Original article

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IDENTIFICATION OF BACTERIAL ISOLATES FROM SOILS OF THE ARAL SEA REGION

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ABSTRACT

The Aral Sea region is an extreme arid-saline environment formed under long-term desiccation, salinization, and degradation of coastal and former seabed soils. The aim of this study was to perform the molecular genetics and taxonomic identification of culturable bacterial isolates recovered from soils of the Aral Sea region. Soil samples were collected from 9 sampling sites located in the coastal zone adjacent to the dried Aral seabed. A total of 42 bacterial isolates were obtained, characterized by colony morphology and microscopy, and identified by 16S rRNA gene sequencing followed by phylogenetic analysis in MEGA11. The isolates were assigned to 23 species belonging to 11 genera, including *Priestia, Bacillus, Planomicrobium, Paenibacillus, Streptomyces, Microbacterium, Sphingobium, Sphingomonas, Pseudomonas, Acinetobacter,* and *Acidovorax*. The species distribution was uneven, with the predominance of *Paenibacillus, Bacillus,* and *Pseudomonas*. Among the most frequent taxa were *Paenibacillus xylanexedens, Paenibacillus barcinonensis, Bacillus subtilis,* and *Pseudomonas putida*. Phylogenetic reconstruction based on 24 nucleotide sequences and 604 aligned positions confirmed clustering according to taxonomic affiliation. The obtained results demonstrate substantial taxonomic heterogeneity of culturable bacteria in Aral Sea soils and provide a basis for further studies of their ecological roles and biotechnological potential**.**

Keywords: soil, Aral Sea, salt tolerance, bacteria, microbiome.

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1. INTRODUCTION

The Aral Sea region represents one of the most pronounced zones of anthropogenically transformed arid ecosystems that have developed as a result of the decades-long desiccation of the Aral Sea. The reduction in the water body area, exposure of the former seabed, increased mineralization, accumulation of gypsum, halite, and other evaporite minerals, as well as the formation of salt-dust emissions, have led to profound changes in the physical, chemical, and biological characteristics of the region's soils [1]. For terrestrial ecosystems, this implies not only the intensification of desertification and secondary salinization processes, but also a radical restructuring of microbial communities, which are among the first to respond to changes in the water-salt regime, organic matter content, and the mineral composition of the substrate. The study of the microbiota of the Aral Sea region is therefore of not only fundamental but also applied importance, as it makes it possible to assess the adaptive potential of microorganisms under conditions of extreme environmental stress [2].

Recent studies have shown that, in the soils and coastal substrates of the Aral Sea region, a pronounced salinity gradient is directly associated with a decrease in microbial diversity and changes in community structure. For the western part of the former Aral Sea, it has been demonstrated that the content of total soluble salts increases from slightly saline areas to hypersaline zones, and that differences among microbial communities are positively correlated with the contents of gypsum and calcite [3]. At the same time, it is precisely the mineral composition and geochemical parameters, rather than the mere presence of plants that act as major factors structuring the soil microbiota. These data are particularly important for the interpretation of results obtained for culturable isolates, since the bacteria and fungi recovered are not random but are formed within a strongly selective environment determined by salinity, moisture deficiency, and the low content of available organic matter [4].

Under saline soil conditions, bacteria play a key role in maintaining biogeochemical cycles, mobilizing nutrients, stabilizing rhizosphere processes, and forming active plant–microbe associations. Salt-tolerant and halotolerant bacteria have been shown to mitigate the negative effects of salt stress on plants through phytohormone synthesis, phosphate solubilization, siderophore production, ACC deaminase activity, and participation in the regulation of the host antioxidant system [5]. Recent reviews emphasize that rhizospheric and endophytic bacteria adapted to salinity are regarded as an import-

ant resource for the restoration of degraded lands and the development of bioformulations for agriculture in arid and saline environments. Therefore, the search for and molecular genetic identification of bacterial isolates from soils of the Aral Sea region represent a promising area not only for describing biodiversity, but also for the subsequent selection of strains with biotechnological potential [6].

Of particular interest, the Aral Sea region is already regarded as a natural testing ground for the search for culturable bacteria associated with halophytes and salt-tolerant plants. For the western Aral Sea region, a high diversity of culturable endophytic bacteria isolated from halophytes has previously been reported, with identification performed on the basis of *16S* rRNA sequencing. The authors note that such bacteria are not only taxonomically diverse, but also exhibit pronounced antagonistic activity against phytopathogenic fungi [7]. Moreover, recent studies from the Aral Sea region have shown that plant-associated culturable bacteria can suppress the development of a broad spectrum of phytopathogens, which further increases interest in isolates from the Aral Sea region as potential biocontrol agents and components of future microbial consortia for saline soils [8].

