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The effect of mineral fertilizers on reproduction of soil saprophytic bacteria

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Abstract. The study of vital activity regulation of soil microorganisms is one of the general problems of soil microbiology. One of the factors influencing the existence and reproduction of bacteria in terrestrial ecosystems are fertilizers introduced into the soil. The effect of mineral fertilizers on the reproduction of bacterial complexes in soils of diverse types at different temperatures (4 ° C and 20 ° C) was studied. Mineral fertilizing promotes the active reproduction of saprophytic bacteria in calcaric cambisol (CCS) and distric cambisol soils (DCS). The variants with fertilizers can be arranged according to their effect on the reproduction of saprophytic bacteria in following order, regardless of the soil type: soil with nitric fertilizer > soil with phosphoric fertilizers > soil with potash fertilizer. Introduction of nitric or phosphoric fertilizers at 20 °C was more successful in CCS. It was found that application of mineral fertilizers positively affects the conservation and reproduction of bacteria in soils of diverse types. Prolonged application of mineral fertilizers reveals a positive effect on bacterial complexes, regardless of the soil type.

1. Introduction

Soil is a multifactor system containing numerous different microorganisms interlinked with each other, which complicates the study of individual components of microbial ecosystems. Thus, the study of vital activity regulation of soil microorganisms is one of the general problems of soil microbiology. The existence of microorganisms in a natural area is determined by other organisms and environmental conditions [1-4]. One of the factors influencing the existence and reproduction of bacteria in terrestrial ecosystems is fertilizers introduced into the soil. The intensive chemicalisation of agriculture with the growing implementation of large volumes of mineral and organic fertilizers increases the complex anthropogenic load on the soil.

The influence of mineral fertilizers on soil biological activity is widely studied in the literature. It is considered [5] that intensive implementation of fertilizers changes microbial communities in the soil and impacts the reproduction and activity of soil microflora.

Some authors have noted a significant increase in biogenesis and intensity of microbiological processes in soil with the introduction of manure, basic types of mineral fertilizers (NPK) and lime [2,6-10]. The literature data on the influence of fertilizers on soil microflora are controversial. Some authors believe that the implementation of mineral fertilizers increases soil biogenesis [11] and others note the neutral [12] or suppressive effect on soil bacteria [13]. There is an opinion that the decrease in

the number of microorganisms with prolonged use of mineral fertilizers occurs due to reduction of the number of asporous bacteria and actinomycetes [14].

In this work, we consider the effect of mineral fertilizers on the reproduction of soil saprophytic bacteria in diverse types of soil (different in their physicochemical properties) at different temperatures (4 ° C and 20 ° C).

2. Materials and methods

The saprophytic bacteria were isolated from natural microbial associations of distric cambisol (DCS) and calcaric cambisol soils (CCS) of Southern Far East Russia. Microorganisms were isolated and cultivated for experiments on Nutrient Agar with 1% peptone (Microgen, Russia). To compare the effect of mineral fertilizers on the growth of microorganisms, the bacteria were cultivated on meat-peptonic (MPA) and vegetable agar (VA) [3].

In total, 20 strains of microorganisms with different cultural and biochemical properties have been isolated from the soil and used in the experiments. According to Bergey's Manual of Systematic Bacteriology (1997) and API-tests (Analytical Profile Index; bioMerieux, France), the following genera have been identified: *Agrobacterium*, *Acinetobacter*, *Aeromonas*, *Micrococcus*, *Pseudomonas*, *Flavobacterium* and *Bacillus.* Additionally, strains that were in doubt when identified by these methods were identified based on the 16S rRNA gene sequences, as described previously [15]. The obtained sequences were compared with the known genes for 16S rRNA of bacteria in the GenBank database using the NCBI BLAST program using the blastn algorithm. Sequence editing was performed using the BioEdit program [16].

For the experiment, cultures of the isolated strains were washed three times with a physiological solution. The preliminarily prepared soil suspensions were inoculated with the bacterial cultures in a concentration of 100 cells per 0.1 mL of suspension. The number of bacterial cells in 1 mL of suspension was determined by the optical standard of turbidity.

The concentration and composition of humus, current acidity, the composition of the soil absorption complex, exchangeable acidity, hydrolytic acidity, exchange bases (Ca^{2+} and Mg^{2+}), cation exchange capacity and the degree of saturation by the bases have been determined for the soil samples [17].

To determine the number of viable bacterial cells in soil ecosystems (control), the soil samples were prepared as suspensions containing 1 g of soil per 100 mL of physiological solution and fractionally sterilized for 20 minutes over 3 days at 100 °C in an autoclave. These suspensions were used further as a control.

