Mobilization hard-to-reach phosphorus compounds in soil and influence growth processes of microbiological association

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Abstract. The article presents results of research of monocultures and association of lactic-oxide bacteria, purple nonsulfur bacteria and yeasts on indicators of stimulation of growth processes, nitrogen fixation and phosphate mobilization for 2 crops: one-year and long-term. The research was carried out in both laboratory and production conditions. It is shown that milk-oxide and purple nonsulfur bacteria stimulate growth processes of wheat variety Nador in aquatic culture by 20-30%, and their consortium with yeasts by 30%. In comparison with control, an increase of 3.4 t/ha in the yield of Golden Delishes apples was detected with 2-fold treatment based on a consortium of lactic oxide, purple nonsulfur bacteria and succromycetes. It was determined that all the strains of the above-mentioned groups of microorganisms studied mobilized hard-to-soluble compounds of phosphorus, and only purple nonsulfur bacteria were capable of nitrogen fixation.

1. Introduction

In the past 20 years, crop yields and quality have declined in many regions of the world, owing to a decrease in the use of mineral fertilizers [1, 2]. Increasing crop productivity is directly dependent on providing plants with nutrients, primarily nitrogen and phosphorus. The main sources of nitrogen for plants are mineral compounds containing only 1-3% of total soil nitrogen in the soil [3]. In many soil types, phosphorus is found in poorly accessible mineral and organic forms [4-7]. Soil pH plays a decisive role in the availability of phosphorus to plants. In soils with a pH above 7.2, 3-substituted calcium, aluminium and iron phosphates are prevalent and are virtually insoluble in water and therefore not available for plants [8-11].

Organic phosphates are mainly found in humus and are not readily available for plants, as microflora binds to these compounds during microbiological decomposition. It is also difficult for plants to assimilate phosphorus at a low rate of diffusion in the soil, ranging from 10-12 to 10-15 m/s, resulting in rapid soil depletion [1, 2]. Phosphorus concentration in soil solution is usually low, so it is one of the most difficult-to-reach soil macroelements, which often limits plant yields.

The problem of optimizing nitrogen and phosphorus food is rather actual, one way of solving it is to increase the availability of nitrogen and phosphorus for plants. Classical agrochemical methods include treatment of seeds with accessible forms of nitrogen and phosphorus and compounds that increase the efficiency of plant absorption of elements, off-site treatment with fertilizers containing...
water-soluble phosphorus and mineral nitrogen, local application of nitrogen and phosphorus fertilizers [12, 13], use of denitrification inhibitors [3, 14].

Advanced techniques such as pre-sowing seeds with mycorrhizal fungi, phosphate mobilizing bacteria and complex microbiological preparations attract the attention of scientists and agricultural producers in present time [15].

Microbiological agents such as azotobacterine, phosphobactone, etc. have a narrow specialization and aim at increasing the content of one element in the soil. Microbiological agents, which are used in agriculture to increase plant biological productivity typically, stimulate plant growth and development by producing plant hormones [6, 16-19]. Drugs based on active antagonist bacteria protect plants from phytopatogens and thus help to preserve crops [17, 20, 21].

Complex microbial associations are of greatest interest, which contain different groups of microorganisms capable of increasing plant yields through the biosynthesis of hormone-like substances, enzymes, antibiotic substances, mobilization of hard-to-reach nutrients, with anti-stress effects.

The aim of the research was to study the influence of the microbiological association of lactic oxide bacteria, purple nonsulfur bacteria and yeast on plant growth processes, increase biological fixation of atmospheric nitrogen and mobilize hard-to-reach phosphorus compounds in soil.

2. Materials and methods
The subjects of research were the microbial associations and the monocultures of the micro-organisms that make up it: mild-oxide homoenzymatic streptobacteria of the genus *Lactobacillus*: *L. plantarum* IMB B-7344 and *L. casei* IMB B-7343, lactic oxide homoenzymatic streptococci *Lactococcus lactis* IMB B-7352, purple nonpurple bacteria *Rhodopseudomonas palustris* IMB B-7334 and *Rh. sphaeroides* IMB B-7335, yeast *Saccharomyces cerevisiae* IMB Y-5046.

The phytoactive activity of monocultures of micro-organisms and their associations have been studied in laboratory experiments. Wheat seeds of Nador variety were used as the test object. Plants were grown in aquatic culture. The seeds were sterilized in 1% of the KMnO₄ solution, and then in a quantity of 30 of them were placed in Petri dishes on filter paper soaked in distilled water (control) or 0.1%, 0.5%, 1.0% of the solutions of the culture liquid of the consortium or monocultures of microorganisms for this purpose. Petri dishes were placed in the thermostat at a temperature of 25±1°C. Three repetitions were used in each variant.

