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Efficacy of *Pseudomonas chlororaphis* subsp. *aurantiaca* SR1 for Improving Productivity of Several Crops

Susana B. Rosas, Nicolás A. Pastor, Lorena B. Guiñazú, Javier A. Andrés, Evelin Carlier, Verónica Vogt, Jorge Bergesse and Marisa Rovera
Laboratorio de Interacción Microorganismo–Planta. Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Campus Universitario. Ruta 36, Km 601, (5800) Río Cuarto, Córdoba Argentina

1. Introduction

The recognition of plant growth-promoting rhizobacteria (PGPR) as potentially useful for stimulating plant growth and increasing crop yield has evolved over the past years. Currently, researchers are able to successfully use them in field experiments. The use of PGPR offers an attractive way to supplement or replace chemical fertilizers and pesticides. Most of the isolates cause a significant increase in plant height, root length and dry matter production of plant shoot and root. Some PGPR, especially if they are inoculated on seeds before planting, are able to establish on roots. Also, PGPR can control plant diseases. These bacteria are a component of integrated management systems, which use reduced rates of agrochemicals. Such systems might be used for transplanted vegetables in order to produce more vigorous seedlings that would be tolerant to diseases for at least a few weeks after transplanting to the field (Kloepper *et al.*, 2004). Commercial applications of PGPR are being tested and are frequently successful. However, a better understanding of the microbial interactions that result in plant growth enhancements will greatly increase the success of field applications (Burr *et al.*, 1984).

In the last few years, the number of identified PGPR has been increasing, mainly because the role of the rhizosphere as an ecosystem has gained importance in the functioning of the biosphere. Several species of bacteria like *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus* and *Serratia* have been reported to enhance plant growth. Of these, the genera that are predominantly studied and increasingly marketed as biological control agents include *Bacillus*, *Streptomyces* and *Pseudomonas* (Glick, 1995; Joseph *et al.*, 2007; Kloepper *et al.*, 1989; Okon & Labandera-González, 1994).

2. Fluorescent *Pseudomonas*

The fluorescent pseudomonads produce a variety of biologically active natural products (Budzikiewicz, 1993; Leisinger & Margraff, 1979), many of which have an ecological function in these gram-negative bacteria. Some of these natural products contribute to the

suppression of plant-pathogenic fungi (Dowling & O'Gara, 1994; Thomashow, 1996) whereas others are important virulence factors against certain plant-pathogenic *Pseudomonas* species (Bender, *et al.*, 1999). Because secondary metabolism significantly contributes to the molecular ecology of several *Pseudomonas* species and has also provided lead compounds for crop protection applications, several gene clusters that encode secondary metabolic pathways in this genus have been sequenced and characterized. For example, some *Pseudomonas* biosynthetic pathways have functionally combined modular, dissociated, or chalcone synthase-like polyketide synthases with adenylating enzymes (pyoluteorin, mupirocin, coronatine) or with components of fatty acid synthases (2,4-diacetylphloroglucinol) (Bender *et al.*, 1999; Guhien *et al.*, 2004). Phenazines are an important type of secondary metabolites. Phenazine antibiotics, nitrogen-containing aromatic compounds, are especially active against lower fungi and most gram-positive and gram-negative phytopathogenic bacteria (Chin-A-Woeng *et al.*, 2003). The most studied phenazinic substances are: phenazine 1-carboxilic acid, phenazine 1 carboxamide, aeruginosin A, pyocyanin, 2-hydroxyphenazine 1-carboxilic acid, 1-hydroxyphenazine (Price Whelan *et al.*, 2006; Raajmakers *et al.*, 1997). The phenazine antibiotics complex of *P. aurantiaca* B-162 consists of 1-oxyphenazine, phenazine, and phenazine-1,6 dicarboxylate (their common precursor) (Feklistova & Maksimova, 2005). Also, Feklistova & Maksimova (2008) reported the production of N-hexanoyl homoserine lactone (HHL) by *P. aurantiaca* B-16.

