Article

Incidence and severity of the common blight in bean plants inoculated with *Rhizobium phaseoli*

José Osvaldo Aguilar Ramírez[1]
Gabriel Gallegos Morales[1]
Francisco Daniel Hernández Castillo[1]
Melchor Cepeda Siller[1]
David Sánchez-Aspeytia[2§]

[1]Department of Agricultural Parasitology-Autonomous Agrarian University Antonio Narro. Buenavista, Saltillo, Coahuila, Mexico. CP. 25315. Tel. 01(844) 4110326. (uaaan-osvaldo@hotmail.com; fdanielhc@hotmail.com; melchoresraza2010@hotmail.com; aspeytia.david@inifap.gob.mx). [2]Experimental Field Saltillo-INIFAP. Carretera Saltillo-Zacatecas km 342+119, núm. 9515, Colonia Hacienda de Buenavista, Saltillo, Coahuila, Mexico. CP. 25315. Tel. 01(844) 2912234.

Corresponding author: ggalmor@uaaan.mx.

Abstract

The common bacterial blight *Xanthomonas axonopodis* pv. *phaseoli* is a disease that attacks the bean crop (*Phaseolus vulgaris*), is present 83% of the areas of seed production and up to 79% in commercial fields and reduces yields up to 55%. The inoculation of *Rhizobium* in plants as a promoter of plant growth, nitrogen fixer, to the synthesis of indoleacetic acid, produces in the plant greater vigor and tolerance to diseases. The behavior of the incidence and severity of common blight of bean plants inoculated with *Rhizobium phaseoli* and *X. axonopodis* pv. *phaseoli*. For this, strains of *Rhizobium* (FM-1, N-2, PS-3, M-4, BJ-5) of root nodules of bean plants and *Xanthomonas* (Xant 1) of necrotic leaf spots were isolated. The isolates were identified based on cellular, colonial and biochemical morphological characteristics. The greenhouse and field trials were carried out on seeds inoculated with 10^6 cfu mL^{-1} of *Rhizobium* and a second application 10 days after planting. Subsequently, 15 days after emergence of the bean, *Xanthomonas axonopodis* was inoculated directly by sprinkling directly into the plant. After 30 days of greenhouse cultivation, it was observed that the severity of common blight was lower in plants with nodules in the root (14.5%), than in the control (46%), it was also found that plants inoculated with *Rhizobium phaseoli* BJ -5 developed greater vigor, stem diameter, dry weight, number of leaves and root length. In the field, the inoculation with strain BJ-5 also showed lower severity (25.6%) compared to the control (55.8%) and it turned out to have better growth in the plant. The inoculation of beans with *Rhizobium phaseoli* allows the crop to be nutritionally and phytosanitarily favored.

Keywords: biological control, phytosanitary, promoter of growth.

Reception date: January 2019
Acceptance date: March 2019

Introduction

The common bacterial blight *Xanthomonas axonopodis* pv. *phaseoli* is a bacterium that attacks the cultivation of bean (*Phaseolus vulgaris*) worldwide (Gent *et al*., 2005). It is present in 83% of the areas of seed production and up to 79% in commercial fields, it reduces yields by 55%, those that increase at temperatures of 27 °C, with high relative humidity (Fourie, 2002; Belete and Basta, 2017). For its management, chemical, biological, cultural and genetic control methods can be used (Francisco *et al*., 2013). The use of chemicals has allowed for greater production, but excessive use causes problems to the environment, so searches for alternatives to the management of diseases are of vital importance (Zavaleta, 2000). Compatibility has been reported in *Pseudomonas* sp., *Bacillus cereus* and *Rhodococcus fascians* with *Rhizobium leguminosarum* bv. *phaseoli*, whose symbiosis helps develop a protective activity against common bean blight (Zanatta *et al*., 2007).

The plant growth promoting rhizobacteria (RPCV), are used as biological control agents, these bacteria are characterized by their ability to directly or indirectly facilitate the development of the root and foliage, the indirect stimulation of plant growth is due to the fact that the bacterium allows fungal action (Essalmani and Lahlou, 2003). Direct stimulation may include nitrogen fixation (Sessitsch *et al*., 2002), the production of hormones (Perrine *et al*., 2004), enzymes (Mayak *et al*., 2004) and the solubilization of phosphates (Rodríguez and Fraga, 1999). The objective of this work was to determine the behavior of bean plants, inoculated with *Rhizobium phaseoli* and *X. axonopodis* pv. *phaseoli* and its relationship with the presence and development of common blight in the plant.

Materials and methods

Isolation of *Xanthomonas* spp.

