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Priestia endophytica bacteria stimulate *Rhodiola rosea* L. *in vitro* growth

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Soil microorganisms, in particular so-called plant growth promoting rhizobacteria (PGPR), can positively affect plants, stimulating growth by changing their metabolism. In addition, these bacteria synthesize hormone-like chemicals that can influence the formation of primary roots. This work was aimed to determine the possibility of using the sterile cell-free cultural medium obtained after the growth of endophytic bacteria *Priestia endophytica* UKM B-7515 strain (test solution) to stimulate the rooting of a valuable medicinal plant *Rhodiola rosea* L. (golden root) shoots in *in vitro* conditions and to study the physiological characteristics of the response of plants to treatment with such a medium. Single treatment of golden root shoots with a sterile cultural fluid has led to stimulation of the growth of the root system. The stimulating effect was manifested already at the beginning of cultivation. In particular, in seven days, the average number of formed roots on one shoot was 3.6 ±0.7 in the control and 7.5 ±2.1 in the experimental variant. After 28 days, the rooting of shoots that were treated with the test solution occurred more intensively. Thus, the number of roots formed on one shoot in the control and experimental variants was 7.9 ±1.2 and 16.2 ±4.8, respectively. Treated plants exceeded the control one in parameters of root weight. In particular, the weight of the roots in the control was 10.1 ±4.0 and in the experimental plants – 18.6 ±6.3. Thus, the culture medium obtained after the cultivation of *P. endophytica* UKM B-7515 bacteria without the microorganisms themselves was able to stimulate the process of rooting. Therefore, such a solution can be used for rooting plant shoots that need to be propagated in *in vitro* culture.

Keywords: *Rhodiola rosea*, *Priestia endophytica*, plant growth stimulation, indole-3-acetic acids, plant-bacteria interaction

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Introduction

The development of methods of cultivation of rare plants with a rather small area is of considerable interest especially if such plants can synthesize a number of valuable biologically active compounds. *Rhodiola* spp. belongs to such plants. The plants grow in the arctic regions of Europe and Asia. Many *Rhodiola* species, including *Rhodiola rosea* L. (golden root), demonstrated numerous bioactivities. In particular, the plants possess antidepressant, anti-inflammatory, adaptogenic, and antitumor properties (Chiang et al., 2015). The cultivation of golden root plants under sterile conditions is a necessary element of the biotechnology of these plants. In particular, such plants can be used to study the peculiarities of the synthesis of biologically active compounds that are characteristic of *R. rosea*. In addition, *in vitro* grown plants are necessary for the development of technologies for the genetic transformation of these plants.

The growing demand for *R. rosea* plants requires the availability of plant material. However, wild harvest cannot meet this demand. That is why methods of microclonal propagation of these plants in *in vitro* culture have been developed. Usually, various plant growth regulators are used to root the shoots formed in sterile conditions. In particular, indole-3-butyric and indolyl-3-acetic acids were used for the stimulation of *R. rosea* shoots rooting *in vitro* (Tasheva and Kosturkova, 2010). At the same time, this process can probably be stimulated by using soil microorganisms, in particular, the so-called plant growth-promoting rhizobacteria (PGPR). This is possible due to the production of plant hormone-like chemicals by these bacteria.

The study of the interaction of microorganisms and plants is one of the important aspects of evaluation ways and mechanisms of adaptation. This is primarily because microorganisms are part of the biocenosis of the soil on which plants grow. Plants synthesize and excrete compounds that are a source of nutrition for microorganisms. At the same time, the latter, in turn, are able to use the compounds contained in the soil, making them bioavailable to plants and thus promoting better plant growth.

Soil microorganisms can positively affect plants, stimulating their growth by changing their metabolism. In particular, it is known that bacteria of the *Bacillus* genus, which are the component of soil microbiocenosis, are able not only to increase the bioavailability of chemical elements for plants (Kang et al., 2014; Kang et al., 2015a; Yousuf et al., 2017) but also increase the resistance of plants to stress factors and pathogenic microflora (Gururani et al., 2013; Bashir et al., 2021) and synthesize growth-stimulating compounds (Kang et al., 2015b). Such features of plant growth-promoting bacteria are the basis for the development of technologies for the use of these microorganisms to stimulate plant growth, protect them from pathogens and increase resistance to stress factors (Sharma et al., 2022).

