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GROWTH-REGULATING PHYTOHORMONES AUXINS AND CYTOKININS OF ENDOPHYTIC SOYBEAN BACTERIA

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Plant growth-promoting bacteria that are able to penetrate in plant tissues and colonize them without causing visible damage or disease symptoms are called bacterial endophytes. Endophytes are believed to be superior to rhizospheric microorganisms in promoting plant growth because they have the ability to colonize the internal parts of plants, where they can quickly sense any changes in the environment and respond quickly to their own needs and those of the plants [1]. Because endophytes are generally not host-specific, endophytes with a desirable set of plant-promoting properties can be easily introduced into plants that are not their natural hosts. In the light of these considerations, the use of endophytes in agriculture can be an economical means of achieving high crop productivity and therefore sustainable agriculture.

It is known that the bacterial endophytic microbiome contributes to the plants growth and development, and its beneficial effects are in many cases indirect and characterized by various metabolic interactions [2]. Recent advances in the study of metabolite synthesis by plant microsymbionts indicate that they can produce a

number of different types of metabolites. These substances play a role in defense and competition, but may also be necessary for specific interaction and communication with the host plant. By directly stimulating plant growth, endophytic bacteria either supply or modulate the levels of major phytohormones, including auxins, cytokinins, ethylene, and gibberellins, or provide plants with nutrients such as phosphates, nitrogen, and iron, etc. [3].

The aim of this work was to research the synthesis of growth-regulating phytohormones auxins and cytokinins by non-diazotrophic endophytic bacteria isolated from soybean nodules.

Strains of non-diazotrophic endophytic bacteria isolated from soybean nodules in the active phase of nitrogen fixation in the middle of the growing season of soybean plants were studied: *Paenibacillus* sp. 1; *Ochrobactrum* sp. 2; *Paenibacillus* sp. 3; *Bacillus* sp. 4; *Brevibacillus* sp. 5; *Pseudomonas* sp. 6. Cultivation was carried out on 2 nutrient media: a glucose-mineral medium and a modified peptone medium. The qualitative and quantitative composition of auxins and cytokinins was analyzed by high-performance liquid chromatography (HPLC) using an Agilent 1200 liquid chromatograph (Agilent Technologies, USA).

The free trial Statistica 14 (TIBCO Software Inc., 2020) was used for statistical processing of the results. All results are presented as mean ± standard error of the mean (M±SE). Analysis of variance was used to compare several groups. Fisher's F test was used to test statistical hypotheses. Differences were considered reliable at a significance level of $p<0.05$.

Using the HPLC method, it was established that the largest amount of synthesized auxins was determined in *Brevibacillus* sp. 5 and *Paenibacillus* sp. 1 on modified peptone medium. Thus, 1.0 g of absolutely dry biomass of these bacteria synthesized 16.5±2.1 mg and 8.2±1.6 mg of auxins, respectively. The best results of auxin's production on glucose-mineral medium in *Ochrobactrum* sp. 2 and *Paenibacillus* sp. 3 – 1.25±0.27 mg and 1.1 ±0.2 mg, respectively.

Bacteria that were more productive on glucose-mineral medium than on modified peptone medium include *Ochrobactrum* sp. 2, *Paenibacillus* sp. 3 and *Bacillus* sp. 4. They synthesized, respectively, 1.2 ±0.2 and 1.1±0.3 mg/g of auxins on the glucose-mineral medium

and 0.34±0.1 and 0.85±0.1 mg/g on the modified peptone medium. *Bacillus* sp. 4 became the least productive strain, and showed results of 0.30 and 0.04 mg/g on the first and second media, respectively.

When comparing the amount of the auxins production of by different bacteria, it can be seen that for 1-4 strains on glucose-mineral medium and all on modified peptone medium, the production of indole-3-acetic acid hydrazide was most active, which exceeded 90% for each of the specified strains from all products of compounds of this class.

It is shown that non-rhizobial soybean microsymbionts *Paenibacillus* sp. 1, *Ochrobactrum* sp. 2, *Paenibacillus* sp. 3, *Bacillus* sp. 4, *Brevibacillus* sp. 5 and *Pseudomonas* sp. 6 also synthesize extracellular phytohormones-stimulators – cytokinins (zeatin, zeatin-riboside, N6-(2-isopentenyl)adenine and N6-(2-isopentenyl) adenosine).

Among the cytokinin metabolites in the culture medium of *Paenibacillus* sp. 1, *Bacillus sp.* 4, *Brevibacillu*s sp. 5 and *Pseudomonas* sp. 6, the largest amount of the transportable physiologically active form of cytokinin – zeatin-riboside (96-105 μg/ml of culture liquid) was identified, which is actively used by plants to regulate their main physiological processes.

The activity of cytokinin production by endophytic bacteria was lower ($p<0.01$, $F=11.2$) compared to the auxins values. However, the strain of *Paenibacillus* sp. 3 synthesized an equal number of different cytokinin compounds on both media ($p<0.1$, $F = 0.66$).

The conclusion made in relation to the auxins production is reliable that the activity of the synthesis of individual cytokinins was higher on the second medium ($p<0.05$, $F=8.4$). The most active synthesis of cytokinins occurred on the second medium by the *Pseudomonas* sp. 6, which as a result was able to accumulate almost 4.9±0.6 mg of growth-regulating substances of this type per 1 g of absolutely dry biomass. This result is significantly greater ($p<0.001$, $F=24.4$) in comparison with the activity of cytokinin production on the first medium of *Pseudomonas* sp. 6, which was 0.064±0.6 mg/g.

In turn, the different activity of the phytohormones production in different environments conditions confirms the researcher's opinion about the influence of external factors on the secretory activity of endophytes and inside plant tissues [4].

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