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PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF MYXOBACTERIA ISOLATED FROM NATURAL BIOTOPES OF THE BLACK SEA

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Abstract. The physiological and biochemical properties of 35 myxobacterial strains isolated from natural biotopes in the Black Sea coastal zone near Odesa were studied. The strains showed variable growth responses to temperature, pH, and NaCl concentration, with 25–30 °C, pH 7.0, and 0.5–1.5 % NaCl being the most favorable conditions for most strains. Strain-dependent variability was also observed in oxidase and catalase activities and enzymatic profiles. Overall, the strains exhibited a limited spectrum of low-molecular-weight substrate assimilation, with the most restricted spectrum among representatives of the genus *Nannocystis*. Taken together, these findings demonstrate substantial physiological and biochemical heterogeneity among the investigated myxobacteria and support their potential for further investigation as producers of biologically active compounds and hydrolytic enzymes.
Keywords: myxobacteria, Black Sea, physiological and biochemical properties.

Myxobacteria are Gram-negative prokaryotes with a unique life cycle, social behavior, large genomes, and high biosynthetic potential. They are active producers of secondary metabolites, which motivates the search for new natural sources for their isolation and investigation (Saggu et al., 2023). The specific physicochemical conditions of marine ecosystems differ substantially from those of terrestrial ecosystems, particularly soil ecosystems, promoting physiological and biochemical adaptations and metabolic characteristics in microorganisms and making marine microbiota, including myxobacteria, a promising source of biologically active compounds and enzymes (Barzkar et al., 2024; Saggu et al., 2023).

The microbial diversity of natural biotopes of the Black Sea remains insufficiently studied, particularly with regard to representatives of *Myxococcota*. Therefore, the aim of

this study was to investigate the physiological and biochemical properties of myxobacterial strains isolated from Black Sea biotopes.

Thirty-five myxobacterial strains isolated from natural substrates of the Black Sea coastal zone near Odesa were investigated. The strains are maintained in the culture collection of microorganisms at Odesa I. I. Mechnikov National University at −80 °C. Physiological properties were assessed based on the ability of the strains to grow on VY/2 agar at temperatures of 10–40 °C, pH values of 4.0–9.0, and NaCl concentrations of 0.0–4.0 % (Zhang et al., 2024). The strains were incubated for 21 days, and swarm diameters were measured on days 7, 14, and 21. Growth intensity was assessed based on the dynamics of swarm diameter expansion over the incubation period (Ahearne et al., 2023). Biochemical properties were determined using the API ZYM and API 20NE test systems (bioMérieux, France) according to the manufacturer's instructions, while oxidase and catalase activities were determined according to Zang et al. (2024).

The studied strains demonstrated variable physiological properties. Growth occurred at 10–35 °C, with most strains showing the highest growth intensity at 25–30 °C; 30 °C was optimal based on the overall dynamics of swarm expansion. Eight strains grew at 15 and 20 °C, two of which also grew at 10 °C, although growth was slower at lower temperatures. At 35 °C, approximately 70.0 % of strains exhibited weak growth, while no growth occurred above 35 °C. All strains formed fruiting bodies, whose coloration and timing of formation depended on temperature: at 25–30 °C, some strains formed pale yellow, lemon-yellow, or orange fruiting bodies, whereas colorless fruiting bodies predominated under suboptimal condition.

Growth occurred at pH 6.0–9.0, with pH 7.0 being optimal for all strains. At pH 6.0, five strains exhibited weak growth, three moderate growth, and one intensive growth, while the remaining strains did not grow. More than 74.0 % of strains grew at pH 7.5–8.5. At pH 9.0, only three strains showed weak growth, with the first signs appearing after 14 days, followed by the formation of colorless fruiting bodies 1–2 days later.

Growth was observed at NaCl concentrations of 0.0–3.0 %. At 0.0–2.5 %, growth intensity varied among strains, with approximately 92.0 % exhibiting intensive growth at 0.5–1.5 % NaCl and approximately 8.0 % showing less intensive growth. These concentrations were the most favorable for growth. Increasing NaCl concentrations resulted in gradual decreases in swarm expansion and growth intensity and, in most cases, earlier formation of colorless fruiting bodies. One strain grew at 3.0 % NaCl, whereas no growth occurred above 3.0 %. No myxobacterial strains unable to grow in the absence of NaCl (0.0 %) were detected, which could indicate an obligate marine origin.

The biochemical properties of the strains showed substantial variability in oxidase and catalase activities, enzymatic profiles, and the spectrum of assimilated substrates. API ZYM revealed activities of phosphatases, esterases, lipases, selected proteases (trypsin, α-chymotrypsin, leucine arylamidase, valine arylamidase, and cysteine arylamidase), and glycosidases, with specific reaction intensities varying substantially among strains. *Nannocystis* representatives showed relatively high activities of selected proteolytic enzymes (trypsin and α-chymotrypsin), lipolytic enzymes, and phosphatases, accompanied by weak or absent glycosidase activity, whereas *Myxococcus* representatives exhibited pronounced activities of alkaline phosphatase, esterase-lipase C8, lipase C14, trypsin, and acid phosphatase, with weak reactions for esterase C4, leucine arylamidase, valine arylamidase, and cysteine arylamidase, α-chymotrypsin, and naphthol-AS-BI-phosphohydrolase. The observed spectrum of enzymatic activities indicates the potential of the studied strains to hydrolyze complex organic compounds. API 20NE revealed an overall limited spectrum of low-molecular-weight substrate assimilation with pronounced strain-dependent variability. *Nannocystis* representatives had the most limited assimilation spectrum among the substrates

included in the API 20NE panel, as evidenced by the absence of or only occasional positive reactions to individual carbon substrates.

Taken together, the results obtained indicate substantial physiological and biochemical heterogeneity among the studied marine myxobacteria, manifested in variability in their temperature, pH, and salt tolerance, enzymatic profiles, and the spectrum of substrates they assimilate. The combination of physiological plasticity and the potential ability to hydrolyze complex organic compounds characterizes the studied strains as promising candidates for further investigation of their biotechnological potential.

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POLYHYDROXYALKANOATES IN SYNTHETIC MICROBIAL COMMUNITIES OF ANTARCTIC ENDOPHYTIC BACTERIA

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Abstract. This study investigates the accumulation dynamics of polyhydroxyalkanoates (PHA) in synthetic microbial communities formed by Antarctic endophytic bacteria isolated from *Deschampsia antarctica* and *Colobanthus quitensis*. We evaluated the influence of biofilm architecture (air-liquid-solid [ALS] vs. liquid-solid [LS]) and wide temperature range (4, 15, 25, 37, and 42 °C) on PHA biosynthesis. Quantitative gravimetric analysis and confocal laser scanning microscopy (CLSM) demonstrated that PHA accumulation is significantly enhanced in ALS multi-species biofilms compared to monocultures, peaking at 4 °C. Higher temperatures led to rapid depletion of energy reserves. Oxygen availability and spatial organization within the biofilm matrix are critical drivers of carbon allocation toward PHA storage in cold-adapted microbial communities.
Keywords: Polyhydroxyalkanoates, Antarctic endophytes, synthetic microbial communities, biofilm architecture, psychrotolerant bacteria, confocal microscopy.

The growing global contamination by synthetic plastics has accelerated the search for eco-friendly, biocompatible alternatives. Polyhydroxyalkanoates (PHA) produced by