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G.K. Kaiyrmanova[1], Sh.Zh. Tapeshova[1], U.T. Shaimerdenova[1], R.B. Magmiyayev[2], A.K. Yernazarova[1*]

[1]Al-Farabi Kazakh National University, Kazakhstan, Almaty
[2]Scientific Research Institute of Ecology Problems,
Al-Farabi Kazakh National University, Kazakhstan, Almaty
*e-mail: aliya.yernazarova@kaznu.kz

IDENTIFICATION OF MICROORGANISMS ISOLATED FROM OIL RESERVOIR WATER OF THE AKINGEN FIELD, KAZAKHSTAN

Currently, there is a continuous growth of deposits in Western Kazakhstan that are in the late stage of development. Deposits often have a complex heterogeneous structure with hard-to-recover reserves, so for their effective development it is necessary to apply methods of increasing oil recovery. One of the most effective methods of enhanced oil recovery is microbial enhanced oil recovery (MEOR). Microorganisms of the developed oil-reservoir waters, adapted to the extreme underground conditions of the reservoirs, are promising objects for the development of microbiological methods for increasing oil recovery, based on their ability to displace and dilute oil. The article presents the identification of aerobic microorganisms, waterlogged oil reservoir waters of the Akingen field. The aim of this study was to identify microorganisms of oil-reservoir waters of the Akingen field isolated under aerobic conditions. Traditional microbiological and genetic methods of identification of microorganisms are used in the research work. The enzymatic activity of the isolated strains (lipase, amylolytic, proteolytic activity) was evaluated. The emulsification index was carried out according to the Cooper method. As a result of microbiological and genetic studies of the nucleotide sequence of the 16S rRNA gene fragment, 14 aerobic strains of microorganisms were identified as representatives of the genus Pseudomonas and Bacillus, in particular, Bacillus paramycoides-M1; B. subtilis subsp. spizizenii-S1; Bacillus sp. – M2, A1, A2, A3, A4, A5, S2, S3, D-1X; P. aeruginosa-D5, D6, D7. The evaluation of emulsifying activity allowed us to identify 4 strains of microorganisms with a high oil emulsification index: P. aeruginosa-D5, D6, D7 (40-49 %) and Bacillus sp. D1X (32 %). Isolates P. aeruginosa – D5, D6, D7 and Bacillus sp. D1X are promising objects for use in enhanced oil recovery technologies.

Key words: microorganisms, oil reservoir water, identification, bacteria Bacillus, Pseudomonas aeruginosa, oil emulsification index.

Г.К. Кайырманова[1], Ш.Ж. Тапешова[1], Ұ.Т. Шаймерденова[1],
Р.Б. Магмияев[2], А.К. Ерназарова[1*]

[1]Әл-Фараби атындағы Қазақ ұлттық университеті, Қазақстан, Алматы қ.
[2]Әл-Фараби атындағы Қазақ ұлттық университетінің
экологиялар мәселелері ғылыми-зерттеу институты, Қазақстан, Алматы қ.
*e-mail: aliya.yernazarova@kaznu.kz

Ақінген мұнай кен орнының мұнай пласт суларынан бөлініп алынған микроорганизмдердің идентификациясы, Қазақстан

Қазіргі уақытта Батыс Қазақстанда мұнай игерудің соңғы сатысында тұрған кен орындары үздіксіз өсуде. Кен орындары көбінесе қиын өндірілетін қорлары бар күрделі гетерогенді құрылымға ие, сондықтан оларды тиімді игеру үшін мұнай шығуын арттыру әдістерін қолдану қажет. Мұнай шығуын арттырудың ең тиімді әдістерінің бірі – микроорганизмдер негізінде мұнай шығуын арттыру (MEOR). Экстремалды жер асты жағдайларына бейімделген мұнай пласт суларының микроорганизмдері мұнайды ығыстыру және сұйылту қабілетіне негізделген пласттардан мұнай шығуын арттырудың микробиологиялық әдістерін әзірлеу үшін перспективті объектілер болып табылады. Мақалада суландырылған Ақінген кен орнының мұнай пласт суларынан білініп алынған аэробты микроорганизмдердің идентификациясы берілген. Зерттеудің мақсаты Ақінген мұнай кен орнының мұнай пласт суларынан аэробты

жағдайда бөлініп алынған микроорганизмдердің идентификациясы анықтау болды. Зерттеу жұмысында микроорганизмдерді идентификациялаудың дәстүрлі микробиологиялық және генетикалық әдістері қолданылды. Бөлінген штаммдардың ферментативті белсенділігіне (липаза, амилолитикалық, протеолитикалық белсенділік) бағалау жүргізілді. Эмульгирлеу индексі Купер әдісімен жүргізілді. Геннің 16S rRNA фрагментінің нуклеотидтер тізбегін микробиологиялық және генетикалық зерттеу нәтижесінде микроорганизмдердің 14 аэробты штаммдары Pseudomonas және Bacillus тұқымдарының өкілдері ретінде анықталды, атап айтқанда Bacillus paramycoides – M1; B. subtilis subsp.spizizenii – S1; Bacillus sp. – M2, A1, A2, A3, A4, A5, S2, S3, D-1x; P. aeruginosa – D5, D6, D7. Эмульгирлеу белсенділікті бағалау барысында мұнай эмульсиясының жоғары индексіне микроорганизмдердің 4 штаммы ие болды: P. aeruginosa – D5, D6, D7 (40-49 %) және Bacillus sp. D1X (32 %). P. aeruginosa изоляттары – D5, D6, D7 және Bacillus sp. D1X пласттардан мұнай шығуын арттыру технологияларында пайдалану үшін перспективті объектілер болып табылады.

Түйін сөздер: микроорганизмдер, мұнай пласт суы, идентификация, Bacillus, Pseudomonas aeruginosa бактериялары, эмульгирлеу индексі.

