(13.38 a.u.), on day 4 – at 4.5 % (17.57 a.u.), and on day 5– again at the 4.5 % NaCl (21.16 a.u.). Under the influence of 6.5 % NaCl, siderophore production remained the lowest throughout the entire study period.

Conclusions. The obtained results indicate that the bacterium *O. rhizosphaerae* IMV B-7956 is capable of producing auxin-like compounds and siderophores under the influence of NaCl.

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OPTIMIZATION OF THE ISOLATION METHOD FOR EXOPOLYSACCHARIDES OF PROBIOTIC *BACILLUS VELEZENSIS* STRAINS

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Abstract. This study focused on the implementation and adaptation of new approaches for extracting exopolysaccharides (EPS) from the probiotic bacterial strains *Bacillus velezensis* UCM B-7242 and *B. velezensis* UCM B-7243. The primary objective of this research was to optimize the EPS isolation method by eliminating the conventional alcohol precipitation stage to achieve the highest possible yield and purity of the exopolysaccharides. In the course of the study, five sequential extraction stages were conducted and optimized, resulting in concentrated EPS samples with a significantly reduced content of co-extracted impurities.
Keywords: exopolysaccharides, ethanol-free extraction, probiotic strains, *Bacillus velezensis.*

Introduction. Exopolysaccharides produced by probiotic bacteria possess significant potential for biomedical applications, serving as an effective natural alternative to synthetic

therapeutics. These biopolymers exhibit potent biological properties, offering promising prospects for their use in the treatment of various diseases (Mouro et al., 2024).

Probiotic bacteria of the genus *Bacillus*, characterized by high biosynthetic activity, serve as valuable producers of EPS. However, obtaining a highly purified EPS preparation remains a challenging task that requires continuous optimization of methodological approaches. Conventional protocols for isolating bacterial EPS rely on precipitation with large volumes of chilled ethanol. However, the use of alcohol significantly increases production costs and introduces severe explosion hazards during process scaling. Furthermore, ethanol extraction non-selectively co-precipitates various matrix contaminants, complicating subsequent sample purification (Riyamol et al., 2026).

In view of the above, the **aim** of this study was to optimize the extraction method for exopolysaccharides from the probiotic strains *B. velezensis* UCM B-7242 and UCM B-7243 without using classical ethanol precipitation, followed by the determination of EPS concentration and extract purity profiles.

Methods and Materials. The probiotic strains *B. velezensis* UCM B-7242 and UCM B-7243 served as the objects of the study. Bacterial cultivation was carried out for 48 h at 37 °C in liquid nutrient medium containing glucose and a mixture of mineral salts as nutrients.

Cell-free supernatant was obtained by centrifuging the culture broth at 5.000 rpm for 30 min at 4 °C. Deproteinization was performed by adding 50 % trichloroacetic acid (TCA) to the supernatant, followed by incubation at 4 °C for 1.5 h. Protein precipitation was achieved by centrifugation at 5.000 rpm for 30 min. The deproteinized solution was neutralized to pH 7. Dialysis was performed against distilled water for 24 h using dialysis tubing with a molecular weight cut-off of 12–16 kDa. Following dialysis, the sample was additionnally centrifuged at 5.000 rpm for 15 min to remove the precipitate. To remove hydrophobic molecules, the solution was passed through an activated charcoal column. The final EPS concentration was performed by evaporating the purified solution using a rotary evaporator at 45 °C until a 5-fold reduction of the initial volume was achieved.

The EPS content in the concentrated sample was determined by the phenol-sulfuric acid method through spectrophotometric measurement of the OD_{490} of the reaction mixture. Concentration calculations were conducted using a calibration curve of D-glucose standards. Sample purity profiles were determined based on the OD_{260}/OD_{280} values of the unconcentrated extracts. Statistical data analysis was performed using Microsoft Excel.

Results and Discussion. As a result of this research, two concentrated EPS extracts were obtained from the cell-free culture supernatants of both studied strains. The EPS concentration in the extract from *B. velezensis* UCM B-7242 was 0.31 mg/mL, whereas for the UCM B-7243, it was 0.55 mg/mL. Accordingly, under the proposed isolation method, the specific EPS yield was 0.021 g/L for strain UCM B-7242 and 0.084 g/L for strain UCM B-7243. The obtained EPS yields are in agreement with global literature data for wild-type *Bacillus* strains cultivated on glucose substrates, which yields an average EPS concentration ranging from 0.05 to 0.2 g/L (Yuan et al., 2025).

The OD_{280} values for both extracts were 0.35, corresponding to the content of residual peptide impurities of no more than 0.005 % in the concentrated extracts. This result demonstrates the high efficiency of TCA-mediated deproteinization. However, the co-extracted nucleic acid background was significant, leading to an artificial overestimation of the OD_{280} value. The OD_{260} value for the unconcentrated extracts was 0.7, corresponding to a DNA and RNA content of approximately 0.016 % in the concentrated extracts. Consequently, the proposed EPS isolation method requires the inclusion of an additional nucleic acid removal step.

Given the physiological and metabolic traits of the used strains, the alcohol-free isolation method was structurally optimized and adapted to the specific technological challenges encountered during the experiments. In particular, an additional post-dialysis centrifugation step was introduced due to the coagulation of destabilized structural peptides. These protein molecules lost their solubility and formed aggregates as a result of a rapid decrease in the ionic strength of the solution. The critical evaporation stage also underwent optimization. To protect the EPS from thermal degradation, this process was carried out at a temperature not exceeding 45 °C, with a reduction in solution volume of no more than 5-fold. Additionally, a filtration step using activated charcoal was performed to remove hydrophobic impurities and pigments, as both strains proved to be active producers of riboflavin.

Conclusions:

1. An alcohol-free scheme for the isolation of EPS from *B. velezensis* strains UCM B-7242 and UCM B-7243 was implemented and optimized.

2. It was established that under the proposed alcohol-free isolation method, the specific EPS yield was 0.021 g/L for *B. velezensis* UCM B-7242 and 0.084 g/L for the UCM B-7243. The content of residual peptide components in the concentrated extracts was strictly limited to 0.005 %, while that of nucleic acids was 0.016 %.

3. The most significant results of the methodological optimization included the enhancement of the deproteinization stage, the determination of the optimal evaporation conditions, and the implementation of an adsorption purification step.

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INFLUENCE OF STRESS-TOLERANT BACTERIA ON THE MORPHOMETRIC AND BIOCHEMICAL PARAMETERS OF SPRING WHEAT GROWN IN SOIL WITH COPPER(II) CHLORIDE

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Abstract. This study examines the influence of inoculating spring wheat (*Triticum aestivum* L., cv. Tybalt) seeds with stress-tolerant bacterial strains isolated from Antarctic substrates, Lviv