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BIOLOGICAL PROPERTIES OF BACTERIA *BACILLUS SUBTILIS* ONU551 AND *AEROMONAS* *ICHTHIOSMIA* ONU552 – PHENOL DESTRUCTORS

Abstract

The biological properties of phenol destruction bacteria *Bacillus subtilis* ONU551 and *Aeromonas ichthiosmia* ONU552 are studied. The strain of *Bacillus subtilis* ONU551 is presented by gram-positive rods that form subterminal spores. A strain of *Aeromonas ichthiosmia* ONU552 is gram-negative direct rod. The features of fat-acid composition of strain of *Aeromonas ichthiosmia* ONU552, *B. subtilis* ONU551 of destructions – to the phenol, that distinguish them from other bacteria – in cellular lipids each of strain presence of fat acids : 16: 1 w7c alcohol, 17: 0 iso, 17: 0 anteiso.

Key words: phenol, purification of water, bacteria of destructors, *Bacillus subtilis* ONU551, *Aeromonas ichthiosmia* ONU552.

Introduction

The risk of phenolic compounds entering the sewage into the environment is due to their toxicity to biological objects and resistance to decomposition.

Microbiological detoxification is a promising method of purifying the environment, during which the cleavage of the aromatic ring occurs and the formation of non-toxic compounds – carbon dioxide and water [1, 2].

In this regard, the current issue is the development of New Environmentally Safe Biotechnologies for the Purification of Sewage from Phenol [3].

The aim of the work was to study the biological properties of bacteria *Bacillus subtilis* ONU551 and *Aeromonas ichthiosmia* ONU552 – phenol destructors promising for use in biotechnology wastewater treatment.

Materials and methods

The objects of the study were strains *Bacillus subtilis* ONU551 and *Aeromonas ichthiosmia* ONU552, isolated from wastewater produced by pharmaceutical preparations.

Morphological properties of strains were investigated using classical bacteriological methods.

The analysis of fatty acid profile of strains was carried out by gas chromatography using the system of identification of microorganisms MIDI Sherlock (MIDI, USA). Cultivation of microorganisms was carried out on Tryptic soy agar, at 24 °C for 24 hours.

Lyses and lipids were washed with 50% CH₃OH and 3.7 M NaOH at



95–100 °C. for 30 minutes, methylation with acidic methanol solution, 80 °C., 10 min, neutralization, 0.3 M NaOH solution. Chromatographic separation was carried out at 170–270 °C with a gradient of 5°C / min.

For the identification of microorganisms, a system for the identification of microorganisms MIDI Sherlock, a library of fatty acid profiles of aerobic microorganisms RSTBA6 Version 6.2 was used.

Results

As a result of the research, it was found that the strain *Bacillus subtilis* ONU551 is represented by mobile, large gram-positive sticks measuring 2.2 × 5.5.0 µm for mangoval endospores that are subterminally placed.

Fatty acid composition of the total bacterial lipids of *Bacillus subtilis* ONU551 representations in Table 1 and Fig. 1

From Table 1 of Fig. 1 shows that in the total bacterial lipids, 14 fatty acids with a predominant content of long chain fatty acids of branched structure 15: 0 (13-methyltetradecanoic acid and 12-methyltetradecanoic acid) and 17: 0 (15-methyl hexadecanoic acid and 14-methylhexadecanoic acid) in the form of iso and anteiso.

The methyltetradecanoic acid iso was in the minor amount, and the anteiso is absent.

Table 1

Fatty acid composition of common lipid bacteria *Bacillus subtilis* ONU551

Fatty acid	% of the total peak areas	Fatty acid	% of the total peak areas
12:0	0.36	16:0 iso	1.85
14:0 iso	0.52	16:1 w11c	1.21
14:0	0.28	16:0	1.30
15:0 iso	34.72	17:1 iso w10c	3.18
15:0 anteiso	33.72	17:0 iso	7.11
15:1 w5c	1.85	17:0 anteiso	10.24
16:1 w7calcohol	1.08	17:1 iso I/ anteiso B	2.57

The mole fraction of other fatty acids in bacteria *Bacillus subtilis* ONU551 is 3% and lower. *Bacillus subtilis* ONU551 hydroxy acids are absent.

On the basis of morphological properties and fatty acid composition of common lipids, the species belonging the ONU551 strain to *Bacillus subtilis* was confirmed.

The strain *Aeromonas ichthiomia* ONU552 is a gram-negative straight stick with rounded ends, measuring 0.5 x 2.5 microns. In smears, they are located individually.

