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A bacterial strain from the biofilm on the surface of poly(ethylene terephthalate) from soil in Chernihiv city (Ukraine)

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INTRODUCTION & AIM

Bacteria take an active part in the degradation of polymeric materials due to their high biochemical activity and ability to form biofilms (Saveliev et al., 2011; Rogers et al., 2020; Zhang et al., 2024).

The following species of soil bacteria have been previously isolated and identified from the soil in the Chernihiv city (Ukraine) from the surface of artificial materials (steel): *Desulfovibrio oryzae* strains NUChC SRB1 and NUChC SRB2, *Peribacillus* (*Bacillus*) simplex strain ChNPU F1, *Streptomyces gardneri* strain ChNPU F3, *Streptomyces canus* strain NUChC F2, *Fictibacillus* sp. strain ZVB1, *Anaerotignum* (*Clostridium*) *propionicum* strain Sat1 (Tkachuk et al., 2020; Tkachuk and Zelena, 2021; Tkachuk and Zelena, 2023).

In order to expand knowledge about the soil bacterial biodiversity of the surface of artificial materials from the soil of Chernihiv city (Ukraine), in this study, a bacterial strain was isolated from the biofilm formed on the poly(ethylene terephthalate) (PET)-bottle material taken from the soil, which was identified by a complex of microbiological and molecular genetic methods.

METHOD

Isolation of a bacterial isolate from a biofilm formed on the surface of poly(ethylene terephthalate)

a b

Fig. 1. Research object and its location: a – poly(ethylene terephthalate) bottle with a formed biofilm; b - place of sample selection (51°31'N 31°17'E, soil, Regional Landscape Park "Yalivshchyna", Chernihiv, Ukraine)

The biofilm was mechanically removed with a sterile scalpel in the sterile medium of Postgate's "C" modified by us, which contained a sample of PET sterilized in 96% ethyl alcohol. After cultivation of microorganisms for 30 days at a temperature of 29 ± 2 °C under aerobic conditions, the biofilm formed on the PET surface was mechanically removed and transferred to test tubes with sterile modified Postgate's "C" medium with PET samples.

Study of cultural-morphological and some physiological-biochemical properties of the selected isolate

Culture purity was checked by microscopy. Light microscopy at magnification (× 400 and ×1000) was used to study the morphology of bacteria. Preparations were made, which were stained according to Gram in Kalina's modification (to determine Gram affiliation) and according to Hansen's method (for staining spores). Morphological analysis of colonies on MPA was carried out according to a conventional scheme. Research on the presence of catalase and oxidase was carried out by conventional methods.

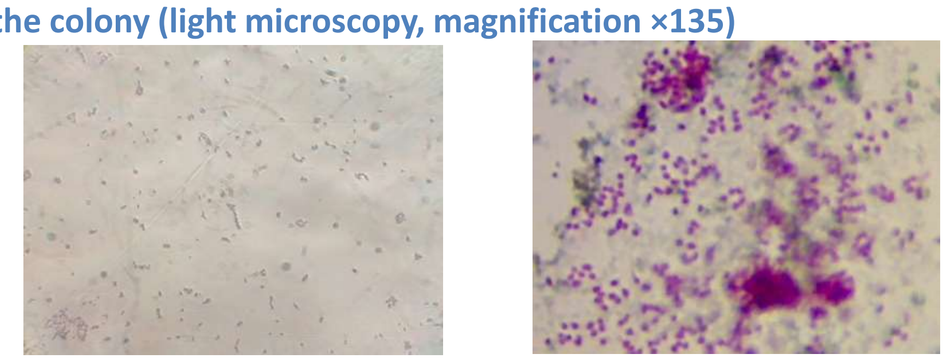
Molecular genetic studies of the selected isolate

Establishing the systematic position of bacteria was carried out according to the nucleotide sequence of the 16S rRNA gene fragment.

RESULTS & DISCUSSION

a b

Fig. 2. Colonies of isolate PET1: a - growth on MPA (2nd day); b – the edge of the colony (light microscopy, magnification ×135)



a b

Fig. 3. Bacterial cells of isolate PET1 (light microscopy, immersion, magnification ×1000): a – preparation-smear (crystal violet staining); b - Gram staining in Kalina's modification

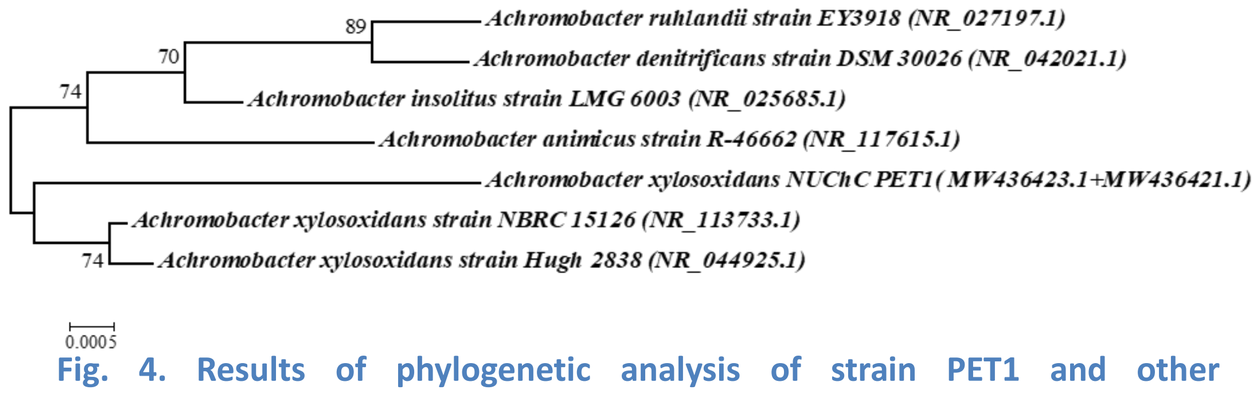


Fig. 4. Results of phylogenetic analysis of strain PET1 and other representatives of the genus *Achromobacter*

CONCLUSION

Thus, the strain NUChC PET1 was isolated from the biofilm that formed on the surface of the PET-bottle, which was located in the soil, on the modified Postgate's "C" medium with PET as the only carbon source. Based on a complex of microbiological and molecular genetic features, the strain NUChC PET1 was identified as *Achromobacter xylosoxidans* (MW436423.1 and MW436421.1 in the GenBank).

The perspective of further research may be the study of the intensity of biofilm formation by this strain on the surface of artificial materials (in particular, plastics) and participation in the processes of their biodegradation.

REFERENCES

Rogers et al., 2020, L&O Letters. 5, 18-36.
Saveliev et al., 2011, Polymer journal. 33(3), 205-217. (in Ukrainian).
Tkachuk et al., 2020, EQ. 31(2), 79-88.
Tkachuk, N., Zelena, L., 2021, Stud. Quat. 38(2), 101-108.
Tkachuk, N., Zelena, L., 2023, Biofouling. 39(3), 327-338.
Zhang et al., 2024, Appl. Soil Ecol. 196, 105312.