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Use of the Phosphate-Solubilizing Bacterial Preparation Polymyxobacterin in Pond Aquaculture

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Abstract

The paper contains results of studies on the application of the bacterial preparation Polymyxobacterin in pond aquaculture for optimization of mineral phosphorus content in water, development of natural forage base, and increase of fish productivity. It was found that in aquatic environment, there are optimal conditions for growth of *Paenibacillus polymyxa* KB bacterium. Polymyxobacterin application contributes to an increase of mineral phosphorus in pond water by 1.3-3.7 times compared to the control and does not exceed the normative values (less than 0.5 mgP/dm^3) that in its turn stimulates phytoplankton vegetation and zooplankton development. After Polymyxobacterin application, due to development of natural forage organisms, fish productivity of nursery and fattening ponds exceeded the control variant by 3.9 and 6.3% for common carp; by 34.1 and 16.7% for silver carp; and by 5.5 and 9.6 for grass carp, respectively

Keywords: Polymyxobacterin, pond aquaculture, aquatic environment.

Introduction

An important component of intensification measures in aquaculture is fertilization of ponds with mineral and organic fertilizers (Green *et al*., 1989; Debeljak *et al*., 1990; Das and Jana 1996; Garg and Bhatnagar, 2000; Das *et al*., 2005) that significantly satisfies the requirements of aquatic organisms in biogenic elements; however, at the same time, some ballast substances (e.g., fluorine and cadmium), which are contained in some superphosphate fertilizers, can be accumulated in water bodies (Velikiy and Mudriy, 1999; Mikanova and Novakova, 2002) that results in deterioration of the state of aquatic ecosystems (Naumov, 2005).

A promising trend in aquaculture development is implementation of new measures for increasing productivity of ponds taking into account the cost-effectiveness and preservation of the environmental balance of water bodies (Vovk and Bazaeva, 2009, Stolovych *et al*., 2003; Khizhnyak and Tsion, 2009). Demand for production of organic agriculture is growing all over the world. Environmentally safe intensification measures for agricultural production are conducted in EU countries, USA, Canada, Japan, India, and South-East Asia, where fish farmers actively use microorganism-based biological fertilizers (Tereshchenko, 2004).

In Ukraine, there is an industry of environmentally safe bacterial preparations based on nitrogen-fixing (Rizobofit, Rizogumin, Diazofit, Diazobacterin, Azotobacterin) and phosphate-solubilizing (Polymyxobacterin, Albobacterin, Agrobacterin, Fosfoenterin) bacteria (Tokmakova and Bliznyuk, 2004; Chaikovs'ka *et al*., 2004; Volkogon *et al*., 2007). Application of such preparations does not result in accumulation of mineral compounds in agricultural products (Volkogon, 2007) that is a necessary condition of their organic origin. An increase in the number of potential consumers of ecologically clean products accordingly widens the field of application of biological fertilizers, in particular the bacterial ones.

The aim of this study was to determine the effect of introduced phosphate-solubilizing bacteria *Paenibacillus polymyxa KB*, which constituted the base of the bacterial fertilizer Polymyxobacterin, on aquatic ecosystems. The preparation was developed at the Institute of Agricultural Microbiology of the NAAS of Ukraine (Chernihiv) in 1997 and was intended for application in agriculture as a biological fertilizer (Patent 2035507; Patent UA 20206). Polymyxobacterin contains *P. polymyxa KB* bacteria (gram-positive bacilli, 1.1-1.3 × 3.0-5.0 µm, isolated from soil on the potato medium №3) at a concentration of 5.0×10^9 cells/g of dry mixture. The mechanism of action of this preparation is based in the ability of phosphate-solubilizing bacteria to produce organic acids and enzymes that contributes to dissolution of poorly soluble phosphoric compounds and to conversion them into soluble inorganic phosphates (Jana, 2007) thereby increasing their availability for phytoplankton, which is the basic component of the aquatic food chain. *P. polymyxa KB* bacteria are not pathogenic for warm-blooded animals (Filatova, 1994) and fish (Vovk and Tokmakova, 1997).

Materials and Methods

The study was conducted during 2006-2010 in two stages: model experiments under laboratory conditions and experiments under fish farm conditions.

Stage I. Dynamics of *P. polymyxa KB* count in pond water in model experiments was determined at temperatures of 18.0-21.0ºC and 10.5-15.5ºC, pH – 7.0-8.3. A 30 L tank was filled with pond water, where the phosphate-solubilizing bacteria were introduced in concentrations of $(4.50\pm0.50[SE]) \times 10^2 - (5.63\pm0.46) \times 10^3$ cfu/ml, except the control. The exposure was for 35 days. Viable count of *P. polymyxa KB* bacteria in experimental containers was determined every 5 days by serial dilution method (Steiner 1979) with further inoculation of respective dilutions in sterile Petri dishes, which contained meat-peptone medium and potato agar, at three replications. Incubation was conducted in an incubator at 28ºC.

