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V.S. Pidgorskyi, T.M. Nogina

Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, 154 Zabolotny St., Kyiv, 03143, Ukraine

BIODEGRADATION OF PETROLEUM HYDROCARBONS BY ACTINOBACTERIA AND ACINETOBACTERIA STRAINS PRODUCING BIOSURFACTANT

Environmental pollution with petroleum hydrocarbons has become one of the most urgent problems worldwide. The effectiveness of bioremediation of oil pollutions is significantly affected by the inherent capabilities of microorganisms and their specific adaptive mechanisms of hydrocarbon assimilation. In this study the hydrocarbon biodegradation efficiency and the surface-active properties of the hydrocarbon-oxidizing strains of Gordonia rubripertincta IMB Ac-5005, Rhodococcus erythropolis IMB B-7012 and Acinetobacter calcoaceticus IMB B-7013 were determined. These strains showed high efficiency of biodegradation of n-hexadecane (82.1–86.7 %), kerosene (72.5–80.3 %), diesel fuel (70.1–74.3 %) and crude oil (63.5–68.9 %). The mixed culture of these strains completely assimilated n-alkanes C_9–C_{21} as well as iso-alkanes C_8–C_{17} and significantly decreased (150–230 times) the amount of n-alkanes C_{22}–C_{26} in the process of cleaning up water from raw oil. The utilization of hydrocarbons by G. rubripertincta IMB Ac-5005 and R. erythropolis IMB B-7012 with low initial hydrophobicity index (2.4 and 9.6 % respectively) was accompanied by the increase in cell surface hydrophobicity (4 and 25 times respectively) and by the synthesis of cell-bound biosurfactants forming stable emulsions of "oil-in-water" type. The dominating components of these biosurfactants were glycolipids: mono- and dimycolates of trehalose. The assimilation of hydrocarbons by highly hydrophobic A. calcoaceticus IMB B-7013 with the initial hydrophobicity index 99.4 % resulted in the decrease of this value by 1.4 times and the synthesis of extracellular biosurfactant forming stable emulsions of "water-in-oil" type. The biosurfactant of A. calcoaceticus IMB B-7013 is similar to emulsans according to its chemical composition and ratio of the main components (carbohydrates, proteins and lipids). The experimental data provide grounds for efficient using of these strains in the process of bioremediation of oil-polluted water and soils.

K e y w o r d s: hydrocarbon-oxidizing microorganisms, surface-active properties, biosurfactants, Gordonia, Rhodococcus, Acinetobacter.

Hydrocarbon compounds are highly persistent in the environment and pose significant threats to human health and natural biodiversity [11]. Currently, microbiological methods of bioremediation of oil polluted ecosystems gain ever increasing popularity owing to their sustainability, relatively low cost, and environmental safety. Bioremediation of polluted environments is based on contaminant biodegradation, that is, metabolic abilities of microorganisms to transform or mineralize organic contaminants into less harmful, nonhazardous substances, which are further integrated into natural biogeochemical cycles [5, 14, 20]. The spectrum of microorganisms used for hydrocarbons degradation mostly includes the actinobacteria of *Rhodococcus*, *Dietzia*, *Gordonia* genera as well as bacteria of *Pseudomonas*, *Arthrobacter* and *Acinetobacter* genera [7, 12, 14, 15]. Many authors have determined that these microorganisms are widely spread in oil-polluted ecosystems where hydrocarbon-oxidizing actinobacteria play a significant role, which is explained by metabolic peculiarities of these

bacteria and their resistance to unfavourable conditions [9, 14]. All data available to date evidence that mixed populations of microorganisms with overall broad enzymatic capacities are required to degrade complex mixtures of hydrocarbons such as crude oil, aromatic and polyaromatic compounds in soil, fresh water, and marine environments [15, 20, 23]. It is known that hydrocarbons utilization by microorganisms is limited by hydrophobic nature of these substances. The microbial oil-degradation activity largely depends on the features of the cell envelope of microorganisms and their ability to synthesize biosurfactants. Among the hydrocarbon-oxidizing bacteria – active producers of biosurfactants – the genera *Rhodococcus* and *Acinetobacter* can be effectively used in environmental technology for bioremediation of oil-contaminated ecosystems [3, 7, 9, 14, 18].

