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Identification of Microorganicms Isolated from Saline Soils and Their Agrobiological Significance

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Abstract **Background/Aim:** The Aral Sea region faces extreme soil salinity due to environmental degradation, necessitating sustainable biological approaches. This study aimed to isolate salt-tolerant microorganisms, identify them, and evaluate their antifungal activity and agrobiological potential. **Materials and Methods:** Soil samples were collected in October 2024 from three depths (0–30 cm, 30–60 cm, 60–90 cm) in Uchsoy, Muynak district, Karakalpakstan. Microorganisms were isolated using serial dilution and cultured on various media (NA, Ashby, Chapek, Pikovskaya, Gauze, starch agar) **Figure 1**. Identification was performed via MALDI-TOF MS (Zybio EXS2600). Antifungal activity was tested using dual culture against Fusarium oxysporum, F. solani, and Alternaria spp. Soil pH, salinity, organic matter, and ion content were also analyzed. **Results:** Twenty-five strains were isolated; twelve were identified as Pseudomonas chlororaphis, Bacillus spp., Brevundimonas aurantiaca, Stenotrophomonas, Mucosa, and Aspergillus. Several strains tolerated up to 10% NaCl and showed strong antifungal effects, especially Pseudomonas and Bacillus species. Salinity and alkalinity influenced microbial diversity and activity. **Conclusion:** Native salt-resistant microorganisms possess significant antifungal potential and stress tolerance, making them suitable as biofertilizers and biocontrol agents for restoring saline-affected ecosystems in the Aral Sea region.

Keywords Saline soil, Microorganicms, Maldi-tof, Agrobiological

1. Introduction

Currently, active research focuses on preserving soil fertility in highly saline, marshy, and sandy desert soils and on increasing biological productivity. Leading scientific centers and universities worldwide to work on developing short-rotation crop rotation systems, introducing biological preparations, cultivating various crops, and improving soil fertility. For example, a study by Zhang et al demonstrated that crop rotation enhances microbial diversity and promotes soil ecosystem multifunctionality. Several studies are also being conducted in our country. Specifically, efforts are focused on improving soil fertility in the dried Aral Sea basin, enhancing the ecological balance of agroecosystems, expanding the area of crops adapted to the soil and climate conditions of the desiccated Aral seabed, increasing land use efficiency, and reducing salt content in soils [1]. All of these tasks require the implementation of numerous practical measures aimed at mitigating the consequences of the Aral disaster, preserving the natural environment—including flora and fauna—for future generations, restoring vegetation in the Aral Sea basin, and creating protective forest plantations within the “Green Belt” initiative. "In the area around the Aral Sea, environmental problems, particularly the increase in soil salinity, have significantly decreased agricultural potential. Thus, the use of microorganisms to ensure ecological sustainability should be explored" [2]. The goal of this study is to isolate microorganisms from saline soils, investigate their biological properties, and evaluate their practical significance for agroecosystems.

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2. Materials and Methods

2.1. Characteristics of the Study Area

This study was conducted in the territory of the Uchsoy settlement (43.9016 ℕ, 58.6803 ℇ), located in the Muynak district of the Republic of Karakalpakstan. This region is an ecosystem crisis zone within the Aral Sea region, a consequence of the long-term drying of the sea, which has resulted in dry, saline, and hydromorphic soils [3]. The area has a sharply continental climate with an annual temperature range of -25℃ to +45℃ and receives only 80–100 mm of precipitation per year, the majority of which falls during the spring and winter [4]. These conditions lead to intense

evaporation and a critical shortage of water resources.

The soils in the Uchsoy area are predominantly sandy loam and sandy, with high salinity (EC) and a distinctly alkaline reaction (pH > 8). The primary types of saline soils are chloride-sulfate and carbonate, with concentrations varying by depth.

Soil samples were collected from five points using the horizontal trench method at depths of 0-30 cm, 30-60 cm, and 60-90 cm. Following the ISO 10381 standard, samples weighing 500 to 1000 grams were taken from each depth, placed in plastic containers, and transported to the laboratory under refrigeration (ISO 10381-1:2002). For each layer, we determined the pH, electrical conductivity (EC), humus content, and microbiological composition [5].

