sciendo

Journal of Horticultural Research 2021, vol. 29(1): 35–46
DOI: 10.2478/johr-2021-0005

INFLUENCE OF MICROBIOLOGICALLY ENRICHED MINERAL FERTILIZERS ON SELECTED GROUPS OF MICROORGANISMS IN THE RHIZOSPHERE OF STRAWBERRY PLANTS

Lidia SAS-PASZT, Urszula SMOLIŃSKA*, Beata KOWALSKA, Magdalena SZCZECH, Anna LISEK, Paweł TRZCIŃSKI, Sławomir GŁUSZEK, Krzysztof GÓRNIK, Edyta DERKOWSKA, Beata SUMOROK

National Institute of Horticultural Research
Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland

Received: August 2020; Accepted: March 2021

ABSTRACT

In recent years, the use of bio-fertilizers enriched with specially selected microorganisms has been used more and more often. The beneficial effects of bio-fertilizers enriched with consortia of microorganisms on strawberry plants have been reported previously. The purpose of the research was to determine the effect of bio-fertilizers containing selected fungal and bacterial strains on the microorganisms living in the rhizosphere of strawberry plants. In the experiments described in this paper, synthetic mineral fertilizers were enriched with selected microorganisms. The fertilizer urea was enriched with the fungi *Aspergillus niger* and *Purpureocillium lilacinum*, while the fertilizers Polifoska 6 and Super Fos Dar 40 with strains of the bacteria *Bacillus* sp., *Bacillus amyloliquefaciens*, and *Paenibacillus polymyxa*. Bacteria and fungi belonging to these species can exert a positive effect on the growth of many plants. The results obtained in this study showed that the application of fertilizers enriched with microorganisms had different effects on the analyzed populations of soil microorganisms in the rhizosphere of strawberry plants. There were evidences of both, an adverse effect of the applied fertilizer and/or microorganisms, but more often, the beneficial effect was found on the abundance of the microorganisms in the rhizosphere of the strawberry. The most effective for the population of *Pseudomonas* bacteria was application of urea and fungi and Polifoska and bacteria. The highest number of phosphorus utilizing bacteria B was scored in the treatments containing NPK, NPK + fungi and urea 60% + fungi. The application of NPK + fungi and urea 100% + fungi as well as Super Fos Dar with bacteria was most beneficial for population of actinomycetes.

Key words: bio-fertilizers, rhizosphere of strawberry, microorganisms, soil

INTRODUCTION

Strawberry fruit, because of their high taste qualities and valuable nutritional properties, is very popular among consumers. In Poland, about 50,000 hectares are under the strawberry cultivation. The implementation of integrated production (IP) methods in strawberry cultivation has reduced the use of synthetic fertilizers and plant protection preparations (Donmez et al. 2011). One of the methods that helps to achieve this goal is the use of bio-fertilizers enriched with specially selected microorganisms (Derkowska et al. 2015; Itelima et al. 2018; Todeschini et al. 2018; Sas-Paszt et al. 2019). In addition to the environmental benefits of this method, the use of plant growth-promoting microorganisms (PGPM), e.g., *Bacillus cereus* KI-2, can have a beneficial effect on the yield of strawberry fruit and, depending on the cultivar, on the sugar content in them (Kurokura et al. 2017).

*Corresponding author:
e-mail: urszula.smolinska@inhort.pl

The great importance of soil microorganisms for plant growth, especially of the species inhabiting the root zone, is well known. In a "healthy" soil, there are diverse, dynamically balanced communities of microorganisms. The bacteria and fungi play an extremely important role in terms of plant health because of, among other things, their antagonistic properties toward pathogens and their role in stimulating plant growth, inducing systemic resistance, and facilitating the absorption of elements essential for plant life (Adesemoye et al. 2009; Ghorbanpour et al. 2018).

In recent years, the dominant trend is to use consortia of microorganisms, instead of individual strains (Nuti & Giovanetti 2015; Sekar et al. 2016; Bradáčová et al. 2018; Woo & Pepe 2018). Researchers work on the assumption that in very different and changing soil conditions, the use of a variety of microorganisms increases their chance to be effective. The use of native isolates exhibiting different modes of action seems to be the most effective approach.

Every year, millions of tons of synthetic fertilizers are applied into the soil, of which a significant part, not used by plants, penetrates to ground and surface waters, or the atmosphere, thus polluting the natural environment. Making use of properly selected microorganisms helps to reduce the amounts of applied synthetic fertilizers (Adesemoye et al. 2009). Indeed, it has been found that in some cases, the application of microorganisms increases the effectiveness of the fertilizers used (Çakmakçı 2019). The beneficial effects of bio-fertilizers based on organic substances enriched with consortia of microorganisms on strawberry plants have been reported by Derkowska et al. (2015). They showed that the fertilizers containing consortia of microorganisms (e.g., Micosat F) had a positive effect on the numbers of bacteria and fungi in the rhizosphere of strawberry plants in greenhouse experiment, in comparison with control (without fertilization) and control + NPK. In recent years, intensive research has been conducted on the interaction between plants and soil and the microorganisms living in it. This is necessary for bio-fertilizers to be effectively used in modern agriculture (Singh et al. 2011; Ishaq 2017).

Relatively little research has been done to determine the impact of the beneficial microorganisms on the effectiveness of mineral fertilizer use (Bargaz et al. 2018). Apart from their main role, which is the decomposition of organic matter and the release of biogenic elements such as carbon, nitrogen and phosphorus into the environment, microorganisms help plants to absorb scarce (under certain conditions) elements, e.g., phosphorus or iron. Many different groups of bacteria, e.g., *Pseudomonas*, *Bacillus*, or *Streptomyces*, are able to transform sparingly insoluble forms of phosphorus compounds into forms readily available to plants by producing organic and inorganic acids and enzymes (phosphatases) (Bargaz et al. 2018). Therefore, in many bio-fertilizers, one of the most commonly included groups of microorganisms are phosphate-solubilizing bacteria (PSB). It has also been observed that the simultaneous application of phosphorus-containing fertilizers and a consortium of microorganisms, containing e.g., *Bacillus subtilis*, had a synergistic effect on plant growth (Duarah et al. 2011; Kaur & Reddy 2015; Bargaz et al. 2018).

