On the development of biological products based on microorganisms from permafrost

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Abstract. The development of phytostimulants based on bacterial cultures isolated from permafrost soils of Western and Eastern Siberia, which have a higher adaptive potential to the peculiarities of the climate and soils of Western Siberia, is an urgent task. At present, the issues of "green" agricultural technology, based on the minimum use of chemicals and the expansion of the use of drugs of biological origin, are being actively considered. However, they are not universally applicable in various agro-climatic zones. Their activity and efficiency is associated with the survival rate in the rhizosphere and rhizoplane of plants and can be significantly reduced when used in areas of risky farming, in particular in Western Siberia. According to the results of morphophysiological studies, it was revealed that during the pre-sowing treatment of seeds of spring soft wheat of the Grenada variety with binary mixtures of pure cultures of some bacterial strains isolated from permafrost soils of three regions of Siberia, an increase in adaptively important plant parameters is noted.

1. Introduction

In modern agricultural technology, to increase the yield of grain crops, mineral fertilizers and chemical plant protection products are most widely used. Their active large-scale use has a negative impact on the environment, increasing the risks of degradation of biocenoses. In this regard, it becomes necessary to search for alternative ways to increase plant productivity, in particular, the use of bioorganic and biotechnological preparations containing microorganisms with the effect of phytostimulants. At present, the issues of “green” agricultural technology, based on the minimum use of chemicals and the expansion of the use of drugs of biological origin, are being actively considered [1, 2, 3]. To protect plants and increase their productivity, there is a wide range of bacterial preparations. The difficulty of their widespread use is that they do not have universality when used in various agro-climatic zones. Their activity and efficiency when used in areas of risky farming, in particular in the conditions of Western Siberia, can significantly decrease due to the fact that it is associated with the survival rate in the rhizosphere and rhizoplane of plants and depends significantly on agroclimatic conditions. This problem can be solved by creating phytostimulants based on bacterial cultures with a higher adaptive potential and adapted to soil and climatic conditions when used in areas of risky farming and in a changing climatic environment. In our opinion, such phytostimulants can be bacterial cultures isolated from permafrost soils and, at present, practically not studied for their biological effect on plants.

The purpose of this study is to assess the effect of binary mixtures of pure cultures of bacterial strains isolated from permafrost soils on the morphological parameters of seedlings of soft spring wheat cultivar Grenada.
2. Materials and methods

The research work was carried out in June 2020 using seeds of spring bread wheat variety Grenada (Triticum aestivum) (provided from the Research Institute of Agriculture of the Northern Trans-Urals, Tyumen). These seeds of the 2019 reproduction have a reduced germination rate of 70%.

From permafrost samples of the Quaternary period taken in the regions of Tarko-Sale (Western Siberia), frozen outcrops of the Chara river bank and frozen outcrops of the Aldan river bank (Eastern Siberia), we isolated bacterial strains that were used in this study. Pure cultures of bacteria were identified by the 16S RNA sequencing method: B1M – B. sp. (cereus), B1T – B. cereus, B2T - B. megaterium, B3T – Achromobacter spanius, B1Ch – B. sp. (megaterium), B2Ch – B. sp. (pumilus), B3Ch - B. cereus. The obtained primary 16S RNA sequences were edited using the BioEdit program [4, 5]. Then they were compared with the GenBank database using the BLAST program [6]. The strains for this work were taken on the basis of our previous studies [7]. In the work, we used in various combinations bacterial preparations in the form of binary mixtures of pure cultures.

