agar medium was selected for the isolation of bacteria. In this study, bacteria were isolated from soil samples using serial dilution and the streak plate method.

Nutrient agar is a general-purpose culture medium designed for the cultivation of microorganisms. It supports the growth of a wide range of non-fastidious organisms, including various bacteria and fungi. Due to its rich composition, nutrient agar provides essential nutrients required for microbial growth and is widely used in microbiological studies.

Serial dilution is a stepwise dilution technique used to reduce the concentration of a substance in a solution. To perform serial dilution, a small volume of a well-mixed sample is transferred into a new container, and an appropriate amount of diluent (such as sterile water or buffer solution) is added to achieve dilution. The resulting diluted sample serves as the basis for subsequent dilutions. When this process is repeated multiple times, a series of solutions with progressively decreasing concentrations is obtained.

The number of dilution steps and the dilution factor depend on the initial concentration and the desired range for analysis. Serial dilutions are commonly performed in tenfold (1:10) or hundredfold (1:100) increments. For example, a 1:10 dilution is prepared by mixing one part of the sample with nine parts of the diluent, whereas a 1:100 dilution involves mixing one part of the sample with ninety-nine parts of the diluent.



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Figure 3. Pure Culture Isolation Process: 1. Selection of the sampling site 2. *Salsola richteri* plant 3. Collection of soil samples 4. Transportation of soil samples to the laboratory 5. Serial dilution method 6. Inoculation (culturing) of microorganisms 7. 24-hour incubation 8. Obtaining a pure culture

The figures were created using BioRender (https://www.biorender.dev/).

For instance, mixing 100 µL of the original sample with 900 µL of water results in a 1:10 dilution. The final volume becomes 1000 µL (1 mL), and the concentration is reduced to one-tenth of the original sample. This is commonly referred to as a tenfold (10×) dilution.

The streak plate method is a widely used technique for isolating pure bacterial colonies from mixed cultures. In this study, a small amount of soil suspension was aseptically collected using a sterile inoculating loop. The inoculum was then streaked across the surface of a pre-prepared nutrient agar plate in a series of successive streaks, enabling gradual dilution of the sample.

The inoculating loop was sterilized between each streak to prevent contamination and ensure effective isolation. The plates were incubated at 37°C for 24–48 hours. Following incubation, well-isolated colonies were selected for subsequent morphological and biochemical analyses.

This method ensures the separation of individual bacterial cells and facilitates the growth of pure colonies suitable for further identification (Figure 3).

The cultivated bacteria were initially differentiated into Gram-positive and Gram-negative groups, and their cellular morphology and arrangement were examined using the Gram staining method developed by HC (1884). A bacterial smear was prepared on a clean glass slide and then gently heat-fixed using a spirit lamp. Subsequently, 2–3 drops of crystal violet were applied to the smear and allowed to stand for one minute. The excess stain was carefully rinsed off with tap water, and the slide was gently dried using absorbent paper.

Thereafter, Gram's iodine was applied for one minute, followed by brief washing. Decolorization was carried out using 96% ethanol for approximately 10 seconds. Next, several drops of safranin were added and left for one minute. The slide was then rinsed again with water to stop the decolorization process and allowed to air dry. Finally, the stained smear was observed and analyzed under a microscope.

The isolated microbial strains were identified using a MALDI-TOF Mass Spectrometry EXS 2600 (Zybio) system. During the analysis, a centrifuge, vortex mixer, pipettes, and a reagent kit (Matrix solution, Microorganism lysate I, and Microorganism lysate II) were utilized, along with distilled water and 96% ethanol.

For the identification procedure, pure and well-isolated colonies grown on nutrient media were selected. A volume of 300 µL of ultrapure water was added to 1.5 mL microcentrifuge tubes, followed by the addition of 1–5 mg of an individual colony. The resulting suspension was thoroughly homogenized using a vortex mixer until a uniform consistency was achieved. Subsequently, 900 µL of absolute ethanol was added to each tube, mixed again, and centrifuged at 12,000 rpm for 2–3 minutes.

After centrifugation, the supernatant was carefully discarded, and 20 µL of Microorganism lysate I reagent was added to the remaining pellet and mixed thoroughly. Then, 20 µL of Microorganism lysate II reagent was added, and the mixture was homogenized using a pipette. The obtained mixture was centrifuged again for 2 minutes.

The MALDI-TOF target plate was disinfected with 96% ethanol prior to sample application. For each sample, 1 µL of the supernatant was spotted onto the designated wells of the plate and allowed to air-dry at room temperature. After complete drying, 1 µL of Matrix solution was added on top of each spot and again allowed to dry.