Mehnaz *et al.* (2009) described the characterization of a newly isolated strain, PB-St2, of *P. aurantiaca* and its main secondary metabolites. This strain showed antifungal activity against strains of *C. falcatum* and *Fusarium* spp., phytopathogens of agricultural significance. Two main antibiotics were isolated and identified to be phenazine-1-carboxylic acid and 2-hydroxyphenazine. In addition, strain PB-St2 produces HHL, a compound that indicates the presence of a quorum-sensing mechanism. Quorum sensing is the major mechanism by which many bacteria regulate production of antifungal factors. Strain PB-St2 produce hydrogen cyanide (HCN) and siderophores as PGPR traits. All of these data suggest that PB-St2 could be used as a potential effective biocontrol agent or biofertilizer to decrease the incidence of plant diseases and promote plant growth (Mehnaz *et al.*, 2009).

Liu *et al.* (2007) detected the production of two main antifungal substances produced by *Pseudomonas chlororaphis* GP72: phenazine-1-carboxylic acid and 2-hydroxyphenazine. This strain also produced the quorum-sensing signalling molecules N-butanoyl-L-homoserine lactone and N-hexanoyl-L-homoserine lactone. In addition, Ramarathnam & Dilantha Fernando (2006) reported that one isolate of *P. aurantiaca*, termed DF200, tested positive for the presence of pyrrolnitrin and phenazine biosynthetic genes.

Several *Pseudomonas* strains have already been marketed as commercial biocontrol products, such as Cedomon (BioAgri AB, Upsala, Sweden), a seed treatment based on a *Pseudomonas chlororaphis* strain providing protection against seedborne diseases in barley. Similarly, Mycolytin is an antifungal biopesticide containing *P. aurantiaca* M-518 (Omelyanets & Melnik, 1987). The genus *Pseudomonas* is well known for producing metabolites which stimulate plant growth and root colonization by beneficial microorganisms. The *Pseudomonas* also synthetize phytohormones and siderophores and solubilize phosphates (Mikuriya *et al.*, 2001; Kang *et al.*, 2006; Arshad & Frankernberger 1991; Rosas *et al.*, 2006). *Pseudomonas aurantiaca* S-1 can serve as a natural source of pesticides towards phytopathogens like *Fusarium oxysporum* P1 and *Pseudomonas syringae* pv. *glycinea* BIM B-

280. This strain was able to produce indole acetic acid and siderophores (Mandryk *et al*., 2007).
Feklistova & Maximova (2009) extracted and identified gibberellins synthesized by *P. aurantiaca* B-162 and proposed a new method for selecting bacteria that produce plant growth-promoting substances. By NG-mutagenesis and a consequent selection on toxic analogues of the gibberellins, regulative analogue-resistant mutants of *P. aurantiaca* were obtained. These were capable of overproducing gibberellic acid (31 mg l^{-1}), that was 2,3 times in excess of that of the master strain.
Thus, research on *Pseudomonas* natural products provides an opportunity to study not only the ecological function of secondary metabolism but also the potential diversity found in secondary metabolic pathways.

3. *Pseudomonas chlororaphis* subsp. aurantiaca SR1

P. chlororaphis subsp. *aurantiaca* SR1 (GenBank accession number GU734089) was isolated from the rhizosphere of soybean in the area of Río Cuarto, Córdoba, Argentina. It was initially classified as *P. aurantiaca* by using the BIOLOG (Biolog Inc., Hayward, CA) system (Rosas *et al*., 2001) and, more recently, by amplification and sequencing of a partial fragment from the 16S rDNA gene. The species *Pseudomonas aurantiaca* was recently reclassified as *P. chlororaphis* subsp. *aurantiaca* (Peix *et al*., 2007).
Strain SR1 inhibits a wide range of phytopathogenic fungal species including *Macrophomina phaseolina*, *Rhizoctonia* spp. T11, *Fusarium* spp., *Alternaria* spp., *Pythium* spp., *Sclerotinia minor* and *Sclerotium rolfsii* (Rosas *et al*., 2001). It produces siderophores, behaves as an endophyte and is capable of promoting plant growth through mechanisms that involve phytohormone-like substances (Rovera *et al*., 2008). For instance, SR1 shows the ability to produce indole 3-acetic acid (IAA). In addition, strain SR1 is able to colonize the root-system of several crops, maintaining appropiate population densities in the rhizosphere area (Rosas *et al*., 2005). SR1 was also shown to produce signal molecules such as acyl homoserine lactones (AHL's).
Recently, PCR assays were carried out to detect *phlD* and *phz*, genes involved in the biosynthesis of 2,4-diacetylphloroglucinol (DAPG) and phenazine-1-carboxylic acid (PCA), respectively, in strain SR1 through the use of primers and protocols described by Raaijmakers *et al.* (1997). Also, PCR assays involving the specific primers to detect *prnD* and *pltC*, genes encoding the production of pyrrolnitrin (PRN) and pyoluteorin (PLT), respectively, were performed as described by De Souza & Raaijmakers (2003). On the other hand, detection of *hcnAB* genes (involved in the biosynthesis of HCN synthetase) was performed by PCR using the primers PM2-F (5′-TGCGGCATGGGCGCATTGCTGCCTGG-3′) and PM2-R (5′-CGCTCTTGATCTGCAATTGCAGGC-3′) (Svercel *et al*., 2007). As a result, fragments of the predicted size for PCA, PRN and HCN were amplified from the DNA of strain SR1.

