Biofilms of Some Anaerobic Bacteria on the Polyethylene Terephthalate Surface

Nataliia Tkachuk[1,*], Liubov Zelena[2]

[1]Department of Biology, T.H. Shevchenko National University "Chernihiv Colehium", Ukraine

[2]Department of Physiology of Industrial Microorganisms, Danylo Zabolotny Institute of Microbiology and Virology National Academy of Sciences of Ukraine, Ukraine

*E-mail: nataliia.smykun@gmail.com

ABSTRACT

The intensity of biofilm formation by sulfate-reducing bacteria *Desulfovibrio oryzae* and organic acid-producing bacteria *Anaerotignum propionicum* on the polyethylene terephthalate (PET) surface was studied. The studied D. *oryzae* strains are able to form a biofilm on the surface of PET, which may contribute to the degradation of this plastic. The weak adhesive properties of *Anaerotignum propionicum* against PET have doubts as to polyethylene terephthalate biodegradation by this species.

Keywords: *Anaerotignum propionicum*; biofilm; *Desulfovibrio oryzae*; polyethylene terephthalate.

INTRODUCTION

Biofilm is a surface model of growth of microorganisms. Today, biofilms of microorganisms are actively studied because they affect most important natural and engineering systems. In particular, the role of biofilms is being studied in medicine, wastewater treatment, biodegradation of toxic compounds, biodamage of materials. Due to the high biochemical activity and the ability to form biofilms, bacteria are actively involved in the degradation of polymeric materials [1-2]. A significant amount of polymeric materials is represented by polyethylene terephthalate (PET), the production of which is growing every year: 2 million tons/year in 2007 and 10 million tons/year in 2012 [3]. Among the microorganisms isolated from the plastic surface, there are representatives of sulfate-reducing bacteria [2]. Previously, anaerobic bacteria were identified as participants in the damage of the metal construction in the soil: organic acid-producing bacteria *Anaerotignum propionicum* [4] and sulfate-reducing bacteria Desulfovibrio oryzae [5], whose ability to form a biofilm on the surface of PET has not been studied.

OBJECTIVE

The aim of this work was to study the intensity of biofilm formation of bacteria *D. oryzae* and *A. propionicum* on the surface of PET.

METHODOLOGY

Five-day pure cultures of *A. propionicum* NUChC Sat1, *D. oryzae* NUChC SRB1 and *D. oryzae* NUChC SRB2 isolated from the sulfidogenic microbial community of soil ferrosphere were used for the research [4-5]. The nucleotide sequences were deposited in the GenBank with accession numbers MG924854.1, MT102713.1 and MT102714.1 respectively. Bacteria were grown in Postgate's "C" liquid medium (without adding Fe (II) salt) under anaerobic conditions, which were created by pouring the medium to the edges of the tubes and closing them with rubber stoppers. Suspensions with an optical density of 0.5 McFarland were prepared from cultures of the studied strains in sterile isotonic sodium chloride solution.

To study the intensity of biofilm formation on the PET surface, 10×10 mm samples were cultured in Postgate's "C" liquid medium (76% of the volume) with the addition of meat-peptone broth (MPB, 22% of the volume) and bacterial cultures (2% of the volume) during 50 days.

A biofilm assay (indirect measurement of bacterial biofilm biomass by crystal violet adsorption/desorption) was used [6]. The strains were classified into adhesion categories as was described by Stepanović *et al*. [6]. Statistical analysis of the obtained results was performed using the statistical module of Microsoft Office Excel 2010.

CONCLUSIONS/RESULTS

The results of the study of the optical density of crystal violet solutions, absorbed by the biofilm formed on PET by the studied strains are presented in the Table **1**.

Table 1. Optical density (OD540) of crystal violet absorbed by a biofilm formed on the surface of PET by some strains of anaerobic bacteria.