In soils and plant-associated microbial communities of the Aral Sea region and adjacent saline territories, a wide range of bacteria typical of arid and salt-affected ecosystems has already been identified. Thus, studies of the dried bottom of the Aral Sea have shown that representatives of the genera *Pseudomonas* and *Bacillus* predominate in solonchak soils, whereas *Aeromonas hydrophila, Arthrobacter* spp., *Agrobacterium radiobacter*, and *Propionibacterium freudenreichii* also play an important role during the early stages of soil formation; overall, 59 bacterial species belonging to 43 genera were reconstructed in these substrates [9]. In contrast, endophytic communities of halophytes from the western Aral Sea region have been reported to contain bacteria belonging to the dominant phyla *Bacillota, Pseudomonadota, Actinomycetota,* and *Bacteroidota*, with particular attention given to strains of *Bacillus swezeyi*, which exhibit pronounced salt tolerance, enzymatic activity, and the potential to interact beneficially with the plant host [10]. Additional studies of the halophyte *Seidlitzia rosmarinus* from saline soils of Uzbekistan revealed the presence of representatives of the genera *Pantoea, Acinetobacter, Pseudomonas, Burkholderia, Kocuria,* and *Bacillus*, further confirming the high taxonomic diversity of bacteria associated with salt-tolerant vegetation in Central Asia. Thus, according to the available data, the bacterial component of the Aral Sea region and neighboring solonchak habitats includes both spore-forming and ecologically resilient members of the genus *Bacillus* and metabolically versatile Gram-negative bacteria such as *Pseudomonas, Acinetobacter, Pantoea,* and *Burkholderia*, as well as actinobacteria and other taxa potentially important for soil formation, organic matter mineralization, and plant adaptation to salt stress [11].

Thus, bacterial and fungal isolates from the Aral Sea region should be regarded as an important component of the microbial pool of extremely transformed soils formed under the influence of chronic salinization, aridization, salt-dust load, and the scarcity of organic matter [12]. Their study makes it possible to address several tasks simultaneously: to clarify the taxonomic structure of the culturable microbiota of the region, to identify microbial adaptation strategies to extreme environmental factors, to evaluate the potential involvement of isolates in rhizospheric and degradative processes, and to provide a basis for the further selection of strains with antagonistic, plant growth-promoting, enzymatic, and remediation-related activities [13]. In this context, the molecular genetic identification of bacterial and fungal isolates from soils of the Aral Sea region is a necessary stage for the subsequent ecological and biotechnological analysis of the local microbiota.

This study aimed to perform the molecular genetics and taxonomic identification of bacterial isolates obtained from soils of the Aral Sea region.

2. MATERIALS AND METHODS

2.1 Study material

Soil samples for the isolation of bacterial strains were collected aseptically from the coastal zone of the Aral Sea region at a depth of 0-10 cm after removal of surface plant residues. At each sampling site, a composite sample was prepared by combining several subsamples, placed into sterile containers, and transported to the laboratory under cooled conditions. For bacterial isolation, 1 g of homogenized soil was suspended in 9-10 mL of sterile physiological saline or sterile water, followed by the preparation of a series of tenfold dilution. Aliquots were then plated onto solid nutrient medium and incubated at 28-30 °C for 24-72 h, after which morphologically distinct colonies were subcultured to obtain pure isolates [14].

2.2 DNA extraction and molecular characterization

The genomic DNA of separate colonies of the microorganisms was isolated using the bacterial DNA isolation kit Biolabmix. The isolation was performed according to the kit instructions. The quality of genomic DNA was monitored by electrophoresis on a 1 % agarose gel. Electrophoresis was carried out in a Max Fill HU10 horizontal electrophoresis chamber and a Consort EV 243 current source. $1^{\times}$ TAE buffer was used as an electrode buffer. The 16S rRNA was amplified using the primer pair: forward *16SrRNA-8F* (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse *16SrRNA-806R* (5'-GGACTACCAGGGTATCTAAT-3') (Sigma-Aldrich, USA). For all used primers, we prepared 20 µlmixture that contained 25 ng of each target DNA. The mixture also contained Taq DNA Polymerase (Fermentas), 0.2 mM of each dNTP, $1^{\times}$ PCR buffer, 2.5 mM $MgCl_2$, and 10 pmol of each primer. The PCR program was run on a Master cycler Gradient, (Eppendorf) amplificator.

2.3 PCR samples purification

PCR samples were purified from oligonucleotide residues by dephosphorylation using alkaline phosphatase (SAP - shrimp alkaline phosphatase) and endonuclease. A mixture was prepared in a total volume of 10 µl for each sample - dH_2O - 7.25 µL, $10^{\times}$ PCR Buffer - 1.0 µl, $MgCl_2$ - 1.0 µl, SAP (5 mM) - 2.5 µl, Exonuclease I (5 units/µL) - 0.125 µl. The resulting mixture was added to each PCR product, placed in a thermal cycler under the following conditions: 37°C - 30 min, 85°C - 15 min, 4°C - ∞. Sample preparation for sequencing carried out by precipitation with an alcohol-acetate mixture.

2.4 DNA sequencing

The components of a standard set of reagents for the sequencing reaction were prepared in a 0.2-ml thin-walled ther-

mocycler tube. A standard set of reagents for cyclic sequencing using *CEQ WellRED* terminator dyes (partially mixed). The following thermal cycle program was chosen: 96°C - 20 sec, 50°C - 20 sec, 60°C - 4 min for 30 cycles and followed by aging at 4°C. The sequencing was done by using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), and the sequence was deposited in GenBank. These sequences were compared with other sequences in the GenBank by using the BLAST analysis. The phylogenetic analysis was carried out with MEGA 11 software.

2.5 Statistical analysis

To analyze the taxonomic structure of the isolated bacterial community, all recovered strains were classified according to their species-level identification obtained from nucleotide sequence analysis. The frequency of occurrence of each identified species was calculated as the number of isolates assigned to a given taxon. The summarized data were then visualized as a bar chart, allowing the species-level distribution of the bacterial isolates recovered from soils of the Aral Sea region to be evaluated.

3. RESULTS

To characterize the study area and the spatial distribution of the soil sampling sites, a schematic map of the research area was prepared. The map shows nine sampling sites located within the Aral Sea region, in the area adjacent to the dried seabed of the Aral Sea (Figure 1).