Among the bacterial isolates used to enrich the fertilizers was the spore-forming *Bacillus amyloliquefaciens*. It was found that isolates belonging to this species were able to colonize roots, increase plant yields, and produce toxic compounds that helped to control pathogens (Borriss 2011; Chowdhury et al. 2015; Ishaq 2017. Seema et al. (2018) have observed that inoculation of strawberry roots with *Bacillus licheniformis* had a positive effect on the vegetative growth of this plant. In the experiments presented in that work, an isolate of *Paenibacillus polymyxa* was also used. As reported by Yi et al. (2019), bacteria of this species, because of their ability to form a biofilm, can have protective properties against plant pathogens.

Another microorganism investigated in our experiment was *Aspergillus niger*. Yin et al. (2015) found that this fungus, used together with *Penicillium oxalicum*, had a positive influence on the biomass of maize plants by dissolving phosphorus compounds. The observed effect was caused, *inter alia*, by the increased production of organic acids,

such as acetic, citric, lactic, formic, and succinic acids, by the applied fungi. Yadav et al. (2011) had observed that *A. niger* BHUAS01 efficiently dissolved calcium phosphate. This strain also produced indole-3-acetic acid (IAA), a plant hormone from the auxin group. Another fungus used in the experiments described in that work was *Purpureocillium lilacinum* (Nagachandrabose 2020). Although this fungus was mainly used to control parasitic nematodes, it became evident that it also had properties stimulating the growth of many plants, including tomato, bean, cotton, and banana (Lan et al. 2017).

A very important objective of studies assessing the effectiveness of biological preparations is to determine their impact not only on cultivated plants, but also on native microorganisms living in the soil. It is particularly important to investigate whether the use of a given bio-fertilizer stimulates the development of beneficial (or adverse) microorganisms that may be of special importance in the cultivation of perennial plants. Soil microorganisms are considered good indicators of soil quality because they respond quickly to changes in the environment, and changes in their abundance and activity are considered an early signal of a deterioration or improvement in the soil quality (Brzezińska 2009). In the present work, the impact of mineral fertilizers enriched with microorganisms was determined by methods involving the plating of soil suspensions onto selective media. Although molecular studies clearly show that these methods allow the isolation of only a small part of soil microorganisms, it has been found that the result representing the population size of "cultivable microorganisms" is correlated with the enzymatic and respiratory activity of soil microorganisms and is a reflection of active and potentially active groups of microorganisms (Blagodatskaya & Kuzyakov 2013). It has also been found that these microorganisms, because of their significant participation in the circulation of elements, rapid growth, and considerable size, are responsible for 80–90% of the bacterial biomass in the soil (Olsen & Bakken 1987).

The purpose of the research presented here was to determine the impact of mineral fertilizers containing selected microorganisms on the populations of microorganisms living in the rhizosphere of strawberry plants.

MATERIALS AND METHODS

The experiment was performed in 2018–2019 in the SGGW Experimental Field in Skierniewice. Strawberry plants were grown in stoneware pots (0.40 m in diameter, 1.20 m in height), containing approximately 120 liters of a podsolic soil with a pH of 6.2 and organic matter content of approximately 1.2%. The stoneware pots were buried in the open field. Three strawberry plants vegetatively propagated cultivar 'Marmolada' were planted in each stoneware pot. Correct soil moisture was maintained by drip irrigation. The strawberry plants were grown in 13 fertilization combinations, with three stoneware pots per combination. Three commercial fertilizers were used in this experiment: 1) Polifoska 6 – 6% nitrogen in NH_4 form; 20% phosphorus (P_2O_5); 30% potassium (K_2O); 7% sulfur trioxide (SO_3); 2) Super Fos Dar 40 (SFD) – 40% phosphorus pentoxide (P_2O_5) soluble in mineral acids and 25% P_2O_5 soluble in neutral citrate solution and water; 10% calcium oxide (CaO); microelements (Cu, Ca, Fe, Mn, Zn); 3) urea – 46% nitrogen (N) in amide form.

Fungi (*Aspergillus niger*, *Purpureocillium lilacinum*) and bacteria (*Bacillus* sp., *Bacillus amyloliquefaciens, Paenibacillus polymyxa*) strains were obtained from the collection of Microbiology and Rhizosphere Department, the National Institute of Horticultural Research, Skierniewice. They were kept frozen in glycerol at −80 °C until use. To prepare inoculum, bacteria were grown on maltodextrin medium. In the treatments enriched with bacteria, 3.82 g of overgrown substrate of concentration 1–2 × 10^8 cfu·g^{-1} were applied per one stoneware pots. The fungal inoculum was produced on the rice and corn meal medium, prepared in the proportion 1 : 8 (in 2018) and 1 : 10 (in 2019).

The selected strains of fungi were applied at a dose of 5.25 g of overgrown substrate per one stoneware pots, at a propagule concentration: 2–3 × 10^8 cfu·g^{-1} (in 2018) and 1 × 10^7 cfu·g^{-1} (in 2019). All the treatments are listed in Table 1.

