Mexican Journal of Phytopathology
ISSN: 2007-8080

Crossref
Open access

Phytopathological Note

Characterization of endophytic bacteria growth-promoting in potato plants (*Solanum tuberosum*)

Rosa María Longoria-Espinoza*, Cristal Leyva-Ruiz, Gloria Margarita Zamudio-Aguilasocho, Universidad Autónoma de Occidente, Unidad Regional Guasave, Departamento de Ciencias Biológicas, Avenida Universidad s/n, CP 81120. Guasave, Sinaloa, México; **Rubén Félix-Gastélum,** Universidad Autónoma de Occidente, Unidad Regional Los Mochis, Departamento de Ciencias Biológicas, Bulevard Macario Gaxiola y Carretera Internacional s/n, CP 81223. Los Mochis, Sinaloa, México.

***Corresponding Author**:
rosa.longoria@uadeo.mx
rosamarialongoria@hotmail.com

Section:
Periodical Issue

Received:
02 October, 2023
Accepted:
10 January, 2024
Published:
23 January, 2024

Citation:
Longoria-Espinoza RM, Leyva-Ruiz C, Zamudio-Aguilasocho GM and Félix-Gastélum R. 2024. Characterization of endophytic bacteria growth-promoting in potato plants (*Solanum tuberosum*). Mexican Journal of Phytopathology 42(2): 10. https://doi.org/10.18781/R.MEX.FIT.2310-4

ABSTRACT

Objective/Background. The purpose of this research was to evaluate the *in vitro* plant growth-promoting activity of endophytic bacteria isolated in tissue from Atlantic variety potato plants from the municipality of Guasave, Sinaloa, Mexico.

Materials and Methods. The bacterial population was isolated in Lb agar culture medium; two bacterial isolates were obtained from the root and two from the stem, all four Gram positive. The bacterial population of the tissue samples was expressed as (CFU/g^{-1}). The phosphate solubilization capacity, production of chitinases and siderophores were qualitatively evaluated.

Results. Partial sequencing of the 16S rDNA gene was performed, allowing the identification of associated bacterial species within the *Firmicutes*. 100% of the strains were identified as *Bacillus* sp. with identities greater than 97%: *B. cereus*, *B. tropicus*, *B. thuringiensis*, *B. fungorum*. The *B. thuringiensis* and *B. cereus* strains showed positive activity in promoting plant growth *in vitro* through phosphate solubilization, production of chitinases and siderophores. *B. cereus* and *B. tropicus* presented inhibitory capacity greater than 50% for *Sclerothium rolfsii.*

Conclusion. It is relevant to continue research carried out in the laboratory, in order to determine its potential in the field, improving the production of potato crops.

Keywords: *Bacillus cereus*, antagonism, siderophores, chitinases phytopathogenic fungi, PGPB.

Introduction

The potato (*Solanum tuberosum*) is the fourth most widely planted food crop worldwide. In Mexico it ranks fourth in terms of planted surface, surpassed only by the basic grains of maize (*Zea mays*), beans (*Phaseolus vulgaris*) and wheat (*Triticum aestivum*) (SADER, 2022). The Mexican potato is highly valued in many countries, due to its nutritional properties and external characteristics. Aside from supplying the internal market, Mexican farmers export over 2,500 tons (SADER, 2022). However, chemical pesticides (insecticides, fungicides, herbicides) are the predominant way to control pests, with a negative impact on the biodiversity of the agro-ecosystems (Chamorro-Anaya *et al*., 2020). Research is currently focused on understanding the diseases where eliminating the pathogen is not the goal, but rather, despite its presence, to achieve good yields for the farmer (Rocha *et al*., 2019). This has increased the development of ecological alternatives such as plant microbiota, composed of a large diversity of bacteria associated with the plants inside the leaf tissue, root, stem and seed without causing any damage; these bacteria are known as endophytic (Hallman *et al.,* 1997; Strobel and Daisy 2003; Huang *et al.,* 2008; Santoyo *et al*., 2016). There is currently a great interest in knowing the beneficial activities, highlighting that the diversity and density of endophytes depends on diverse abiotic and biotic factors (Pérez-Cordero *et al*., 2010; Chamorro-Anaya *et al*., 2020). The endophytic bacteria-plant interaction is related to the promotion of the plant growth and the biocontrol of phytopathogens, which are important characteristics for agro-biotechnological use (Zgadzaj *et al*., 2015; Contreras *et al.,* 2016; Chamorro-Anaya *et al*., 2020). Among the most abundant genera reported as endophytes are *Bacillus, Pseudomonas, Burkholderia, Stenotrophomonas, Micrococcus*, *Pantoea* and *Microbacterium* (Hallman *et al.,* 1997; Rosenblueth and Martínez-Romero, 2006). The north of the state of Sinaloa ranks first nationwide regarding the surface planted with potato, where the main problem is the presence of pests and diseases related to the weather conditions, as well as the abuse of pesticides, which leads to resistance by pests (SADER, 2022). Given that the diverse international regulations to import/export products without agrochemicals, it is necessary to implement strategies that promote plant growth and fight pests in crops. Nevertheless, only a few studies that have reported the diversity of endophytic bacteria in the potato plant. As a consequence, the aim of this investigation was to identify and characterize potential plant growth promoting endophytic bacteria and antagonistic activity for phytopathogenic fungi, thus optimizing the production of this tuber.