It should be noted the perspective of PGPR using to stimulate plant growth *in vitro*. Obviously, the bacteria cannot be used in the case of plant cultivation in sterile conditions. At the same time, this approach is possible due to the fact that culture fluid obtained during the cultivation of the bacteria contains compounds that can affect plant growth (Shao et al., 2015). Thanks to the presence of such compounds as indole-3-acetic acid, the resulting culture fluid can be used for rooting plant shoots *in vitro* and stimulating the growth of the root system. Therefore, this approach is of special interest for the cultivation of valuable medicinal plants in sterile conditions.

Study of the possibility of using free of cells culture medium obtained after the growth of *Priestia endophytica* UKM B-7515 bacteria to stimulate the growth of *R. rosea* in *in vitro* conditions was the purpose of this work. Some biosynthetic parameters of seedlings were also determined (the content of photosynthetic pigments; total content of flavonoids; antioxidant activity).

Material and methodology

Bacterial test solution preparation and plant cultivation

Priestia endophytica UKM B-7515 strain from the Ukrainian collection of microorganisms of the D.K. Zabolotny Institute of Microbiology and Virology NAS of Ukraine was used in the study. The bacteria were cultivated in liquid LB medium at 37 °C for 24 h with periodic stirring (180 rpm). The culture fluid was separated from the cell biomass by centrifugation at 9000 rpm (Eppendorf Centrifuge 5415C) for 10 minutes. The supernatant was sterilized by filtration through a filter with a pore diameter of 0.2 µm (Sartorius, Minisart) and diluted with sterile distilled water up to the concentration of 20% to obtain the test solution.

Biological material

Rhodiola rosea plants from the *in vitro* collection of the Laboratory of Adaptational Biotechnology, Institute

of Cell Biology and Genetic Engineering, NAS of Ukraine, were used in the experiment. The apical parts of the shoots were separated and transferred to Petri dishes with the solidified half-strength Murashige and Skoog nutrient medium (Duchefa Biochemie, Netherlands) containing 2% sucrose. 30 µl of sterile test solution was applied to the lower part of the shoots. In 28 days of cultivation at a temperature of +24 °C the plants were removed from the medium and washed with distilled water. Morphometric and biosynthetic parameters of seedlings were determined (weight of the shoots and roots; content of photosynthetic pigments; total content of flavonoids; antioxidant activity).

Chemicals

For the plants cultivation Murashige and Skoog solid nutrient medium (Duchefa, Netherlands) was used. The reagents ($NaNO_2$, $AlCl_3$, NaOH, 2,2-diphenyl-1-picrylhydrazyl radical) were of analytical grade (Sigma-Aldrich). All solutions were prepared using deinized water.

Total flavonoid content assay

Total flavonoid content was studied by a modified method (Matvieieva et al., 2019). Before this study the plants were homogenized in 70% ethanol, the resulting extracts were centrifuged for 10 min at 10000 rpm (Eppendorf Centrifuge 5415C), and the supernatants were used for flavonoid content assay. The absorbance of the samples was measured at 510 nm using the spectrophotometer Fluorat-02 Panorama. Total flavonoid content was calculated by the calibration plot: C(rutin) = 0.7889D (R^2 = 0.9928) and expressed as milligrams per gram of plant fresh weight in rutin equivalent (mg RE.g^{-1} FW).

Antioxidant activity assay

The plant extracts obtained for the total content of flavonoids study were used for antioxidant activity analysis using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) by the method described in (Brand-Williams et al., 1995). The optical density of the mixture was determined at 515 nm on the Panorama Fluorate-2 spectrophotometer. The inhibition percentage was determined by the formula: $I = [(A_0 - A_1)/A0] \times 100$, where A_0 – absorbance of DPPH*; A_1 – absorbance of the sample in the reaction. Equivalent concentration (EC_{50}) was calculated as the corresponding weight of plant material needs to obtain the extract with a 50% DPPH* inhibition level.

