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

© Irina A. Degtyareva*,, Edward V. Babynin***,*, Tatyana Yu. Motina*, Mansur I. Sultanov***

* Tatar Research Institute of Agrochemistry and Soil Science, the "Kazan Scientific Centre of the Russian Academy of Sciences" Federal Research Centre, Kazan, Russian Federation,
** Kazan National Research Technological University, Kazan, Russian Federation
*** Kazan Federal University, Kazan, Russian Federation

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

И.А. Дегтярева*,, Э.В. Бабынин***,*, Т.Ю. Мотина*, М.И. Султанов***

* Татарский научно-исследовательский институт агрохимии и почвоведения – обособленное структурное подразделение Федерального государственного бюджетного учреждения науки «Федеральный исследовательский центр «Казанский научный центр Российской академии наук»,
г. Казань, Российская Федерация
** Казанский национальный исследовательский технологический университет,
г. Казань, Российская Федерация
*** Казанский (Приволжский) федеральный университет, г. Казань, Российская Федерация

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. SEED viewer использовали для отнесения предсказанных генов к функциональным категориям. Показано, что данный геном содержит 2535 кодирующих белок последовательностей. Большинство аннотированных генов определяет синтез аминокислот и их производных (255), углеводный обмен (195), белковый метаболизм (167), кофакторы, витамины, простетические группы и пигментные образования (87), нуклеозиды и нуклеотиды (78), метаболизм жирных кислот, липидов и изопреноидов (55) и метаболизм ДНК (68). Полногеномное секвенирование и аннотирование генома штамма Staphylococcus warneri подтвердило наличие у него углеводородокисляющих свойств. Выявлено два гена неохарактеризованных 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-диоксигеназу, фумарилацетоацетат-гидролазу и салицилат-1-монооксигеназу, участвующие в биодеградации ароматических углеводородов. Полученные данные последовательности генома позволяют лучше понять механизмы деградации (поглощения) углеводородов штаммом Staphylococcus warneri и его роль в бактериальном консорциуме.*

Ключевые слова: *Staphylococcus warneri*, *геном, секвенирование, биодеградация, 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[1] [8]. The soil suspension was introduced into a Voroshilova-Dianova agar medium followed by the addition of

[1] Koleshko O.I. Ecology of soil microorganisms: laboratory workshop for biological specialities of universities. Minsk: Vysheysh. shkola, 1981. 176 p.

diesel fuel (DF) in concentrations of 1, 3, 5, 7, 10 and 12 %. A sample without microorganisms containing 5 % of DF was used as a control. The cultivation was carried out in stirring (200 rpm) at 30 ºC in a thermostat chamber. The degree of oil film destruction on the medium surface was visually assessed over a period of 30 days. Further, the suspension was subcultured on a freshly-prepared medium. The isolation of pure HCOM cultures was carried out according to Koch's method on a meat-peptone agar medium[2].

Microorganisms identified by culturing on solid nutrient media underwent subsequent species identification by mass spectrometric analysis on a MALDI Microflex time-of-flight mass spectrometer (Bruker Daltonik GmbH, Germany) using the Flex Control program. Decisions concerning the taxonomic affiliations of the microorganisms were made on the basis of the coincidence index value (score value parameter, SV). Here, a SV ≥ 2.3 value corresponded to reliable identification of the species, SV = 2.299–2.000 indicated reliable identification to the extent of genus and probable identification to the extent of species, while an SV value in the range of 1.7–1.999 was taken as a probable identification concerning the extent of genus and SV < 1.7 was considered to be an unreliable result.