The aim of this work was to test the efficiency of biodegradation of oil hydrocarbons by the strains *G. rubripertincta* IMB Ac-5005*, R. erythropolis* IMB B-7012 and *A. calcoaceticus* IMB B-7013, and to determine their surface-active properties and ability to produce biosurfactants.

Materials and methods. *Microorganisms.* The study included three strains of hydrocarbon-oxidizing bacteria: *Gordonia rubripertincta* IMB Ac-5005*, Rhodococcus erythropolis* IMB B-7012 and *Acinetobacter calcoaceticus* IMB B-7013. They are maintained in the Ukrainian Collection of Microorganisms of the Zabolotny Institute of Microbiology and Virology of the National Academy of Science of Ukraine and are deposited in the Depositary of microorganisms of this institute as the destructors of oil and oil products.

*Media and growth conditions***.** Bacteria were grown in liquid mineral medium (g/l): KNO_3 – 3.0, KH_2PO_4 – 0.28, $Na_2HPO_4 \times 12H_2O$ – 1.2, NaCI – 2.0, $MgSO_4 \times 7H_2O$ – 0.2, $CaCl_2 \times 6H_2O$ – 0.0111, $FeSO_4 \times 7H_2O$ – 0.0199, yeast extract – 1.0; tap water – 900 ml; distilled water – 100 ml, pH 6.8–7.0. Crude oil, diesel fuel, kerosene or n-hexadecane 0.5 % (v/v) were used as the only carbon and energy sources. For the determination of surface-active properties, cells were grown on this medium with 1.0 % (v/v) n-hexadecane. Cultures were incubated in 750 ml Erlenmeyer flasks with 100 ml of medium at 28 ºC with agitation at 200 rpm for 5 days. 48 h cultures grown on the mineral medium described above, containing 0.3 % (v/v) n-hexadecane were used as inoculums. Free cell cultures were obtained by centrifugation of culture medium for 30 min at 12.000 g and 4 ºC. Spread plate technique was used for studying the colonial morphologies and determination of colony forming unit (CFU/ml).

Total petroleum hydrocarbon (TPH) concentrations were determined using the laboratory analyzer of the content of petroleum products in water AN-1 (Neftehimavtomatika-SPb, Russia) according to the manufacturer's instructions. The biodegradation efficiency (BE) was evaluated in percent (%) and calculated according to the formula: $BE\ (\%) = 100\ \% - [C_2 \times 100\ \% / C_1]$, where: BE – biodegradation efficiency, %; C_1 – primary hydrocarbon substrate concentration in the sample, mg/l; C_2 – quantity of hydrocarbon in the sample after biological degradation, mg/l.

The hydrocarbon composition of crude oil. Quantitative content of paraffin-naphthenic (PNH), aromatic and asphaltene hydrocarbons of crude oil was studied by columnar liquid adsorption chromatography [21] with the use of granular silica gel KSGK (Reap, Ukraine).

Model experiments on the crude oil biodegradation in water. The study

of crude oil biodegradation was carried out in stationary conditions within 28 days using 250 ml flasks containing 200 ml of the natural pond water (pH – 7.8, hardness – 6.5 mg/l, O_2 – 8.5 mg/l, hydrocarbons < 0.01 μg/l). The crude oil (specific gravity of 0.88 g/ml) of Glynsko-Rozbyshivske field (Poltava region, Ukraine) was added to the final concentration of 4.000 mg/l. Complex mineral fertilizer NPK 16:16:16 (Dniprovsky plant of chemical fertilizers**,** Ukraine) was used as a source of nutrients in such an amount that per 1 g of oil accounted 35 mg of nitrogen. A 9–ml-sample of the liquid cultural mixture of *G.rubripertincta* IMB Ac-5005, *R.erythropolis* IMB B-7012 and *A.calcoaceticus* IMB B-7013 strains (in the ratio 1:1:1) after their separate cultivation on the medium with 0.5 % (v/v) n-hexadecane was used as inoculum. Experiments were carried under aerated conditions using a microprocessor SMD Rework Station AOYUE 852 (Aoyue tongyi electronic equipment factory, China) providing air at 0.5 l/l min. The experiment modification without introducing bacteria was used as control.