Thirty whole potato plants of the Atlantic variety were gathered during the 2022-2023 cycle in a plot of the village of El Gallo located in Guasave, Sinaloa, Mexico, Longitude (dec): 108 Lattitude (dec): 25. The plants were chosen at random and transported to the laboratory for their analysis, separating foliar tissue, root and stem, and washed in running water for 5 min, carefully removing any impurities stuck to them. Later, the plant tissue was disinfested in ethanol at 70% for one minute, washed six times in a 1% sodium hypochlorite solution for 10 min and Tween-80 at 10% (v/v) was added for 1 min; finally, it was washed six times with sterile distilled water. Bacteria were isolated in an Lb agar culture medium in triplicate, using the direct contact technique, with modifications, cutting the tissue transversally and longitudinally under aseptic conditions, with the cut facing the culture medium. As a sterilization control, 100 µL of distilled water were taken from the last wash and planted under the same tissue conditions. Subsequently, it was incubated at 27 °C for eight days (Yang *et al*., 2011). Strains were purified by separating cultures with a different morphology and/or color until cultures were obtained that had similar morphological characteristics. The bacterial population density per tissue was determined by counting directly, expressed as culture forming units (CFU g^{-1}). The identification of possible plant growth promoting bacteria for each of the isolations was carried out in triplicate; qualitatively, the capability of production of siderophores was evaluated in a chrome azurol S (CAS) agar culture medium, where the colonies with yellow/orange zones were considered siderophore-producing strains starting at 20 minutes and for up to 24 h, according to the methodology described by Schwyn and Neilands (1987). Chitinase production was evaluated using the protocol by Shanmugaiah *et al.* (2008); colonies with a clear halo were considered positive. On Pikosvkaya agar plates, the phosphate solubilization capabitily was evaluated, considering those that formed clear halos around the colony as positive (Pikosvkaya, 1948). The strain (B25) *Bacillus cereus* was used as a positive control (Figueroa-López *et al*., 2016).

The *in vitro* antagonistic capability was determined by facing endophytic bacteria isolated from potato plants with phytopathogenic fungi: *Phytophthora capsici, Fusarium oxysporum, Sclerotium rolfsii* and *Colletotrichum coccodes*, isolated from tomato plants (*Solanum lycopersicum*), and jalapeño chili plants (*Capsicum annuum*), reported as the causes of the rotting of roots in tomato plants by Fernández-Herrera *et al*. (2006). The phytopathogenic fungi were donated by the Food and Development Research Center (Centro de Investigación en Alimentación y Desarrollo - CIAD) in Culiacán, Sinaloa. The trial was carried out using the dual cultivation technique in PDA, with three repetitions for every evaluation and the isolations of phytopathogenic fungi in pure culture as a control. The inoculated Petri dishes were incubated at 28 °C for 10 days in a growth chamber (Precision Scientific, Model 6LM, Winchester, U.S.A.); the radial growth of the fungal cultures was measured every 24 h in pathogens and antagonists. The halo of inhibition among cultures in confrontation was measured on day 8 post-incubation (Aquino-Martínez *et al.,* 2008). The antagonism was evaluated by recording the following variables: radial growth of the antagonist (RGA), radial growth of the pathogen (RGP) and the percentage of inhibition of the radial growth (PIRG). The PIRG was determined on day 6 post-incubation using the formula by Ezziyyani *et al*. (2004): PRGI= [(R1- R2)/R1] × 100, where R1= radial growth of the control colony (pathogen) and R2= radial growth of the colony of the pathogen in the *in vitro* confrontation.

