Journal of Siberian Federal University. Biology 2017 10(3): 333-342
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УДК 635:631.45

Introduction of Microbiocenosis in Agroecosystem for Increasing the Plant Productivity

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Received 28.01.2016, received in revised form 14.07.2016, accepted 07.12.2016

The aim of the work was to study the effect of polyfunctional microbiocenosis 'Microbiovit' (MBV) on growth and development of agricultural plants, their productivity and biological activity of soil. Microbiocenosis application increased agricultural crops yield and maintained soil fertility. Treatment with MBV enhanced productivity of wheat, vegetables and potato by 26–56 %. Wheat ripening was reduced by 9 days. MBV application enhanced resistance of potato tubers to infection agents of rots and the shelf-life of the product increased. Concentration of nitrates in the root vegetables (carrot and beetroot) treated with MBV decreased. MBV increased the rate of organic matter mineralization in soil. Experiments under laboratory and field conditions showed that microbiocenosis introduced into agrophytocenoses increased productivity and demonstrated active regulatory, protective, and trophic functions.

Keywords: polyfunctional microbiocenosis, microbial communities, biological activity of soil, productivity of agricultural plants, safe food.

Citation: Somova L.A., Mikheeva G.A., Pechurkin N.S. Introduction of microbiocenosis in agroecosystem for increasing the plant productivity. J. Sib. Fed. Univ. Biol., 2017, 10(3), 333-342. DOI: 10.17516/1997-1389-0002.

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Интродукция микробиоценоза в агроэкосистемы для увеличения продуктивности растений

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Цель работы – изучение влияния полифункционального микробиоценоза (“Microbiovit” (MBV), микробное сообщество) на рост и развитие сельскохозяйственных растений, их продуктивность, а также на биологическую активность почвы. Применение микробиоценоза позволило увеличить урожайность сельскохозяйственных культур и сохранить плодородие почвы. Микробиоценоз способствовал увеличению продуктивности пшеницы, овощей и картофеля на 26–56 %. Срок созревания пшеницы сократился на 9 дней. Обработка клубней картофеля препаратом повышала их устойчивость к инфекционным агентам гнили, что увеличивало срок хранения продукции. Концентрация нитратов в корнеплодах (морковь и свекла), обработанных MBV, в процессе хранения снижалась. Микробиоценоз способствовал увеличению скорости минерализации органического вещества в почве. Эксперименты в лабораторных и полевых условиях показали, что микробиоценоз, интродуцированный в агрофитоценозы, повышает их продуктивность и активирует регуляторные, защитные и трофические функции.

Ключевые слова: полифункциональный микробиоценоз, микробные сообщества, биологическая активность почвы, продуктивность сельскохозяйственных растений.

Introduction

Control of agrophytocenoses productivity is a great challenge to solve the problem of providing food for the mankind. Chemical protection, mineral fertilizers and irrigation, used to control productivity of agricultural plants, considerably increase the crop yield. However, ecological monitoring demonstrates that long-term use of chemical preparations frequently leads to adverse implications. Species diversity and biological activity of soil biota are diminished, soil fertility reduced, pesticides residues and nitrates can accumulate in crop products (Maeder, 2002; Adesemoye and Kloepper, 2009). Indiscriminate use of synthetic fertilizers has led to the pollution and contamination of the soil, has polluted water basins, destroyed microorganisms, making the plants more prone to diseases and reduced soil fertility (Mishra et al., 2013).

Implementation of biological methods offers the mankind great opportunities to improve environmental safety of arable farming, to produce high quality food, and to maintain high yield of agricultural crop (Govedarica et al., 2002; Vissey, 2003; Adesemoye and Kloepper, 2009; Kravchenko et al., 2013). Useful microorganisms of biological preparations enhance growth of plants and protect them from pests and infections (Vissey, 2003; Morgan et al., 2005; Handbook of Microbial Biofertilizers, 2006).

Most biological preparations used in agriculture are highly specialized. They are monocultures of cellulosolythic, phosphate mobilizing microorganisms, nitrogen fixers, and antagonists of infectious agents in plants (Vissey, 2003; Barea et al., 2005; Morgan et al., 2005; Cordell et al., 2009; Patkowska and Konopinsky, 2014). Biopreparation including 2-4 microbial species are labor intensive. On artificial media some microorganism species lose their activity and useful properties. It makes the search for stable microbial communities a crucial task for agricultural biotechnology.

