UDC 579.22:579.64

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FATTY ACIDS COMPOSITION OF *MICROBACTERIUM* GENUS BACTERIA – DESTRUCTORS OF OIL HYDROCARBONS

Aim. *To conduct an identification of microorganisms – destructors of oil hydrocarbons – based on their fatty acid composition. This is one of the chemotaxonomic characteristics that correlate with bacterial molecular genetic features. Fatty acids analysis of cells of bacteria was carried out using automatic system of microorganisms' identification MIDI Sherlock (MIDI, USA) based on gas chromatograph Agilent 7890. For fatty acids profiles test culture were characterized by C_{15}:0 anteiso – 44.26–46.36 %, C_{17}:0 anteiso – 27.04–28.03 %, C_{16}:0 iso – 14.49–16.9 %, C_{15}:0 iso – 5.51– 8.58 %, C_{17}:0 iso – 1.67–2.4 %. Isomers C_{13}:0 anteiso, C_{14}:0 iso, C_{18}:0 iso, C_{19}:0 anteiso identified smaller amounts and ranged from 0.05 to 0.72 % of the total peaks square. It demonstrates the presence of saturated isomers C14:0, C16:0, C17:0 and C18:0, which composition ranged from 0.05 to 1.02 %. By phenotypic characteristics and the composition of fatty acids investigated culture were assigned to type Microbacterium barkeri. Similarity index of fatty acid profiles of the library is 0.685 for strain Microbacterium barkeri OZ-2 and 0.942, for strain Microbacterium barkeri OZ-3.*

K e y w o r d s: composition of fatty acids, identification, Microbacterium barkeri.

For many decades the use of the Zmiiniy Islandin military aims led to a significant oil pollution amounting up to 10 % of soil area at the moment. It is shown that the oil content is up to 112.5 g/kg soil. The problem of soil remediation in this area is complicated due to its chemical composition, specifically by salinity. The total salt content in the upper horizons is around 0.05–0.07 %, increasing with depth to 0.1–0.2 % [1].

Modern methods of soil and water purification from oil and oil products are often developed using bacteria destructors of oil hydrocarbons. Different representatives are describedamong these destructors: for Proteobacteria – *Bacillus, Rhizobium, Arthrobacter* [15], for ascomycetes – *Fusarium, Alternaria* [6, 12] and foractinobacteria – *Rhodoccocus and Microbacterium* [13, 15]. High metabolic flexibility and rapid adaptation to the harsh living conditions allows active utilizing organic compounds including of carbohydrates, by reducing the oil content in the soil to baseline values at low operating costs and low technological complexity of the process of contaminated soil purification [3, 9].

Increased research interest in studying fatty acids' profile of poorly explored microorganisms is explained by the fact that some of their cellular fatty acids (unsaturated and branched) are autoinducers in quorum-sensing system, which provides contacts both between population members and other microorganisms [3].

Fatty acid composition of total cellular lipids is important species and intraspecific chemotaxonomic characteristics [2, 5]. For microorganisms it is used

as chemotaxonomic feature that allows identification of microorganisms using fatty acid profiles' libraries [11].

Previously, as a result of screening microorganisms isolated two strains of bacteria capable of anionic surfactants and oil recycling were isolated from petroleum contaminated soil of the Zmiiniy Island. As these strains were not identified, the aim of the work was to conduct an identification of microorganisms – destructors of oil hydrocarbons – based on their fatty acid composition. This is one of the chemotaxonomic characteristics that correlate with bacterial molecular genetic features.

Materials and Methods. Two strains of bacteria isolated from petroleum contaminated soil of Zmiiniy Island were the objects of this study. To perform analysis of cellular lipids' content, samples were prepared appropriately with bacterial cultures that had been previously incubated at Tryptic soy agar medium (Merck, Germany) at a temperature of 28 ± 1 °C for 24 hours.

The 3 full loop with wet biomass was transferred to glass vials and was added a concentrated solution of NaOH. Suspension was thoroughly mixed heated at 95–100 ºC for 5 min in a water bath. Prepared samples were stored for 25 minutes at a temperature of 95–100 ºC for cellular destroying and saponification of microorganisms' lipids. Methylation of fatty acids was performed by heating the reaction mixture at 80 ºC for 10 minutes after adding acidic methanol solution. Extracted methyl esters of fatty acids were neutralized with 0.3 M solution of NaOH and analyzed by gas chromatography [4].