Alkanes compound analysis of the n-hexane crude oil extract was completed on the Agilent 6890N equipped with a 5973inert mass selective detector and operated in SCAN mode (Agilent Technologies, US). The injection temperature was 250 ºC, column HP–5ms, 30m × 0.25 mm × 0.25 μm (J&W Scientific, USA). The oven temperature program included a 60 ºC hold for 4min ramped to 300 ºC at 4 ºC/min with the final 10 min hold at 300 ºC. Compound identification was performed according to the compound spectra available in the NIST 02 MS library and based on the comparison with the retention times of the standard mixture of saturated hydrocarbons (C_9–C_{24}). When identifying the substances by comparing the experimental and library mass spectra into account only those which have similarity coefficients > 0.85.

Determination of surface-active properties. The emulsification index (E_{24}) of culture samples was determined according to the method of Cooper & Goldenberg [4]. Kerosene (TC-1, Russia) was used as hydrophobic phase. The E_{24} is given as height of the emulsified layer (mm) divided by the total height of the liquid column in a test tube (mm). Cell surface hydrophobicity (CSH) assay was carried out according to the procedure described by Rosenberg et al. [19] with some modifications. Cells were grown on the medium with 1 % (v/v) n-hexadecane as a carbon source and harvested each 24 h during 5 days, then washed twice with phosphate saline buffer (KH_2PO_4 – 3.40, $Na_2HPO_4 \times 12H_2O$ – 8.90, pH – 7.0) and resuspended in the same buffer to reach OD_{540} = 0.5. Acid-washed glass test tubes were filled with 2.5 ml of the cell suspension and 0.5 ml of *n*-hexadecane. The samples were mixed with Vortex FS 16 (BioSan, Latvia) for 60 s. After the phases were allowed to separate, the aqueous phase was carefully removed, and its light absorbance was measured using a Lambda EZ 201 (Perkin Elmer, USA) model spectrophotometer. The percentage of cell adhesion to *n*-hexadecane was used to estimate the hydrophobicity index (HI) calculated as: $HI\ (\%) = [A_1 - A_2) / A_1] \times 100\ \%$, where: A_1 – OD_{540} of the initial cell suspension, A_2 – OD_{540} of the aqueous phase. The surface tension (SFT) of cell-free broth was measured with a digital tensiometer Krüss K6 (Krüss GmbH, Germany) using standard Wilhelmy plate method at room temperature according to the manufacturer's instructions. To determine of SFT the cell-free broth was preliminarily pretreated with hexane to remove of residual *n*-hexadecane, which has surface-active properties.

Chemical nature of biosurfactants. The content of the total lipids *G. rubripertincta* IMB Ac-5005 and *R. erythropolis* IMB B-7012 strains was determined as described by Folch et al. [8]. Determination of lipids carried by thin layer chromatography plates DC-Alufolien Kieselgel 60 (Merck, Germany) in a solvent system (mobile phase): non polar: hexane–diethyl ether (2:1); polar: chloroform–methanol–water (85:15:1). The visualization of the chromatogram was performed with 10 % phosphomolybdic acid solution in ethanol using marker analysis, comparing the R_f of the studied lipids with the R_f of the standard. Peptidoglycolipids were identified according to Kretschmer and Bock [13]. Isolation of surfactant synthesized by *A. calcoaceticus* IMB B-7013 was performed as described by Neufeld and Zajic [16]. The content of carbohydrates in the surfactants was determined by calorimetry in the reaction with phenol and sulfuric acid [6], proteins were identified by the Bradford method [2] and lipids – by the method of Folch et al. [8]. The amount of these components was presented as a percentage of dry matter.

All experiments were carried out in three replicas and statistical analysis was performed using Microsoft Office Excel 2003.

Results. The determination of the hydrocarbon biodegradation efficiency (BE) of strains *G. rubripertincta* IMV Ac-5005, *R. erythropolis* IMV B-7012 and *A. calcoaceticus* IMV B-7013 showed (Table 1) that the highest BE they demonstrated in relation to n-hexadecane (82.1–86.7 %), somewhat smaller – to kerosene (72.5–80.3 %), diesel fuel (70.1–74.3 %) and crude oil (63.5–68.9 %). It was found that the BE of the mixed-culture of these bacteria increased by 7–10 %.