Microorganisms are essential components of all ecosystems, and they function as decomposers and as drivers of local and global nutrient cycles (Taylor and Krings, 2005; Bottjer, 2005; Hartmann et al., 2009). Evolution and adaptation processes of biotic turnover in nature give rise to microbiocenoses, which develop trophic and metabolic relations with plants (Mikheeva and Somova, 2011). Microbiocenosis-based polyfunctional biopreparation considerably increase opportunities to enhance productivity of plants, soil fertility, and production of safe food.

The aim of this work was to study the effect of polyfunctional microbiocenosis on growth and development of agricultural crops (wheat, potato, carrot, beet-root, cabbage, radish, cucumber, tomato), as well as to assess its effect on the soil biological activity.

Materials and methods

Microbiocenosis

Polyfunctional microbiocenosis 'Microbiovit' (MBV) (state registration – 068/002803, TU 9291-002-00517186-2003) has been formed from natural microbial association of koumiss (fermented mare's milk) and cellulosolythic bacteria strains of *Bacillus* (Mikheeva and Somova, 2011).

MBV microorganisms have been identified and indicator groups of microbes have been isolated. Dominant groups are bacteria of *Lactobacillus, Streptococcus, Rhodopseudomonas, Bacillus* genera, yeast of *Kluyveromyces, Saccharomyces, Torulopsis* genera (Mikheeva and Somova, 2011; Mikheeva and Somova, 2013). Nutrient media and optimum cultivation conditions providing stable reproduction of species composition and useful properties of microbiocenosis have been selected. The biological preparation retained stability of stimulating properties for 2 years, longer than other preparations (not more than 1 year) (Mikheeva and Somova, 2009). One milliliter of preparation contained 10^7–10^8 cells of microorganisms. Optimal MBV concentrations, exposure time, periods and processing methods for wheat, vegetables and potato have been established (see below).

Effect of microbiocenosis on germination and heterotrophic growth of seeds of wheat, bean, and vegetable cultures (laboratory tests)

Effect of MBV on heterotrophic growth of two spring wheat cultivars adapted to soil and climate conditions of Krasnoyarsk farming area – Krasnoyarskaya 83 and Angarida – was studied in the experiment. The experimental design for each variety was the following: 1) control – 100 seeds were soaked for 20 minutes in water; 2) treatment – 100 seeds were soaked for 20 minutes in aqueous solution of MBV containing 10^5 or 10^6 cells ml^{-1}. The seeds were germinated on moisturized absorbent paper at 20º–24 ºC in the dark for 7 days. The germination energy was evaluated on day 4 of the experiment by counting the number of germinated seeds. The number and length of roots were measured on day 7. The same method was used to evaluate germination energy of seeds of oat, barley, pea, soybean, radish, cucumber, and carrot.

Effect of microbiocenosis on growth and development of agricultural crop (field experiments)

Effect of MBV on growth and development of plants was studied in field experiments with the following crops: 1 – spring wheat (*Triticum aestivum* L.) cv. *Krasnoyarskaya-83* on dryland; 2 – potato (*Solanum tuberosum* L.) cv. *Adretta* on dryland; 3 – cucumber (*Cucumis sativus* L.) cv. *Novoselsky* on dryland; 4 and 5 – carrot (*Daucus carota* L.) cv. *Nantskaya frantsuzskaya uluchshennaya* on dryland and on irrigated land, respectively; 6 and 7 – beet (*Beta vulgaris* L.) cv. *Bordeaux Detroit* on dryland and on irrigated land, respectively; 8 – cabbage (*Brassica oleracea* L.) cv. *Podarok* on dryland; 9 – radish (*Raphanus sativus var. radicula* L.) cv. *Zhara* on irrigated land; 10 – tomato (*Solanum lycopersicum* L.) cv. *Raketa* on irrigated land.