Plants with irregular necrotic leaf spots surrounded by yellow halo were collected in different areas of the leaves (Fourie, 2002) in the experimental field The Bajío of the Autonomous Agrarian University Antonio Narro (UAAAN). The samples were cut into pieces, disinfected with 1% hypochlorite for 3 min and washed with sterile distilled water, then placed in a sterile mortar where they were macerated in 0.85% saline, then a roast was taken, which was fluted. in YDC culture medium (Schaad, 1988). The plates were incubated at 28 °C for 48 h. At the end of the period, bacterial colonies were selected with yellow color, convex, with whole borders and mucoid appearance (Schaad, 2001) and purified by stria. The identification was made by morphological, colonial and biochemical characterization (Abd-Alla and Bashandy, 2008; Francisco *et al*., 2013; Osdaghi, 2014).

Isolation of *Rhizobium* spp.

Samples of reddish colored nodules of different bean varieties were collected in the prefloration period (Table 1), washed and disinfected with 1% hypochlorite for 3 min, then washed three times with sterile distilled water and placed in a sterile tube. where they macerated, a roast was later taken and plated with the yeast mannitol agar red congo extract (ELMA-RC) (Vincent, 1970;

CIAT, 1988). Plates were incubated at 28 °C for 4 days. The red colonies were reseeded in plates with the same medium, until obtaining pure cultures, each isolate was characterized by physiological, biochemical, morphological and colonial tests.

Table 1. Location coordinates of the study area.

Location	Coordinates	Altitude (m)	Samples	Strains
UAAAN Buenavista, Saltillo, Coahuila.	25° 22” North and 101° 02” West	1 742	Bean, pre-flowering stage	FM-1
San Andres Tlalamac, Edo. México.	18° 58’ 1” North and 98° 48’ 28” West	2 073	Bean, pre-flowering stage	N-2 and M-4
UAAAN Buenavista, Saltillo, Coahuila.	25° 22’ North and 101° 02’ West	1 742	Bean, pre-flowering stage	PS-3 and BJ-5

Bacterial reproduction

To increase the populations of *Xanthomonas* liquid YDC was used, while for *Rhizobium* it was liquid ELMA, propagating in a rotary incubator (C25 Incubator Shaker New Brunswick Scientific) at 28 °C and 150 rpm for 72 h, to obtain each bacterium a cell concentrate

Greenhouse experiment

To determine the relationship between the presence of common blight and its severity in bean plants inoculated with *R. phaseoli*, bean seeds were initially disinfected, for which 1% sodium hypochlorite was used for one minute, washing the seeds three times with sterile water. Each seed was impregnated by spraying with a solution of 1 x 10^6 cells of *R. phaseoli*, later they were placed in unicel containers of 1 liter capacity with ¾ parts of *peat moss*-pearlite (in proportion 3:1), a second application it was done 10 days after sowing. Each plant represented an experimental unit, being ten plants per treatment (Table 2).

Table 2. Experimental design to evaluate the behavior of *Phaseolus vulgaris* to the inoculation of *R. phaseoli* and common blight.

Treatments	Strains					
	FM-1	N-2	PS-3	M-4	BJ-5	Xant-1
I. *Rhizobium* (FM-1)	+	-	-	-	-	+
II. *Rhizobium* (N-2)	-	+	-	-	-	+
III. *Rhizobium* (PS-3)	-	-	+	-	-	+
IV. *Rhizobium* (M-4)	-	-	-	+	-	+
V. *Rhizobium* (BJ-5)	-	-	-	-	+	+
VI. Irrigated only with uninoculated water, absolute control (CA)	-	-	-	-	-	+

+= applied; -= not applied.

The pathogen (*Xanthomonas axonopodis*) was inoculated to fifteen days of growth of the culture, with a suspension of 1.6 x 10^6 cells, applied by spray to the foliage (Pastor, 1991; Abeysinghe, 2009). The evaluation was made thirty days later to determine the incidence and severity of common blight. according to the scale of the degree of yellowness and necrosis of the inoculated plants: 0= absence of symptoms; 1= from 1 to 12.5% of damaged area; 2= from 13 to 25.5%; 3 from 26 to 38.5%; 4= from 39 to 51.5%; 5= from 52 to 64.5%; 6= from 65 to 77.5%; 7= from 78 to 90.5%; 8= from 91 to 100% of damaged leaf area (Gilberston *et al*.,1988; Osdaghi *et al*., 2009).

Field experiment

To determine the behavior of bean plants inoculated with *R. phaseoli* and the presence of common blight in the field, an experimental plot of 10 m long with five furrows at 80 cm distance between them was established. Each *Rhizobium* isolate was applied as a treatment (Table 2), to inoculate the seed and the plant the same procedure was followed for the greenhouse experiment.

Subsequently, the inoculated seeds were sown at a depth of 3 cm interspersed every 10 cm in the furrow in the whole plot established with a drip irrigation system. Fifteen days after sowing *Xanthomonas axonopodis* was applied at a concentration of 1.6 x 10^6 cfu mL^{-1} to the foliage of the crop (Pastor, 1991) and 50 days later the incidence and severity of the common blight with the scale was determined (Gilberston *et al*., 1988; Osdaghi *et al*., 2009).

