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SCREENING OF MARINE SPORE-FORMING BACTERIA DEGRADING POLYETHYLENE TEREPHTHALATE

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Abstract. *The ability of 60 cultures of marine spore-forming bacteria to degrade polyethylene was studied. The activity of microorganisms was evaluated preaccording to formation of clear zone around colonies on LB agar containing bis(hydroxyethyl)terephthalate (BHET). A total of 24 active strains were found with the prevalence of species *Bacillus subtilis*. Strains of *Bacillus reuszeri*, *Bacillus licheniformis* and *Priestia megaterium* showed the highest activity.*

Keywords: *polyethylene terephthalate, biodegradation, spore-forming bacteria, Black Sea.*

Introduction. The problem of plastic pollution is actual and requires finding new solutions [Sadler et al., 2021]. It is known that microorganisms can use waste from various industries as substrates for bioconversion and decompose them into monomers. Therefore, marine spore-forming bacteria were screened to determine their ability to degrade polyethylene terephthalate.

Materials and methods. The objects of the study were sixty cultures of spore-forming bacteria isolated from the bottom sediments of the Black Sea. All studied strains are represented by nine genera. The species affiliation of another 12 strains is unknown.

Cultivation of bacteria was carried out on an agarized LB nutrient medium with the following composition (g/l): tryptone – 10; NaCl - 5; yeast extract - 5; agar - 15. 5 mM bis(hydroxyethyl)terephthalate (BHET) was added to the medium. BHET consists of terephthalic acid and ethylene glycol residues, that are PET monomers. Therefore, it is convenient to use it to check the presence of PET hydrolytic activity [Weber et al., 2021].

All cultures were plated in duplicate for each strain. Cultivation was carried out in incubators with temperature regimes of 30 °C and 37 °C within 1 week.

The ability of the cultures to decompose polymer additive was evaluated by the formation of a transparent zone around the colonies.

Results and discussion. The strains were assigned numbers from B1 to B60. A total of 24 strains showed a positive result. The most active were 6 strains (B1, B8, B10, B12, B16 and B19) with zones of 2 mm and more. Average activity was shown by three strains (B26, B31 and B33) that formed zones of 1.5 mm. All other strains showed low activity, forming zones 1 mm or smaller.

No clear correlation between BHET decomposition and temperature was found. Fifteen strains degraded BHET at both temperatures, four strains only at 30 °C, and five strains only at 37 °C. In 10 strains, the activity increased with increasing cultivation temperature, in 7 – on the contrary, it was higher at 30 °C, in 7 strains it did not depend on temperature.

A total of 21 strains out of 24 active ones belonged to seven species of microorganisms: *Priestia megaterium* (3 strains), *Shouchella clausii* (1 strain), *Brevibacillus reuszeri* (1 strain), *Bacillus licheniformis* (1 strains), *Bacillus subtilis* (10 strains), *Bacillus atrophaeus* (2 strains), *Sutcliffiella halmapala* (1 strain). The species affiliation of three other strains is unknown.

The highest prevalence of the ability to decompose BHET was found among representatives of the species *B. subtilis* and was equal to 83%. But they showed low activity, the average size of transparent zones did not exceed 1 mm.

Strains belonging to the species *B. reuszeri* and *B. licheniformis* showed an average prevalence of 43 and 50%, respectively. Among the *B. reuszeri* strains, one strain (B8) showed high activity, and among the *B. licheniformis* strains, two strains (B12 and B19) showed high activity.

P. megaterium strains showed a reduced frequency of prevalence (27%), but at the same time high activity (two active strains B1 and B16). This bacterium was found in a consortium capable of degrading PET [Taniguchi et al., 2019]. Results demonstrate that *P. megaterium* decompose PET degradation products such as BHET.

The species *S. clausii*, *B. atrophaeus* and *S. halmapala*, which were each represented by one strain, did not show significant activity.

The genus *Bacillus* is the most widespread degrader among all studied (3 species and 15 strains). The literature describes the ability of *Bacillus* to decompose polyethylene terephthalate, but with low activity, which is confirmed by experimental data.

Conclusions. The ability of marine spore-forming bacteria to decompose polyethylene terephthalate was proven, activity was shown by 24 strains out of 60. Six strains showed high activity. No clear dependence was found between the activity of enzymes that hydrolyze BHT and the temperature regime.

Five genera of bacteria (*Priestia*, *Shouchella*, *Bacillus*, *Sutcliffiella* and *Brevibacillus*) have been shown to be capable of degrading

bis(hydroxyethyl)terephthalate. Representatives of the *Bacillus subtilis* species (ten strains) were most often found among the active cultures, but none of them showed high activity.

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СКРИНІНГ МОРСЬКИХ СПОРОУТВОРЮЮЧИХ БАКТЕРІЙ, ЗДАТНИХ ДО БІОДЕГРАДАЦІЇ ПОЛІЕТИЛЕНТЕРЕФТАЛАТУ

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Анотація. Досліджено здатність 60 культур морських спороутворюючих бактерій до деградації поліетилентерефталату (ПЕТ). Активність мікроорганізмів оцінювали за утворенням прозорих зон навколо колоній на середовищі LB з біс(гідроксіетил)терефталатом (БГЕТ). Виявлено 24 активних штами, більшість яких належать до виду *Bacillus subtilis*. Найвищу активність проявили культури *Bacillus reuszeri*, *Bacillus licheniformis* та *Priestia megaterium*.

Ключові слова: поліетилентерефталат, біодеградація, спороутворюючі бактерії, Чорне море.

References

1. Sadler J. C. Microbial synthesis of vanillin from waste poly(ethylene terephthalate) / J. C. Sadler, S. Wallace // *Green Chemistry*. – 2021. – V. 23, № 13. – P. 4665–4672.
2. Weber G. Enzymatic Plastic Degradation / G. Weber, U. T. Bornscheuer, R. Wei // *Elsevier Science*. – 2021. – V. 7. – P. 145–151.
3. Taniguchi I. Biodegradation of PET: Current Status and Application Aspects / I. Taniguchi, S. Yoshida, K. Hiraga, K. Miyamoto, Y. Kimura, K. Oda // *Catalysis*. – 2019. – V. 9, № 5. – P. 4089–4105.