Available online: www.notulaebotanicae.ro
Print ISSN 0255-965X; Electronic 1842-4309
Not Bot Horti Agrobo, 2016, 44(1):296-301. DOI:10.15835/nbha44110252
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Original Article

Growth and Nutrient Uptake of Orchardgrass (*Dactylis glomerata* L.) and Meadow Fescue (*Festuca pratensis* Huds.) as Affected by Rhizobacteria

Olivera STAJKOVIĆ-SRBINOVIĆ[1*], Dušica DELIĆ[1], Djordje KUZMANOVIĆ[1], Biljana SIKIRIĆ[1], Nataša RASULIĆ[1], Biljana NIKOLIĆ[2], Jelena KNEŽEVIĆ-VUKČEVIĆ[2]

[1]*Institute of Soil Science, Teodora Drajzera 7, 11000 Belgrade, Serbia; oliverastajkovic@yahoo.com (*corresponding author); vukmirdusica@yahoo.com; djorkuz@yahoo.com; biljana-s@sbb.rs; nrasulic@yahoo.com*
[2]*University of Belgrade, Faculty of Biology, Belgrade, Serbia; biljanan@bio.bg.ac.rs; jelenakv@bio.bg.ac.rs*

Abstract

A diverse group of soil bacteria found in the rhizosphere which can colonize plant roots and improve plant growth are designated as plant growth promoting rhizobacteria. The aim of this study was isolation and screening of different rhizobacterial strains for plant growth promoting characteristics and their ability to improve growth of two grass species, orchardgrass (*Dactylis glomerata* L.) and meadow fescue (*Festuca pratensis* Huds.). The strains investigated, belonging to the genera *Azotobacter*, *Bacillus*, *Pseudomonas* and rhizobial bacteria, showed various plant growth promoting traits, such as phosphate solubilisation, siderophore production, and indole-3-acetic acid (IAA) production. Co-inoculation of meadow fescue with *Azotobacter chroococcum* A2 and *Sinorhizobium meliloti* or *Pseudomonas* sp., and *A. chroococcum* A5 with *S. meliloti*, significantly increased shoot dry weight (SDW)(25-33%), as well as total N (26-33%), P (24-31%) and K (26-28%) contents in plants (mg pot^{-1}), compared to uninoculated control. In addition, inoculation of orchardgrass with *A. chroococcum* strain A1, as well as co-inoculation with *B. megaterium* and *A. chroococcum* A1 or A31, significantly increased SDW (51-59%) and total N (54-59%), P (51-74%) and K (49-55%) contents, compared to uninoculated control. Nitrogen percentage in SDW was slightly higher than sufficiency ranges, while K percentage was optimal in all treatments in both species. Phosphorous percentage was lower than sufficiency ranges as a consequence of very low soil P content. The results emphasize the potential of particular rhizobacteria to improve the growth of forage grasses.

Keywords: grasses, inoculation, plant nutrition, plant growth promoting rhizobacteria, yield increase, soil fertility

Introduction

Perennial grasses include variety of widely spread species, adaptable to different agro-ecological conditions, which represent basis for sustainable livestock production as a main food for ruminants (Tomić *et al.*, 2007). Among perennial grasses, orchardgrass (*Dactylis glomerata* L.) and meadow fescue (*Festuca pratensis* Huds.) are considered to be of great importance due to their high productivity and nutritive values, as well as durability, vitality, modest soil requirements and tolerance to moderate drought stress (Sosnowski *et al.*, 2015). Both species are very productive, orchardgrass dry matter yields ranged from 5-6 t ha^{-1} to 15 t ha^{-1}, while meadow fescue realised similar yield of 13.5 t ha^{-1} (Tomić *et al.*, 2007). They are suitable for mixed sowing with perennial legumes, alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.) or white clover (*Trifolium repens* L.). The legume-grass mixtures are of great importance, considering that legumes perform the biological fixation of nitrogen, with consequent economic and ecological benefit. In addition, being deep-rooted, both grass species may be used as a ground cover, in order to control soil erosion and in rehabilitation programmes of sites disturbed by mining (Baran *et al.*, 2015).

