ISSN 2518-1483 (Online),
ISSN 2224-5227 (Print)

2020 • 6

ҚАЗАҚСТАН РЕСПУБЛИКАСЫ
ҰЛТТЫҚ ҒЫЛЫМ АКАДЕМИЯСЫНЫҢ

БАЯНДАМАЛАРЫ

ДОКЛАДЫ

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК
РЕСПУБЛИКИ КАЗАХСТАН

REPORTS

OF THE NATIONAL ACADEMY OF SCIENCES
OF THE REPUBLIC OF KAZAKHSTAN

PUBLISHED SINCE 1944

ALMATY, NAS RK

ҚАЗАҚСТАН РЕСПУБЛИКАСЫ
ҰЛТТЫҚ ҒЫЛЫМ АКАДЕМИЯСЫНЫҢ
БАЯНДАМАЛАРЫ **2020 • 6**

«Қазақстан Республикасы Ұлттық ғылым академиясының баяндамалары»
ISSN 2518-1483 (Online),
ISSN 2224-5227 (Print)

Меншіктенуші: «Қазақстан Республикасының Ұлттық ғылым академиясы» Республикалық қоғамдық бірлестігі (Алматы қ.).
Қазақстан Республикасының Ақпарат және қоғамдық даму министрлігінің Ақпарат комитетінде 29.07.2020 ж. берілген **№ KZ93VPY00025418** мерзімдік басылым тіркеуіне қойылу туралы куәлік.

Тақырыптық бағыты: *наноматериалдар алу, биотехнология және экология саласындағы бірегей зерттеу нәтижелерін жариялау.*

Мерзімділігі: жылына 6 рет.
Тиражы: 500 дана.

Редакцияның мекенжайы: 050010, Алматы қ., Шевченко көш., 28; 219, 220 бөл.; тел.: 272-13-19, 272-13-18,
http://reports-science.kz/index.php/en/archive

Типографияның мекенжайы: «NurNaz GRACE», Алматы қ., Рысқұлов көш., 103.

ДОКЛАДЫ **2020 • 6**
НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК
РЕСПУБЛИКИ КАЗАХСТАН

Доклады Национальной академии наук Республики Казахстан»
ISSN 2518-1483 (Online),
ISSN 2224-5227 (Print)

Собственник: Республиканское общественное объединение «Национальная академия наук Республики Казахстан» (г. Алматы).
Свидетельство о постановке на учет периодического печатного издания в Комитете информации Министерства информации и общественного развития Республики Казахстан **№ KZ93VPY00025418**, выданное 29.07.2020 г.

Тематическая направленность: *публикация оригинальных результатов исследований в области получения наноматериалов, биотехнологии и экологии.*

Периодичность: 6 раз в год.
Тираж: 500 экземпляров

Адрес редакции: 050010, г.Алматы, ул.Шевченко, 28; ком. 219, 220; тел. 272-13-19, 272-13-18,
http://reports-science.kz/index.php/en/archive

Адрес типографии: «NurNaz GRACE», г. Алматы, ул. Рыскулова, 103.

REPORTS **2020 • 6**
OF NATIONAL ACADEMY OF SCIENCES OF THE
REPUBLIC OF KAZAKHSTAN

Reports of the National Academy of Sciences of the Republic of Kazakhstan.
ISSN 2224-5227
ISSN 2518-1483 (Online),
ISSN 2224-5227 (Print)

Owner: RPA "National Academy of Sciences of the Republic of Kazakhstan" (Almaty).
The certificate of registration of a periodical printed publication in the Committee of information of the Ministry of Information and Social Development of the Republic of Kazakhstan **No. KZ93VPY00025418**, issued 29.07.2020.

Thematic scope: *publication of original research results in the field of obtaining nanomaterials, biotechnology and ecology.*

Periodicity: 6 times a year.
Circulation: 500 copies.

Editorial address: 28, Shevchenko str., of. 219, 220, Almaty, 050010, tel. 272-13-19, 272-13-18,
http://reports-science.kz/index.php/en/archive

Address of printing house: «NurNaz GRACE», 103, Ryskulov str, Almaty.