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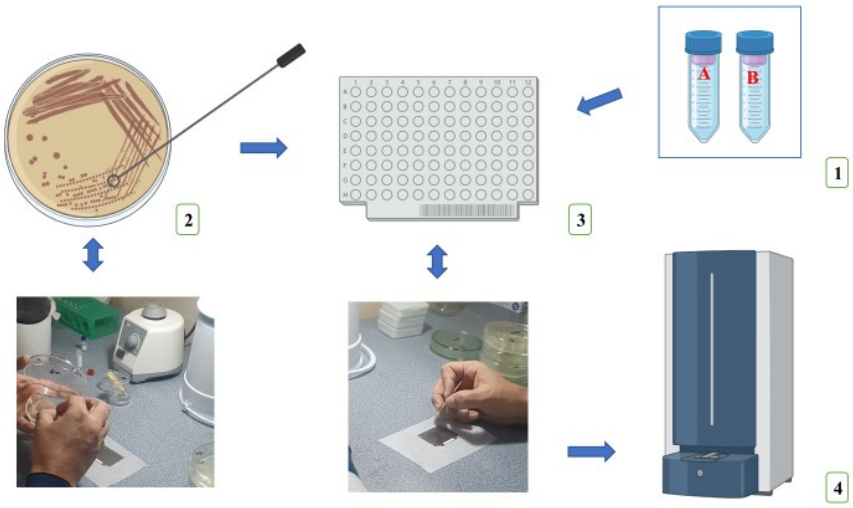


Figure 4. Identification process using a MALDI-TOF instrument: 1 – Treatment with reagents, 2 – Processing of samples obtained from pure cultures, 3 – Drying of the target plate, 4 – Placement into the MALDI-TOF device followed by microbial identification
The figures were created using BioRender (https://www.biorender.dev/).

Finally, the prepared target plate was inserted into the MALDI-TOF instrument, and microbial identification was performed (Figure 4).

Results

During the course of the study, a total of 13 bacterial isolates were obtained from soil samples collected from the rhizosphere of selected plants growing on the dried bottom of the Aral Sea (Figure 6).

The isolated colonies exhibited significant morphological diversity in terms of colony color, shape, size, texture, and growth characteristics under saline conditions. Such morphological variability among the isolates indicates a diverse microbial community adapted to the extreme environmental conditions of the dried Aral Sea region, particularly high salinity and low moisture content. The observed differences in salt tolerance among the isolates further suggest that these microorganisms possess different physiological adaptation mechanisms to saline environments.

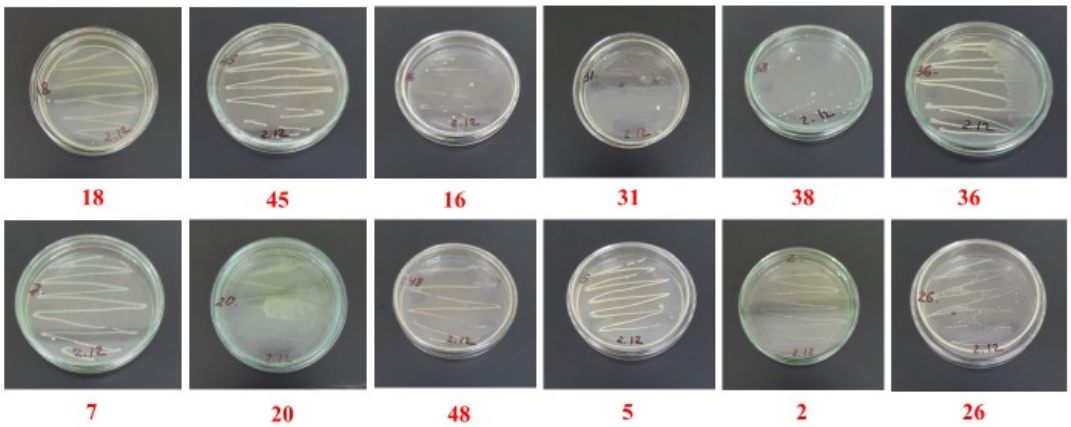


Figure 5. Isolates obtained from the roots of certain plants and the surrounding rhizosphere soil collected from the dried bottom of the Aral Sea

According to the Gram staining results, 6 (60.0%) of the isolated strains were identified as Gram-negative bacteria, while 7 (40.0%) were identified as Gram-positive bacteria. Microscopic examination revealed that the isolates were predominantly rod-shaped and partially coccoid in morphology (Table 1).