4. Effect of inoculation with strain SR1 on agronomically important crops

4.1 Wheat (*Triticum aestivum* L.)

In these studies, we evaluated the effect of inoculating wheat seeds with strain SR1 on plant growth, under field conditions (Carlier *et al*., 2008; Rosas *et al*., 2009). Experiments were conducted with a complete randomized block design with seven blocks. Each block consisted of six plots (one per treatment and each of 7.20 m^2). Plots were separated in

between by a distance of 1 m. Six rows (separated by 0.20 m) per block were sowed by using a plot seed drill. The sowing density was 120 kg ha^{-1} of seeds. The six treatments were: (1) uninoculated seeds in unfertilized soil (control); (2) uninoculated seeds in soil fertilized with 80 kg ha^{-1} of urea - 60 kg ha^{-1} of diammonium phosphate (100% dose); (3) uninoculated seeds in soil fertilized with 40 kg ha^{-1} of urea - 30 kg ha^{-1} of diammonium phosphate (50% dose); (4) seeds inoculated with SR1 in unfertilized soil; (5) seeds inoculated with SR1 in soil fertilized with the 100% dose; (6) seeds inoculated with SR1 in soil fertilized with the 50% dose. Seeds were inoculated with a formulation manufactured by *Laboratorios Biagro S.A.* containing strain SR1 at 10^9 CFU g^{-1} of peat. Briefly, 40 g inoculant, 20 g S2 adherent (*Laboratorios Biagro S.A.*), and 5 g cell protector S1 (*Laboratorios Biagro* S.A.) were mixed in 80 ml of water. Then, 12 g of this mixture was added to 1 kg wheat seeds to obtain a colony count of 10^5 CFU g^{-1} seeds.

Growth and yield parameters were recorded at the growth stages termed 1.5 (5 leaves), 3.0 (tillering), and 11.4 (ripe for harvest) (Feekes International Scale—Large 1954). At Feekes 1.5, the number of seedlings emerging per m^2 was evaluated. At Feekes 1.5 and 3.0, shoot length, root length, number of tillers, root volume (cm^3), shoot and root dry weight (72 h at 60 ^oC) were assessed. Yield parameters evaluated were: kg ha^{-1}, weight of 1,000 grains, number of spikes per plant, and number of grains per spike.

Inoculation had no effect on emergence of plants, as compared to control. On the other hand, the number of plants per m^2 was higher for inoculation treatments than for fertilization without inoculation. Increases in mean shoot length (14%) were observed for the inoculated/unfertilized treatment and for fertilization with a 50% dose (8%) during Feekes 1.5, compared to control plants. By comparison, a 60% increase in shoot length, relative to control plants, was observed during Feekes 3.0 in plants inoculated and fertilized with a 100% dose. Plants inoculated with strain SR1 showed increases between 47 and 78% in root length during Feekes 1.5 and between 65 and 75% during Feekes 3.0, compared to control. Also, root volume significantly increased during Feekes 1.5 after inoculation and fertilization with the 100% dose (Table 1).