Table 1

Hydrocarbons biodegradation by the tested strains

Strains	Biodegradation efficiency, %			
	crude oil	diesel fuel	kerosene	*n*-hexadecane
G. rubripertincta IMB Ac-5005	65.4±2.9	71.9±2.8	80.3±2.9	85.3±2.6
R. erythropolis IMB B-7012	68.9±2.7	74.3±2.7	75.4±2.8	86.7±2.7
A. calcoaceticus IMB B-7013	63.5±2.8	70.1±2.9	72.5±2.7	82.1±2.8
Mixed-culture of strains (1:1:1)	76.0±3.4	82.2±3.3	86.6±3.2	97.8±3.1

Note: Determination was performed after 120 h of cultivation of the strains in medium with the initial hydrocarbon concentration of 0.5 %.

For the model experiments on oil biodegradation in water by the mixed culture (1:1:1 v/v) of *R. erythropolis* IMB B-7012, *G. rubripertincta* IMB Ac-5005 and *A. calcoaceticus* IMB B-7013 crude oil was used. It contained 72.8 % PNH as the main components, 23.6 % of aromatics and a small amount (3.9 %) of asphaltics. It was shown that the BE of the mixed-culture at the end of the experiment reached 80.5 % which exceeds the control 1.7 times. Chemical analysis of the hexane extracts of PNH showed that they are represented by continuous homologous series of the n-alkanes with intermediate peaks of iso-alkanes (Fig. 1A). An undivided complex of cycloalkanes and aromatic hydrocarbons is located at the base of the peaks.

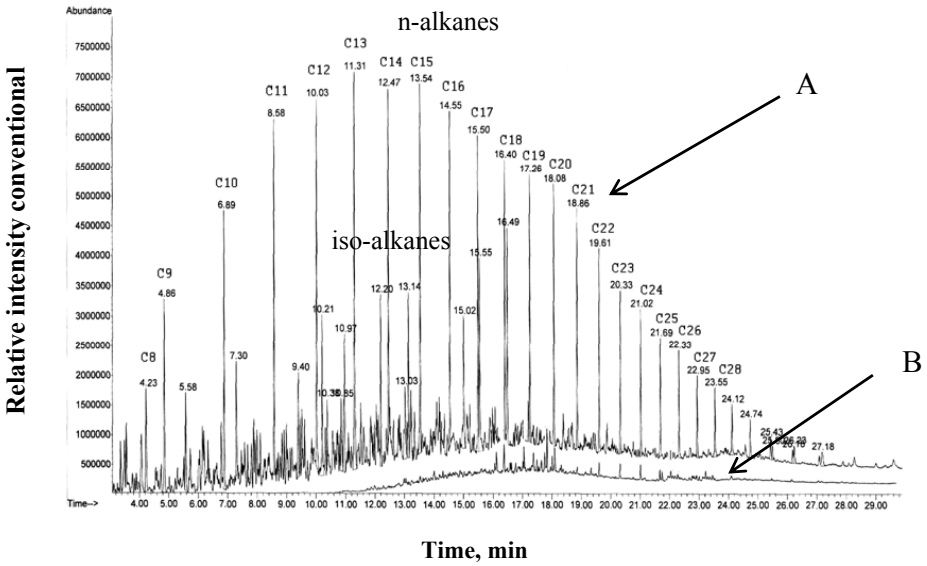

Fig. 1. Chromatogram of the hexane extracts of paraffin-naphthenic hydrocarbons in the process of purification by the stains of water contaminated with crude oil. A – the control sample containing untreated oil-polluted (4.000 mg/l) water; B – the sample of water treated with the mixed-culture of *G. rubripertincta* IMB Ac-5005, *R. erythropolis* IMB B-7012 and *A. calcoaceticus* IMB B-7013 for 30 days

The composition of the PNH included the n-alkanes with carbon chain length C_9–C_{30}, with the prevalence of C_{10}–C_{21} and dominance of C_{11}–C_{17}. It was found that the iso-alkanes PNH mainly included methylated derivatives of the n-alkanes iso-C_8–iso-C_{17}. The mixed-culture of the tested strains almost completely utilized these oil fractions (Fig. 1B). The strains completely assimilate n-alkanes C_9–C_{21} as well as iso-alkanes C_8–C_{17} and significantly decreased n-alkanes C_{22}–C_{26} (150–234 times) in comparison to the initial amount.