Environmental monitoring in the region has shown that soil salinization significantly reduces biological activity. However, these soils still host microorganisms adapted to such harsh conditions, particularly salt-resistant bacteria and fungi. The isolation, identification, and further study of these organisms offer promising potential for developing environmentally friendly biological products. Such products could enhance plant resistance to salinity, improve nutrient uptake, and ultimately contribute to the biological recovery and sustainability of agroecosystems [6,7,10].

2.2. Isolation of Microorganicms from Soil

For the isolation and identification of microorganisms, the soil was initially subjected to serial dilutions using sterile water and sterile physiological saline solution (0.9% NaCl). To extract microorganisms from soil, including azotobacter, oligotrophs, actinomycetes and micromycetes, were grown on the particular solid selective nutrient media under conditions of isolation. Soil samples were collected for microbiological analysis. To accomplish this, 10 grams of soil were added to 90 milliliters of sterile water and then shaken for 10 minutes. Out of the suspension, 1 milliliter was taken with the help of a pipette. The remaining 9 milliliters of sterilized water was added to a test tube. The procedure of making a series of dilutions followed where the dilution factor was 1: 1 000 000 the whole process repeated three times to maintain the exactitude. Subsequently, in order to obtain isolates of single types of microbes of all generations, the right amount of suspension from each stage of dilution was inoculated onto the appropriate solid selective nutrient media through three replications, i.e., the process accounted for a total of twelve procedures of inoculation. The following culture media were used: meat peptone agar (MPA) for azotobacter, Ashby medium for oligotrophs, starch-ammonia agar (SAA) for actinomycetes, and Czapek-Dox medium for micromycetes. For all four kinds of media, the 4th dilution served as the inoculum, and the final determination was made after the growth of colonies. Cultures, seeded using the lawn plating method in Petri dishes, were incubated at a temperature of 28 ±2 ℃ for 2–7 days. Each colony was isolated based on morphological characteristics, purified, and then subjected to primary experiments using the Gram staining method [12,13,14].

2.3. Identification

The isolated microorganisms were identified using MALDI-TOF mass spectrometry (EXS2600, Zybio). Each pure colony was fixed in 70% ethanol, treated with formic acid and acetonitrile, and then subjected to spectral analysis by laser ionization. The identification results were processed using the MALDI Biotyper program, and based on the data obtained, the species, genus, and families of bacteria were identified [8]. The results of the study revealed the existence of several microorganisms, including Enterobacter asburiae, Acinetobacter johnsonii, Pseudomonas aeruginosa, rhizomatous cocuria (Kocuria rhizophila), Pseudomonas chlororaphis Pseudomonas aeruginosa, Stenotrophomonas sp., and lichen -like Bacillus licheniformis). The antagonistic activity of the isolated microorganisms against phytopathogenic fungi such as Fusarium oxysporum, Fusarium solani, and Alternaria spp. was studied by double cultivation (crop rotation) in Petri dishes. Each microorganicm and pathogen was simultaneously plated on the same nutrient medium. After 7 days, the growth zones were measured, and the antagonistic index (%) was calculated based on the size of the zones [9]. Determination of Soil Physicochemical Properties: The pH of the soil was determined using the potentiometric method, and the soil salinity was assessed by measuring electrical conductivity (EC). The humus content was determined using the Tyurin method (Tyurin). Average values were obtained from three replicates for each depth [15].

Antifungal Activity: The antagonistic activity of the isolated microorganisms against Fusarium oxysporum, Fusarium solani, and Alternaria was evaluated using the dual culture method in Petri dishes. Each colony was placed at the center of the dish, and the pathogenic fungus was seeded around it. The growth inhibition zones were measured by diameter (mm) ***Table 1***. The antagonistic activity against Fusarium solani, Fusarium oxysporum, and Alternaria species, as well as the tolerance of microbial strains to NaCl at concentrations of 1%, 2%, 5%, 10%, and 15%, were studied and analyzed based on the data presented in ***Table 2***.

Soil Composition Study

A sample weighing 50.41 grams was taken for the study of soil composition, and the amount of absorbed water was 252.05 mL.