The use of plant growth promoting rhizobacteria (PGPR), to increase soil fertility and improve growth and yield of agronomically important crops is a significant alternative to chemical fertilizers in sustainable agriculture (Saia *et al.*, 2015). Increases in growth and yield of crops in response to inoculation with PGPR have been repeatedly reported (Baris *et al.*, 2014; Dinesh *et al.*, 2015; Imran *et al.*, 2015; Saia *et al.*, 2015). PGPR exert positive effect on plant growth by direct mechanisms, such as biological N_2 fixation, phosphate solubilisation and production of growth regulators (phytohormones), or by indirect mechanisms, such as prevention of the deleterious effects of plant pathogens, production of inhibitory substances (siderophore, antibiotics), or increase of natural resistance of the host (Glick, 1995). Positive growth effects of non-symbiotic nitrogen fixing bacteria of the genera *Azotobacter*, *Azospirillum*, *Bacillus*, *Klebsiella* etc. associated with different cereals and some grasses have been reported (Boddey and Dobereiner, 1995; Steenhoudt and Vanderleyden, 2000; Kennedy *et al.*, 2004). In these studies, plant growth enhancement is considered not only

Received: 29 Jan 2016. Received in revised form: 17 May 2016. Accepted: 20 May 2016. Published online: 14 June 2016.

the result of biological nitrogen fixation, but also of other mechanisms, such as phytohormones, etc. (Steenhoudt and Vanderleyden, 2000). Therefore, rhizobacteria with multiple mechanisms of action could be successful in the production of forage grasses. Rhizobial strains, known as legume symbiotic nitrogen-fixing bacteria, can also promote non-legume plant growth by mechanisms independent of N_2 fixation, which can be essential in legume-grass mixture inoculation (Hilali *et al*., 2001; Vargas *et al*., 2009, Souza *et al*., 2013). Soil physical and chemical properties, (such as pH, water availability, temperature, salinity, etc.) can affect plant growth and microflora and fauna in the rhizosphere (Antoun and Prevost, 2005). Therefore, the isolation of PGPR tolerant to these factors is also important.

Although there are some studies showing the potential of rhizobacteria inoculation to increase yield of perennial grasses, a limited number is available regarding PGP rhizobacteria effects on the growth and nutrient accumulation in orchardgrass and especially in meadow fescue. The use of indigenous PGPR can be an added advantage since they can easily acclimatize to the natural conditions (Verma *et al*., 2013). Therefore, the aim of the research was to isolate native rhizobacterial strains with multiple plant growth-promoting traits and evaluate their effects on growth and nutrient uptake (N, P, K) of orchardgrass and meadow fescue.

Materials and Methods

Bacterial strains

Seven strains of *Azotobacter chroococcum* (A1, A2, A3, A4, A5, A31 and A136v), used in this study, were isolated from arable land of different localities. Strains of *Bacillus megaterium* 4148pk, LR1K and SNji, strains of *Pseudomonas* sp. luc2, LG, L1K and L2Cr previously isolated as root nodules endophytes of alfalfa (Stajković *et al*., 2009; 2011), as well as two rhizobial strains, *Sinorhizobium meliloti* 218 and *Rhizobium leguminosarum* bv. *trifolii* 459, were also used. *Azotobacter* strains were cultivated in the N free mannitol broth, *Pseudomonas* strains in King B medium (KB), *Bacillus* strains in nutrient broth (NB), and rhizobial strains in yeast mannitol (YM) medium.

Phosphate solubilisation

Strain phosphate solubilising ability was examined on Pikovskaya medium (1948). The appearance of clear zone around the colony (halo), after 15 days of incubation at 28 °C, indicated P solubilisation ability of strains. Halo size was calculated by subtracting colony diameter from the total diameter.

Siderophore production

Siderophore production was determined in plates with CAS-blue agar and appropriate medium according to the procedure Milagres *et al.* (1999). The distance of colour change from blue to orange was measured on the 7th, 14th and 21st day of incubation.

Indole-3-acetic acid production

For indole-3-acetic acid (IAA) detection, the strains were grown in appropriate liquid medium (YM, KB, NB or N free mannitol broth) supplemented with L-tryptophan (2 mg ml^{-1}). Cultures were centrifuged and 1 ml of supernatant was mixed with 2 ml of reagent, which consisted of 4.5 g of $FeCl_3$ per litre in 10.8 M H_2SO_4 (Glickmann and Dessaux, 1995). The appearance of pink colour indicates IAA production and IAA concentration was determined by spectrophotometer at 530 nm.

Tolerance to some environmental factors

Tolerance to NaCl, temperature and pH were examined according to Somasegaran and Hoben (1994).

Vegetative pot experiment

To evaluate plant promoting potential, pots were field with 2 kg of non-sterile soil with the following chemical characteristics: $pH_{(KCl)}$ 3.95, $pH_{(H2O)}$ 5.1, humus 2.83%, N 0.15%, P 28.1 mg kg^{-1}, K 15.94 mg kg^{-1}. Fifty seeds of each grass species were planted in every pot. The experiment was carried out with 6 replications in a completely randomised system and the pots were kept in greenhouse conditions. Orchardgrass (variety K24) and meadow fescue (variety K21) seeds were inoculated with single strains or with their mixture. Inoculums were prepared mixing 2 g of sterile peat with 0.5 ml of liquid single strain culture containing >10^9 cells ml^{-1} or with strains mixture in 1:1 ratio. For every grass species there were 6 different treatments with inoculation (single or co-inoculation) and uninoculated control plants (Ø). Plants were harvested after seven weeks.