Treatment	**Root length (cm)**		**Root volume (ml)**	
	1.5	3.0	1.5	3.0
Control	101b	204b	0.9c	2.7b
Uninoculated seeds in soil fertilized with the 100% dose	87b	357a	1.2b	4.2a
Uninoculated seeds in soil fertilized with the 50% dose	159a	235b	1.2b	2.4b
Seeds inoculated with SR1 in unfertilized soil	160a	339a	1.7a	4.4a
Seeds inoculated with SR1 in soil fertilized with the 100% dose	181a	346a	2.0a	3.9a
Seeds inoculated with SR1 in soil fertilized with the 50% dose	150a	359a	1.6a	3.6a

Values in each column with different letters are significantly different according to the LSD test ($P<0.05$)

Table 1. Root length and root volume of wheat plants during Feekes 1.5 and 3.0

All mean values of shoot dry weight from inoculation and/or fertilization treatments were higher than those of control plants during Feekes 1.5, but differences were not significant. The higher mean value was obtained after inoculating and fertilizing with the 50% dose, which increased shoot dry weight by 64 mg when compared to control. Throughout Feekes 1.5 and Feekes 3.0, root dry weight was significantly increased by inoculation with strain SR1 alone, as compared to control (Table 2). In addition, the number of tillers increased between 31 and 50 % at Feekes 3.0 in inoculated plants, with or without fertilization, compared to control plants.

Treatment	Dry weight (mg) at Feekes 1.5		Dry weight (mg) at Feekes 3.0	
	Shoot	Root	Shoot	Root
Control	198a	80d	790c	326c
Uninoculated seeds in soil fertilized with the 100% dose	217a	128bcd	1,510a	498ab
Uninoculated seeds in soil fertilized with the 50% dose	228a	109cd	1,080bc	377bc
Seeds inoculated with SR1 in unfertilized soil	223a	190a	1,310ab	536a
Seeds inoculated with SR1 in soil fertilized with the 100% dose	252a	183ab	1,310ab	420abc
Seeds inoculated with SR1 in soil fertilized with the 50% dose	262a	156c	1,370ab	512a

Values in each column with different letters are significantly different according to the LSD test ($P<0.05$)

Table 2. Shoot and root dry weight of wheat plants during Feekes 1.5 and 3.0

When considering the yield parameters, the value of kg ha^{-1} was significantly higher in plants inoculated with SR1 and fertilized with a 50% dose, as compared to control. Regarding number of grains per spike, values for inoculation treatments were always higher than for control. The highest value was observed after inoculation and fertilization with the 50% dose (40% more than the control) (Table 3).

Treatment	Yield (kg ha^{-1})	Number of grains per spike
Control	2,005b	32c
Uninoculated seeds in soil fertilized with the 100% dose	2,169ab	39b
Uninoculated seeds in soil fertilized with the 50% dose	2,264ab	40b
Seeds inoculated with SR1 in unfertilized soil	2,249ab	42b
Seeds inoculated with SR1 in soil fertilized with the 100% dose	1,776b	41b
Seeds inoculated with SR1 in soil fertilized with the 50% dose	2,641a	45a

Values in each column with different letters are significantly different according to the LSD test ($P<0.05$)

Table 3. Parameters of wheat yield

There are few reports on the contribution of inoculation of wheat seeds with *Pseudomonas* strains for improving plant growth and yield under field conditions. For instance, Shaharoona *et al.* (2007) tested several *Pseudomonas* spp. strains in the field to determine their efficacy to increase growth and yield of this crop plant. Their results revealed that all of the strains significantly increased plant height compared to uninoculated control. Strain *P. fluorescens* biotype F caused the maximum increase (16%). This strain also significantly increased the number of grains per spike (11.7% more than the uninoculated control). Another strain, *P. fluorescens* biotype G increased the number of tillers per m^2 by 9%, compared to uninoculated control plants. The maximum increase in 1,000-grain weight was recorded with *P. fluorescens* (ACC_{50}) (34% higher than the uninoculated control). They also reported that inoculation with strain *P. fluorescens* (ACC_{50}) increased grain yield by 39% when compared to the uninoculated control.

4.2 Maize (*Zea mays* L.)

The application of a SR1 formulation on maize seeds allowed us to evaluate its effectiveness as maize growth promoter in the field (Rosas *et al.*, 2009). For these experiments, plots were arranged in a completely randomized design, with four replicates of 156 m^2 for each treatment. Two treatments were included: 1. Seeds inoculated with strain SR1 and 2. Uninoculated seeds. Soil was fertilized with 100 kg ha^{-1} of diammonium phosphate at the sowing time and 100 kg ha^{-1} of urea during V7-8 stages for both treatments.