Table 1. Gram staining results of the isolates

Isolates obtained from the roots of selected plants and the surrounding rhizosphere soil from the dried bottom of the Aral Sea	2	Gram-negative
	5	Gram-positive
	7	Gram-negative
	26	Gram-negative
	45	Gram-positive
	7	Gram-negative
	16	Gram-positive
	18	Gram-negative
	20	Gram-positive
	31	Gram-positive
	36	Gram-positive
	38	Gram-positive
	48	Gram-negative

The identification of the bacterial isolates was performed using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). This method allows rapid and reliable identification of microorganisms based on protein spectral profiles. According to the obtained mass spectra and database matching results, the isolated microorganisms were assigned to the genera *Bacillus*, *Providencia*, *Pseudomonas*, *Rhodococcus*, and *Enterobacter*.

Among the identified bacteria, the most frequently detected species were *Rhodococcus ruber* and *Enterobacter cancerogenus*, indicating that these species are widely distributed in the rhizosphere and root-associated soil of plants growing in the dried Aral Sea region. The presence of representatives of the genera *Bacillus* and *Pseudomonas* is particularly important, as these microorganisms are known for their plant growth-promoting properties, stress tolerance, and ability to survive under saline and arid environmental conditions.

The identification results suggest that the microbial community associated with the roots of desert plants consists of ecologically adapted and physiologically active bacteria, which may play an important role in plant adaptation to extreme environmental conditions such as high salinity, drought, and nutrient deficiency (Table 2).

Table 2. Identification of the isolates by MALDI-TOF MS

2	Rhizosphere soil of *Salsola richteri*	***Providencia rettgeri***	Bacteria	2.42
5	Shuvoq tuproq	***Rhodococcus ruber***	Actinomycetes	2.12
7	Root of *Salsola richteri*	***Enterobacter cloacae***	Bacteria	2.25
26	Root of *Salsola richteri*	***Enterobacter cancerogenus***	Bacteria	2.40
45	Rhizosphere soil of *Salsola richteri*	***Bacillus cereus***	Bacteria	2.34
7	Root of *Salsola richteri*	***Enterobacter cloacae***	Bacteria	2.25
16	Rhizosphere soil of *Aristida pennata*	***Bacillus infantis***	Bacteria	2.38
18	Stem of *Lugaion aphyllum*	***Pseudomonas azotoformans***	Bacteria	2.12
20	Stem of *Lugaion aphyllum*	***Bacillus subtilis***	Bacteria	2.25
31	Rhizosphere soil of *Aristida pennata*	***Bacillus simplex***	Bacteria	2.13
36	Rhizosphere soil of *Aristida pennata*	***Bacillus atrophaeus***	Bacteria	2.17
38	Rhizosphere soil of *Aristida pennata*	***Bacillus firmus***	Bacteria	2.06
48	Rhizosphere soil of *Haloxylon aphyllum*	***Pseudomonas koreensis***	Bacteria	2.16

The salt tolerance of the isolated microorganisms was evaluated by culturing the strains on nutrient media supplemented with different NaCl concentrations (7%, 10%, 12%, and 14%). The results showed that the salt tolerance of the isolates varied significantly depending on the genus and species of microorganisms.

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Most of the isolates demonstrated growth at 7% NaCl, indicating their ability to tolerate moderately saline conditions. Among the tested strains, *Rhodococcus ruber* showed the highest salt tolerance, as it was able to grow at all tested NaCl concentrations (7–14%), indicating its high adaptability to saline environments. Similarly, *Providencia rettgeri* and *Enterobacter cloacae* showed growth up to 12% NaCl, but no growth was observed at 14% NaCl, suggesting that these strains belong to the group of moderate halophilic or halotolerant microorganisms.

Species of the genus *Bacillus* showed different levels of salt tolerance. In particular, *Bacillus subtilis*, *Bacillus firmus*, and *Bacillus cereus* were able to grow at 7–10% NaCl, while growth inhibition was observed at higher salt concentrations (12–14% NaCl). *Bacillus infantis* demonstrated limited salt tolerance and grew mainly at 7% NaCl, with weak or no growth at higher salt concentrations.

In contrast, isolates belonging to the genus *Pseudomonas*, particularly *Pseudomonas azotoformans* and *Pseudomonas koreensis*, showed low salt tolerance and did not grow at high NaCl concentrations, indicating that these bacteria are likely non-halophilic or weakly halotolerant microorganisms.

Based on the obtained results and according to the Kushner classification, most of the isolated strains can be classified as moderate halophiles or halotolerant microorganisms, as they were able to grow at NaCl concentrations between 7% and 10%, and some strains up to 12–14% NaCl. These results indicate that microorganisms isolated from the rhizosphere of plants growing in the dried Aral Sea region possess significant adaptive potential to saline soil conditions and may be promising for use in agricultural biotechnology under saline environments (Table 3).

