REVIEW ARTICLE

Pseudomonas chlororaphis subsp. *aurantiaca* SR1: isolated from rhizosphere and its return as inoculant. A review

Susana B. Rosas

Affiliation:

Departamento de Biología Molecular, Universidad Nacional de Río Cuarto, Campus Universitario, Ruta 36, Km 601, 5800 Río Cuarto, Cba, Argentina

Corresponding e-mail address: srosas@exa.unrc.edu.ar

Abstract

Most pseudomonas isolated from the plant rhizosphere favor the growth of plants through direct and indirect mechanisms. These bacteria produce phytohormones that promote plant growth and produce secondary metabolites that inhibit the growth of pathogenic bacteria and fungi, ensuring crop health. The present review is a compilation on the characterization of *Pseudomonas aurantiaca* SR1 and its role in the promotion of growth on several crops and its capacity to produce secondary metabolites involved in the control of pathogenic fungi. *Pseudomonas aurantiaca* SR1, a subspecies of *Pseudomonas chlororaphis*, produces IAA, HCN, siderophores, phenazines, and quorum, among other abilities. It has shown antifungal activity against several pathogenic strains, among them, *Fusarium*, *Pythium*, *Rhizoctonia* and *Sclerotium spp*. The SR1 *aurantiaca* strain has been shown to be a plant growth promoter for various crops, such as alfalfa, wheat, soybean, maize, carob, sugar cane, as well as promoting germination and obtaining vigorous and healthy seedlings. *Pseudomonas chlororaphis* subsp. *aurantiaca* SR1 is currently marketed as PSA liquid from BIAGRO-BAYER Laboratory.

Keywords: *Pseudomonas aurantiaca* SR1, growth promotion, biocontrol, inoculant, crops, seedling

1. Introduction

Natural agricultural ecosystems depend directly on beneficial microorganisms that are present in the soil rhizosphere and help crops to reach higher productivities. The beneficial microorganisms of the rhizosphere are important determinants of plant health and soil fertility because of their participation in several key processes such as those involved in the biological control of plant pathogens, nutrient cycling and seedling establishment [1].

The interactions between the plants and the microorganisms have undoubtedly big effects on the development of humanity, given that man for his survival needs to increase every day the agricultural productions for nutritive ends, between others.

At the global level, phytopathogenic fungi are the most economically important group in terms of frequency of appearance and damage that they can cause. The damage they cause not only refers to the loss of

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economic production, but also to the losses of biological production [2] and have caused havoc and major social changes. But agricultural diseases, although arising from a mode of interaction between plants and microorganisms, are not the only example of such interactions. A large number of beneficial relationships make a tremendous importance for the development of sustainable agriculture. Moreover, through beneficial associations between plants and microorganisms, attempts are made to displace the diseases that affect plants.

The rhizosphere is called the zone adjacent to the root system of plants influenced by root exudates [3].The rhizosphere is a site of intense and complex microbiological activity. It is an absolutely dynamic environment due to the constant change in the root structures, the exudate profile of the root and the balance between the different microbial communities. The promotion of plant growth mediated by PGPR (Plant growth promoting rhizobacteria) is caused by the interaction of the entire microbial community in the rhizosphere through the production of various substances [4]. In general, PGPR promote plant growth directly either facilitating the acquisition of resources (nitrogen, phosphorus and minerals) or modulating hormone levels plant or indirectly inhibiting deleterious effects of various pathogens on growth and development of the plants in the form of biological control agents [5]. Losses in plant production caused by fungal diseases are an important economic factor to be considered. Historically, control over plant diseases has been carried out through the use of resistant varieties and agronomic practices, including the application of synthetic fungicides and other agrochemicals. However, ecological problems arose with risks to human health, contamination of water and selection of pathogens with chemical resistance associated with the use of agrochemicals. This raises the urgent need to replace, at least partially, the practices used by new methods.

Although PGPRs have been classified according to their mechanism to promote plant growth or to control pathogens, they exert their beneficial effect by employing a combination of mechanisms to stimulate growth and maintain plant health [6].

Fluorescent pseudomonades have received the most attention as candidates for biocontrol agents and biofertilizer due to their ability to colonize surfaces and internal tissues of roots and stems at high densities. These bacteria can compete successfully with soil microorganisms. They are well known for promoting the growth of several plants due to their ability to produce indoleacetic acid (IAA) and to solubilize phosphate [7, 8, 9]. Pseudomonas also have a great capacity to produce secondary antifungal metabolites. It is known that more than twenty species of Pseudomonas synthesize more than 100 antibiotics and aromatic antibiotics [10]. Some well known antibiotics are phenazine-1-carboxylic acid (PCA), phenazine-1-carbo-xamide (PCN), 2,4-diacetyl-phloroglucinol (Phl), pyro-cyanine, 2-aceta-midophenol, pyrrolnitrine, pyoluteorin [11].