The planted area of wheat, cucumber, potato, beet, carrot and cabbage was 200 m^2, while that of radish and tomato was 40 m^2 (both in control and treatment). The soil in experiments 1–8 and 10 was ordinary leached chernozem, in experiment 9 – sod-podzol, medium clay-loamy. The concentrated MBV was diluted in water with a ratio of 1:300. The soil was irrigated and the plants were sprayed with a preparation. The application dose of the solution was 300 liter ha^{-1}. The wheat was treated twice, when plants were at the seedling stage and at the start of the tillering stage. Vegetable crops were treated upon emergence of fully germinated sprouts and 15 days after it. Potato was treated three times: at the seedling stage, during budding and flowering.

At the end of the experiment for all crops the whole-plants dry weight was determined. Besides, influence of MBV on duration of stages of organogenesis for *Triticum aestivum* was studied over the entire vegetation period. The wheat crop was harvested during the last ten days of August. Prior to harvesting the number of productive footstalks and seed yield (the weight of 1000 grains and the number of grains per ear) were measured.

Storage of root crops

Root vegetables (potato, carrot and beet-root) before storage were treated by spraying with a concentrated solution of the MBV. The vegetables without treatment were used as control. The percentage of potato tubers with putrefactive damage was determined visually after 7 months of storage in a typical vegetable store (Dementieva, Vygonskoye, 1988; Borisov et al., 2003). Also, it was determined the content of nitrates in carrot and beet-root by Machigin (Piskunov, 2004; Soil sampling and methods of analysis, 2006).

Biological activity of soil

a) Cellulosolythic activity of soil

Effect of MBV on cellulosolythic activity of soil was evaluated in laboratory experiment. Standard cellulose 0.54 g filters were submerged 5-10 cm deep in pots containing 1.5 kg of ordinary chernozem. Control samples were irrigated with water; experimental samples were treated with the MBV in dilution 1:1000. Application rate of the solution of the biopreparation was equivalent to 1000 liter ha^{-1}. Incubation conditions: soil moisture content 60 %, temperature 18–25 ºC. The mass of preserved cellulose was evaluated after 46 days.

b) Mineralization of organic matter in the soil

Mineralization capacity of MBV was studied in ordinary chernozem samples with humus content 8.2 %, total nitrogen – 115 mg kg^{-1} of soil, nitrate nitrogen – 76 mg kg^{-1}, soluble forms of phosphorus – 15 mg 100 g^{-1} of soil, potassium – 12.4 mg 100 g^{-1} of soil, pH_{KCl} – 7.0. Soil samples were treated with MBV biopreparation at a dilution of 1:300. The solution was introduced in a volume equivalent to 300 liter ha^{-1}. For two months moisture content in the samples was maintained at 60 %. At the beginning and at the end of experiment nitrate nitrogen, P_2O_5, K_2O were determined by Machigin (Piskunov, 2004; Soil sampling and methods of analysis, 2006).

Statistical analysis of data

All experiments were performed in triplicate. Figures show the mean values and standard deviations. The Student's *t*-test was used to determine the significance of the differences between control and treatment (confidence level = 95 %).

Results and discussion

Effect of microbiocenosis on germination rate, heterotrophic growth of seeds of grain, bean, and vegetable cultures

Germination of wheat seeds treated with MBV was 100 %, while in the control 83 ± 2 %. Morphological parameters of wheat seedlings depended on the concentration of the applied product. MBV dose of 10^5 cells ml^{-1} induced a statistically significant increase in the average amount of roots from 4.2 to 5 compared with the control, without affecting their length. The higher concentration of MBV (10^6 cells ml^{-1}) significantly increased the average number of roots from 4.2 to 6, and their length from 8.1 cm to 9 cm compared with the control. Germination of seeds treated with MBV significantly increased by an average: for oat – 15 %, barley – 9 %, pea – 5 %, soybean – 16 %, radish – 7 %, cucumber – 12 %, carrot – 22 %.

We guess that the stimulating effect of a biological product under conditions of heterotrophic growth of seeds is associated with the participation of different groups of plant hormones (Costacurta and Vanderleyden, 1995; Badr and Vivanco, 2009).