Concentration of major ions at a depth of 1–30 cm of the studied area:

Chloride (Cl^-): 8600 mg/kg — high salinity; Sulfate (SO_4^{2-}): 960 - 4800 mg/kg — medium-high level; Calcium (Ca^{2+}): 990 - 3000 mg/kg — medium level; Magnesium (Mg^{2+}): 450 - 1495 mg/kg - medium-high level; Potassium (K^+): 100 - 300 mg/kg — sufficient; sodium (Na^+): 1495- 4000 mg/kg — high mineralization; pH: 7.5 – 8.5 — slightly alkaline; Mineralization: 4 - 8 mg/m2 — saline soil; Humus (organic matter): 1.0 – 2.0% — good level.

Concentration of basic ions at a depth of 30-60 cm in the study area:

Chloride (Cl^-): 9000 mg/kg — high salinity; Sulfate (SO_4^{2-}): 500 - 2960 mg/kg — average level; Calcium (Ca^{2+}): 800 - 1950 mg/kg — average level; Magnesium (Mg^{2+}): 387 - 1217 mg/kg — average level; Potassium (K^+): 80-200 mg/kg — sufficient; Sodium (Na^+): 975 - 1290 mg/kg — presence of salinity; pH: 8.0 – 9.0 — alkaline; Mineralization: 3 - 5 g/m2 — saline soil; Humus (organic matter): 0.8 – 1.5% — average level.

The concentration of basic ions at a depth of 60-90 cm of the studied area:

Chloride (Cl^-): 7,100 mg/kg — salinity is still high; Sulfate (SO_4^{2-}): 300 - 2000 mg/kg — average; Calcium (Ca^{2+}): 550 - 1510 mg/kg — average; Magnesium (Mg^{2+}): 300 - 990 mg/kg - average; Potassium (K^+): 50 - 150 mg/kg — relatively low level; Sodium (Na^+): 800 - 2000 mg/kg — causes salinity; pH: 8.5 – 9.5 — highly alkaline; Mineralization: 2 - 4 dS/m — saline soil; Humus (organic matter): 0.5 – 1.0% — low level.

Table 1. Antifungal Activity Statistics

Bacterium / Isolate	Target Fungus	Rep1	Rep2	Rep3	Mean zone (mm)	Std Dev
Enterobacter asburiae	F. solani	8.0	7.0	8.2	7.73	0.64
Enterobacter asburiae	F. oxysporum	8.0	9.0	8.8	8.6	0.53
Enterobacter asburiae	Alternaria sp.	11.0	11.5	12.0	11.5	0.5
Acinetobacter johnsonii	F. solani	4.0	4.2	4.5	4.23	0.25
Acinetobacter johnsonii	F. oxysporum	5.0	5.3	5.7	5.33	0.35
Acinetobacter johnsonii	Alternaria sp.	6.0	6.6	7.0	6.53	0.5
Pseudomonas aeruginosa	F. solani	7.0	7.0	8.0	7.33	0.58
Pseudomonas aeruginosa	F. oxysporum	8.8	9.0	10.0	9.27	0.64
Pseudomonas aeruginosa	Alternaria sp.	11.0	12.0	12.4	11.8	0.72
Kocuria rhizophila	F. solani	0.2	1.0	2.0	1.07	0.9
Kocuria rhizophila	F. oxysporum	2.0	3.0	4.0	3.0	1.0
Kocuria rhizophila	Alternaria sp.	3.0	4.0	5.0	4.0	1.0
Pseudomonas chlororaphis	F. solani	9.0	9.5	10.0	9.5	0.5
Pseudomonas chlororaphis	F. oxysporum	10.0	11.0	11.7	10.9	0.85
Pseudomonas chlororaphis	Alternaria sp.	12.0	13.0	13.5	12.83	0.76
Stenotrophomonas sp.	F. solani	5.5	6.0	6.6	6.03	0.55
Stenotrophomonas sp.	F. oxysporum	6.0	6.5	7.0	6.5	0.5
Stenotrophomonas sp.	Alternaria sp.	7.0	7.8	8.0	7.6	0.53
Bacillus licheniformis	F. solani	7.3	7.7	7.8	7.6	0.26
Bacillus licheniformis	F. oxysporum	8.0	9.0	9.5	8.83	0.76
Bacillus licheniformis	Alternaria sp.	10.5	11.0	11.2	10.9	0.36
Brevundimonas aurantiaca	F. solani	0.2	1.0	2.0	1.07	0.9
Brevundimonas aurantiaca	F. oxysporum	2.0	3.0	4.0	3.0	1.0
Brevundimonas aurantiaca	Alternaria sp.	4.0	4.9	6.0	4.97	1.0
Bacillus pumilus	F. solani	9.2	9.8	10.0	9.67	0.42
Bacillus pumilus	F. oxysporum	10.5	11.0	11.5	11.0	0.5
Bacillus pumilus	Alternaria sp.	12.0	12.5	13.0	12.5	0.5
Bacillus atrophaeus	F. solani	12.0	12.5	13.0	12.5	0.5
Bacillus atrophaeus	F. oxysporum	13.5	14.0	14.2	13.9	0.36
Bacillus atrophaeus	Alternaria sp.	15.0	15.6	16.0	15.53	0.5
Bacillus vallismortis	F. solani	12.0	12.4	12.9	12.43	0.45
Bacillus vallismortis	F. oxysporum	13.8	13.3	14.0	13.7	0.36
Bacillus vallismortis	Alternaria sp.	14.0	14.5	15.0	14.5	0.5
Metabacillus halosaccharovorans	F. solani	2.0	3.0	4.0	3.0	1.0
Metabacillus halosaccharovorans	F. oxysporum	5.0	6.0	7.0	6.0	1.0
Metabacillus halosaccharovorans	Alternaria sp.	7.0	8.0	9.0	8.0	1.0