REPORTS OF THE NATIONAL ACADEMY OF SCIENCES OF THE REPUBLIC OF KAZAKHSTAN
ISSN 2224-5227 **https://doi.org/10.32014/2020.2518-1483.141**
Volume 6, Number 334 (2020), 96 – 102

UDC 551.461:502.36
ISTIC 34.35.33

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USE OF MODERN METHODS OF IDENTIFICATION OF HYDROCARBON CONTAINING MICROORGANISMS ISOLATED FROM THE MARINE ENVIRONMENT OF THE CASPIAN SEA

Abstract. Currently, the problem of the negative impact of petroleum hydrocarbons in the Caspian Sea has become catastrophic. Intensive pollution of marine waters by oil and oil products, from production, transportation and storage of hydrocarbons leads to the oppression of the sea ecosystem. Mechanical and physico-chemical methods for cleaning the marine environment are characterized by low efficiency, secondary pollution and high cost. The most promising is the use of microbiological methods for cleaning waters from oil pollution. In the article presents the results of the identification of reactive oxidizing bacteria isolated from the marine environment of the Caspian Sea, for further use in new biopreparation from oil pollutions.

From the sea water in bulk berths Aktau sea port and the port of Bautino (North Caspian) allocated 27 hydrocarbon isolates of microorganisms having the ability to oil degradation of these strains selected the 4 most active cultures, which were identified as Bacillus cereus (2 strains), strain Bacillus sr.13 and Acinetobacter sr.10.

Article is written by results of the scientific project of grant financing of Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan "Activization of the self-clearing ability of sea water of the Caspian Sea from oil products". Article is issued thanks to the international project Tempus of IV IEMAST of "Establishing Modern Master-level Studies in Industrial Ecology".

Key words: identification, morphological, cultural and biochemical properties of oil destructors, sequencing, pure culture.

Introduction. The North Caspian is a unique water area, the hydrological and hydrochemical regime of which is formed by complex processes due to frequent storm activity, water convergence, shallow water, salinity variability and the influence of river flow.

In the general chain of human-hydrosphere interaction, an important link belongs to the management of the marine environment, where high economic activity is carried out and flows of pollutants coming from both the land and the sea face. Recently, water areas have suffered most from oil pollution, mostly of anthropogenic origin. Intensive marine pollution by oil and oil products, from production, transportation and storage of hydrocarbons lead to the oppression of the sea ecosystem. The most promising is the use of microbiological methods for cleaning waters from oil pollution. This is because the microorganisms effectively, quickly and without additional damages to the ecosystem can remove.

Microflora is a sensitive indicator of changes in the conditions of their environment, which is formed from various physiological groups of microorganisms. The number and activity of microflora are largely dependent on environmental and geographical factors. The processes taking place in water bodies are closely related to the number of microorganisms.

It is in a situation of developing oil fields that it is of interest to monitor studies, identify patterns of distribution of microscopic organisms, and use highly active strains to create biological products for cleaning the marine environment from oil products.

Main part. For the most complete and quick purification of waters from oil products cheaper to use aboriginal strains of microorganisms that do not need to adapt to the environment into which they are introduced. These bacteria begin destruction of oil faster than those microorganisms that allocated from other biotopes.

For the emergence of biologics to allocate initially from seawater active bacteria-oil destructors, determine their hydrocarbon-oxidizing activity, make an active consortium of microorganisms, identify, etc.

The aim of this study was the identification of active oil destructors strains isolated from seawater near the port of Bautino.

To achieve this goal, the following tasks were:

1. Conducting primary identification of the strains by culture-morphological, physiological and biochemical properties;

2. Phylogenetic identification of strains carrying-based sequencer variable regions of the genes encoding 16S rRNA;

The object of the research were 4 strains of microorganisms: Bacillus sp. 7 (B-1), Arhtrobacter sp. 13 (P-7), Bacillus sp. 27 (B-4) and Serratia sp. 10 (S-8) which was obtained from seawater near the Caspian Sea Bautino port.

To identify strains used cytochemical methods and biochemical researches.

Preliminary identification of the isolated microorganisms was performed by culture-morphological, physiological and biochemical characteristics, using the work of many authors [1-8].

For the identification of genetic strains were sent to the "State Research Institute of Genetics" PA on solid medium and M9 as well as IIB liquid medium, cell titers was 106 CFU / ml. The species identification was performed by polymerase chain reaction and further sequencing of PCR fragments of 16S rRNA gene using a universal primer system [9-21].