Г.К. Кайырманова[1], Ш.Ж. Тапешова[1], Ұ.Т. Шаймерденова[1],
Р.Б. Магмияев[2], А.К. Ерназарова[1*]

[1]Казахский национальный университет имени аль-Фараби, Казахстан, г. Алматы
[2]НИИ Проблем Экологии Казахского национального университета
имени аль-Фараби, Казахстан, г. Алматы
*e-mail: aliya.yernazarova@kaznu.kz

Идентификация микроорганизмов, выделенных из нефтепластовых вод нефтяного месторождения Акинген, Казахстан

В настоящее время непрерывно растет месторождений, в Западной Казахстане которые находятся в поздней стадии разработки. Месторождения часто имеют сложную неоднородную структуру с трудноизвлекаемыми запасами, поэтому для их эффективной разработки необходимо применять методы увеличения нефтеотдачи. Одним из наиболее эффективных методов повышения нефтеотдачи является микробное повышение нефтеотдачи (MEOR). Микроорганизмы разработанных нефтепластовых вод, адаптированные к экстремальным подземным условиям пластов, являются перспективными объектами для разработки микробиологических методов увеличения нефтеотдачи пластов, основанные на их способности вытеснять и разжижать нефть. В статье дана идентификация аэробных микроорганизмов, заводненных нефтепластовых вод месторождения Акинген. Целью настоящего исследования явилась идентификация микроорганизмов нефтепластовых вод месторождения Акинген, выделенных в аэробных условиях. В исследовательской работе использованы традиционные микробиологические и генетические методы идентификации микроорганизмов. Проведена оценка ферментативной активности выделенных штаммов (липазная, амилолитическая, протеолитическая активность). Индекс эмульгирования проводилась по методу Купера. В результате микробиологических и генетических исследований нуклеотидной последовательности фрагмента 16S rRNA гена, идентифицированы 14 аэробных штаммов микроорганизмов как представители рода Pseudomonas и Bacillus, в частности, Bacillus paramycoides -M1; B. subtilis subsp.spizizenii -S1; Bacillus sp. – M2, A1, A2, A3, A4, A5, S2, S3, D-1X; P. aeruginosa – D5, D6, D7. Оценка эмульгирующей активности позволила выделить 4 штамма микроорганизмов с высоким индексом эмульгирования нефти: P. aeruginosa – D5, D6, D7 (40-49 %) и Bacillus sp. D1X (32 %). Изоляты P. aeruginosa – D5, D6, D7 и Bacillus sp. D1X являются перспективными объекты для использования в технологиях повышения нефтеотдачи пластов.

Ключевые слова: микроорганизмы, нефтепластовая вода, идентификация, бактерии Bacillus, Pseudomonas aeruginosa, индекс эмульгирования.

Introduction

One of the rapidly developing areas of modern microbiology is the study of the microflora of underground ecosystems [1]. Interest in the microorganisms of deep ecosystems is determined by the need to clarify the features of microbial diversity and establish the lower boundary of the biosphere, as well as the patterns of geochemical activity of microorganisms in the lower layers of the earth for the rational management of biogenic processes [2, 3].

Microbiological methods in the oil industry attract attention with low investment demand, high efficiency and environmental safety. Oil reservoir microorganisms have great biotechnological poten-

tial and are used in technologies for microbiological enhanced oil recovery [4].

Microorganisms of oil reservoirs have been the object of research since 1926. Oil reservoirs are generally characterized by oxygen-free conditions or low oxygen content. Anaerobic acetogens and methanogens of sulfate-, sulfur-, thiosulfate-, iron-reducing and fermenting prokaryotes have been identified in reservoir waters [5, 6, 7]. Aerobic bacteria of oil reservoirs are relatively poorly studied. However, aerobic microorganisms also live in oil reservoirs, where they usually enter with injected water, drilling mud, and as a result of natural hydrodynamic flows [8]. The injected water often contains dissolved oxygen, and an aerobic or microaerobic zone is created in part of the reservoir, where a group of bacteria can develop [9, 10]. In oil fields that are exploited using flooding, aerobic microorganisms are the initial link in the aerobic-anaerobic microbial trophic chain that performs microbial transformation of oil [11, 12]. It is known that the vital activity of microorganisms in reservoir waters with the formation of oil-substituting compounds, apparently, is the basis of microbiological methods for increasing oil recovery in flooded reservoirs [13]. Microbiological methods of enhanced oil recovery can increase current oil production by 10-15 %, which is comparable to the discovery of a new field [14]. Microbiological oxidation of residual heavy oil and its conversion to oil-displacing agents such as carbon dioxide, methane, and surfactants such as polysaccharides, alcohols, and fatty acids increase the mobility of the oil. These substances form an oil shaft and migrate through the reservoir with the water flow, displacing oil from the host rocks [15]. An important stage in the development of such technologies is the selection of bioagents capable of active life in extreme conditions of oil reservoirs.

The aim of this work was to identify the microorganisms isolated from the flooded reservoir water of the Akingen field.

Materials and methods

The Akingen oil field is located in the Atyrau region of Western Kazakhstan. The field was discovered in 1980, development began on September 1, 1992, and the productive horizon of the oil reservoir is located at a depth of 700-900 m. Oil reservoir water sample collected in spring 2018 From an active well in the Akingen oil field. The initial reservoir pressure is 6.2-12.8 MPa, the temperature is 34-47^{0}C, the pH of the oil reservoir water is 6.34±0.31. The oil density is 842-905 kg/m^3. Low-sulfur oils (0.15-0.28%), low-paraffin oils-0.88%. The regime of oil deposits is elastic-water-pressure. Formation waters of the chlorocalcium type, with a density of 1078-1105 kg/m^3 and a mineralization of 127.1-162.5 g/l. It is currently in the late stage of development, and the remaining amount of oil in the reservoirs is up to 60% of the original reserve.

