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Effect of secondary bacterial metabolites from permafrost on photosynthetic activity of potato (*Solanum tuberosum* L.) in vitro culture

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Abstract. The influence of secondary metabolites of bacteria from perennially frozen rocks on the activity of photosynthesis processes in potato leaves cultivated in vitro has been studied. Early meristemous potato plants of Zhukovsky variety were used as an object of research. The concentration of photosynthetic pigments of chlorophylls *a*, *b* and carotenoids was determined by spectrophotometry. Calculation of concentrations of chlorophylls *a*, *b* and carotenoids by Wellburn formula. It was found that when potato microplants are grown together in the Murashige-Skoog nutrient medium with metabolites of bacteria of *Bacillus cereus* 9-08-CH9 and *Achromobacter spanius* 10-50TS2 strains, introduced at the moment of drawing in a dose of 250 pl, under conditions of sufficient and optimal nutrition the process of photosynthesis is stimulated. First of all: by activating the light-gathering ability of the plant, by increasing the synthesis of chlorophyll *b* (on day 20), as well as increasing the resistance of plants to adverse environmental factors, through the synthesis of carotenoids (on day 30). The obtained results can be used for creation of biopreparations on the basis of microbial metabolites in order to increase efficiency of microclonal reproduction technology of potatoes.

1. Introduction

Improving the efficiency of in vitro cultivation of plant cells and tissues is an important challenge for the development of agro-biotechnology. Traditional approaches to this problem are based on the search for chemical, physical or genetic cultivation parameters. Biological factors such as associative bacteria and their metabolites are much less well studied [1]. A way out of this problem could be the creation of phytostimulants based on bacteria isolated from perennially frozen rocks (permafrost). Microorganisms that have preserved their viability for a long time under the conditions of low temperatures and slow metabolism, forced to adapt to adverse environmental factors can possess high adaptability to the soil and climatic conditions of Western Siberia [2]. Previously, it was found that bacteria from MMP have a positive effect on morphophysiological, biochemical and adaptive parameters of cultivated cereal plants [2, 3]. To date, studies on the effects of bacterial metabolites from MMPs on meristem plants of potato are sparse in the literature available to us. These studies are necessary to identify bacterial strains and their metabolites, which can be used in biotechnological production of potato micropropagation. In connection with the above, the relevance of research aimed at studying the impact of secondary metabolites of bacteria on meristem potato plants is beyond doubt.

The aim of this study is to evaluate the effect of secondary metabolites of bacteria isolated from perennially frozen rocks on the activity of photosynthetic processes in meristem potato plants.



2. Materials and methods

The effect of secondary metabolites was investigated on meristem potato cultivars of the variety 'Zhukovsky early', a selection of the Russian Potato Research Center.

Three strains of bacterial cultures were used: *Bacillus cereus* 875 TS, *Achromobacter spanius* 10-50TS2, isolated from mud cores from borehole drilling in Tarko-Sale area (Russia, Yamalo-Nenets Autonomous District, Purovsky region) and *Bacillus cereus* 9-08-CH9, isolated from mud samples of coastal outcrops of the Chara River. (Russia, Transbaikalia Territory, Kalarskii District). The strains were identified by 16S pPNA and deposited in VKPM of FSUE GosNIIGenetika (Moscow): 875TS (B-12242) - *Bacillus cereus*; 9-08-CH9 (B-12401) - *Bacillus cereus*; 10-50TS2 (B-12405) - *Achromobacter spanius*. The bacterial strains were cultured as described below. Bacterial strains were seeded in test tubes on standard method of oblique nutrient agar (GRM-agar, Obolensk, TU 9398-020-78095326-2006) and cultivated in thermostat for 48 hours at $t^{\circ}=26^{\circ}\text{C}$. Then, the microorganisms were washed out of each tube with 5 ml of distilled water [2]. Concentration of microorganisms was determined by the culture method of serial dilutions by the number of CFU on agarized nutrient medium in Petri dishes [4]. After determining the number of bacterial cells in the initial matrix suspension, the density of cultures was adjusted to a working concentration of 1×10^9 microbial cells in 1 ml of distilled water. The cell suspension was then frozen for 8 hours at $t^{\circ} -15^{\circ}\text{C}$, after which it was thawed at $t^{\circ} +22^{\circ}\text{C}$ for 16 hours. The freeze-thaw cycle was repeated 3 times. According to our experimental data this method increases the yield of secondary metabolites of bacteria into the aqueous medium by 2 times. The total mass of peptide complexes in the filtrates was 200 $\mu\text{g/ml}$. The mass of peptide complexes was determined by the biuret method [5] and by liquid preparative chromatography on a Gilson chromatograph.

