SHORT COMMUNICATION

Plant growth promoting rhizobacteria in the production of English ryegrass

D. Stamenov, M. Jarak, S. Đurić, D. Milošev, T. Hajnal-Jafari

Department of Field and Vegetable Crops, Faculty of Agriculture, University of Novi Sad, Novi Sad, Serbia

ABSTRACT

The effect of inoculation with *Pseudomonas fluorescens* and *Bacillus subtilis* on the yield of fresh and dry mass of English ryegrass (*Lolium perenne* L.) as well as on the number of rhizospheric microorganisms was studied. The microorganisms were introduced into the soil before sowing. The control plots were not inoculated. The number of microorganisms was determined after the third mowing. The yield was determined after the first, second and third mowing. In comparison with the control, after the first and second mowing, there was a statistically significant increase in the fresh and dry mass in both inoculated variants whereas after the third mowing, a statistically significant increase in the yield of fresh mass was recorded only in the variant with *B. subtilis*. The use of *B. subtilis* had a better effect on the total yield of the fresh and dry mass of English ryegrass. The number of the investigated groups of microorganisms, apart from actinomycetes, increased in the inoculated variants. Inoculation of *P. fluorescens* affected the increase of total number of bacteria and aminoheterotrophs whereas inoculation of *B. subtilis* affected the increase of the number of azotobacter and fungi.

Keywords: *Pseudomonas fluorescens*; *Bacillus subtilis*; *Lolium perenne* L.; yield; rhizospheric soil

Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surface and in association with roots. They can improve the extent or quality of plant growth directly and/or indirectly. Most popular bacteria studied and exploited as biocontrol agents include the species of fluorescent *Pseudomonas* and *Bacillus* (Joseph et al. 2007).

Pseudomonas ssp. produces a variety of biologically active substances among which growth-promoting compounds represent a keen interest. It was shown that many strains of pseudomonads are able to solubilize phosphorous in soil and increase its availability to plants (Sundara et al. 2002). Some strains of pseudomonads produce siderophores, with high affinity for Fe absorption (Kloepper et al. 1980).

Bacillus ssp. PGPR activity of some of these strains has been known for many years. There are a number of metabolites that are released by these strains, which strongly affect environment by increasing nutrient availability of the plants (Barriuso et al. 2008). *Bacillus* is also found to have a potential to increase the yield and growth of different plants (Orhan et al. 2006).

The objective of this work was a comparative study of the PGPR effect (*Pseudomonas fluorescens* and *Bacillus subtilis*) on the yield of English ryegrass and on the number of microorganisms in the rhizospheric soil.

MATERIAL AND METHODS

The experiment was conducted in the soil having the following characteristics: 3.53% $CaCO_3$; 4.51% humus; 0.3% N; 20.89 mg/100 g P; 19.68 mg/100 g K; pH in H_2O 8.11; pH in KCl 7.59. According to FAO classification, the experiment was conducted on the chernozem soil.

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The experiment was set up folowing randomized block system. The size of the experimental plot was 5 m^2. Each variant was conducted in four repetitions. From each repetition one sample was taken for analysis, each was analyzed in three repetitions.

Plant growth promoting bacteria *Pseudomonas fluorescens* and *Bacillus subtilis* (from the collection of the Department of Microbiology, Institute of Soil Science, Belgrade, Serbia) were used as inoculants. English ryegrass (*Lolium perenne* L., cv. Calibra) was taken from the collection of Institute of Forage Crops, Kruševac, Serbia. The variants of the experiment were the following: 1. *P. fluorescens*, 2. *B. subtilis*, 3. control – no inoculation.

P. fluorescens was cultivated on the King's B medium (King et al. 1954) whereas *B. subtilis* was cultivated on nutrient agar. Before sowing, 50 mL with 10^8 CFU/mL of *B. subtilis* as well as 50 mL with 10^8 CFU/mL of *P. fluorescens* cells was introduced into 5 L of tap water that was sprayed on the plot surface. The sowing was performed manually with 20 kg of English ryegrass per ha.

The number of microorganisms was determined after the third mowing, using the dillution method (Trolldenier 1996). Appropriate nutrient media were used (Hi Media Laboratories Pvt. Limited, Mumbai, India): nutrient agar for the total number of bacteria, synthetic agar for the number of actinomycetes, potato dextrose agar for the number of fungi, meat peptone agar for the number of aminoheterotrophs, and azotobacter medium with manitol for the number of azotobacter. The yield of fresh and dry mass was measured after the first, second and third mowing.

The data were statistically processed by means of Statistics 10 programme (Hamburg, Germany). The significance of the difference between the applied treatments was determined using the Fisher's *LSD* test.