Statistical analysis

The behavior of the variables analyzed in each experiment, as well as the incidence and severity of common bean blight in each plant, was processed under a completely randomized design, where each plant was considered as an experimental unit, taking data from 10 plants by treatment. The variance between the data (Anova) was analyzed through means comparison according to Tukey (<0.05) processing the data in the statistical package SAS Statistical Analysis System version 9.0.

Results and discussion

Five isolates of *Rhizobium* spp. were recovered from radicular nodules of bean plants, in the Table 3 shows the results of different biochemical tests and the characteristics of shape, edge, elevation, appearance and color, which indicate that the isolates have the characteristics of a good inoculant of the *Rhizobium phaseoli* type, which agrees with studies carried out by (Cuadrado *et al*., 2009; Berrada *et al*., 2012; Granda *et al*., 2013).

Coloration of the colonies of the isolates obtained with a beige color at 72 h, which becomes intense red after 8 days of incubation (Rojas *et al*., 2009) to rule out similarity, biochemical tests were performed with the genus *Agrobacterium*, the medium mannitol yeast added bromothymol blue (LMA-ABT) was used, where the *Rhizobium* isolates acidified the medium turning it yellow while the reports indicate that *Agrobacterium* alkalizes the medium turning it blue (Cuadrado *et al*., 2009).

Table 3. Biochemical, morphological and colonial behaviors of R. phaseoli.

Strains	Biochemical and morphological							Colonial				
	Gram stain	Oxidase	Catalase	Growth in NaCl 2, 3 and 5%	Growth in pH 4, 5, 7 and 9	MacConckey growth	Growth AML-ABT	Shape	Edge	Elevation	Appearance	Colour
FM-1	-	-	-	+	+	+	+	Circulate	Wavy	Convex	Mucoid	Red
N-2	-	-	+	+	+	+	+	Circulate	Wavy	Convex	Mucoid	Red
PS-3	-	-	+	+	+	+	+	Circulate	Wavy	Planoconvex	Mucoid	Red
M-4	-	-	+	+	+	+	+	Circulate	Wavy	Planoconvex	Mucoid	Red
BJ-5	-	-	+	+	+	+	+	Circulate	Wavy	Convex	Mucoid	Red

From leaves with symptoms of common blight, strains were identified that presented characteristics typical of the genus *Xanthomonas*, such as yellow colonies with a whole, convex edge and a mucoid appearance, which agrees with what was described by (Corzo *et al*., 2015). Isolated strains were negative for gram stain, levana production and fluorescent pigments, while peroxidase, catalase, oxidative and fermentative metabolism of glucose and starch hydrolysis tests were positive, indicating that the recovered strain corresponds to *Xanthomonas axonopodis* and agrees with the characteristics reported by (Schaad, 2001; Francisco *et al*., 2013).

Greenhouse experiment

The inoculation of *Rhizobium phaseoli* in bean plants induced a greater tolerance to leaf expression of common blight. In Figure 1, it is shown that the strain *Rhizobium phaseoli*-BJ-5 showed lower severity (14.5%) to this disease compared to the absolute control inoculated with *Xanthomonas axonopodis* that registered 46% damage.

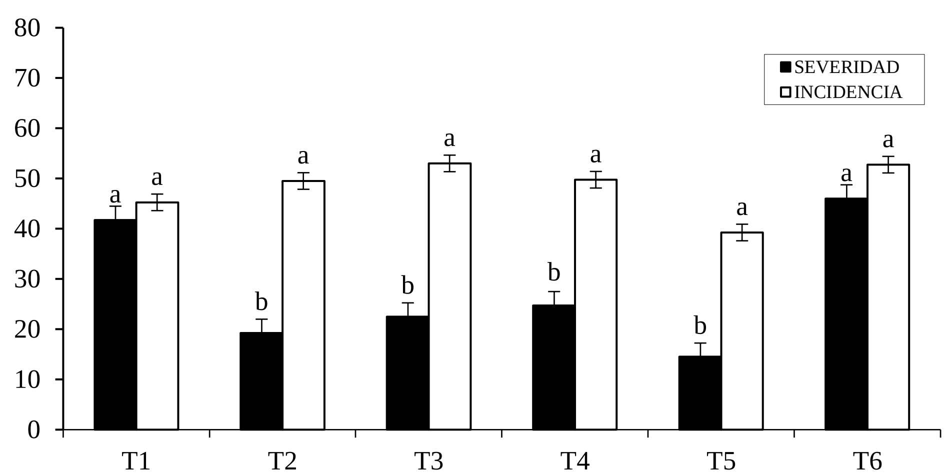

Figure 1. Effect of the inoculation of *Rhizobium phaseoli* on *Phaseolus vulgaris* and the presence and development of common blight in the greenhouse. T_1= *Rhizobium* (FM); T_2= *Rhizobium* (N); T_3= *Rhizobium* (PS); T_4=*Rhizobium* (M); T_5=*Rhizobium* (BJ) and T_6= absolute control (CA).