The objects of the study were 14 strains of microorganisms isolated by the Koch method under aerobic conditions from the flooded reservoir waters of the Akingen field. The field, in turn, is at a late stage of development and needs to develop microbial methods to improve oil recovery.

To study the biological properties of microorganisms, the following nutrient media were used: *Nutrient Agar, Actinomycete Isolation Agar, Sabouraud Dextrose Agar, Nutrient Broth, MPG, Ashby medium* (HiMedia Laboratories, Mumbai, India). Aerobic microorganisms were cultured in stationary conditions at a temperature of 40 °C for 24-48 hours.

The study of the isolated microorganisms: morphological, cultural, physiological and biochemical properties of microorganisms. The purity of the isolated cultures of microorganisms was controlled by the generally accepted methods of 3-segment depletion stroke [16]. The phenotypic properties of microorganisms (macro -, micromorphology of microorganisms, cell morphology, motility, presence of spore formation, Gram color, physiological and biochemical characteristics) were studied using traditional (standard) methods [17, 18]. Microscopic studies were performed using a MOTIC B1-220A light binocular microscope (Spain) [19]. The identification of microorganisms was carried out by determining the direct nucleotide sequence of the 16S rRNA gene fragment, followed by determining the nucleotide identity with the sequences deposited in the international Gene Bank database, as well as constructing phylogenetic trees with the nucleotide sequences of the reference strains. Genomic DNA from bacterial cultures was isolated using the method of K. Wilson [20]. Quantitative DNA analysis was performed using a NanoDrop ND 2000 spectrophotometer at a wavelength of 260 nm. For amplification of the 16S RNA site, a reaction mixture of 25 µl was prepared: 12.5 µl Q5 ® Hot Start High-Fidelity 2X Master Mix (New England Biolabs Ins., USA); a pair of universal primers: 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 806R (5'-GGACTACCAGGGTATCTAAT-3') of 1.2 µl at 10 µM concentration; DNA matrix and water up to 25 µl. The aplication mode consisted of the fol-

lowing cycles: 95°C for 5 minutes, then: 95°C for 30 seconds, 55°C for 40 seconds, 72°C for 50 seconds for 30 cycles; elongation at 72°C for 10 minutes. PCR products were separated in 1 % agarose gel, the strips were stained with ethidium bromide and visualized in a UV transilluminator. A 1xTBE buffer was used as the electrode buffer. The PCR product was purified using the CleanSweep ™ cleaning reagent (ThermoFicher Scientific, USA). The 16S rRNA gene fragments were sequenced using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) according to the manufacturer's protocol [BigDye ® Terminator v3.1 Cycle Sequencing Kit Protocol Applied Biosystems USA]. To clean the sequencing products, the BigDye ® XTerminator ™ Purification Kit was used according to the manufacturer's protocol. Capillary phoresis was performed on a genetic analyzer ABI 3500 DNA Analyzer (Applied Biosystems, USA). The sequencing results were processed in the SeqA (Applied Biosystems) program. The search for homologous nucleotide sequences of 16S rRNA genes was carried out using the BLAST program (Basic Local Alignment Search Tool) in the International Gene Bank Database of the US National Center for Biotechnology Information (htpp://www.ncbi. nlm. nih.gov). The nucleotide sequences of 16S rRNA gene fragments obtained during the study of 14 bacterial strains are placed in GenBank under the numbers: MW386831; MW386832; MW386833; MW386834; MW386835; MW386836; MW386837; MW386838; MW386839; MW386840; MW386841; MW386842; MW386843; MW386844;

Phylogenetic analysis was performed using MEGAX software. The alignment of the nucleotide sequences was performed using the ClustalW algorithm. To construct phylogenetic trees, the Neiighbor-Joining (NJ) method was used [21].

The emulsification index was carried out according to the Cooper method [22]. Synthetic glycerin and sterile crude oil from the Akingen field were used to determine the oil emulsification index by microorganisms.

Experimental studies were conducted on the basis of an accredited laboratory of the Research Institute of Ecology of Problems.

Results

The Akingen oil field is located in the Atyrau region of Western Kazakhstan, in the south-eastern part of the Caspian Basin. Visual characteristics of the oil reservoir water of the Akingen field two-phase liquid, the upper layer is oil, brown color, the lower one is water. The initial reservoir pressure is 6.2-12.8 MPa, the temperature is 34-47^{0}C. Formation water of the chlorocalcium type, with a density of 1078-1105 kg/m^3 and a mineralization of 127.1-162.5 g/l.

Previously, as a result of studies conducted under aerobic conditions, 14 strains of microorganisms were isolated from the flooded oil reservoir of the Akingen field. Table 1 presents the results of morphological and cultural characteristics of microorganisms of oil-reservoir waters.

Table 1 – Differentiating characteristics of aerobic microorganisms isolated from water-flooded

№	Strains	The conditions of cultivation, t 40 ^{0}C	Cell shape	Gram stain	Spore formation	Mobility	Colony morphology
1	M1	aerobic	Long sticks, in pairs	G+	+	-	Shape round, surface smooth, flat, shiny, color white, edge smooth, 10 mm
2	M2	aerobic	Long and thin sticks that exist separately	G+	+	+	The edges of the colonies are smooth, round, the color is white, shiny, the top is flat, 3mm
3	A1	aerobic	Long sticks that exist separately, in pairs, and strepto	G+	+	+	The edges of the colony are rhizoid, cream color, forms mucus, the surface is convex, 10 mm
4	A2	aerobic	Short sticks that exist separately	G+	+	-	The colony is round, the color is white with a yellow tinge, the edges of the colony are smooth, the surface is smooth, shiny
5	A3	aerobic	Short sticks, separately and in pairs	G+	+	-	Colony with irregular edges, cream color, uneven surface, mucus forms, 7 mm