Chromatographic separation of methyl FA esters was conducted at Agilent 7890 gas chromatograph (Agilent Technologies, USA) with a capillary column ULTRA 2 and flame ionization detector. Samples of 2 ml volume were transferred into the evaporator in the mode split with a coefficient 40:1, evaporator's temperature – 250 ºC. The separation was carried out in temperature programming mode – initial temperature of 170 ºC with a gradient of 5 ° C/min to 270 ºC. Fatty acid content was expressed as a percentage to total sum of peaks' squares.

The MIDI Sherlock 4.5 software together with fatty acids profiles' library of aerobic microorganisms RSTBA6 version 6.21, were used for identification of studied strains' fatty acid profile.

Statistical analysis of the results of research was performed in a computer program MS Excel with determination of Student's *t*-criteria. A statistically significant difference was considered as $P < 0.05$.

Results and Discussion. Previous studies have shown that isolated microorganisms hold the destructive ability to petroleum hydrocarbons. The level of oil spot destruction (oil 10 mg / 10 ml of bacterial culture) at the M-9 medium for 20 days reached 35 %. Strains gave moderate grow that the "hungry" agar containing 1 % sodium dodecyl sulfate. This indicated at their ability to destructive activity forward anionic surfactants (AS).

According to phenotype features (morphological, physiological-biochemical, cultural), that were determined by classical bacteriological methods and API 50 CHB Medium test-systems (bioMerieux, France), isolated bacterial strains were primary assigned to the *Microbacterium* genus.

It is known that *Microbacterium* group is heterogeneous. Majority of its' representatives were detected in environmental samples – water, soil, plants, dairy products, as well as in human clinical isolates in some described cases

[8, 10]. A common feature for *Microbacterium* representatives and relative genera is the ability to form yellow pigmented colonies [7]. The *Microbacterium* genus representatives distinguish from related genera such as *Aureobacterium, Curtobacterium* due by growth under anaerobic conditions and impossibility to move [7, 8]. The absence of cellulose activity and specter of general cellular fatty acids takes apart them from representatives of *Cellulomonas*. Thus *Microbacterium* representatives are characterized by a dominance of C15:0 anteiso – and C17:0 anteiso isomers, the total content of which may compose up to 70 % of the total fatty acid pool . Other members of this group are characterized by shorter isomers [10, 14].

Chromatographic analysis of fatty acids profiles (Fig.1, Fig. 2.) for studied bacterial cells detected typical high content of branched saturated isomers with amount of carbon atoms from C13 to C19. For cells of bacteria *Microbacterium* sp. OZ-2 (table 1) there's demonstrated the presence of C_{15}:0 anteiso – 44.26 %, C_{17}:0 anteiso – 28.03 %, C_{16}:0 iso – 14.49 %, C_{15}:0 iso – 8.58 %, C_{17}:0 iso – 2.4 %. Isomers C_{13}:0 anteiso, C_{14}:0 iso, C_{18}:0 iso, C_{19}:0 anteiso were detected in lower amounts and consisted from 0.05 to 0.58 % of the total peaks' squares. There is also observed the presence of saturated isomers C14:0, C16:0, C17:0 and C18:0, which content varied between 0.05 and 0.75 %.

For cells of bacteria *of Microbacterium* sp. OZ–3 (table 1) there's established the presence of C_{15}:0 anteiso – 46.36 %, C_{17}:0 anteiso – 27.04 %, C_{16}:0 iso – 16.9 %, C_{15}:0 iso – 5.51 %, C_{17}:0 iso – 1.67 %. Isomers C_{13}:0 anteiso, C_{14}:0 iso, C_{18}:0 iso, C_{19}:0 anteiso are detected in lower amounts and consisted from 0.05 to 0.72 % of the total peaks' squares. There are also found saturated isomers C14:0, C16:0, C17:0 and C18:0 whose content varied from 0.05 to 1.02 %.