It was also observed that all the treatments behaved statistically equal ($p < 0.05$) with respect to the incidence of common blight with respect to the control, which indicated that the pathogen is active in all infested plants and there is no difference between them regarding their presence, but not in the degree of response or damage in the manifestation of the disease where two of the inoculants of *Rhizobium phaseoli* T2 and T5, induced or reduced the level of damage (Figure 1) of the common blight with respect to the control statistically ($p < 0.05$), similar experimental results were reported by Zanatta *et al*. (2007) who reports the induction of resistance generated by *Rhizobium* spp. in bean plants.

While Osdagui *et al*. (2011) report results similar to those of the present study, where they show significant differences in the manifestation of common blight in plants inoculated with *Rhizobium leguminosarum* pv *phaseoli* compared with plants fertilized with urea and the control.

Table 4 shows the response of *Phaseolus vulgaris* infested with *Xanthomonas axonopodis* and with five inoculants of *R. phaseoli*; through vegetable biomass produced, where it is observed that in most of the quantified variables the inoculated plants showed greater weight, number of leaves and root length than the uninoculated control, coupled with higher nodulation by rhizobia when using them as inoculants favored a greater number and the presence of nodules in the plants within the experiment.

Table 4. Behavior of agronomic variables in bean plants inoculated with *Rhizobium phaseoli* and *Xanthomonas axonopodis* in greenhouse.

Treatments	Fresh weight (g)	Dry weight (g)	No. of leaf	Root length (cm)	Stem diameter (mm)	No. of nodules
Rhizobium FM-1	35.05 a	5.6 a	31.8 ab	20.6 abc	4.06 b	199 a
Rhizobium N-2	25.75 ab	4.2 a	26.8 c	21.6 a	4.32 b	120.2 b
Rhizobium PS-3	25.34 ab	4.4 a	27.6 bc	21.2 ab	5.22 a	116 b
Rhizobium M-4	25.25 ab	3.6 a	28 abc	19.2 bc	4.08 b	73 bc
Rhizobium BJ-5	33.09 a	5.6 a	32.6 a	21 abc	4.44 b	228 a
Absolute control	16.82 b	3.6 a	29 abc	18.8 c	4.08 b	48 c
SE ±	1.38	0.25	0.57	0.27	0.09	12.61
CV	18.78	27.51	8.41	5.73	7.73	19.36

Values with different letters have statistical differences according to Tukey < 0.05.

The strains of *Rhizobium* spp., used in this study induced the improvement to the crop possibly due to the ability of the rhizobia to produce hormones such as indole acetic acid and gibberellic acid growth regulating substances in the plants, observing an increase in emergence, vigor, biomass and development of the root system (Dey *et al*., 2004; Perrine *et al*., 2004; Lugtenberg and Kamilova, 2009). Another study showed that *Phaseolus vulgaris* inoculated with *Rhizobium etli* had 100% germination or emergence in 4 and 8 days, respectively, values with statistical difference compared to 62.5% in 7.5 days with *Phaseolus vulgaris* without inoculation and as absolute control (García *et al*., 2016).

Field experiment

As shown in Figure 2, the presence of *X. axonopodis* in the leaves of the bean plant clearly decreased by the inoculation of *Rhizobium phaseoli* strain BJ-5 (18.4%) followed by strain FM-1 (19.8%) with with respect to absolute control (CA).

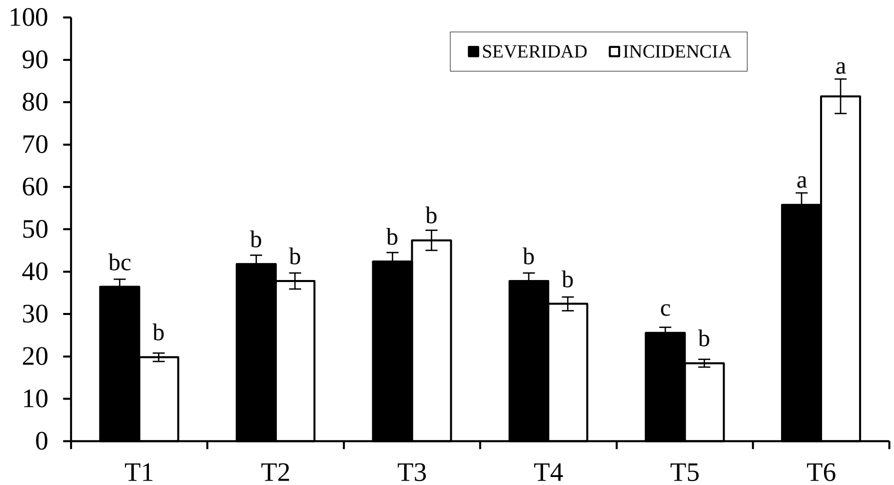

Figure 2. Effect of the inoculation of *Rhizobium phaseoli* on *Phaseolus vulgaris* and the presence and development of common blight in the field. T_1= *Rhizobium* (FM); T_2= *Rhizobium* (N); T_3= *Rhizobium* (PS); T_4= *Rhizobium* (M); T_5= *Rhizobium* (BJ) and T_6= absolute control (CA).

