Phototrophic microorganisms for agricultural technology and food security

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Abstract. The main idea of the study was to find cyanobacteria for the development of fertilizers, plant protection products and as well as for the use in agro-biotechnology. The physiological and biochemical potential of 22 strains of cyanobacteria and its influence on the efficiency of plant-microbe interactions were studied. Promising cyanobacterial strains Nostoc sp. ACSSI 57, Desmonostoc muscorum ACSSI 149, Desmonostoc muscorum ACSSI 091 can be recommended for development of biofertilizer. Strain Nostoc calcicola ACSSI 82 is promising for the development of biopreparations with herbicidal action. Strains Nostoc sphaeroides ACSSI 150 and Nostoc linckia ACSSI 271 can be used as a basis for biological stimulants and inhibitors of plant growth and development. It depends on the level of physiological parameters during cultivation in artificial media. Bacterization with one-month culture of a strain Nostoc linckia ACSSI 271 and a homogenate of the strain Nostoc sphaeroides ACSSI 150 significantly increased seed germination of Triticum aestivum L. (by 22.2 and 11.0%, p < 0.05). Strains of cyanobacteria Nostoc linckia ACSSI 271, Nostoc calcicola ACSSI 82 and Nostoc sphaeroides ACSSI 150 are able to affect the enzymatic activity and productivity of Ambrosia artemisiifolia L. Phytomass of plants after bacterization essentially (the least significant difference (0.5) is 2.31) reduced by 1.4-1.6 times (28.7-39.3%) compared to control (12.59 g/plant) and depending on the strain and its form of preparation. This suggests that bacterization with these strains is a powerful inhibitory factor for Ambrosia artemisiifolia L.

1. Introduction

Currently, in the agricultural practice worldwide, there are a number of solutions aimed at a qualitatively new level of agricultural production, such as Intensive Agriculture, Precision Agriculture, low-cost and environmentally friendly agriculture – Organic Agriculture. We focus on a high culture of agriculture, competent use of the laws of nature, scientific achievements aimed at maintaining the stability of agro-ecosystems, eco-friendly nature management, soil improvement, cost reduction and improvement of the quality of agricultural products. The interstate standard of the Russian Federation for obtaining organic products in the section "Land-use and fertilization" says that it is allowed using preparations based on microorganisms and their products to increase the content of nutrients in the soil or crop and improve the overall condition of the soil [1]. In this regard, the use of microorganisms and their associations in biotechnology is promising because of the possibility the creating of polyfunctional microbial preparations of a new generation, which belong to the category of biofertilizers and plant protection products (PPP).
The main objective of the study was to find phototrophic microorganisms for the development of fertilizers and plant protection products, as well as for the use in agro-biotechnology. Cyanobacteria are a complex group of phototrophic microorganisms with unique morphology and physiology, wide ecological valence and plasticity [2]. They are actively used to solve various biotechnological problems. Due to the ability to convert nutrients into an easily digestible form for plants, biofertilizers for plant nutrition were developed on the basis of soil cyanobacteria. Nitrogen-fixing cyanobacteria show phyto stimulating activity on rice [3, 4], wheat [5], tomato [6], etc. Cyanobacteria can be used for soil and seed treatment against phytopathogenic fungi that cause root diseases of plants [7]. They can survive in drought, at very low and very high temperatures resist strong insolation, be tolerant to high concentrations of salts, action of toxicants, etc. [8].

2. Methods and conditions
The research was carried out in 2016-2019. 22 strains of cyanobacteria from the Crimean Collection of Microorganisms of Federal State Budget Scientific Institution "Research Institute of Agriculture of Crimea" (https://niishk.ru/innovacionnye-razrabotki/kollekciya-mikroorganizmov/) and Algal Collection of Soil Science Institute (ACSSI) of the Institute of Physicochemical and Biological Problems of Soil Science RAS (http://acssi.org/index.php/catalogue) were used in the study. The numbers of strains are listed according to registration in the collection of the ACSSI.