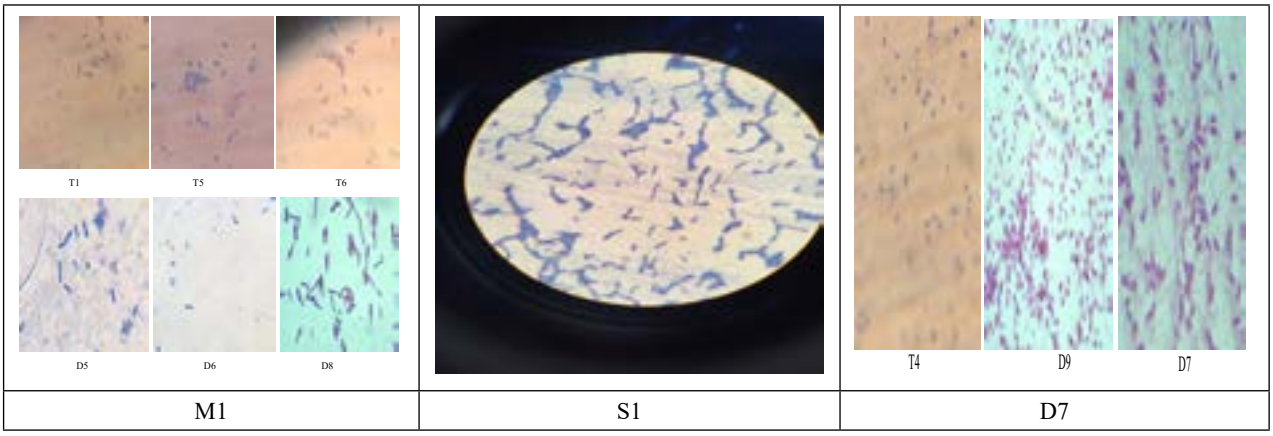
Table continuation

№	Strains	The conditions of cultivation, t 40 ^{0}C	Cell shape	Gram stain	Spore formation	Mobility	Colony morphology
6	A4	aerobic	Long sticks that exist separately	G+	+	+	Colony with irregular edges, color white, matte, surface smooth, 5 mm
7	A5	aerobic	Long sticks that exist separately	G+	+	-	The colony is round, the edges are smooth, matte, the color is white, the surface is smooth, 8 mm
8	S1	aerobic	Short sticks that exist separately, in pairs, and strepto	G+	+	-	The edges of the colonies are uneven, wavy, the surface is bumpy, the colony is matte, 4 mm
9	S2	aerobic	Long sticks that exist separately	G+	+	-	The colony is round with irregular edges, the surface is smooth, the colony is shiny
10	S3	aerobic	Long sticks that exist separately	G+	+	-	The edges of the colonies are wavy, matte, white color, smooth surface, 6 mm
11	D-1X	aerobic	Short sticks that exist separately, in pairs	G+	+	-	Colony round, folded surface, matte, slimy, wavy edge, 3 mm
12	D5	aerobic	Short sticks existing separately and in pairs	G-	-	+	Colonies are irregular, convex, shiny, slimy, with blue-green pigment, 2 mm
13	D6	aerobic	Short sticks that exist separately	G-	-	+	Colonies are irregular, convex, shiny, slimy 2-3 mm
14	D7	aerobic	Short sticks that exist separately	G-	-	+	Colonies are irregular, convex, shiny, slimy, with blue-green pigment, 2 mm

Note: «+» – the sign is present, «-» – the sign is absent

As can be seen, from 14 aerobic strains of microorganisms isolated from the reservoir water of the Akingen field: 11 strains of spore-forming, gram-positive (M1, M2, A1, A2, A3, A4, A5, S1, S2, S3, D1X) and 3 strains of non-spore-forming, gram-negative (D5, D6, D7), mono -, diplobacteria with different cell connections (Fig. 1). Cell motility is observed in 6 strains (M2, A1, A4, D5, D6, D7). Aerobic microorganisms were cultured in stationary conditions at a temperature of 40 °C for 24-48 hours

Table 2 shows the results of the physiological and biochemical characteristics that are necessary to determine the systematic position of various representatives of bacteria.



M1 S1 D7

Figure 1 – Morphology of gram-negative (D7) and gram-positive (M1, S1) bacterial cells (*nutrient Broth medium*, 48 hours of growth, type of Gram staining, 1000x)

Table 2 – Physiological and biochemical characteristics of aerobic microorganisms isolated from water-flooded

Signs \ Strain	M1	M2	A1	A2	A3	A4	A5	S1	S2	S3	D1X	D5	D6	D7
Catalase activity	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Amylolytic activity	+	+	+	+	+	+	+	+	+	+	+	-	-	-
Proteolytic activity	+	-	-	+	+	-	+	-	-	-	+	+	+	+
Use of molecular nitrogen	-	-	+	-	-	+	+	-	+	-	+	+	+	+
Lipolytic activity	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidaseactivity	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Note: «+» – the sign is present, «-» – the sign is absent														

As can be seen, all 14 aerobic strains of microorganisms are catalase-positive. 11 strains (M1, M2, A1, A2, A3, A4, A5, S1, S2, S3, D1 X) hydrolyze starch, hydrolysis zones vary from 1-7 mm, 3 strains (D5, D6, D7) do not hydrolyze starch. Proteolytic activity is observed in 8 strains (M1, A2, A3, A5, D1 X, D5, D6, D7), 6 strains of non-diluting gelatin (M2, A1, A4, S1, S2, S3). Molecular nitrogen uses 8 strains (A1, A4, A5, S2, D1, D5, D6, D7). Oxidase activity was detected in 3 strains (D5, D6, D7), oxidase-negative-in 11 strains (M1, M2, A1, A2, A3, A4, A5, S1, S2, S3, D1). Lipolytic activity was not detected in all 14 strains.