The severity of the disease in the foliar leaves of the bean plant in the crop was classified as mild, given that the strain BJ-5 (25.6%) the numerical values in this phase, were statistically different compared to the non-inoculated or CA with 55.8%, the effect shown by the inoculation of *R. phaseoli* in the bean crop in the presence and severity of the disease in the leaves, emphasizing the decrease in strain BJ-5.

Similar results have been reported by Osdagui *et al*. (2011). Those who report that the severity of the disease in the field evaluated at 50 days was very severe (complete necrosis of the leaves) in the susceptible cultivar and line that were fertilized with urea and in the control group, with very severe loss in the yield, but in the plants inoculated with *R. leguminosarum* pv *phaseoli*, the severity of the disease was relatively low (p< 0.01). According to Bartsev *et al*. (2004) *Rhizobium* spp., can induce resistance in common beans and limit the presence of bacterial common blight. Other studies mention that the treatment of *Rhizobium* spp., reduces stem and root rot compared to non-inoculated plants (Becquer, 2011; Rahman *et al*., 2017).

Table 5 presents the response of *P. vulgaris* in the yield of biomass at the *Rhizobium* seedling level in the field where, like the greenhouse higher yield of fresh, dry weight, number of leaves, stem diameter and root length was observed in inoculated plants, than those not inoculated, highlighting the strain *Rhizobium phaseoli* BJ-5 where these variables had higher values in yield.

Table 5. Behavior of agronomic variables in plants of *Phaseolus vulgaris* inoculated with *Rhizobium phaseoli* and *Xanthomonas axonopodis* in the field.

Treatments	Fresh weight (g)	No. of leaves	Root length (cm)	Stem diameter (mm)	No. of nodules
Rhizobium FM-1	404 a	68.2 a	17.4 ab	8.72 a	54.2 ab
Rhizobium N-2	290 ab	61.4 ab	18.68 ab	9.8 a	34 b
Rhizobium PS-3	390 a	63 ab	17.62 ab	8.76 a	34.2 b
Rhizobium M-4	312 ab	51 ab	15.34 b	7.9 a	39.2 ab
Rhizobium BJ-5	535 a	69.8 a	19.98 ab	9.78 a	79.8 a
Absolute control	136 b	43.8 b	20.2 a	8.34 a	12 b
SE ±	31.21	2.39	0.5	0.22	5.3
CV	37.51	16.87	13.26	12.77	51.78

Values with different letters have statistical differences according to Tukey < 0.05.

The plant growth promoting effect of *Rhizobium phaseoli* in *P. vulgaris* coincides with that reported by Yadegari (2008); Moreno *et al*. (2007). Other studies indicate that *Rhizobium* spp., increases the nodulation in the roots and provides greater nitrogen fixation, productivity and fertility in the crops, even under adverse soil conditions, providing an alternative to be used as a replacement for nitrogen fertilizers (Solaiman *et al*., 2005; Bhattacharjee and Sharma, 2012).

These results also coincide with those of Colas *et al*. (2014) who mention that all plants co-inoculated with *Rhizobium* spp., produce higher yields and numbers of nodules than in non-inoculated ones, which demonstrates the potential of the use of mixed inoculate to improve the productivity of common bean.

Conclusions

The use of symbiotic inoculants of the *Rhizobium phaseoli* type has the potential to reduce and prevent the damages caused by *Xanthomonas axonopodis* and the appearance of symptoms associated with the disease in the culture, such as chlorosis and common bacterial blight of beans, considered an advantage to avoid the use of bactericides and chemical nitrogen fertilizers. The use of microbial agents could be an alternative in bean cultivation due to its activity as a promoter of plant growth and solve problems of a nutritional and phytosanitary nature.

Cited literature

Abd, A, M. H. and Bashandy, S. R. 2008. Bacterial wilt and spot of tomato caused by *Xanthomonas vesicatoria* and *Ralstonia solanacearum in* Egypt. World J. Microbiol. Biotechnol. 24:291-292. Doi 10.1007/s11274-007-9385-8.