The strains were cultured in the recommended for the cultivation of cyanobacteria non-nitrogen liquid mineral medium [9] in the climatic chamber at temperatures of +23-25°C with a density of photosynthetic photon flux PPFD (PPF) – 115 µmol/m²/sec and power light (PPF) to 24.5 W/m² with four zones of the spectrum: blue (400-500 nm), green (500-600 nm), red (600-700 nm), infrared (700-780 nm), the photoperiod was 12 h. At the same time, cyanobacteria were cultivated during the year under natural lighting to use the potential of daylight. Physiological parameters of cyanobacterial cultures were measured every a half month. Physiological and biochemical parameters of the strains were determined before bacterization of plants: biomass of cells on absolutely dry mass (a.d.m.) mg/ml of medium by gravimetric method on an analytical scales “PA Pioneer”; pH was measured with pH-meter “Mettler Toledo Seven Go 2”; the enzymatic activity of oxidoreductases (catalases, peroxidases, and polyphenol oxidase) and antioxidants (ascorbic acid and glutathione) in the biomass of the strains were identified according to conventional biochemical methods of research of plants and soils [10]. The nitrogen-fixing activity of cyanobacteria was measured by acetylene method using gas chromatograph "Chrom-5" with flame ionization detector. Exposition – 24 hours.

The efficiency of bacterization was evaluated in a laboratory experiment on test–plants of Triticum aestivum L. Plant was cultivated in the climatic chamber in receptacles (volume of 200 ml) with a perforated bottom on a sterile substrate – mineral class – silicates, mica crumb (1-5 mm fraction), fertilized with a nutrient mix of Pryanishnikov D. N. [11]. Seedlings were inoculated with strains of cyanobacteria of different ages at the amount of 10 ml/plant to identify the stimulating / inhibitory effect on the growth and development of plants. Bioherbicide properties of the strains were evaluated in pot experiments carried out on the test–plants of Ambrosia artemisiifolia L. using southern chernozems. Plants were treated with 10 ml/plant of a suspension of promising strains of cyanobacteria or their homogenate preparative forms (g), which was obtained by mixing disperse systems with liquid medium with fast-spinning rotor homogenizer. Distilled water was used as a control. The effectiveness of bacterization was assessed by the accumulation of plant phytomass. Experiments were repeated six times.

The following indicators of cyanobacteria and plants were used to identify the relationship between the physiological parameters of strains and the effect of stimulation / inhibition of plant growth:

a. Productivity of cells biomass of cyanobacteria (g/ml) and changes in the culture medium pH (pH).

b. Age of culture of cyanobacterial strain during cultured (Age, months).
c. Quantitative content of chlorophyll A (Chl, ω-mass fraction), carotenoids (Kr, ω) and phycocyanine (F, ω). These are indicators of physiological state of photosynthetic cells of cyanobacteria strains.

d. Nitrogen-fixing (nitrogenase) activity of cells of strains (NA, nmol of ethylene/day). They provide the recovery of atmospheric nitrogen to ammonium and the formation of nitrogen-containing organic molecules in cells.

e. Enzymatic activity of oxidoreductase such as catalase (Kt, mmol H₂O₂/g/min), peroxidases (PA, μmol guaiacol/g biomass cell) and polyphenol oxidase (PFA, C₆H₅O₆ μmol/g/min). These enzymes are catalyzing the decomposition reaction of toxic peroxides and control the adaptive capacity and participant in the regulation of oxidative processes in cells of bacteria and the plant.

f. Antioxidants of cellular systems of cyanobacteria strains such as glutathione (G, mg/g biomass cell) and ascorbic acid (Aa, mg/g biomass cell). They are regulate the growth of plants and participate in protective reactions under adverse effects.

g. Above-ground biomass (Mv, g/) and root biomass (Mr) of plants.

Correlation and dispersion analyses, multivariate exploration analysis and computer programs Excel, Statistica 10 were used to find functional dependencies of various factors and processes.