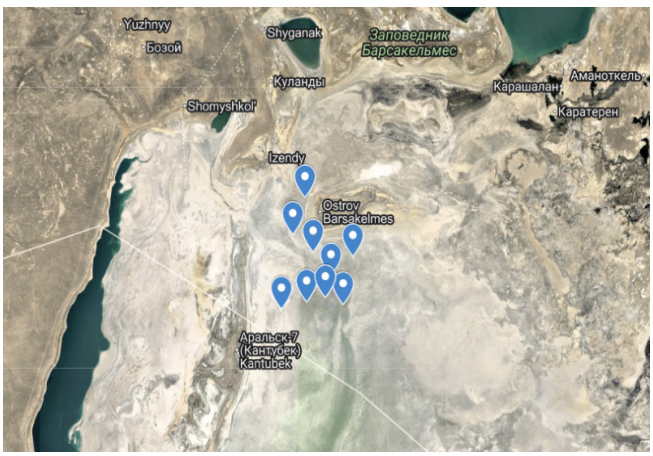

Figure 1 – Map of the soil sampling area in the Aral Sea region showing the locations of the nine sampling sites.

As shown in Figure 1, the soil sampling sites were located within the arid zone of the Aral Sea region, encompassing the area of the dried Aral seabed and adjacent territories. The spatial distribution of the nine sampling sites covered areas differing in salinity, moisture regime, and substrate characteristics, thereby ensuring the collection of representative samples for the subsequent isolation and molecular genetic identification of bacterial isolates. The selection of multiple sampling sites across the study area was intended to assess the taxonomic diversity of culturable microorganisms in the ecologically heterogeneous soils of the Aral Sea region. The soil samples collected from the nine sampling sites served as the source for the isolation of bacterial strains. Subsequent examination of the obtained cultures revealed differences in their cultural and morphological (Figure 2 and 3) characteristics.

Figure 2 illustrates differences in the growth and colony morphology of bacterial isolates recovered from soil samples collected in the Aral Sea region when cultivated on solid nutrient medium. The isolates exhibited considerable variation in cultural characteristics, including colony size, shape, pigmentation, surface texture, margin structure, and degree of elevation. The observed differences indicate the morphological heterogeneity of the isolated bacterial community and further support the taxonomic diversity of the microorganisms.

For a more detailed characterization of the isolated strains, their microscopic features were examined in addition to their cultural characteristics. The results of the microscopic analysis of the bacterial cells are presented in Figure 3.

Bacillus spp. Cells are rod-shaped, Gram-positive, and occur singly, in pairs, or in short chains. Members of this genus are characterized by endospore formation, and the cells are generally larger than those of most other bacterial genera.

Paenibacillus spp. Cells are rod-shaped, Gram-positive or Gram-variable, and occur singly or in short chains. Members of this genus are also capable of forming endospores, and the cells are often elongated.

Pseudomonas spp. Cells are straight or slightly curved rods, Gram-negative, and usually occur singly. They do not form spores; many representatives of the genus are characterized by small cell size and an even distribution in the smear.

Microbacterium spp. Cells are small, Gram-positive, rod-shaped or coryneform, often short and sometimes slightly curved. In smears, they may occur singly, in pairs, or in small aggregates.

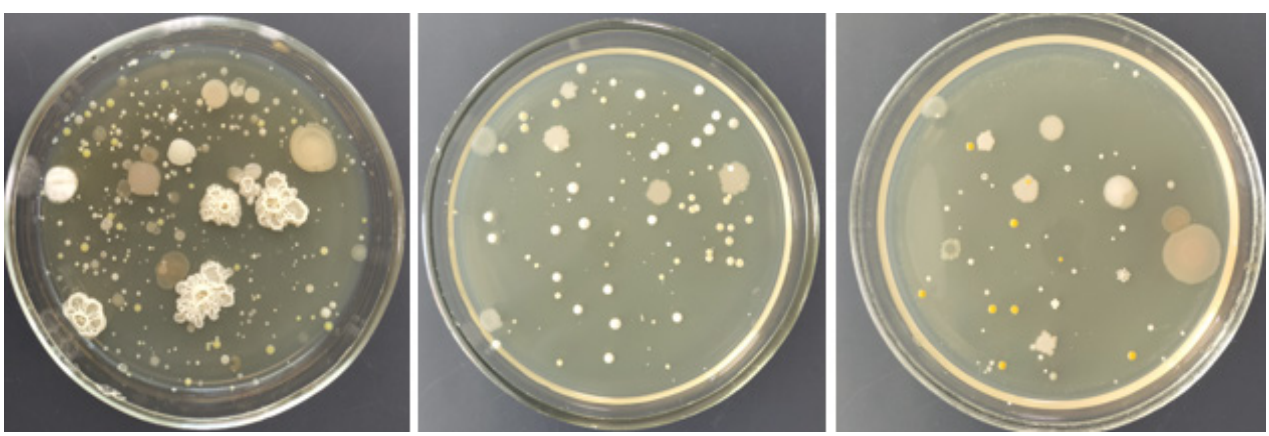

Figure 2 – Colony morphology of bacterial isolates grown on nutrient medium.