For the experiment with fertilizers, mixtures of 1 kg of DCS and CCS soils and 0.3 g of one of the mineral fertilizers — nitric ($CO(NH_2)_2$), phosphoric (Ca_2HPO_4) or potash (K_2CO_3) — were prepared. The prepared mixture (1 g) was placed into a flask with distilled water (100 mL). The obtained suspensions were fractionally sterilized for 20 minutes over 3 days at 100 °C in an autoclave.

The studies were carried out at periodic cultivation. The initial dose in the control inoculation from the soil suspension was 100 CFU/0.1 mL. The growth curve was plotted to determine the dynamics of the change of the number of bacteria by periodic inoculation of 0.1 mL of bacterial culture (with necessary serial dilution) of the MPA and VA. The stages of the growth curve and maximum concentration of bacteria were determined by counting the number of colonies grown on a Petri dish (in colony-forming units, CFU).

To identify the significance of the temperature factor, all experiments were performed at 20 and 4 °C.

The experiments were performed in three reproductions. Mathematical processing of the results was performed by using the statistical functions in Microsoft Excel 2007 and Statistica 10.0 software.

3. Results

At the first stage of this study, the growth dynamics of bacteria in different types of soils supplied with fertilizers were investigated. It was found that the reproduction of bacteria in soils depends on both the

type of soil and its properties, and the biological properties of the bacterial strain. Active multiplication of the majority of the investigated bacterial strains (especially bacteria of genus Aeromonas, 1, 5, 10, 20 strains) was observed in CCS (5.4 lg), whereas a considerable decline in the number of bacteria up to their full destruction was noted by the fifth day in DCS (figure 1).

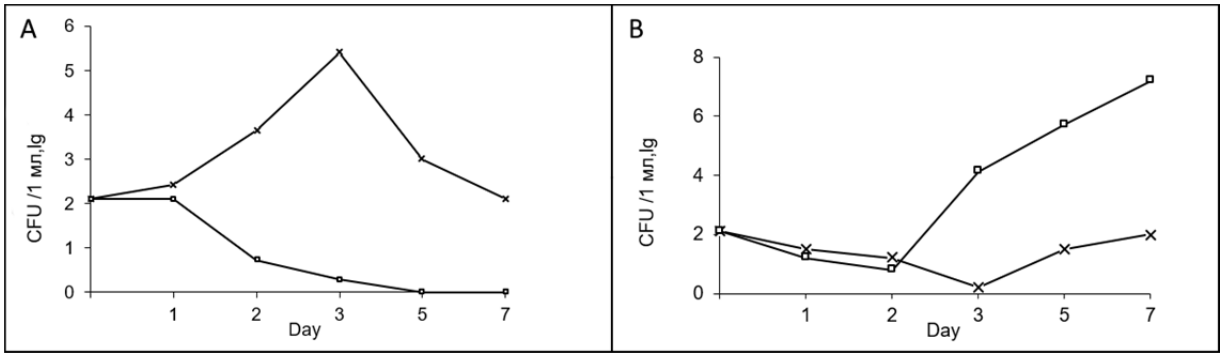

Figure 1. Reproduction of *Aeromonas*, strain 1 (A) *P. fluorescens* strain 6 (Б) in distric cambisol (-□-) and calcaric cambisol (-x-) under 20-22^{0}C (n = 5, p ≥ 5).

In the second stage, the influence of mineral fertilizers on the reproduction of saprophytic bacteria was studied. It was found that applied soil fertilizers (a mixture of nitric, phosphorus and potash) positively affected the reproduction of saprophytic bacteria.

The most active reproduction of saprophytic bacteria was observed in experiments with nitric fertilizer, regardless of the soil type (figure 2). The variants with fertilizers can be arranged according to their effect on the reproduction of saprophytic bacteria in the following order, regardless of the soil type: soil with nitric fertilizer > soil with phosphoric fertilizers > soil with potash fertilizer. Bacteria of the genus Bacillus mainly reproduced in the presence of nitric and phosphoric fertilizers (6.2 and 5.4 lg, respectively), and the growth of bacteria reached the maximum (4.0 lg) in the presence of potash fertilizers, regardless of the soil type. It should be noted that similar results were found for both “psychrophilic” and “thermophilic” strains.