For inoculation of wheat seeds, we used an aqueous suspension of pure cultures of bacterial strains from permafrost, which were prepared according to the method described earlier [7]. Pure cultures of bacterial strains in a working concentration (1x10^7 microbial cells in 1 ml of distilled water) were mixed in a ratio of 1/1 in 15 different combinations. The obtained mixes of bacterial suspensions were used for pre-sowing treatment (inoculation) of wheat seeds (n = 100) by soaking them for 1 hour in 50 ml of a mixture of bacterial cultures. In the control variant (C), pure water was used to soak the seeds. Sowing of seeds was carried out in the sand of fraction No. 2 sterilized by temperature treatment in 10-liter growing cuvettes, to a depth of 2 cm. The seeds were germinated under laboratory conditions under natural light and a temperature of 22 ± 1.5 °C. In the course of the experiment, the following were determined: germination energy (on the 3rd day); laboratory seed germination (on the 7th day); sprout length and weight; coleoptile length; length, number and weight of roots (on the 20th day).

Statistical processing of the obtained data was carried out using the statistical program "IBM SPSS Statistics 21" (mean, variance of means, Student's t-test and frequency analysis).

3. Results and discussion

According to GOST [8], in relation to sowing and varietal qualities, seed material is characterized by such characteristics as germination energy and germination capacity. For the study, we deliberately took seeds of soft spring wheat variety Grenada with a reduced germination rate of 70% to determine how the preparations of bacterial cultures affect their germination energy and germination capacity. It was found that during pre-sowing inoculation with bacterial mixes of spring wheat seeds of the Grenada cultivar, according to these parameters, changes in various variations of the experiment were recorded (Table 1). In 3 variants, when the seeds were treated with mixes of bacterial cultures from permafrost, the seed germination energy significantly (p <0.05) exceeded this indicator in the control.

In decreasing order of values for this indicator, they were distributed as follows: No. 9, 11, 8, (B3Ch x B2Ch, B3T x B1M, B3Ch x B1M). In other variants, no significant differences with the control were found, however, in variants No. 3, 5, 6, 7, 12, 14, there is a tendency to an increase in the studied indicator. In the control variant (C), laboratory germination was 70.54 ± 4.48%. A significant increase (p <0.05) of seed germination during their presowing treatment with mixes of bacterial strains was established only in option No. 12.

However, in options No. 7, 5, 2, 3, 8, 11, there is a tendency to an increase in the studied indicator. It should be noted that mixes of bacterial cultures differ markedly from pure cultures of bacteria [7] in their effect on germination energy and seed germination. Mixing pure cultures that showed the best positive results in experimental studies does not always justify the expectation of a positive effect, which is probably due to the interaction of bacterial strains and their metabolites in the mixes.
Table 1. Influence of mixtures of pure cultures of bacterial strains from permafrost soils on the germination energy and laboratory germination when they inoculate the seeds of spring wheat variety Grenada, %.

| Option No. | Experience Option | Sprout length, cm | Coleoptile length, cm | Root length, cm | Number of roots, pcs | Sprout weight, g | Root weight, g |
|------------|------------------|-------------------|----------------------|----------------|---------------------|----------------|---------------|
| 0 (C)      | Control          | 27 ± 4.45         | 70 ± 4.43            |
| 1          | B1T x B3Ch       | 22 ± 4.16         | 68 ± 4.69            |
| 2          | B1T x B3T        | 25 ± 4.35         | 74 ± 4.41            |
| 3          | B1T x B2T        | 32 ± 4.69         | 74 ± 4.41            |
| 4          | B1T x B1M        | 21 ± 4.09         | 66 ± 4.76            |
| 5          | B1T x B2Ch       | 35 ± 4.79         | 76 ± 4.29            |
| 6          | B3Ch x B3T       | 32 ± 4.69         | 64 ± 4.82            |
| 7          | B3Ch x B2T       | 32 ± 4.69         | 77 ± 4.23            |
| 8          | B3Ch x B1M       | 41 ± 4.94*        | 74 ± 4.41            |
| 9          | B3Ch x B2Ch      | 42 ± 4.96*        | 67 ± 4.73            |
| 10         | B3T x B2T        | 25 ± 4.35         | 67 ± 4.73            |
| 11         | B3T x B1M        | 42 ± 4.96*        | 74 ± 4.41            |
| 12         | B3T x B2Ch       | 31 ± 4.65         | 79 ± 4.09            |
| 13         | B2T x B1M        | 34 ± 4.76         | 71 ± 4.56            |
| 14         | B2T x B2Ch       | 31 ± 4.65         | 70 ± 4.61            |
| 15         | B1M x B2Ch       | 33 ± 4.73         | 58 ± 4.96            |