The bacteria *Aeromonas ichthiomia* ONU552 grow at 20–30 °C, pH 7.0 a simple nutrient media – MAA.

The spectrum of fatty acids of strain *Aeromonas ichthiomia* ONU552 is presented in Table 2.

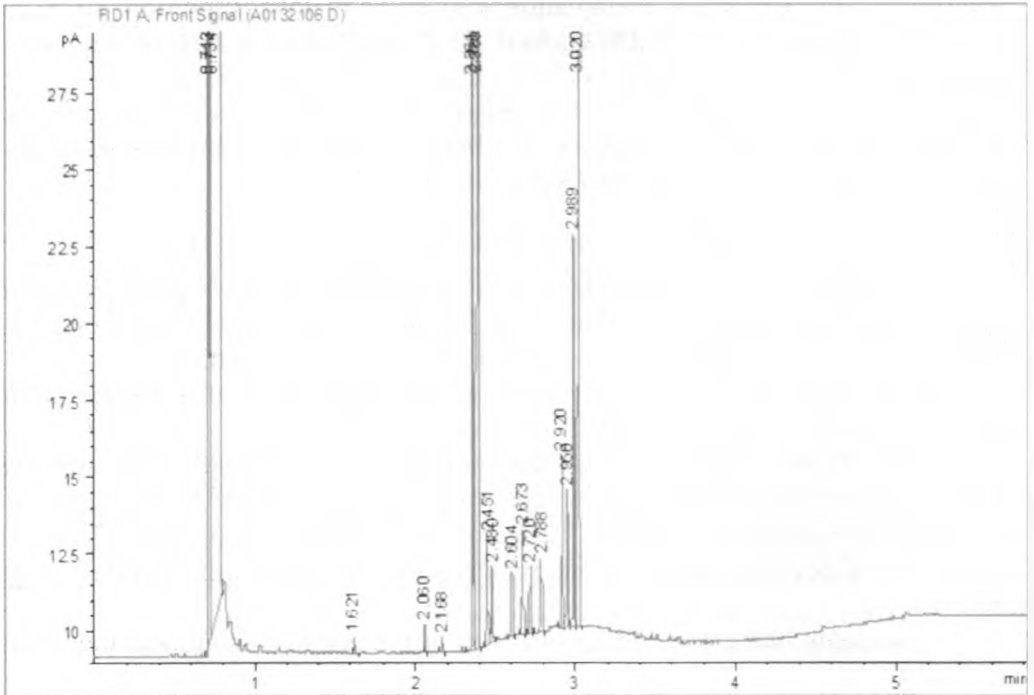


Fig. 1. Fatty acid spectrum of *Bacillus subtilis* ONU551 strain

Table 2

Fatty acid composition of common lipid bacteria *Aeromonas ichthiosmia* ONU552

Fatty acid	% of the total peak areas	Fatty acid	% of the total peak areas
10:0	0.17	∑16:1 w7c/16:1 w6c	36.89
12:0	6.94	16:1 w5c	0.12
12:0 3OH	0.23	16:0	21.84
13:0 iso	0.20	17:1 iso w9c	1.35
13:0	0.15	17:0 iso	1.49
14:0	3.77	17:0 anteiso	0.27
∑14:0 3OH/16:1 iso I	6.59	17:1 w8c	0.60
15:0 iso	0.97	17:1 w6c	0.26
15:0 iso 3OH	3.85	17:0	0.33
15:0 3OH	0.35	18:1 w7c	8.53
16:1 w7c alcohol	3.45	18:0	0.30
16:0 N alcohol	1.34		

It is evident from the data in Table 2 that in the total of the lipid bacteria *Aeromonas ichthiosmia* ONU552 23 fatty acids were found with 16: 0 (hexadecanoic acid), the sum of (9-hexadecenoic acid and 10-hexadecenoic acid) hexadecenoic acids and 11-octadecenoic acid. The content of other acids was at a level of 7% or less.



Aeromonas ichthiosmia ONU552 hydroxy acids present and they are biomarkers for the differentiation of this strain at the generic level.

Conclusions

1. The morphological properties of two strains isolated from sewage – *Bacillus subtilis* ONU551 strain represented by gram-positive rods, which form subterminally located endospores, strain *Aeromonas ichthiomia* ONU552 – gram-negative straight rods.
2. Specific feature of the fatty acid composition of strains *A. ichthyosis* ONU552, *B. subtilis* ONU551 destructors – phenol that distinguish them from other bacteria – in cellular lipids of each strain the presence of fatty acids: 16: 1 w7c alcohol, 17: 0 ISO, 17: 0 anteiso.

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