A sterile solution of metabolites was obtained by filtering bacterial suspensions through Millipor filters with a pore diameter of 0.22 μm (Durapore membrane filters, type 0.22 μm GV). The obtained filtrates containing bacterial metabolites were used for further work. To study the effect of bacterial metabolites on meristem development of potato plants, the metabolites in an amount (dose) of 250 μl . (50 μg in peptide complexes) were digested (applied) to the surface of Murashige-Skoog nutrient medium, poured into 5 ml tubes. In the control version, filtered flush from the surface of nutrient agar for cultivation of microorganisms was added in the same volume. After 60 minutes, potato microtransplants were planted in these tubes.

In vitro propagation of potato plants by microclonal cuttings was carried out in a sterile box. After the plants grew from the apical meristems to the formation of 5-6 leaves, they were removed from the test tube with forceps and cut in a sterilized Petri dish into cuttings, including part of the stem with one leaf. The cuttings were planted in test tubes with Murashige-Skoog nutrient medium to the depth of the internode. Test-tubes with planted plants were placed in a special room with a temperature of $20-22^{\circ}\text{C}$ and illumination of 5000-8000 lux with a photoperiod of 16 hours [6].

The laboratory experiment scheme consisted of 4 variants:

- 1 - control;
- 2 - secondary metabolites of *Bacillus cereus* strain 9-08-CH9;
- 3 - secondary metabolites of *Achromobacter spanius* strain 10-50TS2;
- 4 - secondary metabolites of *Bacillus cereus* strain 875TS.

To assess the activity of photosynthesis, process the chlorophyll *a* and *b* and carotenoid content were determined by the following procedure. Leaves of meristem potato plants were thoroughly ground in a porcelain mortar with a small amount of 100% acetone (2-3 ml), pure quartz sand and chalk. After standing (2-3 min), the extract was transferred to a glass filter № 3 and filtered into a Bunsen flask connected to a vacuum pump KNF-UN 035.3 TTP (USA). Extraction of pigments by small portions of pure solvent was repeated 3-4 times on the filter until the pigments were completely extracted. The analysis was performed using a spectrophotometer (Agilent Cary 60, USA). The concentration of pigments was calculated by equations based on specific absorption coefficients for 100% acetone according to the Wellburn method [7].

Determinations of chlorophyll *a*, *b* and carotenoid content were carried out on days 20 and 30 of the experiment.

Statistical processing of the results of the study was carried out according to international requirements for the processing of scientific research data, using SPSS 11.5 for Windows software for personal computers (mean value of photosynthetic pigments, parametric comparison by Student's *t*-criterion).

3. Results and discussion

Pigments (chlorophyll *a* and *b*, carotenoids) are known to play a crucial role in photosynthesis and, ultimately, plant productivity in general [8-12]. Chlorophyll *a* is the main functional pigment that serves as an energy donor for photosynthetic reactions. Chlorophyll *b* is a component of the photosynthetic apparatus of higher plants, the main function of which is considered to increase light-collecting capacity [11,13,14]. Chlorophyll content can be used as an important diagnostic indicator for the study of plant growth [15]. Carotenoids play a role as auxiliary pigments. They transfer additional energy to chlorophylls, performing a light-collecting function, and withdraw excess energy from chlorophylls, performing a light-protective function [16].

