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HALOTOLERANT RHIZOBACTERIA FROM BLACK SAXAUL RHIZOSPHERE IN THE ARAL SEA REGION: NITROGEN-FIXING POTENTIAL

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Abstract: Bacterial isolates obtained from the rhizosphere of black saxaul (*Haloxylon aphyllum*) in the dried bottom areas of the Aral Sea were screened to select halotolerant strains, and their nitrogen-fixing ability was investigated. Isolates capable of growth at 15% NaCl were tested in nitrogen-free Ashby medium. As a result, the isolates Qsi 1/3, Qs root 1/3, and Qst 1/2 were found to possess nitrogen-fixing ability. These isolates are expected to directly fix nitrogen in areas exposed to stress conditions.

Keywords: halotolerant bacteria, nitrogen fixation, Aral Sea region, *Haloxylon aphyllum*, Esbi medium

Introduction

The desiccation of the Aral Sea has caused extensive ecological degradation in Central Asia. Plants growing in extreme environments, particularly *Haloxylon aphyllum*, survive through symbiotic interactions with rhizosphere microbiota. Among the microorganisms in the rhizosphere, free-living nitrogen-fixing bacteria are of particular importance, as they convert atmospheric molecular nitrogen into a form that can be assimilated by plants. Identification of halotolerant rhizobacteria capable of functioning in saline soils and studying their functional traits is crucial for restoring degraded ecosystems.

Another study conducted in the Aralkum desert analyzed the halophilic and halotolerant microbiome of the soils and assessed the potential of microbial inoculation to enhance the populations of salt-tolerant plants in the region [1]. Several studies in this area selected 22 salt-tolerant strains for detailed morphological analysis, most of which were rod-shaped, occurring singly or in chains, and mainly belonging to the families Bacillaceae and Pseudomonadaceae [2]. Key plant-interacting organisms in this region include nitrogen-fixing bacteria, mycorrhizal fungi, and plant growth-promoting rhizobacteria (PGPR) [3]. Under hypersaline conditions, the most commonly identified bacterial phyla include Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria, Deinococcus-Thermus, and Verrucomicrobia [4].

Methods

As research material, bacterial strains isolated from the rhizosphere of black saxaul (*Haloxylon aphyllum*) growing in the dried bottom areas of the Aral Sea were used. In the study, samples were taken from the root rhizosphere of *Haloxylon aphyllum* at a depth of 25–30 cm. Soil and root samples were collected in November 2025. The map of the Aral Sea and the location of the sampling site

are shown in Figure 1. During the study, meteorological and weather conditions remained stable. Throughout the observation period, daytime air temperature was approximately 22 °C, with consistently sunny weather.



Figure 1. Map of the Aral Sea (A) and the location of the sampling site

Bacterial strains isolated under laboratory conditions were cultivated in media containing 5, 7, 10, 12, and 15% NaCl [5]. Isolates that exhibited stable growth at 15% NaCl were inoculated into nitrogen-free Esbi medium [6]. The procedure for cultivating rhizosphere bacterial isolates is as follows:

Esbi medium (g/L): mannitol 20.0, K_2HPO_4 0.2, $MgSO_4$ 0.2, NaCl 0.2, K_2SO_4 0.1, $CaCO_3$ 5.0, agar 20.0; pH 7.1–7.3 The medium was incubated at 28 °C for 7 days [7].