Note: * - the reliability of the difference in indicators in the experiment when compared with indicators in the control (p < 0.05)

When analyzing the influence of mixes from pure cultures of bacteria on the process of seedling development, the following changes were recorded: an increase in the length of the sprout up to 13.37%, the length of the coleoptile up to 11.3% and the length of the root up to 43.47%, relative to the control (table 2).

Table 2. Morphological parameters of wheat seedlings of the Grenada variety during inoculation of seeds with mixes from pure cultures of bacterial strains from permafrost.

| Option No. | Sprout length, cm | Coleoptile length, cm | Root length, cm | Number of roots, pcs | Sprout weight, g | Root weight, g |
|------------|-------------------|----------------------|----------------|---------------------|----------------|---------------|
| 0 (C)      | 26.4±0.67         | 3.9±0.07             | 11.2±0.39       | 4.5±0.14            | 0.14±0.008     | 0.13±0.009    |
| 1          | 29.0±1.05*        | 3.9±0.12             | 11.0±0.45       | 4.7±0.12            | 0.18±0.010*    | 0.15±0.011    |
| 2          | 29.9±0.96*        | 4.1±0.14             | 11.5±0.45       | 4.9±0.05*           | 0.18±0.010*    | 0.16±0.009    |
| 3          | 29.3±1.02*        | 3.8±0.14             | 15.2±0.96*      | 4.9±0.11            | 0.17±0.011*    | 0.15±0.009    |
| 4          | 30.0±0.77*        | 3.8±0.13             | 12.8±0.57*      | 4.9±0.11*           | 0.16±0.010*    | 0.15±0.007    |
| 5          | 27.9±0.69         | 3.9±0.11             | 12.8±0.46*      | 4.5±0.15            | 0.14±0.008     | 0.14±0.010    |
| 6          | 27.1±0.62         | 4.1±0.17             | 12.2±0.55       | 4.6±0.13            | 0.14±0.008     | 0.15±0.009    |
| 7          | 27.4±0.65         | 4.1±0.15             | 12.5±0.56       | 4.9±0.10*           | 0.13±0.008     | 0.12±0.009    |
| 8          | 27.7±0.59         | 4.1±0.16             | 12.1±0.37       | 5.0±0.05*           | 0.13±0.008     | 0.15±0.006    |
| 9          | 28.1±0.70         | 4.3±0.13*            | 12.8±0.66*      | 4.7±0.14            | 0.15±0.009     | 0.18±0.014    |
| 10         | 26.8±0.92         | 4.0±0.15             | 11.0±0.45       | 5.0±0.15*           | 0.12±0.008     | 0.14±0.008    |
| 11         | 27.9±0.54         | 4.0±0.19             | 13.2±0.76*      | 4.7±0.16            | 0.14±0.007     | 0.16±0.010    |
| 12         | 25.8±0.76         | 4.1±0.12             | 16.1±0.95*      | 4.8±0.19            | 0.117±0.009    | 0.16±0.013    |
| 13         | 29.8±0.89*        | 4.4±0.10*            | 13.5±0.45*      | 4.9±0.10*           | 0.15±0.008*    | 0.17±0.008    |
| 14         | 29.5±0.58*        | 4.3±0.08*            | 15.2±0.65       | 4.9±0.15*           | 0.16±0.007*    | 0.17±0.009    |
| 15         | 27.0±0.77         | 4.0±0.16             | 10.3±0.39       | 4.8±0.12            | 0.14±0.008     | 0.14±0.011    |
Note: * - the reliability of the difference in indicators in the experiment when compared with indicators in the control (p < 0.05).