Effects of *P. polymyxa KB* on fish were determined under laboratory conditions by biological test (Bauer *et al*., 1981). The experiments were conducted in 40 L tanks at water temperatures of 18.0-20.5^{o}C, pH – 7.5. Common carp, *Cyprinus carpio* L., and silver carp, *Hypophthalmichthys molitrix* (Valenciennes), larvae were kept in water with Polymyxobacterin at concentrations of $(2.36\pm0.17) \times 10^4 - (5.01\pm0.15) \times 10^6$ cfu/ml (0.01; 0.1; 1.0 ml/L of the native preparation) that corresponded to 15, 150, and 1500 L/ha, respectively. To see if Polymyxobacterin can have effects on fish health, yearlings of common carp were intraperitoneally injected with $(4.83\pm0.17) \times 10^5$, $(5.10\pm0.15) \times 10^6$, and $(1.23\pm0.22) \times 10^7$ cfu/ml of *P. polymyxa KB* bacteria (Bauer *et al*., 1981). In the control, the fish were injected with 1 ml of sterile saline solution. Exposure was for 20 days. During the experiment, the fish were observed for their behavior and health by an ichthyopathologist to see if they changed after the injections. The laboratory experiments were conducted from May to September of 2006.

Stage II. Experiments at aquaculture facilities of the JSC "Chernihivrybgosp" (site 1) were conducted in three earthen nursery ponds of 8, 10, and 13 ha area with depths from 1.2 to 1.5 m. Polymyxobacterin was introduced into the experimental ponds on the floor 3 days before filling (8 ha pond) or on the water surface 3 days after filling (10 ha pond) at a rate of 1 L of the native preparation per 1 ha of the floor or water surface that constituted $0.5\text{-}7.5\times10^2$ cfu/ml of *P. polymyxa KB* in 1 L of water. The preparation was applied using a spraying device. The 13 ha pond without Polymyxobacterin application was used as the control. Three-day old larvae of common carp, silver carp, and grass carp, *Ctenopharyngodon idella* (Valenciennes) were stocked in the ponds for their raising in polyculture. The common carp stocking density was 50×10^3 fish/ha; silver carp – 4×10^3 fish/ha; grass carp – 9×10^3 fish/ha. During the culture season, all cultured fish were fed with a standard premixed feed for carps with protein content not less than 26% (KP-R-AG24, Dneprovsky feed factory, Ukraine). This experiment was conducted in triplicate from May to October (2007, 2008, 2009).

For experiments at the fish farm "Nyvka" (site 2), 4 ponds of 0.05 ha area with depths from 0.5 to 1.5 m were stocked with yearlings of common, silver, and grass carps. The stocking density for common carp (mean body weight of 30.2±1.7 g) was 1000 fish/ha; silver carp (46.4±2.1 g) – 200 fish/ha; grass carp (55.2±1.1 g) – 400 fish/ha. Polymyxobacterin was applied on the water surface 3 days after filing at a rate of 1 L of the native preparation per 1 ha of the water surface that constituted $0.5\text{-}7.5\times10^2$ cfu/ml of *P. polymyxa KB* in 1 L of water and in a complex with ammonified superphosphate (9% of the active substance) at a rate of 5 L/ha (3.7×10^3 cfu/ml). The required quantity of superphosphate (for achieving the optimum P concentration of 0.5 mg/dm^3) was determined based on water chemical tests by reducing it two-fold according to recommendations on effective application of microbial preparations combined with traditional fertilizers (Volkogon, 2007). The first pond was fertilized with Polymyxobacterin, the second with Polymyxobacterin + superphosphate, the third with superphosphate only, the forth was used as a control. This experiment was conducted in duplicate from May to October (2009, 2010).

Following hydrochemical measurements were taken: water temperature, dissolved oxygen (DO), pH, water hardness, carbonates, sulfates, chlorides, phosphorus, nitrogen, calcium, magnesium, potassium, sodium (Alekin, 1970; Bessonov and Privezentsev, 1987). Hydrochemical samples were taken every week at five sampling sites for each pond during the culture periods. Water temperature, DO, and pH values were recorded on the spot using oxygen meter HI 3810 and pH-meter SX 620, ULAB. Other hydrochemical parameters were examined following the standard methods (Alekin, 1970; Bessonov and Privezentsev, 1987; APHA, 1998). Water quality values were presented as an annual average values.