Results

Among the isolated strains, several bacterial strains capable of growth at 15% NaCl were identified. The experiment was continued based on their growth activity in nitrogen-free Ashby medium. According to the results, the isolates Qsi 1/3, Qs root 1/3, and Qst 1/2 exhibited growth characteristics in this medium. Bacterial isolates grown in Esbi nutrient medium **Figure 2.**

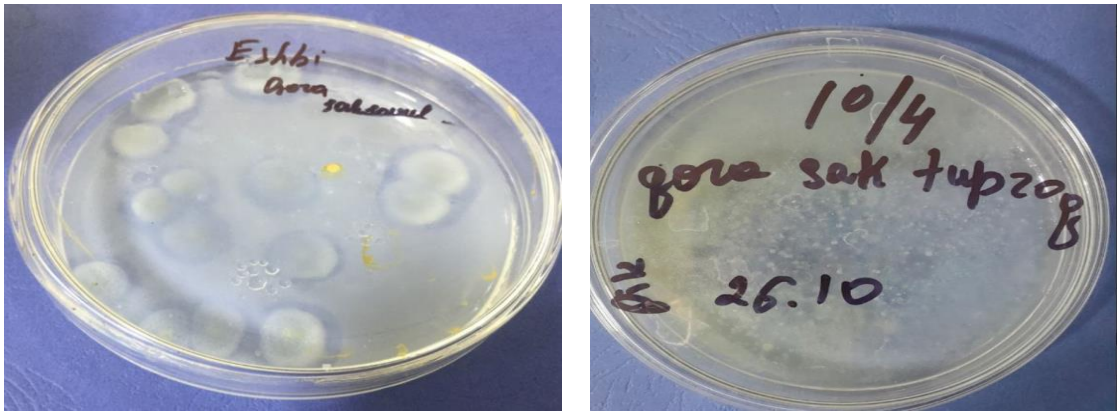


Figure 2. Bacterial isolates grown in Esbi nutrient medium

The growth of these isolates under nitrogen-free conditions indicates their ability to biologically fix atmospheric nitrogen. In the remaining isolates, the nitrogen-fixing trait was expressed only weakly.

Discussion

The obtained results indicate that certain bacteria occurring in the rhizosphere of *Halocnemum strobilaceum* (black saxaul) are adapted to high salinity conditions and maintain their metabolic activity. Although a 15% NaCl concentration represents a stressful environment for many microorganisms, the isolates identified in this study demonstrated active growth and nitrogen-fixing ability under these conditions. These findings provide new opportunities for the identification of halotolerant rhizobacteria in the dried areas of the Aral Sea and for ecological restoration research. Experiments can be conducted with these isolates to promote plant growth biologically and to restore ecosystems in saline-affected regions.

References:

1. Mamarasulov B. et al. Soil Microbiome of the Dried Aral Sea Soils

Promotes Wheat Growth Under Salinity //Egyptian Journal of Soil Science. – 2025. – T. 65. – №. 4.

2. Abdusamatov S. et al. Study of the antagonistic properties of bacterial strains selected from the rhizosphere of halophytic plants //E3S Web of Conferences. – EDP Sciences, 2025. – T. 648. – C. 02006.
3. MusF.CrookM. B.GarciaK. N.Garcia CostasA.GeddesB. A.KouriE. D.et al (2016). Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes.*Appl. Environ. Microbiol*.82:3698. 10.1128/AEM.01055-16
4. Zhang, G.; Bai, J.; Zhai, Y.; Jia, J.; Zhao, Q.; Wang, W.; Hu, X. Sho'rlangan tuproqlardagi mikrobial xilma-xillik va funktsiyalar: Biogeokimyoviy nuqtai nazardan sharh. *J. Adv. Res.* **2024** , *59* , 129–140. [Google Scholar] [CrossRef] [PubMed]
5. Pérez-García L. A. et al. Plant growth promoting rhizobacteria enhances germination and bioactive compound in cucumber seedlings under saline stress //Ecosistemas y recursos agropecuarios. – 2025. – T. 12. – №. 2.
6. Boomer S. Exploring and Enriching for Nitrogen Cycling Microbes: Nitrogen Fixation, Ammonia Oxidizing Lithotrophy, and Anaerobic Nitrate Reduction. – 2006.
7. Van Chuong N., Le Kim Tri T. Isolation and Characterization Identification of Edophytic Nitrogen-Fixing Bacteria from Peanut Nodules //International Journal of Microbiology. – 2024. – T. 2024. – №. 1. – C. 8973718.