Plant samples analyses

Plant shoots were separated from roots and dried in an oven at 70 °C till constant weight and the average dry weight per plant were calculated. The percentage of shoot N was determined from dried and ground plant samples, using the CNS analyzer, and the percentage was used to calculate total N content. In plant material samples, P and K contents were determined by dry ashing at 550 °C and acid digestion, after which, P was determined colorimetrically and K by flame photometry (Egner *et al.*, 1960).

Statistical analysis

The effect of inoculation was evaluated using analysis of variance (ANOVA) (statistical program COSTAT), and differences between means were tested for significance by Duncan's multiple range test.

Results and Discussion

Strains of *A. chroococcum* showed optimal growth in the pH range of 6 to 12, and weaker growth at pH 5, while strains of *Pseudomonas* sp. and *B. megaterium* grew well at pH 5, but they tolerated only pH 9 (Table 1). Similar results for *Azotobacter* strains were obtained by Ninawe and Paulraj (1997), who detected their growth in the pH range of 5-10, with the optimum about 7.5, while the growth and N-fixation declined with further pH increase or decrease. Detected pH tolerance for *Pseudomonas* and *Bacillus* also corresponds to the previous reports (Mishra *et al.*, 2011; Peter and Pandey, 2014).

Strains of *A. chroococcum* showed the lowest NaCl tolerance, with the good growth in the presence of 0.5% NaCl, and visibly weaker growth going up to 2% NaCl. *Pseudomonas* sp. strains grew well up to 3% NaCl, while strains of *B. megaterium* grew up to 7% NaCl, and the most tolerant strain LR1K, showed moderate growth in the presence of 9% NaCl (Table 1). As expected, *B. megaterium* strains tolerated the highest temperature, showing moderate growth at 50 °C, while other strains could grow up to 35 °C. The lowest temperature for

Table 1. Effect of different NaCl concentration, pH, and temperature on the strain growth

	Azotobacter							*Bacillus*			*Pseudomonas*			
	A1	A2	A3	A4	A5	A31	A136V	LR1K	4148pk	SNji	L2Cr	LG	luc2	L1K
Temperature (°C)														
4	-	-	-	-	-	-	-	-	-	-	±	±	±	±
12	±	±	±	±	±	±	±	±	±	±	±	±	±	±
28	+	+	+	+	+	+	+	+	+	+	+	+	+	+
35	+	+	+	+	+	+	+	+	+	+	+	+	+	+
45	-	-	-	-	-	-	-	+	+	+	-	-	-	-
50								±	±	±				
55								-	-	-				
NaCl tolerance (%)														
0.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1	±	±	±	±	±	±	±	+	+	+	+	+	+	+
1.5	±	±	±	±	±	±	±	+	+	+	+	+	+	+
2	±	±	±	±	±	±	±	+	+	+	+	+	+	+
3	-	-	-	-	-	-	-	+	+	+	+	+	+	+
5								+	+	+	-	-	-	-
7								+	+	+	-	-	-	-
9								±	-	-				
								-	-	-				
pH tolerance														
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	±	±	±	±	±	±	±	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	-	-	-	-	-	-	-
11	+	+	+	+	+	+	+							
12	+	+	+	+	+	+	+							
13	-	-	-	-	-	-	-							

+ Good growth; ± moderate growth; - no growth

Table 2. Plant growth promoting traits of the strains

	Azotobacter							*Bacillus*			*Pseudomonas*				Rhizobia	
	A1	A2	A3	A4	A5	A31	A136V	LR1K	4148pk	SNji	L2Cr	LG	luc2	L1K	218	459
P solubilisation (halo in mm)																
	-	10	-	nt	7	9	-	1	1	1.5	12	13	10	10	4	10
Siderophore production (color change in mm)																
7th day	-	-	-	-	-	-	-	8	9	7	18	20	17	16	-	-
14th day	-	-	-	-	-	-	-	21	28	14	25	28	25	26	-	-
21st day	-	-	-	-	-	-	-	30	35	15	34	35	35	35	-	-
IAA production(μg ml^{-1})																
24^{h}	28	51	28	29	21	31	36	59	54	76	67	89	90	nt	nt	10
48^{h}	56	143	56	76	63	103	72	35	43	68	122	128	120		200	66
72^{h}	105	141	105	97	106	141	141									141
96^{h}	146	105	146	97	121	146	146									