Growth indicators (the length of the root system, the height of the above-ground part) were measured in seven-day sprouts using a metal ruler at 0.05 cm and expressed in % control.

Nitrogen fixation and phosphate mobilization of strains of micro-organisms were studied both in the laboratory and in the field. The nitrogen-free Ashby medium was used to detect the nitrogen-fixing capacity of micro-organisms [22]. Nitrogen fixation and phosphate mobilization of strains of micro-organisms were studied both in the laboratory and in the field. The nitrogen-free Ashby medium was used to detect the nitrogen-fixing capacity of micro-organisms.

Menkina’s medium was used to observe the mobilization of phosphorus from organic phosphates [23]. Studies on the transformation of inorganic phosphates were carried out on the Muromtsev’s medium [12]. Three-substituted calcium, iron and aluminium phosphates were introduced as the sole source of phosphorus in the modification of the medium [12]. In the study of phosphate mobilizing capacity of lactic oxide bacteria, an additional 0.5% yeast autolysate was introduced into nutrient medium [24]. Crop activity was estimated by calculating the phosphate solubility index (ratio of the diameter of the dilution zone of dissolved phosphorus around the colonies to the diameter of the microorganism colony) [25].

The organic acid content of the culture fluid of the microbiological consortium was determined by methods of high-efficiency liquid and gas chromatography [26].

Field experiments on the soil content of insoluble and affordable nitrogen and phosphorus compounds in the soil and plant yields were carried out on Golden Delishes root fruit-bearing apples. The soils of the plot are represented by black land by the southern small humus medium powerful. The combined sample was obtained by taking soil samples with a soil drill at a depth of 20 and 50 cm
during fruit growth and after application, at maturity, at 18 points over a total area of 2 hectares. The apple orchard was soil-fed twice during the growth of the fruit, with a 2-week interval, with a 5-day microbiological consortium culture fluid of 5 l/ha. Phosphorus in soil was determined by the Machigine method, nitrogen by Kjeldal [27].

Statistical analysis of experimental data was carried out using Excel (Microsoft, USA) and Statistica 6.0. The results of the study were considered statistically reliable at p <0.05 [28].

3. Results and discussion
The influence of different concentrations of culture fluid of lactic oxide bacteria, purple nonsulfur bacteria and saccharomicetes has shown that the studied microorganisms stimulate growth processes of wheat varieties Nador (Tab. 1).

Table 1. Morphometric indicators of wheat varieties Nador grown in the cultivation of milk-oxide bacteria, purple nonsulfur bacteria and saccharomicetes.

| Variants experience | Concentration of cultural liquid, % | Root length to control, % | Length above ground to control, % |
|---------------------|------------------------------------|---------------------------|-------------------------------|
| Control (water)     | -                                  | 100                       | 100                           |
| L. casei B-7343     | 0.1                                | 114.2                     | 113.5                         |
|                     | 0.5                                | 121.7                     | 119.4                         |
|                     | 1.0                                | 128.3                     | 121.5                         |
| L. plantarum B-7344 | 0.1                                | 128.3                     | 124.7                         |
|                     | 0.5                                | 131.2                     | 129.2                         |
|                     | 1.0                                | 134.7                     | 132.4                         |
| L. lactis B-7352    | 0.1                                | 108.3                     | 117.9                         |
|                     | 0.5                                | 117.1                     | 106.2                         |
|                     | 1.0                                | 82.3                      | 86.7                          |
| R. palustris B-7334 | 0.1                                | 108.2                     | 114.5                         |
|                     | 0.5                                | 120.5                     | 119.4                         |
|                     | 1.0                                | 131.6                     | 121.2                         |
| R. sphaeroides B-7335 | 0.1                                | 113.6                     | 113.2                         |
|                     | 0.5                                | 115.5                     | 118.4                         |
|                     | 1.0                                | 122.1                     | 125.1                         |
| S. cerevisiae Y-5046 | 0.1                                | 103.4                     | 105.3                         |
|                     | 0.5                                | 105.0                     | 107.4                         |
|                     | 1.0                                | 105.9                     | 108.5                         |
| microbial association | 0.1                                | 125.4                     | 117.4                         |
|                     | 0.5                                | 131.7                     | 121.4                         |
|                     | 1.0                                | 135.3                     | 126.9                         |

The three studied concentrations of culture fluid of L. casei B-7343 strain, a maximum of 28.3% root growth stimulation and 21.5% escape induced 1.0% solution. A similar trend was observed in the phytostative activity of the L. plantarum B-7344 strain: the length of the root and the above-ground part in the treatment of 1.0% of the culture fluid solution is greater than the control by 34.7% and 32.4% respectively. The strains of lactic oxide bacteria, independent on concentration, stimulated growth processes in the root more strongly than in the above-ground part of the plants.