As a result of studying the main morphological, cultural, physiological, and biochemical characteristics of 14 strains isolated under aerobic conditions from oil-reservoir waters, we were able to identify them to their generic identity, so 11 strains of microorganisms (M1, M2, A1, A2, A3, A4, A5, S1, S2, S3, D1) were assigned to the genus *Bacillus,* 3 strains of microorganisms (D5, D6, D7) to the genus *Pseudomonas.*

To determine the species identity of these isolates, genotyping was performed at the conservative 16S rRNA locus (Table 3). Taking into account the literature data (Kumar et al., 2018), indicating the presence of GeneBank nucleotide sequences in international banks (http://www.ncbi.nlm.nih.gov/), we additionally constructed phylogenetic trees with the nucleotide sequences of the 16S rRNA gene of the reference strains of these species. The analysis included the nucleotide sequences of the 16S rRNA gene, the most phylogenetically related to microorganisms (Fig. 2, 3). Table 3 shows the results of identification of strains of microorganisms.

Table 3 – The results of the identification of strains by fragment 16S rRNA gene

№	Strains	Closest taxon (GenBank accession no.)	Percent identity %	Identification
1	*M1*	NR_157734.1	92	*Bacillus paramycoides*
2	*M2*	NR_041794.1 NR_148786.1	97	*Bacillus sp.* *B.safensis/* *B. pumilus/*
3	*A1*	NR_118996.1 NR_157609.1	99	*Bacillus sp.* *B. licheniformis/* *B. haynesii*
4	*A2*	NR_157609.1 NR_137421.1 NR_118996.1	100	*Bacillus sp.* *B. haynesii/ B.paralicheniformis/ B. licheniformis/*
5	*A3*	AY842869.1 NR_157609.1	98	*Bacillus sp.* *B. licheniformis/* *B. haynesii*

№	Strains	Closest taxon (GenBank accession no.)	Percent identity %	Identification
6	*A4*	NR_137421.1 NR_116023.1 NR_157609.1	99	*Bacillus sp.* *B.paralicheniformis/ B. licheniformis/* *B. haynesii*
7	*A5*	NR_075005.2 NR_118383.1	100	*Bacillus sp.* *B. velezensis/* *B. subtilis*
8	*S1*	NR_112686.1	99	*Bacillus subtilis subsp. spizizenii*
9	*S2*	NR_157609.1 NR_116023.1	98	*Bacillus sp.* *B. haynesii/* *B. licheniformis*
10	*S3*	NR_157609.1 NR_113993.1	99	*Bacillus sp.* *B. haynesii/* *B. sonorensis*
11	*D1X*	NR_113945.1 NR_112637.1 NR_148786.1	99	*Bacillus sp.* *B.safensis/B. pumilus/* *B.zhangzhouensis*
12	*D5*	NR_113599.1	100	*P.aeruginosa*
13	*D6*	NR_117678.1	97	*P.aeruginosa*
14	*D7*	NR_117678.1	99	*P.aeruginosa*

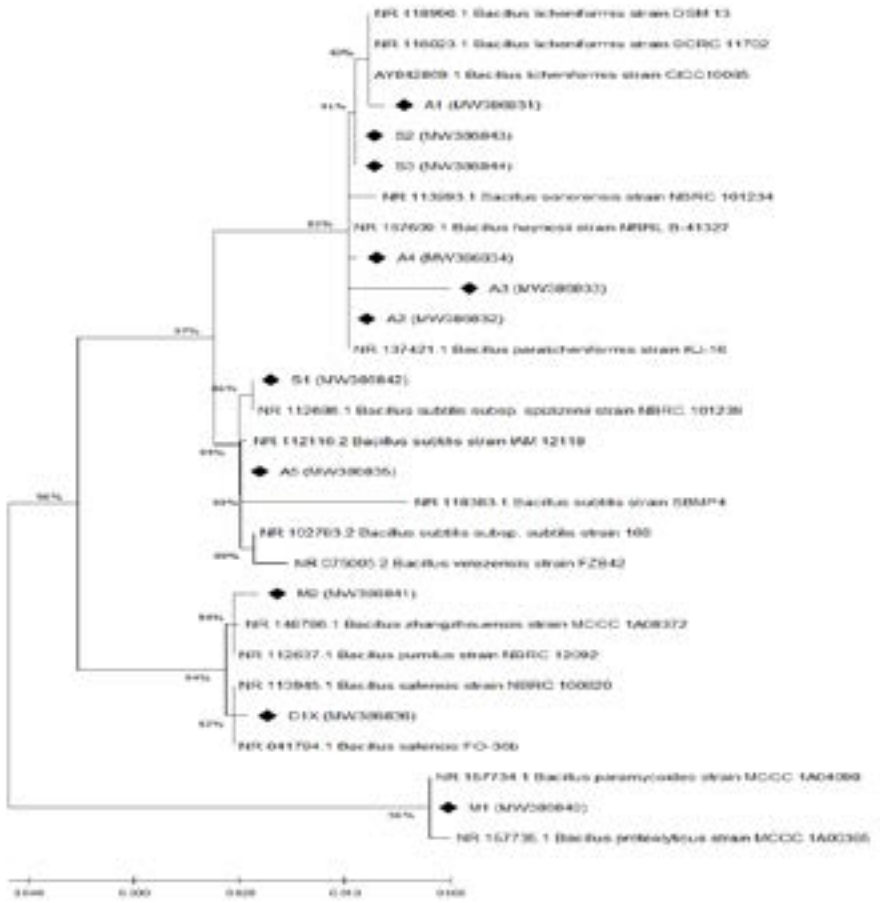


Figure 2 – Phylogenetic tree that reflects the position of nucleotide sequences of isolated organisms among related sequences of the genus Bacillus. The sequences obtained in the course of the work are highlighted in bold. The sequence numbers in GenBank are shown in parentheses

As can be seen from the tabular data, genotyping of 14 aerobic strains of microorganisms based on the analysis of the nucleotide sequence of the 16S rRNA gene 1 strain S1-*Bacillus subtilis subsp. spizizenii* (99 %) and 1 strain M1-*Bacillus paramycoides* (92 %); 9 strains A1, A2, A3, A4, A5, S3, S2, M2, D1X – *Bacillus sp.* (97-100%); 3 strains D5, D6, D7 – *P. aeruginosa* (97-100%).