Soil samples for microbiological analyses were collected from the rhizosphere of strawberry plants in September 2018 and 2019. Soil was taken from each stoneware pot individually using a 1.5 cm diameter, 20 cm long, soil sampling stick, which was driven into the soil in 3–4 places as close as possible to the plant's stem (and roots). Then, the collected soil with small root fragments was emptied out into a plastic bag. The samples were stored in a cold at 5 °C for about 24 hours. Before microbiological analyses, the soil was thoroughly mixed, ground in a mortar, and four 10 g portions were weighed out. Three of them were poured into flasks containing 100 ml of saline solution with glass balls each, the fourth 10-gram amount was placed in a thermostat and dried at 104 °C for 24 hours. The soil in the flasks was shaken on a shaker for 20 min., and then was plated (100 µl suspension per dish) directly onto the previously prepared selective media.

The overall bacterial population was determined on 1/10 strength tryptone soya agar (TSA). The number of *Pseudomonas* bacteria was determined on the Gould's medium (Gould et al. 1985), while the number of *Pseudomonas* secreting fluorescent dyes also on the same medium but under UV light. Populations of fungi were assessed on the Rose-Bengal Chloramphenicol Agar commercial medium (BTL). Spore-forming bacteria with the ability to dissolve calcium phosphate were analyzed on the Pikovskaya medium (Pikovskaya 1948). The colonies of bacteria exhibiting these properties were divided into two groups, depending on the size of the "halo" zone. Phosphate-solubilizing bacteria (PSB) **A** – the number of bacterial colonies forming clear translucent "halo" zones that form when calcium phosphate is dissolved. PSB **B** – the number of all bacterial colonies that can grow on the Pikovskaya medium. Included here were also bacteria with poor calcium phosphate-solubilizing ability. The number of actinomycetes was determined on colloidal chitin agar (Hsu & Lockwood 1975). The results illustrating the populations of these bacteria are presented as follows: actinomycetes **A** – the number of colonies of actinomycetes forming a distinct "halo", resulting from the decomposition of chitin; actinomycetes **B** – colonies that did not form a distinct "halo", but exhibited morphological features like actinomycetes. This distinction was caused by an attempt to avoid the error caused by the difficulty of assessing the size of degraded chitin zone. It also allowed to include actinomycetes whose intensity of chitin decomposition was low.

The obtained results were analyzed statistically by means of analysis of variance for univariate experiments. The Newman–Keuls test in the statistical program Statistica 13.1 was used to assess differences between means.

RESULTS

Analysis of the results showed that enriching the soil with the mineral fertilizers and/or selected microorganisms increased in some cases the number of different groups of microorganisms in the rhizosphere of strawberry plants comparing with the treatments without their addition. An influence was largely dependent on the year of study. In both years of study, the addition of NPK decreased total number of bacteria and number of *P. fluorescens* but increased total number of *Pseudomonas* and in 2019 number of *P. fluorescens* (Table 2). Application of fungi as a sole slightly decreased the number of bacteria. Fungi given in a combination with mineral fertilizer did differently. The highest number of total *Pseudomonas* bacteria was counted when urea was added together with fungi.

Effect of bacteria application mostly increased the number of total and *Pseudomonas* bacteria. Addition of bacteria together with Polifoska and Super Fos Dar 40 (SFD) in some treatment increased greatly bacteria number (for example, total *Pseudomonas* in 2018 and 2019 as well as *P. fluorescens* in 2019). In 2018, the overall population of bacteria in the soil taken from the rhizosphere of strawberry plants was positively affected by fertilization with urea 60% + fungi and Polifoska 6 60% + bacteria (Table 2). The numbers of bacteria of the genus *Pseudomonas* did not differ statistically between the combinations although combination of fungi with urea and Polifoska with bacteria instantly stimulated bacteria population (Table 2). The highest number of *P. fluorescens* in 2018 was counted in the treatments where fungi with mineral fertilizers were applied. In 2019, their number was stimulated by NPK given as single and SFD given with bacteria (Table 2).

Table 1. The list of treatments (dose of fertilizers per one stoneware)

Treatment no.	Treatments	Mineral fertilizers	Microorganisms
1	control	0	0
2	control + NPK	NPK (12 g Super Fos Dar 40, 50 g potassium salt, 35 g urea before planting and 12 g in midsummer)	0
3	control + fungi	0	Fungi (*A. niger* and *P. lilacinum*)
4	control + NPK + fungi	NPK (as in 2)	Fungi (as above)
5	urea 100% + fungi	NPK, except urea in midsummer, 20 g	Fungi (as above)
6	urea 60% + fungi	NPK, except urea before planting, 20 g and 12 g in midsummer	Fungi (as above)
7	control + bacteria	0	Bacteria (*Bacillus* sp., *B. amyloliquefaciens*, *P. polymyxa* added before planting)
8	control + NPK + bacteria	NPK (as in 2)	Bacteria (as above)
9	Polifoska 6 100% + bacteria	Polifoska 6, 26 g, 33 g potassium salt, 30 g urea before planting and 20 g in midsummer	Bacteria (as above, incorporated into the fertilizer during production of granules)
10	Polifoska 6 100% + bacteria*	as in 9	Bacteria (as above but added to the soil before planting)
11	Polifoska 6 60% + bacteria	Polifoska 6 decreased to 14 g and urea decreased to 18 g before planting, 30 g potassium salt	Bacteria (as in 7)
12	Super Fos Dar 40 100% + bacteria	NPK (as in 2)	Bacteria (as in 7)
13	Super Fos Dar 40 60% + bacteria	NPK (as in 2 but Super Fos Dar 40 decreased to 7 g, potassium salt decreased to 30 g and urea decreased to 20 g before planting	Bacteria (as in 7)

