KM-4 strain: *L. plantarum* KM-4 + *K. marxianus* F21 (pH 4.22 ± 0.19, 21.25 ± 2.47 °T), *L. plantarum* KM-4 + *Y. lipolytica* FF21 (pH 3.92 ± 0.01, 26.5 ± 0.7 °T), *L. plantarum* KM-4 + *S. cerevisiae* UCM Y-2519 (pH 4.12 ± 0.21, 19.5 ± 0.7 °T).

Thus, the results obtained demonstrate that selected strains of lactic acid bacteria and yeast are promising candidates for pumpkin juice fermentation to obtain a functional drink. Further research is needed to study the spectrum of biologically active compounds synthesised by microorganisms during fermentation and their interaction, and the effect of fermentation on the antioxidant potential of the pumpkin juice.

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EFFECT OF CULTIVATION PARAMETERS ON ANTIBIOTIC PRODUCTION IN ACTINOMYCETES OF THE GENUS *MICROMONOSPORA*

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Abstract. The effect of the composition of the ISP-9 synthetic medium on the growth and antimicrobial activity of strains of the genus *Micromonospora* was investigated. It was found that the use of arginine as a nitrogen source resulted in the highest level of activity at a concentration of 2.5 g/L. As the phosphate concentration increased, antimicrobial activity decreased, with the highest activity observed at a concentration of 1 mM. A dependence of pigment formation on the phosphate content in the medium was also observed.
Keywords: *Micromonospora,* secondary metabolism, culture medium, antimicrobial activity.

The spread of antibiotic resistance highlights the critical necessity of searching for new classes of antimicrobial compounds (Murray et al., 2022). In this context, members of the Micromonosporaceae family are regarded as extremely promising producers; however, a significant proportion of their biosynthetic gene clusters (BGCs) are cryptic, and their expression is strictly dependent on cultivation conditions (in accordance with the OSMAC concept) (Yan et al., 2022). Since traditional complex culture media often cause catabolite repression of secondary metabolism due to an oversupply of nutrients, they are unable to provide an optimal balance between biomass growth and the induction of antimicrobial activity (Ruiz-Villafán et al., 2022). Overcoming this limitation requires the use of chemically defined (synthetic) media, which allow for the control of trophic parameters, the targeted removal of repression of biosynthetic pathways, and a reliable assessment of the effect of each component on antibiotic production (Li et al., 2019). The aim of the study was to develop an optimised composition of a culture medium to enhance the biosynthetic potential of strains belonging to the *Micromonosporaceae* family, in particular those isolated from mine tailings.

ISP-9 medium was selected as the base medium for modifying the culture conditions. A combination of glucose (10 g/L) and glycerol (5 g/L) was used as carbon sources. This choice is justified by the two-phase growth model: readily available glucose provides rapid biomass accumulation in the early stages of cultivation, whilst the slower assimilation of glycerol maintains the cells' energy balance during the stationary phase, which is critical for the initiation of secondary metabolite biosynthesis. To maintain osmotic homeostasis and provide the enzyme systems with the necessary cofactors, the medium was supplemented with inorganic salts of $MgSO_4$, NaCl and $CaCl_2$ at a concentration of 0.5 g/L. Magnesium and calcium ions play a key structural and regulatory role, stabilising cell membranes and ensuring the functioning of ATP-dependent enzymes in biosynthetic cascades. A key step in the optimisation process was the targeted replacement of the nitrogen source. In the standard ISP-9 formulation, this role is fulfilled by ammonium sulphate ($(NH_4)_2SO_4$). However, for our work, it was replaced with the amino acid arginine. The theoretical basis for this decision was the need to avoid nitrogen catabolite repression (NCR). According to the literature, an excess of readily available ammonium ions activates the global transcription regulator GlnR, which, in turn, represses the expression of secondary metabolism genes and inhibits the synthesis of antimicrobial compounds (He et al., 2016). The use of arginine as a slowly assimilated source of nitrogen allows one to bypass GlnR-mediated repression and create conditions of moderate trophic stress, which acts as a trigger for the activation of 'silent' biosynthetic clusters. In order to find the optimal balance between biomass accumulation and maximum antimicrobial activity, the arginine concentration was tested within the range of 0.25–3.0 g/L. Based on the screening results, the working concentration of this component was set at 2.5 g/L, which ensured the highest level of bioactive substance production.

The next stage of the research involved optimising the phosphate content (in the form of KH_2PO_4 and K_2HPO_4 salts). The ISP-9 basal medium is characterised by high levels of these compounds, which often act as a limiting factor for the synthesis of bioactive substances. An excess of inorganic phosphorus acts as a trophic signal that stimulates primary metabolism and biomass accumulation, but at the same time represses the expression of genes responsible for the synthesis of antibiotics (a process known as phosphate repression). To establish an optimal balance between culture growth and the production of secondary metabolites, phosphates were added to the medium in the form of a potassium phosphate buffer with a molar concentration ranging from 1 to 50 mM. The screening results revealed a clear trend towards a decrease in antimicrobial activity as the phosphate concentration in the medium increased. The largest zones of growth inhibition in the test cultures were observed at the lowest concentration tested (1 mM). This concentration proved to be the

optimal trigger for the induction of biosynthetic processes; therefore, it was selected as the working concentration for further optimisation. Furthermore, during cultivation, a change in colony pigmentation was observed depending on the phosphate level. Strains that actively produced pigment under phosphate-limiting conditions completely lost this ability following an increase in phosphate concentration in the medium. Since pigment formation, like antibiotic synthesis, is a classic manifestation of secondary metabolism, the results obtained indicate a close interrelationship between these processes. It is likely that the concentration of inorganic phosphorus exerts a pleiotropic (multifactorial) effect, simultaneously regulating both biosynthetic pathways via shared molecular mechanisms (Hobbs et al., 1990).

Therefore, optimising the ISP-9 synthetic medium by incorporating arginine and limiting phosphate (to 1 mM) enabled us to effectively overcome catabolite repression. This ensured an optimal balance between culture growth and maximum activation of secondary metabolite synthesis, in particular antimicrobial compounds and pigments.

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Romanov S., Tsyrulnyk A., Dzanaeva L., Fayura L., Fedorovych D., Dmytruk K., Sibirny A.

SEF2 PROTEIN NEGATIVELY REGULATES RIBOFLAVIN PRODUCTION IN *CANDIDA FAMATA*

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