Effect of microbiocenosis (MBV) on growth and development of agricultural crop

MBV effect on growth, development and productivity of wheat, potato and vegetable cultures was studied under field conditions. Introduction of microbiocenosis into soil and on vegetating plants significantly increased the dry matter of potato, carrot (on irrigated land), beet, cabbage, and tomato (Fig. 1). The increase of plants growth was considerable and depending on the plant species and water supply constituted 10–56 % (Fig. 1). Effect of microbiocenosis to a large extent depended on water supply for carrot and beet plants. In experiment without irrigation the increase due to biopreparation was 26–32 %, with irrigation – 39–56 % (Fig. 1).

Growth and development of wheat was monitored in the different stage of growth (Fig. 2). Shortening of phenological phases of development under the influence of MBV was marked. The blooming phase was 10 days earlier in MBV treated plants. Full ripeness of wheat was in the control in 90 days and in experiment in 81 days, i.e. wheat grain matured 9 days earlier. The number of productive footstalks and yield of wheat tended to increase after the treatment with MBV, but insignificantly (Table).

In recent years, a large number of different microbial preparations were produced, which carry out several functions providing an increase in crop yield. Effect of biological preparations on the growth and development of plants depends on many factors, such as species and varieties of the plants, composition and properties of the soil, agro-climatic conditions, etc. For this reason, we compared yields of some crops treated with MBV and other preparations in percentage terms.

Comparison of the influence of MBV and other biopreparations on yield of different agricultural crops showed the advantage of MBV

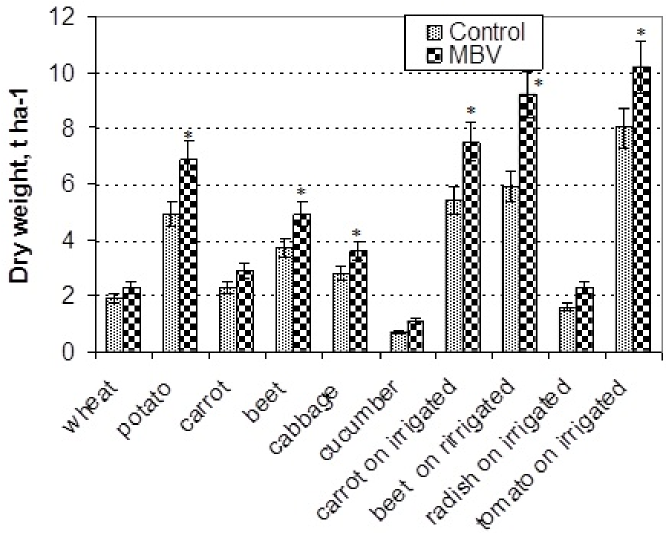

Fig. 1. Effect of 'Microbiovit' application on dry weight of wheat, potato, and vegetable cultures. Data are shown as the mean ± SD (n=3). Asterisks above bars indicate significant difference between treatment and control (Student's test, $p < 0.05$)

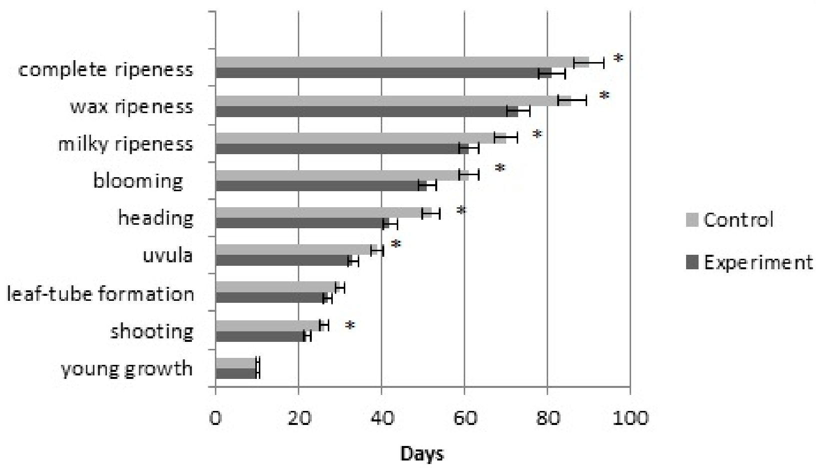

Fig. 2. Influence of 'Microbiovit' application on duration of stages of organogenesis of wheat (*Triticum aestivum*). Data are shown as the mean ± SD (n=3). Asterisks above bars indicate significant difference between treatment and control (Student's test, $p < 0.05$)