3. Results and Discussion

The dynamics of cyanobacteria biomass accumulation and changes of pH in artificial media were studied during cultivation. The strains of ACSSI 57, ACSSI 82, ACSSI 149, 150 ACSSI, ACSSI 091, ACSSI 271 were highly productive and accumulated 0.1-0.65 µg a.d.m./ml media after nine months of cultivation. The efficiency of cell biomass accumulation of other strains was lower by 1-2 orders (p < 0.05). The strains ACSSI 091, ACSSI 150, ACSSI 47, ACSSI 10 continued to accumulate biomass after 9.0 months of cultivation in comparison with other studied strains, which either grew slowly or died in an artificial media.

The results of pH measurements during cultivation showed that all strains differed in this indicator. However, most strains during cultivation accumulated alkaline metabolic products in medium. Strain ACSSI 82 showed an alkaline reaction only at the end of the year of cultivation. Strains ACSSI 231, ACSSI 47, and ACSSI 57 produced both acidic and alkaline products. Strains ACSSI 37 and ACSSI 115 acidified the medium during the cultivation period.

All strains had nitrogen-fixing activity to varying degrees. High peak indicator – 39.0-143.1 nmol of ethylene/day was observed in five strains (23% of all studied) after 7.5 months of cultivation; 52.9-327.7 nmol of ethylene/day was detected in nine strains (40% of all studied) after 6.0 months; 106.9 nmol of ethylene/day was detected in the strain ACSSI 43 after 4.5 months of cultivation.

 Peroxidase activity differed little in strains and did not depend on cultivation conditions. Polyphenol oxidase activity of three strains: ACSSI 46, ACSSI 82 and ACSSI 115 was higher (111.8; 119.6; 123.7 μmol guaiacol/g of biomass cells, p < 0.05), respectively, after 4.5, 6.0 and 7.5 months of cultivation than the same of the other strains. Long cultivation affected the amount of carotenoids in photosynthetic systems for eleven strains (50% of all studied) and reduced their mass fraction 2-100 times (p < 0.05).

According to the results of multivariate exploratory analysis, correlations of physiological and biochemical parameters of cyanobacterial strains with plant phytomass were determined (Table 1). The analysis of the obtained data identified 11 promising strains of cyanobacteria for development of biofertilizers and bioherbicides.

The catalase activity of a strain of cyanobacteria Nostoc sphaeroides ACSSI 150 correlated with above-ground and root mass of bacterized plants (r = -0.85; -0.82, p < 0.05) (Table 1). After visualization of such interactive links, the ranges of effects of inhibition and stimulation of plant growth and development were calculated (Figure 1, Table 1).
Table 1. Correlations in the system "strain of cyanobacteria – *Triticum aestivum* L.".

| Strain                          | Indicator of strain | Indicator of plant | Correlation (p < 0.05) |
|---------------------------------|---------------------|--------------------|------------------------|
| *Desmonostoc muscorum* ACSSI 091| mg/ml               | Mr                 | 0.77                   |
|                                 | Kr                  | Mr                 | -0.95                  |
|                                 | Kt                  | Mr                 | 0.82                   |
| *Nostoc* sp. ACSSI 231          | PA                  | Mr                 | 0.79                   |
|                                 | PA                  | Mv                 | -0.93                  |
| *Desmonostoc muscorum* ACSSI 149| Kr                  | Mv                 | -0.76                  |
|                                 | G                   | Mr                 | -0.76                  |
| *Nostoc sphaeroides* ACSSI 150  | Kt                  | Mr                 | -0.82                  |
|                                 | PA                  | Mv                 | -0.93                  |
| *Nostoc punctiforme* ACSSI 37   | Kr                  | Mv                 | -0.76                  |
| *Nostoc* sp. ACSSI 46           | Chl                 | Mv, Mr             | 0.79                   |
| *Nostoc* sp. ACSSI 57           | PA                  | Mv                 | 0.81                   |
| *Nostoc calcicola* ACSSI 82     | Age                 | Mv                 | -0.88                  |
|                                 | Age                 | Mr                 | -0.87                  |
| *Nostoc* sp. ACSSI 113          | G                   | Mv, Mr             | 0.92                   |
| *Nostoc* sp. ACSSI 115          | Kr                  | Mv                 | 0.78                   |
| *Nostoc linckia* ACSSI 271      | pH                  | Mv                 | -0.81                  |
|                                 | Aa                  | Mv                 | -0.72                  |