Length and dry weight of shoot were determined during the V2, V5, V13, R3 and R6 phenological stages. In addition, the following parameters were recorded during the first stages (V2 and V5): root length, root surface (Díaz Vargas *et al.*, 2001) and root volume (Carley & Watson, 1966). The weight of 1,000 grains and grain yield (kg ha^{-1}) were evaluated at the harvest time.

During V2 and V5, the beneficial effect of inoculation with strain SR1 was evidenced at the root system level. Root length increased 28% during V2 and 32% during V5 in inoculated plants. Similar results were obtained with root volume (42% and 36%, respectively) and root surface (39% and 34%, respectively). Shoot dry weight determinations indicated that inoculation with strain SR1 impacted favourably during the whole cycle of the crop. For instance, we observed a 22% increase in shoot dry weight during stage R3, as compared to control plants. Such beneficial effect was also observed for yield parameters. To illustrate, the weight of 1,000 grains and grain yield (kg ha^{-1}) were 11 and 20% higher in inoculated plants.

Egamberdiyeva *et al.* (2002) reported on the effect of a *Pseudomonas fluorescens* strain, termed PsIA12, and three *Pantoea agglomerans* strains (370320, 020315 and 050309) on the growth of maize in the field. Inoculation with these bacterial strains was found to significantly increase the root and shoot growth of maize grown in loamy sand at 16 ^oC. Also, K content was significantly increased in all treatments. More recently, Naveed *et al.* (2008) assessed the performance of an organic fertilizer and three *Pseudomonas* strains prepared as bio-fertilizers for improving growth and yield of maize in the field. Their results revealed that application of bio-fertilizers significantly improved the growth and yield of this crop. Indeed, plant height increased between 4 and 9% after inoculation with the bio-fertilizers and only 2% after treatment with the organic fertilizer, compared to control. Similarly, they observed that total biomass was enhanced between 21 and 39% by bio-fertilizers and 11.4% by the organic fertilizer, compared to control plants. The increases obtained for grain yield (t ha^{-1}) were

14.2% by the organic fertilizer and between 21 and 30% by treatment with the bio-fertilizers. Finally, bio-fertilization caused increases between 14 and 19% in 1,000 grain weight, as compared to control plants.

4.3 Soybean (*Glycine max* (L.) Merril)

Treatment of soybean seeds with strain SR1 was studied to determine the effect of inoculation on plant growth, under greenhouse conditions. At the present time, soybean is the most important oleaginous seed worldwide. In Argentina, soybean cultivation was introduced in the 1970's and it has been characterized by an incredible rate of adoption and growth. Indeed, Argentina is one the main exporters of soybean flour (27% of the world exports) as well as soybean oil (30% of the world exports) (Penna & Lema, 2002).

Soybean seeds were inoculated with a peat-based formulation prepared and packed by *Laboratorios Biagro S.A.* containing strain SR1 at 2.4 x 10^9 CFU g^{-1} peat. Then, plastic pots were filled with sterile soil and 4 inoculated seeds were placed into the soil surface in each pot. The four treatments were: (1) uninoculated seeds (control); (2) seeds inoculated with strain SR1 at 10^7 CFU g^{-1}; (3) seeds inoculated with SR1 at 10^8 CFU g^{-1}; (4) seeds inoculated with SR1 at 10^9 CFU g^{-1}. The inoculant containing 10^8 and 10^7 CFU g^{-1} was obtained by diluting the original formulation with sterile peat. Pots were incubated in a greenhouse. Shoot and root length as well as shoot and root dry weight (120 h at 60 ^oC) was recorded from each treatment after 25 days. Pots were arranged in a completely randomized design with five replicates per treatment.

SR1 at 10^{-9} CFU g^{-1} enhanced shoot length by 31%, as compared to control plants. There were no significant differences in root length. Although there were no significant differences among the three inoculation doses for shoot dry weight, the optimum inoculation dose proved to be 10^8 CFU g^{-1}. Compared to control plants, SR1 at 10^8 CFU g^{-1} increased shoot and root dry weight of inoculated soybean plants by 53 and 14%, respectively (Table 4).

