

Biofertilizers of rhizobacteria in the growth of Poblano chili seedlings

Vivian F. Quiroz-Sarmiento¹
Juan J. Almaraz-Suarez^{2§}
Gabriela Sánchez-Viveros¹
Rosalba Argumedo-Delira³
Apolinar González-Mancilla⁴

¹Postgraduate in Agricultural Sciences-Veracruzana University. Circuit Gonzalo Aguirre Beltrán s/n, Xalapa, Veracruz. CP. 91000. (quirozvi@yahoo.com.mx; gabsanchez@uv.mx). ²Postgraduate College-Montecillo *Campus*. Mexico-Texcoco Highway km 36.5, Montecillo, State of Mexico. CP. 56230. Tel. 595 9520200, ext. 1280. ³Support Services Unit in Analytical Resolution-Veracruzana University. Luis Castelazo Anaya s/n, Col. Industrial Animas, Xalapa, Veracruz. CP. 91190. (rosasusana13@hotmail.com). ⁴Faculty of Agriculture and Zootechnics-Universidad Juárez de Durango. Gómez Palacio-Tlahualilo road km 35, Ejido Venecia, Durango. CP. 35170. (apolinar.gonzalez@uied.mx).

§Corresponding author: jalmaraz@hotmail.com.

Abstract

Poblano chili (*Capsicum annuum* L.) is a widely consumed variety in our country, but there are several problems that affect its cultivation and production. Considering the above, the objective of this research was to elaborate and compare the effect of two biofertilizers with rhizobacteria on the growth of Poblano chili seedlings. The research was conducted at the Postgraduate College, Montecillo *Campus* in the years 2015-2016. The work was carried out in three stages: 1) selection of culture media and growth dynamics of four strains of rhizobacteria; 2) elaboration of two bioformulations using alginate and peat pearls as carriers; also, the viability of the rhizobacteria was evaluated for five months; and 3) evaluation of the effect of bioformulations in promoting the growth of Poblano chili seedlings. *Serratia* strains showed the highest growth in the TBS culture medium, while *Pseudomonas* strains had high growth in Luria-Bertani broth. The stationary phase of the *Pseudomonas* strains was reached between 24-48 h and that of the *Serratia* strains between 12-44 h. The shelf life of the two formulations was maintained from 1×10^{12} to 1×10^9 CFU for five months. The application of alginate beads in the seedlings stimulated seedling growth up to 35% with respect to the control. The application of biofertilizers based on alginate and peat pearls is a good option to promote the growth of chili seedlings.

Keywords: *Capsicum annuum*, biotechnology, inoculants.

Reception date: August 2019

Acceptance date: October 2019

Introduction

The demand for the use of biological inputs in agriculture has increased, due to the need to reduce environmental pollution and produce healthy and safe food. An alternative is the use of microbial biofertilizers as a means to increase yield, improve crop profitability, reduce the impact of agrochemicals on the environment and decrease the presence of contaminants in the food we eat (Soria *et al.*, 2001; Dinesh *et al.*, 2010).

In our country, the production of chili has declined in recent years due to several problems, among which are the poor quality of seedlings that are transplanted in the field (Huerta *et al.*, 2007). In the case of the cultivation of Poblano chili in Mexico, there are few studies on the use of plant growth promoting rhizobacteria in the greenhouse or field. At the seedbed level, rhizobacterial inoculation can be used to obtain seedlings with greater growth, better health and nutrition that allow them to establish a rapid transplant in the field (Vessey, 2003).

The production of an inoculant begins with the isolation of rhizobacterial strains from the rhizosphere in specific media, followed by the selection of the best growth promoting strains in laboratory, greenhouse and field tests (Bhattacharyya and Jhan, 2012). The use of an appropriate methodology is what makes it possible to obtain an optimal microbial culture, either for the production of bacterial cells or their metabolites (Gómez and Batista, 2006). For which the selection of economic culture media is of vital importance in the production of bioinoculants.

However, the scaling processes of a biofertilizer can increase production costs due to the type of formulation and the culture media used for the multiplication of bacteria (Herrmann and Lesueur, 2013). Among the processes for the elaboration of a microbial inoculant, the choice of the carrier is of the utmost importance. The support (carrier) is the main portion of the inoculant. The materials of which the support is composed and the type of formulation vary from liquid, suspension, powder, granular and gels.

The support has the function of preventing the loss of viability of microorganisms and having the ability to provide the correct number of viable cells in good physiological conditions at the time of release in the greenhouse or field (Bashan *et al.*, 2014; Maheshwari *et al.*, 2015). The raw materials used in most carriers are substrates of organic or inorganic origin, for example peat, grass, talc, lignite, vermiculite, etc. (Albareda *et al.*, 2008; Ardakani *et al.*, 2010; Angayarkanni *et al.*, 2014).

Another viable option is the use of alginate beads as carriers. The encapsulates are not toxic, they easily degrade in the soil, the release of the bacteria is slow and their shelf life can be over 14 years (Lebsky *et al.*, 2001; Bashan *et al.*, 2002; Yubur *et al.*, 2007). Therefore, the main objective of this research was to develop two biofertilizers based on rhizobacteria (*Serratia* and *Pseudomonas*) using moist alginate pearls and peat as a carrier to stimulate the growth of Poblano chili seedlings. The hypothesis was that alginate pearls increase the survival of rhizobacteria resulting in greater effectiveness in seedlings of Poblano chili.