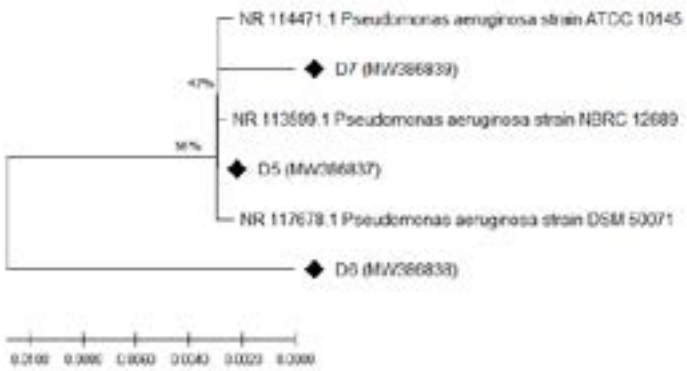


Figure 3 – Phylogenetic tree reflecting the position of nucleotide sequences of isolated strains Pseudomonas aeruginosa. The sequences obtained in the course of the work are highlighted in bold. The sequence numbers in GenBank are shown in parentheses

Figure 2 shows a phylogenetic tree based on the analysis of the 16S rRNA nucleotide sequences of the isolated strains among the related sequences of the genus *Bacillus.*

Figure 3 shows a phylogenetic tree based on the analysis of the 16S rRNA gene nucleotide sequences from the strains that had the maximum identity to *Pseudomonas aeruginosa* when identified in BLAST.

Figure 3 shows that the nucleotide sequences from samples D5, D6, and D7 are located on the same branch as representatives of *Pseudomonas aeruginosa*. Taking into account the maximum percentage of coincidence of the analyzed sequence in the international database using the BLAST algorithm, as well as the results of phylogenetic analysis (Fig. 2, 3), it was found that 14 aerobic microorganisms isolated from oil-reservoir water, 11 strains belong to the genus *Bacillus*, in particular: S1 (MW386842) – *Bacillus subtilis subsp. spizizenii*; M1 (MW386841) – *Bacillus paramycoides;* M2 (MW386840), A1 (MW386831), A2 (MW386832), A3 (MW386833), A4 (MW386834), A5 (MW386835), S2 (MW386843), S3 (MW386844) D1X (MW386836) – Bacillus sp. 3 strain D5 (MW386837), D6 (MW386838), D7 (MW386839) – *P. aeruginosa*.

In their vital activity, microorganisms form metabolites that have the necessary properties to increase oil production. Metabolites: acids, gases (CO2, CH4, N2), solvents, bioavailants that affect the processes of oil displacement in reservoir systems, including reservoir fluids and porous media [3].

It is known that the presence of hydrocarbons in the culture medium, some microorganisms synthesize surfactants that can affect the processes of oil displacement by forming the smallest oil emulsions. Such microorganisms-producers of bioemulsifiers of petroleum hydrocarbons contribute to the dispersion of oil, which increases the effectiveness of the contact of bacteria with oil. Determination of the emulsifying activity of the culture liquid is an important characteristic of microbial strains as producers of surfactants.

The ability of microorganisms to form surfactants was evaluated by the emulsification index, based on the property of various microbial surfactants to form the smallest emulsions at the “liquid-hydrophobic substrate”phase boundary. The determination of the emulsification index is an important characteristic of microbial strains as producers of surfactants. Biofuels dilute the oil, making it more mobile and reducing the tension between the oil and the reservoir [3, 23].

Earlier in our studies, it was shown that the studied strains of microorganisms have endogenous emulsifying activity, i.e., the ability of microorganisms to form cell-bound biosurfactants. Oil was used as hydrophobic substrates for the determination of emulsifying activity.

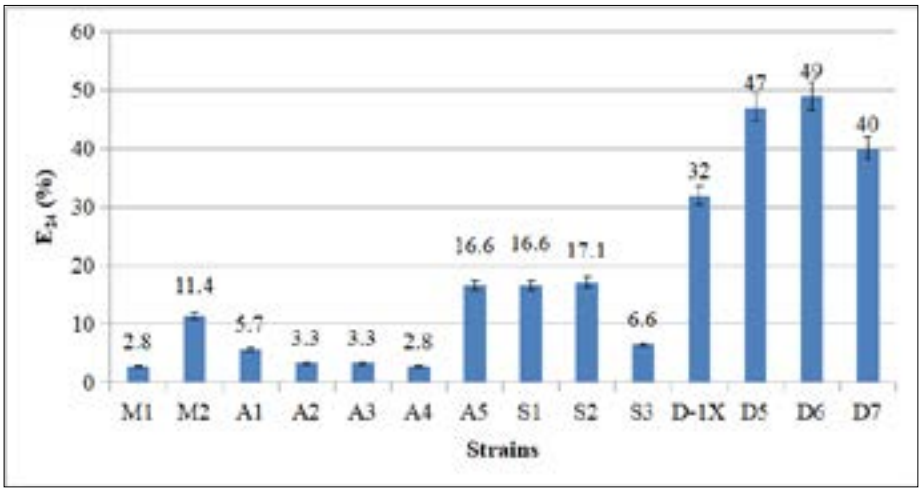


Figure 4 – Definition of the emulsification index

The ability of microorganisms to produce bioemulsifiers that disperse oil, increasing the bioavailability of petroleum hydrocarbons for microorganisms, was studied by determining the endogenous oil-emulsifying activity after 24 hours in a culture liquid without centrifuging the cells of microorganisms [22]. The results of the study of the oil-emulsifying properties of the studied bacterial strains are shown in Fig. 4.

