DOI 10.66073/ReFocus-Conf/3010025

ISOLATION, METAGENOMIC PROFILING, AND BIOTECHNOLOGICAL POTENTIAL OF INDIGENOUS METHANOTROPHIC BACTERIA IN UZBEKISTAN

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Methanotrophs are a unique group of bacteria possessing the remarkable ability to utilize methane as their sole source of carbon and energy. They are widely distributed in nature and have been isolated from a variety of environments, including soils, sediments, freshwater, marine sediments, seawater, acidic peat bogs, hot springs, and cold environments such as Antarctica. Methane represents a promising next-generation carbon feedstock for industrial biotechnology, as it is abundant and cost-effective. The biological conversion of methane into high-value products offers a potential solution to mitigate greenhouse gas (GHG) emissions associated with methane releases. Single-cell proteins (SCP) produced from these microorganisms contain 30–80% protein, as well as essential amino acids, vitamins, and minerals, making them highly effective feed additives in livestock and poultry diets. Soil and substrate samples were collected from diverse ecological regions of Sirdaryo, Qashqadaryo, and Surxondaryo regions to isolate and cultivate resilient methanotrophic bacterial strains of biotechnological importance. After 10–15 days of incubation in serum bottles and desiccators inoculated into NMS (Nitrate Mineral Salts) liquid medium under a CH_4: air (1:1 and 1:4) gas-phase mixture, signs of microbial growth, including turbidity of the culture medium and an increase in sample biomass, were observed. Application of a high-temperature regime (45 °C) inhibited the growth of mesophilic companion microbiota and enriched the population of target thermotolerant methanotrophic bacteria. Total metagenomic DNA was extracted from cumulative and enriched liquid cultures using a commercial kit (DNeasy PowerSoil Pro Kit, Qiagen, Germany) in accordance with the manufacturer's instructions. The concentration and purity of the extracted DNA were evaluated using a Qubit 4.0 Fluorometer and a NanoDrop spectrophotometer. A metagenomic library was prepared, and high-throughput shotgun sequencing was performed on the Illumina platform. The obtained raw reads underwent quality control and were filtered using bioinformatic software tools (FastQC, Trimmomatic). For taxonomic classification and determination of relative abundance, sequences were aligned against the NCBI and GTDB (Genome Taxonomy Database) databases. According to the analysis results, members of the class Gammaproteobacteria (phylum Proteobacteria) exhibited absolute dominance in both samples, accounting for 74.04% in the sample collected from the gas mines of the Qashqadaryo region (H1) and 75.37% in the sample from the Sirdaryo region (H2). The second most abundant class was Betaproteobacteria (15.99%) in sample H1 and Alphaproteobacteria (16.15%) in sample H2. The obtained results confirm that the cultivation conditions successfully enriched the population of methanotrophic bacteria belonging to the class Gammaproteobacteria.

Analysis of the 16S rRNA gene of methanotrophic bacteria isolated from samples collected in the Surxondaryo region (oil wells of the Boysun district) revealed a 99.4% sequence identity with *Methylocaldum gracile*.

FOR CITATION
Sherbekova N.A., Kadirova Z.A., Karimov H.X. Isolation, metagenomic profiling, and biotechnological potential of indigenous methanotrophic bacteria in Uzbekistan // Proceedings of the Republican Scientific and Practical Conference “Status and Development Prospects of Fundamental and Applied Microbiology: The Viewpoint of Young Scientists”. – Tashkent, 2026. – P. 100–101.
DOI: https://doi.org/10.66073/ReFocus-Conf/3010025

Based on the obtained results, scientific research is consistently ongoing. At the present stage, applied research is being conducted to evaluate the biocatalytic and biotechnological potential of the isolated thermotolerant methanotrophic bacterial strains, specifically focusing on the efficiency of methane biotransformation and microbial biomass (protein) biosynthesis.

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