Materials and methods

The experiment was carried out in the laboratory and greenhouse of the Area of Soil Microbiology, of the Edaphology Program of the Postgraduate College, Montecillo *Campus*, in the period from April 2015 to October 2016. Four strains of rhizobacteria were used: *Serratia liquefaciens* CPAC53, *Serratia plymuthica* CPPC55, *Pseudomonas tolaasii* P61 and *Pseudomonas yamanorum* OLSf5 from the microbial collection of the Soil Microbiology Laboratory. These strains were isolated from different hosts and are characterized as promoters of plant growth of chili crops (González *et al.*, 2017; Angulo-Castro *et al.*, 2018).

Stage 1: selection of the culture medium and growth dynamics of each bacterial group

The means were used: nutritive broth (CN) and Luria-Bertani broth (LB) for the four strains of rhizobacteria and a specific medium for each bacterial genus; King B broth (CK) for *Pseudomonas* and tryptone soy broth (TBS) for *Serratia*. Glass flasks with a capacity of 250 mL were used, to which a volume of 150 mL of each medium was placed. Roast was taken from the strain and placed in each of the bottles under aseptic conditions in a laminar flow hood.

The bottles were incubated on a Thermo Scientific™ MaxQ™ 40000® orbital shaker at 180 rpm and a temperature of 28 °C. Bacterial growth was quantified by optical density, using a Bio Tek model Synergy 2® spectrophotometer at a wavelength of 600 nm. For this, 200 µL aliquots were taken in triplicate of each of the bacterial cultures, deposited in each of the wells of a microplate (Costar® 3596, Corning Incorporated, NY) and its optical density was measured; the samples were taken at 24, 48 and 72 h of incubation. An experiment was established for each genus of bacteria (*Pseudomonas* or *Serratia*), where growth was evaluated in three culture media, this in a completely randomized experimental design with 9 repetitions per treatment.

A statistical analysis was performed, using the SAS package for Windows (SAS Institute Inc. 2002), where the study variable was optical density. Analysis of variance and comparison test of means by Tukey ($\alpha=0.05$) were made. Once the culture medium was selected for each bacterial genus, the next step was to know the growth dynamics of each of the four strains of rhizobacteria. For the *Serratia* genus the TBS medium was used and for the *Pseudomonas* genus the LB medium. The methodology used for this phase was similar to that used for the selection of culture media.

The strains were inoculated in the media and the optical density was measured every four hours for 48 hours and finally one last reading at 72 hours, giving a total of 14 readings. The experiment was established using a completely randomized experimental design. The treatments were the 4 strains of rhizobacteria with 9 repetitions. The 2 strains of *Serratia* were grown in TBS medium and the 2 strains of *Pseudomonas* in LB medium. The measured variable was the optical density. The data obtained were analyzed using the SAS statistical package for Windows (SAS Institute Inc. 2002), performing an analysis of variance and Tukey test ($\alpha=0.05$).

Stage 2: elaboration of biofertilizers and survival of rhizobacteria

The methodology used to make the pearls was the one proposed by Bashan (1986), with some modifications. The same culture media indicated above were used to prepare the inoculum of the rhizobacterial strains. To bottles with 750 mL of broth 2% sodium alginate was added and sterilized at 18 lb for 18 min. Three roasts of the bacterial strain were inoculated into the jars and incubated on a Thermo Scientific™ MaxQ™ 40000® orbital shaker at 180 rpm, at a temperature of 28 °C for 48 h.

The pearls were made using a peristaltic pump (Watson-Marlow 120U/DV®), for which one end of the pump hose was placed inside the bottle containing the liquid culture medium and pumped at 72 rpm, the other end of the hose was placed on top of a 2 L beaker containing 1.5 L of a 0.1 M solution of calcium chloride (CaCl₂), under constant stirring on a stir plate (Corning PC-420®) where they were going depositing the drops that would subsequently form the pearls, for which they were left in the solution under stirring for a period of three hours.

After that time the beads were rinsed three times with a saline solution (0.85% NaCl) and placed in a strainer to remove excess moisture. The whole process of pearl elaboration was carried out in a laminar flow hood. Finally, the pearls were placed in sterile ziploc bags and kept refrigerated at 4 °C. In the case of peat biofertilizer, it was neutralized with calcium carbonate and a pinch of activated carbon in order to adsorb any toxic that could be released. The peat was sterilized in an autoclave at 18 lb, at intervals of three hours for three days.

After the sterilization time was placed in a drying oven (FELISA, model 242-A®) at a temperature of 72 °C for 48 h. Once the peat was sterile and dried, 1 kg was placed in a disinfected plastic bag and approximately 100 mL of inoculum was added with a concentration of 109 CFU mL⁻¹. The mixture was perfectly homogenized, without moisture saturation. Once the peat was impregnated, the bag was sealed and incubated at 28 °C for 15 days (Ferrera-Cerrato *et al.*, 1993).

After the incubation time, 100 g of the biofertilizer were placed in metallic polypropylene bags and sealed under vacuum with a sealer (Food Saber 3800® series) and stored under refrigeration at 4 °C.

The shelf life (survival) of the bacteria in the alginate beads and in the peat was evaluated once a month, for a period of five months. To quantify the bacterial load, the plate dilution and counting technique was used, in the case of alginate beads 1 g of them was placed in a dilution tube containing 9 mL (10⁻¹ dilution) of a phosphate Buffer solution (solution A (NaH₂PO₄) + solution B (NaPO₄) at 0.1 M with pH 6) and allowed to stir for 24 h in order to dissolve the beads completely.