Photosynthetic pigments assay

Pigments were determined spectrophotometrically after extraction with 70% ethanol. The material was triturated in a mortar and centrifuged for 10 min at 10000 rpm (Eppendorf Centrifuge 5415C). The supernatant was used in the study. The total concentration of green pigments (C_{a+b}) in the extracts was determined at a wavelength of 652 nm and calculated by the formula $C_{a+b} = 29\ D_{652}$ (mg.L^{-1}), where D_{652} is the optical density of the solution at a wavelength of 652 nm. The concentrations of chlorophyll *a* (C_a) and *b* (C_b) were determined at a wavelength of 665 and 649 nm and calculated by the formulas $C_a = 11.63\ D_{665}$ (mg.L^{-1}) and $C_b = 20.11\ D_{649} - 5.18\ D_{665}$ (mg.L^{-1}), where D_{665} and D_{649} were the optical densities of the solution at a wavelength of 665 and 649 nm, respectively. Carotenoids (C_c) concentration was determined at a wavelength of 440 nm by the formula C_c = 4,695 D_{440} – 0,268 C_{a+b} (mg.L^{-1}), where D_{440} was the optical density of the solution at a wavelength of 440 nm. The content of pigments was calculated according to their concentration in solution and the weight of the starting material in mg.g^{-1} of leaf weight.

Statistical analysis

All analyses were carried out in triplicate; growth experiments were provided 7–9 times. Values were represented as mean and standard deviation (SD). The data were analyzed for statistical significance using Student's *t*-test. P values less than 0.05 were considered significant. The linear regression method was applied and the coefficient of determination (R^2) was calculated for establishing the relationship between the values.

Results and discussion

Treatment of the shoots with culture fluid has led to rapid root formation. In particular, after seven days, the average number of formed roots on one shoot was 3.6 ±0.7 in the control and 7.5 ±2.1 in the experimental variants ($p < 0.016$) (Figure 1 a, b). After 28 days, the number of roots naturally increased in both variants. However, the rooting of shoots that were treated with the test solution occurred more intensively. Thus, the number of roots formed on one shoot in the control and experimental variants was 7.9 ±1.2 and 16.2 ±4.8, respectively ($p < 0.010$) (Figure 1 c, d).

The weight of the roots of the control plants in 28 days of cultivation was significantly ($p < 0.025$) lower than the same parameter of the roots of the treated plants and was 10.1 ±4.0 and 18.6 ±6.3, respectively (Figure 2). The shoots of the treated plants also had a greater

Figure 1 Formation of the roots on the control and treated *Rhodiola rosea* L. plants in 7 (a, b) and 28 (c, d) days after treatment with the test solution