Kr – content of carotenoids in the biomass of the strains; Kt – activity of catalase; PA – peroxidase activity in the biomass of the strains; G – glutathione, Aa – ascorbic acid in the biomass of the strains; Chl – content of chlorophyll «A» in the biomass of the strains; PFA – polyphenoloxidase activity in the biomass of the strains; Age – age of the strains in artificial media; Mv – above-ground biomass of wheat plants; Mr – root biomass of wheat plants.

To obtain the herbicidal effect, it is necessary to use a culture of this strain of cyanobacteria with catalase activity in the range of 69.1-63.8 μmol H₂O₂/g/min, which corresponds to the 4.5-6.0-month culture of ACSSI 150. An increase in plant phytomass can ensure bacterization with this strain with minimal catalase activity after a month of cultivation.

Figure 1. Nonlinear interactions in the system «physiological and biochemical potential of a strain of *Nostoc sphaeroides* ACSSI 150 – productivity of plant» (coordinate axis: z – Mv, g/plant; y – Kt, mmol H₂O₂/g/min; x – Mr, g/plant).
We assessed the potential of the cyanobacterial strain *Nostoc linckia* ACSSI 271. The effectiveness of bacterization by this strain of cyanobacteria had a high inverse correlation between the medium pH during cultivation and the content of ascorbic acid in its cells ($r = -0.81$; $-0.72$, $p < 0.05$) (Table 1). Analyzing the main component, we established the ranges of influence of physiological parameters of the strain to obtain the effects of inhibition and stimulation of plant growth and development. The stimulating effect was observed in pH range 6.9–7.14 with a decrease in the content of ascorbic acid from 0.81 to 0.25 mg/100 g a.d.m., which corresponded to the one-month culture. The inhibitory effect was observed at high alkalinity (pH 7.9) and maximum content of ascorbic acid of 3.1 mg/100 g a. d. m., which corresponded to the 10-month culture.

The effectiveness of bacterization of plants by other promising strains similarly depended on their physiological and biochemical status, which influenced the plant-microbial interaction. Taking into account the technological effectiveness of the studied strains, we recommend strains ACSSI 57, ACSSI 149, and ACSSI 091 for development of biofertilizers and microbial preparations with stimulating action. Strain ACSSI 82 is recommended for bioherbicides development. Strains ACSSI 150 and ACSSI 271 can be used as a basis for biological stimulants and inhibitors of plant growth and development. It depends on the level of physiological parameters during cultivation in artificial media.

We studied the effect of strains on the germination of wheat seeds. The analysis of the obtained data showed a high functional dependence of seed germination and plant biomass on physiological and biochemical parameters of cyanobacterial strains and their homogenized preparative forms.

A strong positive relationship between seed germination and biomass of the cells of the strains ($r = 0.91$, $p < 0.05$) and the content of glutathione ($r = 0.83$, $p < 0.05$) was found after the bacterization of plants with one-month ACSSI 82, ACSSI 150 and ACSSI 271. At the same time, the accumulation of plant biomass negatively correlated with the content of ascorbic acid and enzymatic activity of peroxidases in the culture medium ($r = -0.95$ and -0.89, $p < 0.05$).