RESULTS AND DISCUSSION

Different PGPR strains have a stimulative effect on the growth and development of plants by means of synthesis of phytohormones (Vessey 2003), siderophore production (Tian et al. 2009), transformation of inaccesible P forms into forms accesible to plants (Chen et al. 2006) and other ways. To what extent this stimulative effect will affect the plant yield depends on the type of soil, effectivenes of the indigenous and introduced strains of microorganisms, plant species, agrotechnical measures etc. This research focused on examining the effect of *P. fluorescens* and *B. subtilis* which produce indole-3-acetic acid and siderophores and decompose tricalcium phosphate (TCP) (Jošić et al. 2010). In comparison with the control, after the first and second mowing, a statistically significant increase in the yield of fresh and dry mass was recorded in both variants (Table 1). After the third mowing, a statistically significant increase in the yield of fresh mass was recorded only in the variant with *B. subtilis*. The yield of dry mass after the third mowing in both inoculated variants was not changed in comparison with the control. There was no significant statistical difference in their effect on the yield between *B. subtilis* and *P. fluorescens*, except after the second mowing when the yield of fresh mass was statistically higher in the variant with *B. subtillis* than in the variant with *P. fluorescens* (Table 1).

Table 1. The effect of inoculation on the yield of fresh and dry mass of English ryegrass

Yield (t/ha)		Control	*Pseudomonas fluorescens*	*Bacillus subtilis*
I mowing	fresh mass	3.00^{b}	8.0^{a}	6.6^{a}
	dry mass	0.86^{b}	1.8^{a}	1.5^{a}
II mowing	fresh mass	5.33^{c}	8.0^{b}	9.0^{a}
	dry mass	2.66^{b}	5.0^{a}	6.0^{a}
III mowing	fresh mass	8.00^{a}	8.6^{ab}	11.0^{b}
	dry mass	1.63^{a}	1.6^{a}	2.0^{a}
Total yield	fresh mass	16.30^{b}	24.6^{a}	26.6^{a}
	dry mass	5.15^{b}	8.4^{a}	9.5^{a}
Total yield (%)	fresh mass	100	151	163
	dry mass	100	163	184

The different letter above the number indicates a significant difference at $P < 0.05$

Table 2. The effect of inoculation on the number of microorganisms in the rhizosphere of English ryegrass

Variants	The number of microorganisms in 1 g of absolutely dry soil (CFU/g)				
	total bacteria number (10^6)	fungi (10^4)	actinomycetes (10^5)	aminoheterotrophs (10^6)	azotobacter (10^2)
Pseudomonas fluorescens	76.71^a	5.68^b	11.37^a	67.47^a	177.57^b
Bacillus subtilis	34.68^b	14.90^a	7.80^a	6.38^b	223.45^a
Control	25.74^b	5.30^b	15.90^a	4.54^b	162.83^b

The different letter above the number indicates a significant difference at $P < 0.05$

The use of *B. subtilis* had a better effect on the total yield of fresh and dry mass of English ryegrass. The yield of fresh mass of the plants inoculated with *B. subtilis* was by 63% higher than in the control, whereas the yield of dry mass was by 84% higher (Table 1). The yield of fresh mass of plants inoculated with *P. fluorescens* was by 51% higher than in the control whereas the yield of dry mass was by 63% higher.

The use of bacteria in plant production increases the number and enzymatic activity of microorganisms which enhaces the productive capability of soil (Nannipieri et al. 2003). This research showed that the number of the investigated groups of microorganisms, apart from the number of actinomycetes, increased in both variants in comparison with the control (Table 2). The use of *P. fluorescens* had a better effect on the increase in the total number of microorganisms and aminoheterotrophs whereas the use of *B. subtilis* had a better effect on the increase in the number of azotobacter and fungi.

Comparing the two variants, it was noticed that the number of aminoheterotrophs and the total number of bacteria were statistically larger in the variant with *P. fluorescens*, whereas the number of azotobacter and fungi was larger in the variant with *B. subtilis*.

Similarly, several other authors pointed to the positive effect of bacilli and pseudomonads on the plant yield. Garcia et al. (2004) noticed a positive effect of inoculation with *B. licheniformis* on the plant yield. Han et al. (2006) improved the biological properties of soil by introducing *Bacillus* sp. According to Kloepper et al. (2004), the use of *Pseudomonas* sp. can increase the plant yield by even 144%. According to the results by Rokhzadi et al. (2008), the use of *P. fluorescens* has a positive effect on the yield and growth of the plant. Menhaz et al. (2009) observed the positive effect of the bacterium on the fresh and dry mass of the plant. Comparing the effect of *Bacillus* sp. and *Pseudomonas* sp. on the yield and dry mass of tomato and spinach, Adesemoye et al. (2008) did not establish a statistically significant difference between these two inoculants.

The increase in the number of microorganisms in soil and the positive effect of inoculation on the plant confirm the fact that the use of *P. fluorescens* and *B. subtilis* can result in a better yield of forage crops, especially in organic production, where mineral fertilizers are not used.

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Corresponding author:

Dragana Stamenov, Msci, University of Novi Sad, Faculty of Agriculture, Department of Field and Vegetable Crops, Novi Sad, Serbia
e-mail: draganastamenov@yahoo.com