The surface-active properties of the strains tested were determined in the process of their utilization of n-hexadecane. All strains demonstrated good growth on this substrate. By the end of cultivation (5 days) the cell titres of strains increased from 10^6 CFU/ml to 2.1×10^8 – 3.4×10^8 CFU/ml (Fig. 2). Our study showed that the initial level of cell-surface hydrophobicity in *R. erythropolis* IMB B-7012 and *G. rubripertincta* IMB Ac-5005 before the contact with hydrocarbon substrate had low indices (2.4 and 9.6 % accordingly) (Fig. 2, a, b).

During the cultivation on n-hexadecane the hydrophobicity index (HI) of *R. erythropolis* IMB B-7012 increased by more than 20 times and reached the maximum level of 60 % at the end of the exponential growth phase (Fig. 2, a). It was accompanied with the reduction of S-forms of this strain and the increase of R-forms with higher hydrophobicity of cell surface and activity of hydrocarbon assimilation. In *G. rubripertincta* IMB Ac-5005 which forms only S-variants of colonies cells hydrophobicity increased 3.9 times (Fig. 2, b). In contrast to actinobacteria strains, *A. calcoaceticus* IMB B-7013 cells possessed high initial HI (99.4 %), reducing down to 72.0 % at the end of the exponential growth on n-hexadecane (Fig. 2, c).

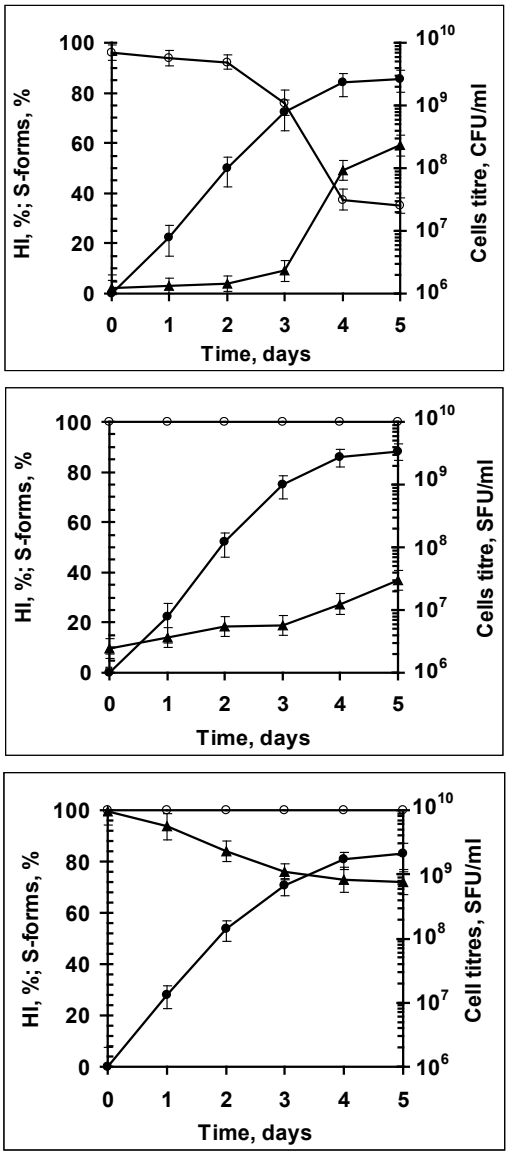

Fig. 2. The change of a cell surface hydrophobicity, amount of S-forms dissociants and titre cells of the tested strains during the growth on n-hexadecane. ▲ – HI (hydrophobicity index); ○ – S-forms; ● – colony forming units (CFU/ml). a – *R. erythropolis* IMB B-7012; b – *G.rubripertincta* IMB Ac-5005; c – *A. calcoaceticus* IMB B-7013

The study of the strains ability to produce of biosurfactants showed that the cell suspensions of *R. erythropolis* IMB B-7012 and *G. rubripertincta* IMB Ac-5005 had high emulsification index (E_{24} = 51.0 and 54.0 % respectively) and free cell culture had low E_{24} – 4–5 % (Table 2). These results indicate that these strains produced cell-bound biosurfactants. The presence of small quantities of biosurfactants in free cell culture can be explained by lipid nature of these substances that are easily dissolved in hydrocarbons and can be extracted by them from cells. The surface tension in the cultivation medium was reduced by these strains to 50–46 mN/m.

