ABSTRACT

Dissertations for the degree of Doctor of Philosophy (PhD) in the specialty 6D060700-Biology

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Biological properties and oil-liquefying potential of microorganisms reservoir water of the Akingen field

General description of the research. The dissertation work is devoted to the study of biological (morphological, physiological-biochemical) and molecular-genetic properties of microorganisms isolated from flooded oil waters of the conserved oilfield «Akingen» by determining their phylogenetic genus and genes *lchAA, rhlA, srfA*, responsible for the formation of associated biosurfactants with oil-diluting properties, for the subsequent development of microbial methods of enhanced oil recovery (EOR).

Significance of the research. In the world, reserves of heavy and high-viscosity oil are 5 times higher than the volume of recoverable oil reserves of low and medium viscosity, therefore hard-to-recover oil is the main reserve of world oil production.

Currently, most of the oil fields in Kazakhstan, with the exception of large projects, have already passed the peak of production, are at the stage of late development and are characterized by high viscosity and water-cut of oil, which classifies their reserves as hard-to-recover, i.e. the problem of Kazakhstan and all oil-producing countries is not the lack of reserves, but the difficulty of extracting them to the surface. The products remaining in the subsoil after primary and secondary oil production methods, due to the high level of water cut in the reservoir, is 60-70%.

Currently, scientific, experimental and industrial research on the extraction of heavy hydrocarbon raw materials is especially relevant. Tertiary methods of oil production are being developed for the economically viable development of hard-to-recover oil reserves. One such method is the use of microorganisms, which has enormous potential. Microbial methods of enhancing oil recovery from reservoirs can increase oil recovery by 10-15%, which is comparable to the discovery of a new field and are resource-saving, environmentally friendly technologies.

In oil-reservoir waters, the vital activity of microorganisms is accompanied by the formation of oil-displacing compounds, which is the basis of microbial methods for extracting raw materials from flooded formations. The high biochemical activity of microorganisms increases the yield of their metabolic products (gases, surfactants, acids, alcohols, etc.), which have oil-diluting and oil-displacing properties, which contributes to an increase in the mobility of oil in the reservoir and additional extraction of raw materials.

The purpose of the research. study of the morphological, physiological, biochemical and oil-diluting properties of microorganisms of reservoir waters of the «Akingen» field, located in the Atyrau region of the Republic of Kazakhstan

To achieve this goal, the following **tasks** were set:

1. To identify the composition of the oil-reservoir waters of the Akingen field, including total mineralization, pH, hardness, and the content of basic salts.
2. To establish the quantitative and qualitative microbiological composition of the oil-reservoir waters of the Akingen field.
3. Determine the morphological and physiological, biochemical properties of microorganisms isolated from oil reservoir waters.
4. Carry out phylogenetic identification of microorganisms based on 16S rRNA nucleotide sequences.
5. Determine the presence of *lchAA, rhlA, srfA* genes in microorganisms isolated from reservoir waters, which are responsible for the production of biosurfactants involved in oil emulsification.
6. Selection of microorganisms with high oil-diluting and oil-displacing properties.
7. Create associations of microorganisms with high oil-diluting and oil-displacing properties.

Objects of research: the oil-reservoir waters of the preserved Akingen field located in the Atyrau region and 31 cultures of microorganisms isolated from the oil-reservoir waters of the Akingen field were used in the work.

Research methods. In the course of the work, basic microbiological (Koch method, microscopy methods, perpendicular strokes method, etc.), genetic (sequencing of a *16S RNA* gene fragment) and physico-chemical methods (Cooper method, potentiometric method, spectrophotometric method, electrometric method, titrimetric method, complexometric method, liquid chromatography) were used.