Sequencing was performed on the basis of the Interdisciplinary Centre for Collective Use of Kazan Federal University. The isolate was cultured for 24 h at 37 ºC on an agar medium with Luria broth (LB), followed by the isolation of genomic DNA using the ZymoBIOMICS DNA Miniprep kit (Zymoresearch, USA) with preliminary lysozyme treatment. The genomic DNA fragments were obtained by sonication using a Covaris S220 instrument in accordance with the manufacturer's recommendations (Covaris, USA). Using the obtained fragments, a DNA library was created by NEBNext Ultra II kits (NEB, USA) according to the manufacturer's instructions. Quality assessment was carried out on 2100 Bioanalyser chips (Agilent Technologies, USA). The resulting libraries were sequenced on the MiSeq Illumina platform (Illumina, USA) using the MiSeq® Reagent Kit v3 sequencing reagent kit.

RESULTS AND DISCUSSION

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 xylosoxidans, 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.

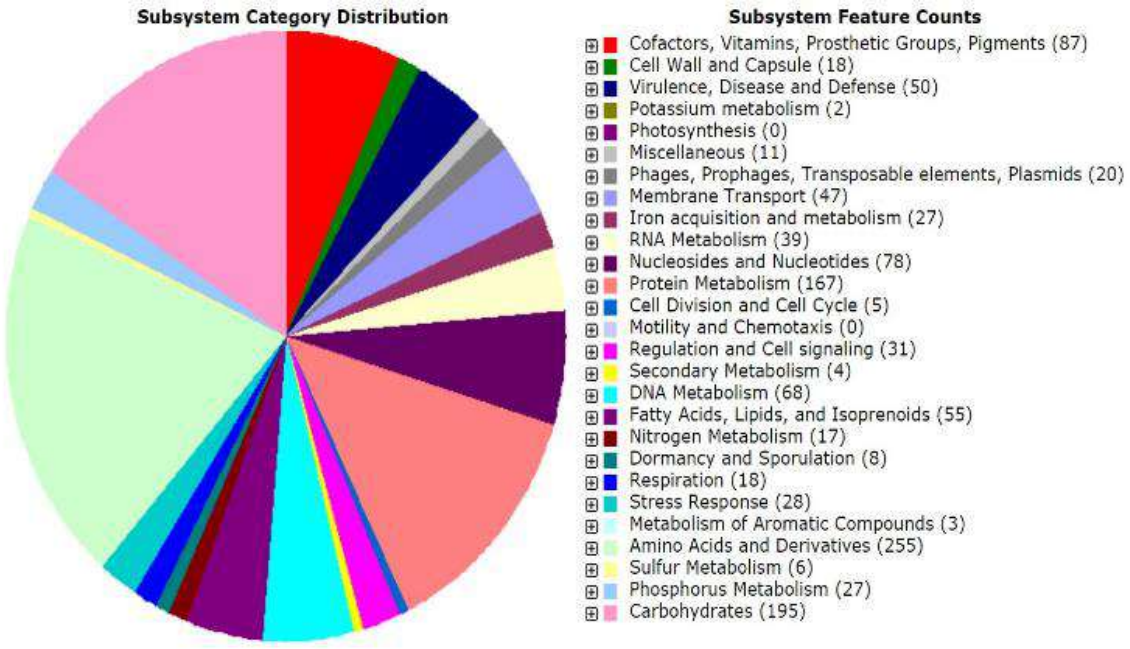
In the sequencing of the *S. warneri* genome, 1075522 readings of 300 bp were yielded, with an average GC content of 32.7 %. Genomic sequence assembly, analysis and automatic reporting performed using SPAdes v. 3.11.1 [15] resulted in a total of 54 contigs with 26 sized over 500 bp.

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 hydrolase and salicylate-1-monooxygenase 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 monooxygenases likely to be involved in biodegradation of alkanes. Biodegradation of alkanes takes place in several stages.

[2] Methods of soil microbiology and biochemistry: textbook / ed. D.G. Zvyagintsev. Moscow: Publishing House of Moscow State University, 1991. 304 p.



Subsystem category distribution for protein-coding genes of Staphylococcus warneri (based on RAST annotation server)

Распределение генов Staphylococcus warneri, кодирующих белки, по функциональным категориям (на основе сервера аннотаций RAST)

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, Tatyana 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, Tatyana 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.