Abeysinghe, S. 2009. Induced systemic resistance (ISR) in bean (*Phaseolus vulgaris* L.) mediated by rhizobacteria against bean rust caused by *Uromyces appendiculatus* under greenhouse and field conditions. Arch. Phytopathol. Plant Protec. 42(11):1079-1087. Doi: 10.1080/03235400701622154.

Bartsev, A.; Kobayashi, H. and Broughton, W. J. 2004. Rhizobial signals convert pathogens to symbionts at the legume interface. *In*: Plant Microbiol. (Gillings, M. y Holmes, A. (Ed.). BIOS Scientific Publishers, Oxford, UK. 19-31 p.

Bécquer, C.; Salas, Á.; Palmero, L. y Nápoles, J. 2011. Efecto de la inoculación con rizobios procedentes de Alberta, Canadá, en sorgo (*Sorghum bicolor* L. Moench), en condiciones de campo. Pastos y Forrajes 34: 303-311. http://scielo.sld.cu/pdf/pyf/v34n3/pyf06311.pdf.

Belete, T. and Bastas, K. K. 2017. Common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*) of Beans with Special Focus on Ethiopian Condition. J. Plant Pathol. Microbiol. 8:403. doi: 10.4172/2157-7471.1000403.

Berrada, H.; Nouioui, I.; Iraqui, H. M.; Ghachtouli, N.; Gtari, M. and Fikri, B. K. 2012. Phenotypic and genotypic characterizations of *rhizobi*a isolated from root nodules of multiple legume species native of Fez, Morocco. Afr. J. Microbiol. Res. 6:5314-5324. Doi: 10.5897/AJMR11.1505.

Bhattacharjee, S. and Sharma, G. D. 2012. Effect of dual inoculation of arbuscular *mycorrhiza* and *Rhizobium* on the chlorophyll, nitrogen and phosphorus contents of pigeon pea (*Cajanus cajan* L.). Adv. Microbiol. 2:561-564. Doi: 10.4236/aim.2012.24072.

CIAT. 1988. Centro Internacional de Agricultura Tropical. Simbiosis leguminosas *Rhizobium*. Manual de métodos evaluación, selección y manejo. Sección microbiología de suelos. Programa de pastos tropicales y Programa de fríjol. CIAT, Cali-Colombia 178 p. http://pdf.usaid.gov/pdf_docs/PNABE088.pdf.

Colás, S. A.; Torres, G. R.; Cupull, S. R.; Rodríguez, U. A.; Fauvart, M.; Michiels, J. and Vanderleyden, J. 2014. Effects of co-inoculation of native *Rhizobium* and *Pseudomonas* strains on growth parameters and yield of two contrasting *Phaseolus vulgaris* L. genotypes under Cuban soil conditions. Eur. J. Soil Biol. 62:105-112. Doi: 10.1016/j.ejsobi.2014.03.004.

Corzo, L. M.; Rivero, G. D.; Zamora, G. L.; Martinez, Z. Y. y Martínez C. B. 2015. Detección e identificación de nuevos aislados de *Xanthomonas axonopodis* pv. *phaseoli* en cultivares de frijol común (*Phaseolus vulgaris* L.) en la provincia Mayabeque, Cuba. Rev. Protección Vegetal. 30 (2):97-103. http://scielo.sld.cu/pdf/rpv/v30n2/rpv03215.pdf.

Cuadrado, B.; Rubio, G. y Santos, W. 2009. Caracterización de cepas de *Rhizobium* y *Bradyrhizobium* (con habilidad de nodulación) seleccionadas de los cultivos de frijol caupí (*Vigna unguiculata*) como potenciales bioinóculos. Rev. Colomb. Cienc. Químico-Farmaceuticas, 38(1):78-104. http://www.scielo.org.co/pdf/rccqf/v38n1/v38n1a06.pdf.

Dey, R.; Pal, K.; Bhatt, D. M. and Chauhan, S. M. 2004. Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting *rhizobacteria*. Microbiol. Res. 159: 371-394. DOI: 10.1016/j.micres.2004.08.004.

Essalmani, H. et Lahlou, H. 2003. Mécanismes de bioprotection des plantes de lentile par *Rhizobium leguminosarum* contre *Fusarium oxysporum* sp. *Lentis*. C.R. Biologies. 326:1163-1173. Doi: 10.1016/j.crvi.2003.10.003.

Fourie, D. 2002. Distribution and severity of bacterial diseases on dry beans *(Phaseolus vulgaris* L.) in South Africa. J. Phytopathol. Phytopathol. Zeitschrift. 150:220-226. Doi: 10.1046/j.1439-0434.2002.00745.

Francisco, F. N.; Gallegos, M. G.; Ochoa, F. Y. M.; Hernández, C. F. D.; Benavides, M. A. y Castillo, R. F. 2013. Aspectos fundamentales del tizón común bacteriano (*Xanthomonas axonopodis* pv. *phaseoli* Smith): Características, patogenicidad y control. Rev. Mex. Fitopatol. 31(2):147-160. http://rmf.smf.org.mx/Vol3122013/articulosrevision/ar_aspectos.pdf.