Treatments	Shoot length (cm)	Root length (cm)	Shoot dry weight (mg)	Root dry weight (mg)
Control	34.2b	8.1a	430a	140a
Seeds inoculated with SR1 at 10^7 CFU g^{-1}	41.2b	6.3a	500a	160a
Seeds inoculated with SR1 at 10^8 CFU g^{-1}	41.7b	8.5a	660a	160a
Seeds inoculated with SR1 at 10^9 CFU g^{-1}	45.0a	6.6a	420a	110b

Values in each column with different letters are significantly different according to the Scheffé test ($P<0.05$)

Table 4. Soybean growth parameters

In addition, we evaluated strain SR1 for control of *Macrophomina phaseolina* (Tassi) Goid. in soybean, under greenhouse conditions. *Macrophomina* (the cause of charcoal rot, dry root rot and damping-off of many crop plants) is one of the most destructive plant pathogenic fungal genera. It prevails in the tropics and sub-tropics, inciting diseases in a wide range of hosts (Anjaiah, 2004). Significant yield losses of soybean are reported every year due to charcoal rot fungus *M. phaseolina* (Tassi) Goid. (Senthilkumar *et al.*, 2009). During biocontrol

assays, soybean seeds were inoculated with strain SR1 prior to planting into infested soil. Growth parameters of soybean plants were recorded after 25 days. Compared to pathogen controls, strain SR1, inoculated at 10^7 CFU g^{-1}, increased shoot and root length by 277 and 290%, and shoot and root dry weight by 275 and 375%, respectively. Results suggest that strain SR1 provides effective control of *M. phaseolina* and that it might be applied as a biological control agent to protect soybean plants from this phytopathogen.

Wahyudi *et al.* (2011) isolated *Pseudomonas* sp. from the rhizosphere of soybean and tested them for promotion of seed growth. As a result, they found that two isolates (Crb-44 and 63) exhibited promoting activity for all of the measured parameters (length of primary root, shoot length and number of lateral roots). Also, they reported that other 15 *Pseudomonas* isolates showed promotion of soybean seed growth at varying degrees. After their experiments, they concluded that the *Pseudomonas* sp. isolates could be applied as inoculants of soybean plants because of their excellent growth promotion and biocontrol activities.

4.4 Alfalfa (*Medicago sativa* L.)

Finally, we also studied strain SR1 in co-inoculation with *Sinorhizobium meliloti* strain 3DOh13 to determine their effects on nodulation and growth of alfalfa plants (Rovera *et al.*, 2008). In our studies, both SR1 and *S. meliloti* strain 3DOh13 were cultured on tryptic soy broth (TSB) medium at 28 ± 1 ºC. Their optical cell densities were 0.22 and 0.36 at 600 nm (OD_{600}), which corresponded to approximately 4.5 x 10^8 CFU ml^{-1} and 6.8 × 10^8 CFU ml^{-1} for SR1 and *S. meliloti* 3DOh13, respectively. The inoculant was prepared by mixing strain SR1 and *S. meliloti* 3DOh13 in a 1:1 ratio (v v^{-1}). The optical cell density at 600 nm (OD_{600}) was 0.25, which corresponded to approximately 6.6 × 10^8 CFU ml^{-1} of *S. meliloti* 3DOh13 and 6.3 × 10^8 CFU ml^{-1} of SR1. One gram of sterilized seeds was inoculated with the mixed bacterial suspension.

SR1, when inoculated alone, stimulated shoot and root length of alfalfa by 82 and 57%, respectively, compared to control plants. Co-inoculation of strain SR1 and *S. meliloti* 3DOh13 stimulated shoot and root length of alfalfa by 140 and 96%, respectively, as compared to control. Additionally, co-inoculation of alfalfa seeds with strain SR1 and *S. meliloti* 3DOh13 caused a significant increase in dry weight of shoot and root (Table 5). Finally, co-inoculation significantly enhanced nodulation and total N content, compared to inoculation with *S. meliloti* 3DOh13 alone or uninoculated control.