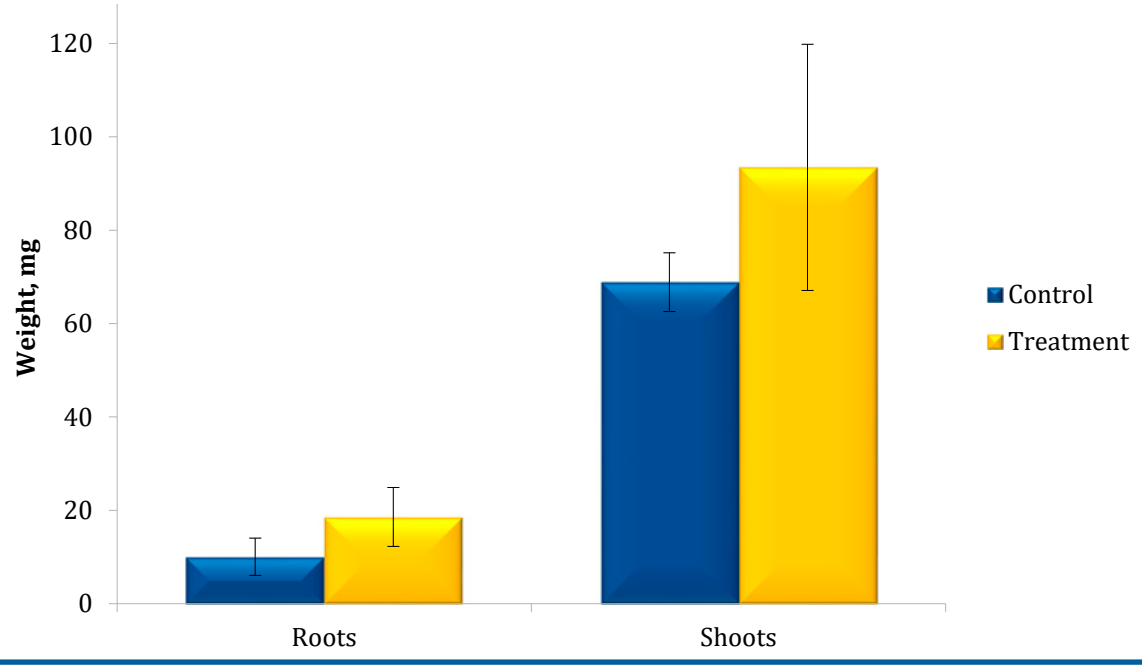

Figure 2 Weight of the roots and shoots of the control and treated *Rhodiola rosea* L. plants in 28 days of *in vitro* growth

(p <0.062) weight, which was 93.5 ±26.4, while the weight of the shoots of the control plants was equal to 68.9 ±22.1. Thus, a single treatment of *R. rosea* shoots with a cultural fluid in *in vitro* conditions has led to a significant stimulation of the growth of the root system. The stimulating effect was manifested already at the beginning of cultivation.

We used the bacterial strain isolated from cotton plants and described earlier (Reva et al., 2002). Previously, the chemical composition of the culture medium was determined after one day of cultivation of the bacteria. In particular, indol-3-butyric and indole-3-acetic acids (IAA), which were synthesized in the process of bacterial growth, were found in the nutrient medium for 59 and 757 μg.L^{-1}, respectively. These chemicals are known as the most common hormones of plants (auxins) which take part in the regulation of various plant growth processes. The compounds can be synthesized also in different microorganisms. In particular, *Pseudomonas*, *Rhizobium*, *Azospirillum*, *Enterobacter*, *Azotobacter*, *Klebsiella*, *Alcaligenes*, *Pantoea* and *Streptomyces* were found to produce IAA (Apine and Jadhav, 2011). IAA affects the induction of lateral and adventitious root formation in plants (McSteen, 2010). Park et al. (2017) demonstrated that adding IAA exhibited the highest levels of shoot and root and the fresh weight of common buckwheat plants. IAA also affected increasing the size of xylem cells (Uggla et al., 1996) and stimulated plant growth (Chhetri et al., 2022). This hormone demonstrated the properties of signaling molecules in plant-microbe interactions, taking part in the symbiosis between microorganisms and host plants (Spaepen et al., 2007; Malhotra and Srivastava, 2009; Duca et al., 1014). Based on the above data, it can be assumed that the obtained in our experiments effect of stimulating the growth of *R. rosea* plants, in particular, increasing the weight of the root system, may be associated with the presence of IAA in the test solution. Some biochemical parameters of the plant of both experimental and control variants were studied (Figure 3).

In the study of Sharma et al. (2022), treatment of plants with *Priestia endophytica* SK1 bacteria stimulated not only plant growth but also increased nitrogen and phosphorus content, as well as the concentration of phenolic compounds in fenugreek plants. In our experiments, there we no statistical differences in total flavonoid content (p <0.5) and antioxidant activity (p <0.95) in the control and treated plants. In particular, the content of flavonoids in the control plants was 1.2 0.3 mg RE.g^{-1} FW, and in treated plants – 1.0 ±0.1 mg RE.g^{-1} FW. A similar absence of significant changes was found in the analysis of the content of photosynthetic pigments and carotenoids. This lack of change can be explained by the fact that the test solution was used in a small amount (only 30 μl per shoot). Apparently, this amount was sufficient to stimulate the process of shoot formation, probably due to the presence of IAA. However, higher concentrations of active compounds are required to initiate changes in the synthesis of secondary metabolites. This assumption is confirmed, in particular, by the results of studies on the effect of