Thus, held primary identification obtained pure cultures. Research was performed on liquid or solid nutrient mediums are shown in (table 1).

Table 1 – Growth of pure cultures in various mediums

Name of culture	Growth in the BCH	Growth in the IPA	Growth on M9 with oil
Bacillus sp. 7 (B-1)	Abundant growth in the form of flakes of cotton, lumps suspended	Colonies larger than 2 cm in diameter, grow on agar, with not even a fringed edge, folded, convex, dull, gray	colony point, convex, gray, dull, flat edge of the colonies, mucous consistency
Serratia sp. 10 (S-8)	Weak growth film on a surface of the medium	colony of large, round with smooth edge, slimy, transparent, beige	colony point, convex, glossy, gray-pink, mucous
Arhtrobacter sp. 13(P-7)	Uniform turbidity environment	colonies are round with a straight edge, slimy, beige, black pigment allocates to meduim	point, convex, glossy, gray
Bacillus sp. 27 (B-4)	Weak growth film on a surface of the medium	colonies are round with no flat edge eroded, convex, dull, gray, gray pigment forms	colony point, convex, gray, frosted

A result of research noted that the two strains (S-8, B-4) with an increase of the liquid medium is observed weak growth to form a film on the surface of the medium. In strain R-7 noted uniform turbidity environment. The most intensive growth to form flakes and lumps noted for strain B-1 (table 1).

When grown in IPA noted that among the studied cultures for two strains (P-7 and B-4) is characterized by the appearance of the pigment on the surface of nutrient agar, colonies of all strains are characterized by large size, intensively proliferating medium surface. Strains B-1 and B-4 are characterized by a matte surface of the colonies, strains S-8 P-7 and surface gloss. When grown in M9 medium for the growth of all the strains noted point convex colonies. Strains B-1 and B-4 M9 medium matte, and strains S-8 P-7 and glossy.

When studying the cytochemical properties of the strains noted that three strains lack of acid resistance characteristic of the cell walls. The results are shown in (table 2).

Table 2 – Results of the morphological properties of the pure cultures of

Name of culture	Gram stain	Determination of acid-fast bacilli by Ziehl-Nielsen	Stained bacterial spores by Ziehl-Nielsen modified by Mueller
Bacillus sp.7 (B-1)	T + Sticks, 0.8 x 2.7 m	Acid-fast, painted in red	Spores, painted in bright red color. Vegetative cells painted over in blue
Serratia sp. 10 (S-8)	T-short rods and cocci, 1x2 m	Acid-resistant, painted in red color	No controversy, only vegetative cells painted in blue color
Arhtrobacter sp. 13(P-7)	T + sticks, 0.6 x3, 5 microns	Acid-resistant, painted in red color	Spores, painted in bright red color
Bacillus sp. 27 (B-4)	T + large sticks, 1,2 x 4 m	Acid-resistant, painted in red color	Spores, painted in bright red color

A result of research noted that the 3 strains are spore-forming rods + T (table 2). All test strains are aerobic and one can assume that the 3 strains belong to the group of Gram-positive rods, endospore forming the genus Bacillus. Furthermore, the strain Serratia sp. 10 (S-8) and does not form a acid-enodospor strain.

Thus, as a result of studying the cultural-morphological properties of pure cultures found that 3 strains (B-1, B-4, F-7) is identified as the genus Bacillus, a strain of S-8 belongs to the expectation number Bergey's Manual of Determinative Bacteriology 4 gram-negative aerobic / microaerophilic rods and cocci.

Study of the biochemical properties of pure cultures of the following results. Assimilation of different carbohydrates by pure cultures as a result of crop on semi-solid medium Hiss presented in (table-3).