Scientific novelty of the research. For the first time, the quantitative and qualitative microbiological characteristics of the oil - reservoir waters of the Akingen field are given: it is shown that the aerobic microflora of reservoir waters is $96.1x10^7$ CFU/ml, and the content of anaerobes is much less than $–14x10^4$ CFU/ml, the qualitative composition is represented by *Pseudomonas* and *Bacillus*, quantitatively dominated by representatives of the genus *Bacillus* – $13x10^3$ CFU/ml. Isolated and identified 31 cultures of bacteria, of which 17 cultures of bacilli: *B. subtilis subsp. spizizenii* S1; *Bacillus paramycoides* M1*; B. subtilis* A5; *B. haynesii* S3, *B. safensis* D7X; *Brevibacillus borstelensis* SR3, 2 strains *B. pumilus* (M2, D1X)*;* 8 strains *B. licheniformis* (A1, A2, A3, A4, S2 SR1, SR2, CL1, CL2)

14 strains of *Pseudomonas* - *P. aeruginosa* (D5; D6; D7; D1; D2; D3; D8; T1; T2; T3; T4; T5; T6; D4).

For the first time, the nucleotide sequence of genomes of 31 strains of bacteria isolated from the oil reservoir waters of the mothballed Akingen field were determined and published in the international database *GenBank.* The presence of genes (*lchAA, rhlA, srfA*) responsible for the production of biosurfactants involved in oil emulsification has been shown:

srfA gene - in 10 strains of *P. aeruginosa* - D2, D3, D4, D5, D6, D7, D1, T2, T4, T6;

rhlA gene - in 12 strains of *P. aeruginosa* - T2, T3, T4, T5, T6, D2, D3, D4, D5, D6, D7, D1;

lchAA gene - in 10 *Bacillus* strains: of which 2 strains *B. licheniformis* (SR-2, CL-2) and 8 strains *Bacillus subtilis* (A1, S2, S3, M2, A3, A4, SR-1 , CL-1).

For the first time, the oil-diluting potential of microorganisms in the oil-formation waters of the Akingen field was determined, in particular, microorganisms with high oil-emulsifying properties (with an emulsification index of more than 50%) were selected and microbial associations were created on their basis, promising for the development of microbial methods for increasing oil recovery.

Scientific and practical significance of the research.

To develop methods for increasing oil recovery, promising associations of microorganisms with high oil-liquefying and oil-displacing properties were created on the basis of identified microorganisms from the reservoir waters of the Akingen field.

The selected 31 strains of microorganisms are included in the collection of hydrocarbon-oxidizing microorganisms of the Al-Farabi Kazakh National University for their further use in biotechnologies.

The *16S rRNA* nucleotide sequences of 31 bacterial cultures were registered and published in GenBank. Accession numbers for strains: *B. subtilis subsp. spizizenii S1* - MW386842; *B. paramycoides M1* - MW386841; *B. pumilus M2* - MW386840; *B. licheniformis A1* - MW386831; *B. licheniformis* A2 - MW386832; *B. licheniformis A3* - MW386833; *B. licheniformis A4* - MW386834; *B. subtilis A5* - MW386835; *B. licheniformis S2* - MW386843; *B. haynesii S3* - MW386844; *B.pumilus D1X* - MW386836; *P. aeruginosa D5* - MW386837; *B. licheniformis CL1* - MW600501; *B. licheniformis CL2* - MW600502; *B. safensis D7X* - MW600506; *B. licheniformis SR1* - MW600508; *B. licheniformis SR2* - MW600509; *Brevibacillus borstelensis SR3* - MW600510; *P. aeruginosa D8* - MW600507; *P. aeruginosa D6* - MW386838; *P. aeruginosa D7* - MW386839; *P. aeruginosa D1*- MW600503; *P. aeruginosa D2* - MW600504; *P. aeruginosa D3* - MW600505;*P. aeruginosa T1* - MW617329; *P. aeruginosa T2* - MW617330; *P. aeruginosa T3* - MW617331; *P. aeruginosa T4* - MW617332; *P. aeruginosa T5* - MW617334; *P. aeruginosa T6* - MW617335; P. *aeruginosa D4* - MW617336.