Table. Yield and yield structure of spring wheat (*Triticum aestivum*) in control and after treatment with polyfunctional biopreparation 'Microbiovit'

Data	Control	Treatment	*t*	*P*
The number of productive stems, pcs. m^{-2}	410 ± 23	502 ± 35	2.19	>0.05
The weight of 1000 grains, g	37.2 ± 1.5	36.8 ± 1.5	0.19	>0.05
The number of grains per ear of wheat, pcs.	18 ± 2	17 ± 2	0.35	>0.05
Yield, t ha^{-1}	2.68	3.14	-	-

application. For example, MBV treatment can increase wheat, potato, tomato, and carrot yield by 10-17 %, 40 %, 37 %, and 80 %, whereas yields of these crops processed with other preparations rose by 5-15 %, 18-42 %, 22 % and 25-31 %, respectively (Zavalin, 2005; Sokolova et al., 2008; Antypkina, 2015).

Storage of root crops

Storage of vegetables is an important task for the farmers. Microbiocenosis enhanced resistance of potato to infections during prolonged storage. The treated potato tubers had no infection (potato blight). In control 6–24 % of tubers exhibited pinpoint sites of this infection. Thus, the biopreparation inhibited growth and development of pathogenic microorganisms on potato during long storage.

In control samples of carrot and beet content of nitrates was 65 ± 3 μg g^{-1} w.w. and 88 ± 5 μg g^{-1} w.w., respectively. In samples of these vegetables processed with MBV nitrates were detected in trace amounts. It may be supposed, that the root crops transformed this nitrogen compound into organic substances more efficiently under the influence of MBV.

Soil biological activity

Being introduced in agrocenoses, MBV gets involved in complex processes of soil biota.

The biopreparation manifested its effect in increased indicators of biological activity of soil. Intensified mineralization of organic substances promoted optimization of nutrient conditions for plants. Degradation of cellulose in MBV treated soil was 90 % and in control it was 76 %. Nitrate nitrogen content in MBV treated soil increased by 80 %, labile phosphorus increased by 42 %, while the potassium content did not change significantly (Fig. 3). Moreover, it was shown earlier that intensity of carbon dioxide generation by soil treated with BMV increased 6-7.5 times (Mikheeva and Somova, 2009).

Nutrient cycling is fundamental not only for primary production but for the long-term functioning of ecosystems (Aon et al., 2001; Gaofei et al., 2010). Microorganisms play an important role in soil processes that determine plant productivity. Experiments under laboratory and field conditions showed that microbiocenosis introduced into agrophytocenoses increased productivity and demonstrated active regulatory, protective and trophic functions.

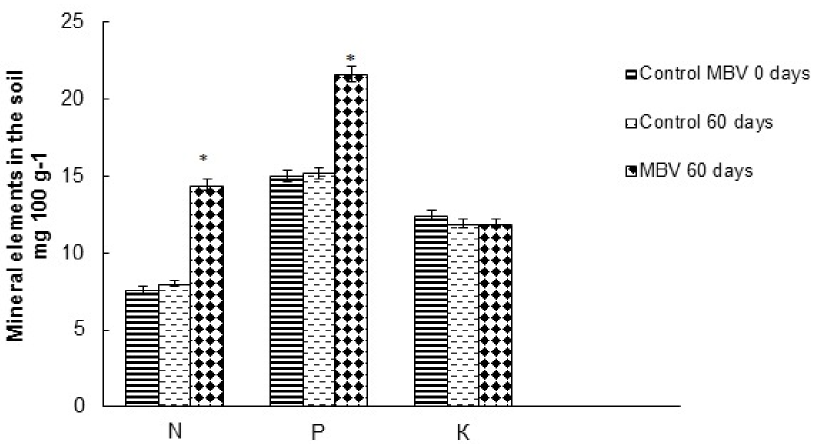

Fig. 3. The content of chemical elements in the soil treated with 'Microbiovit' and without treatment (control). Data are shown as the mean ± SD (n=3). Asterisks above bars indicate significant difference between treatment and control (Student's test, p < 0,05)