Table 3 – The ability of pure cultures of fermentable carbohydrates

carbohydrate	B-1	B-4	P-7	S-8
arabinose	++	+	++	+++
xylose	++	+++	++	+++
glucose	+++	+++	+++	+++
levulose	++	++	+++	-
galactose	+++	+++	+++	+++
saccharose	+++	+++	+++	-
maltose	+++	+++	+++	-
lactose	++	+	++	++
dextrin	++	+	+	+
starch	++	++	++	+
cellulose	+	-	-	-

"+ + +" Strong, "+ +" Medium, "+" weak intensity bacterial growth, "-" no growth of bacteria

As a result of studies on the ability of the strains for fermentation of carbohydrate with "mottled number" indicated that all tested strains actively use glucose and galactose. Strains B-1, B-4, F-7 is also active against sucrose and maltose. The average intensity of the use of these strains is characteristic of carbohydrates arabinose, levulose, lactose and starch. Low activity splitting characterized dextrin. It has been established that fiber is not cleaved strains B-4 and R-7. For strain S-8 also characterized by intensive use of arabinose and xylose carbohydrates, less widely used lactose. Weak growth rate on media marked with dextrin and starch, and levulose, maltose, sucrose and cellulose does not cleave this strain (table 3).

The results of the study of the proteolytic activity, the ability to form ammonia, hydrogen sulfide and indole are shown in (table 4).

Table 4 – The ability of the strains to the formation of a protease, ammonia, hydrogen sulfide and indole

property	B-1	B-4	P-7	S-8
protease Activity	+	+	+	-
the formation of ammonia	+	+	+	+
formation of indole	-	-	-	-
formation of hydrogen sulfide	+	-	+	-
Attitude to oxidase	+	+	+	+
catalase	+	+	+	+

"+" - The bacterial strain showing the property, "-" - no part of the properties of the strain.

The study showed found that strains B-1, B-4, R-7, showing the ability to synthesize proteases formation of ammonia, oxidase and catalase do not form indole. Strains B1 and R 7 are capable of forming hydrogen sulphide, the strains B-4 S-8, and do not form hydrogen sulfide. Strain S-8 is able to form ammonia, catalase and oxidase positive, the protease does not produce hydrogen sulfide and indole.

For genetic identification of pure cultures were sent to the "State Research Institute of Genetics" (Moscow, Russian Federation), where strains were performed primary screening studies to identify pathogenic, opportunistic and pathogenic species. As a result of primary screening of the strains B-1 and B-4 are related to an Bacillus cereus. This kind of relates to microorganisms pathogenic microflora capable of causing foodborne diseases, produce enterotoxins. This type of bacteria is not used in the composition of biologics without additional toxicity studies strains and their metabolites in warm-blooded animals. Accordingly, these strains for further studies can not be used and their sequencing was conducted.

During the sequencing of variable regions of 16S rRNA strains R-7 and S-8 nucleotide sequences obtained for both strains.

As a result, primary screening nucleotide sequence of strain R-7 on the GenBank database, and RDP-II found that the investigated strain belongs to the following taxonomic groups of Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus, and homology with some species of the genus Bacillus is 98%.

Sequences were aligned with the corresponding sequences of a species of bacteria nearest available from the database GenBank.

According to the analysis of phylogenetic tree was constructed with homologous strains figure 1.

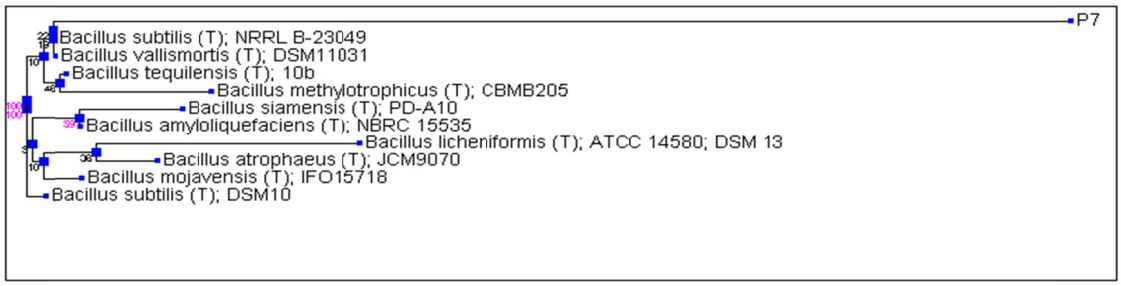

Figure 1 – Phylogenetic position of strain R-7

Criterion for classifying a microorganism to a particular type of homology is considered no less than 97%. By this criterion, the test strains can be attributed to several species of the genus Bacillus.