With respect to the peat biofertilizer, 1 g of this was weighed and placed in a dilution tube containing 9 mL of sterile distilled water (10⁻¹ dilution), the tube was stirred for 40 min. In both cases, 100 µL of the suspension was taken and added to a tube containing 900 µL of sterile distilled water (dilution 10⁻²), the tube was agitated and from this dilution successive dilutions were made up to 10⁻⁹. A 100 µL aliquot of dilutions 10⁻⁷, 10⁻⁸ and 10⁻⁹ was taken and placed in the center of the Petri dishes with nutritive agar plates were raked with a glass rod and incubated at 28 °C for 48 h. This procedure was performed in triplicate.

The experiment was established using a completely randomized experimental design. The treatments were four strains of rhizobacteria (CPAC53, CPPC55, P61 and OLSf5) with three repetitions, both for the alginate beads and for the peat formulation, giving a total of 12 experimental units for each formulation. The variable evaluated was the survival of the bacteria in the two formulations. The CFU count was expressed in logarithmic units. The data obtained were analyzed using the SAS statistical package for Windows (SAS Institute Inc. 2002) and an analysis of variance and Tukey test ($\alpha=0.05$) was performed.

Stage 3: Establishment of the greenhouse test

A substrate containing agrolite, peat moss and vermiculite was used in a 1:1:1 ratio. Polypropylene containers with 30 cavities were used. For the biofertilizer of alginate beads, the containers were covered at half capacity with the substrate. 10 g of pearls were used per 100 g of substrate, which corresponded to placing 0.33 g per cavity of the seedbed, after placing the pearls the containers were filled entirely with substrate. In the case of peat biofertilizer, 10 g of the bioformulation was mixed with every 100 g of substrate. The two formulations contained a concentration of 109 CFU g⁻¹.

Seeds of a creole variety of Poblano chili from the Sierra Nevada region were used, specifically from the municipality of San Matias Tlalancaleca, Puebla. The seeds were provided by Mr. Leopoldo Ramírez Morales, who has practiced a selection process on his variety during the last 20 years. The variety is representative of the region given that Mr. Ramírez, supplies seedlings to the vast majority of producers in that area. The cycle of the variety from the seedling to the last harvest is approximately seven months.

The seeds were disinfected with a 1% sodium hypochlorite solution for five min and subsequently rinsed with sterile distilled water five times. Two seeds were placed for each cavity of the seedbed. The seedlings were watered with distilled water every three days and once a week 10% Steiner's nutrient solution (Ca (NO₃) 0.106 g, KNO₃ 0.0312, K₂SO₄ 0.0492 g, KH₂PO₄ 0.013 and micronutrient mix 0.017 per liter) was applied, to all treatments including the control.

After 60 days the seedlings were harvested and the agronomic variables were evaluated: 1) number of leaves in each seedling; 2) seedling height; 3) the leaf area was determined with a Li-cor[®] model LI-3100C meter; and 4) dry biomass, where the plant material was dried in a FELISA oven, model 242-A[®] for 48 h at 70 °C and weighed on a Sartorius analytical balance, Analytic Ac 2105[®] model. An analysis of the nutrient content was also carried out: phosphorus (P) and potassium (K) were determined using an optical emission spectrophotometer with inductive coupling plasma brand Varian model 725-ES[®] and the nitrogen content (N) was determined by digestion wet by the method of Kjeldahl (Bremner, 1996).

The treatments used were 4 strains of rhizobacteria in 2 bioformulations (alginate and peat beads); a control was included without inoculation, all treatments had 15 repetitions and the experimental design used was randomized blocks. The data obtained from all the variables studied (number of leaves, height, biomass, leaf area, N, P, K) were analyzed using the SAS statistical package for Windows (SAS Institute Inc. 2002), performing an analysis of variance and test of comparison of means by Tukey ($\alpha=0.05$).

Results and discussion

Selection of the culture medium and growth dynamics

Regarding the growth of rhizobacteria in the different culture media, significant differences ($p \leq 0.01$) were found in both bacterial genera. The strains of the genus *Pseudomonas* presented the highest growth in the LB broth with 1.3 units of optical density, while the strains of the *Serratia* genus in TBS broth with a growth of 1.4 units of optical density at 72 h respectively (Figure 1a and 1b).