The most stimulating effect on the root length compared to control was 0.5% solution of the cultural liquid L. lactis B-7352 – 117.1%, and runoff – 0.1% solution – 117.9%. 1.0% of B-7352 lactococcal fluid has an influence on the wheat growth.

The strains of purple nonsulfur bacteria showed a high phytostative effect similar to that of lactic oxide bacteria. Thus, 1.0% of the 5-day deep culture of the R. palustris B-7334 strain in the liquid
medium stimulated root growth by 31.65% compared to control and shoots by 21.24%. 1.0% of the solution of the culture fluid \textit{R. sphaeroides} B-7335 stimulated the length of the above-ground part by 25.1% compared to control, and the root 22.1%. \textit{R. palustris} B-7334, like milk-oxide bacteria, had the greatest phytostatic effect on the root of the plant and \textit{R. sphaeroides} B-7335-on the shoot.

A strain of \textit{S. cerevisiae} Y-5046, among all microorganisms studied, had the least stimulating effect on the morphometric indicators of Nador wheat varieties - 3-8% compared to control, which may be caused by the metabolites of sucromycetes, mainly vitamins and amino acids. A significant phytostatic effect of lactic oxide bacteria and purple nonsulfur bacteria - 20-30% compared to control - may be related to the biosynthesis of these phytohormon microorganisms.

When testing a microbial association on Nador wheat sprouts, the maximum effect - more than 30% - is achieved by using 1.0% of the culture fluid.

Increase in crop yields is directly related to the nitrogen and phosphorus content of the soil. Most nitrogen is captured by soil microorganisms. A study of the fixation of atmospheric nitrogen by purple nonsulfur bacteria showed that \textit{R. palustris} B-7334 and \textit{R. sphaeroides} B-7335 grow on a nitrogen-free Ashby medium. This is circumstantial evidence of atmospheric nitrogen fixation by purple bacteria [29, 30]. No saccharomicetes or lactic bacteria were detected by nitrogen.

Laboratory studies of the mobilization of plant-inaccessible forms of phosphorus are presented in table 2. Yeast \textit{S. cerevisiae} Y-5046, purple bacteria \textit{R. palustris} B-7334 and \textit{R. sphaeroides} B-7335 show the use three-substituted calcium phosphates, aluminium and iron as the sole source of phosphorus in the medium. After the introduction of yeast autolysate into the Muromtsev’s medium, the strains of lactic-oxide bacteria also showed visible growth at day 5 (tab. 2).

| Microorganism strains | Phosphate solubility index | organic compounds of phosphorus | Ca$_3$(PO$_4$)$_2$ | Al$_3$(PO$_4$)$_2$ | Fe$_3$(PO$_4$)$_2$ |
|-----------------------|---------------------------|-------------------------------|-------------------|-------------------|-------------------|
| \textit{L. casei} B-7343 | -                         | 1.6±0.3                       | 1.4±0.4           | 1.7±0.8           |
| \textit{L. plantarum} B-7344 | -                         | 1.8±0.6                       | 1.8±0.8           | 1.7±0.5           |
| \textit{L. lactis} B-7352 | -                         | 2.1±0.8                       | 1.9±0.3           | 2.2±0.3           |
| \textit{R. palustris} B-7334 | 3.2 ±0.6                  | 3.3±0.4                       | 3.1±0.6           | 3.2±0.7           |
| \textit{R. sphaeroides} B-7335 | 2.3±0.3                   | 2.5±0.6                       | 2.4±0.5           | 2.6±0.5           |
| \textit{S. cerevisiae} Y-5046 | 1.7±0.1                   | 2.0±0.3                       | 1.8±0.7           | 1.9±0.6           |

During sowing of strains of micro-organisms studied on a Menkina’s medium with organic compounds of phosphorus, it was shown that only purple nonsulfur bacteria are able to remove phosphoric acid, forming a zone of melting of chalk around the colonies. The main mechanism by which phosphates are dissolved by micro-organisms is their released by organic acids [31-33]. We have therefore studied the composition of the organic acids produced by the micro-organisms that are part of the microbial consortium being studied (table. 3).
Table 3. Organic acid content of microbial association culture fluid

| Acid           | Quantity, mg/l |
|----------------|---------------|
| Lactyc acid    | 1030.00       |
| Acetic acid    | 540.00        |
| Capric acid    | 8.77          |
| Caprillic acid | 29.51         |
| Oily acid      | 4666.46       |
| Fumaric acid   | 8.06          |

In the culture fluid of the microbial association monocarboxylic acids are mainly found. The presence of dicarboxy fumaric acid in the metabolites of micro-organisms suggests that phosphate mobilization can occur, not only through reduced pH in the medium, but also through the chelation of metal ions [31].