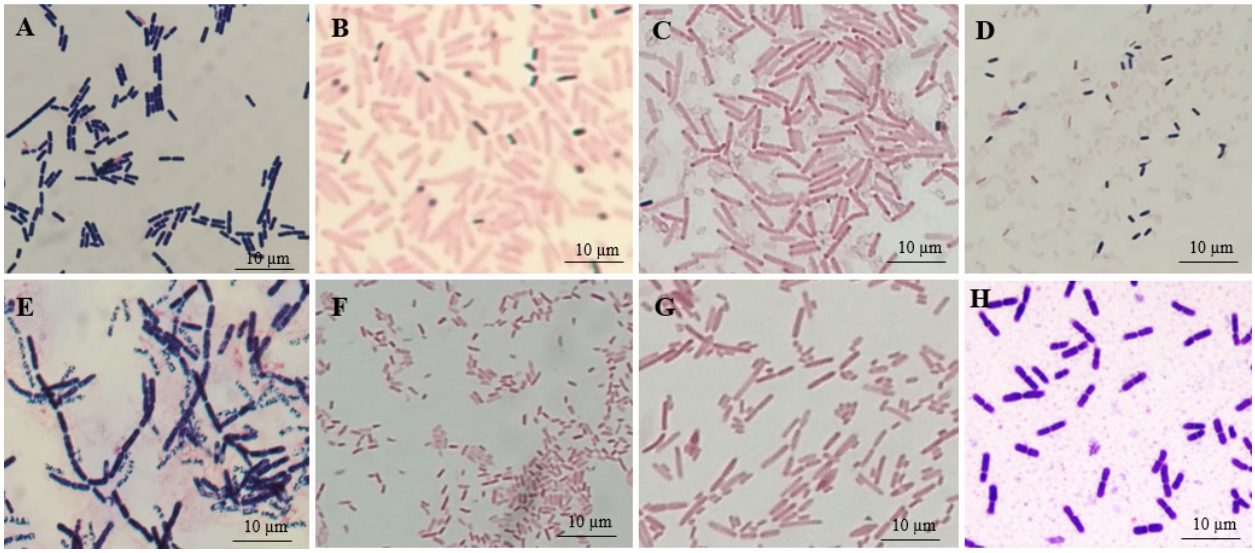

Figure 3 – Microscopic characteristics of the isolated bacterial strains, bar ×100: A – *Bacillus* spp.; B – *Paenibacillus* spp.; C – *Pseudomonas* spp.; D – *Microbacterium* spp.; E – *Streptomyces* spp.; F – *Planomicrobium* spp.; G – *Sphingobium* spp.; H – *Priestia* spp.

Streptomyces spp. Cells are represented by branched filamentous structures forming a mycelium. Members of this genus are characterized by thin hypha-like elements and fragmentation of filaments with the formation of spore chains.

Planomicrobium spp. Cells are coccoid, short rod-shaped, or oval, Gram-positive, and occur singly, in pairs, or in small groups. Members of this genus are characterized by small cell size and a compact morphology.

Sphingobium spp. Cells are small Gram-negative rods, usually straight or slightly curved, and predominantly occur singly. They do not form spores; in smears, they appear as short, slender cells.

Priestia spp. Cells are rod-shaped, Gram-positive, and occur singly or in short chains. Members of this genus are capable of forming endospores and are microscopically similar to species previously assigned to the genus *Bacillus*.

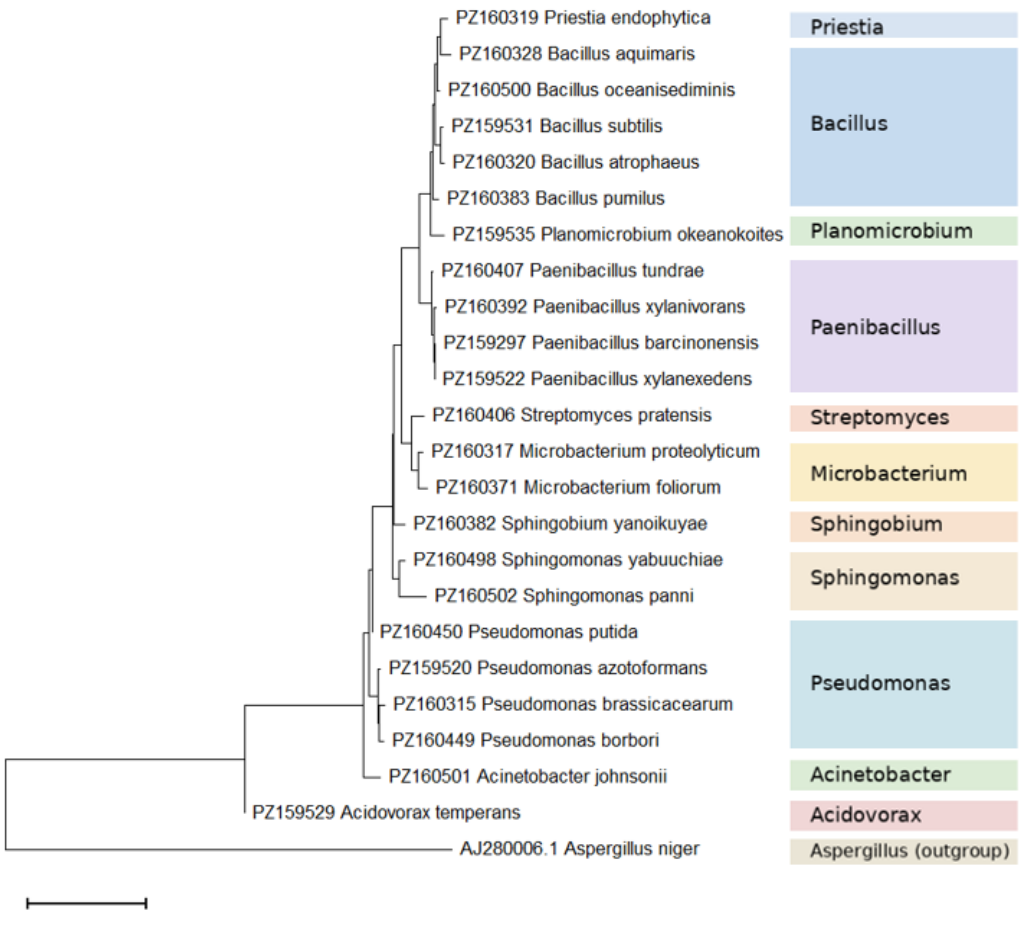

Figure 4 – Neighbor-Joining phylogenetic tree of bacterial isolates recovered from soils of the Aral Sea region.