Table 2

Surface-active properties of the tested strains

Strains	Emulsification index E_{24} (%)		Surface tension of free cell culture (mN/m)
	Cell suspension	Free cell culture	
R. erythropolis IMB B-7012	51.0 ± 2.0	5.0 ± 0.6	50.0 ± 0.4
G. rubripertincta IMB B-5005	54.0 ± 3.2	4.0 ± 0.5	46.0 ± 0.5
A. calcoaceticus IMB B-7013	4.0 ± 0.6	67.0 ± 2.6	40.0 ± 0.6

Note: The growth was carried out at 28 °C for 5 days on an orbital shaker (220 rpm) on the liquid medium with 1 % (v/v) n-hexadecane as carbon source.

It was revealed that in comparison with cells suspension free cell culture of *A. calcoaceticus* IMB B-7013 possessed, considerably higher emulsification index (E_{24} = 67.0 %). It testified to its capability to synthesise extracellular biosurfactants. According to our data, this strain reduced the surface tension in the cultivation broth down to 40.0 mN/m. It was shown that investigated strains also differed from each other by the type of emulsion they formed: *Rhodococcus* and *Gordonia* strains formed stable emulsions of "oil-in-water" type while *Acinetobacter* strain produced "water-in-oil"-like emulsions.

Determination of the chemical nature of biosurfactants from the tested strains showed that glycolipids, identified as trehalose monomycolate and trehalose dimycolate, as well as peptidoglycolipids dominate in the polar lipid fraction of actinobacteria. Among the non-polar lipids of *R. erythropolis* IMB B-7012 and *G. rubripertincta* IMB Ac-5005 strains mycolic acids, cetyl alcohol, palmitic acid and fatty acid methyl esters are found. In the content of extracellular biosurfactants of *A. calcoaceticus* IMB B-7013 carbohydrates (43.5 %) and proteins (39.6 %) were found in prevailing quantities and lipids (14.1 %) – in small amounts. Our experiments showed that the isolated from the growth medium crude emulsan contains a complex which exhibits high emulsifying activity (E_{24} = 76.5 %) on hydrophobic substrates such as kerosene. The value of E_{24} of an individual fraction of this biosurfactants was much lower. So, E_{24} of the carbohydrate-protein fraction was equal to 54.7 % and the lipid fraction – to 44.0 %. This indicates that the emulsifying properties of this biosurfactant are related to the presence of both of these fractions.

Discussion. It is known that the natural processes of bioremediation of oil-contaminated water and soil occurs with the active participation of microorganisms. More than 20 families of bacteria and 10 families of fungi capable of biological degradation of various hydrocarbons have been described [15]. This study represents the results of investigation of the biodegradation potential of the species *G. rubripertincta* IMB Ac-5005, *R. erythropolis* IMB B-7012 and *A. calcoaceticus* IMB B-7013. Representatives of these genera are often detected in oil-contaminated soil or water and each plays a role in the transformation of hydrocarbons [9, 14]. This study showed that the susceptibility of hydrocarbons to microbial attack decreases in the following order: n-hexadecane > kerosene > diesel fuel > crude oil. The mixed culture of the strains showed the most effective destruction of these substrates. These results are confirmed by other authors, who suggest that an associations of

microorganisms exhibit a higher BE of oil hydrocarbons than monocultures [20, 23]. Of the various oil fractions, n-alkanes of the intermediate length (C_{10}–C_{20}) are the preferred substrates and tend to be the most readily degradable. Long chain n-alkanes (C_{20}–C_{40}) are hydrophobic solids which makes them difficult to be degraded due to their poor water solubility and bioavailability and branched-chained alkanes are also degraded more slowly than normal alkanes [10]. In the present study the mixed-culture of the tested strains has shown maximal degradation (80.5 %) of 0.4 % crude oil and complete assimilation of n-alkanes C_9–C_{21}, iso-alkanes C_8–C_{17} and also significantly decreased the amount of n-alkanes C_{22}–C_{26} (150–230 times) after 30 days of purification of contaminated water. The ability to utilize short chain n-alkanes (C_9 to C_{12}) as well as a broad range of long chain n-alkanes (C_{19} to C_{32}) present in crude oil is also exhibited by other rhodococci and acinetobacteria strains [7, 14]. The high biodegradation activity of the strains tested in this work in relation to iso-alkanes and long chain n-alkanes indicates that they can be useful in degrading complex compounds present in crude oil.