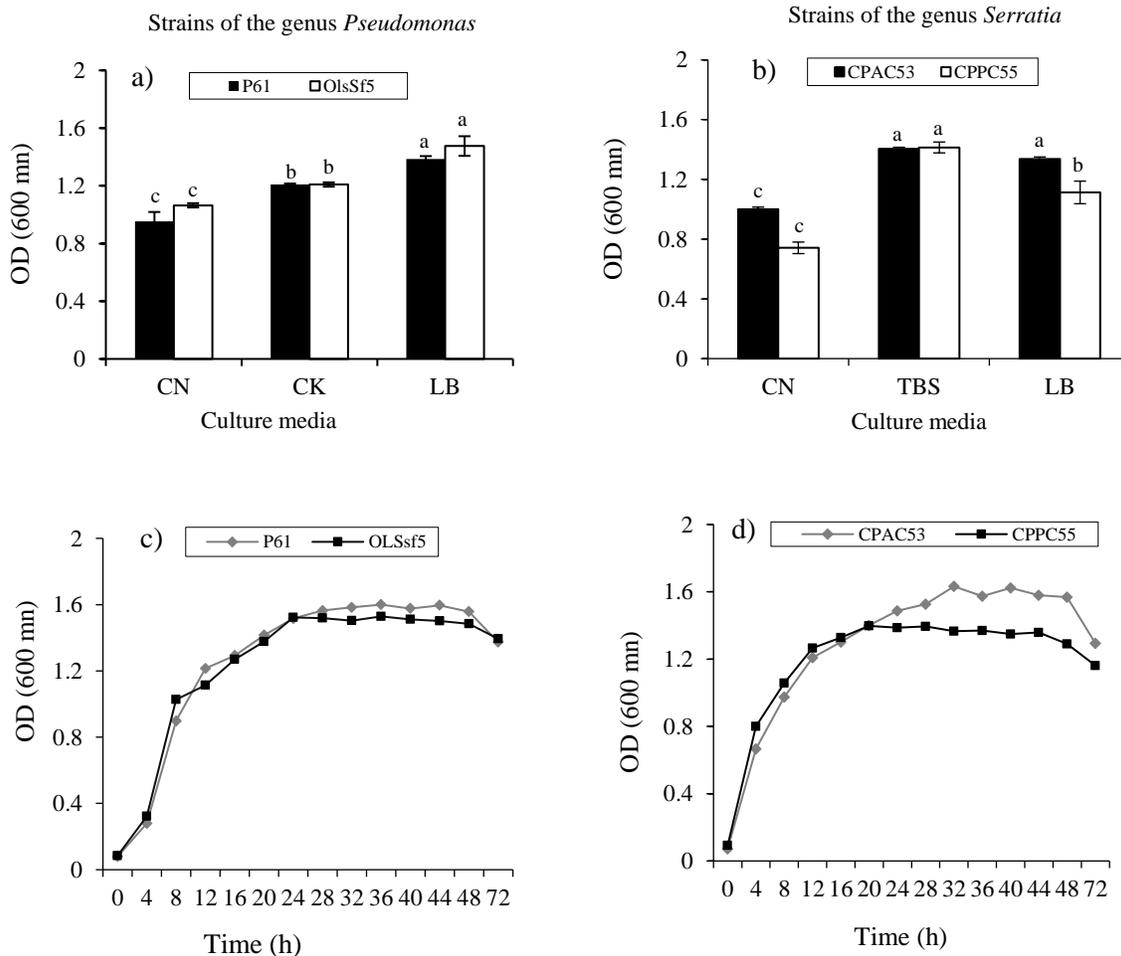


Figure 1. Selection of liquid culture media (a and b). The bars represent the average of nine repetitions and the different letters indicate significant differences. CN= nutrient broth; TBS= Tryptone Broth Soy; LB= Luria Bertani broth and CK= King B broth; and growth dynamics of the strains in the selected media (c and d). The optical density technique measured at 600 nm (OD) was used for both methods. Tukey ($\alpha = 0.05$).

The growth dynamics of the two strains of *Pseudomonas* were evaluated in the LB medium, which was the best for this bacterial genus. It was observed that the growth increased from a value of 0.3 units at 4 h to a value of 1 unit of optical density at 8 h, this period is known as an exponential

phase, since it reaches the maximum speed of increase. After the above, a pre-stationary phase was observed between 8 and 24 h of culture, which is a growth retardation phase, reaching a value of 1.5 units of optical density.

The stationary phase was presented in the period from 24 to 48 h, in this period there was no growth, and after this time the growth decreased (Figure 1c). In the case of the strains of the genus *Serratia*, the culture medium used was the TBS broth. An exponential growth phase was observed in the period of 0 and 8 h with 0.8 optical density units, followed by the pre-stationary phase between 8 and 12 h with a reached value of 1.2 optical density units, while the phase stationary occurred between 12 noon and 44 h with an average value of 1.3.

Units of optical density in strain CPPC55 and 1.6 units of optical density in strain CPAC53 and finally after 48 h cell death occurred (Figure 1d). *Pseudomonas* and *Serratia* are two highly versatile bacteria genus in the use of substrates as a carbon source and do not require vitamins, which largely explains their viability in a variety of culture media (Holt *et al.*, 1994). The *Serratia* genus does not require many growth factors; it can grow at temperatures between 10-36 °C, with a pH of 5-9 and in the presence of NaCl (Grimont and Grimont, 1978).

In this work it was observed that the four strains of rhizobacteria grow well in the three culture media used, although the two strains of the *Pseudomonas* genus reached their highest growth in LB broth, and the two strains of the *Serratia* genus in TBS broth (Figure 1). This indicates that the rhizobacteria used are not demanding in their nutritional requirements, as indicated by the aforementioned authors.

The TBS medium has been used for the propagation of strains of the *Serratia* genus in several investigations, among its components are casein peptone, soy peptone, NaCl, KH₂PO₄ and glucose, which favors the development of bacteria. For example, Czajkowski *et al.* (2012) used this medium in the propagation of a strain of *Serratia plymuthica* to use as a soft rot controller in potato crops.

With respect to the two strains of the *Pseudomonas* genus, the highest growth was obtained in LB broth. This medium is mainly composed of tryptone, yeast extract and NaCl, it is one of the most used for the cultivation of bacterial species, because it is rich in nutrients and is easy to prepare (Garboza *et al.*, 2011). The nutrient broth is one of the most widely used culture media for the activation and spread of different bacterial genus. However, of the three-culture media used in this study, the nutrient broth proved inefficient for the growth of the strains of the *Serratia* and *Pseudomonas* genus (Figure 1).