Table 2. Salt tolerance

Bacterium / Isolate	1% NaCl	2% NaCl	5% NaCl	10% NaCl	15% NaCl
Enterobacter asburiae	+++	+++	++	-	-
Acinetobacter johnsonii	+++	+++	++	-	-
Pseudomonas aeruginosa	+++	+++	++	+	-
Kocuria rhizophila	+++	+++	++	+	-
Pseudomonas chlororaphis	+++	+++	++	++	-
Stenotrophomonas sp.	+++	+++	+++	++	-
Bacillus licheniformis	+++	+++	+++	+++	++
Brevundimonas aurantiaca	+++	++	-	-	-
Bacillus pumilus	+++	+++	+++	+++	+
Bacillus atrophaeus	+++	+++	+++	+++	+
Bacillus vallismortis	+++	+++	+++	++	+
Metabacillus halosaccharovorans	+++	+++	+++	++	+

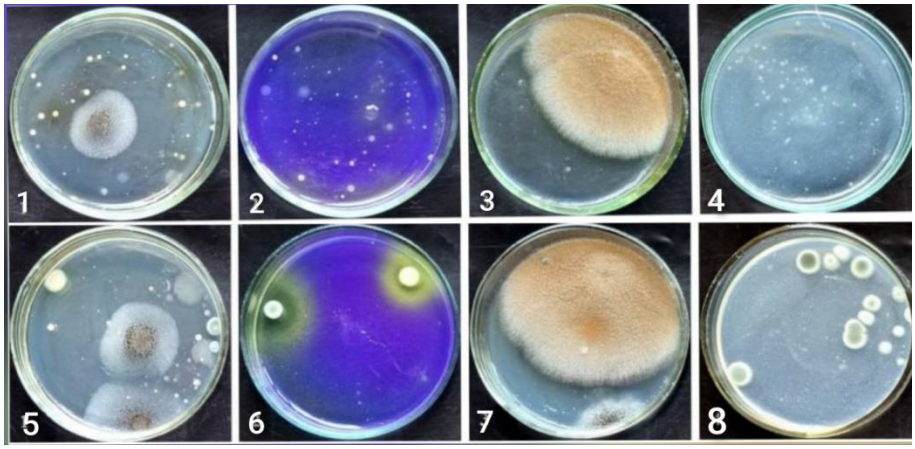

Figure 1. Total count and morphology of the main physiological groups of microorganisms grown on different nutrient media at soil depths of 0 – 30 cm and 30 – 60 cm in the Aral Sea region