- no production/solubilisation; nt not tested

moderate growth was 12 °C for *Azotobacter* and *Bacillus*, while *Pseudomonas* strains showed moderate growth at 4 °C. *Azotobacter* is likely more sensitive to salinity and high temperature with no growth at 45 °C and 1% or 2.5% of NaCl (Sharma and Rai, 2013; Nosrati *et al*., 2014). However, some literature data indicate that most *Azotobacter* strains grew slowly in the presence of 4% NaCl, while 6% NaCl completely stopped their growth. Moreover, some *Azotobacter* strains grew in the presence of even 10% NaCl (Akhter *et al*., 2012). *Bacillus* strains tolerated 50 °C, as well as salinity of 7% NaCl, in contrast to genera *Pseudomonas* with only few strains tolerant to these factors (Kumar *et al*., 2014).

Three *A. chroococcum* strains A2, A5 and A31 solubilized phosphates in PVK medium (7-10 mm halo) (Table 2). Among *Pseudomonas* strains, P solubilisation ability was detected for L1K, but it was also confirmed for strains L2Cr, LG, luc2 (Stajković *et al*., 2011; 2014). *B. megaterium* strains confirmed week ability of phosphate solubilisation previously detected (Stajković *et al*., 2009). Numerous literature data also indicate good phosphate solubilisation ability of *Azotobacter* strains (Garg *et al*., 2001; Farajzadeh *et al*., 2012; Nosrati *et al*., 2014) as well as *Pseudomonas* and *B. megaterium* strains (Bhakthavatchalu *et al*., 2013; Deshwal and Kumar, 2013). Siderophore production was not detected for *A. chroococcum* strains, while all *B. megaterium* strains showed this ability, but with lower efficiency (lower diameter of colour change) compared to *Pseudomonas* sp. strains (Table 2). Siderophore production is not such a common characteristic of *Azotobacter*; although some strains possessed this feature (Muthulselvan and Balagurunathan, 2013), research conducted by Joseph *et al.* (2007) indicated that none of the 40

Table 3. Effect of different rhizobacteria on growth of meadow fescue (*Festuca pratensis* Huds.)

Treatment	Plant height (cm)	Shoot dry weight (g pot^{-1})	N %	Total N content (mg pot^{-1})	P %	Total P content (mg pot^{-1})	K %	Total K content (mg pot^{-1})
A2	21.4^{b}	0.926^{bc}	4.28^{b}	39.63^{b}	0.18^{a}	1.66^{b}	3.36^{a}	31.11^{bc}
A2+218	20.82^{b}	1.289^{a}	4.13^{b}	53.24^{a}	0.17^{a}	2.19^{a}	3.48^{a}	44.86^{a}
A2+L2Cr	24.35^{a}	1.208^{a}	4.19^{b}	50.62^{a}	0.18^{a}	2.17^{a}	3.67^{a}	44.33^{a}
A5	21.27^{b}	0.777^{c}	4.61^{a}	35.82^{b}	0.19^{a}	1.47^{b}	3.53^{a}	27.43^{c}
A5+218	24.22^{a}	1.268^{a}	4.18^{b}	53.00^{a}	0.18^{a}	2.28^{a}	3.50^{a}	44.38^{a}
A5+L2Cr	20.64^{b}	1.137^{ab}	4.10^{b}	46.62^{ab}	0.17^{a}	1.93^{ab}	3.41^{a}	38.77^{ab}
Ø	21.1^{b}	0.969^{bc}	4.12^{b}	39.92^{b}	0.19^{a}	1.84^{b}	3.61^{a}	34.98^{bc}

Treatments: A2- *A. chroococcum* A2; A2+218 - *A. chroococcum* A2+*S. meliloti* 218; A2+L2Cr - *A. chroococcum* A1+*Pseudomonas* sp. L2Cr; A5- *A. chroococcum* A5; A5+218 - *A. chroococcum* A5+ *S. meliloti* 218; A5+L2Cr - *A. chroococcum* A5+ *Pseudomonas* sp. L2Cr; Ø- uninoculated control plants; a-d: Means in a column followed by the same letter are not significantly different according to Duncan's multiple range test (P≤0.05).

Table 4. Effect of different rhizobacteria on growth of orchardgrass (*Dactylis glomerata* L.)