The selection of a culture medium must guarantee increases in the yields of fermentation processes quickly and economically (Gómez and Batista 2006). The economic cost of each of the reagents to be used is a point to consider. Costs in the multiplication of rhizobacteria can be reduced by replacing reactive grade inputs of the reference medium with alternative nutritional sources such as agroindustrial wastes (Rivera and Botía, 2008) and plant extracts (Khalil *et al.*, 2016), which can help reduce costs.

Survival of rhizobacteria in biofertilizers

The storage time of the pearls was for 150 days. The four strains showed significant differences ($p \leq 0.01$) in survival during storage in the two formulations. The initial bacterial load of the four strains in the alginate beads was around $12 \text{ Log}_{10} \text{ CFU g}^{-1}$ and the population of almost all remained almost unchanged throughout the five months of storage, except for the CPAC53 strain that had a slight fall with a value of $11.4 \text{ Log}_{10} \text{ CFU g}^{-1}$.

With respect to the biofertilizer based on peat, the two strains of *Serratia* had an initial load greater than $12 \text{ Log}_{10} \text{ CFU g}^{-1}$ and the *Pseudomonas* strains had an initial load of about $11 \text{ Log}_{10} \text{ CFU g}^{-1}$. In this bioformulation the fall of the population of the four strains after five months of storage was higher than in the alginate beads, reaching values between 10.1 to $9 \text{ Log}_{10} \text{ CFU g}^{-1}$, where the OLSf5 strain was the one that presented the smaller population. In general, survival was higher in alginate beads than in peat (Figure 2).

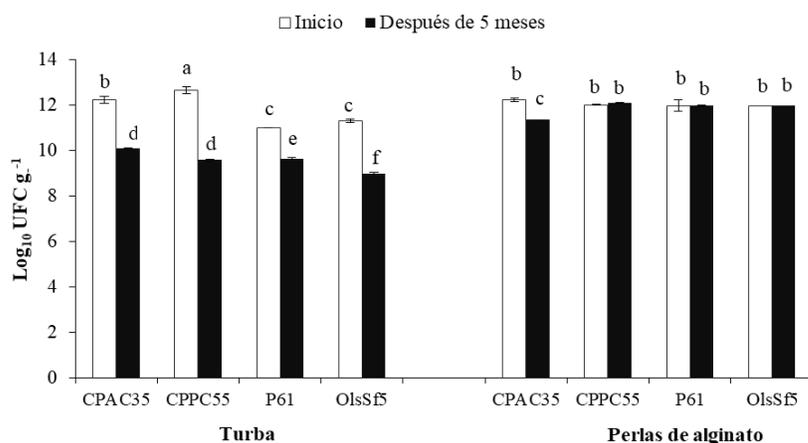


Figure 2. Evaluation of the shelf life of the rhizobacteria in the two biofertilizers (peat and alginate beads) kept refrigerated for five months. The bars represent the average of 15 repetitions. The different letters indicate significant statistical difference. Tukey ($\alpha = 0.05$).

Alginate is the polymer with the greatest potential for encapsulation of bacteria because the polymer chain of mannuronic and guluronic acids can be linked by calcium ions to form the gels. This conformation determines alginate properties such as solubility, viscosity and the ability to exchange ions with divalent metals (Yabur *et al.*, 2007; De-Bashan and Bashan, 2008). Alginate beads are easy to biodegrade and harmless; In addition, due to their properties, they allow high survival and a gradual release of *Azospirillum* bacteria in the field (Bashan, 1998; De-Bashan *et al.*, 2007).

In the present study, the greatest survival of bacteria was achieved with alginate beads. The characteristics of the strains are one of the attributes that must be considered to choose the carrier since not all microorganisms are suitable for encapsulation. The use of wet alginate beads is not reported in other studies, so this research could open the pattern to consider this formulation as a good alternative in the production of biofertilizers using rhizobacteria of the *Pseudomonas* and *Serratia* genus.

Although *Azospirillum* is one of the most studied and used bacteria in bioformulations (Fuentes-Ramírez and Caballero-Mellado, 2005), there are also other bacteria that have been used. For example, Ivanova *et al.* (2005) observed that *P. corrugata* is more sensitive to storage than *Azospirillum*, in alginate pearls its bacterial load decreased to 10^3 after six months in storage. Instead, *Azospirillum* maintained a load of up to 10^8 CFU. This same behavior was observed by Trivedi *et al.* (2005) with the strain *Bacillus subtilis*.

Peat is an ideal carrier, which provides nutrients to microorganisms and has a large specific surface area, as well as a high moisture retention and damping capacity, which provides microorganisms with protection from adverse environmental conditions in the field (De-Bashan *et al.*, 2007). Apart from the peat, there are several materials that can be used as supports in the formulations, among these are: bagasse, manure, compost, coconut powder, vermicompost, rice bran, perlite, rock phosphate, coal, lignite, bentonite, talc and clays (Albareda *et al.*, 2008; Ardakani *et al.*, 2010; Bashan *et al.*, 2014). Of the four strains of rhizobacteria used in this investigation, *S. plymuthica* CPPC55 was the one that presented the highest viability value during the storage period.

