

INHIBITORY ACTIVITY OF CELL-FREE SUPERNATANT OF MARINE MICROBIOTA ISOLATED FROM MUSSELS IN BLACK SEA

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Introduction. Antibiotics resistance has become a challenge for scientists in recent decades. Therefore, the development of new effective medicine against bacterial infections is needed. Researchers have already used living things from the environment in the medical field, but there are still many things to be discovered, and the sea is an excellent place to start with. Marine microbiota produces various chemical substances, and it became the main interest of study upon the presence of antibiotic activity of secondary metabolites that will lead to the production of new effective antibiotic drugs. In our research, bacteria from the Black Sea were used to study their metabolites for antibiotics activity.

Materials & methods. Samples were isolated from the surface of mussels in the harbour of Odesa Bay in the Black Sea. Marine microbiota has been identified using MIDI Sherlock™ fatty acid analysis as *Pseudomonas aeruginosa* (M1, M3, M4) — group 1, *Bacillus subtilis* (MC3), *B. atrophoeus* (MH4) and *Alcaligenes faecalis* (AF) — group 2. Liquid media Iso-sensitest broth Oxoid™ and Lennox broth (LB) were used. Gram(-) bacteria (*Pseudomonas aeruginosa* PA01 and *Escherichia coli* K12) and gram(+) (both clinical strains of *Staphylococcus aureus* and *Mycobacterium smegmatis*) were used as test strains. Cultivation was performed separately and in pairs (group 1 with group 2) in plastic tubes with 2 ml of ISO:LB 1:1 (v/v) with 1% sea salts for 20 hours at +37C with shaking at 200 rpm. The supernatant was filtered using 0.2 µm filters and stored at -20C. Inhibition activity was studied by dropping 30 µL of cell-free supernatant on the paper disks that were placed on the bacterial lawn of test strains. The inhibition zone was identified after incubation.

Results & conclusions. Bacterial inhibition was observed from the MH4 strain and its pair with M3 on *S. aureus* and *M. smegmatis*. No inhibition was shown on PA01 and K12 indicating that active substance in supernatant had action towards Gram(+) bacteria. The results from the cultivation of MH4 with other strains from group 2 showed no inhibition, indicating that MH4 could produce metabolite with antibiotic activity just with some bacterial strains.

In conclusion, the MH4 strain of marine microbiota of the Black Sea produces substances that demonstrate the inhibitory effect against Gram(+) bacteria. The inhibitory effect was observed by mono-culture and in pair with M3, and this effect mainly occurred by MH4, and perhaps this strain plays the main role in antibiotis production.

References

1. Marmann, A., Aly, A. H., Lin, W., Wang, B., & Proksch, P. (2014). Co-cultivation--a powerful emerging tool for enhancing the chemical diversity of microorganisms. *Marine drugs*, 12(2), 1043–1065. <https://doi.org/10.3390/md12021043>
2. Bayona, L. M., de Voogd, N. J., & Choi, Y. H. (2022). Metabolomics on the study of marine organisms. *Metabolomics: Official journal of the Metabolomic Society*, 18(3), 17. <https://doi.org/10.1007/s11306-022-01874-y>
3. Isnansetyo, A., & Kamei, Y. (2009). Bioactive substances produced by marine isolates of *Pseudomonas*. *Journal of industrial microbiology & biotechnology*, 36(10), 1239–1248. <https://doi.org/10.1007/s10295-009-0611-2>