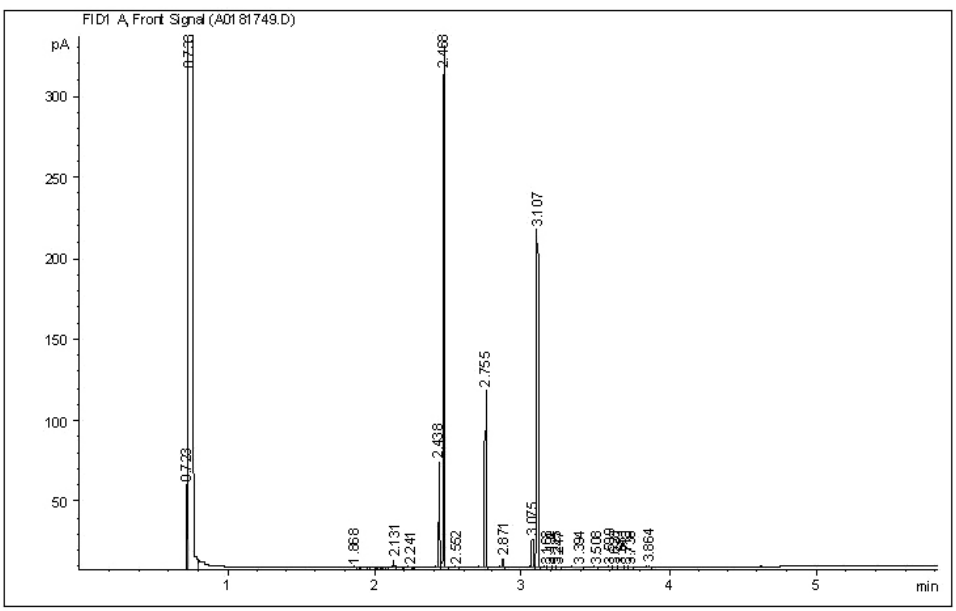

Fig. 1. Fatty acids' chromatogram of culture of Microbacterium sp. OZ-2 general cellular lipids

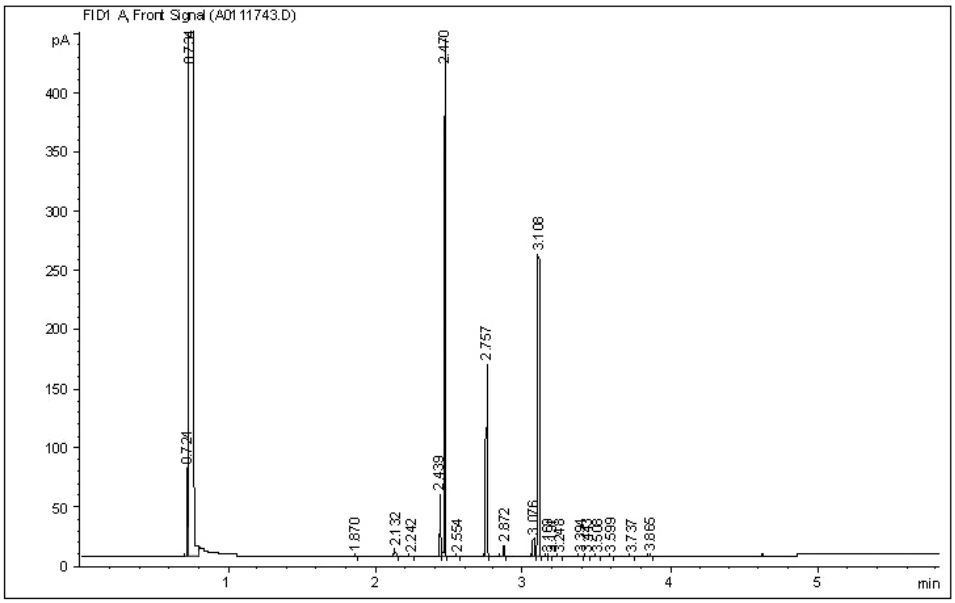

Fig. 2. Fatty acids' chromatogram of culture of Microbacterium sp. OZ-3 general cellular lipids

Table 1

Fatty acids (%) cells of bacteria of *Microbacterium* spp. OZ-2 and OZ-3

Fatty acid	Percentage of the total peaks square (%)	
	***Microbacterium* sp. OZ-2**	***Microbacterium* sp. OZ-3**
C_{13}:0 anteiso	0.05	0.05
C_{14}:0	0.05	0.06
C_{14}:0 iso	0.58	0.72
C_{15}:0 iso	8.58	5.51
C_{15}:0 anteiso	44.26	46.36
C_{16}:0	0.75	1.02
C_{16}:0 iso	14.49	16.90
C_{17}:0	0.11	0.08
C_{17}:0 iso	2.40	1.67
C_{17}:0 anteiso	28.03	27.04
C_{18}:0	0.05	0.05
C_{18}:0 iso	0.08	0.06
C_{19}:0 anteiso	0.05	0.03
C_{17}:1 w5c	0.08	0.07
C_{18}:0 10-metyl	0.04	----
C_{18}:1 w9c	----	0.03
C_{16}:1 2OH	0.05	----
C_{16}:0 izo 3OH	0.06	0.08
C_{17}:0 2OH	0.10	0.11
C_{17}:0 3OH	0.05	----
C_{18}:1 2OH	0.14	0.16

Note: «----» – not found.