Effect of biofertilizers in Poblano chili seedlings

There were significant statistical differences ($p \leq 0.01$) between the strains of the two biofertilizers for the agronomic variables evaluated. It was observed that all treatments were superior to the control. With respect to the leaf area, the highest values were obtained with the CPAC53 and OLsSf5 strains in wet alginate beads with values between 18.3 and 18.2 cm², while the lowest values were recorded in the OLsSf5 strain with 12.8 cm² in peat and the control with 12.6 cm². The highest number of average leaves was presented with strain CPAC53 in alginate beads and the lowest number was obtained in the control.

The highest height value of the seedlings was presented with the CPAC53, CPPC55 and OLsSf5 strains in wet alginate beads with an average of 13.3 cm, and the lowest was in the control with 10.4 cm. The highest dry biomass was presented with the CPAC53, P61 and OLsSf5 strains in wet alginate beads with values between 151.3 and 155.3 mg. The control had a dry biomass of only 91 mg (Table 1). With respect to nutrients, the highest phosphorus value was presented with the strain OLsSf5 in alginate beads with 2.4 µg and the lowest in the control with 1.7 µg.

Table 1. Agronomic variables evaluated in the Poblano chili seedlings in the two biofertilizers.

Treatments	Strain	Foliar area (cm ²)	Number of leaves	Height (cm)	Biomass (mg)	P (µg)	K (µg)	N (µg)
Control	S/inoculate	12.6±0.5 _c	5.7±0.2 _c	10.4±0.4 _d	91.0±2.9 _c	1.7±0.06 _e	14.9±0.6 _c	6.7±0.04 _d
Peat	CPAC53	17.2±0.6 _{ab}	6.7±0.2 _{ab}	12.7±0.2 _{ab}	131.1±4.9 _{ab}	1.9±0.06 _{cd}	18.6±0.6 _b	7.5±0.4 _{cd}
	CPPC55	16.5±0.5 _{ab}	6.5±0.1 _{abc}	12.3±0.3 _{abc}	121.5±4 _b	2.1±0.05 _{bc}	19.6±0.4 _{ab}	8.1±0.06 _{bc}
	P61	15.6±0.5 _b	5.8±0.2 _{bc}	10.9±0.4 _{cd}	129.1±6.9 _{ab}	2.2±0.04 _{bc}	18.1±0.4 _b	8.1±0.3 _{bc}
	OLsSf5	12.8±0.6 _c	5.8±0.2 _{bc}	11.0±0.4 _{cd}	101.9±3.9 _{bc}	1.9±0.1 _d	16.1±0.7 _c	7.3±0.3 _d

Treatments	Strain	Foliar area (cm ²)	Number of leaves	Height (cm)	Biomass (mg)	P (μg)	K (μg)	N (μg)
Pearls from alginate	CPAC53	18.3±0.6 _a	7.2±0.24 _a	13.3±0.3 _a	155.3±6.5 _a	2.2±0.06 _b	21.0±0.7 _a	8.7±0.2 _{ab}
	CPPC55	17.4±0.4 _{ab}	6.7±0.1 _{ab}	13.3±0.3 _a	128.4±6.3 _{ab}	2.1±0.02 _{bcd}	18.1±0.4 _b	7.2±0.06 _d
	P61	16.8±0.5 _{ab}	6.6±0.1 _{abc}	11.4±0.4 _{bcd}	154.8±8 _a	2.1±0.1 _{bcd}	18.5±0.8 _b	8.7±0.4 _{ab}
	OLsSf5	18.2±0.5 _a	6.5±0.1 _{abc}	13.3±0.2 _a	151.3±11.3 _a	2.4±0.05 _a	21.1±0.6 _a	9.2±0.2 _a
CV (%)		13.7	13.2	10.3	19.7	8.2	7.7	7.6

The data represent the mean ± standard deviation n= 15. Different letters within the same column represent significant differences, Tukey ($\alpha=0.05$); CV= coefficient of variation.

The highest potassium values were presented with the CPAC53 and OLsSf5 strains in alginate beads with an average of 21 and the lowest in the control with 14.9 μg. Finally, the highest nitrogen value was presented in the seedlings treated with the OLsSf5 strain in alginate beads with 9.2 μg and the lowest with the CPPC 55 strain in the same formulation with 7.2 μg, and the control with 6.7 μg (Table 1). The Poblano chili seedlings presented a response similar to inoculation with the BPCV strains in both formulations, except for the OLsSf5 strain that presented its greatest effect on the seedlings when applied in alginate beads.

In general, a tendency for a slightly higher effect on seedlings was found when the alginate pearl formulation was used compared to the use of peat. In the agricultural field, polymers are widely used for different purposes, for example, to make the application of pesticides more efficient in the control of crop pathogens and as bioremediators of contaminated soils (Puoci *et al.*, 2008). In the case of biofertilizers in agriculture, the use of alginate beads is an option for the gradual delivery of microorganisms to plant roots (Bashan *et al.*, 2014).

In our case, the alginate pearl formulation was used in Poblano chili seedlings, showing that it is equally effective that the peat formulation, with the advantage that protects the bacteria and gradually releases them. In Poblano chili there are few studies on the quality of seedlings. Obtaining healthy and vigorous plants in seedlings is vital so that they can be established in the field after transplantation, which can be achieved with proper handling of the substrate, fertilization and seed health (García *et al.*, 2011).

The combination of management practices in seedlings and the use of bioformulations can lead to success in the establishment of plants in the field. However, the use of inoculants is not included in the management practices of Poblano chili (Huerta *et al.*, 2007). The use of these in seedlings can reduce crop costs, combat phytopathogenic damage and increase seedling vigor.