The results obtained in the course of the scientific research are included in the content of the academic subject «Microbial preparations and products of ecosystem restoration» of the specialty «6M070100-Biotechnology» of the Al-Farabi Kazakh National University (Application A).

The main provisions submitted for the defense of the dissertation:

1. Selected and identified on the basis of the phenotypic and genetic properties of 31 cultures of bacteria in the oil reservoir waters of the Akingen field.

2. The oil-emulsifying properties of microorganisms in the oil-reservoir waters of the Akingen field are associated with the presence of the *lchAA, rhlA, srfA* genes, which are responsible for the production of biosurfactants involved in oil emulsification.

3. 16 strains of microorganisms, included *B.safensis* D7X, *B. subtilis* A5, *B. subtilis subsp. spizizenii* S1, 2 strains *B. pumilus* (D1X, M2), 5 strains *Bacillus*

licheniformis (S2, SR1, SR2, CL1, CL2) and 6 strains *P. aeruginosa (*D5, D6, D7, D8, T2, T3*)* strains, isolated from oil-reservoir waters, they have high oil-displacing and oil-liquefying properties with an emulsification index above 51% and are capable of abundant gas formation and acidification of the medium with the addition of molasses.

4. Active associations of microorganisms with high oil-liquefying and oil-displacing properties have been created, which can be used to develop microbial methods for increasing oil recovery of flooded reservoirs.

Main research results and conclusions:

The results obtained in this work allow us to draw the following conclusions:

1. It is shown that the oil-formation waters of the Akingen field are highly mineralized, the pH was 6.34 units, sodium and chlorine ions predominate and belong to the sodium-chloride type of formation waters.

2. It has been established that the aerobic microflora of the oil reservoir waters of the Akingen field is $96.1x10^7$ CFU/ml, while the content of anaerobes is much lower - $14x10^4$ CFU/ml, the qualitative composition is represented by *Pseudomonas* and *Bacillus*, moreover, representatives of the genus *Bacillus* are quantitatively dominant - $13x10^3$ CFU/ml

3. Selected and identified on the basis of morphology, physiological and biochemical properties and analysis of the nucleotide sequence of *16S rRNA* genes of 31 cultures bacterial, of which 14 strains belong to *P. aeruginosa aeruginosa* –D1, D2, D3, D4, D5, D6, D7, D8, T1, T2, T3, T4, T5, T6; 17 cultures of *bacilli – B. subtilis subsp. spizizenii* S1; *B. paramycoides* M1*; B. subtilis* A5; *B. haynesii* S3; *B.safensis* D7X; *Brevibacillus borstelensis* SR3, 2 strains *B. pumilus* (M2, D1X)*;* 9 strains *B. licheniformis* (A1, A2, A3, A4, S2, SR1, CL1, CL2, SR2).

The nucleotide sequences of *16S rRNA* 31 cultures of bacteria were registered and published in *GenBank*, accession registration numbers: *B.subtilis subsp. spizizenii S1* - MW386842; *B.paramycoides M1* - MW386841; *B.subtilis M2* - MW386840; *B.subtilis A1* - MW386831; *B.subtilis* A2 - MW386832; *B.subtilis A3* - MW386833; *B.subtilis A4* - MW386834; *B.subtilis A5* - MW386835; *B.subtilis S2* - MW386843; *B.subtilis S3* - MW386844; *B.subtilis D1X* - MW386836; *B.subtilis CL1* - MW600501; *B.licheniformis CL2* - MW600502; *B.subtilis D7X* - MW600506; *B.subtilis SR1* - MW600508; *B.licheniformis SR2* - MW600509; *Brevibacillus borstelensis SR3* - MW600510; для псевдомонад: *P. aeruginosa D1*- MW600503; *P. aeruginosa D2* - MW600504; *P. aeruginosa D3* - MW600505; *P. aeruginosa D8* - MW600507; *P. aeruginosa D5* - MW386837; *P. aeruginosa D6* - MW386838; *P. aeruginosa D7* - MW386839; *P. aeruginosa T1* - MW617329; *P. aeruginosa T2* - MW617330; *P. aeruginosa T3* - MW617331; *P. aeruginosa T4* - MW617332; *P. aeruginosa T5* - MW617334; *P. aeruginosa T6* - MW617335; P. *aeruginosa D4* - MW617336.