Treatment	Plant height (cm)	Shoot dry weight (g pot^{-1})	N (%)	Total N content (mg pot^{-1})	P (%)	Total P content (mg pot^{-1})	K (%)	Total K content (mg pot^{-1})
A1	21.67^{a}	1.294^{a}	3.67^{a}	47.50^{a}	0.20^{a}	2.59^{abc}	3.15^{a}	40.76^{a}
A1+459	19.33^{a}	0.978^{ab}	3.92^{a}	38.44^{ab}	0.20^{a}	1.96^{cd}	3.04^{a}	29.73^{bc}
A1+LR1K	21.95^{a}	1.292^{a}	3.88^{a}	47.61^{a}	0.23^{a}	2.97^{a}	3.13^{a}	40.44^{ab}
A31	19.27^{a}	1.014^{ab}	3.87^{a}	39.21^{ab}	0.20^{a}	2.02^{bcd}	3.10^{a}	31.43^{abc}
A31+459	19.74^{a}	0.705^{b}	3.95^{a}	27.87^{b}	0.22^{a}	1.55^{d}	3.21^{a}	22.63^{c}
A31+LR1K	21.73^{a}	1.227^{a}	3.80^{a}	49.10^{a}	0.22^{a}	2.70^{ab}	3.18^{a}	39.02^{ab}
Ø	20.32^{a}	0.813^{b}	3.70^{a}	30.08^{b}	0.21^{a}	1.71^{d}	3.22^{a}	26.18^{c}

Treatments: A1- *A. chroococcum* A1; A1+459 - *A. chroococcum* A1+ *R. leguminosarum* bv. *trifolii* 459; A1+LR1K- *A. chroococcum* A1+ *B. megaterium* LR1K; A31- *A. chroococcum* A31; A31+459 - *A. chroococcum* A31+ *R. leguminosarum* bv. *trifolii* 459; A31+LR1K- *A. chroococcum* A31+ *B. megaterium* LR1K; Ø- uninoculated control plants; a-d: Means in a column followed by the same letter are not significantly different according to Duncan's multiple range test (P≤0.05).

tested *Azotobacter* strains produced siderophores. Indole-3-acetic acid (IAA) production, the most physiologically active auxin, is a major property of rhizobacteria which promote plant growth (Hayat *et al*., 2010). Production of IAA was detected in all *Azotobacter* strains and quantified in these and *Bacillus* and *Pseudomonas* strains. Strains of *B. megaterium* showed the lowest IAA production, while strains of *A. chroococcum* reached 146 μg ml^{-1}. Rhizobial strains (218 and 459) did not produce siderophores, but they solubilized phosphates and produced significant amount of IAA.

Inoculation of both grasses with some rhizobacterial strains, increased height, shoot dry weight and total N, P and K contents significantly (Tables 3 and 4). Inoculation of meadow fescue with single *A. chroococcum* strains A2 and A5, did not influence any significant changes in the parameters investigated, compared to uninoculated control Ø. However, co-inoculation with *A. chroococcum* A2 + *Pseudomonas* sp. L2Cr, A2 + *S. meliloti* 218 and A5 + *S. meliloti* 218 significantly increased SDW and total N, P and K contents (mg pot^{-1}) compared to uninoculated control Ø. The SDW increase was 25%, 33% and 31%, respectively, compared to control Ø (100%). Considering that *A. chroococcum* did not increase SDW individually, it is possible that the increased SDW in co-inoculated plants is the result of *S. meliloti* or *Pseudomonas* sp. strain. Content of N (%) in SDW was increased only in the A5 treatment, which realised the lowest SDW, while content of P and K (%) did not significantly differ among treatments. Mineral nutrient contents in plants depend on plant genotype (cultivars), and to a lesser extent, on mineral nutrition and ecological factors.

Inoculation of orchardgrass with single *A. chroococcum* A1 strain and co-inoculation with A1+ *B. megaterium* LR1K and A31+ *B. megaterium* LR1K increased SDW for about 60% compared to control Ø (100%). In these treatments total N, P and K contents were also increased, compared to control Ø. Contents of N, P and K (%) did not significantly differ among treatments.

Nitrogen percentage in SDW of meadow fescue and orchardgrass ranged between 4.10-4.61% and 3.67-3.95% respectively, which is slightly higher than sufficiency ranges, 2.5-3.5% for both species (Schwab *et al*., 2007). The P content was 0.17-0.19% for meadow fescue and 0.20-0.23% for orchardgrass which is lower than sufficiency ranges of 0.26-0.4% (actively growing plants of tall fescue) and 0.25-0.35% (for 5 week old plants) respectively. Higher N% could be the consequence of plant development early phase and limited plant growth, while lower P content was possibly caused by low P soil content. The K content was optimal in both plants in all treatments.