García, R. V. E.; García, O. V. R.; Escareño, H. J. J. y Sánchez, Y. J. M. 2016. Respuesta de *Phaseolus vulgaris* a microorganismos promotores de crecimiento vegetal. Sci. Agropec. 7(3):313-319. Doi: 10.17268/sci.agropecu.2016.03.20.

Gent, D. H.; Lang, J. M. and Schwartz, H. F. 2005. Epiphytic survival of *Xanthomonas axonopodis* pv. *Allii* and *X. axonopodis* pv. *Phaseoli* on leguminous hosts and onion. Plant Dis. 89:558-564. Doi: 10.1094/PD-89-0558.

Gilberston, R. L.; Rand, R. E.; Carlson, E. and. Hagedorn, D. J. 1988. The use of dry-leaf inoculum for establishment of common bacterial blight of beans. Plant Dis. 72:385-389. Doi: 10.1094/PD-72-0385.

Granda, M. K.; Paccha C. H.; Campoverde S. C. y Torres G. R. 2013 Variabilidad de aislados diazotróficos simbióticos en diferentes condiciones agroecológicas del sur del Ecuador. Centro de Biotecnología 2:6-15. http://revistas.unl.edu.ec/index.php/biotecnologia /article/view/83.

Hassan, D. G. H.; Zargar, M. Y. and Beigh, G. M. 1997. Biocontrol of *Fusarium* Root Rot in the Common Bean (*Phaseolus vulgaris* L.) by using Symbiotic *Glomus mosseae* and *Rhizobium leguminosarum*. Microbial Ecol. 34:74-80 https://link.springer.com/article/10.1007/s002489900036.

Kuykendall, D.; Young, J.; Martinez, E.; Kerr, A. and Sawada, H. 2005. *Rhizobium*. *In*: bergey's manual of systematic bacteriology. Second Edition. Volume 2 The Proteobacteria. Part C The *Alpha*-, *Delta*-, and *Epsilonproteobacteria*. Brenner, D. J.; Krieg, N. R. and Staley, J. T. (Eds.). Garrity, G. M. (Ed.). Springer Science and Business Media Inc, New York, USA. 325-340 p.

Lugtenberg, B. and Kamilova, F. 2009. Plant-growth-promoting rhizobacteria. The Ann. Rev. Microbiol. 63:541-556. DOI: 10.1146/annurev.micro.62.081307.162918.

Martínez, P. M.; Castañeda, V. H.; Martínez, M. J. M.; Ruiz L. M. y Álvarez, M. C. 2005. Aislamiento e identificación de *Rhizobium* en *Lupinus* silvestres por la técnica de reacción en cadena de la polimerasa (PCR). Rev. Sci. 7(2):175-181. https://www.researchgate.net/publication/279941626.

Mayak, S.; Tirosh, T. and Glick, B. 2004. Plant growth promoting bacteria confer resistance in tomato plants to salt stress**.** Plant Physiol. Biochem. 42:565-572. Doi: 10.1016/j.plaphy.2004.05.009.

Moreno, S. N.; Moreno, R. L. F. y Uribe, D. 2007. Biofertilizantes para la agricultura en Colombia. *In*: Izaguirre-Mayoral, M. L.; Labandera-C. and Sanjuan, J. (Eds.). Biofertilizantes en Iberoamerica: visión técnica, científica y empresarial. Denad Internacional, Montevideo. Vol. 1. 38-45 pp.

Osdaghi, E.; Alizadeh, A.; Shams-bakhsh M. and Lak, R. M. 2009. Evaluation of common bean lines for their reaction to the common bacterial blight pathogen. Phytopathol. Mediterranea. 48:461-468. https://www.researchgate.net/publication/279596913_evaluation_of-common_bean_lines_for_their_reaction_to_the-common_bacterial_blight-pathogen.

Osdaghi, E.; Shams-bakhsh, M.; Alizadeh, A.; Lak, R. M. and Maleki, H.H. 2011. Induction of resistance in common bean by *Rhizobium leguminosarum* bv.*phaseoli* and decrease of common bacterial blight. Phytopathol. Mediterranea. 50:45-54. https://www.researchgate.net/publication/258689140_Induction_of_resistance_in_common_bean_by_rhizobium_leguminosarum_bv_phaseoli_and_decrease_of_common_bacterial_blight.

Osdaghi, E. 2014. Occurrence of common bacterial blight on mungbean (*Vigna radiata*) in Iran caused by *Xanthomonas axonopodis* pv. *phaseoli*. New Disease Reports 30. 9. Doi: 10.5197/j.2044-0588.2014.030.009.