When plants were bacterized by homogenates based on cyanobacteria strains, statistically significant ($p < 0.05$) values of correlation coefficients between seed germination and ascorbic acid content ($r = 0.98$, $p < 0.05$) and culture medium pH ($r = 0.99$, $p < 0.05$) were revealed, as well as with the cell biomass of strains ($r = 0.99$, $p < 0.05$) and culture medium pH ($r = 0.99$, $p < 0.05$). A significant positive relationship was established between the accumulation of plant biomass and the content of glutathione ($r = 0.98$, $p < 0.05$), while the accumulation of biomass inversely correlated with the enzymatic activity of cyanobacterial cultures peroxidases ($r = -0.97$, $p < 0.05$).

Thus, the established reliable links between a number of indicators of the studied plant and indicators of culture media with cyanobacteria allow us to state that the used strains have a powerful physiological and biochemical potential, which is very promising in terms of increasing the yield of *Triticum aestivum* L.

**Table 2.** The efficiency of bacterization of *Triticum aestivum* L. with strains of cyanobacteria and their homogenates

| Variant of the experiment | Germination, % $X \pm SE$ | Plant height, cm $X \pm SE$ | Plant biomass, g $X \pm SE$ |
|---------------------------|---------------------------|-----------------------------|-----------------------------|
| Control (water)           | 60.00±0.10                | 23.90±2.98                  | 0.44±0.03                   |
| *Nostoc sphaeroides* 150  | 60.00±0.10                | 23.90±5.27                  | 0.43±0.06                   |
| *Nostoc calcicola* 82     | 40.00±0.05                | 24.10±2.26                  | 0.46±0.05                   |
| *Nostoc linckia* 271      | 73.30±0.10                | 24.60±4.45                  | 0.42±0.04                   |
| *Nostoc sphaeroides* 150 (g) | 66.60±0.10            | 23.48±1.77                  | 0.41±0.04                   |
| *Nostoc calcicola* 82 (g) | 60.00±0.10                | 21.31±3.65                  | 0.41±0.04                   |
| *Nostoc linckia* 271 (g)  | 60.00±0.10                | 22.80±2.98                  | 0.48±0.03                   |

$g$ – homogenate.
The results of the experiment indicated the prospects for the use of cyanobacteria for the development of microbial stimulants. Bacterization of plants with *Nostoc linckia* ACSSI 271 and a homogenate of the strain *Nostoc sphaeroides* ACSSI 150 significantly increased wheat seeds germination by 22.2 and 11.0%, respectively (p < 0.05). But the plants' height and phytomass were at the level of control (within the error of experiment) (Table 2).

Treatment with the strain of *Nostoc calcicola* ACSI 82 reduced seed germination by 50% (p < 0.05). This confirms the potential of this strain for development biopreparation with herbicidal action.

In pot experiments on southern chernozems, we evaluated the activity of oxidoreductases in *Ambrosia artemisiifolia* L. after bacterization it with *Nostoc sphaeroides* ACSSI 150 (6-month culture), *Nostoc linckia* ACSSI 271 (10-month culture), *Nostoc calcicola* 82 (9-month culture) and their homogenates.

Peroxidase is sensitive to external influences. It participates in protective reactions of the plant and can be considered as a stress enzyme for the plant [12, 13]. In our studies, bacterization increased the level of peroxidases in the green mass of *Ambrosia artemisiifolia* L. 1.6-2.6 times (p < 0.05) in compared to the control (Table 3).

**Table 3. Efficiency of Ambrosia artemisiifolia L. bacterization with cyanobacteria of the genus Nostoc (pot experiments on the southern chernozem).**

| Variant of the experiment | Peroxidases, µmol guaiacol/g, X ± SE | Polyphenol oxidase, C₆H₈O₆ µmol/g/min, X ± SE | Catalase, mmol H₂O₂/g/min, X ± SE | Phytomass, g/plant |
|---------------------------|--------------------------------------|--------------------------------|--------------------------------|---------------------|
| Control (water)           | 3.6±0.01                             | 4.0±0.01                        | 1061.4±0.01                    | 12.59               |
| *N. sphaeroides* 150      | 5.7±0.01                             | 7.8±0.01                        | 1472.6±0.01                    | 11.21               |
| *N. calcicola* 82         | 9.3±0.01                             | 48.4±0.01                       | 1222.0±0.01                    | 8.18                |
| *N. linckia* 271          | 9.3±0.01                             | 15.5±0.01                       | 1973.2±0.01                    | 8.98                |
| *N. sphaeroides* 150 (g)  | 5.9±0.01                             | 79.6±0.01                       | 1018.8±0.01                    | 7.64                |
| *N. linckia* 271 (g)      | 6.6±0.01                             | 5.5±0.01                        | 1172.6±0.01                    | 13.18               |
| The least significant (0.5) | –                                   | –                               | –                              | –                   |