No	A variant of the experiment	OD_{540} of crystal violet solution	Strain adhesion category
1.	Culture medium without bacteria	0.0380±0.0004	Negative control
2.	SRB1	0.1390±0.0048*	Moderately adhesive
3.	SRB2	0.1180±0.0016*	Moderately adhesive
4.	Sat1	0.0530±0,0014*	Weakly adhesive
5.	SRB1+Sat1	0.1480±0.0126*	Moderately adhesive

Note: the differences are significant * compared to the control at $p \leq 0.05$

It was found that SRB1 strain significantly formed a stronger biofilm on PET than SRB2 strain. Both strains were classified as moderately adhesive, because the optical density of crystal violet solutions absorbed by SRB biofilms was 3.7 times (SRB1) and 3.1 times (SRB2) higher than in the negative control.

It was revealed that *A. propionicum* NUChC Sat1 was weakly adhesive - the optical density of crystal violet, absorbed by its biofilm is only 1.4 times greater than in the negative control. Co-cultivation of SRB1 with Sat1 did not show increasing of biofilm formation by the studied bacteria, the association was moderately adhesive.

Thus, the studied strains of sulfate-reducing bacteria *Desulfovibrio oryzae* are able to form a biofilm on the surface of PET, which indicates the potential for participation of these bacteria in the degradation of this plastic. The weak adhesive properties of *Anaerotignum propionicum* to PET cast doubt on the possibility of this species participating in the biodegradation of polyethylene terephthalate.

REFERENCES

1. Савельєв, Ю В та ін. "Створення та застосування деградуючих за умов навколишнього середовища поліуретанів на основі вуглеводів." [Savelyev, Yu V *et al*. Stvorennia ta zastosuvannia dehraduiuchykh za umov navkolyshnoho seredovyshcha poliuretaniv na osnovi vuhlevodiv. Creation and application of polyurethanes, degrading under environmental conditions, based on carbohydrates] Polimernyi zhurnal vol. 33, no 3 (2011):205-217. (in Ukrainian).
2. Rogers, Kesley L *et al*. "Micro-by-micro interactions: How microorganisms influence the fate of marine microplastics." Limnology and Oceanography Letters, vol. 5 (2020):18-36. doi:10.1002/lol2.10136.
3. Андрушків, Богдан та ін. "Удосконалення економічного інструментарію пошуку нових ресурсів в умовах пострадянського суспільства." [Andrushkiv, Bogdan *et al*. "Udoskonalennia ekonomichnoho instrumentariiu poshuku novykh resursiv v umovakh postradianskoho suspilstva. Improving the economic tools for finding new

resources in post-Soviet society]. Halytskyi ekonomichnyi visnyk. no 3(36) (2012):80-85. URL: http://elartu.tntu.edu.ua/bitstream/123456789/2166/1/GEB_2012_v36_No3-B_Andrushkiw_I_Vovk_O_Pohaydak-Improving_economic_tools_to_find_new__80.pdf (in Ukrainian).

4. Ткачук, Н та ін. "Виділення та ідентифікація анаеробного супутника сульфатвідновлювальних бактерій." [Tkachuk, N *et al*. Vydilennia ta identyfikatsiia anaerobnoho suputnyka sulfatvidnovliuvalnykh bakterii. Isolation and identification of anaerobic satellite of sulfate-reducing bacteria.] «Shevchenko's spring: achievements of biological science / BioScience Advances»: collection of abstracts of the XVI International scientific conference of students and young scientists (Kyiv, April 24-27, 2018). Kyiv: Palivoda, A.V. (2018):106-107. (in Ukrainian).
5. Tkachuk, N *et al*. "Genotypic, physiological and biochemical features of Desulfovibrio strains in a sulfidogenic microbial community isolated from the soil of ferrosphere." Ecological questions, vol. 31, no 2 (2020):79-88. doi:10.12775/EQ.2020.016.
6. Stepanović, S *et al*. "A modified microtiter-plate test for quantification of staphylococcal biofilm formation." Journal of Microbiological Methods, vol. 40, no 2 (2000):175-179. doi:10.1016/s0167-7012(00)00122-6.