BIOTECHNOLOGICAL APPLICATION OF MICROORGANISMS

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EFFECT OF *AZOTOBACTER VINELANDII* AND *BACILLUS SUBTILIS*, THE COMPONENTS OF THE AZOGRAN BIOPREPARATION, ON THE FORMATION OF WHEAT ROOT EXUDATES

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Abstract. The effect of different concentrations of *Azotobacter vinelandii* and *Bacillus subtilis*, the bacterial components of the Azogran biopreparation, on the formation of wheat root exudates under sterile hydroponic conditions was investigated. Increasing the concentration of each bacterial strain in the culture medium to 10^7 CFU/mL resulted in the highest accumulation of root exudate dry matter accompanied by a decrease in total carbohydrate content, suggesting intensive utilization of exuded carbohydrates by the bacteria. Gas chromatography-mass spectrometry (GC-MS) revealed alterations in the monosaccharide composition of root exudates, including increased proportions of galactose and mannose as well as the appearance of several unidentified compounds absent from the control. These findings indicate that bacterial concentration in the growth medium is an important factor regulating the metabolism of wheat root exudates and plant-microorganism interactions. The most pronounced effects were observed at bacterial concentrations of 10^6-10^7 CFU/mL.
Keywords: wheat, root exudates, *Azotobacter vinelandii*, *Bacillus subtilis*, carbohydrates, monosaccharides.

Objective. The aim of this study was to investigate the effect of different concentrations of *Azotobacter vinelandii* and *Bacillus subtilis*, the bacterial components of the Azogran biopreparation, on dry matter accumulation, total carbohydrate content, and the monosaccharide composition of wheat root exudates.

Materials and Methods

Experiments were conducted using wheat (*Triticum aestivum* L.) cv. Shestypalivka. Seeds were surface-sterilized and germinated on potato agar. Sterile seedlings were transferred onto stainless-steel mesh supports placed in 1.5-L glass vessels containing 80 mL of Fåhraeus medium. Plants were cultivated hydroponically in the presence of a mixed culture of *A. vinelandii* and *B. subtilis* at concentrations ranging from 10^5 to 10^7 CFU/mL for each strain. Plants grown without bacterial inoculation served as the control.

At the end of cultivation, the culture medium containing root exudates was separated by centrifugation, freeze-dried, and the dry matter yield was determined. Total carbohydrates were quantified by the phenol–sulfuric acid method (Dubois et al., 1956). Monosaccharide composition was determined by gas chromatography–mass spectrometry after acid hydrolysis according to Albersheim et al. (1967).

Results. The concentration of the bacterial inoculum significantly affected both the accumulation of root exudate dry matter and their carbohydrate composition. In the control

treatment, the dry matter yield was 19.0 mg (0.059 mg/mL of exudate). Inoculation with the *A. vinelandii-B. subtilis* consortium progressively increased dry matter accumulation depending on bacterial concentration. At inoculum concentrations of 10^5-10^6 CFU/mL, dry matter increased to 66.0–70.0 mg, whereas at 10^7 CFU/mL it reached a maximum of 256.0 mg (1.1 mg/mL of exudate), indicating enhanced physiological and biochemical activity of plants during intensive interactions with the introduced bacteria.

Simultaneously, total carbohydrate concentration in the culture medium decreased markedly from 0.67 μg/mL in the control to 0.019–0.015 μg/mL in inoculated treatments. This reduction is most likely associated with active bacterial utilization of root-derived carbohydrates as the primary carbon source. Similar patterns have been reported for rhizosphere associations where plant growth-promoting bacteria accelerate carbon exchange between plants and microorganisms (Badri & Vivanco, 2009; Canarini et al., 2019; Zhalnina et al., 2018).

GC-MS analysis identified rhamnose, fucose, mannose, galactose, glucose, and several unidentified compounds (X_1-X_5) in wheat root exudates. Galactose predominated in the control treatment (31.91 %), whereas the remaining monosaccharides were detected in relatively low amounts. In the presence of bacteria, the monosaccharide profile was substantially modified. At an inoculum concentration of 10^6 CFU/mL, the proportion of galactose increased to 62.89 %, while at 10^5 CFU/mL mannose content increased to 18.17 %. Moreover, unidentified compounds X_1-X_5 were detected only in inoculated treatments, suggesting the formation of new plant- or microbially derived metabolites during active plant-bacteria interactions.