**g** – homogenate, «–» – not determined.

Catalase protects cellular structures from destruction by hydrogen peroxide. Catalase activity in green mass of *Ambrosia artemisiifolia* L increased by 10.4-85.9% (p < 0.05). However, bacterization with *Nostoc sphaeroides* ACSSI 150 (g) reduced the activity of catalase in green mass of this plant by 4.0% compared to control.

High activity of the polyphenol oxidase was observed by bacterization namely by 1.4-19.9 time (p < 0.05) than in the control. This indicated a decrease in the intensity of respiration, inhibition of growth processes and increased resistance of plants to adverse stress factors.

The correlations of peroxidase and polyphenol oxidase activity with plant phytomass (r = -0.59 and -0.84, p < 0.05) were established. Phytomass of plants after bacterization with *Nostoc linckia* ACSSI 271, *Nostoc calcicola* 82 and homogenate of *Nostoc sphaeroides* ACSSI 150 significantly (the least significant difference (0.5) is 2.31) reduced 1.4-1.6 times (28.7-39.3%) than in the control (12.59 g/plant).

Thus, to obtain the maximum herbicidal effect (inhibition of plants), it is necessary to achieve prolonged exposure of strains. One of the solutions can be to study the herbicidal activity of strains with different inoculation load of cells of a certain physiological age and to control their physiological parameters during bacterization of *Ambrosia artemisiifolia* L.
4. Conclusion
Promising cyanobacterial strains *Nostoc* sp. ACSSI 57, *Desmonostoc muscorum* ACSSI 149, *Desmonostoc muscorum* ACSSI 091 can be recommended for the development of biofertilizers and microbial preparations with stimulating action; strain *Nostoc* sp. ACSSI 82 – for preparations with herbicidal action. Strains *Nostoc sphaeroides* ACSSI 150 and *Nostoc linckia* ACSSI 271 show both stimulating and inhibiting effect on plants, depending on the physiological status of the bacterial culture.

Each of the studied cyanobacterial strains is unique in nature and has a range of physiological features that determine the degree and direction of influence (stimulation / inhibition) on bacterized plants in plant-microbial interaction.

In the study, we found a significant correlation between some physiological and biochemical parameters of three strains of cyanobacteria (cells biomass and culture medium pH; peroxidase activity; content of antioxidants: glutathione and ascorbic acid) and seed germination and productivity of bacterized plants of *Triticum aestivum* L. Bacterization with one-month culture of a strain *Nostoc linckia* ACSSI 271 and a homogenate of the strain *Nostoc sphaeroides* ACSSI 150 significantly increased seed germination (by 22.2 and 11.0%).

Strains of cyanobacteria *Nostoc calcicola* ACSSI 82, *Nostoc sphaeroides* ACSSI 150 and *Nostoc linckia* ACSSI 271 are able to affect the enzymatic activity and productivity of *Ambrosia artemisiifolia* L. Phytomass of plants after bacterization essentially reduced 1.4-1.6 times (28.7-39.3%) depending on the strain and its form of preparation. Statistically significant (p < 0.05) correlations between the activity of peroxidases and polyphenol oxidases and the phytomass of bacterized plants (r = -0.59 and -0.84, p < 0.05) were established. This suggests that bacterization by these strains is a powerful inhibitory factor for *Ambrosia artemisiifolia* L. plants.

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