Hydrobiological samples, which included bacteria, phytoplankton, and zooplankton, were taken for qualitative and quantitative analysis 2-3 times per month during the culture period (June-August). Dynamics of phosphate-solubilizing bacteria in water and bottom sediments of the experimental ponds was examined according to standard microbiological techniques (Labinskata, 1978) using Muromtsev's medium (Steiner, 1979). Phytoplankton samples (0.5 L) were taken by the sedimentation technique. Zooplankton samples were taken by filtering 50 L of pond water through an Apstein plankton net (Salazkin 1984; Arsan *et al*., 2006). Fish productivity was determined using methods recommended by Sherman and Tovstyk (2004). At the fish farm "Nyvka", it was possible to determine fish productivity only for the ponds fertilized with Polymyxobacterin and for control ponds.

All results were expressed as mean ± S.E., if applicable, and they were statistically analyzed by one-way ANOVA. If the main effect was found significant, the ANOVA was followed by a LSD (least significant difference) test. All statistical tests were considered significant at 5% probability level. The statistical analysis was performed using Statistica 6.0 package.

Results

In model tests in laboratory conditions, it was found that aquatic environment has necessary conditions for development of phosphate-solubilizing bacteria *P. polymyxa KB*, and temperature had direct effect of their count. At water temperature of 18.0-21.0°C, bacterial development occurred more actively than at 10.5-15.5°C ($P<0.05$).

At the initial bacterial count of $(4.50\pm0.50) \times 10^2$ and $(4.86\pm0.82) \times 10^2$ cfu/ml, their maximum count at temperatures of 18.0-21.0°C was observed in 18 days and composed $(6.33\pm1.20) \times 10^8$ cfu/ml. At temperatures of 10.5-15.5°C, the bacterial count attained $(9.68\pm0.14) \times 10^4$ cfu/ml on the Day 17 and gradually decreased later (Figure 1).

Variation of pH values within 7.0-8.3 did not have any effect on the development of phosphate-solubilizing bacteria *P. polymyxa KB* ($P>0.05$).

After exposing larvae of common and silver carps in the pond water with *P. polymyxa KB* at concentrations of $(2.36\pm0.17) \times 10^4$ and $(5.01\pm0.15) \times 10^2$ cfu/ml, the mortality rate in both experiment and control was 7.5-12.5% that did not exceed the allowable norms of natural mortality 20-25% according to Luzhin (1976).

Intraperitoneal injections of *P. polymyxa KB* at concentrations of $(4.83\pm0.17) \times 10^5$ to $(1.23\pm0.22) \times 10^7$ cfu/ml did not cause clinical signs of diseases or pathological changes of internal organs that was one of conditions for the application of Polymyxobacterin in aquaculture.

Regardless of the method of application of Polymyxobacterin in fish ponds (on the floor or water surface), concentrations of major chemical elements were usually below maximum allowable values indicated in the Standard of Ukraine (SOU 05.01-37-385:2006. Water for fish farms. General requirements and norms). During the study period, DO values in the experiment were 5.4-7.2 mgO_2/dm^3 and 5.6-7.3 mgO_2/dm^3 in the control that was in the range of normal values for fish ponds (5.0-7.0 mgO_2/dm^3). The pH values in the experiment were in the range of 6.5-7.7. The content of free ammonium (NH_3) in water did not exceed the maximum allowable level (0.05 mgN/dm^3). Total iron concentrations (Fe^{2+} and Fe^{3+}) in fish ponds after Polymyxobacterin application were in the range of 0.63-0.81 mg Fe/dm^3 and 0.75-0.79 mg Fe/dm^3 in control ponds (maximum allowable level is 1.0 $mgFe/dm^3$). Magnesium, sodium, and potassium ions as well as sulfates and chlorides were at about the same level before and after the experiment and they were: Mg^{2+} – 12.2 mg/dm^3; $Na^+ + K^+$ (combined value) – 2.8 mg/dm^3; SO_4^{2-} – 10.7 mg/dm^3, Cl^- – 16.2-