Number of reports have proven that *Azotobacter* application, as a free-living nitrogen-fixer, improves the yield of different plants, including grass species, wheat, English ryegrass, Italian ryegrass, etc. (Delić *et al*., 2012; Stamenov *et al*., 2012; Miri *et al*., 2013). In this research we have identified only one (A1) of 7 isolated *A. chroococcum* strains, which is able to promote grass (orchardgrass) growth individually (about 60% SDW increase compared to control Ø).

It is well-known that rhizobial strains, besides N_2 fixation with legumes, can promote growth of non-legume plants by mechanisms independent of biological N_2 fixation (mainly through phytohormones production). Previous studies showed that rhizobium can stimulate the growth of non-legumes, such as wheat (Hilali *et al*., 2001), rice (Vargas *et al*., 2009; Souza *et al*., 2013) and oats (Stajković-Srbinović *et al*., 2014). In this study, *S. meliloti* 218 strain showed significant growth promotion of meadow fescue in co-inoculation with *A. chroococcum* A2 and A5, but *R. leguminosarum* bv. *trifolii* 459 did not have any positive effects on orchardgrass. Similarly, the inoculation of orchardgrass with *Beijerinckia* or *Azospirillum* did not significantly increase dry mass during a two-year period (Dragomir and Moisuc, 2007). However, in the same research, when orchardgrass was grown in the mixture with alfalfa and co-inoculated with rhizobium *Beijerinckia* and *Azospirillum*, an increase in dry mass was noted in the second year, indicating a benefit inter-species relationship. The ability of *S. meliloti* strain 218 to promote grass growth could be of special importance, since grasses are often grown in mixture with alfalfa, a host plant of *S. meliloti*.

Strains of *Pseudomonas* and *Bacillus* are among the most efficient PGPR and promoted growth and yield of variety of plants (Hayat et al., 2010). Single inoculation with *P. fluorescens* or *B. subtilis* showed a statistically significant increase in the yield of fresh and dry mass of English ryegrass (Delić *et al*., 2012; Stamenov *et al*., 2012a). In our research *B. megaterium* LR1K showed some PGP potential, since co-inoculation with *A. chroococcum* A31 increased all parameters of orchardgrass in respect to control Ø, while single inoculation with *A. chroococcum* A31 strain did not. It is possible that the PGP effect of LR1K could not be visible in co-inoculation with A1, due to the good effect of A1 strain alone. Previously the strain *Pseudomonas* sp. L2Cr increased oats and barley growth in single inoculation (Stajković-Srbinović *et al*., 2014), while in this research co-inoculation of meadow fescue with *A. chroococcum* A2 and L2Cr also increased plant growth.

In the research presented it is difficult to connect positive influence of particular strains on growth promotion with their PGP mechanisms detected *in vitro*. All the strains used showed more mechanisms that might be involved in plant growth promotion, and within each genera (*Azotobacter, Bacillus, Pseudomonas, Rhizobium*), there were almost no differences in PGP characteristics. Most of the strains belong to nitrogen-fixing bacteria, produced IAA, could solubilise phosphates and differed only in siderophore production ability. Therefore, the presence of specific growth promoting traits does not guarantee that an isolate will promote plant growth.

Conclusions

All rhizobacterial strains tested showed some PGP characteristics, with IAA production as a dominant characteristic. Different rhizobacterial strains improved the growth of both investigated forage grasses. Shoot dry weight of inoculated orchardgrass increased significantly up to 59%, compared to the uninoculated control plants, while the increase in SDW of inoculated meadow fescue went up to 33% over uninoculated control plants. Total content of N, P or K in shoot dry weight increased in some treatments, depending on the strains applied and plant species. The results demonstrate that grass seed inoculation could be of practical benefit in sustainable agricultural practices.

Acknowledgments

This research was supported by the Ministry of Education and Science and Technological Development of the Republic of Serbia, Projects III46007 and TR37006.

References

Antoun H, Prevost D (2005). Ecology of plant growth promoting rhizobacteria. In: Siddiqui ZA (Ed). PGPR: Biocontrol and Biofertilization. Springer Netherlands pp 1-39.

Akhter MS, Hossain JS, Amir-Hossain SK, Datta RK (2012). Isolation and characterization of salinity tolerant *Azotobacter* sp. Greener Journal of Biological Sciences 2:43-51.

Baran S, Wójcikowska-Kapusta A, Żukowska G, Bik-Małodzińska M, Wesołowska-Dobruk S (2015). Influence of sludge-ash composts on some properties of reclaimed land. Archives of Environmental Protection 41:82-88.

Baris O, Sahin F, Turan M, Orhan F, Gulluce M (2014). Use of plant-growth-promoting rhizobacteria (PGPR) seed inoculation as alternative fertilizer inputs in wheat and barley production. Communications in Soil Science and Plant Analysis 45:2457-2467.