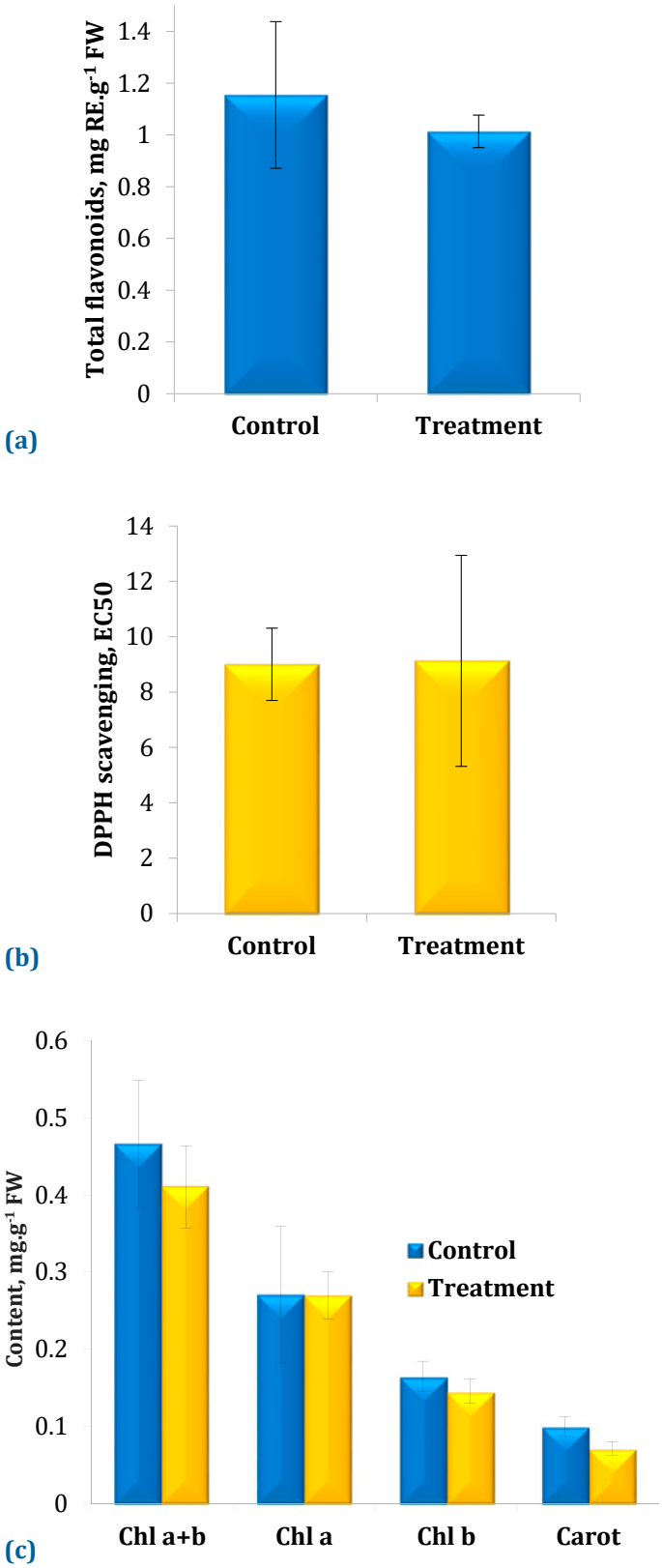

Figure 3 Total flavonoid content (a), DPPH scavenging activity (b), and photosynthetic pigments content (c) in the shoots of the control and treated *Rhodiola rosea* L. plants in 28 days of *in vitro* growth

IAA on plant growth and on the secondary metabolism of buckwheat (Park et al., 2017). Authors found that the treatment of plants with IAA at a concentration of up to 1.0 mg.L^{-1} has led not only to the stimulation of root growth, but also to an increase in the content of the total phenolic compounds and some flavonoids, in particular, 4-hydroxybenzoic acid, catechin, chlorogenic acid, caffeic acid, epicatechin, rutin, and quercetin. Treatment of tomato plants with *Bacillus licheniformis*, *Priestia megaterium*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens* resulted in an increase in dry weight, the photosynthetic rate, and the content of carotenoids (Katsenios et al., 2021). However, the above studies used bacteria and not sterile culture fluid. Obviously, this can explain the lack of effect of treatment with the test solution on the content of flavonoids and photosynthetic pigments in our experiments.

Conclusions

Thus, it was established that a single treatment of *R. rosea* shoots with a sterile culture medium obtained after the cultivation of *Priestia endophytica* UKM B-7515 bacteria allows stimulating significantly the formation of roots. However, this treatment did not affect the synthesis of flavonoids, the level of antioxidant activity, and the content of photosynthetic pigments. The result indicates the possibility of using free of bacterial cells solution for quick and effective rooting of shoots of valuable medicinal plants in *in vitro* conditions.

Conflict of Interests

Authors declare any Conflict of Interests.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

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