These results are consistent with current concepts of the dynamic nature of root exudation. According to Zhalnina et al. (2018), rhizosphere bacteria not only utilize specific exudate components but also modify their composition by inducing the synthesis of novel metabolites involved in signaling and regulation of rhizosphere microbial community assembly. The elevated galactose content observed at 10^6 CFU/mL may reflect enhanced synthesis of cell wall polysaccharides, whereas increased mannose levels may indicate intensified glycosylation processes associated with plant-bacterial interactions.

Overall, inoculation of wheat with *A. vinelandii* and *B. subtilis*, the bacterial components of the Azogran biopreparation, stimulated the accumulation of root exudate biomass and substantially modified both carbohydrate and monosaccharide composition. The strongest response was observed at inoculum concentrations of 10^6–10^7 CFU/mL, corresponding to maximal metabolic activity of the plant–microorganism system and the greatest diversity of root exudate components.

Conclusions. Application of *A. vinelandii* and *B. subtilis* effectively modifies the composition of wheat root exudates. The highest dry matter yield (256.0 mg) and the lowest total carbohydrate concentration (0.015 μg/mL) were obtained at an inoculum concentration of 10^7 CFU/mL, indicating high bacterial metabolic activity. Bacterial concentrations of 10^6–10^7 CFU/mL produced the greatest changes in the monosaccharide profile of root exudates and may therefore be considered optimal for promoting beneficial interactions between wheat plants and rhizosphere microorganisms.

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BIOTECHNOLOGICAL APPLICATIONS OF LACCASE AND LACCASE-LIKE NANOZYMES FOR BIOSENSOR DEVELOPMENT

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Abstract. This study explores the bioanalytical application of natural laccase, surface-displayed recombinant cells, and laccase-like nanozymes for biosensor development. Trametes zonata 1525 laccase was characterized and effectively utilized for the spectrophotometric and electrochemical determination of 4-hydroxyindole and 5-hydroxyindole-3-acetic acid. A cell-based biosensor featuring genetically modified yeast with surface-displayed laccase enabled sensitive 5-HIAA quantification in urine samples. Furthermore, laccase-mimicking nanomaterials (nAuCePt, nAuCu, nPtCu, nCu-HCF, nPtMn, Ag/MnO_2@PDA–B, FeMn-MOF and nMnO_2) demonstrated high catalytic efficiency for dopamine, adrenaline, 5-HIAA, 4-HI and phenolic metabolite detection. The nAuCePt nanozyme achieved a 380-fold increase in sensitivity compared to natural enzyme-based sensors. Overall, combining natural laccases with artificial mimetics offers a robust platform for modern diagnostic biosensors.
Keywords: laccase, laccase mimetics, fungi, hydroxyindole derivatives, analysis, electrochemical biosensors.

Laccases (EC 1.10.3.2) are copper-containing oxidases with a broad substrate specificity, capable of catalyzing the oxidation of a wide range of phenolic and aromatic compounds using molecular oxygen as the terminal electron acceptor. These properties have stimulated considerable interest in laccases as biocatalysts for enzymatic analytical methods, including electrochemical biosensors. In particular, laccases are promising for the determination of biologically important catechol and indole compounds, while laccase-like artificial catalysts can serve as alternatives to natural enzymes.

In our studies, laccase from *Trametes zonata* 1525, selected from 38 basidiomycete strains as the most productive strain for extracellular laccase production, was characterized. Optimization of cultivation conditions and the use of an inducer resulted in a higher enzyme productivity. The purified laccase was shown to oxidize indole compounds, including 4-hydroxyindole (4-HI) and 5-hydroxyindole-3-acetic acid (5-HIAA), with catalytic efficiencies of $1.1 \cdot 10^4$ and $5.4 \cdot 10^3$ $M^{-1} \cdot s^{-1}$, respectively. A laccase-based spectrophotometric method for