The results of our research are the same with the literature, which suggests that mild-oxide bacteria, when developed in the soil, may participate in processes related to the dissolution of phosphorous compounds [24, 32, 33]. According to a number of authors, purple bacteria form a significant amount of polyphosphates as substitute nutrients [34]. Therefore, this group of micro-organisms must have a phosphate enzyme that hydrolyzes the ether compounds of phosphoric acid.

Soil analysis data showed that by applying a microbiological association to the root system of apple trees, the exchange phosphorus content at a depth of 20 cm decreased by a factor of 1.6 and at a depth of 50 cm by a factor of 1.7. The content of available phosphorus after treatment decreased at a depth of 20 cm by 2.4 times, at a depth of 50 cm – 7.1 times. Nitrate nitrogen content has increased by a factor of 1.2 since the application of the microbial association culture fluid in the 20 cm zone, while at a depth of 50 cm it remained unchanged. However, the ammonium content did not change significantly (table 4).

Studies have shown that the increase in exchange phosphorus content in soil following the application of a microbiological consortium was due to the transfer of inaccessible phosphate forms into soluble compounds. During fruit ripening, nutrients are actively removed from the soil, especially phosphorus, which explains the decrease in soluble phosphorus compounds. The greatest removal of phosphorus is at a depth of 50 cm. This area contains the suction area of tree roots.

Table 4. Nitrogen and phosphorus content in soil treated by microbial association.

| Depth of sampling | Nitrogen and phosphorus content in soil, mg/100g |
|-------------------|-----------------------------------------------|
|                   | before use drug | after use drug |
| P₂O₅ water-solube |                 |                |
| 20 cm             | 12.18±0.69      | 11.77±0.55     |
| 50 cm             | 7.60±1.61       | 6.7±0.62       |
| P₂O₅ exchange     |                 |                |
| 20 cm             | 2.71±0.31       | 2.42±0.49      |
| 50 cm             | 1.15±1.08       | 0.34±0.08      |
| NO₃ (nitrate)     |                 |                |
| 20 cm             | 10.83±0.94      | 12.67±1.08     |
| 50 cm             | 10.33±0.88      | 10.5±1.42      |
| NH₄ (ammonium)    |                 |                |
| 20 cm             | 1.00±0.00       | 1.00±0.17      |
| 50 cm             | 1.00±0.00       | 1.05±0.06      |

The increase in nitrate nitrogen at a depth of 20 cm is due to the fact that the site is well aerated and purple sulfur bacteria fix atmospheric nitrogen. At deeper depths, the nitrate content does not change. As depth increases, anaerobic processes in the soil increase. Ammonium nitrogen in the soil is
produced by the ammonium process. The ammonium nitrogen content remained unchanged during the experiment, due to the fact that the consortium micro-organisms did not increase the mineralization of the organic residues.

As a result of the use of a consortium based on lactic oxide bacteria, purple nonsulfur bacteria and yeast brings its positive effects on the quality and quantity of apple yields, that are shown below (table 5).

| Table 5. Yield of apples. |
|---------------------------|
| Consortium performance indicators | control | microbial association |
| Number of questions, p. /tree | 113.5 | 120.2 |
| Average fruit weight, g | 170 | 175 |
| Crop, kg/tree | 19.3 | 21.0 |
| Yield, t/ha | 38.6 | 42.0 |
| Crop share of 2 varieties, % | 8 | 5 |

There is more harmonious harvest. The number of fruit per tree has increased by an average of 7, and the average weight of the fruit has increased by 5 grams compared to control. As a result of the microbiological consortium, the yield from one tree increased by 700 grams compared to control. Yields increased from 38.6 to 42.0 t/ha, with a 3% decline in the second sort for apples (5% in the investigated variant compared to 8% in the control).

So our research has shown that a microbiological consortium of lactic oxide bacteria, purple nonsulfur bacteria and yeast converts inaccessible forms of phosphorus into soluble compounds, increases nitrate nitrogen in the soil, which has a positive impact on the mineral nutrition of plants and the increase of crop yields.

4. Conclusion

As a result of the research is carried out, that it has been established that milk-oxide and purple nonsulfur bacteria stimulate growth processes of wheat variety Nador by 20-30% compared to control. The microbial association had a maximum stimulating effect on the wheat Nador wheat fruits - more than 30% when using 1.0% liquid associative culture.

It is shown, that a consortium of lactic oxide, purple nonsulfur bacteria and sugaromicetes convert forms of three-substituted phosphates that are not available for plants into soluble compounds. It has been determined that purple nonsulfur bacteria fix atmospheric nitrogen.

As a result of the microbiological consortium, the exchange phosphorus content in the soil decreased by a factor of 1.6 and nitrate nitrogen by a factor of 1.2 in well aerated soils compared to the reference values.

A consortium based on a consortium of lactic oxide, purple nonsulfur bacteria and sugaromicetes increased the yield of Golden Delishes apples by 3.4 t/ha compared to control.

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