Table 2. Isolation of bacteria from soil of strawberry rhizosphere

Treatment no.	Treatments	Bacteria total count (cfu × 10^6·g DW^{-1} of soil)	*Pseudomonas* total count (cfu × 10^3·g DW^{-1} of soil)	*Pseudomonas fluorescens* (cfu × 10^3·g DW^{-1} of soil)	Bacteria total count (cfu × 10^6·g DW^{-1} of soil)	*Pseudomonas* total count (cfu × 10^3·g DW^{-1} of soil)	*Pseudomonas fluorescens* (cfu × 10^3·g DW^{-1} of soil)
		2018			**2019**		
1	control	39.2 ± 7.4 ab	59.4 ± 26.8 a	27.4 ± 10.1 a	13.6 ± 3.7 e	13.9 ± 2.3 b	11.6 ± 1.8 c
2	control + NPK	30.9 ± 2.2 ab	76.5 ± 14.8 a	13.7 ± 3.9 a	7.1 ± 1.1 e	69.9 ± 2.3 a	60.3 ± 2.1 a
		+ Fungi					
3	control + fungi	28.4 ± 6,3 ab	42.7 ± 14.1 a	20.6 ± 10.0 a	10.9 ± 0.0 e	9.0 ± 1.9 b	8.3 ± 1.6 c
4	control + NPK + fungi	7.2 ± 1.5 b	61.2 ± 20.3 a	78.9 ± 1.6 a	8.4 ± 0.1 e	42.9 ± 2.9 ab	20.4 ± 1.0 c
5	urea 100% + fungi	43.8 ± 13.6 ab	162.4 ± 58.0 a	78.4 ± 32.0 a	10.1 ± 0.2 e	41.9 ± 5.1 ab	32.4 ± 5.4 bc
6	urea 60% + fungi	61.7 ± 15.3 a	129.6 ± 32.2 a	77.7 ± 24.4 a	54.0 ± 0.6 b	50.1 ± 11.9 ab	26.3 ± 1.2 c
		+ Bacteria					
7	control + bacteria	39.7 ± 10.1 ab	100.9 ± 39.5 a	54.2 ± 18.7 a	32.6 ± 6.6 d	35.4 ± 1.8 ab	12.2 ± 2.9 c
8	control + NPK + bacteria	18.2 ± 0.7 b	72.6 ± 29.9 a	48.8 ± 14.4 a	7.4 ± 0.8 e	28.5 ± 6.1 ab	22.9 ± 5. c
9	Polifoska 6 100% + bacteria	25.1 ± 6.7 ab	172.6 ± 54.9 a	24.3 ± 2.9 a	69.3 ± 10.2 a	41.6 ± 8.3 ab	34.6 ± 7.7 bc
10	Polifoska 6 100% + bacteria*	36.9 ± 5.3 ab	80.3 ± 31.2 a	32.0 ± 0.3 a	31.6 ± 1.2 cd	49.3 ± 17.2 ab	15.4 ± 1.6 c
11	Polifoska 6 60% + bacteria	61.5 ± 2.3 a	138.2 ± 41.5 a	47.2 ± 16.1 a	7.7 ± 0.2 e	54.6 ± 14.8 ab	40.0 ± 7.5 abc
12	Super Fos Dar 40 100% + bacteria	34.2 ± 14.5 ab	79.2 ± 30.9 a	54.4 ± 19.7 a	21.2 ± 0.5 de	66.3 ± 18.1 a	56.6 ± 17.1 ab
13	Super Fos Dar 40 60% + bacteria	29.8 ± 2.9 ab	92.9 ± 23.3 a	39.6 ± 12.2 a	43.5 ± 1.0 c	38.1 ± 11.5 ab	19.7 ± 8.0 c

Values marked with the same letter in the columns do not differ significantly according to the Newman–Keuls test (p = 0.05)

Population of actinomycetes A and B was in both years greater in comparison with control in the treatment, where sole NPK was added (Table 3). The number of both types of actinomycetes increased further in the treatment where both NPK and fungi were applied. The highest number of actinomycetes in 2018 was in the treatment where sole bacteria were applied, but this result was not repeated in 2019. The population of actinomycetes B increased in 2019 where bacteria with SFD were added together with bacteria.

The population of filamentous fungi was significantly higher in 2018, where sole fungi and fungi with urea 60% were applied (Table 3).

Population of PSB A was in 2018 higher than in control, when urea 100% was applied with fungi and Polifoska 100% with bacteria. In 2019 the highest population of PSB was observed in the treatment with urea 60% with fungi. PSB B population was slightly stimulated with application of NPK and fungi, and in 2019, by NPK and urea 60% with fungi (Table 4).

Table 3. Isolation of actinomycetes and filamentous fungi from soil of strawberry rhizosphere