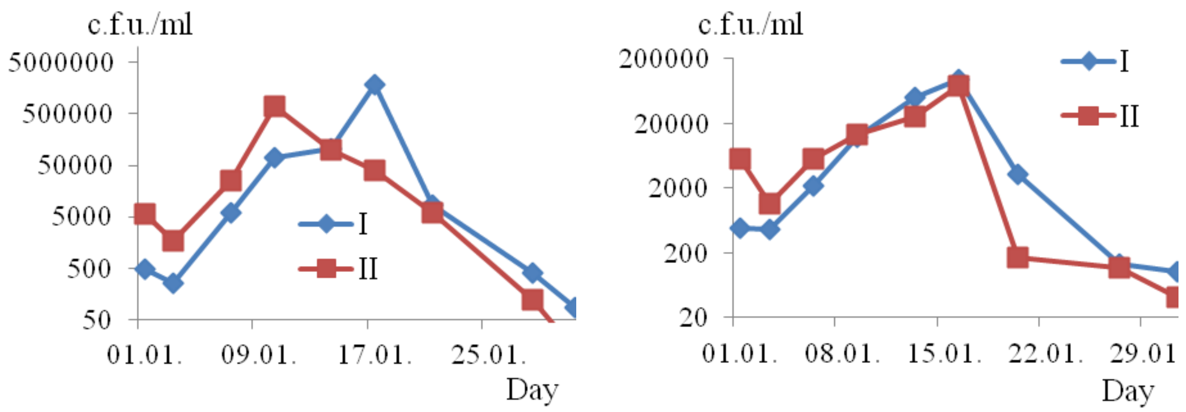

Figure 1. Dynamics of *P. polymyxa KB* count in pond water at temperatures of 18.0-21.0ºC (A) and 10.5-15.5ºC (B), (mean±SE, n=3)
Note: Initial *P. polymyxa KB* count: A – (4.5 ± 0.50) and $(4.9\pm0.82) \times 10^2$ cfu/ml; B – $(2.7\pm0.38) \times 10^3$ cfu/ml.

17.5 mg/dm^3. As for calcium ions, their content in water before Polymyxobacterin application was 80.2 mg/dm^3 and exceeded the normal value (50-65 mg/dm^3) that is common for Polissya zone of aquaculture. Later on, its content gradually dropped to 48.1-52.1 mg/dm^3 in the experimental ponds and 46.1-60.1 mg/dm^3 in the control ponds. Concentrations of nitrates and nitrites in water of experimental ponds were within normal values (0.1 and 2.0 mg N/dm^3). Their content was 0.02-0.03 and 0.05-0.06 mg N/dm^3 in the experiment, respectively, and 0.03 and 0.07-0.09 mg N/dm^3 in the control, respectively. At the same time, an increase of mineral phosphorus was observed in water in experimental variants (Figure 2).

After introducing Polymyxobacterin into experimental ponds of the fish farm JSC "Chernihivrybgosp" on the floor and the water surface at a concentration of 1.0 L/ha with an initial mineral phosphorus content of 0.18±0.02 and 0.19±0.006 mgP/dm^3, respectively in water, a gradual increase of its concentration was observed. The maximum content of mineral phosphorus in water after introducing the bacterial fertilizer on the floor was registered in the second week of June (0.48±0.007 mg P/dm^3); on the water surface – at the end of June (0.44±0.005 mgP/dm^3), exceeding the control values by 1.3-1,6 and 1.3-2.3 times ($P<0.05$), respectively, while the maximum allowable value is 0.5 mgP/dm^3.

After introducing Polymyxobacterin (1.0 L/ha) into fattening ponds of the "Nyvka" with initial mineral phosphorus content of 0.24±0.005 mgP/dm^3 in water, its concentration increased sharply up to 0.33±0.004 mg P/dm^3; while in the control it increased up to 0.25±0.011 mgP/dm^3 ($p<0.05$). After introducing bacterial fertilizer at a concentration of 5.0 L/ha ("Nyvka"), the mineral phosphorus content in water also increased up to 0.58±0.013 mgP/dm^3 versus 0.32±0.08 mgP/dm^3 in the control ($P<0.05$).

After applying Polymyxobacterin, the mineral phosphorus content in water gradually increased and remained at a sufficiently high level (0.44±0.005 mg P/dm^3) during the culture season as compared with a short-term (2-3 days) increase of its content after introducing of superphosphate.

A study of a possibility of a complex application of bacterial and mineral fertilizer superphosphate showed that from an initial phosphorus content in water of 0.19±0.006 mgP/dm^3, its concentrations increased rapidly up to 0.37±0.04 mgP/dm^3 and up to 0.30±0.02 mgP/dm^3 immediately after repeated introduction of superphosphate (Figure 3). After application of Polymyxobacterin in fish ponds, the salt composition (Ca, Mg, K, SO_4 ions, hydrocarbonates, chlorides); the content of organic substances (according to the permanganate and dichromate values), and pH met the Ukrainian requirements for fish ponds.

An increase of *P. polymyxa KB* count after Polymyxobacterin application was observed in water and bottom sediments. After introducing of the bacteria into fish ponds at concentrations of 5.0 × 10^2 cfu/ml, their number in aquatic environment increased up to (1.7±0.12) × 10^4 cfu/ml; in bottom sediments up to (0.9±0.69) × 10^6 cfu/ml; that resulted in an increase of phosphate-solubilizing bacteria by 1.4-4.5 and 1.8-2.6 times, respectively, versus the control ($P<0.05$).

Phytoplankton: No significant differences in taxonomical structure of phytoplankton were found in the experimental and control ponds.

