

Halophilic bacteria of the genera *Halomonas* and *Marinobacter* from petroleum reservoirs and their possible applications in biotechnology

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Motivation and Aim: An exploitation of many oil fields becomes complicated because of high salinity of formation waters. Increased salt concentration makes difficulties in oil production, purification and disposal of oil-contaminated waters. The use of microbial technologies on oil fields with high salinity formation water is limited due to the low solubility of oxygen and the negative impact of salt on microorganisms, including the destruction of the cell walls and denaturation of enzymes [1]. Bacteria of the genera *Halomonas* and *Marinobacter* are often presented in microbial communities of these habitats, including marine reservoirs, deep underground horizons, oil reservoirs, and wastewaters from these sites [2, 3]. Thus, the aim of this study was to determine the composition of the microbial communities of oil reservoirs with highly mineralized formation waters, to isolate halophilic hydrocarbon-oxidizing bacteria, to study their physiological and genomic characteristics and their role in oil biotransformation.

Methods and Algorithms: Bacterial strains TAT1 and KAZ22 were isolated from bed 302 of the Romashkinskoe oil field (Republic of Tatarstan, Russian Federation) and from the Uzen oil field (Republic of Kazakhstan) respectively. Strains were identified using the 16S rRNA gene sequencing with an ABI 3730 DNA Analyzer sequencer and the ABI PRISM BigDye™ Terminator v 3.1 reagent kit (Applied Biosystems, USA). The alkane monooxygenases genes (*alkB*, *ladAα*, *ladAβ*, *ladB*, *CYP153A*) were detected using bacterial strains from the collection of the Research Center of Biotechnology RAS and a strain of *Geobacillus icigianus* G1w1^T provided by the staff of the Institute of Cytology and Genetics SB RAS [4]. The strain genomes were sequenced using the Illumina HiSeq 2500 system (USA). Microbial communities' composition was determined using high-throughput sequencing of the V3–V4 regions of the 16S rRNA genes. Reconstruction of probable metabolic pathways was performed using the NCBI, PATRIC and KEGG online services.

Results: The presence of bacteria of the genus *Halomonas* in the Romashkinskoe oil field, characterized by low temperature and high sulfide content in reservoir fluids, and bacteria of the genus *Marinobacter* in the high-temperature Uzen oil reservoir was shown using high-throughput sequencing. Halophilic bacteria *Halomonas titanicae* TAT1 and *Marinobacter lutaoensis* Kaz22 were isolated from oil reservoirs, their physiological characteristics were determined, and their genomes were sequenced. Both strains utilized a wide range of oil hydrocarbons with the formation of biosurfactants in a liquid media with salinity higher than 100 g NaCl/L, and differed in the temperature ranges for growth, which were 4–42°C for the *Halomonas* strain and 22–55°C for the

Marinobacter strain. The genomes of the strains contained genes that determine resistance to high salinity and genes for alkane hydroxylases responsible for the degradation of *n*-alkanes with different chain length. In *H. titanicae* TAT1 *alkB* gene was detected and in *M. lutaoensis* KAZ22 *alkB1*, *alkB2*, *alkB3*, and *almA* genes were detected. Several genes responsible for the production of osmoprotectants were presented in both strains. The *betIBA* and *ectABC* gene clusters and separate *ectD1* and *ectD2* genes were found in the *Halomonas* strain, so it was presumably able to produce betaine, ectoine, and hydroxyectoine. The *Marinobacter* strain did not show the ability to synthesize betaine, but possessed the *ectAB* gene cluster with an aspartate kinase gene and separately located *ectC* and *ectD* genes, that is, it was also a potential producer of ectoine and hydroxyectoine. Strain TAT1 grew heterotrophically under anaerobic conditions with organic substrates reducing nitrate to nitrite. This ability makes a possibility of using this strain in biotechnology to suppress the formation of hydrogen sulfide in an oil reservoir. The strain *M. lutaoensis* KAZ22 was incapable of nitrate reduction, and nitrate reductase genes in its genome were absent.

Conclusion: Two bacterial strains, *Halomonas titanicae* TAT1 and *Marinobacter lutaoensis* KAZ22, are isolated from the highly mineralized Romashkinskoe (Russian Federation) and Uzen (Republic of Kazakhstan) oil fields respectively, their physiology is studied, and their genomes are sequenced. In the genome of the *Halomonas* strain the *alkB* gene for alkane-1-monooxygenase is found; in the *Marinobacter* strain two genes *alkB1* and *alkB2* and the flavin-dependent monooxygenase *almA* gene are found as well. All these genes belong to the separate phylogenetic clusters. *Halomonas titanicae* TAT1 strain is potentially capable of synthesizing osmoprotectants betaine, ectoine and hydroxyectoine; strain *Marinobacter lutaoensis* KAZ22 is able to produce ectoine and hydroxyectoine. The *Halomonas* strain reduces nitrate with nitrite accumulation, which indicates the possibility of its use to suppress the formation of hydrogen sulfide in the reservoir. Both *Halomonas* and *Marinobacter* strains oxidize oil alkanes under high salinity conditions with the formation of biosurfactants, which indicates the possibility of their application in biotechnologies for cleaning oil pollutions and microbial enhancement of oil recovery.

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