Treatment no.	Treatments	Actinomycetes A (cfu × 10^5·g DW^{-1} of soil)	Actinomycetes B (cfu × 10^5·g DW^{-1} of soil)	Filamentous fungi (cfu × 10^4·g DW^{-1} of soil)	Actinomycetes A (cfu × 10^5·g DW^{-1} of soil)	Actinomycetes B (cfu × 10^5·g DW^{-1} of soil)	Filamentous fungi (cfu × 10^4·g DW^{-1} of soil)
		2018			**2019**		
1	control	20.0 ± 2.9 b	25.0 ± 3.1 b	15.5 ± 3.0 c	7.7 ± 2.5 d	24.6 ± 6.7 b	20.3 ± 1.1 ab
2	control + NPK	36.9 ± 13.2 b	50.0 ± 15.8 b	11.7 ± 2.7 c	18.6 ± 0.9 bcd	50.6 ± 12.7 ab	22.8 ± 8.8 ab
				+Fungi			
3	control + fungi	12.8 ± 0.8 b	16.1 ± 1.1 b	**41.5 ± 0.1 a**	14.3 ± 1.4 bcd	28.4 ± 1.0 ab	18.5 ± 3.5 ab
4	control + NPK + fungi	54.3 ± 6.5 b	68.3 ± 10.0 b	37.8 ± 3.7 ab	34.8 ± 6.3 abc	52.2 ± 6.1 ab	23.2 ± 5.9 ab
5	urea 100% + fungi	42.7 ± 10.8 b	52.0 ± 15.1 b	30.1 ± 6.8 b	52.1 ± 5.6 a	74.6 ± 8.7 a	30.9 ± 8.0 a
6	urea 60% + fungi	35.9 ± 14.2 b	50.8 ± 19.6 b	**46.7 ± 0.7 a**	12.7 ± 1.4 bcd	22.8 ± 0.3 b	8.0 ± 0.3 b
				+Bacteria			
7	control + bacteria	103.9 ± 7.8 a	110.7 ± 8.5 a	13.5 ± 2.8 c	18.2 ± 1.1 bcd	36.5 ± 0.3 ab	7.8 ± 1.6 b
8	control + NPK + bacteria	34.4 ± 11.7 b	42.5 ± 13.7 b	10.1 ± 2.3 c	11.0 ± 1.7 cd	19.0 ± 1.7 b	12.1 ± 1.3 ab
9	Polifoska 6 100% + bacteria	23.4 ± 6.1 b	36.6 ± 15.0 b	10.3 ± 1.9 c	38.5 ± 0.1 ab	55.1 ± 0.5 ab	22.1 ± 4.5 ab
10	Polifoska 6 100% + bacteria*	21.6 ± 4.7 b	28.3 ± 4.3 b	13.5 ± 0.3 c	18.9 ± 3.4 bcd	27.6 ± 5.4 ab	9.3 ± 2.6 ab
11	Polifoska 6 60% + bacteria	49.9 ± 16.0 b	55.3 ± 15.6 b	18.7 ± 2.8 c	32.1 ± 3.7 abcd	55.8 ± 15.1 ab	15.4 ± 3.3 ab
12	Super Fos Dar 40 100% + bacteria	28.7 ± 5.9 b	33.2 ± 4.5 b	16.7 ± 0.9 c	36.0 ± 11.4 abc	68.1 ± 16.7 ab	18.8 ± 4.3 ab
13	Super Fos Dar 40 60% + bacteria	23.4 ± 1.8 b	32.6 ± 0.2 b	6.2 ± 0.8 c	30.9 ± 12.1 abcd	61.7 ± 19.5 ab	28.3 ± 0.2 ab

Values marked with the same letter in the columns do not differ significantly according to the Newman–Keuls test (p = 0.05)

DISCUSSION

The composition and abundance of microorganisms living in the soil is determined by many factors. One of the most important of these is the plant species (Klimek et al. 2010; Jankowska & Swędrzyńska 2016; Jacoby et al. 2017). No less important is the type of soil, especially its acidity, and abiotic factors, such as soil moisture and temperature. All these factors, combined with agrotechnical methods, especially fertilization, affect soil microorganisms directly and indirectly, determining plant growth and the quantity and quality of chemical compounds secreted by the roots into the soil (Ishaq 2017), which serve as nutrition for microorganisms living in the soil.

Table 4. Isolation of phosphorus bacteria from soil of strawberry rhizosphere

Treatment no.	Treatments	Phosphate-solubilizing bacteria A (cfu × 10^4·g DW^{-1} of soil)	Phosphate-solubilizing bacteria B (cfu × 10^4·g DW^{-1} of soil)	Phosphate-solubilizing bacteria A (cfu × 10^4·g DW^{-1} of soil)	Phosphate-solubilizing bacteria B (cfu × 10^4·g DW^{-1} of soil)
		2018		**2019**	
1	control	6.8 ± 0.7 bc	51.3 ± 7.1 a	14.6 ± 1.4 bc	36.1 ± 7.5 b
2	control + NPK	9.6 ± 1.8 bc	38.4 ± 0.8 a	28.5 ± 4.5 abc	54.7 ± 10.9 ab
			+Fungi		
3	control + fungi	5.3 ± 1.3 bc	49.1 ± 18.4 a	18.9 ± 4.3 bcd	45.0 ± 9.0 ab
4	control + NPK + fungi	13.2 ± 0.8 ab	74.3 ± 21.5 a	31.4 ± 0.8 ab	60.1 ± 3.6 ab
5	urea 100% + fungi	18.6 ± 5.2 ab	56.0 ± 9.3 a	14.8 ± 2.3 bc	34.4 ± 7.5 b
6	urea 60% + fungi	7.0 ± 1.7 bc	66.6 ± 13.9 a	35.2 ± 2.9 a	75.5 ± 5.2 a
			+Bacteria		
7	control + bacteria	6.7 ± 0.8 bc	62.9 ± 7.1 a	20.8 ± 3.2 bcd	37.3 ± 4.8 b
8	control + NPK + bacteria	7.0 ± 3.2 bc	48.7 ± 3.2 a	12.5 ± 1.6 d	27.8 ± 2.0 b
9	Polifoska 6 100% + bacteria	9.3 ± 2.0 bc	49.3 ± 6.2 a	25.7 ± 1.5 abc	52.4 ± 4.7 ab
10	Polifoska 6 100% + bacteria*	25.5 ± 0.1 a	52.2 ± 4.7 a	16.6 ± 4.3 cd	31.9 ± 6.7 b
11	Polifoska 6 60% + bacteria	3.0 ± 0.8 c	64.5 ± 6.7 a	20.8 ± 2.0 bcd	51.9 ± 3.3 ab
12	Super Fos Dar 40 100% + bacteria	14.3 ± 5.9 ab	88.0 ± 27.7 a	20.4 ± 3.2 bcd	46.0 ± 13.2 ab
13	Super Fos Dar 40 60% + bacteria	14.0 ± 3.7 ab	54.5 ± 12.6 a	17.8 ± 3.9 bcd	43.1 ± 12.6 ab

Values marked with the same letter in the columns do not differ significantly according to the Newman–Keuls test (p = 0.05)

So, there is a close relationship between the condition of the plant, the size of the root system, and the intensity of the impact on soil-inhabiting microorganisms (De-la-Peña & Loyola-Vargas 2014; Jankowska & Swędrzyńska 2016; Haldar & Sengupta 2015).