In the experiment, when studying the development of the aerial part of seedlings, it was found that when seeds are inoculated with mixes No. 1, 2, 3, 4, 13, 14, an increase in the length of the sprout is observed from 10.8% to 13.3% (p < 0.05). For the rest of the variants, no significant differences were found.

In variants No. 1, 2, 3, 4, 13, 14, under the influence of bacterial mixes, a significant increase in the mass of the shoot relative to the control variant is noted (p < 0.05).

Field germination of wheat seeds significantly depends on the length of the coleoptile, which performs a protective function in the early stages of seedling development in the soil. Considering the significance of this indicator, we evaluated the effect of pre-sowing inoculation of seeds with mixes of bacterial cultures on it. A significant increase (p < 0.05) in the length of the coleoptile by 9.0 - 11.3% is observed in options: No. 9, 13, 14.

The indicators of the intensity of growth and development of seedlings are, in particular, the parameters of the length and weight of the roots. Both plant resistance to dry periods and lodging resistance depend on the development of the root system. A significant increase (p < 0.05) in the length of the root from 14% to 43% is observed in options: No. 3, 4, 5, 9, 11, 12, 13, 14. An increase in the number of roots (p < 0.05) from 8 % up to 11% is observed in options: No. 2, 3, 4, 7, 8, 10, 13, 14. An increase in the mass of roots (p <0.05) by 15-30% is observed in options: No. 2, 3, 4, 6, 11, 12, 13, 14. A simultaneous increase in all three indicators of the development of the root system is recorded in experimental variants No. 3, 4, 13, 14. In terms of the effect on the development of the aerial part of the sprout and its root system, the best results are observed with pre-sowing inoculation of wheat seeds with bacterial mixes No. 3, 4, 13, 14. Note that the B2T strain is present in 3 mixes with a pronounced positive effect. At the same time, 10 bacterial mixes No. 1, 5, 6, 7, 8, 9, 10, 11, 12, 15 do not have a positive effect on the studied parameters of plants. Five of them contain the B3Ch strain (No. 1, 6, 7, 8, 9), three contain the B3T strain (No. 10, 11, 12) and three contain the B2Ch strain (No. 5, 12, 15). To characterize the effect of pre-sowing treatment of wheat seeds with bacterial cultures on plant development, we calculated the symmetry coefficient (SC) of seedlings and the coefficient of root supply (CRS).

**Table 3.** Coefficients of symmetry and root supply in seedlings of spring wheat cultivar Grenada under the influence of mixes from bacterial strains from permafrost.

| Option No | Processing option | SC1       | SC2       | CRS        |
|-----------|-------------------|-----------|-----------|------------|
| 0 (C)     | Control           | 2.42±0.07 | 55.01±2.42| 0.92±0.04  |
| 1         | B1T x B3Ch        | 2.70±0.13*| 57.27±2.71| 0.83±0.05  |
| 2         | B1T x B3T         | 2.68±0.16 | 54.81±4.20| 0.88±0.03  |
| 3         | B1T x B2T         | 2.05±0.14*| 41.70±2.75*| 0.88±0.04 |
| 4         | B1T x B1M         | 2.40±0.09 | 48.72±1.65 | 0.93±0.04  |
| 5         | B1T x B2Ch        | 2.21±0.08 | 50.20±2.33 | 1.00±0.07  |
| 6         | B3Ch x B3T        | 2.28±0.10 | 49.61±2.22 | 1.07±0.08* |
| 7         | B3Ch x B2T        | 2.25±0.10 | 46.03±1.88*| 0.92±0.05  |
| 8         | B3Ch x B1M        | 2.32±0.07 | 45.94±1.48*| 1.24±0.06* |
| 9         | B3Ch x B2Ch       | 2.33±0.16 | 49.24±3.01 | 1.17±0.07* |
| 10        | B3T x B2T         | 2.51±0.15 | 50.29±2.91 | 1.16±0.08* |
| 11        | B3T x B1M         | 2.22±0.11 | 48.82±4.12 | 1.14±0.05* |
| 12        | B3T x B2Ch        | 1.69±0.11*| 36.95±3.21*| 1.36±0.07* |
| 13        | B2T x B1M         | 2.24±0.09 | 45.90±1.90*| 1.13±0.04* |
| 14        | B2T x B2Ch        | 2.00±0.08*| 41.34±2.48*| 1.06±0.05  |
| 15        | B1M x B2Ch        | 2.65±0.08 | 55.48±1.53 | 1.00±0.07  |