Siderophores include salicylic acid, piocheline and pioverdine, which chelate ions and contribute to disease control by limiting the availability of trace minerals required for microbial growth [12].

2. *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 [13, 14]. It was initially classified as *P. aurantiaca* by using the BIOLOG (Biolog Inc., Hayward, CA) system [15] and by amplification and sequencing of a partial fragment from the 16S rDNA gene. The

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species *Pseudomonas aurantiaca* was reclassified as *P.chlororaphis* subsp. *aurantiaca* 16].

It produces siderophores, behaves as an endophyte and is capable of promoting plant growth through mechanisms that involve phytohormone-like substances [17]. For instance, SR1 shows the ability to produce indole 3-acetic acid (16,5 ug. ml^{-1}). In addition, strain SR1 is able to colonize the root-system of several crops and behaves as an excellent growth promoter in wheat, alfalfa, soybean, sugarcane, corn [18, 19, 20] maintaining appropriate population densities in the rhizospheric area [21]. Its production capacity for chitinases has been evaluated, proving that it does not present this property, nor does it present conserved secretion protein genes type III although polyacrylamide gels have been shown to be active in secretion of extracellular. The electrophoretic profiles of the LPS show a model similar to that of the pseudomonas genus with a multiband profile. In addition, the ability to fix N2 in *P. aurantiaca* SR1 free life was studied, obtaining a positive ARA at 24, 48 and 72 hours of incubation under microaerobiosis conditions. The strain produces signals Acil type Homoserine lactones (AHL's) [22].

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* [15]. In addition, strain SR1 is able to colonize the root-system of several crops, maintaining appropriate population densities in the rhizosphere area [21]. 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.[23]. 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 and Raaijmakers [24]. On the other hand, detection of hcnAB genes (involved in the biosynthesis of HCN synthetase) was performed by PCR using the primers PM2-F5′-TGCGGCATGGGCGC ATTGCTGCCTGG3′) and PM2-R (5′-CGCTCTTGATCTGCAATTGCAGGC-3′) [25]. As a result, fragments of the predicted size for PCA, PRN and HCN were amplified from the DNA of strain SR1.

2.1. Pigment production by *P. aurantiaca* in different culture media

The carbon sources normally found in root exudates have a differential influence on the spectrum of antibiotics produced by the strains involved in biological control [26, 27, 28]. It is important to note that many other variables affect biological control, some of which reside particularly in soil physicochemical characteristics such as moisture, temperature, pH, soil texture and mineral nutrients; hence the importance of the in vitro study of the effects of the chemical composition of the culture medium and growth conditions on the antifungal activity.

Macrophomina phaseolina, due to its high sensitivity to *P. aurantiaca*, was selected to evaluate the biocontrol in the media used for the detection of antifungal activity and the correlation with the presence of pigment.

The presence of pigment and a marked inhibition (greater than 60%) of fungal growth in TSA medium with presence of SRI was observed. This percentage inhibition was maintained when 5.5 mM glucose was added to the medium but was reduced to 30% when added 27.5 mM and 5% when 55.5 mM glucose was added.

The addition of $FeCl_3$ revealed a decrease in pigment color intensity as well as in biological activity, where percentages of

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inhibition were maintained between 20 and 30%. In the YEM medium, in which no pigment was produced, the inhibitory activity did not exceed 30%, being clearly lower to the one observed in the TSA medium. The addition of glucose to the YEM medium did not modify the antagonistic capacity of *P. aurantiaca* SR1.

The antifungal activity was not observed in minimal medium alone or supplemented with yeast extract. The addition of tryptone allowed to observe an important antifungal activity, with approximately 60 % of inhibition of the growth of *Macrophomina phaseolina* and appearance of pigment.

When the minimal medium was supplemented with different amino acids, no pigment production was observed. However, inhibition of about 20% was detected when supplemented with methionine, histidine and tryptophan. Fungal inhibition was not observed with the addition of arginine.

The addition of mannitol and sucrose reduced the biocontrol capacity to 17% and 7% respectively.