Considerable amounts of mineral fertilizers used in agriculture are lost due to various physical, chemical, and biological processes, e.g., leaching, absorption, volatilization (denitrification), etc. The economic and environmental damage that these processes cause is widely known. The use of bio-fertilizers may be useful to limit the amounts of synthetic mineral fertilizers and ensure their more effective use. In the experiment described here, synthetic mineral fertilizers were enriched with selected microorganisms. Bacteria and fungi genera used in our experiment can exert a positive influence on the growth of many plants (Lan et al. 2017; Ishaq 2017; Yin et al. 2015).

Actually, more and more researchers are paying attention to increasing the effectiveness of PGPM by using consortia of microorganisms with different modes of action. The results obtained in the present study showed that the application of fertilizers enriched with microorganisms consortia had different effects on the analyzed populations of soil microorganisms in the rhizosphere of strawberry plants. It should be noted, however, that in the most cases, this effect was positive.

The beneficial effect of the selected strains of microorganisms applied to soil can be illustrated with more than five-fold increase in the abundance of actinomycetes in the soil in the combination control + bacteria. In 2018, the addition of fungi caused a significant increase in the populations of filamentous fungi in the combinations control and control + NPK, compared to the combinations without additives. Unfortunately, in 2019, the differences between the combinations were not statistically significant.

The results of the experiment are evidence that the most beneficial effect on soil microorganisms in the strawberry root zone was produced by the treatment with urea 60% + fungi. For this combination, significant increases in the size of populations of bacteria (total counts for 2018 and 2019), actinomycetes (2019), filamentous fungi (2018 and 2019), and PSB B (2019) were observed. Although the number of bacteria of the genus *Pseudomonas* in this combination was not statistically significant, it was markedly higher than in control in both years. Urea 100% + fungi increased populations of filamentous fungi and PSB in 2018. Therefore, it follows that the use of urea reduced by 40% in combination with *Aspergillus niger* and *Purpureocillium lilacinum* had a positive effect on the analyzed populations of soil microorganisms.

The fertilizer Polifoska 6 60% + bacteria had a positive effect on total soil bacteria and bacteria of the genus *Pseudomonas*; however, the differences were not significant. The other combinations in which Polifoska 6 100%, + bacteria were used had a positive effect on the overall number of bacteria in 2019. Super Fos Dar 40 100% + bacteria increased almost fivefold the total number of *Pseudomonas* bacteria and also fluorescens *Pseudomonas* compared to control.

The analyses performed in this experiment determined the number of microorganism groups that have long been recognized as soil quality indicators because their significance from the point of view of broadly understood soil fertility is considered to be extremely important (Garbeva et al. 2004). One of the analyzed groups are bacteria of the genus *Pseudomonas*. They are bacteria that colonize the soil effectively because they compete strongly for nutrients with other microorganisms. Some species of *Pseudomonas* can stimulate plant growth because they have the ability to produce phytohormones such as auxins, gibberellins, and cytokinins, a large number of biologically active compounds (including antibiotics, HCN, lytic enzymes), and is known as a inducer of systemic resistance (ISR) in plants. *Pseudomonas* bacteria have a positive influence on soil structure because they produce large amounts of sticky polymers that contribute to the aggregation of soil particles (Pathma et al. 2011; Pociejowska et al. 2014). The highest numbers of these bacteria, both overall and of those producing fluorescent dyes, were observed in 2019 in the combinations control NPK and Super Fos Dar 40 100% + bacteria.

Another group of bacteria commonly found in the soil are actinomycetes (Lenart-Boroń & Banach 2014). They play an important role because of their ability to break down various chemical compounds and participation in the mineralization of organic substances (cellulose, chitin, lignin, and others). They produce a vast number of antibiotic compounds that have antibacterial and antifungal properties. The results obtained by analyzing the soil from strawberry rhizosphere indicate that enrichment of the soil with the selected microorganisms or treatment with fertilizers enriched with these microorganisms had a positive effect on the numbers of actinomycetes. The lowest populations of these bacteria were found in the soil from control, but significantly higher in control + bacteria. In 2019, most actinomycetes were found in the soil samples taken from the combinations with urea 60% + fungi.

One of the important aspects taken into account in the effective use of microorganisms is selection for those that can improve the condition of plants by providing them with certain nutrients. Researchers have long focused their attention on bacteria with the ability to dissolve insoluble phosphorus compounds and transform them into forms readily available to plants (Kurek & Ozimek 2008; Wang et al. 2018). These bacteria may be of particular importance in Poland because acidic soils, which are prevalent in this country, are conducive

to the formation of insoluble compounds that reduce the pool of mobile (available) phosphorus. Some bacteria (e.g., *Bacillus*) found in the soil can produce significant amounts of organic acids that dissolve sparingly available forms of phosphorus. The ability of bacteria to dissolve these compounds varies greatly because individual species secrete different organic acids that may affect that process with varying intensity (Ciopińska & Bezak-Mazur 2018). The results obtained in the study showed that the use of Polifoska 6 100% + bacteria was the most beneficial for increasing the number of PSB in 2018, while in the second year, the highest numbers of these bacteria were observed in the combinations urea 60% + fungi.

To conclude, it can be stated that the fertilizers used in the study had, in some cases, a beneficial influence on microorganisms in the rhizosphere of strawberry plants. This experiment was conducted in the field conditions, and many different factors (biotic and abiotic) may affect obtained results, so the study requires further investigations.