In the test with Polymyxobacterin application

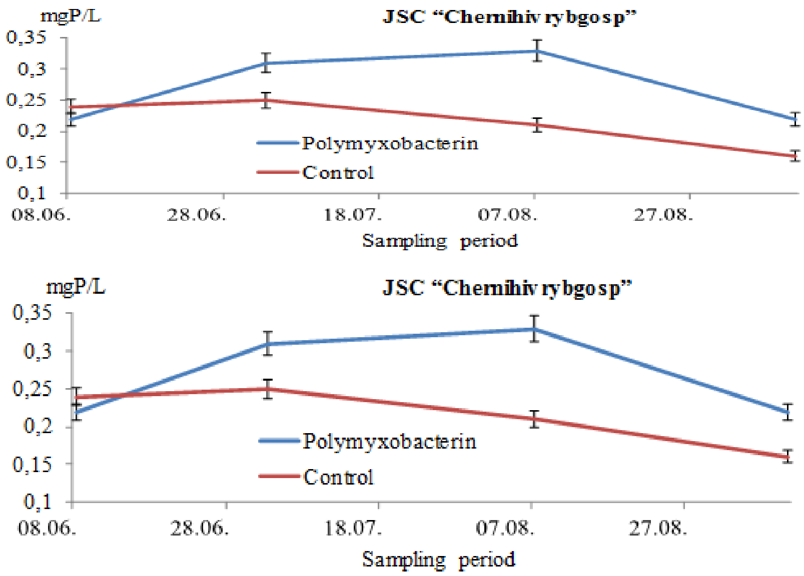

Figure 2. Dynamics of mineral phosphorus content in water of experimental ponds after introducing Polymyxobacterin on the floor (A) and water surface (B), (mean±SE, n=5)

(JSC "Chernihivrybgosp") during the culture period, the phytoplankton biomass was in the range of 9.80-22.63 mg/dm^3 and 7.17-8.42 mg/dm^3 in the control. The average seasonal phytoplankton biomass in the experimental variant was 14.34 mg/dm^3 that exceeded the control (7.94 mg/dm^3) by 1.8 times ($P<0.05$) (Figure 4).

In experimental ponds of the fish farm "Nyvka" during the culture period, phytoplankton biomass fluctuated in the range 1.25-6.44 mg/dm^3 after applying the bacterial fertilizer and 0.60-4.12 mg/dm^3 in the control ($P<0.05$). An increase of phytoplankton biomass by 2.2 times as compared with the control was also observed in the case of combined application of bacterial and mineral fertilizers.

Zooplankton: No significant difference in zooplankton species composition was observed during the culture period in the experimental and control ponds.

The average seasonal zooplankton biomass after introducing bacterial fertilizer (JSC "Chernihivrybgosp") was 1.247 g/m^3 that exceeded the control (0.305 g/m^3) by 4.1 times ($P<0.05$) mainly due to active development of rotifers (Figure 5). When applying Polymyxobacterin in the experimental ponds of the fish farm "Nyvka", zooplankton biomass was in the range of 0.315-5.766 g/m^3, and 0.918-1.592 g/m^3 in the control. The average seasonal zooplankton biomass in the experimental ponds was 3.198 g/m^3 that exceeded the control (1.337 g/m^3) by 2.4 times ($P<0.05$).

Fish Productivity

After using the bacterial fertilizer Polymyxobacterin in fish ponds, an increase in their fish productivity was observed (Figures 6 and 7).

Common carp productivity of the experimental fattening ponds (JSC "Chernihivrybgosp") (1191 kg/ha) exceeded that of the control (1146 kg/ha) by 3.9%. That was probably associated with more active development of zooplankton at the beginning of the culture period. Silver carp productivity in the experimental ponds was 220 kg/ha exceeding that of the control (164 kg/ha) by 34.1%. An increase of fish

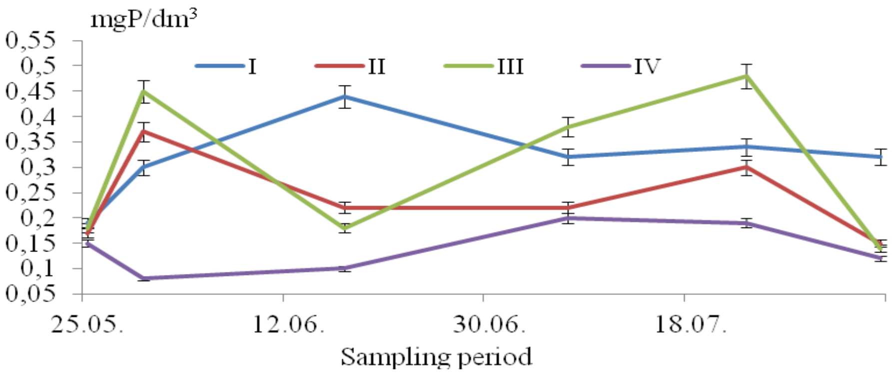

Figure 3. Dynamics of mineral phosphorus content in water of experimental ponds after introducing Polymyxobacterin with superphosphate, (mean±SE, n=5) [I – Polymyxobacterin; II – Polymyxobacterin + superphosphate; III – superphosphate; IV – control ($P<0.001$)]