It was observed that *P. aurantiaca* produced pigment at the tested temperatures (4, 10, 16, 18, 28 and 32°C). It seems that the temperature does not affect pigment production and biocontrol capacity.

It was shown that the greater antagonistic effect of *P. aurantiaca* is associated with the production of a pigment and is affected when the media is supplemented with different carbon sources, with glucose being one of the most influential carbohydrates in the biological action. In the presence of continuous light, pigment is not produced in the culture media that promote it, although already produced it is not affected.

High temperatures, extreme pH and the action of proteases did not affect antifungal activity.

In the medium supplemented with the pigment, the growth of all the rhizospheric strains tested was observed, which allows to infer that the pigment produced by *P. auriantiaca* SR1 does not interfere with the normal development of other bacteria; These data may reflect that the strain in a rhizosphere environment would not affect the development of beneficial micro-organisms such as those that play a major role in the rhizobium-legume symbiosis.

3. Effect of radical exudates on pigment induction

Exudates of soybean, extracted at different times of the development of the seedling, were used, in sterile form, to supplement plates of cultures with minimal medium, where *Pseudomonas aurantiaca* does not form pigment, in order to observe if they are able to trigger the formation of said compound.

Early tests indicate that exudates do not induce pigment formation.

The test of biocontrol activity with phytopathogenic fungi is necessary for the tests to be complemented since it has been observed that there is a fraction that is not part of the pigment that shows biocontrol capacity.

4. Production of rhizosphere pigment

In rhizospheric soil *Pseudomonas aurantiaca*, previously pelleted with soybean seed, was able to produce pigment and to establish effectively on root and rhizospheric area.

The extraction of pigment was carried out using soil agarized with the same protocol used for the plate extractions.

The TLC chromatography detected the same pattern of bands as the pigment under dark conditions, with the band at Rfs 0.35 and 0.37 corresponding to those of antifungal activity.

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

P. aurantiaca SR1 has been inoculated in several crops and growth promotion in these crops has been reported [17, 18, 19, 20, 21, 22, 29]. In order to evaluate its growth promotion effect in greenhouse and field conditions, *P. aurantiaca* SR1 was formulated as inoculant and applied on seeds at the sowing time.

5.1. Alfalfa (*Medicago sativa*)

In vitro nodulation tests showed that alfalfa seeds pelleted with the formulated inoculant of *Pseudomonas aurantiaca* (10^9 CFU g^{-1}) inhibited the development of the plant while being co-inoculated with *Sinorhizobium meliloti* 3DOh13, inhibition decreased showing excellent root development and greater number of nodules than the control treatment. When the tests were carried out in pots supplemented with soil: sand: perlite (2: 1: 1) the first effect observed when the alfalfa seed was pelleted with the Pseudomonas inoculant is attenuated. The results indicate that it is the actual concentration of the inoculant that determines the effect on germination and development of the plant. The optimal concentration to inoculate is between 10^6 and 10^7 CFU g^{-1} of the bioformulation [22]. 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 [17]. 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 CFUml^{-1} of *S. meliloti* 3DOh13 and 6.3×10^8 CFU ml^{-1} of *P. aurantiaca* SR1.

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 1).

Table 1. Effect of co-inoculation with *P. aurantiaca* SR1and *S. meliloti* 3DOh13 on alfalfa growth. Means with different letters in the same column differ significantly at P *≤0.05 (Bonferroni test)*

Treatment	Shoot length (cm)	Root length (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

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Finally, co-inoculation significantly enhanced nodulation and total N content, compared to inoculation with *S. meliloti* 3DOh13 alone or uninoculated control.

Biological nitrogen fixation is the most important biochemical process after photosynthesis. It can contribute substantial amounts of N2 to plants and soil, reducing the need for industrial fertilizers [30, 31].

The use of PGPR in combination with rhizobia is an interesting alternative to facilitate and optimize the nitrogen fixation of legume crops [32, 33, 34 ,35]. The effects of PGPR can influence the activity of rhizobia to compete with indigenous populations for nodulation [36].The effects of PGPR co-inoculated in legume symbiosis include increases in nodule numbers and/or nodule weight and in some cases increased nitrogen fixation or N accumulation [35]. A variety of mechanisms have been proposed for the observed responses of symbiotic legumes to co-inoculation of PGPR, including phytohormonal root growth stimulation [37]. The production of indoleacetic acid is of particular interest, since this phytohormone stimulates root lengthening and increased density of both root and lateral roots [38]. As roots are the starting point for nodule formation, increased growth may result in more rhizobial colonization sites [39].