Table 3. Salt tolerance of the isolates at different NaCl concentrations

T/r	Microorganisms	7%	10%	12%	14%
2	***Providencia rettgeri***	+	+	+	-
5	***Rhodococcus ruber***	+	+	+	+
7	***Enterobacter cloacae***	+	+	+	-
16	***Bacillus infantis***	+	+	-	-
18	***Pseudomonas azotoformans***	-	-	-	-
20	***Bacillus subtilis***	-	-	-	-
26	***Bacillus infantis***	+	-	-	-
31	***Pseudomonas azotoformans***	+	-	-	-
36	***Bacillus subtilis***	+	+	+	-
38	***Bacillus firmus***	+	+	-	-
45	***Bacillus cereus***	+	-	-	-
48	***Pseudomonas koreensis***	-	-	-	-

Discussion

The dried bottom of the Aral Sea (Aralkum), characterized by hypersaline and anthropogenically disturbed soils, represents an extreme ecological filter for microbial communities. In our study, the predominance of isolates belonging to the genera *Bacillus*, *Enterobacter*, and *Rhodococcus*, obtained from the rhizosphere of *Salsola richteri*, is directly associated with the unique geochemical and mineralogical properties of this environment.

As reported by Jiang et al. (2021), the concentration of total soluble salts (TSS) in soils increases with distance from the Aral Sea shoreline, resulting in a significant reduction in microbial diversity. The occurrence of the bacterial isolates identified in our study in association with evaporite minerals such as gypsum and calcite indicates that these microorganisms are adapted not only to high salinity but also to the mineralogical composition of the soil. In particular, the presence of representatives of the genus *Bacillus* in all samples confirms their ability to survive under adverse environmental conditions due to their endospore-forming capability, which allows long-term persistence in extreme habitats.

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The isolated strains, particularly *Rhodococcus ruber* and *Enterobacter cloacae*, demonstrated the ability to grow under high NaCl concentrations (12–14%). As explained by Kumawat (2022), such bacteria employ a “compatible solute” strategy, synthesizing organic osmolytes such as betaine, ectoine, or trehalose within the cell. This mechanism helps balance the osmotic pressure between the intracellular environment and the external medium, thereby preventing cellular dehydration and maintaining metabolic activity under saline stress conditions.

Halophytic plants such as *Salsola richteri* form specialized microbiomes in the rhizosphere that enable them to survive in highly saline soils. The bacteria identified in our study are capable of producing the enzyme ACC deaminase, which reduces stress-induced ethylene levels in plants and promotes root system development under saline conditions. Furthermore, the ability of *Enterobacter* and *Bacillus* species to solubilize phosphate and produce siderophores provides a scientific basis for their application in phytomelioration programs in nutrient-poor environments such as the Aral Sea region.

Although MALDI-TOF MS used in this study enabled rapid identification of culturable microorganisms, multi-omics approaches such as metagenomics and transcriptomics are required to fully elucidate the ecological roles of unculturable microorganisms in the Aralkum ecosystem. The integration of these advanced molecular approaches will significantly enhance the potential for biotechnological utilization of biological resources from the dried Aral Sea region in the future.

Conclusions

This study was devoted to investigating the composition of microorganisms inhabiting the root and rhizosphere zones of *Salsola richteri*, a halophytic plant growing under the extremely saline and arid conditions of the dried bottom of the Aral Sea, and to their precise identification using MALDI-TOF mass spectrometry. During the study, a total of five pure bacterial isolates were obtained using classical microbiological methods, and their morphological characteristics, Gram staining properties, and modern mass spectrometric analyses were carried out.

The results obtained demonstrated that the rhizosphere and root zone of *Salsola richteri* harbor a diverse community of microorganisms, among which bacteria belonging to the genera *Bacillus*, *Enterobacter*, and *Providencia*, as well as actinomycetes belonging to the genus *Rhodococcus*, were predominant. In particular, the high occurrence frequency of *Rhodococcus ruber* and *Enterobacter cancerogenus* indicates that these microorganisms are well adapted to saline and arid environmental conditions.

The identified microorganisms may play an important ecological role in increasing plant tolerance to extreme environmental conditions, maintaining rhizosphere stability, and reducing soil degradation processes. Furthermore, these isolates can be considered as promising biological resources for future applications in phytomelioration, development of microbial biopreparations, restoration of saline soils, and other biotechnological applications.

The results of this study provide an important scientific basis for the investigation of microbial resources in the Aral Sea region, identification of microorganisms associated with the rhizosphere of halophytic plants, and the development of sustainable ecological technologies based on these microorganisms.

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Conflict of Interest

The authors declare no conflict of interest.

Information

Artificial intelligence tools were used during the academic editing of this manuscript to improve language and clarity.

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