Pastor, C. M. 1991. Técnica, materiales y métodos utilizados en la evaluación de frijol por su reacción a las enfermedades. *In*: frijol: investigación y producción. López, M.; Fernández, F. y Schoonhoven, A. (Eds.). 2da reimpresión. Centro Internacional de Agricultura Tropical (CIAT). Cali, Colombia. 157-168 pp.

Pérez, G.; Gómez, G.; Nápoles, M. y Morales, B. 2008. Aislamiento y caracterización de cepas de rizobios aisladas de diferentes leguminosas en la región de Cascajal, Villa Clara. Pastos y Forrajes. 31(2):151-159. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0864-03942008000200005.

Perrine, F.; Rolfe, B.; Hynes M. and Hocart, C. 2004. Gas chromatography-mass spectrometry analysis of indolacetic acid and tryptophan following aquerous chloroformate derivatisation of *Rhizobium* exudates. Plant Physiol. Biochem. 42:723-729. Doi: 10.1016/j.plaphy.2004.07.008.

Prakash, V. J.; Yadav, J.; Tiwari, K. and Kumar, A. 2013. Effect of indigenous *Mesorhizobium* spp. and plant growth promoting rhizobacteria on yields and nutrients uptake of chickpea (*Cicer arietinum* L.) under sustainable agriculture. Ecological Engineering. 51:282-286. Doi: 10.1016/j.ecoleng.2012.12.022.

Rahman, M.; Ali, M. E.; Alam, F.; Islam, M. N. and Bhuiyan, M. A. H. 2017. Combined effect of arbuscular mycorrhiza, *Rhizobium* and *Sclerotium rolfsii* on grass pea (*Lathyrus sativus*). The Agriculturists. 15(1):143-155. Doi:10.3329/agric.v15i1.33438.

Rodríguez, H. and Fraga, R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances. 17:319-339. Doi: 10.1016/S0734-9750(99)00014-2.

Rojas, T. D. F.; Garrido, R. M. F. y Bonilla, B. R. R. 2009. Estandarización de un medio de cultivo complejo para la multiplicación de la cepa C50 de Rhizobium sp. Corpoica-Ciencia y Tecnología Agropecuaria. 10(1):70-80. Doi: 10.21930/rcta.vol10_num1_art:131.

Saddler, G. S. and Bradbury, J. F. 2005. *Xanthomonadales* ord. nov. Bergey's manual of systematic bacteriology. *In*: validation of the publication of new names and new combinations previously effectively published outside the IJSEM, List no.106. Inter. J. Sys. Evol. Microbiol. 55:2235-2238. Doi: 10.1099/ijs.0.64108-0.

Schaad, N. W. 1988. Laboratory guide for identification of plant pathogenic bacteria. APS press. 2nd ed. The American Phytopathological Society; St Paul, Minnesota. 84-107 pp.

Schaad, N. W.; Jones, J. B. and Chun, W. 2001. Laboratory guide for identification of plant pathogenic bacteria. APS press. 3rd (Ed.). American Phytopathological Society, St Paul, Minnesota. 218 p.

Sessitsch, A.; Howieson, J. G.; Perret, X.; Antoun. H. and Martínez-Romero, E. 2002. Advances in Rhizobium research. Critical Rev. Plant Sci. 21:323-378. Doi:10.1080/0735-260291044278.

Solaiman, A. R. M.; Rabbani, M. G. and Molla, M. N. 2005. Effects of inoculation of *Rhizobium* and arbuscular mycorrhiza, poultry litter, nitrogen, and phosphorus on growth and yield in chickpea. The Korean Soc. Crop Sci. 50(4):256-261. https://www.researchgate.net /publication/264148718.

Vincent, J. M. 1970. A manual for practical study of root nodule bacteria. *In*: international biology program handbook No. 15. Blackwell Scientific Publishers, Oxford. 164 p. http://www.sciepub.com/reference/103923.

Yadegari, M.; Rahmani, H.A.; Noormohammadi G. and Ayneband, A. 2008. Evaluation of bean (*Phaseolus vulgaris*) seeds inoculation with *Rhizobium phaseoli* and plant growth promoting rhizobacteria on yield and yield components. Pak. J. Biol. Sci. 11:1935-1939. Doi: 10.3923/pjbs.2008.1935.1939.

Zanatta, Z. G. C. N.; Moura, A. B.; Maia, L. C. y dos Santos, A. S. 2007. Biossay for selection of biocontroller bacteria against bean common blight (*Xanthomonas axonopodis* pv. *phaseoli*). Braz. J. Microbiol. 38:511-515. Doi:10.1590/S1517-83822007000300024.

Zavaleta, E. 2000. Alternativa de manejo de las enfermedades de las plantas. Revista Mexicana de la Ciencia del Suelo. Terra Latinoam. 17:201-207. https://www.redalyc.org/pdf /573/57317304.pdf.