The effect of secondary bacterial metabolites on the photosynthetic pigment content of meristem potato leaves on day 20 of the experiment is shown in Table 1.

Table 1. Influence of secondary metabolites of bacteria on the content of pigments of photosynthesis in the leaves of meristem plants of potatoes (day 20).

№	Option	Content of pigments, mg/g raw weight				Relationship	
		Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Sum of chlorophylls	Carotenoids	<i>a/b</i>	<i>a+b/</i> carotenoids
1	Control	0,227±0,00067	0,139±0,00033	0,366±0,00088	0,038±0,00033	1,625	9,642
2	Bacillus cereus 9-08-CH9	0,225±0,00033	0,193±0,00068 [#]	0,418±0,00058 [#]	0,034±0,00272	1,163 [#]	12,243
3	Achromobacter spanius 10-50TS2	0,236±0,00058*	0,180±0,00033 [#]	0,416±0,00067 [#]	0,035±0,00200	1,309 [#]	11,935
4	Bacillus cereus 875TS	0,219±0,00033	0,158±0,00033 [#]	0,377±0,00033	0,025±0,00088 [#]	1,392 [#]	15,247 [#]

Note: * - validity of experience with control difference ($p < 0,05$); # - validity of experience with control difference ($p < 0,01$).

Analysing the data shown in table 1, we see that on the 20th day the content of chlorophyll *a* in variant 3 (Achromobacter spanius 10-50TS2) was significantly ($p < 0,05$) higher than that of the control variant. Values of other variants have no significant differences with the control ($p < 0,05$). The secondary metabolites of Achromobacter spanius strain 10-50TS2 stimulated chlorophyll *a* synthesis by 3,9% relative to control. Secondary metabolites of other strains studied have no effect on chlorophyll *a* content.

The values of chlorophyll *b* content in almost all variants were significantly ($p < 0,01$) higher than the control values. The maximum value was observed in variants with strain Bacillus cereus 9-08-CH9 where the content of chlorophyll *b* exceeded the control level by 38,8 %, and in variants with strains Achromobacter spanius 10-50TS2 and Bacillus cereus 875TS - by 29,5 % and 13,7 % respectively. Under the influence of secondary metabolites of the studied bacterial strains a stimulation of chlorophyll *b* production was observed, indicating an increase in the light-gathering capacity of the plant.

The total value of chlorophyll *a* and *b*, characterizing the activity of photosynthesis in the variants № 2 (Bacillus cereus 9-08-CH9) and № 3 (Achromobacter spanius 10-50TS2) significantly ($p < 0,01$) above the control values. Secondary metabolites of strain Bacillus cereus 875TS (variant № 4) had no reliable ($p < 0,01$) effect on the analyzed index. Due to increase of chlorophyll *b* synthesis high activity

of photosynthesis process was noted in meristem potato plants influenced by secondary metabolites of strains *Bacillus cereus* 9-08-CH9 and *Achromobacter spanius* 10-50TS2.

Another component of the pigment system is carotenoids. In the given research it is established that the quantitative content of carotenoids in potato plants is authentically ($p < 0,01$) lower, in comparison with the control, on 28,9 % under the influence of secondary metabolites of strain *Bacillus cereus* 875TS. The other variants had no effect on the studied index ($p < 0,01$). Carotenoid synthesis under the influence of secondary metabolites of the bacterial strains studied is not observed.

Many modern studies show the possibility of using the ratio of chlorophyll *a* and *b*, chlorophylls and carotenoids as indicators of the physiological state of plants [17]. To analyze the degree of stressability and stress tolerance of plants under the influence of secondary metabolites of bacteria, such indicators as the ratio of chlorophyll *a* to chlorophyll *b* and the ratio of the sum of chlorophylls to carotenoids were used.

In the course of experiment on 20 day of experiment it has been established that the ratio of chlorophyll *a* to chlorophyll *b* in meristem potato plants, in all variants authentically ($p < 0,01$) below indicators of control. It specifies low level of stressability of meristem potato plants, under the influence of secondary metabolites of bacteria.