Bhakthavatchalu S, Shivakumar S, Sullia SB (2013). Characterization of multiple plant growth promotion traits of *Pseudomonas aeruginosa* FP6, a potential stress tolerant bio-control agent. Annals of Biological Research 4:214-223.

Boddey RM, Dobereiner J (1995). Nitrogen fixation associated with grasses and cereals: Recent progress and perspectives for the future. Fertilizer Research 42:241-250.

Delić D, Stajković-Srbinović O, Kuzmanović D, Rasulić N, Maksimović S, Radović J, Simić A (2012). Influence of plant growth promoting rhizobacteria on alfalfa, *Medicago sativa* L. yield by inoculation of a preceding Italian ryegrass, *Lolium multiflorum* Lam. In: Barth S, Milbourne D (Eds). Breeding strategies for sustainable forage and turf grass improvement, Springer Netherlands pp 333-339.

Deshwal VK, Kumar P (2013). Production of plant growth promoting substance by *Pseudomonads*. Journal of Academia and Industrial Research 2:221-225.

Dinesh R, Anandaraj M, Kumar A, Bini YK, Subila KP, Aravind R (2015). Isolation, characterization, and evaluation of multi-trait plant growth promoting rhizobacteria for their growth promoting and disease suppressing effects on ginger. Microbiological Research 173:34-43.

Dragomir C, Moisuc A (2007). Bacterial inoculation effect upon yield capacity in alfalfa and orchardgrass. Lucrări Ştiinţifice, Facultatea de Agricultură USAMVB Timişoara 38:275-278.

Egnér H, Riehm H, Domingo WR (1960). Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Böden. II. Chemische Extraktionsmethoden zur Phosphor- und Kaliumbestimmung, [Studies concerning the chemical analysis of soils as background for soil nutrient assessment. II Chemical extracting methods to determinate the phosphorous and potassium content of soil] Kungliga Lantbrukshögskolans Annaler 26:199-215.

Farajzadeh D, Yakhchali B, Aliasgharzad N, Bashir NS, Farajzadeh M (2012). Plant growth promoting characterization of indigenous *Azotobacteria* isolated from soils in Iran. Current Microbiology 64:397-403.

Garg SK, Bhatnagar A, Kalla A, Narula N (2001). In vitro nitrogen fixation, phosphate solubilization, survival and nutrient release by *Azotobacter* strains in an aquatic system. Bioresource Technology 80:101-109.

Glick BR (1995). The enhancement of plant growth by free-living bacteria. Canadian Journal of Microbiology 41:109-117.

Glickmann E, Dessaux Y (1995). A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Applied and Environmental Microbiology 61:793-796.

Hayat R, Ali S, Amara S, Khalid R, Ahmed I (2010). Soil beneficial bacteria and their role in plant growth promotion: a review. Annals of Microbiology 60:579-598.

Hilali A, Prevost D, Broughton WJ, Antoun H (2001). Effects of inoculation with *Rhizobium leguminosarum* biovar *trifolii* on wheat cultivated in clover crop rotation agricultural soil in Morocco. Canadian Journal of Microbiology 47:590-593.

Imran A, Mirza MS, Shah TM, Malik KA, Hafeez FY (2015). Differential response of kabuli and desi chickpea genotypes toward inoculation with PGPR in different soils. Frontiers in Microbiology 6:article 859.

Joseph B, Patra RR, Lawrence R (2007). Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arietinum* L.). International Journal of Plant Production 1:141-152.

Kennedy IR, Choudhury ATMA, Kecskés ML (2004). Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited?. Soil Biology and Biochemistry 36(8):1229-1244.

Kumar GP, Hassan Ahmed SKM, Desai S, Amalraj ELD, Rasul A (2014). *In vitro* screening for abiotic stress tolerance in potent biocontrol and plant growth promoting strains of *Pseudomonas* and *Bacillus* spp. International Journal of Bacteriology 195946, 6, doi:10.1155/2014/195946.

Milagres AMF, Napoleao D, Machuca A (1999). Detection of siderophore production from several fungi and bacteria by modification of chrome azurol S (CAS) agar plate assay. Journal Microbiology Methods 37:1-6.

Miri MR, Tohidi Moghadam HR, Ghooshchi F, Zahedi H (2013). Effect of *Azotobacter* and arbuscular mycorrhizal colonization enhance wheat growth and physiological traits under well-watered and drought conditions. Advances in Environmental Biology 7:4630-4636.

Mishra RR, Prajapati S, Das J, Dangar TK, Das N, Thatoi H (2011). Reduction of selenite to red elemental selenium by moderately halotolerant *Bacillus megaterium* strains isolated from Bhitarkanika mangrove soil and characterization of reduced product. Chemosphere 84:1231-7.