The use of biofertilizers is not current, but in the case of the cultivation of Poblano chili, there are no specific biofertilizers in the market for this crop to promote growth and health.

The wet and peat alginate formulations used significantly promoted the growth of Poblano chili seedlings at the seedling level. These formulations may be an option in the production of healthy and vigorous chili seedlings for transplantation. In the case of alginate beads, they slowly release bacteria into the soil, which can provide an advantage over peat when applied to crops.

Conclusions

Serratia strains had the highest growth in TBS broth and *Pseudomonas* strains in LB broth. Of the two formulations, wet alginate beads maintained the highest population for five months. The formulations that had the greatest effect on Poblano chili were from *S. liquefaciens* CPAC53, *P. tolaasii* P61 and *P. yamanorum* OLSf5 strains in wet alginate beads or peat. Bioformulations stimulated seedling growth up to 35% with respect to the control in seedling. Both bioformulations can be used to increase the growth of Poblano chili seedlings (*Capsicum annuum* L.) and optimize nutrient application.

Cited literature

- Albareda, M.; Rodríguez-Navarro, D. N.; Camacho, M. and Temrano, F. J. 2008. Alternatives to peat as carrier for rhizobia inoculants: Solid and liquid formulations. *Soil Biol. Biochem.* 40(11):2771-2779.
- Angayarkanni, T.; Subash, A. and Kamalakannan, A. 2014. Efficacy of talc-based formulation of *Pseudomonas fluorencens* on the management of leaf spot disease of *Stevia rebaudiana* Bertoni. *Helix.* 4(1):460-467.
- Angulo-Castro, A.; Ferrera-Cerrato, F.; Alarcón, A.; Almaraz-Suárez, J. J.; Delgadillo-Martínez, J.; Jiménez-Fernández, M. y García-Barradas, O. 2018. Crecimiento y eficiencia fotoquímica del fotosistema II en plántulas de 2 variedades de *Capsicum annuum* L. inoculadas con rizobacterias u hongos micorrizicos arbusculares. *Rev. Argentina de Microbiología.* 50(2):178-188.
- Ardakani, S. S.; Heydari, A.; Tayebi, L. and Mohammadi, M. 2010. Promotion of cotton seedlings growth characteristics by development and use of new bioformulations. *Inter. J. Bot.* 6(2):95-100.
- Bashan, Y. 1986. Alginate beads as synthetic inoculant carriers for the slow release of bacteria that affect plant growth. *Appl. Environ. Microbiol.* 51(5):1089-1098.
- Bashan, Y. 1998. Inoculants of plant growth-promoting bacteria for use in agriculture. *Biotechnology Advances.* 16(4):729-770.
- Bashan, Y.; De Bashan, E. L.; Hernández, P. J.; Puente, E. M.; Bacilio, M. y Leyva, A. L. 2008. Inoculantes microbianos sintéticos: ¿son el futuro?. *In: Díaz-Franco, A. y Meyek-Pérez, N. (Ed.). La Biofertilización como tecnología sostenible. México, Consejo Nacional de Ciencia y Tecnología. Editorial Casa y Valdés. México, DF. 167-189 pp.*
- Bashan, Y.; De-Bashan, L. E.; Prabhu, S. R. and Hernández, J. P. 2014. Advances in plant growth bacterial inoculant technology: formulations and practical perspectives (1998-2013). *Plant Soil.* 378(1-2):1-3.
- Bashan, Y.; Hernández, J. P. and Leyva-Macario, L. A. 2002. Alginate microbeads as inoculant carriers for plant growth-promoting bacteria. *Biol. Fertility Soils.* 35(5):359-368.
- Bhattacharyya, P. N. and Jha, D. K. 2012. Plant growth-promoting rhizobacteria (PGPR) emergence in agriculture. *World Journal of Microbiology and Biotechnology.* 28(4):1327-1350.
- Bremner, J. M. 1996. Nitrogen total. *In: Sparks, D. L. (Ed.). Methods of soil analysis. Part 3: Chemical Methods, Series 5. Madison, Wisconsin. Soil Science Society of America. 1085-1122 pp.*