Acknowledgments

Publication was co-financed by the National Centre for Research and Development under the BIOSTRATEG program, contract number BIOSTRATEG3/347464/5/NCBR/2017.

REFERENCES

Adesemoye A.O., Torbert H.A., Kloepper J.W. 2009. Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microbial Ecology 58: 921–927. DOI: 10.1007/s00248-009-9531-y.

Bargaz A., Lyamlouli K., Chtouki M., Zeroual Y., Dhiba D. 2018. Soil microbial resources for improving fertilizers efficiency in an integrated plant nutrient management system. Frontiers in Microbiology 9; 1606; 26 p. DOI: 10.3389/fmicb.2018.01606.

Blagodatskaya E., Kuzyakov Y. 2013. Active microorganisms in soil: Critical review of estimation criteria and approaches. Soil Biology and Biochemistry 67: 192–211. DOI: 10.1016/j.soilbio.2013.08.024.

Borriss R. 2011. Use of plant-associated *Bacillus* strains as biofertilizers and biocontrol agents in agriculture. In: Maheshwari D.K. (Ed.), Bacteria in Agrobiology: Plant Growth Responses. Springer, Berlin, pp. 41–76. DOI: 10.1007/978-3-642-20332-9_3.

Bradáčová K., Florea A.S., Bar-Tal A., Minz D., Yermiyahu U., Shawahna R. et al. 2018. Microbial consortia versus single-strain inoculants: An advantage in PGPM-assisted tomato production? Agronomy 9; 105; 23 p. DOI: 10.3390/agronomy9020105.

Brzezińska M. 2009. The use of microbial eco-physiological indicators for evaluation of soil quality. Advances in Agricultural Sciences 61(1): 39–51. [in Polish with English abstract]

Çakmakçı R. 2019. A review of biological fertilizers current use, new approaches, and future perspectives. International Journal of Innovative Studies in Sciences and Engineering Technology 5: 83–92.

Chowdhury S.P., Hartmann A., Gao X., Borriss R. 2015. Biocontrol mechanism by root-associated *Bacillus amyloliquefaciens* FZB42 – a review. Frontiers in Microbiology 6; 780; 11 p. DOI: 10.3389/fmicb.2015.00780.

Ciopińska J., Bezak-Mazur E. 2018. Phosphorus solubilizing bacteria – review article. Structure and Environment 10: 278–287. DOI: 10.30540/sae-2018-027.

De-la-Peña C., Loyola-Vargas V.M. 2014. Biotic interactions in the rhizosphere: A diverse cooperative enterprise for plant productivity. Plant Physiology 166: 701–719. DOI: 10.1104/pp.114.241810.

Derkowska E., Sas Paszt L., Trzciński P., Przybył M., Weszczak K. 2015. Influence of biofertilizers on plant growth and rhizosphere microbiology of greenhouse-grown strawberry cultivars. Acta Scientiarum Polonorum, Hortorum Cultus 14(6): 83–96.

Donmez M.F., Esitken A., Yildiz H., Ercisli S. 2011. Biocontrol of *Botrytis cinerea* on strawberry fruit by plant growth promoting bacteria. Journal of Animal and Plant Sciences 21: 758–763.

Duarah I., Deka M., Saikia N., Deka Boruah H.P. 2011. Phosphate solubilizers enhance NPK fertilizer use efficiency in rice and legume cultivation. 3 Biotech 1: 227–238. DOI: 10.1007/s13205-011-0028-2.

Garbeva P., van Veen J.A., van Elsas J.D. 2004. Microbial diversity in soil: Selection of microbial populations by plant and soil type and implications for disease suppressiveness. Annual Review of Phytopathology 42: 242–270. DOI: 10.1146/annurev.phyto.42.012604.135455.

Ghorbanpour M., Omidvari M., Abbaszadeh-Dahaji P., Omidvar R., Kariman K. 2018. Mechanisms underlying the protective effects of beneficial fungi against plant diseases. Biological Control 117: 147–157. DOI: 10.1016/j.biocontrol.2017.11.006.

Gould W.D., Hagedorn C., Bardinelli T.R., Zablotowicz R.M. 1985. New selective media for enumeration and recovery of fluorescent pseudomonads from various habitats. Applied and Environmental Microbiology 49: 28–32. DOI: 10.1128/aem.49.1.28-32.1985.

Haldar S., Sengupta S. 2015. Plant-microbe cross-talk in the rhizosphere: Insight and biotechnological potential. Open Microbiology Journal 9: 1–7. DOI: 10.2174/1874285801509010001.

Hsu S.C., Lockwood J.L. 1975. Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. Applied Microbiology 29: 422–426. DOI: 10.1128/am.29.3.422-426.1975.

Ishaq S.L. 2017. Plant-microbial interactions in agriculture and the use of farming systems to improve diversity and productivity. AIMS Microbiology 3(2): 335–353. DOI: 10.3934/microbiol.2017.2.335.

Itelima J.U., Bang W.J., Sila M.D., Onyimba I.A., Egbere O.J. 2018. A review: Biofertilizer – a key player in enhancing soil fertility and crop productivity. Journal of Microbiology and Biotechnology Reports 2: 22–28.

Jacoby R., Peukert M., Succurro A., Koprivova A., Kopriva S. 2017. The role of soil microorganisms in plant mineral nutrition – Current knowledge and future directions. Frontiers in Plant Science 8; 1617; 19 p. DOI: 10.3389/fpls.2017.01617.

Jankowska M., Swędrzyńska D. 2016. Analysis of the interactions of microorganisms in soil environment. Kosmos 65: 49–55. [in Polish with English abstract]

Kaur G., Reddy M.S. 2015. Effects of phosphate-solubilizing bacteria, rock phosphate and chemical fertilizers on maize-wheat cropping cycle and economics. Pedosphere 25: 428-437. DOI: 10.1016/s1002-0160(15)30010-2.