Muthulselvan I, Balagurunathan R (2013). Siderophore production from *Azotobacter* sp. and its application as biocontrol agent. International Journal of Current Research and Review 5:23-35.

Ninawe AS, Paulraj R (1997). Effect of pH on growth and nitrogen fixation of *Azotobacter* spp. Journal of Aquaculture 5:23-29.

Nosrati R, Owlia P, Saderi H, Rasooli I, Malboobi MA (2014). Phosphate solubilization characteristics of efficient nitrogen fixing soil *Azotobacter* strains. Iranian Journal of Microbiology 6:285-295.

Peter JK, Pandey N (2014). Bioprospecting phosphate solubilisation and PGP Activities of native strains of *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* from Bamboo (*Bambusa bamboo*) rhizosphere: International Journal of Research 1:702-717.

Pikovskaya RI (1948). Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. Mikrobiologiya 17:362-370.

Saia S, Rappa V, Ruisi P, Abenavoli MR, Sunseri F, Giambalvo D, Frenda AS, Martinelli F (2015). Soil inoculation with symbiotic microorganisms promotes plant growth and nutrient transporter genes expression in durum wheat. Frontiers in Plant Science 6:article815.

Schwab GJ, Lee CD, Pearce R (2007). Sampling Plant Tissue for Nutrient Analysis. Dept Plant and Soil Sciences, WO Thom, Div Regulatory Services. http://www2.ca.uky.edu/agc/pubs/agr/agr92/agr92.pdf.

Sharma T, Rai N (2013). Study of *Azotobacter species* isolated from different soil samples of Tarai region of Uttarakhand. Biotechnology International 6:69-73.

Somasegaran P, Hoben HJ (1994). Handbook for rhizobia. Methods in legume *Rhizobium* technology. Springer New York.

Sosnowski J, Jankowski K, Domański P, Herda D (2015). Dry matter yield of different varieties of *Dactylis glomerata* and *Festuca pratensis*. Journal of Ecological Engineering 16:211-216.

Souza R, Beneduzi A, Ambrosini A, Costa PB, Meyer J, Vargas LK, Schoenfeld R, Passaglia LMP (2013). The effect of plant growth-promoting rhizobacteria on the growth of rice (*Oryza sativa* L.) cropped in southern Brazilian fields. Plant and Soil 366(1-2): 585-603.

Stajković O, De Meyer S, Miličić B, Willems A, Delić D (2009). Isolation and characterization of endophytic non-rhizobial bacteria from root nodules of alfalfa (*Medicago sativa* L.). Botanica Serbica 33:107-114.

Stajković O, Delić D, Jošić D, Kuzmanović Đ, Rasulić N, Knežević-Vukčević J (2011). Improvement of common bean growth by co-inoculation with *Rhizobium* and plant-growth promoting bacteria. Romanian Biotechnological Letters 16:5919-5926.

Stajković-Srbinović O, Delić D, Kuzmanović Đ, Protić N, Rasulić N, Knežević-Vukčević J (2014). Growth and nutrient uptake in oat and barley plants as affected by rhizobacteria. Romanian Biotechnological Letters 19:9429-9436.

Stamenov D, Jarak M, Đurić S, Hajnal-Jafari T (2012). Microbial inoculation effect on the yield of English ryegrass and number of rhizosperic microorganisms. Plant Soil and Environment 58:477-480.

Stamenov D, Jarak M, Đurić S, Hajnal-Jafari T, Anđelković S (2012a). The effect of *Azotobacter* and *Actinomycetes* on the growth of English ryegrass and microbiological activity in its rhizosphere. Research Journal of Agricultural Science 44:93-99.

Steenhoudt O, Vanderleyden J (2000). *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. FEMS Microbiology Review 24:487-506.

Tomić Z, Lugić Z, Radović J, Sokolović D, Nešić Z, Krnjaja V (2007). Perennial legumes and grasses stable source of quality livestock fodder feed. Biotechnology in Animal Husbandry 23:559-572.

Vargas LK, Lisboa BB, Schlindwein G, Granada CE, Giongo A, Beneduzi A, Passaglia LMP (2009). Occurrence of plant growth-promoting traits in clover-nodulating rhizobia strains isolated from different soils in Rio Grande do Sul State. The Revista Brasileira de Ciência do Solo 33:1227-1235.

Verma JP, Yadav J, Tiwari KN, Kumar A (2013). Effect of indigenous *Mesorhizobium* spp. and plant growth promoting rhizobacteria on yields and nutrients uptake of chickpea (*Cicer arietinum* L.) under sustainable agriculture. Ecological Engineering 51:282-286.