Molecular identification was carried out with oligonucleotides F2C (5'-AGAG-TTTGATCATGGCTC-3') and C (5'-ACGGGCGGTGTGTAC-3') (Shi *et al.*, 1997), amplifying the gene that codifies subunit 16S of the rDNA. The purification of the PCR product was carried out using the Wizard® _SV Gel kit and the PCR Clean-Up System. It was then sent to the National Genomics Laboratory for Biodiversity (Laboratorio Nacional de Genómica para la Biodiversidad - LANGEBIO) in CINVESTAV-IPN to be sequenced. The sequences obtained were compares with those deposited in the National Center for Biotechnology Information (NCBI) GenBank using the program BLASTn (http://www.ncbi.nlm.nih.gov/BLAST/). The bases were aligned in the program Clustal W; phylogenetic inferences were obtained using the Neighbor Joining method, based on the model kimura-2-paremeter with a bootstrap test in the program MEGA X.

Four morphologically different strains were obtained from the potato plant tissue (two siolated from the root and two from the stem), all of which were Gram positive and according to the partial sequencing of gene 16S rDNA, it helped identify species associated within the *Firmicutes*. All four strains were identified as *Bacillus* sp. with identities of over 97%: *B. cereus*, *B. tropicus*, *B. thuringiensis* and *B. fungorum* (Figure 1). The results coincide with studies on diversity in endophytic communities in maize roots, where the associated bacteria are *Firmicutes* (*Bacillus*), *Gammaproteobacteria* (*Pseudomonas*) and *Burkholderia* spp. (Pereira *et al*., 2011; Ikeda *et al*., 2013; Sánchez-Bautista *et al*., 2018). The ability of *B. cereus* PR2 and *B. thuringiensis* PR1 to promote plant growth *in vitro* coincides with reports by Barboza-García *et al*. (2023), evaluating the *in vitro* plant growth promoting activity of endophytic bacteria isolated from different tissues of rice varieties, finding strains of the genera *B. cereus* and *B. thuringiensis,* which displayed the ability to promote growth in rice crops by the solubilization of phosphate and the production of siderophores, coinciding with results of this investigation (Table 1). *Bacillus* is of great interest in the scope of investigation, due to its great physiological diversity; members of this genus have been isolated from wild species of commercial interest, such as potato, wheat (*Triticum* spp.), rice (*Oryza sativa*) and sugarcane (*Saccharum officinarum*) (Wang *et al*., 2019; Hassan, 2017). The growth promoting activity is mainly related to the increase in the mobilization of phosphate, with a positive effect on the promotion of plant growth, antagonistic activity, the production of enzymes and siderophores, that allow these