Thus, the fatty acids profile analysis of investigated culture using automated MIDI Sherlock identification system assigned studied strains to species *Microbacterium barkeri* with high similarity indices 0.685 for *Microbacterium barkeri* OZ-2 strain and 0.942 for *Microbacterium barkeri* OZ-3 strain.

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СКЛАД ЖИРНИХ КИСЛОТ БАКТЕРІЙ РОДУ *MICROBACTERIUM* – ДЕСТРУКТОРІВ ВУГЛЕВОДНІВ НАФТИ

Р е з ю м е

Мета. Провести ідентифікацію бактерій – деструкторів вуглеводнів нафти, використовуючи жирнокислотний склад як одну із хемотаксономічних ознак, яка корелює з молекулярно-генетичними показниками. Жирнокислотний аналіз клітин бактерій здійснювали з використанням автоматичної системи ідентифікації мікроорганізмів MIDI Sherlock (MIDI, USA) на базі газового хроматографа Agilent 7890. Для жирнокислотних профілів досліджуваних культур була характерна наявність C_{15}:0 антеізо – 44,26–46,36 %, C_{17}:0 антеізо – 27,04–28,03 %, C_{16}:0 ізо – 14,49–16,9 %, C_{15}:0 ізо – 5,51–8,58 %, C_{17}:0 ізо – 1,67–2,4 %. Ізомери C_{13}:0 антеізо, C_{14}:0 ізо, C_{18}:0 ізо, C_{19}:0 антеізо виявлені в менших кількостях та складали від 0,05 до 0,72 % від загальної суми площ піків. Показана присутність насичених ізомерів C14:0, C16:0, C17:0 та C18:0, вміст яких коливався від 0,05 до 1,02 %. За фенотиповими ознаками та складом жирних кислот досліджувані культури віднесено до виду *Microbacterium barkeri*. Індекс схожості жирнокислотних профілів із бібліотечними складає 0,685 для штаму *Microbacterium barkeri* ОЗ-2 та 0,942, для штаму *Microbacterium barkeri* ОЗ-3.

Ключові слова: склад жирних кислот, ідентифікація, *Microbacterium barkeri*.

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СОСТАВ ЖИРНЫХ КИСЛОТ БАКТЕРИЙ РОДА *MICROBACTERIUM* – ДЕСТРУКТОРОВ УГЛЕВОДОРОДОВ НЕФТИ

Р е з ю м е

Цель. Провести идентификацию микроорганизмов – деструкторов углеводородов нефти, используя жирнокислотный состав как один из хемотаксономических признаков, который коррелирует с молекулярно-генетическими показателями. Жирнокислотный анализ клеток бактерий осуществляли с использованием автоматической системы идентификации микроорганизмов MIDI Sherlock (MIDI, USA) на базе газового хроматографа Agilent 7890. Для жирнокислотных профилей исследуемых культур было характерно наличие C15:0 антеизо – 44,26–46,36 %, C17:0 антеизо – 27,04–28,03 %, C16:0 изо – 14,49–16,9 %, C15:0 изо – 5,51– 8,58 %, C17:0 изо – 1,67–2,4 %. Изомеры C13:0 антеизо, C14:0 изо, C18:0 изо, C19:0 антеизо обнаружены в меньших количествах и составляли от 0,05 до 0,72 % от общей суммы площадей пиков. Показано присутствие насыщенных изомеров C14:0, C16:0, C17:0 и C18:0,

содержание которых колебалось от 0,05 до 1,02 %. По фенотипическим признакам и составу жирных кислот исследуемые культуры относятся к виду *Microbacterium barkeri*. Индекс сходства жирнокислотных профилей с библиотечными составляет 0,685 для штамма *Microbacterium barkeri* OZ-2 и 0,942, для штамма *Microbacterium barkeri* OZ-3.

Ключевые слова: состав жирных кислот, идентификация, *Microbacterium barkeri.*

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Отримано 11.07.2016