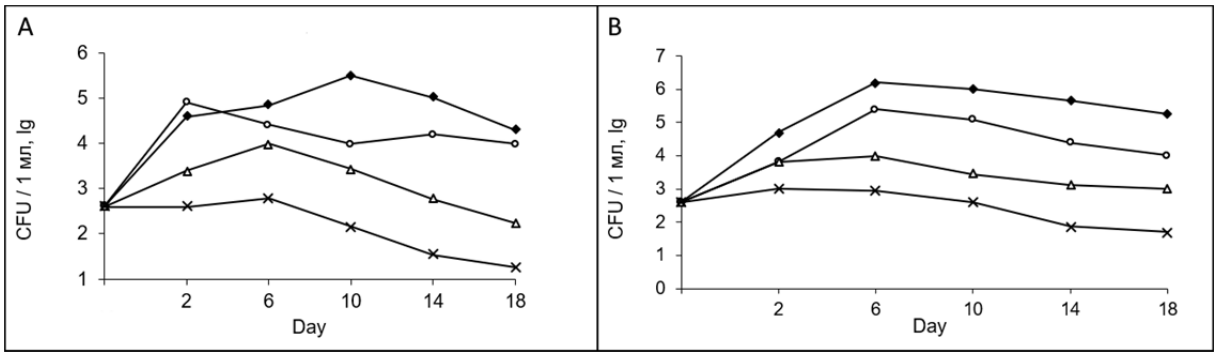

Figure 2. Reproduction of *Bacillus cereus* strain 18 under 20-22^{0}C in distric cambisol (A) and calcaric cambisol (Б) with different types of fertilizers: -■- – nitrogen, -○- – phosphorus, -Δ- – potassic, -x- - control (n = 5, p ≥ 5).

It should be noted that the temperature significantly affected the inoculation of the studied bacterial strains isolated from experimentally infected soil. The dynamics of bacterial reproduction in soils with different fertilizers at 4 and 20 °C, for example, Micrococcus strain 3, is shown in figure 3. Thus, the maximum reproduction of these bacteria (6.2 lg, CCS) was registered on the sixth and first day at 20 °C, whereas at 4 °C, it only reached 4.0 lg (CCS) by the eighteenth day (observation period).

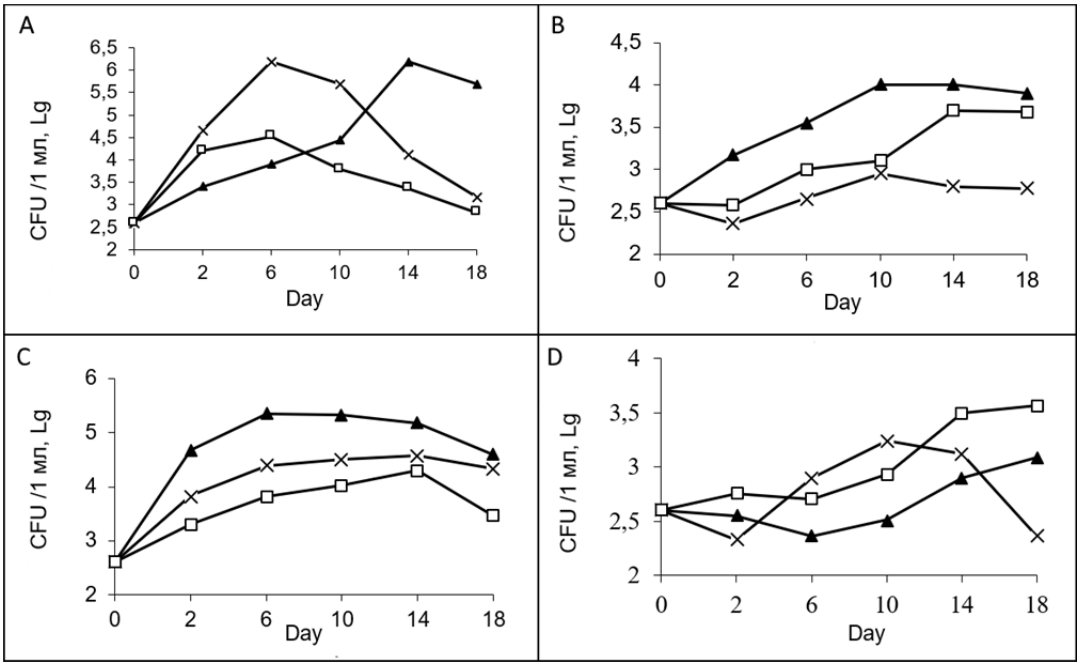

Figure 3. Reproduction of *Micrococcus roseus* strain 3 in calcaric cambisol soil under 20-22^{0}C (A), under 4-6^{0}C (Б) and in districc cambisol soil under 20-22^{0}C (B), under 4-6^{0}C (Г) with fertilizers: -▲- – nitrogen, -□- – phosphorus, -x- – potassic

4. Discussion

Active multiplication of the majority of the investigated bacterial strains is related to the fact that CCS contains higher concentrations of calcium and magnesium cations in the soil absorption complex, which, according to Kim and Gadd [18], are essential for normal reproduction of bacteria. In addition, this soil is characterised as having the optimal pH for the growth of saprophytic bacteria (pH 7.0–7.4) and a sufficient concentration of organic carbon (table 1). The strains of *P. fluorescens* 6, 7, 13 and 14, which actively proliferated in DCS (7.2 lg), were an exception.