Figure 2. Fungi isolated from saline soils in the research area

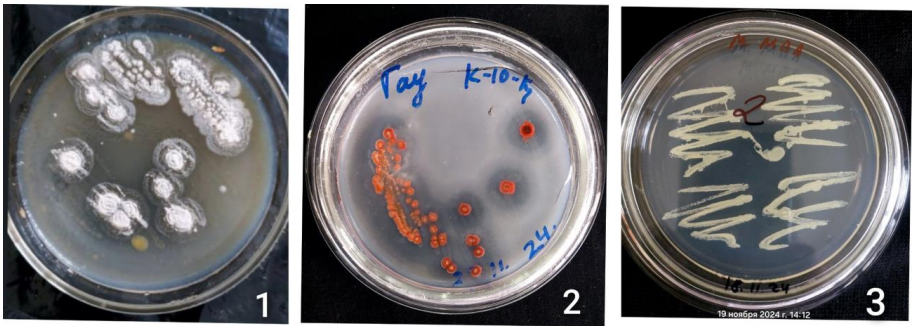

Figure 3. Actinomycetes isolated from the research area

3. Results

During the study, 25 microorganisms were isolated, 12 of which were identified as follows: Some of the bacterial strains found include Enterobacter asburiae, infections caused by Acinetobacter johnsonii, Pseudomonas aeruginosa, Pseudomonas aeruginosa chlororaphisa, Stenotrophic bacillus, lichen bacillus, Brevundimona aurantiaca, Metabacillus halosaccharovorans, pumilus, atrophic bacillus, vallicmortis, Megatherium bacillus, a tiny stick. The main fungi include Mucor, Aspergillus niger, Aspergillus flavus and Penicillium chrysogenum Figure 2. Actinomycetes: Streptomyces roseus, Kocuria rhizophilus Figure 3.

Some of the isolated microorganisms demonstrated high antifungal reaction. The analysis showed that both bacteria and fungi exhibited distinct growth characteristics under varying salinity conditions. Notably, Metabacillus halosaccharovorans and Pseudomonas chlororaphis demonstrated high salt tolerance and strong antifungal reaction.

Physicochemical Properties of the Soil.

The pH and electrical conductivity (EC) values varied significantly depending on the depth of occurrence, in the saline soil samples taken in the Uchsoy area. The pH of the upper layer (0-30 cm) ranged from 7.5 to 8.5, which was slightly alkaline, and, on the other hand, concentrations of dissolved substances in the range from 4 to 8 dS/m2 were observed. As the depth increased, both of these variables had a significant positive effect: at a depth of 60 - 90 cm, a pH of 8.5 – 9.5 meant that the alkali content in the soil was very high, and the salinity was 2-4 dS/m, which is a sign of brackish soil. In the 0 -30 cm layer, the humus content was 0.89%, while in the 30 - 60 cm layer it was 0.41%, which means a decrease in microbial activity and less decomposition of organic matter at great depths. These data indicate the presence of hydromorphic and accumulative salt layers.

4. Discussion

The isolated microorganisms, among which the most active role was played by species of Bacillus and Pseudomonas chlororaphis, demonstrated high biological activity. Due to their antifungal and stress-resistant properties, they can become the main components in the production of bio-cork and bio-humus for saline soils [11]. The identification of salt-tolerant microbial strains forms the basis for the development of microbiological fertilizers adapted for these territories. Under conditions of strong salinization, such as in the Aral region, microorganisms developed their physiological and metabolic adaptation strategies. The ecological adaptation of salt-resistant microorganisms is of great agrobiological importance, as they contribute to improving plant growth, nutrition, and disease resistance. In particular, Bacillus and Pseudomonas species play an important role in the development of bio preparations due to their activity in the rhizosphere, production of siderophores, phosphate solubilization, and combat against phytopathogens [4].

5. Conclusions

The results of this study showed that in saline soils of the Aral region, there are microorganisms with ecological and agrobiological significance. Based on soil samples taken at different depths in the Uchsoy area of the Moynak district, 12 species of microorganisms were isolated, which were identified using the MALDI-TOF mass spectrometry method. Among the isolated microorganisms, species such as Pseudomonas chlororaphis, Bacillus subtilis, Bacillus pumilus, Brevundimonas aurantiaca, Stenotrophomonas maltophilia, Aspergillus, and Mucor predominated. The isolated microorganisms are adapted to conditions of high salt content, and most of them showed high antagonistic reaction against phytopathogenic fungi. In particular, Pseudomonas and Bacillus species formed effective inhibition zones against Fusarium oxysporum, F. solani, and Alternaria spp. This demonstrates not only the biological role of these microorganisms as agents of biological control but also their potential as biofertilizers.

The evaluation of the physicochemical properties of the soil at various depths, in combination with the microbial composition, confirms the direct influence of salinity and organic matter content on the diversity of microorganisms.

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