Although the cultural, morphological, and microscopic characteristics of the isolates provided important preliminary information, reliable species-level identification required molecular genetic confirmation. Therefore, the selected isolates were subjected to molecular genetic analysis, and the obtained sequence data were used to construct a phylogenetic tree, which allowed the taxonomic position of the studied strains to be clarified (Figure 4).

The constructed phylogenetic tree showed that the studied isolates formed distinct clades corresponding to their taxonomic affiliation. The isolates were grouped according to the genera *Priestia, Bacillus, Planomicrobium, Paenibacillus, Streptomyces, Microbacterium, Sphingobium, Sphingomonas, Pseudomonas, Acinetobacter,* and *Acidovorax*. The obtained tree topology confirmed the results of the molecular genetic identification and demonstrated the phylogenetic differentiation of the analyzed isolates at both the genus and species levels. *Aspergillus niger* was used as an outgroup. The evolutionary history was inferred using the Neighbor-Joining method [15]. The tree is drawn to scale, with branch lengths corresponding to the evolutionary distances used to infer the phylogenetic relationships. Evolutionary distances were computed using the Maximum Composite Likelihood method [16] and are expressed as the number of base substitutions per site. This analysis involved 24 nucleotide sequences. Codon positions included were 1st, 2nd, 3rd, and non-coding positions. All ambiguous positions were removed for each sequence pair using the pairwise deletion option. A total of 604 positions were retained in the final dataset. Evolutionary analyses were conducted in MEGA11 [17].

To assess the taxonomic structure of the isolated bacterial community, the distribution of the obtained isolates by identified species was analyzed. The results of this analysis are presented in Figure 5.

As shown in Figure 5, the bacterial isolates recovered from soils of the Aral Sea region were assigned to 23 species. The distribution of isolates by species was uneven, with the predominance of representatives of the genera *Paenibacillus*, *Bacillus*, and *Pseudomonas*. Among the most frequently identified taxa were *Paenibacillus xylanexedens*, *Paenibacillus barcinonensis*, *Bacillus subtilis*, and *Pseudomonas putida*, indicating that these bacteria constitute a substantial part of the culturable microbial community of the studied soils. In contrast, several other species, including *Planomicrobium okeanokoites*, *Priestia endophytica*, *Acinetobacter johnsonii*, *Acidovorax temperans*, and *Streptomyces pratensis*, were represented by a smaller number of isolates. Overall, the graph demonstrates the taxonomic heterogeneity of the isolated bacterial community and reflects the species-level diversity of culturable bacteria in soils of the Aral Sea region.

4. DISCUSSION

The present study demonstrated that culturable bacterial communities recovered from soils of the Aral Sea region are taxonomically heterogeneous and include at least 23 species belonging to 11 genera, with the clearest predominance of *Paenibacillus*, *Bacillus*, and *Pseudomonas*. In addition to these dominant groups, the isolate collection contained repre-