4. The presence of genes responsible for the production of biosurfactants, responsible for the oil emulsifying properties of bacteria, was revealed: *srfA* - in 10 strains of *Pseudomonas aeruginosa* (D2, D3, D4, D5, D6, D7, D1, T2, T4, T6); *rhlA* gene - in 12 strains of *Pseudomonas aeruginosa* (T2, T3, T4, T5, T6, D2, D3, D4,

D5, D6, D7, D1); *lchAA* gene - in 10 cultures of bacilli: *B. haynesii* S3 *B. pumilus* M2 and 8 strains *B. licheniformis* (A1, A3, A4, S2, SR1, SR2, CL1, CL2).

5. Selected 16 strains of microorganisms with high target activity on the E8 medium with the addition of molasses: the emulsification index was above 51 *%*, capable of abundant gas formation and acidification of the medium.

6. For the construction of highly active associations of microorganisms, 5 strains of microorganisms were selected based on the study of antagonistic relationships of 16 strains: *P. aeruginosa D5* - oil emulsifier, acid-forming agent, gas-forming agent, *P. aeruginosa D6* - oil emulsifier, acid-forming agent, gas-forming agent, *Bacillus sp. D1X*-oil emulsifier, gas-forming agent, *B. licheniformis SR1* – acid-forming agent, gas-forming agent and *B. licheniformis CL1* - acid-forming agent, gas-forming agent.

7. From the studied 12 associations of microorganisms, the following 5 associations of microorganisms were selected according to the coincidence of at least 4 indicators out of 6 target properties-oil emulsification, acid formation, gas formation under aerobic and anaerobic conditions, of these: 2 associations consisting of 2 strains-D6: SR 1; D6:CL1; 2 associations of 3 strains - D6:SR1:CL1; D6:CL1:D1X and 1 association of 4 strains D6 : SR1: CL1 : D1X.

The research tasks were completed in full.

Personal contribution of the author. The author independently conducted an analysis of the literature data on the topic of the study, experimental studies, statistical processing and analysis of the results obtained, as well as writing and feeding the dissertation work.

Connection with the plan of the main scientific work. The dissertation work was carried out within the framework of the project AR 05134797 «Creation of a technological scheme for improving oil recovery by microbiological method» No. 188RK00166 (2018-2020).

Approbation of the research. The materials of the dissertation were presented and discussed at the following international scientific conferences:

- XXXV International Scientific Conference «Development of Science in the XXI Century», May 16, 2018, Kharkiv, Ukraine;
- MATERIALS of the international scientific conference of students and young scientists "FARABI ALEMI" April 9-10, 2019, Almaty, Kazakhstan;
- International Scientific and Practical Conference "Actual problems of Biodiversity and Biotechnology", dedicated to the Year of Youth in the Republic of Kazakhstan, October 1, 2019, Nur-Sultan, Kazakhstan;

Publications. The main results of the dissertation are published in 9 printed scientific papers, including 3 articles in domestic periodicals recommended by the Committee for Control in the field of Education and Science of the Republic of Kazakhstan; 1 article in a high-level scientific journal included in the *Scopus* database; 4 theses in the materials of a domestic international conference; 1 article in the materials of a foreign international conference.

Structure of the dissertation. The dissertation work is written on 110 text pages and consists of the following sections: designations and abbreviations, introduction, literature review, materials and methods of research, research results

and their discussion, conclusion and list of sources used from 200 titles; contains 21 figure, 22 table and 2 applications.