Table 1. Physicochemical properties of soils.

Soil type	pH		Exchange acidity	Hydrolytic acidity	Cation exchange capacity	Exchangeable bases		Base saturation, %	C, %
	H_2O	KCl				Ca^{2+}	Mg^{2+}		
	mg eq./ 100 g of soil								
districc cambisol	5,85	5,00	3,25	7,20	44,70	30,3	7,5	83,9	1,1
calcaric cambisol	7,36	6,36	1,15	1,74	80,74	65,1	14,2	97,8	2,6

The positive effect of moderate doses of mineral fertilizers on the soil nutrient regime and its agrochemical properties is known and reveals by extension the variety of soil microorganisms, enhancement of enzymatic activity of soils and intensive production of soil carbon dioxide [5].

It was found that applied soil fertilizers (a mixture of nitric, phosphorus and potash) positively affected the reproduction of saprophytic bacteria. Implementation of mineral fertilizers in the DCS, which is unfavourable for the majority of the strains, caused the active reproduction of the studied

bacteria and an increase in concentration by 3–4 lg compared with soil without fertilizers. Clearly, the acidity of this type of soil, which is low for bacterial reproduction (5.85), plays a significant role (table 2). Additional fertilizer increases the pH to optimal values for reproduction of the studied bacteria. Their intensive reproduction was noted in CCS in both tested (with fertilizers) and control samples (without fertilizers). However, some increase in bacterial population was noted in all variants, by 2–3 lg compared to the control samples.

Table 2. Soil acidity.

Soil type	pH_{H2O}			
	soil without fertilizer	soil with $CO(NH_2)_2$	soil with Na_2HPO_4	soil with K_2CO_3
distric cambisol	5,85	8,01	7,56	8,31
calcaric cambisol	7,36	7,8	7,49	8,08

According to Table 2, the introduction of phosphoric and nitric fertilizers into the soil has a positive effect on the reproduction of saprophytic bacteria of different strains at the optimal soil acidity (pH 7.2–7.4). Introduction of potash fertilizers, which alkalise the soil until pH 10, is less favourable for reproduction of the studied bacteria.

It is known that nitrogen and phosphorus are extremely important biogenic elements, which are included in the main polymers of any living cell, including structural proteins, enzymes, nucleic and adenosinephosphoric acids. In microorganisms, nitrogen is used for the synthesis of amine ($-NH_2$) and iminic (-NH-) groups in amino acids, purines and pyrimidines, nucleic acids and other substances included in the different structures of a cell. Phosphorus is also part of some important organic compounds of the cells (nucleic acids, phospholipids, co-enzymes and others), and is used in living organisms as an energy accumulator and released during the oxidation process.

In addition, it is known that the use of mineral fertilizers increases the mineralisation activity of microorganisms and results in the decomposition of soil organic matter [3,14,19]. This results in an increase of the amount of calcium and magnesium ions, which are required for the normal growth of any microorganism [18].

Therefore, the active reproduction of saprophytic bacteria in soils with fertilizers depends primarily on the type of fertilizer, the temperature of the environment and the characteristics of the bacterial strains. The most successful conditions for bacterial reproduction include CCS and introduction of nitric and phosphoric fertilizers at 20 °C. Prolonged implementation of mineral fertilizers reveals the positive effect on the bacterial complexes in the different types of soil. The results of the experiment indicate the active reproduction of saprophytic bacteria in soils with fertilizers.

5. Conclusion

As a result of research it was found that the implementation of mineral fertilizers ($CO(NH_2)_2$, Ca_2HPO_4, K_2CO_3, individually and in combination) promoted the active reproduction of saprophytic bacteria in CCS and DCS. Introduction of nitric or phosphoric fertilizers is the most favourable. The physicochemical properties of soils affect the reproduction of saprophytic bacteria such bacteria mainly reproduced in fertilized CCS compared with DCS. The temperature influenced the reproduction of saprophytic bacteria in soils with mineral fertilizers. The reproduction of bacteria occurred more intensively at 20 °C than at 4 °C.

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