Analysis of phylogenetic relationships, built using the type strains of closely related bacteria showed that the closest to the test strain is the type Bacillus subtilis. The level of 16S rRNA sequence similarity of strain R-7 with a view Bacillus subtilis was 97%.

As a result, primary screening nucleotide sequence of strain S-8 to the GenBank database, and RDP-II found that the investigated strain belongs to the following taxonomic groups of Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Acinetobacter.

Sequences were aligned with the corresponding sequences of a species of bacteria nearest available from the database GenBank.

According to the analysis of phylogenetic tree was constructed with homologous strains figure 2.

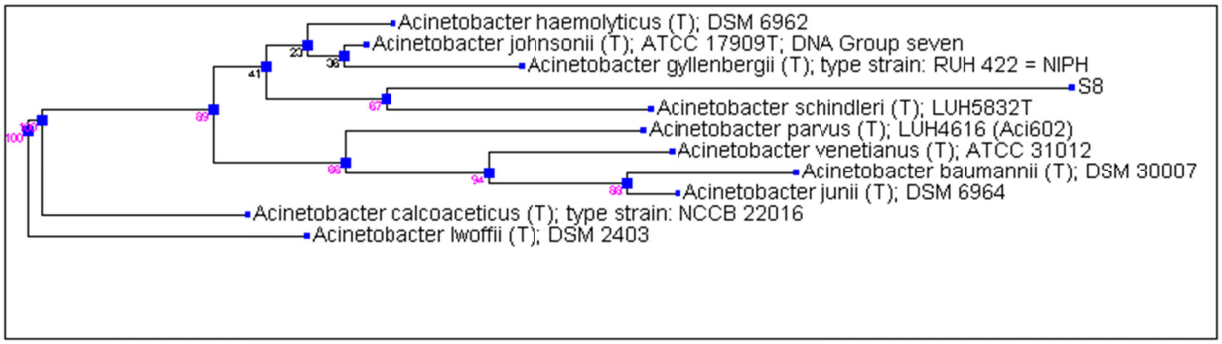

Figure 2 – Phylogenetic position of strain S-8

Criterion for classifying a microorganism to a particular type of homology is considered no less than 97%. By this criterion, the test strains can be attributed to several species of the genus Acinetobacter.

Analysis of phylogenetic relationships, built using the type strains of closely related bacteria showed that the closest to the test strain is the kind of Acinetobacter johnsonii. The level of 16S rRNA sequence similarity of the strain S-8 overlooking Acinetobacter johnsonii was 98%.

The analysis Sequence variable regions of genes encoding 16S rRNA for further work to study the ability of the strains to oil degradation are 2 strain: Bacillus subtilis and Acinetobacter johnsonii.

Conclusion. As a result, the primary screen to identify accessory to pathogenic strains, pathogenic and pathogenic microflora found that strains of B-1 and B-4 are members of the species Bacillus cereus. These representatives were opportunistic and therefore can not be used as a basis for a biological product.

Strains R-7 and S-8 were subjected Sequence analysis of variable regions of the genes encoding 16S rRNA. As a result of this study showed that the strain F-7 97% is representative of species Bacillus subtilis, a strain of S-8 98% is representative species Acinetobacter johnsonii.

For these strains are written passports for national deposit procedures. In addition, these strains will be used to create a domestic biological product.

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КАСПИЙ ТЕҢІЗІНІҢ ОРТАСЫНАН БӨЛІНГЕН КӨМІРСУТЕГІ БАР МИКРООРГАНИЗМДЕРДІ СӘЙКЕСТЕНДІРУДІҢ ҚАЗІРГІ ЗАМАНҒЫ ӘДІСТЕРІН ПАЙДАЛАНУ