Treatment	Shoot lenght (cm)	Root lenght (cm)	Shoot dry weight (mg)	Root dry Weight (mg)
Control	3.4c	7.5c	4c	4c
N_2 Control	5.3c	9.7c	5c	5b
S. meliloti 3DOh13	7.0a	13.2b	26a	9b
P. aurantiaca SR1	6.2b	11.8b	19b	3c
Co-inoculation	8.2a	14.7a	29a	14a

Means with different letters in the same column differ significantly at P ≤ 0.05 *(Bonferroni test)*

Table 5. Effect of co-inoculation with *P. aurantiaca* SR1 and *S. meliloti* 3DOh13 on alfalfa growth

Ogata *et al.* (2008) sampled strains of *Pseudomonas* sp, *Rhizobium* sp, *Bradyrhizobium* sp, *Azotobacter* sp and actinomycetes from the rizosphere of the tara tree (*Caesalpinia spinosa*) and used them as seed inoculants for alfalfa, tara and bean (*Phaseolus lunatus* var. Sieva, *Phaseolus vulgaris* var. Camanejo and var. Caraota) to study the effects of these microorganisms on seed germination. A *Rhizobium* strain (rP2N3) significantly increased the germination percentage of alfalfa (154.9%) when compared to the control without inoculation. However, strain *Pseudomonas* spp. ps52b increased the germination percentage of alfalfa by 83.4%. These authors concluded that increase in germination percentage as well as production of IAA and phosphate solubilization by these bacteria outlines their potential as microbial inoculants for alfalfa, tara and bean.

5. Conclusions

Pseudomonas chlororaphis subsp. *aurantiaca* SR1 colonized the root system of the studied crops, persisted at appropriate population densities in the rhizosphere area and showed a significant plant growth-promoting effect that was reflected in the yield. In general, the promoting effect on growth parameters was observed throughout all of the phenological stages of the crops. A relevant finding was that wheat plants, after inoculation with SR1, presented higher yields with fertilization doses lower than those conventionally applied. This outlined the potential use of SR1 as a reasonable alternative for wheat production, with a minimization of negative environmental impacts.

In addition, maize yield values, expressed as weight of 1,000 grains, showed an 11% increase and were similar to others studies. Strain SR1 behaved as a nodulation-promoting rhizobacterium in alfalfa and soybean and was isolated as endophyte from both these crops.

Strain SR1 mobilizes nutrients and produces IAA, PCA, PRN and HCN. These compounds might be contributing to the observed increase in growth parameters.

Our group's current goals are (1) to evaluate the influence of root exudates from different plant species on the expression of secondary metabolites in strain SR1 and (2) to determine SR1's effects on different plants under water and salinity stress conditions for being able to extend Argentina's cultivation areas.

Currently, a commercial formulation containing strain SR1, termed *Liquid PSA*, is registered with the Argentina's National Service for Agricultural Health (SENASA) for wheat growth promotion. *Liquid PSA* is produced by *Laboratorios Biagro S.A.*

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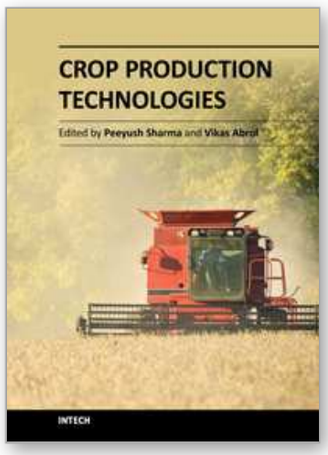

Crop Production Technologies

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Crop production depends on the successful implementation of the soil, water, and nutrient management technologies. Food production by the year 2020 needs to be increased by 50 percent more than the present levels to satisfy the needs of around 8 billion people. Much of the increase would have to come from intensification of agricultural production. Importance of wise usage of water, nutrient management, and tillage in the agricultural sector for sustaining agricultural growth and slowing down environmental degradation calls for urgent attention of researchers, planners, and policy makers. Crop models enable researchers to promptly speculate on the long-term consequences of changes in agricultural practices. In addition, cropping systems, under different conditions, are making it possible to identify the adaptations required to respond to changes. This book adopts an interdisciplinary approach and contributes to this new vision. Leading authors analyze topics related to crop production technologies. The efforts have been made to keep the language as simple as possible, keeping in mind the readers of different language origins. The emphasis has been on general descriptions and principles of each topic, technical details, original research work, and modeling aspects. However, the comprehensive journal references in each area should enable the reader to pursue further studies of special interest. The subject has been presented through fifteen chapters to clearly specify different topics for convenience of the readers.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元

Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
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