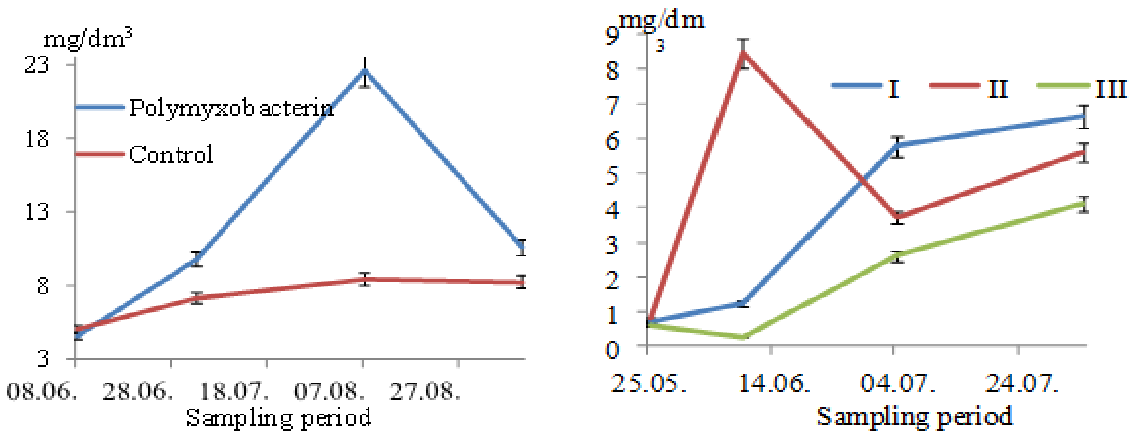

Figure 4. Dynamics of phytoplankton biomass in experimental ponds of the JSC "Chernihivrybgosp" (A) and "Nyvka" (B) (I – Polymyxobacterin; II – Polymyxobacterin + superphosphate; III – control)

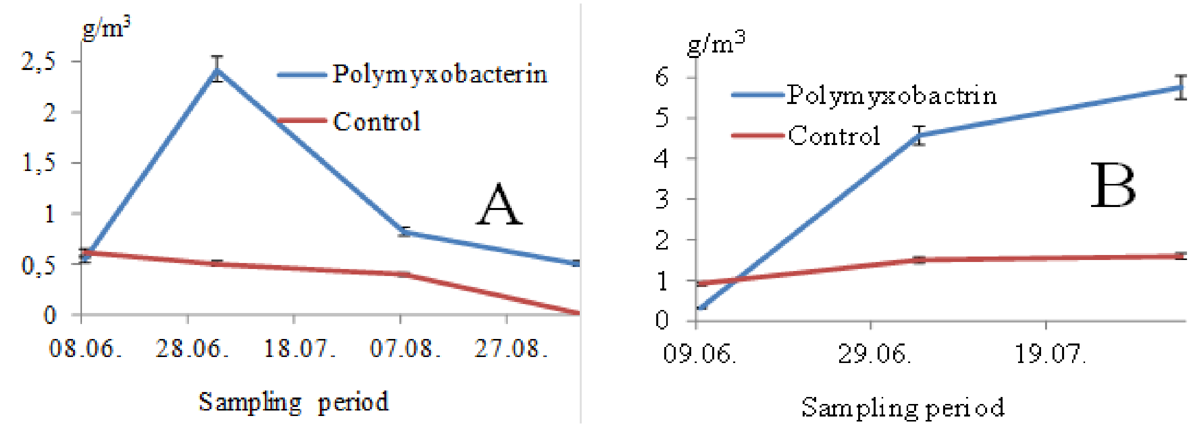

Figure 5. Dynamics of zooplankton biomass in experimental ponds of the JSC "Chernihivrybgosp" (A) and "Nyvka" (B)