5.2. Wheat (*Triticum aestivum* L.)

In these studies, was evaluated the effect of inoculating wheat seeds with strain SR1 on plant growth, under field conditions [18, 19, 40].

Six treatments were performed: (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 2).

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Table 2. Root length and root volume of wheat plants during Feekes 1.5 and 3.0
Values in each column with different letters are significantly different according to the LSD test ($P<0.05$)

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

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 3). 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.

Table 3. Shoot and root dry weight of wheat plants during Feekes 1.5 and 3.0
Values in each column with different letters are significantly different according to the LSD test ($P<0.05$)

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

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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 4).

Table 4. Parameters of wheat yield

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

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

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 [41] 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.

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

This study investigated the ability of the SR1 strain to promote legume growth and biocontrol capacity over the pathogenic fungus *Macrophomina phaseolina.*

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) [42].

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

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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 5).

Table 5. Soybean growth parameters
Values in each column with different letters are significantly different according to the Scheffé test ($P<0.05$)

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

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 [43]. Significant yield losses of soybean are reported every year due to charcoal rot fungus *M. Phaseolina* (Tassi) Goid. [44]. 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.[45] isolated *Pseudomonas spp.* 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

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of soybean seed growth at varying degrees. After their experiments, they concluded that the *Pseudomonas spp*. isolates could be applied as inoculants of soybean plants because of their excellent growth promotion and biocontrol activities.

5.4. 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 [40]. 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 [46] and root volume [47], 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 favorably 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.* [48] 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.* [49] 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.

5.5. Carob tree (*Prosopis* L)

Within a plan of reforestation and protection of regional species was worked with different clones of Prosopis (mesquite). *Prosopis* clones are susceptible to *Fusarium spp*. and within the Environmental Program where the project was developed, Santiago del Estero, Argentina, it was decided to displace the use of agrochemicals and to use plant growth promoting microorganisms (PGPR), including *Pseudomonas* SRI, which produce hormones and have biocontrol capacity[50]. *Pseudomonas aurantiaca* SR1

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was formulated as an inoculant for this purpose.

Six easy-rooting Prosopis: 3 clones (B2F17A2, B5F9A2, B6F5A2) and 3 difficult root clones (B7F6A4, B1F5A3, B2F4A3) were used. All clones were susceptible to *Fusarium* sp. For rooting, the clones were treated with Ac Indol butyric, Ac Naphthalene Acetic and Thiamine-HCl as a chemical treatment and inoculated with *Pseudomonas aurantiaca* as a biological treatment (UFC $10^7 g^{-1}$ of inoculant).

The parameters evaluated were the number of roots, maximum long root, percentage of rooting and sanity.

Rooting percentage: There was no significant difference in Pseudomonas treatment in the easy rooting clones. In difficult rooting clones, 30% higher rooting is observed when clones are treated with Pseudomonas.

The authors conjecture that this is a hormonal balance that favors rooting in certain clones. Hard rooting clones contain an endogenous group of lower hormones, the optimal concentration is brought about by bacteria.

In the presence of excess hormones, there is no response or inhibition occurs.

As for the number of roots, the same anterior profile is observed, accentuated in clone B1F52A3, when treated with Pseudomonas. As for the length of the root the same response is observed. When the clones were subjected to chemical treatment, although three of them achieve rooting, they only have a 5% survival due to the attack of *Fusarium spp.*

The treatment of the same with *Pseudomonas aurantiaca* allowed the rooting of some clones and the protection of the fungal attack in 70%. Easy-to-root clones are more susceptible to Fusarium attack.

It is inferred that *Pseudomonas aurantiaca* acts as a biological control agent, either by antibiosis and/or ISR (induction of systemic resistance) and is a producer of substances similar to phytohormones such as IAA. In tests performed with Azospirillum, there was no rooting capacity greater than with Pseudomonas, except clone B2F17A2.

5.6. Sugarcane (*Saccharum officinarum* L)

Micropropagation is a technique of in vitro culture of vegetal tissues that allows the obtaining of seed sugarcane of quality. The seedlings obtained were acclimatized in greenhouses, optimizing the management of sanitation, environmental and nutritional conditions, in order to adapt to ex vitro conditions.

Sugarcane was of importance in the NOA (Argentine Northwest), representing more than 13% of GDP. The area cultivated in Tucumán, Argentina was 242,000 ha [51]. Plants of sugarcane (*Saccharum officinarum* L. cvs CP 65-357 and TUC 77-42) from vitroplants were inoculated with *Pseudomonas aurantiaca* SR1 (inoculant formulated by BIAGRO SA, stage experimentation) in Tucumán, Argentina.