On day 20 of the experiment, there was a significant increase ($p < 0,01$) in the chlorophyll to carotenoid ratio, compared with controls, indicating that the level of stress tolerance of meristem plants has increased.

The effect of secondary bacterial metabolites on photosynthetic pigment content in meristem potato leaves on day 30 of the experiment is shown in Table 2.

Table 2. Influence of secondary metabolites of bacteria on the content of pigments of photosynthesis in the leaves of meristem plants of potatoes (30 days).

№	Option	Content of pigments, mg/g raw weight				Relationship	
		Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Sum of chlorophylls	Carotenoids	<i>a/b</i>	<i>a+b/</i> carotenoids
1	Control	0,199±0,00033	0,152±0,00088	0,351±0,00066	0,025±0,00009	1,303	14,125
2	<i>Bacillus cereus</i> 9-08-CH9	0,186±0,00033#	0,129±0,00058#	0,315±0,00033#	0,031±0,00009*	1,446#	10,134#
3	<i>Achromobacter spanius</i> 10-50TS2	0,166±0,00033#	0,135±0,00058#	0,301±0,00033#	0,030±0,00014*	1,231	9,900#
4	<i>Bacillus cereus</i> 875TS	0,132±0,00033#	0,125±0,00033#	0,257±0,00033#	0,025±0,00015	1,058#	10,343#

Note: * - validity of experience with control difference ($p < 0,05$); # - validity of experience with control difference ($p < 0,01$).

Analyzing the data shown in Table 2, we see that on day 30 of the study, before planting of meristem plants in protected ground, there was a general decrease in chlorophyll *a* and *b* content in all test variants ($p < 0,01$) and, correspondingly, there was a significant decrease in photosynthesis intensity (sum of chlorophyll *a* and *b*). This decrease is characterized by depletion of Murashige-Skoog nutrient medium.

The quantitative content of carotenoids in plants was significantly ($p < 0,05$) higher compared to the control under the influence of secondary metabolites of *Bacillus cereus* strain 9-08-CH9 and *Achromobacter spanius* 10-50TS2. The other secondary bacterial metabolites had no effect on the indicator studied ($p < 0,05$). The increase in carotenoid content under the influence of secondary metabolites of *Bacillus cereus* 9-08-CH9 and *Achromobacter spanius* 10-50TS2, indicates the activation of protective and light harvesting function.

Due to the depletion of the nutrient medium on day 30 of the experiment, the plants were under stress. As a consequence, the ratio of chlorophyll *a* to chlorophyll *b* significantly ($p < 0,01$) increased under the influence of secondary metabolites of *Bacillus cereus* strain 9-08-CH9. This indicates plant stress tolerance to unfavorable environmental factors. At the same time significantly ($p < 0,01$) low

index of stressability was observed under the influence of secondary metabolites of strain *Bacillus cereus* 875TS.

At the final stage of the experiment (day 30), changes in the chlorophyll-carotenoid ratio were revealed. Under the influence of all secondary metabolites of bacteria there was a reliable ($p < 0.01$) decrease in this ratio, relative to the control. It speaks about decrease in stress tolerance of meristem potato plants.

4. Conclusions

It was found that at a joint cultivation of potato microplants in Murashige-Skoog nutrient medium with bacterial metabolites of *Bacillus cereus* 9-08-CH9 and *Achromobacter spanius* 10-50TS2 strains, introduced at the moment of cuttings in a dose of 250 μ l, under conditions of sufficient and optimum nutrition the photosynthesis process is stimulated. The light harvesting ability of the plant is activated on day 20 by increasing the synthesis of chlorophyll *b*. Resistance of meristem plants to adverse environmental factors increases on day 30 through the synthesis of carotenoids. The results obtained can be used to create biopreparations based on microbial metabolites to increase the efficiency of potato micropropagation technology.

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