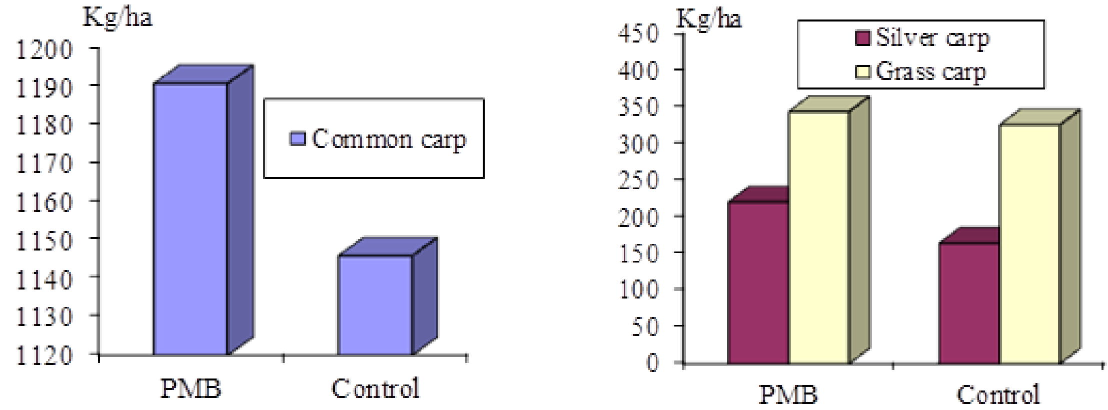

Figure 6. Common, silver, and grass carp productivity in experimental ponds (JSC "Chernihivrybgosp"), (mean±SE, n=100) (PMB = Polymyxobacterin)

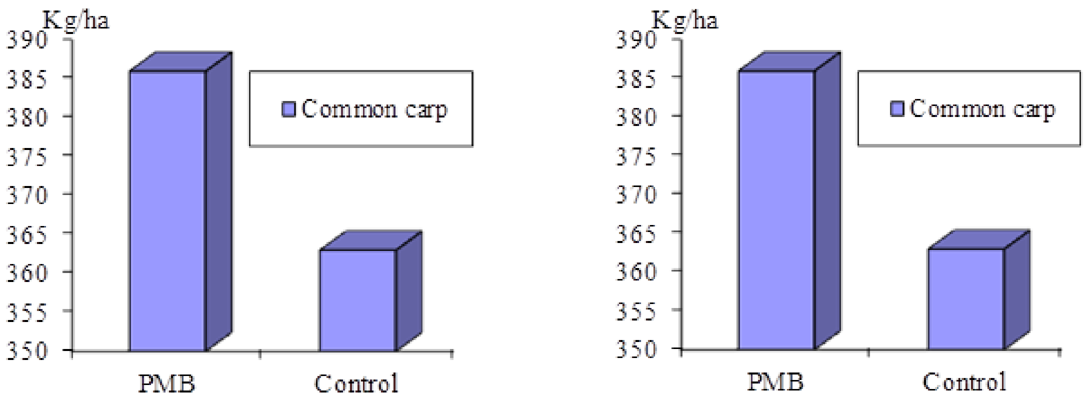

Figure 7. Common, silver, and grass carp productivity in experimental ponds ("Nyvka"), (mean±SE, n=20) (PMB = Polymyxobacterin)

productivity by 5.5% after applying Polymyxobacterin was also noted for grass carp (343 kg/ha in the experimental ponds; 325 kg/ha in the control). Thus, the total fish productivity after using bacterial fertilizer was 1754 kg/ha that exceeded that of the control (1635 kg/ha) by 7.3%.

Common carp productivity in the experimental ponds of the fish farm "Nyvka" (Figure 7) was 386 kg/ha that exceeded the control (363 kg/ha) by 6.3%; silver carp productivity was 91 kg/ha that exceeded the control (78 kg/ha) by 16.7%; grass carp productivity was 171 kg/ha that exceeded the control (156 kg/ha) by 9.6%. The total fish productivity after applying Polymyxobacterin was 648 kg/ha exceeding the control (597 kg/ha) by 8.5%.

Cost efficiency of Polymyxobacterin Application

The cost of Polymyxobacterin under the condition of its introduction into fish ponds at a rate of 1-5 L/ha for increasing the mineral phosphorus in water up to the optimal value (0.50 mg P/dm^3) were lower by 25.0-34.7% as compared with application of the traditional mineral fertilizer superphosphate.

Discussion

The results obtained indicate the possibility of application of the phosphate-solubilizing bacterial fertilizer Polymyxobacterin in pond aquaculture as a way of intensification. In model experiments, it was found that aquatic environment is quite favorable for *P. polymyxa KB* development and temperature has a direct effect on their count. The more the water temperature approaches the optimum (28^{o}C) for these bacteria, the more active their development is. At the same time, pH values in the range of 7.0-8.3 did not have any effect on *P. polymyxa KB* count. In their turn, the bacteria did not cause significant changes in active reaction of water, which was at the optimum level for fish rearing (6.4-7.5). Due to aerobic nature of *P. polymyxa KB*, somewhat lower DO values were observed in the experiment in comparison with those in the control, however this difference was not statistically significant (P>0.05). When developing actively in aquatic medium, phosphate-solubilizing *P. polymyxa KB* did not affect fish organisms that were a necessary precondition for their application in fish culture.

An increase of phosphate-solubilizing bacteria in water of fish ponds results in corresponding increase of concentrations of their metabolites (organic acids and enzymes), which in turn contributes to solubilization of poorly soluble phosphates increasing the concentration of mineral phosphorus accessible by hydrobionts. It was found that after Polymyxobacterin application, the mineral phosphorus content in water of fish ponds gradually increased and remained at sufficiently high level (0.44±0.0005 mgP/dm^3) during the culture period in contrast to its short-term increase after superphosphate application (from 0.48±0.010 to 0.18±0.06 mgP/dm^3 in 2-3 days).