Klimek B., Stefanowicz A.M., Woch M.W., Jaźwa M. 2010. Is there a link between biodiversity of plants and soil microorganisms? Kosmos 59(3–4): 589–598. [in Polish with English abstract]

Kurek E., Ozimek E. 2008. Biological methods to increasing the content of bioavailable phosphorus in soil. Postępy Nauk Rolniczych 60(2): 85–95. [in Polish with English abstract]

Kurokura T., Hiraide S., Shimamura Y., Yamane K. 2017. PGPR improves yield of strawberry species under less-fertilized conditions. Environmental Control in Biology 55: 121–128. DOI: 10.2525/ecb.54.121.

Lan X., Zhang J., Zong Z., Ma Q., Wang Y. 2017. Evaluation of the biocontrol potential of *Purpureocillium lilacinum* QLP12 against *Verticillium dahliae* in eggplant. BioMed Research International 2017; 4101357; 8 p. DOI: 10.1155/2017/4101357.

Lenart-Boroń A., Banach T. 2014. Actinobacteria *Streptomyces* spp in the heavy metal-contaminated environment. Kosmos 63(1): 87–93. [in Polish with English abstract]

Nagachandrabose S. 2020. Management of potato cyst nematodes using liquid bioformulations of *Pseudomonas fluorescens*, *Purpureocillium lilacinum* and *Trichoderma viride*. Potato Research 63: 479–496. DOI: 10.1007/s11540-020-09452-2.

Nuti M., Giovannetti G. 2015. Borderline products between bio-fertilizers/bio-effectors and plant protectants: The role of microbial consortia. Journal of Agricultural Science and Technology A 5(5): 305–315. DOI: 10.17265/2161-6256/2015.05.001.

Olsen R.A., Bakken L.R. 1987. Viability of soil bacteria: Optimization of plate-counting technique and comparison between total counts and plate counts within different size groups. Microbial Ecology 13: 59–74. DOI: 10.1007/bf02014963.

Pathma J., Kamaraj Kennedy R., Sakthivel N. 2011. Mechanisms of fluorescent pseudomonads that mediate biological control of phytopathogens and plant growth promotion of crop plants. In: Maheshwari D.K. (Ed.), Bacteria in Agrobiology: Plant Growth Responses. Springer, Berlin, pp. 77–105. DOI: 10.1007/978-3-642-20332-9_4.

Pikovskaya R.I. 1948. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. Mikrobiologiya 17: 362–370.

Pociejowska M., Natywa M., Gałązka A. 2014. Stimulation of plant growth by bacteria PGPR. Kosmos 63(4): 603–610. [in Polish with English abstract]

Sas-Paszt L., Sumorok B., Derkowska E., Trzciński P., Lisek A., Grzyb Z.S. et al. 2019. Effect of microbiologically enriched fertilizers on the vegetative growth of strawberry plants in container-based cultivation at different levels of irrigation. Journal of Research and Applications in Agricultural Engineering 64(2): 38–46.

Seema K., Mehta K., Singh N. 2018. Studies on the effect of plant growth promoting rhizobacteria (PGPR) on growth, physiological parameters, yield and fruit quality of strawberry cv. Chandler. Journal of Pharmacognosy and Phytochemistry 7: 383–387.

Sekar J., Raj R., Prabavathy V.R. 2016. Microbial consortial products for sustainable agriculture: Commercialization and regulatory issues in India. In: Singh H.B., Sarma B.K., Keswani C. (Eds.), Agriculturally Important Microorganisms. Springer, Singapore, pp. 107–131. DOI: 10.1007/978-981-10-2576-1_7.

Singh J.S., Pandey V.Ch., Singh D.P. 2011. Efficient soil microorganisms: A new dimension for sustainable agriculture and environmental development. Agriculture, Ecosystems and Environment 140: 339–353. DOI: 10.1016/j.agee.2011.01.017.

Todeschini V., AitLahmidi N., Mazzucco E., Marsano F., Gosetti F., Robotti E. et al. 2018. Impact of beneficial microorganisms on strawberry growth, fruit production, nutritional quality, and volatilome. Frontiers in Plant Science 9; 1611; 22 p. DOI: 10.3389/fpls.2018.01611.

Wang X., Wang Ch., Sui J., Liu Z., Li Q., Ji Ch. et al. 2018. Isolation and characterization of phosphofungi, and screening of their plant growth-promoting activities. AMB Express 8; 63; 12 p. DOI: 10.1186/s13568-018-0593-4.

Woo S.L., Pepe O. 2018. Microbial consortia: Promising probiotics as plant biostimulants for sustainable agriculture. Frontiers in Plant Science 9; 1801; 6 p. DOI: 10.3389/fpls.2018.01801.

Yadav J., Verma J.P., Tiwari K.N. 2011. Plant growth promoting activities of fungi and their effect on chickpea plant growth. Asian Journal of Biological Sciences 4(3): 291–299. DOI: 10.3923/ajbs.2011.291.299.

Yi J., Zhang D., Cheng Y., Tan J., Luo Y. 2019. The impact of *Paenibacillus polymyxa* HY96-2 *luxS* on biofilm formation and control of tomato bacterial wilt. Applied Microbiology and Biotechnology 103: 9643–9657. DOI: 10.1007/s00253-019-10162-0.

Yin Z., Shi F., Jiang H., Roberts D.P., Chen S., Fan B. 2015. Phosphate solubilization and promotion of maize growth by *Penicillium oxalicum* P4 and *Aspergillus niger* P85 in a calcareous soil. Canadian Journal of Microbiology 61: 913–923. DOI: 10.1139/cjm-2015-0358.