Аннотация. Қазіргі уақытта Каспий теңізі аумағындағы мұнай көмірсутектерінің теріс әсер ету проблемасы апатты жағдайға жатады. Адам мен гидросфераның өзара әрекеттесуінің жалпы тізбегінде маңызды байланыс теңіз ортасын басқаруға кіреді, онда жоғары экономикалық қызмет жүзеге асырылады және құрлықтан да, теңізден де ластаушы заттардың ағыны пайда болады. Соңғы уақытта су айдыны антропогендік ластанудан қатты зардап шекті. Көмірсутекті шикізатты өндіру, тасымалдау және сақтау нәтижесінде теңіз акваторияларының мұнай және мұнай өнімдерімен қарқынды ластануы теңіз экожүйесінің тежелуіне әкеледі. Теңіз ортасын тазартудың механикалық және физика-химиялық әдістері төмен тиімділік, қайталама ластану және қымбаттығымен сипатталады. Су айдынын мұнайдың ластығынан тазартудың микробиологиялық әдістерін қолдану неғұрлым перспективалы болып келеді. Микрофлора микроорганизмдердің әртүрлі физиологиялық тобынан қалыптасатын қоршаған орта жағдайының өзгеруінің сезімтал көрсеткіші болып саналады. Микрофлора саны мен белсенділігі көбінесе экологиялық және географиялық факторларға байланысты. Су объектілерінде жүретін процестер микроорганизмдердің санымен тығыз байланысты. Мұнай кен орындарын игеру жағдайында зерттеу мониторингі, микроскопиялық организмдердің таралу заңдылықтарын анықтау, теңіз ортасын мұнай өнімдерінен тазартуда биологиялық өнімдер жасау үшін жоғары белсенді штамдарды пайдалану қызығушылық тудырады. Мақалада мұнаймен ластануда жаңа биопрепаратты одан әрі пайдалану үшін Каспий теңізінің ортасынан бөлінген мұнай тотықтырғыш бактериялардың белсенді түрлерін сәйкестендіру нәтижелері келтірілген.

Бір-бірімен тығыз байланысты бактериялардың типтік штамдарын қолдану арқылы салынған филогенетикалық байланыстарды талдау Acinetobacter johnsonii түрі сыналған штамға жақын екенін көрсетті. S-8 штаммының 16S рРНҚ тізбегінің Acinetobacter johnsonii-мен ұқсастық деңгейі 98% құрады.

рРНҚ 16S кодтайтын геннің ауыспалы аймақтарының реттілігіне талдау жасалды, штамдардың мұнайдың деградациясына қабілеттілігін зерттеу үшін 2 штамм бөлінді: бацилла-субтилис және Acinetobacter johnsonii тудырған инфекциялар.

Теңіз суынан Ақтау теңіз порты мен Баутино (Солтүстік Каспий) портының құю айлақтары ауданында мұнайды деструкциялауға қабілеті бар 27 көмірсутекті қышқылдайтын микроорганизм оқшаулағышы бөлінді, осы штамнан 4 неғұрлым белсенді дақыл іріктелді, олар Bacillus cereus (2 штамм), Bacillus sp штамы ретінде сәйкестендірілді (13 және Acinetobacter sp.10).

Патогенді және патогенді микрофлораның патогенді штамына тиістілігін анықтау үшін бастапқы скрининг нәтижесінде в-1 және в-4 штаммдары Bacillus cereus түрлерінің өкілдері екендігі анықталды. Бұл өкілдер оппортунистік болып саналады, сондықтан оларды биологиялық өнім үшін негіз ретінде пайдалану мүмкін емес. R-7 және S-8 штамы 16S рРНҚ-ны кодтайтын геннің ауыспалы аймағының тізбегі талданды. Осы зерттеу нәтижесінде F-7 штамы 97% Bacillus subtilis түрінің өкілі, ал S-8 штамы 98% Acinetobacter johnsonii түрінің өкілі екендігі көрсетілді.

Мақала Қазақстан Республикасы Білім және ғылым министрлігі Ғылым комитетінің «Каспий теңізінің теңіз суының мұнай өнімдерінен өздігінен тазаланатын қабілетін жандандыру» гранттық қаржыландыру ғылыми жобасының нәтижелері бойынша және Tempus IV IEMAST «Өнеркәсіптік экологиядағы заманауи магистрлік зерттеулерді құру» халықаралық жобасының негізінде жарияланды.

Түйін сөздер: сәйкестендіру, морфологиялық-мәдени және биохимиялық қасиеттер, май деструкторы, жүйелеу, таза дақылдар.