Experiments with combined application of bacterial and mineral fertilizers showed that the mineral phosphorus concentration rapidly increased immediately after repeated application of superphosphate. At the same time, in this variant, the content of mineral phosphorus decreased slower than that after application of superphosphate alone, which was probably related to mobilization of its phosphoric compounds by *P. polymyxa KB* bacteria.

It is necessary to note that after introducing bacterial fertilizer on the floor, the mineral phosphorus content in aquatic environment increased more rapidly (15-17 days) than after introducing on the water surface (25-30 days) that should be taken into account when selecting the method of the bacterial fertilizer application.

Irrespective of the category of fish ponds, more active phytoplankton development mainly of green algae (Chlorophyta), which predominate in the silver carp diet, was observed after Polymyxobacterin application. Green algae in experimental ponds in average composed from 69.0% (“Nyvka”) to 89.6% (JSC “Chernihivrybgosp”) of the total phytoplankton biomass. Thus, the average seasonal phytoplankton biomass in experimental variants was 4.17 mg/dm^3 (“Nyvka”) and 14.34 mg/dm^3 (JSC “Chernihivrybgosp”) exceeding the control by 1.8 times. Following combined application of the bacterial and mineral fertilizers, an increase of phytoplankton biomass was observed immediately after superphosphate application, it being 8.48 mg/dm^3. This value reduced later (3.72 mg/dm^3) until next application of the mineral fertilizer, following which it increased again up to 4.12 mg/dm^3.

In contrast to combined application of Polymyxobacterin and superphosphate, in the pond, where the bacterial fertilizer was used alone, phytoplankton biomass increased gradually, with a delay of 15-20 days after mineral phosphorus content increased in water. The maximum phytoplankton biomass (6.44 mg/dm^3) in this variant was observed during summer (June-July) after an increase of water temperature up to 26.0^{o}C and active development of phosphate-solubilizing bacteria, which contributed to an increase of mineral phosphorus content in water.

Irrespective of the way of application of Polymyxobacterin in fish ponds (on the pond floor or water surface), no mass development of cyanobacteria, which causes “algal blooms”, was observed.

In its turn, active vegetation of planktonic algae contributed to an increase of zooplankton biomass. This increase in nursery ponds was mainly due to active development of rotifers (Rotatoria), which are the necessary food organisms for fish juveniles of different species at early stages. It is necessary to note that zooplankton development in experimental ponds was similar after Polymyxobacterin application on both the pond floor and water surface.

Similar results were obtained in studies of other bacterial preparations, Azotobacterin (*Azotobacter* culture) and AMB (complex B microflora), on microbiological processes in water bodies. Application of Azotobacterin in fish ponds had effects on *Azotobacter* count both in water and vegetation, which serves as a substrate for bacterial development. While in control ponds, the *Azotobacter* count in the zone of plant fertilizers did not exceed 1000 cells/ml; in ponds, where Azotobacterin was introduced, *Azotobacter* count reached 10-100×10^3 cells/ml (Rodina, 1954) Application of *Azotobacter* (including its phosphate-solubilizing strain) in fish ponds alone and in combination with inorganic fertilizers enhanced the rate of phosphate solubilization and nitrogen fixation that resulted in significant increase of pond productivity and fish biomass in experiments in India (Garg *et al*., 1998; Garg and Bhatnagar,, 1999; Garg and Bhatnagar, 2002). In other studies carried out in India other bacteria such as *Bacillus subtilis* and *Bacillus polymyxa* showed promising results for enhancement of rock phosphate solubilization with the goal of reducing the fish production costs (Sahu and Jana, 2000). Such bacteria can be used as an ecofriendly low-cost aqua-biofertilizer for sustainable aquaculture (Tripathy and Ayyappan, 2005).

Thus, according to our results and literature data on positive effect of bacterial fertilizers on the development of phyto- and zooplankton, this method of intensification with the use of Polymyxobacterin seems to be advisable. The studies conducted in conditions of fish ponds showed an increase of fish productivity irrespective of the technology of raising of fish. It was found that application of Polymyxobacterin in fish ponds when raising multiple-age common carp in polyculture with silver and grass carps, had a positive effect on their growth. The highest weight and fish productivity in the experimental ponds were obtained for silver carp due to active phytoplankton development (mainly green algae).

Introduction of Polymyxobacterin into fish ponds enables significant reduction in the application of phosphate fertilizers, thereby resulting in reduction of pollution and improvement of ecological state of these water bodies, increase in their productivity and production of organic fish as well as cutting down the costs of fish growing.

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