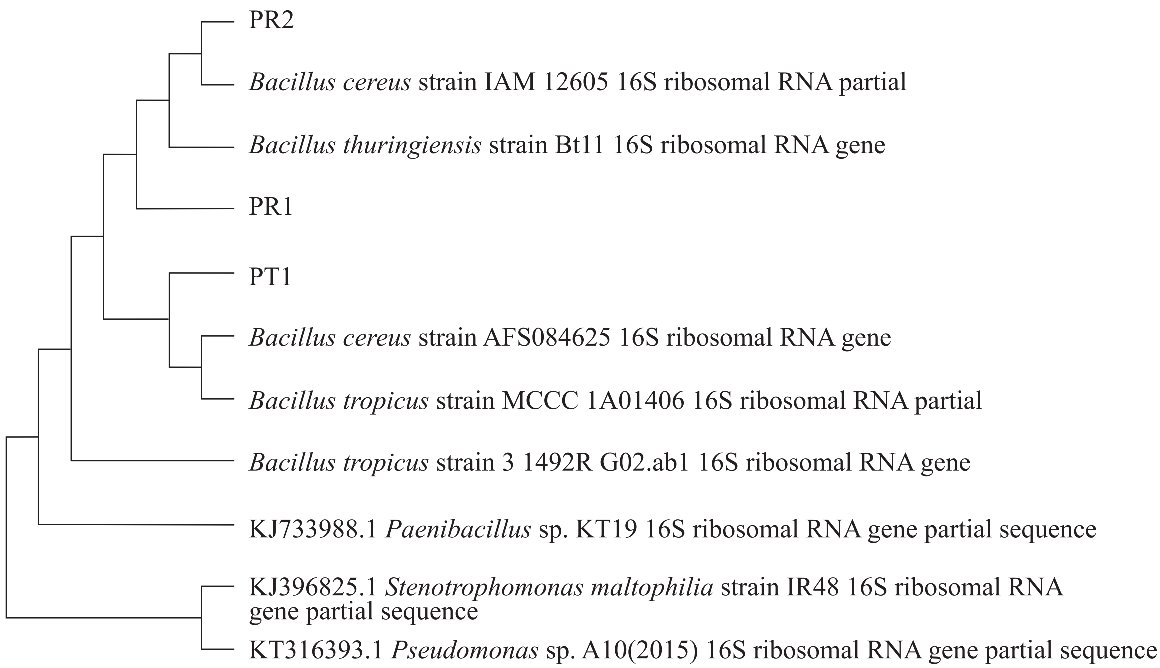

Figure 1. Neighbor-Joining Dendogram from the sequences of the gene that codifies the 16S subunit of the rDNA of endophytic bacteria.

Table 1. Related characteristics in promoting plant growth *in vitro* of bacteria isolated from tissue (root, stem) in potato plants collected in plots of Ejido El Gallo, Municipality of Guasave, Sinaloa, Mexico.

Isolated	Origin	Chitinase	Siderophores	Phosphates
B. cereus [z](B25)	Corn	+	+	+
B. cereus (PR2)	Root	++	++	+++
B. thuringiensis (PR1)	Root	++	++	-
B. tropicus (PT1)	Stem	+	-	-
B. fungorum (PT2)	Stem	-	-	+

Negative activity (-); +< 3mm; ++>3<4mm; +++ >4mm. The letter P stands for potato, R for root and T for stem. [z]Positive control.

microorganisms to exert their biocontrolling and inhibiting abilities, among others otras (Kloepper *et al.,* 2004; Tejera-Hernández *et al.,* 2011; Chamorro-Anaya *et al*., 2020; Barboza-García *et al*., 2023). On the other hand, *B. thuringiensis* has been reported as endophytic bacteria with the capability of solubilizing phosphate and producing siderophores, along with diverse metabolites such as antibiotics and extracellular enzymes such as proteases and chitinases, key compounds for the suppression of pathogens, coinciding with the results of this work. In addition, several *B. thuringiensis* strains have reported to be efficient as a biological control against *Sclerotinia sclerotiorum,* insects and nematodes that cause severe problems in crops of economic interest (Martínez *et al*., 2020; Crickmore *et al*., 2020; Wang *et al*., 2020; Barboza-García *et al.,* 2023).

In this study, the antagonistic effect of the isolations (Table 2) revealed that *B. cereus* PR2 displayed an inhibiting capability of over 50% for *P. capsici* and *S. rolfsii;* and *B. tropicus* PT1, 83.8% against *S. rolfsii,* according to reports by Govin-Sanjudo *et al*. (2019), where they evaluated the antagonistic activity of endophytic *Bacillus* strains from *Leucocroton havanensis*, the nickel-hyperaccumulating plant, against the fungi *Alternaria alternata* and three species of the genus *Fusarium*, where

Table 2. Antagonistic activity against phytopathogenic fungi represented in percentage; of bacteria isolated from tissue (root, stem) in potato plant collected in plots of Ejido El Gallo, Municipality of Guasave, Sinaloa, Mexico.

Isolated	*C. coccodes*	*P. capsici*	*F. oxysporum*	*S. rolfsii*
B. cereus (PR2)	35.03	67.51	6.61	55.29
B. thuringiensis (PR1)	8.12	45.01	20.96	19.22
B. tropicus (PT1)	10.33	13.81	11.85	83.87
B. fungorum (PT2)	5.42	6.04	4.32	21.69

The letter P stands for potato, R for root and T for stem.

the strain *Bacillus* sp. ER11 displayed the highest percentage of inhibition against fungi, with values of over 70% in all cases. There are few studies on endophytic bacteria, diversity, control of phytopathogens using antagonistic microorganisms and their relation with productivity in the potato crop. This study shows that isolated endophytic bacteria inhibit the growth of phytopathogenic fungi, making them an option in the comprehensive management of this crop. Therefore, it is important to continue with the investigations carried out in the laboratory to determine their potential on the field.