The hydrophobic nature of petroleum hydrocarbons is a strong limiting factor in microbial degradation. Therefore, among the basic parameters that could affect the effectiveness of hydrocarbon degradation by microorganisms there are such important properties as the cell-surface characteristics and the ability of strains to synthesize biosurfactants. Literature sources note that the main mechanisms of microorganisms' adaptation to assimilation of hydrophobic substrates are: an increased in level of hydrophobicity of cell surface providing direct contact with hydrocarbon droplets and/or the synthesis of biosurfactants emulsifying the hydrophobic substrate in medium and providing mediated contact with hydrocarbon droplets [3, 9, 14]. The unique property of actinobacteria, belonging to the mycolata taxa in which *Rhodococcus* spp. and *Gordonia* spp. are included, is the external lipid barrier formed by 2-alkyl-3-hydroxy fatty acids of high molecular mass (mycolic acids) and their ethers with trehalose, permeable for hydrophobic substrates [22]. The obtained results coincide with the data cited in the literature about the dynamics of increasing hydrophobicity of rhodococci cell cultures grown on the medium with hydrocarbons and cell-surface hydrophobicity of strains with different colonial morphologies [3, 24]. It was determined in this study that the process of utilization of hydrocarbons by low hydrophobic actinobacteria cells is accompanied by the cell surface hydrophobicity increase and the production of cell-bound glycolipid biosurfactants promoting the adhesion of cells to hydrocarbons. As it is known such substances are the main glycolipid components of cell walls of these bacteria and are responsible for surface-active properties [22].

The assimilation of hydrocarbons by highly hydrophobic acinetobacteria cells resulted in the hydrophobicity index decrease and the formation of emulsan-like extracellular metabolites with surface-active and emulsifying properties. According to the literature, high level of hydrophobicity of *A. calcoaceticus* is one of the main characteristics of this species, contributing to active degradation of hydrocarbons that mediates the attachment of cells to hydrocarbon droplets and stimulates the induction of enzymes for their assimilation [16, 17]. The composition of these substances was close to emulsans typical for this species and includes heteropolysaccharide containing fatty acids

with noncovalently bound proteins [1, 18]. The literature sources also note that the emulsifying activity of extracellular biosurfactants of hydrocarbon-degrading *Acinetobacter* species are largely determined by the presence of lipid and protein components [1, 18].

Thus, the results of this work demonstrated that *G. rubripertincta* IMB Ac-5005, *R. erythropolis* IMB B-7012 and *A. calcoaceticus* IMB B-7013 show high efficiency of oil hydrocarbons biodegradation and exhibit different mechanisms of adaptation to hydrocarbon assimilation. The findings indicate the great bioremediation potential of these strains for the cleaning of oil-polluted environments.

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В.С. Підгорський, Т.М. Ногіна

Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України, вул. Академіка Заболотного, 154, Київ, 03143, Україна