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INFORMATION ABOUT THE AUTHORS

Irina A. Degtyareva,
Dr. Sci. (Biology), Chief Researcher,
Tatar Research Institute of Agricultural Chemistry and Soil Science – Subdivision of the Federal Research Center "Kazan Scientific Center of Russian Academy of Sciences",
20a Orenburg trakt, Kazan 420059,
Russian Federation;
Professor,
Kazan National Research Technological University,
68 Karl Marx St., Kazan 420015,
Russian Federation,
✉ e-mail: peace-1963@mail.ru

Edward V. Babynin,
Cand. Sci. (Biology), Associate Professor,
Kazan Federal University (Institute of Fundamental Medicine and Biology),
18 Kremlin St., Kazan 420008,
Russian Federation;
Senior Researcher,
Tatar Research Institute of Agricultural Chemistry and Soil Science – Subdivision of the Federal Research Center "Kazan Scientific Center of Russian Academy of Sciences",
20a Orenburg trakt, Kazan 420059,
Russian Federation,
e-mail: edward.b67@mail.ru

Tatyana Yu. Motina,
Cand. Sci. (Biology), Senior Researcher,
Tatar Research Institute of Agricultural Chemistry and Soil Science – Subdivision of the Federal Research Center "Kazan Scientific Center of Russian Academy of Sciences",
20a Orenburg trakt, Kazan 420059,
Russian Federation,
e-mail: motina.tatyana@mail.ru

СВЕДЕНИЯ ОБ АВТОРАХ

Дегтярева Ирина Александровна,
д.б.н., доцент, главный научный сотрудник,
Татарский научно-исследовательский институт агрохимии и почвоведения – обособленное структурное подразделение Федерального государственного бюджетного учреждения науки «Федеральный исследовательский центр «Казанский научный центр Российской академии наук»,
420059, г. Казань, Оренбургский тракт, 20а,
Российская Федерация;
профессор,
Казанский национальный исследовательский технологический университет,
420015, г. Казань, ул. Карла Маркса, 68,
Российская Федерация,
✉ e-mail: peace-1963@mail.ru

Бабынин Эдуард Викторович,
к.б.н., доцент,
Институт фундаментальной медицины и биологии,
Казанский (Приволжский) федеральный университет,
420008, г. Казань, ул. Кремлевская, 18,
Российская Федерация,
старший научный сотрудник,
Татарский научно-исследовательский институт агрохимии и почвоведения – обособленное структурное подразделение Федерального государственного бюджетного учреждения науки «Федеральный исследовательский центр «Казанский научный центр Российской академии наук»;
старший научный сотрудник,
420059, г. Казань, Оренбургский тракт, 20а,
Российская Федерация;
e-mail: edward.b67@mail.ru

Мотина Татьяна Юрьевна,
к.б.н., старший научный сотрудник,
Татарский научно-исследовательский институт агрохимии и почвоведения – обособленное структурное подразделение Федерального государственного бюджетного учреждения науки «Федеральный исследовательский центр «Казанский научный центр Российской академии наук»,
420059, г. Казань, Оренбургский тракт, 20а,
Российская Федерация,
e-mail: motina.tatyana@mail.ru

Mansur I. Sultanov,
Junior Researcher,
Tatar Research Institute of Agricultural Chemistry and Soil Science – Subdivision of the Federal Research Center "Kazan Scientific Center of Russian Academy of Sciences",
20a Orenburg trakt, Kazan 420059,
Russian Federation,
e-mail: niiaxp2@mail.ru

Султанов Мансур Ильдарович,
младший научный сотрудник,
Татарский научно-исследовательский институт агрохимии и почвоведения – обособленное структурное подразделение Федерального государственного бюджетного учреждения науки «Федеральный исследовательский центр «Казанский научный центр Российской академии наук»,
420059, г. Казань, Оренбургский тракт, 20а,
Российская Федерация,
e-mail: niiaxp2@mail.ru