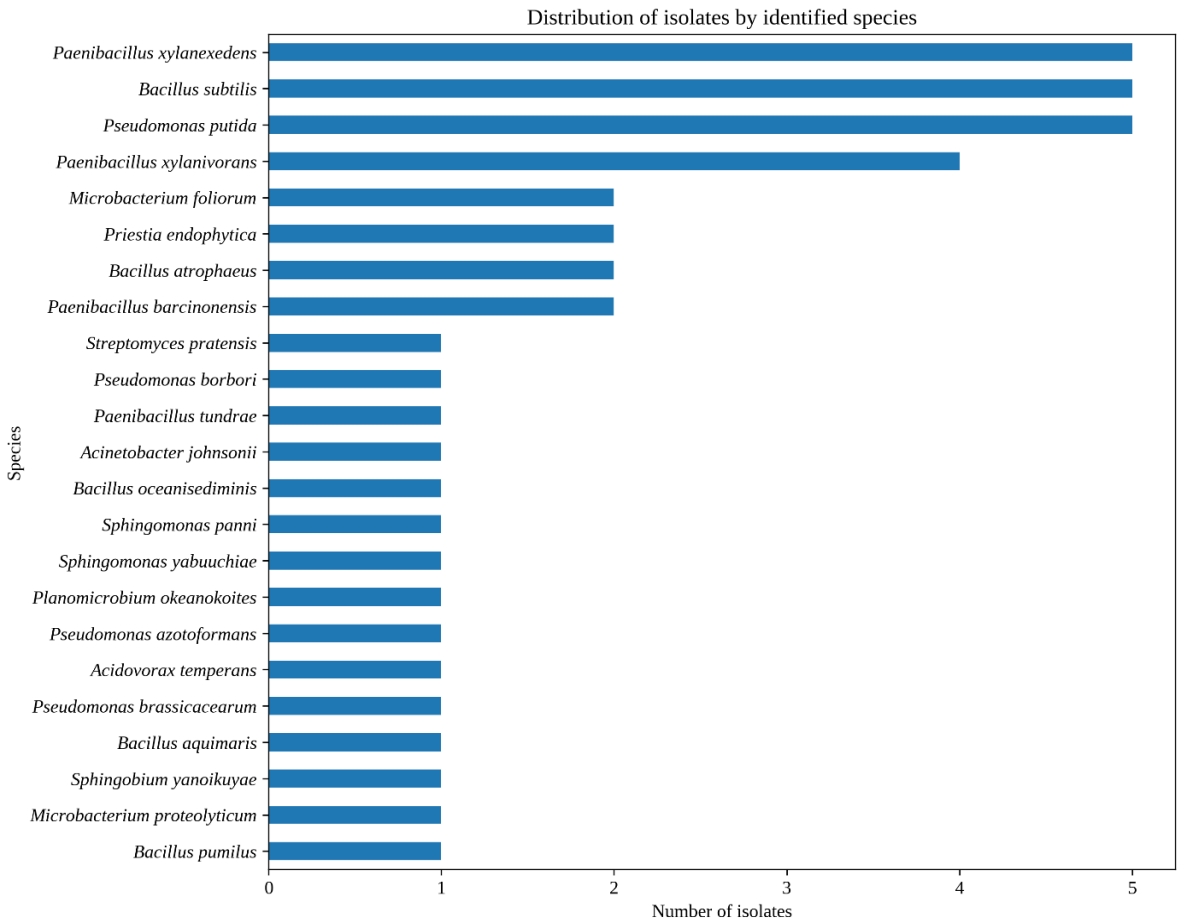

Figure 5 – Distribution of bacterial isolates recovered from soils of the Aral Sea region by identified species.

sentatives of *Priestia, Planomicrobium, Streptomyces, Microbacterium, Sphingobium, Sphingomonas, Acinetobacter,* and *Acidovorax*. The uneven distribution of taxa and the dominance of a relatively small number of genera indicate that the culturable fraction of the microbiota is shaped by strong environmental filtering, most likely associated with salinity, aridity, and substrate heterogeneity across the sampled coastal soils [18]. Contemporary reviews of saline soils likewise show that salinization generally reduces overall microbial diversity while enriching for stress-tolerant taxa capable of persisting under osmotic stress, ion toxicity, and nutrient limitation [19].

A similar molecular study has previously been conducted in Kazakhstan on the epipelagic microbiome of the Small Aral Sea. In that work, metagenomic analysis showed that the identified sequences were dominated by the large bacterial groups *Terrabacteria* and *Actinobacteria*, accounting for approximately 40% and 37% of the total reads, respectively. In addition, representatives of *Proteobacteria, Bacteroidetes, Patescibacteria* were detected, whereas the presence of *Deinococcus–Thermus, Armatimonadetes,* and *Chloroflexi* was considered less expected for the epipelagic zone. The authors also reported signatures of strict anaerobes, including *Ignavibacteria*, hydrogen-oxidizing bacteria, and methanogenic archaea, which were interpreted as evidence of intensive mixing of water masses originating from different ecological niches of the Aral–Syrdarya basin. At the genus level, the Small Aral Sea microbiome was especially enriched in *Streptomyces, Pseudomonas, Mycolicibacterium, Actinobacterium, Bacillus, Paenibacillus, Acinetobacter, Rhodococcus, Vibrio, Burkholderia, Prevotella, Corynebacterium,* and *Bacteroidales*, as well as representatives of *Candidatus Pelagibacter*. These taxa were interpreted as reflecting the ecological complexity and heterogeneity of the Small Aral Sea microbiome. Importantly, several genera revealed in that Kazakhstan-based aquatic metagenomic study, including *Bacillus, Paenibacillus, Pseudomonas, Streptomyces,* and *Acinetobacter*, are consistent with the results of our culture-dependent analysis of soils from the Aral Sea region, indicating that these bacterial groups may represent stable and ecologically significant components of the regional microbiota across different habitats [20].

A notable feature of the recovered collection is the prominence of endospore-forming and stress-resistant *Bacillota*, especially *Bacillus*, *Paenibacillus*, and *Priestia*. This pattern is ecologically plausible for the Aral Sea region, where repeated desiccation, salt accumulation, and temperature fluctuations are expected to favor bacteria capable of dormancy, persistence, and rapid recolonization after episodic moisture inputs. Similar patterns have been reported from other saline and desert environments, where culturable communities are often enriched in *Bacillus*-like taxa and other robust heterotrophs [21, 22]. The occurrence of *Bacillus subtilis, B. pumilus, B. atrophaeus,* and *B. aquimaris* in the present material is particularly noteworthy, because members of these groups are widely recognized as environmentally resilient bacteria with potential roles in nutrient turnover, rhizosphere competence, and stress mitigation. Salt-tolerant *Bacillus* strains, including *B. atrophaeus*, have also been shown to improve plant performance in saline soils, which supports the ecological and applied relevance of the taxa recovered here [23].

The predominance of *Paenibacillus* is also biologically meaningful. In this study, *Paenibacillus xylanexedens, P. barcinonensis,* and *P. xylanivorans* were among the most frequent taxa, indicating that this genus forms a substantial part of the culturable bacterial pool in the investigated soils. Members of *Paenibacillus* are frequently associated with organic matter decomposition, polysaccharide turnover, rhizosphere colonization, and plant growth promotion in stressful soils. Their repeated isolation from the Aral Sea samples may therefore reflect both ecological fitness under saline-arid conditions and an ability to exploit heterogeneous carbon resources in degraded soils. This interpretation is consistent with recent work showing that *Paenibacillus* strains can remain functionally relevant in stress-prone soils and may contribute to crop performance under adverse edaphic conditions [24, 25].