As can be seen, all strains have the properties of oil emulsification. The maximum index of emulsification is observed in 3 strains: *P. aeruginosa*-D5, D6, D7 and is 40-49%, in other strains the index of emulsification is in the range of 2.8-32%. It should be noted that, among others, the maximum emulsification index was 32% for the *Bacillus* sp. D1X strain.

According to the literature data, microorganisms with an emulsification index of more than 50% are considered promising producers of surfactants. Since the microbial cell oxidizes hydrocarbons by adsorbing on their surface, as a result, the oil-oxidizing activity of cultures depends on its ability to utilize the hydrocarbon substrate [24].

Discussion

Representatives of the following microorganisms of the genus Pseudomonas, Bacillus were determined under aerobic conditions.

It is known that allochthones bacteria coming from the injected water and native microflora are present in the developed oil reservoirs [3].

The presence of microorganisms of the genus Bacillus shows in the developed oil reservoirs is due to the fact that they are facultative anaerobes. Representatives of this genus have a significant potential for use in processes affecting oil reservoirs, including due to their ability to form spores, and are also resistant to stressful changes in environmental conditions that are inevitable when microorganisms are injected from the surface into the oil reservoir [23, 25].

In high-temperature oil reservoirs of Russia, Kazakhstan and China, hydrocarbon-oxidizing bacteria are essential components of the biocenosis of the bottom-hole zone of injection wells, through which dissolved oxygen enters the reservoir [26].

The presence of the bacterium *P. aeruginosa* indicates an active gas exchange of the layers with the surface. Fluorescent aerobic bacteria from the genus *Pseudomonas* have been found in the reservoir waters of many oil fields. Many authors have shown that members of the genus *Pseudomonas* are permanent inhabitants of reservoir waters. Bacteria of this genus are well adapted to exist in aerobic conditions [11, 27].

Conclusion

The results obtained indicate that aerobic microorganisms live in the reservoir waters. It was revealed that 14 microorganisms isolated under aerobic conditions from the developed oil-reservoir waters of the Akingen field, according to morphological, cultural, physiological, and biochemical properties and the determination of the direct nucleotide sequence of the 16S rRNA fragment of the gene, made it possible to identify strains S1 (MW386842) – *Bacillus subtilis subsp. spizizenii;* M1 (MW386841) – *Bacillus paramycoides;* M2 (MW386840), A1 (MW386831), A2 (MW386832), A3 (MW386833), A4 (MW386834),

A5 (MW386835), S2 (MW386843), S3 (MW386844) D1X (MW386836) – Bacillus sp. 3 strain D5 (MW386837), D6 (MW386838), D7 (MW386839) – *P. aeruginosa.*

It was found that the evaluation of the emulsifying activity allowed us to identify 4 strains of microorganisms with a high oil emulsification index: *Ps. aeruginosa*-D5, D6, D7 (40-49 %) and *Bacillus* sp. D1X (32 %).

The isolates of *Ps. aeruginosa*-D5, D6, D7 and *Bacillus* sp. D1X are promising objects for use in microbial enhanced oil recovery.

Conflict of interest

All authors have read and are familiar with the contents of the article and have no conflict of objectives.

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References

1 Griebler C. and Lueders T. Microbial biodiversity in groundwater ecosystems // Freshwater Biology. – 2009. – Vol. 54. – P. 649–677.

2 Nilsen R.K., Beeder J., Thorstenson T., Torsvik T. Distribution of thermophilic marine sulfate reducers in North Sea oil field water and oil reservoirs // Appl. Envirom. Microboal. – 1996. – Vol. 62(5). – P. 1793-1798.

3 Nazina T. N., Pavlova N. K., Tatarkin Yu. V., Shestakova N. M., Babich T. L., Sokolova D. Sh, Ivoilov V. S., Khisametdinov M. R., Ibatullin R. R., Tourova T. P., Belyaev S. S., Ivanova M. V. Microorganisms of the Carbonate Petroleum Reservoir 302 of the Romashkinskoe Oilfield and Their Biotechnological Potential // Microbiology. – 2013. – Vol. 82(2). – P. 190–200.

4 Lin J., Hao B., Cao G., Wang J., Feng Y., Tan X. and Wang W. A study on the microbial community structure in oil reservoirs developed by waterflooding // Journal of Petroleum Science and Engineering. – 2014. – Vol. 122. – P. 354–359.

5 Francy D.S. Thomas J.M., Raymond R.L., Ward C.H. Emulsification of hydrocarbons by subsurface bacteria // J. Ind. Microbiol. – 1991. – Vol. 8. – P. 237-246.

6 Pannekens M., Kroll L., Müller H., Tall Mbow F. and Meckenstock R. U. Oil reservoirs, an exceptional habitat for microorganisms // New BIOTECHNOLOGY. – 2019. –Vol. 49. – P. 1–92.

7 Yernazarova A.K., Kayirmanova G.K., Baubekova A.S., Zhubanova A.A. Microbial Enhanced Oil Recovery // Book: Enhanced oil recovery. – 2016. – P. 145-167.

8 Havemann G.D., Clement B.G., Kozicki K.M., Meling T., Beeder J., Sunde E. New Microbial Method Shows Promise in EOR // J Pet Technol. – 2015. – Vol. 67 (03). – P. 32–35.