Significant differences were observed in stem length and total weight in the variety CP 65-357 and varTuc 77-42, relative to Control. In both varieties was also observed a higher production of roots.

In biocontrol experiments carried out in vitro, *Pseudomonas aurantiaca* SR1 controlled *Fusarium subglutinans* up to 60% of micellar development which would indicate that the pigment produced would have antibiotic action, siderophore and induction of systemic resistance, which confirms the antagonistic effect of this bacterium on different pathogenic fungi, including *Fusarium spp*.[15, 52] In vivo, a high health index was observed corroborating the in vitro effect. *Fusarium subglutinans* was isolated in the first year,

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and in the second year *Fusarium subglutinans*, *Pythium spp*. and *Rhizoctonia spp.*, were controlled. The quality of the plants given by the increase of fresh weight of plants, development of adventitious roots and length of roots, would be due to the action of substances of type auxins in addition to other hormones that significantly affect the growth of them.

The inoculation with *Pseudomonas aurantiaca* SR1 in sugarcane plants obtained by tissue culture in vitro improves survival, total weight of plants and decreases the incidence of diseases caused by *Fusarium subglutinans*, the most frequent pathogen.

There are differences between sugarcane genotypes in response to the action of *Pseudomonas aurantiaca* SR1. As for growth promotion, comparative experiments were performed with *Azospirillum brasilense*, both bacteria show a similar promoter behavior, superior to the control. [53]

5.7. Garlic (*Allium sativum*)

Over the years 2012/13, white garlic was planted under field conditions, with and without inoculation of *Pseudomonas fluorescens*, *Pseudomonas aurantiaca* SR1 and *Azospirillum spp*. in the concentration of 1×10^9 CFUml^{-1}, to observe the effect of the application of these commercial biofertilizers on the ontogeny of the plant.

In laboratory and greenhouse, two trials were carried out: one on the effect produced by the aqueous extract of garlic on the growth of the bacteria used and another on the effect of the inoculation on the shoot and the development of the roots of the plants. The results indicate that concentrated aqueous extract of garlic does not inhibit the growth of the strains used and that compared to the control but affects pigment production in *Pseudomonas aurantiaca* SR1. *Pseudomonas fluorescens* and *Pseudomonas aurantiaca* cause a decrease in the number of roots, their weight and length at the concentration used, effect already commented. At a concentration of 10^5 CFU ml^{-1}, Pseudomonas and *Azospirillum spp*. increase the number of roots and shoots without affecting other parameters

In the field trial of 2012 that was affected by a high and severe incidence of white rust and excessive precipitation, it was observed that treatment with *Azospirillum spp*. produced the highest weight and quality of bulbs. However, inoculation with *Pseudomonas aurantiaca* increased yield by ensuring garlic health [54].

5.8. Pepper and tomato seedlings

The use of quality seedling is a precondition to increase yield and quality of vegetable crops. Liquid fertilizers including nitrogen are used during seedling production. However, much of the fertilizer applied contributes potentially to the contamination of surface water, producing detrimental environmental effects. The use of plant growth promoting rhizobacteria (PGPR) with the goal of improving nutrients availability for plants has been suggested to be important and necessary for agriculture [55]. Plant growth promoting rhizobacteria have been used for the production of seedlings.

There are important positive effects of bacteria on the yield and growth of vegetables such as, rocket, tomatoes, cucumber, cauliflower, pepper, potato, radish and lettuce [56, 57, 58, 59, 60, 61, 62] but little is known about the mechanism(s) involved in their effect on seedling performance [62, 63]. The production of vegetable crops with planting transplants is recently preferred by growers as the time required for cultivation is reduced and also provides a low seed cost to establish a good and homogeneous planting of vegetables, a more efficient use of fertilizers and irrigation water during the early stages of growth. The use of a quality transplant is important to

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achieve uniform growth of plants, increase the yield and quality of horticultural crops and effectively protect against environmental stress, insect diseases and pathogens.

Pepper (*Capsicum annuum* L.) has a great demand in the international market and its success is that it is a crop with several consumption destinations: fresh consumption, universal condiment and canned. Among the conditions required to increase production and yield of this crop is to achieve a high percentage of germination and obtaining vigorous seedlings [64].