The recovery of several *Pseudomonas* species, especially *Pseudomonas putida, P. azotoformans,* and *P. brassicacearum*, further strengthens the conclusion that the studied soils harbor bacteria with broad ecological plasticity. *Pseudomonas* species are metabolically versatile, often efficient colonizers of nutrient-poor substrates, and commonly linked to plant-associated and rhizosphere functions in drylands and saline soils. In the present study, *P. putida* was one of the most frequently identified taxa, suggesting that it is well adapted to the environmental mosaic of the Aral Sea coastal zone. Experimental studies from saline and desert-related systems have shown that *Pseudomonas* can contribute to stress alleviation, including salinity mitigation and modulation of plant physiological responses, which makes the occurrence of this genus in the present isolate collection especially relevant from an applied standpoint [26, 27].

Although less abundant, the additional genera recovered in this study considerably expand the ecological interpretation of the results. The presence of *Streptomyces* indicates that filamentous *Actinomycetota* also contribute to the culturable microbiota of Aral Sea soils. This is important because halotolerant *Streptomyces* are increasingly recognized as functionally important soil bacteria in stressed environments, with reported roles in plant growth promotion, antagonism against phytopathogens, and adaptation to saline-alkaline conditions [28, 29]. Likewise, the detection of *Microbacterium* suggests the presence of small Gram-positive heterotrophs that are often associated with oligotrophic survival and plant-associated habitats. The occurrence of *Sphingobium* and *Sphingomonas* is also noteworthy, as these Alphaproteobacteria are often linked to environmental resilience, biodegradation capacity, and plant-interactive functions in stressed soils [30, 31].

The phylogenetic results are consistent with the taxonomic interpretation based on sequence similarity. The Neighbor-Joining tree resolved the isolates into distinct clades corresponding to *Priestia, Bacillus, Planomicrobium, Paenibacillus, Streptomyces, Microbacterium, Sphingobium, Sphingomonas, Pseudomonas, Acinetobacter,* and *Acidovorax*, supporting the robustness of the genus-level assignments. The placement of *Priestia* as a separate lineage is particularly relevant in light of the recent taxonomic revision of the Bacillaceae, in which several former *Bacillus* lineages were reassigned to newly delimited genera, including *Priestia*. Thus, the separation of *Priestia endophytica* from the main *Bacil-*

lus clade in the present tree is taxonomically coherent rather than incidental [32].

At the same time, the sequence dataset suggests that not all assignments are equally secure at the species level. While many isolates showed 99.7-100% similarity to reference sequences, several records had noticeably lower similarity values, including *Acidovoraxtemperans* at 84.25%, *Sphingobium yanoikuyae* at 92.54%, *Bacillus aquimaris* at 97.84%, *Paenibacillus xylanivorans* at 97.76% in one isolate, and *Planomicrobium okeanokoites* at 98.60%. In practical terms, this means that the genus-level placement is likely reliable for most of these isolates, whereas some species-level assignments should be treated as provisional. Such borderline or low-similarity matches are not unusual in saline and desert systems and may reflect insufficient representation of closely related taxa in reference databases, microdiversity within named species, or the presence of locally differentiated lineages. Similar issues have been noted in studies of culturable bacteria from saline desert habitats, where 16S rRNA-based identification often resolves the dominant genera well but may be less definitive for species delimitation in unusual or understudied taxa [33].

Another important point is that the present results describe the culturable fraction of the bacterial community rather than the total microbiome. This distinction is methodologically important, because culture-based approaches favor organisms able to grow on the selected nutrient medium under laboratory conditions and therefore enrich for metabolically flexible and fast-growing taxa. The dominance of *Bacillus, Paenibacillus,* and *Pseudomonas* in the present study is therefore informative, but it should not be interpreted as a complete representation of the natural community. Instead, the recovered collection should be viewed as a biologically relevant subset of the local microbiota that is both viable under standard cultivation conditions and potentially useful for downstream functional screening. Comparative studies have repeatedly shown that cultured and total communities overlap only partially, but the culturable fraction remains particularly valuable when the aim is strain isolation, physiological testing, or biotechnological application [34].

Overall, the findings indicate that soils of the Aral Sea region harbor a culturable bacterial assemblage dominated by ecologically resilient taxa well suited to saline-arid conditions. The coexistence of endospore-formers, metabolically versatile Gram-negative rods, filamentous actinobacteria, and less common Alphaproteobacteria suggests that the local bacterial pool combines persistence strategies with functional breadth. From an applied perspective, this is encouraging because such communities may contain strains useful for salt-stress mitigation, rhizosphere support, or environmental biotechnology. The present work therefore provides not only a taxonomic inventory of culturable bacteria from the Aral Sea region, but also a rational starting point for subsequent screening of salt tolerance, plant growth-promoting traits, enzymatic activity, and biocontrol potential [35].

5. CONCLUSION

In conclusion, the soils of the Aral Sea region contain a taxonomically diverse collection of culturable bacteria, including 23 identified species from 11 genera, with *Paenibacillus*, *Bacillus*, and *Pseudomonas* as the dominant groups. Morphological, microscopic, and molecular genetic analyses revealed mutual consistency, while phylogenetic reconstruction confirmed the clustering of the studied isolates at the genus level and substantiated their taxonomic position.

The predominance of spore-forming bacilli and metabolically versatile Gram-negative bacteria indicates that the culturable community is strongly influenced by environmental selection related to salinity, aridity, and substrate heterogeneity. At the same time, the discovery of less common genera, such as *Streptomyces*, *Microbacterium*, *Sphingobium*, *Sphingomonas*, *Acinetobacter*, and *Acidovorax*, expands the known spectrum of viable bacteria inhabiting Aral Sea soils and indicates functional heterogeneity in the local microbial pool.

