

**Gorshkova O.G., Voliuvach O.V., Kolomiets O.G., Bebyk V.V.,
Khadzhy V.D., Lagovskaya L.S.**

Odessa I.I. Mechnikov National University,
Dvoryanskaya str., 2, Odessa, 65082, Ukraine; E-mail: tgudzenko@ukr.net

BIOMARKER VALUES OF NONPATHENOUS MICROORGANISMS WITH POLYFUNCTIONAL BIOTECHNOLOGICAL PROPERTIES

Abstract

Isolated from the oil-contaminated soil of Zmeinyi island and the marine environment are four non-pathogenic biochemically active strains of microorganisms identified by the fatty acid composition of their cell lipids as *Microbacterium barkeri* OZ-3, *Bacillus megaterium* OZ-5 (isolated from soil), *Pseudomonas fluorescens* ONU-328, *Pseudomonas maltophilia* ONU-329 (separated from the marine environment). The strains possess polyfunctional biotechnological properties: sorption-accumulating ability relative to heavy metal ions, oxidizing activity with respect to petroleum products, anionic surfactants and phenolic compounds. Biomarker values for distinguishing the strains of microorganisms studied (*Microbacterium barkeri* OZ-3, *Bacillus megaterium* OZ-5, *Pseudomonas fluorescens* ONU-328, *Pseudomonas maltophilia* ONU-329) are proposed at the species level.

Key words: biomarker values, microorganisms, non-pathogenic, polyfunctional.

Introduction

In most countries in the world, biological methods are preferred from known methods for purifying water and soil from pollutants of different nature. They are more effective, universal and ecologically safe than non-pathogenic microorganisms in comparison with physico-chemical methods, they do not cause secondary pollution. Therefore, today, biotechnologists are faced with the pressing need to produce new generation biologics intended for cleaning the environment from a wide range of toxic pollutants - heavy metal ions, petroleum products, synthetic surfactants, phenolic compounds. The search and identification of new biochemically active microorganisms, which are non-pathogenic, and have polyfunctional biotechnological properties, continues. The aim of the work is to isolate from non-polluting sources non-pathogenic biochemically



active microorganisms with polyfunctional biotechnological properties and to analyze the fatty acid spectra of cellular lipids to offer biomarker values for their differentiation at a species level.

Materials and methods

The subjects of the study were four biochemically active strains of microorganisms isolated from the oil-polluted soil of Zmeiniy island and from a polluted marine environment. Fatty acid analysis was carried out on an Agilent Technologies 7890 gas chromatograph using an automatic microorganism identification system.

Results and discussions

Specially selected biochemically active microorganisms from the obtained fatty acid composition (the spectra of which are deciphered using the RTSBA6 6.2 library database of the Sherlock MIDI program with a high index of similarity of Sim Index ≥ 0.7) are assigned to *Microbacterium barkeri* OZ-3, *Bacillus megaterium* OZ-5 (isolated from oil contaminated soil of Zmeiniy island) and *Pseudomonas fluorescens* ONU328, *Pseudomonas maltophilia* ONU329 (isolated from the marine environment). According to the decoding of chromatographic spectra of common cell lipids of the investigated ground and sea microorganisms, fatty acids C15:0 anteiso and C15:0 iso were found to prevail among the available fatty acids of the branched structure (in total they constituted the largest percentage of the total of the total areas of the peaks). To distinguish microorganisms at the generic level, we suggest considering the ratio of branched fatty acids as biomarker values (Table 1).

Table 1

Biomarker values of some marine and soil strains of microorganisms of broad biotechnological use

Biomarker values	Strains of soil microorganisms		Strains of marine microorganisms	
	M.barkeri O3-3	B.megaterium O3-5	P.maltophilia ONU-329	P.fluorescens ONU-328
[C ₁₅ :0 anteiso/ C ₁₅ :0 iso]	8,4	1,2	0,54	----
[C ₁₇ :0 anteiso/ C ₁₇ :0 iso]	16,2	1,4	----	----
[C ₁₅ :0 anteiso/ C ₁₇ :0 anteiso]	1,7	14,7	----	----



In the fatty acid profiles of soil strains *M. barkeri* OZ-3 and *B. megaterium* OZ-5, in contrast to the fatty acid profiles of marine strains *P. maltophilia* ONU329 and *P. fluorescens* ONU328 were present from branched fatty acids 15-methylhexadecanoic (C17:0 iso) and 14-methylhexadecanoic acid (C17:0 anteiso), which allowed them to calculate the ratios [C17:0 anteiso / C17:0 iso], [C15:0 anteiso / C17:0 anteiso].

The calculated data obtained in the analysis and comparison of the fatty acid profiles of soil strains *M. barkeri* OZ-3 and *B. megaterium* OZ-5 showed that the value of [C17:0 anteiso/C17:0 iso] is an order of magnitude higher for strain *M. barkeri* OZ-3; and vice versa, the biomarker value [C15:0 anteiso / C17:0 anteiso] during the transition from strain *B. megaterium* OZ-5 to strain *M. barkeri* OZ-3 decreases by an order of magnitude from 14.7 to 1.7.

In the fatty acid spectrum, the saturated fatty acids of the strain *M. barkeri* OZ-3 lacked saturated cycloalkanoic acids, and from the saturated fatty acids of the normal structure were not fixed, as in the composition of the cell lipids of strains *B. megaterium* OZ-5, *P. maltophilia* ONU-329, *P. fluorescens* ONU328 [2], decanoic C10:0, dodecanoic C12:0 and pentadecanoic acid C15:0. Dodecanoic acid was observed only in the fatty acid profile of *P. fluorescens* ONU328 strain. The fatty acid composition of the *M. barkeri* OZ-3 strain compared to *B. megaterium* OZ-5, *P. maltophilia* ONU-329, *P. fluorescens* ONU-328 strains is, That in its fatty acid pool, although in a small amount biomarker heptadecanoic acid is fixed. In the study, strains of microorganisms differ in the quantitative content in their fatty acid profile of hydroxy acids [1, 2]. The metal-resistant ability of microorganisms to highly toxic heavy metal ions [Pb (II), Zn (II), Cr (VI) [3-5] has been experimentally established and the ability to decompose oil: the degree of biodegradation of oil with a concentration of 10 mg/10 ml of bacterial suspension for 20 days of exposure was 40-45% when using soil strains of microorganisms [1] and 70-75% when using marine strains of microorganisms. A good growth of microorganisms on «hungry» agar with 1% sodium dodecyl sulfate was noted, which allows them to be proposed for use in biotechnologies for cleaning the environment from organic compounds. When compiling a new biopreparation on the basis of the association of the strains of microorganisms under study, we established their synergistic adsorption action with respect to Cr (VI) [5] and oxidizing activity with respect to petroleum hydrocarbons and phenolic compounds.



Conclusions

Proposed biomarker values for distinguishing at a species level non-pathogenic strains of *M. barkeri* OZ-3, *B. megaterium* OZ-5, *P. fluorescens* ONU-328, *P. maltophilia* ONU-329. They possess a sorption-accumulating ability with respect to heavy metal ions, oxidizing activity with respect to petroleum products, anionic surfactants and phenolic compounds, do not inhibit each other, and therefore can be successfully used for the preparation of a bacterial association for a wide biotechnological purpose.

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