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ИСПОЛЬЗОВАНИЕ СОВРЕМЕННЫХ МЕТОДОВ ИДЕНТИФИКАЦИИ УГЛЕВОДОРОДОСОДЕРЖАЩИХ МИКРООРГАНИЗМОВ, ВЫДЕЛЕННЫХ ИЗ МОРСКОЙ СРЕДЫ КАСПИЯ

Аннотация. В настоящее время проблема негативного воздействия нефтяных углеводородов на территории Каспийского моря приобрела катастрофический характер. В общей цепи взаимодействия человека и гидросферы важное звено принадлежит управлению морской средой, где осуществляется высокая хозяйственная деятельность и возникают потоки загрязняющих веществ, поступающих как с суши, так и с моря. В последнее время акватории наиболее сильно пострадали от нефтяного загрязнения, в основном антропогенного происхождения. Интенсивное загрязнение морских акваторий нефтью и нефтепродуктами в результате добычи, транспортировки и хранения углеводородного сырья приводят к угнетению экосистемы моря. Механические и физико-химические методы очистки морской среды характеризуются низкой эффективностью, вторичным загрязнением и дороговизной. Наиболее перспективным является использование микробиологических методов очистки акваторий от нефтяных загрязнений. Микрофлора является чувствительным индикатором изменения условий окружающей их среды, которая формируется из различных физиологических групп микроорганизмов. Численность и активность микрофлоры в значительной степени зависят от экологических и географических факторов. Процессы, происходящие в водных объектах, тесно связаны с численностью микроорганизмов. Именно в условиях освоения нефтяных месторождений представляет интерес мониторинг исследований, выявление закономерностей распространения микроскопических организмов, использование высокоактивных штаммов для создания биопрепаратов по очистке морской среды от нефтепродуктов. В статье представлены результаты идентификации активных форм нефтеокисляющих бактерий, выделенных из морской среды Каспия, для дальнейшего их использования в новом биопрепарате от нефтяных загрязнений.

Анализ филогенетических связей, построенных с использованием типовых штаммов близкородственных бактерий, показал, что наиболее близким к тестируемому штамму является вид Acinetobacter johnsonii. Уровень сходства последовательностей 16S рРНК штамма S-8 с Acinetobacter johnsonii составил 98%.

Проведен анализ последовательности вариабельных участков генов, кодирующих 16S рРНК, для дальнейшей работы по изучению способности штаммов к деградации нефти выделены 2 штамма: Bacillus subtilis и Acinetobacter johnsonii.

Из морских вод в районе наливных причалов Актауского морского порта и порта Баутино (Северный Каспий) выделено 27 углеводородокисляющих изолятов микроорганизмов, обладающих способностью к деструкции нефти, из данных штаммов отобрано 4 наиболее активные культуры, которые были идентифицированы как *Bacillus cereus* (2 штамма), штамм *Bacillus sp.*13 и *Acinetobacter sp.*10.

В результате первичного скрининга для выявления принадлежности к патогенным штаммам патогенной и патогенной микрофлоры установлено, что штаммы в-1 и в-4 являются представителями вида Bacillus cereus. Эти репрезентативы были условно-патогенными и поэтому не могут быть использованы в качестве основы для биологического продукта. Штаммы R-7 и S-8 подвергали анализу последовательности вариабельных участков генов, кодирующих 16S рРНК. В результате этого исследования было показано, что штамм F-7 на 97% является представителем вида Bacil-lus subtilis, а штамм S-8 на 98% является представителем вида Acinetobacter johnsonii.

Статья написана по результатам научного проекта грантового финансирования Комитета науки Министерства образования и науки Республики Казахстан «Активизация самоочищающейся способности морской воды Каспийского моря от нефтепродуктов». Статья опубликована благодаря международному проекту Tempus IV IEMAST «Создание современных магистерских исследований в промышленной экологии».

Ключевые слова: идентификация, морфолого-культуральные и биохимические свойства, нефтедеструкторы, секвенирование, чистые культуры.

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ISSN 2518-1483 (Online), ISSN 2224-5227 (Print)

http://reports-science.kz/index.php/en/archive

Редакторы: *М. С. Ахметова, Д. С. Аленов, А. Ахметова*

Верстка на компьютере *А. М. Кульгинбаевой*

Подписано в печать 04.12.2020.
Формат 60x881/8. Бумага офсетная. Печать – ризограф.
8,25 п.л. Тираж 500. Заказ 6.