In order to increase and accelerate the seed germination process and to obtain a high production and homogeneity of seedlings, pepper and tomato seeds were inoculated with SR1 strain at a concentration of 10^7CFU ml^{-1} to observe their effect on germination. Early germination was observed in both plants, being highly significant in the case of pepper, with very slow germination and emergence, in which a certain germination energy disparity can be observed in the same lot. Several substances have been tested to optimize germination, with KNO3 (0.2%) as recommended by ISTA [65]. According to International Seed Testing Association standards [66], the first count of germinated pepper seeds should be done at 7 days and the final count at 14 days after the start of treatment for the percentage of total germination. Thus, the statistical analysis of the mean comparison between treatments was made with the data obtained during this period, in which no significant differences were obtained between the treatments, in terms of the number of germinated seeds. However, it was observed that with the inoculation of the SR1 strain, a higher final percentage of germinated seeds was obtained. The germinated pepper seeds (36%) with *Pseudomonas aurantiaca* were detected three days after the test, presenting statistically significant differences in relation to water control (23%) and KNO3 (6%). On the seventh day, the treatments reached values between 70 and 78% of germination. Despite these differences in germination onset until the seventh day, there were no significant differences between treatments.

At 14 days, the seedlings were transplanted and inoculated again with the same dose. The height of the seedlings at 20 days was evaluated from the beginning, with a slight preponderance of the inoculated treatments, which was accentuated as a function of time with a final result at 40 days, in which a highly significant statistical difference was defined for this treatment. Greater development in shoots and roots was also observed.

Similar results were obtained with tomato seeds, where germination was more uniform but higher in the inoculated treatment. Pseudomonas increased the biomass of tomato plants and roots (42 and 35% respectively). These seedlings were more vigorous than those that were not inoculated.

5.8.1. Development of pepper inoculated on soils infested with *Fusarium moniliforme*

Pepper seeds were inoculated with *Pseudomonas aurantiaca* SR1 (10^7, 10^8, 10^9 CFU g^{-1} in soil infested with *Fusarium moniliforme* (10^4, 10^5, 10^6 spores g^{-1} soil) to observe the biocontrol capacity of the proposed strain. At 15 days of sowing, the fresh weight and dry weight of the stem and roots were determined.

Seeds inoculated at a dose of 10^7 CFU g^{-1} Pseudomonas showed improvements over control in soils infested with 10^6 spores g^{-1}. Spore concentrations 10^4 and 10^5 produced no damage to seedlings

6. Conclusions

The plant genotype affects the response to PGPR inoculation because it affects root colonization by introduced bacteria as well as the total population size of the microbial

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communities in the plant and probably also affects the composition of those communities [67]. The effect of the introduction of PGPRs in the rhizospheric community has not been intensively studied, since many experiments have been carried out under gnotobiotic or greenhouse conditions. However, recent studies strongly suggest that increases in plant growth can be attributed to changes in the microbial community of the rhizosphere due to the presence of PGPR microorganisms inoculated in soils [68].

There is extensive evidence and research in the literature indicating that PGPR organisms can be a true success story in sustainable agriculture. In fact, through their numerous direct or indirect mechanisms of action, PGPRs can allow a significant reduction in the use of chemical pesticides and fertilizers. These beneficial events that produce biological control of diseases and pests, promoting plant growth, increasing crop yields, and improving quality can take place simultaneously or sequentially. The age of the plant and the chemical, physical and biological properties of the soil will greatly influence the result of PGPR inoculation.

Among strains of *P. aurantiaca*, SR1 had been extensively studied for the promotion of plant growth with different cultures at field level [28, 69, 70].

Pseudomonas chlororaphis subsp. *aurantiaca* SR1 colonized the root system of the tested crops, persisted at appropriate population densities in the rhizosphere area and showed a significant effect of plant growth which was reflected in yield. In general, the promoter effect was observed on the growth parameters in all the phenological stages of the cultures. A relevant finding was that wheat plants, after inoculation with SR1, showed higher yields with lower fertilization doses than those applied conventionally. This described the potential use of SR1 as a reasonable alternative for wheat production, with a minimization of negative environmental impacts. *Pseudomonas chlororaphis* subsp. *aurantiaca* SR1 demonstrated sufficient merit that a commercial formulation containing the SR1 strain, called PSA liquid, is currently registered with the National Agricultural Health Service (SENASA) of Argentina for the promotion of wheat growth. PSA Liquid was produced by Laboratorios Biagro S.A., currently acquired by Bayer.

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