Full-genome sequencing of the *Staphylococcus warneri* strain isolated from oil-contaminated soil

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Abstract: Bacterial consortium bioremediation presents itself a promising approach to the treatment water, soil and the atmosphere for pollution by oil and its derivatives. In the territory of the Romashkinskoye oil field, Republic of Tatarstan, various decomposers were isolated from oil-contaminated black soil, including three that demonstrated oil resistance and ability to produce biosurfactants. The genome sequencing of the *Staphylococcus warneri* strain isolated in a consortium of decomposers was performed on the MiSeq Illumina platform. The average content of GC pairs in the genome comprised 32.7%. Genome annotation was performed using the RAST server. The SEED viewer was applied for subsystem category distribution of predicted genes. The sequenced genome of *Staphylococcus warneri* strain was identified as containing 2535 protein coding sequences. The majority of annotated genes govern the synthesis of amino acids and their derivatives (255), carbohydrate (195) and protein metabolism (167), cofactors, vitamins, prosthetic groups and pigmented formations (87), nucleosides and nucleotides (78), fatty acid metabolism, lipids and isoprenoids (55), as well as DNA metabolism (68). The full-genome sequencing and genome annotation of the *Staphylococcus warneri* strain confirmed its hydrocarbon-oxidising properties. The yddN and yceB genes of uncharacterised proteins were identified as similar to alkanal monooxygenases likely to be involved in the biodegradation of alkanes. The three genes detected in this strain code the catechol-2,3-dioxygenase, fumarylacetoacetate hydrolase and salicylate-1-monooxygenase enzymes involved in the biodegradation of aromatic hydrocarbons. The obtained genome sequence data help to provide a better understanding of the process of hydrocarbon degradation (absorption) by the *Staphylococcus warneri* strain and its role in the bacterial consortium.

Keywords: *Staphylococcus warneri*, genome, sequencing, biodegradation, hydrocarbons

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Полногеномное секвенирование штамма *Staphylococcus warneri*, изолированного из загрязненной нефтью почвы

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Резюме: Биоремедиация с использованием бактериальных консорциумов представляет собой перспективный подход к очистке вод, грунтов и атмосферы от загрязнений нефтью и ее производными. Из нефтезагрязненной черноземной почвы на территории Ромашкинского месторождения Республики Татарстан выделены микроорганизмы-деструкторы, три из которых отобраны по устойчивости к нефти и способности к производству биосурфактантов. Секвенирование генома штамма Staphylococcus warneri, выделенного в составе консорциума микроорганизмов-деструкторов, проведено на платформе MiSeq Illumina. Среднее содержание GC-пар в геноме составило 32,7 %. Аннотирование генома выполнено с использованием сервера RAST. Показано, что данный геном содержит 2535 кодирующих белок последовательностей. Большинство аннотированных генов определяет синтез аминокислот и их производных (255), углеводный обмен (195), метаболизм жирных кислот, липидов и изопреноидов (55) и метаболизм ДНК (68). Полногеномное секвенирование и аннотирование генома штамма Staphylococcus warneri подтверждает наличие у него углеводородокисляющих свойств. Выявлено два гена нехарактеризованных белков yddN и yceB, имеющих сходство с алканал-монооксигеназами, которые, вероятно, принимают участие в биодеградации алканов. Три гена, обнаруженные в этом штамме, кодируют ферменты катехол-2,3-дидроксигеназу, фумарилациозетат-гидролазу и салицилат-1-монооксигеназу, участвующие в биодеградации ароматических углеводородов. Полученные данные послеволнительности генома позволяют лучше понять механизмы деградации (поглощения) углеводородов штаммом Staphylococcus warneri и его роль в бактериальном консорциуме.

Ключевые слова: Staphylococcus warneri, геном, секвенирование, биоремедиация, углеводороды

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INTRODUCTION

Crude oil pollution is regularly recorded around the world, representing a serious environmental problem. Sources of pollution involve all types of activities in the oil industry, including exploration, production, transportation, clean-up and the management of oily wastes [1]. Due to the poor degradability of crude oil, pollutants remain in soil and water systems for a long time, adversely affecting the physical, chemical and biological condition of the polluted ecosystem [2].

Various methods are used for the remediation of oil-contaminated soils. Unlike expensive and often environmentally-harmful physical and chemical purification methods, decomposer bioremediation turns out to be preferable in many cases [3, 4]. Free-living or immobilised bacteria with a high ability to decompose xenobiotics are used individually or in a consortium to carry out bioremediation processes. However, the effectiveness of these processes is limited by the complex and toxic nature of oil and oil products, as well as the poor adaptation and survivability of microorganisms in an adverse environment. In this connection, the isolation of microorganism cultures from oil-contaminated natural media is the primarily method used in the search for organisms capable of decomposing hydrocarbons [5, 6]. Advances in genome-wide sequencing have created access to the genetic information of microorganisms belonging to different taxonomic groups and capable of decomposing hydrocarbons by various electron acceptors [7]. A complete genome analysis of the bacterial decomposer strains provides useful information on both the mechanism of hydrocarbon uptake and the genetic adaptation of the strains for propagation in an environment exposed to oil and oil products.

The present study was aimed at the full-genome sequencing and annotation of the Staphylococcus warneri strain genome isolated from black soil as a part of a decomposer consortium in the territory of the Romashkinskoye oil field, Republic of Tatarstan.