9 Venkata R. K., Chaiyulu N.C., Karanth N.I. Mathematical Model for the Production of Bio-surfactants by Pseudomonas aeruginosa CFTR-6: Production of Biomass // J. of Chemical Technology and Biotechnology. – 1991. – Vol. 51(4). – P. 525-538.

10 Muslimov R., Kandaurova G., Wagner M. et al. Microbial Improved Oil Recovery in Carbonate Reservoirs – Preparation of Pilot Project in the Oil Field Romashkino, Republic of Tatarstan (CIS) // Proc. of VII European Symposium on Enhanced Oil Recovery. Moscow. – 1993. – P. 496-506.

11 Cui Q, Sun S, Luo Y, Yu L, Zhang Z. Comparison of in-situ and ex-situ microbial enhanced oil recovery by strain Pseudomonas aeruginosa WJ-1 in laboratory sand-pack columns // Petroleum. Science and Technology. – 2017. – Vol. 35(21). – P. 2044-2050.

12 Watanabe Y. Review on microbial ecology in the deep subterranean environment // Quart. Abstr. Cent. Res. Inst. Elec. Power Ind. – 1997. – Vol. 77. – P. 11-12.

13 El-Sheshtawy H.S., Aiad I., Osman M.E., Abo-Elnasr A.A., Kobisy A.S. Production of biosurfactant from Bacillus licheniformis for microbial enhanced oil recovery and inhibition the growth of sulfate reducing bacteria // Egypt J Pet. – 2016. – Vol. 24. – P.155–162.

14 Shibulal B., Al – Bahry S. N., Al – Wahaibi Y. M., Elshafie A. E., Al – Bemani A. S., Joshi S. J. Microbial enhanced heavy oil recovery by the aid of inhabitant spore- forming bacteria: an insight review // Sci. World J. – 2014. – Vol. 12. P. 342-356.

15 da Cunha C.D., Rosado A.S., Sebastian G.V., Seldin L., von der Weid I. Oil biodegradation by Bacillus strains isolated from the rock of an oil reservoir located in a deep-water production basin in Brazil // Appl Microbiol Biotechnol. – 2006. – Vol. 73(4). – P. 949-59.

16 Егоров Н. С. Практикум по микробиологии // Под ред. Егоров Н. С. Учебное пособие. М., Изд-во Моск. ун-та. – 1976. – 307 с. – С. 119-147. [Egorov N. S. Workshop on Microbiology // Ed. Egorov N. S. Textbook. M., Publishing house Mosk. University. – 1976. – 307 p. – P. 119-147 (in Russian)].

17 Егоров Н.С. Руководство к практическим занятиям по микробиологии. М.: МГУ. – 1995. – 224 с. [Egorov N.S. Guide to practical classes in Microbiology. Moscow: MSU. – 1995. – 224 p. (in Russian)]

18 Теппер Е.З., Шильникова В.К., Переверзева Г.И. Практикум по микробиологии. – М.: Дрофа. – 2004. – 256 с. [Tepper E. Z., Shilnikova V. K., Pereverzeva G. I. Practical work on Microbiology. – Moscow: Drofa. – 2004. – 256 p. (in Russian)]

19 Нетрусов А.И., Егорова М.А., Захарчук Л.М. и др. Практикум по микробиологии / Под ред. А.И. Нетрусова. – М.: Академия. – 2005. – 608 с. [Netrusov A. I., Egorova M. A., Zakharchuk L. M. et al. Workshop on Microbiology / Ed. by A. I. Netrusov, Moscow: Akademiya. – 2005. – 608 p. (in Russian)]

20 Wilson, K. Preparation of Genomic DNA from Bacteria // Current Protocols in Molecular Biology. – 2001. – Vol. 56(1). – P. 2.4.1–2.4.5.

21 Kumar S., Stecher G., Li M., Knyaz C., and Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms // Molecular Biology and Evolution. – 2018. – Vol. 35(6). – P. 1547-1549.

22 Cooper D.G. and Goldenberg B.G. Surface-Active Agents from Two Bacillus Species // Appl Environ Microbiol. – 1987. – Vol. 53 (2). – P 224–229.

23 Nazina T, Sokolova D, Grouzdev D, Semenova E, Babich T, Bidzhieva S, Serdukov D, Volkov D, Bugaev K, Ershov A, Khisametdinov M, Borzenkov I. The Potential Application of Microorganisms for Sustainable Petroleum Recovery from Heavy Oil Reservoirs // Sustainability. – 2020. – Vol. 12(1). – P. 15.

24 Desai J. and Banat I. Microbial production of surfactants and their commercial potential // Microbiol and Molecular Biology Reviews. – 1997. – Vol. 61, №1. – P. 47-64.

25 Nazina T. N., Sokolova D. S., Grigoryan A. A. et al. Geobacillus jurassicus sp. nov. a new thermophilic bacterium isolated from a high-temperature petroleum reservoir, and the validation of the Geobacillus species // Systematic and Applied Microbiology. – 2005. – Vol. 28(1). – P. 43-53.

26 Pelger J.W. Microbial Enhanced Oil Recovery Treatment and Wellbore Stimulation Using Microorganisms to Control Paraffin, Emulsion, Corrosion and Scale Formation // Microbial Enhanc. Oil Recov. Recent Adv.: Proc. Int. Conf., Norman., Okla, Amsterdam. – 1991. – P. 451-466.

27 Zhao F, Li P, Guo C, Shi R, Zhang Y. Bioaugmentation of oil reservoir indigenous Pseudomonas aeruginosa to enhance oil recovery through in-situ biosurfactant production without air injection // Bioresource Technology. – 2018. – Vol. 251. – P. 295-302.