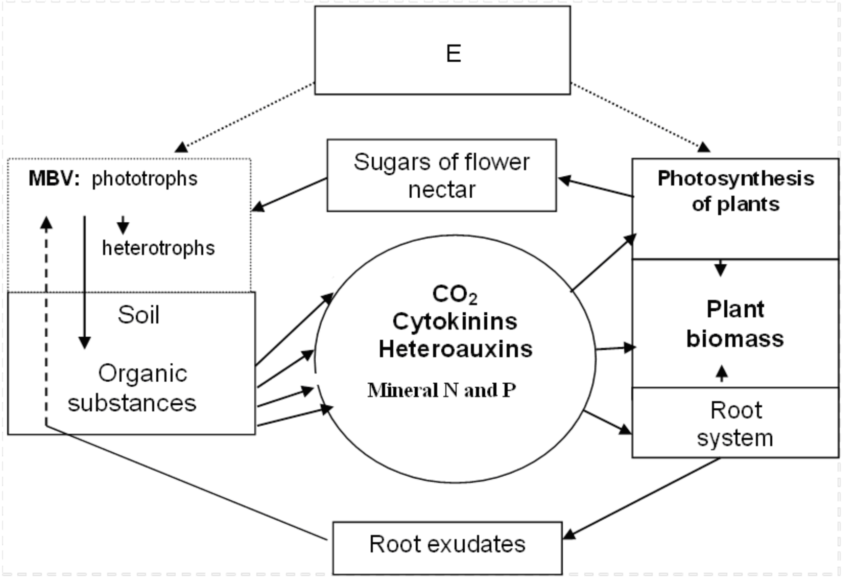

Fig. 4. Hypothetical interaction links in "plants – soil – MBV" agrophytocenoses. Denotations: E – light energy, N – nitrates, P – soluble forms of phosphates

Hypothetical interactions in agrophytocenoses "plant – soil – microbiocenosis" are shown in Fig. 4. The diagram shows a production of carbon dioxide in "soil" and the biosynthesis of cytokinins by introduced microbiocenoses, which contributes to the activation of photosynthesis in plants (Somova et al., 2003). The diagram shows that heteroauxins producing by polyfunctional microbiocenosis stimulate the growth and development of the root system. The exudates of roots and sugars of flower nectar supply nutrients for the microbial community and stimulate growth of MBV microorganisms and soil microflora in the root layer.

Photosynthesizing bacteria of *Rhodopseudomonas* genus use carbon dioxide, reduced forms of sulfur, the energy of light and heat to synthesize organic substances and sustain reproduction of heterotrophs of the microbiocenosis. Plant roots strongly influence C and N availability in the rhizosphere via rhizodeposition and uptake of nutrients (Butler et al., 2003). Mineralization of humus to soluble forms of nitrogen and phosphorus provide additional nutrition in the rhizosphere. On the whole stimulation of photosynthesis and growth of roots makes possible to considerably increase the plant biomass and plant productivity. Microorganisms' enzymes, despite their relatively low amounts, play a crucial role in keeping nutrient cycling in soils such as C, N, P, and S (Aon et al., 2001; Aon and Colaneri, 2001). Disclosed properties of polyfunctional microbiocenosis MBV make possible to attribute microorganisms contained in it to Plant Growth Promoting Rhizobacteria (PGPR) (Vissey, 2003).

Conclusion

Experiments under laboratory and field conditions showed that polyfunctional microbiocenosis introduced into agrophytocenoses demonstrates regulatory, protective and trophic functions:

- MBV introduced into soil and on plants increased productivity of wheat, vegetable cultures and potato. Effect of MBV on plants can be increased by maintaining optimum water supply for the agrocenoses.
- During storage root crops treated with MBV were resistant to infectious agents of rots. MBV substantially reduced concentration of nitrates in the vegetables.
- MBV accelerated mineralization of organic substances in soil to easily digested forms of nitrogen and phosphorus, and allowed to sustain soil fertility.

Acknowledgement

We thank the two anonymous reviewers whose comments and suggestions helped improve and clarify this manuscript.

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