EXPERIMENTAL PART

The isolation of hydrocarbon-oxidising microorganisms (HCOM) was carried out according to the modified method provided in [8]. The soil suspension was introduced into a Voroshilova-Dianova agar medium followed by the addition of

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When cultured on the Dianova-Voroshilova medium, a consortium of microorganisms was obtained that showed resistance to high concentrations of hydrocarbons (up to 10 % of oil). In verifying the ability of the consortium to grow on a medium containing various hydrocarbon pollutants (diesel fuel, fuel oil, vacuum gasoil, hexane, phenol, toluene), the microorganisms were shown to develop well in the presence of all hydrocarbons except phenol.

The species affiliation of the strains included in the consortium was identified using matrix-activated laser desorption/ionisation technology (MALDI-TOF-MS). The consortium was established as including three strain types: *Achromobacter xylosidans*, *Pseudomonas stutzeri* and *Staphylococcus warneri*.

*Staphylococcus warneri* strains of a gram-positive commensal bacterium were repeatedly detected among hydrocarbon-oxidising microorganisms isolated from oil-contaminated soils [9–12]. However, the role of this bacterial type in the degradation of hydrocarbons remains poorly understood. Recent studies have demonstrated that some secondary metabolites, produced by certain species of *Staphylococcus* isolated from the natural environment, have biotechnological and biomedical significance [13], including those involved in the production of biosurfactants [14].

In addition to phenotypic studies, genetic studies of industrially-promising strains using various molecular genetic approaches – including those based on nucleic acid sequencing – are of high current relevance.

In the sequencing of the *S. warneri* genome, 1075522 readings of 300 bp were yielded, with an average GC content of 32.7 %. Genomic sequence assembly, analysis and automatic reporting performed using SPAdes v. 3.11.1 [15] resulted in a total of 54 contigs with 26 sized over 500 bp.

Gene search and annotations were performed for all contigs longer than 500 bp using the RAST server [16]. The SEED viewer was used subsystem category distribution of predicted genes [17]. The genome was determined to contain 2535 protein coding sequences. Here, most annotated genes govern the synthesis of amino acids and their derivatives (255), carbohydrate (195) and protein metabolism (167), cofactors, vitamins, prosthetic groups and pigmented formations (87), nucleosides and nucleotides (78), fatty acid metabolism, lipids and isoprenoids (55), as well as DNA metabolism (68) (see figure).

According to the obtained annotation, three genes coding the catechol-2,3-dioxygenase, fumarylacetoacetate hydratase and salicylate-1-monoxygenase enzymes in the *S. warneri* genome are involved in the biodegradation of aromatic hydrocarbons [18, 19].

Additionally, among the genes of uncharacterised proteins, the *yddN* and *yceB* genes were identified as being similar to alkanal monoxygenases likely to be involved in biodegradation of alkanes. Biodegradation of alkanes takes place in several stages.
After their oxidation by monooxygenases, genes come into play coding enzymes for the oxidation of alcohols (alcohol dehydrogenase), aldehydes (aldehyde dehydrogenase) and completing the second and third stages of mineralisation, respectively. In addition to aldehyde dehydrogenase (adh), the genome of the studied strain contains nine genes coding uncharacterised proteins belonging to the family of short-chain alcohol dehydrogenases, as well as three putative aldehyde dehydrogenases.

Since the substrate specificity of dehydrogenase alcohol is not limited to aliphatic alcohols, the physiological significance of this enzyme is likely to be emphasised by its ability to metabolise xenobiotic aromatic and aliphatic hydroxyls along similar pathways. Aldehyde dehydrogenase is known to play an important role in the detoxification of toxic aldehydes formed along various cellular metabolic pathways and is considered as an indispensable enzyme in decomposing a wide variety of hydrocarbon compounds [20].

**CONCLUSION**

The full-genome sequencing and annotation of the *Staphylococcus warneri* strain genome isolated in a decomposer consortium confirmed the presence of hydrocarbon-oxidising properties of this strain. Information obtained by annotating the genome of *Staphylococcus warneri* strain provides for a better understanding both of the hydrocarbon degrading mechanism of the strain and its role in the bacterial consortium.

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**Contribution**

Irina A. Degtyareva, Edward V. Babynin, Tatyan Yu. Motina, Mansur I. Sultanov carried out the experimental work, on the basis of the results summarized the material and wrote the manuscript. Irina A. Degtyareva, Edward V. Babynin, Tatyan Yu. Motina, Mansur I. Sultanov have equal author’s rights and bear equal responsibility for plagiarism.

**Conflict of interests**

The authors declare no conflict of interests regarding the publication of this article.

The final manuscript has been read and ap-proved by all the co-authors.

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**Критерии авторства**

Дегтярева И.А., Бабынин Э.В., Мотина Т.Ю., Султанов М.И. выполнили экспериментальную работу, на основании полученных результатов провели обобщение и написали рукопись. Дегтярева И.А., Бабынин Э.В., Мотина Т.Ю., Султанов М.И. имеют на статью равные авторские права и несут равную ответственность за плагиат.

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I.A. Degtyareva, E.V. Babynin, T.Yu. Motina, et. al. Full-genome sequencing ...

I.A. Дегтярева, Э.В. Бабынин, Т.Ю. Мотина и др. Полногеномное секвенирование штамма ...

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