- Czajkowski, R.; de Boer, W. J.; Van Veen, J. A. and Vander Wolf, J. M. 2012. Studies on the interaction between the biocontrol agent, *Serratia plymuthica* A30, and blackleg-causing *Dickeya* sp. (biovar 3) in potato (*Solanum tuberosum*). *Plant Pathology*. 61(4):677-688.
- De-Bashan, L. E. and Bashan, Y. 2008. Joint Immobilization of plant growth-promoting bacteria and green microalgae in alginate beads as an experimental model for studying plant-bacterium interactions. *Appl. Environ. Microbiol.* 74(21):6797-6802.
- De-Bashan, L. E.; Holguin, G.; Glick, B. R. and Bashan, Y. 2007. Bacterias promotoras de crecimiento en plantas para propósitos agrícolas y ambientales. *In: Ferrera-Cerrato, R. y Alarcón, A. (Ed.). Microbiología agrícola: hongos, bacterias y macrofauna, control biológico, planta microorganismo. México. Capítulo 8. Editorial Trillas. 170-224 pp.*
- Dinesh, R.; Srinivasan, V.; Hamza, S. and Manjusha, A. 2010. Short-term incorporation of organic manures and biofertilizers influences biochemical and microbial characteristics of soils under an annual crop [Turmeric (*Cucurma longa* L.)]. *Bio. Technol.* 101(12):4697-4702.
- Ferrera-Cerrato, F.; González, C. M. C. y Rodríguez, M. M. N. 1993. Manual de agromicrobiología. México. Editorial Trillas. 141 p.
- Fuentes-Ramírez, L. E. and Caballero-Mellado, J. 2005. Bacterial biofertilizers. *In: Siddiqui, Z. A. (Ed.). PGPR: biocontrol and biofertilization. The Netherlands, Springer, Dordrecht. 143-172 pp.*
- Garboza, F.; Frontado, R.; Noguera, N.; Avila, H.; Ojeda, L.; Ramirez, N.; Triana, J. y Triana F. 2011. Uso de medios alternativos a base de hidrolizado de caseína y extracto de *Aspergillus niger* y su efecto sobre la expresión genética de una cepa de *Escherichia coli*. *Rev. Sociedad Venezolana de Microbiología.* 31(2):138-143.
- García, M. C.; Taboada, G. O. R.; López, S. H.; López, P. A.; Mora, A. G. y Tlapal, B. B. 2011. Calidad de plántulas de chile ‘Poblano’ en la Sierra Nevada de Puebla, México. *Rev. Fitotec. Mex.* 34(2):115-121.
- Gómez, G. y Batista, C. 2006. Optimización de medios de cultivos para microorganismos, una valiosa estrategia para la producción de biopreparados de interés agrícola. *Cultivos Tropicales.* 27(3):17-25.
- González, M. A.; Almaraz, S. J. J.; Ferrera, C. R.; Rodríguez, G. M. P.; Taboada, G. O. R.; Trinidad, S. A.; Alarcón, A. y Arteaga, G. R. I. 2017. Caracterización y selección de rizobacterias promotoras de crecimiento en plántulas de chile Poblano (*Capsicum annum* L.). *Rev. Inter. Contam. Amb.* 33(2):463-474.
- Grimont, P. A. and Grimont, F. 1978. The genus *Serratia*. *Ann. Rev. Microbiol.* 32:221-248.
- Herrmann, L. and Lesueur, D. 2013. Challenges of formulation and quality of biofertilizer for successful inoculation. *Appl. Microbiol. Biotechnol.* 97(20):8859-8873.
- Holt, J. G.; Krieg, N. R.; Sneath, P. H.; Staley, J. T. and Williams, S. T. 1994. *Bergey’s manual of determinate bacteriology.* Baltimore, USA. 754 p.
- Huerta, D. A.; Rojas, S. F. y Fletes, I. O. 2007. Manual de chile Poblano: importancia económica y sociocultural. México. Colegio de Postgrados. Campus Puebla, Fundación Produce Puebla, AC. 80 p.
- Ivanova, E.; Teunou, E. and Poncelet, D. 2005. Alginate based macrocapsules as inoculants carriers for production of nitrogen fixing biofertilizers. *In: Nikolova, M. and Donev, A. (Ed). Proceedings of the Balkan Scientific Conference of Biology. Bulgaria. 90-108 pp.*
- Khalil, S.; Ali, T. A.; Skory, C.; Slininger, P. J. and Schisler, D. A. 2016. Evaluation of economically feasible, natural plant extract-based microbiological media for producing biomass of the dry rot biocontrol strain *Pseudomonas fluorescens* P22Y05 in liquid culture. *World J. Microbiol. Biotechnol.* 32(2):1-11.

- Lebsky, V. K.; Gonzalez-Bashan, L. E. and Bashan, Y. 2001. Ultrastructure of interaction in alginate beads between the microalga *Chlorella vulgaris* with its natural associative bacterium *Phyllobacterium myrsinacearum* and with the plant growth-promoting bacterium *Azospirillum brasilense*. Canadian J. Microbiol. 47(1):1-8.
- Maheshawari, D. K.; Dubey, R. C.; Agarwal, M.; Dheeman, S.; Aeron, A. and Bajpai, V. K. 2015. Carrier based formulations of bioenotic consortia of disease suppressive *Pseudomonas aeruginosa* KRP1 and *Bacillus licheniformis* KRB1. Ecol. Eng. 81:272-277.
- Puoci, F.; Iemma, F.; Gianfranco, S. U.; Cirillo, G.; Curcio, M. and Picci, N. 2008. Polymer in agriculture: a review. Am. J. Agric. Biol. Sci. 3(1):299-314.
- Rivera, B. D. M. y Botía, D. M. R. 2008. Optimización de un medio de cultivo para la producción de un inoculante con base en *Azospirillum brasilense* C16. No. PDF 089.
- SAS Institute Inc. 2002. SAS System for Windows Computer Program, Version 9.00. SAS Institute Inc., Cary.
- Soria, F. M. J.; Ferrera-Cerrato, R.; Barra, J. E.; González, G. A.; Santos, J. T.; Gómez, L. B. y Pérez, G. P. 2001. Producción de biofertilizantes mediante biodigestión de excreta líquida de cerdo. Terra. 19(4):353-362.
- Trivedi, P.; Pandey, A. and Palni, L. M. S. 2005. Carrier-based preparations of plant growth promoting bacterial inoculants suitable for use in cooler regions. World J. Microbiol. Biotechnol. 21(6-7):941-945.
- Vessey, J. K. 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil. 255(2):571-586.
- Yubur, R.; Bashan, Y. and Hernández-Carmona, G. 2007. Alginate from the macroalgae *Sargassum sinicola* as a novel source for microbial immobilization material in wastewater treatment and plant growth promotion. J. Appl. Phycol. 19(1):43-53.