БІОДЕГРАДАЦІЯ ВУГЛЕВОДНІВ НАФТИ ШТАМАМИ АКТИНОБАКТЕРІЙ І АЦИНЕТОБАКТЕРА, ЯКІ СИНТЕЗУЮТЬ БІОСУРФАКТАНТИ

Резюме

Забруднення навколишнього середовища нафтовими вуглеводнями є однією з найактуальніших проблем в усьому світі. Ефективність біоремедіації нафтових забруднень у значній мірі залежить від природних особливостей мікроорганізмів та їх специфічних адаптивних механізмів засвоєння вуглеводнів. У цій роботі визначена ефективність біодеструкції і поверхнево-активні властивості вуглеводень-окиснювальних штамів *Gordonia rubripertincta* IMB Ac-5005, *Rhodococcus erythropolis* IMB B-7012 і *Acinetobacter calcoaceticus* IMB B-7013. Ці штами проявляли високу ефективність біодеструкції н-гексадекану (82,1–86,7 %), керосину (72,5–80,3 %), дизельного палива (70,1–74,3 %) і сирої нафти (63,5–68,9 %). Змішана культура цих штамів повністю асимілювала н-алкани C_9–C_{21}, а також ізо-алкани C_8–C_{17} і значно зменшувала (у 150–230 разів) вміст н-алканів C_{22}–C_{26} у процесі очищення води від сирої нафти. Засвоєння вуглеводнів штамами *G. rubripertincta* IMB Ac-5005 і *R. erythropolis* IMB B-7012 із низьким початковим рівнем індексу гідрофобності (2,4 і 9,6 % відповідно) супроводжувалось збільшенням гідрофобності клітинної поверхні (у 4 і 25 разів відповідно) та синтезом клітинно-зв'язаних біосурфактантів, які утворювали стійкі емульсії типу «масло у воді». Домінуючими компонентами цих біосурфактантів були гліколіпіди: моно- і диміколати трегалози. Засвоєння вуглеводнів високо гідрофобним штамом *A. calcoaceticus* IMB B-7013 з початковим індексом гідрофобності 99,4 % супроводжувалось зменшенням цього показника в 1,4 рази і синтезом позаклітинного біосурфактанту, який утворював стабільні емульсії типу «вода у маслі». За хімічним складом і співвідношенням основних компонентів (вуглеводи, білки та ліпіди) біосурфактант *A. calcoaceticus* IMB B-7013 близький до емульсанів. Експериментальні дані дають підстави для ефективного використання цих штамів у процесі біоремедіації забруднених нафтою води і ґрунту.

Ключові слова: вуглеводень-окиснювальні мікроорганізми, поверхнево-активні властивості, біосурфактанти, *Gordonia*, *Rhodococcus*, *Acinetobacter*.

В.С. Подгорский, Т.М. Ногина

Институт микробиологии и вирусологии им. Д.К. Заболотного НАН Украины, ул. Академика Заболотного, 154, Киев, 03143, Украина

БИОДЕГРАДАЦИЯ УГЛЕВОДОРОДОВ НЕФТИ ШТАММАМИ АКТИНОБАКТЕРИЙ И АЦИНЕТОБАКТЕРА, СИНТЕЗИРУЮЩИМИ БИОСУРФАКТАНТЫ

Резюме

Загрязнение окружающей среды нефтяными углеводородами является одной из самых актуальных проблем во всем мире. Эффективность биоремедиации нефтяных загрязнений в значительной степени зависит от природных особенностей микроорганизмов и их специфических адаптивных механизмов усвоения углеводородов. В этой работе определена эффективность биодеструкции и поверхностно-активные свойства углеводородокисляющих штаммов *Gordonia rubripertincta* IMB Ac-5005, *Rhodococcus erythropolis* IMB B-7012 и *Acinetobacter calcoaceticus* IMB B-7013. Эти штаммы проявляли высокую эффективность биодеструкции н-гексадекана (82,1–86,7 %), керосина (72,5–80,3 %), дизельного топлива (70,1–74,3 %) и сырой нефти (63,5–68,9 %). Смешанная культура этих штаммов полностью ассимилировала н-алканы C_9–C_{21}, а также изо-алканы C_8–C_{17} и значительно уменьшала (в 150–230 раз) содержание н-алканов C_{22}–C_{26} в процессе очистки воды от сырой нефти. Усвоение углеводородов штаммами *G. rubripertincta* IMB Ac-5005 и *R. erythropolis* IMB B-7012 с низким начальным индексом гидрофобности (2,4 и 9,6 % соответственно) сопровождалось увеличением гидрофобности клеточной поверхности (в 4 и 25 раз соответственно) и синтезом клеточно-связанных биосурфактантов, которые образовывали устойчивые эмульсии типа «масло в воде». Доминирующими компонентами этих биосурфактантов были гликолипиды: моно- и димиколаты трегалозы. Усвоение углеводородов высоко гидрофобным штаммом *A. calcoaceticus* IMB B-7013 с начальным индексом гидрофобности 99,4 % сопровождалось уменьшением этого показателя в 1,4 раза и синтезом внеклеточного биосурфактанта, образующего стабильные эмульсии «вода в масле». По химическому составу и соотношению основных компонентов (углеводы, белки и липиды) биосурфактант *A. calcoaceticus* IMB B-7013 близок к эмульсанам. Экспериментальные данные дают основания для эффективного использования этих штаммов в процессе биоремедиации загрязненных нефтью воды и почвы.

Ключевые слова: углеводородокисляющие микроорганизмы, поверхностно-активные свойства, биосурфактанты, *Gordonia*, *Rhodococcus*, *Acinetobacter*.

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