Note: * - the reliability of the difference in indicators in the experiment when compared with indicators in the control (p < 0.05).
The symmetry coefficient is calculated as the ratio of the average sprout length to the average root length (L\text{sprout} / L\text{root}). In the work, it is designated as the coefficient of symmetry 1 (SC1). With the harmonious development of the plant, the optimal symmetry coefficient has values of 0.8-1.1. At SC1 less than 0.7, the sprout is less developed than the root, at SC1 more than 1.2, the development of the sprout prevails over the development of the root system. In agricultural technology, SC1 is important for determining the timing of sowing seeds into the soil; at earlier dates, seeds with a SC of more than 1.2 are sown. Another SC calculation was proposed [9], which takes into account the average number of roots in a seedling, "since the number of roots determines the survival of plants in the initial phases of plant development, the density of productive stems, and therefore is one of the most important indicators of seed yield." The authors point out that the lower this symmetry coefficient, the higher the potential seed yield. In the work, it is designated as the symmetry coefficient 2 (SC2). Symmetry coefficient 2 was calculated by the formula \( C_{\text{symmetry}} = \frac{L_{\text{sprout}} \times 100}{L_{\text{root}} \times N_{\text{root}}} \). Where \( L_{\text{sprout}} \) - average length of shoots in seedlings, cm; \( L_{\text{root}} \) - the average length of roots in seedlings, cm; \( N_{\text{root}} \) - average number of roots, 100 - conversion factor. SC2 at the level of control values is noted in variants of experiment No. 1, 2, 15. In other variants, a decrease in this indicator relative to the control variant is noted. Analysis of SC1 shows that in all variants of the experiment, including control, the development of the aboveground part of the plant prevails over the development of the root system.

4. Conclusions and suggestions

It is obvious that the B3Ch strain impairs the properties of all the studied mixes to which it is included. And strains B3T and B2Ch show no activity both when mixed with each other and in a mixture with strain B1M. Strain B3T is also incompatible with strain B2T, and B2Ch is incompatible with strain B1T. The experimental results indicate that when the strains are mixed, in some variants, the effect of reducing the biological activity can be observed. The analysis of the root supply coefficient, in the calculation of which the weight of the roots in seedlings is taken into account (which is formed not only due to the length of the root, but also their number), shows that according to this indicator, the most balanced ratio of the development of the sprout and the root system have the following options: No. 5, 6, 14, 15, 14, 15. Their RSC is close to or equal to 1. A more developed root system than in the control is observed in variants No. 9, 10, 11, 13. Variants No. 8 and 12 have the highest RSC values. A more developed root system in the early period of plant growth gives the advantage of better water and nutrient absorption in the early stages of the growing season.

Thus, according to the results of morphophysiological studies on the effect of binary mixtures of pure cultures of the studied bacterial strains, it can be stated that the best results are manifested when pre-sowing inoculation of seeds of spring bread wheat variety Grenada (\textit{Triticum aestivum}) with mixes of pure cultures of bacterial strains numbered 3, 4, 13, 14. (B1T x B2T; B1T x B1M; B2T x B1M; B2T x B2Ch). In 3 of these variants, the B2T strain is present as a component.

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