Some isolates showed very high similarity to reference sequences, while others demonstrated lower similarity values and should be considered as requiring further taxonomic detail. Therefore, this study provides a solid foundation for the characterization of cultivable bacteria from the Aral Sea region. Such subsequent analyses will be necessary to identify strains with the greatest potential for use in saline soil remediation, plant growth promotion, and other biotechnological applications.

AUTHORS' CONTRIBUTIONS

Conceptualization, V.K., A.S.; methodology, A.S. and R.U.; validation, A.S., V.K., R.U.; formal analysis, V.K., R.U., and A.S.; investigation A.S, R.U..; resources, V.K.; data curation, A.S.; writing–original draft preparation, A.S., R.U., V.K.; writing-review and editing, V.K. and R.U.; visualization, A.S. R.U.; project administration, A.S., V.K.; funding acquisition, V.K. All authors have read and agreed to the publication of the final version of the manuscript.

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FUNDING

Not applicable.

CONFLICT OF INTEREST

There are no conflicts of interest to declare.

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ИДЕНТИФИКАЦИЯ БАКТЕРИАЛЬНЫХ ИЗОЛЯТОВ ИЗ ПОЧВ РЕГИОНА АРАЛЬСКОГО МОРЯ

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АННОТАЦИЯ

Регион Аральского моря представляет собой экстремальную засушливо-соленую среду, сформировавшуюся в результате длительного высыхания, засоления и деградации прибрежных и бывших донных почв. Целью данного исследования была молекулярно-генетическая и таксономическая идентификация культивируемых бактериальных изолятов, выделенных из почв региона Аральского моря. Образцы почвы были собраны с 9 участков отбора проб, расположенных в прибрежной зоне, прилегающей к высохшему дну Аральского моря. В общей сложности было получено 42 бактериальных изолята, охарактеризованных по морфологии колоний и микроскопии, и идентифицированных с помощью секвенирования гена 16S рРНК с последующим филогенетическим анализом в MEGA11. Изоляты были отнесены к 23 видам, принадлежащим к 11 родам, включая *Priestia, Bacillus, Planomicrobium, Paenibacillus, Streptomyces, Microbacterium, Sphingobium, Sphingomonas, Pseudomonas, Acinetobacter* и *Acidovorax.* Распределение видов было неравномерным, с преобладанием *Paenibacillus, Bacillus* и *Pseudomonas.* Среди наиболее часто встречающихся таксонов были *Paenibacillus xylanexedens, Paenibacillus barcinonensis, Bacillus subtilis* и *Pseudomonas putida*. Филогенетическая реконструкция, основанная на 24 нуклеотидных последовательностях и 604 выровненных позициях, подтвердила кластеризацию в соответствии с таксономической принадлежностью. Полученные результаты демонстрируют существенную таксономическую гетерогенность культивируемых бактерий в почвах Аральского моря и служат основой для дальнейших исследований их экологической роли и биотехнологического потенциала.

Ключевые слова: почва, Аральское море, солеустойчивость, бактерии, микробиом.

АРАЛ ТЕҢІЗІ АЙМАҚ ТОПЫРАҚТАРЫНАН БАКТЕРИЯЛЫҚ ИЗОЛЯТТАРДЫ АНЫҚТАУ

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АННОТАЦИЯ

Арал теңізі аймағы – жағалаудағы және бұрынғы теңіз түбіндегі топырақтардың ұзақ мерзімді кептірілуі, тұздануы және деградациясы кезінде қалыптасқан өте құрғақ-тұзды орта. Бұл зерттеудің мақсаты Арал теңізі аймағының топырақтарынан алынған өсіруге жарамды бактериялық изоляттарды молекулалық-генетикалық және таксономиялық

анықтау болды. Топырақ үлгілері кептірілген Арал теңізінің түбіне жақын жағалау аймағында орналасқан 9 сынама алу орнынан алынды. Колония морфологиясы мен микроскопиясы арқылы сипатталған және 16S рРНҚ гендік секвенирлеу арқылы анықталған, содан кейін MEGA11-де филогенетикалық талдау жүргізілген барлығы 42 бактериялық изолят алынды. Изоляттар *Priestia*, *Bacillus*, *Planomicrobium*, *Paenibacillus*, *Streptomyces*, *Microbacterium*, *Sphingobium*, *Sphingomonas*, *Pseudomonas*, *Acinetobacter* және *Acidovorax* сияқты 11 туысқа жататын 23 түрге жатқызылды. Түрлердің таралуы біркелкі болмады, *Paenibacillus*, *Bacillus* және *Pseudomonas* басым болды. Ең жиі кездесетін таксондардың қатарында *Paenibacillus xylanexedens*, *Paenibacillus barcinonensis*, *Bacillus subtilis* және *Pseudomonas putida* болды. 24 нуклеотидтік тізбекке және 604 тураланған позицияға негізделген филогенетикалық реконструкция таксономиялық сәйкестікке сәйкес кластерленуді растады. Алынған нәтижелер Арал теңізі топырақтарындағы өсірілетін бактериялардың айтарлықтай таксономиялық гетерогенділігін көрсетеді және олардың экологиялық рөлі мен биотехнологиялық әлеуетін одан әрі зерттеуге негіз болады.

Кілт сөздер: топырақ, Арал теңізі, тұзға төзімділік, бактериялар, микробиом.