Acknowledgments

Universidad Autónoma de Occidente, Unidad Regional Guasave, Sinaloa, Mexico.

Literature Cited

Aquino-Martínez JG, Vázquez-García LM y Reyes-Reyes BG. 2008. Biocontrol *in vitro* e *in vivo* de *Fusarium oxysporum* Schlecht. f. sp. *dianthi* (Prill. y Delacr.) Snyder y Hans, con hongos antagonistas nativos de la zona florícola de Villa Guerrero, Estado de México. Revista Mexicana de Fitopatología 26:127-137. https://doi.org/10.18781/r.mex.fit.1904-3

Barboza-García A, Pérez-Cordero A y Chamorro-Anaya L. 2023. Bacterias endófitas aisladas de cultivo de arroz (*Oryza sativa* L.) con actividad promotora de crecimiento vegetal. Revista Biotecnología en el Sector Agropecuario y Agroindustrial 21(1): 28-39. https://doi.org/10.18684/rbsaa.v21.n1.2023.1728

Chamorro-Anaya LM, Chamorro-Anaya L M y Pérez-Cordero A. 2020. *Bacillus cereus* bacteria endófita promotora de crecimiento vegetal. Revista Colombiana Biotecnología 22(18): 18-23. https://doi.org/10.15446/rev.colomb.biote.v22n2.81723

Crickmore N, Berry C, Panneer SS, Mishra R, Connor T and Bonning B. 2020. Structure-based nomenclature for *Bacillus thuringiensis* and other bacteria-derived pesticidal proteins. Journal of Invertebrate Pathology 10(1): 74-38. https://doi.org/10.1016/j.jip.107438

Contreras M, Loeza PD, Villegas J, Farias R and Santoyo G. 2016. A glimpse of the endophytic bacterial diversity in roots of blackberry plants (*Rubus fruticosus*). Genetic Molecular Research 15(3):1-10. https://doi.org/10.4238/gmr.15038542

Ezziyyani M, Pérez-Sánchez C, Requena ME, Rubio L and Candela-Castillo ME. 2004. Biocontrol por *Streptomyces* rochei-Ziyani de la podredumbre del pimiento (*Capsicum annuum* L.) causada por *Phytophthora capsici*. Anales de Biología 26: 69-78. http://dx.doi.org/10.31428/10317/4019

Fernández-Herrera E, Acosta-Ramos M, Ponce-González F y Manuel-Pinto V. 2007. Manejo biológico de *Phytophthora capsici* Leo., *Fusarium oxysporum* Schlechtend.:Fr. y *Rhizoctonia solani* Kühn en jitomate (*Lycopersicon esculentum* Mill.). Revista Mexicana de Fitopatología 25:35- 42.

Figueroa-López AM, Cordero-Ramírez JD, Martínez-Álvarez JC, López-Meyer M, Lizárraga-Sánchez GJ, Félix-Gastélum R, Castro-Martínez C and Maldonado-Mendoza IE. 2016. Rhizospheric bacteria of maize with potential for biocontrol of *Fusarium verticillioides*. Springer Plus 5: 330. https://doi.org/10.1186/s40064-016-1780-x

Govin-Sanjudo A, Leal-Sanabria G y López-Hernández D. 2019. Actividad antagónica de bacterias endófitas de *Leucocroton havanensis* Borhidi frente a hongos fitopatógenos. Revista de Protección Vegetal 34(2): e10.

Hallman JA, Quadt-Hallman WF, Mahaffee and Kloepper JW. 1997. Bacterial endophytes in agricultural crops. Canadian Journal of Microbiology 43: 895- 914. https://doi.org/10.1139/m97-131

Hassan S. 2017. Plant growth-promoting activities for bacterial and fungal endophytes isolated from medicinal plant of *Teucrium polium* L. Journal of advanced research 8(6): 687–695.https://doi.org/10.1016/j.jare.09.001

Huang ZJ, Cai XL, Shao CL, She ZG, Xia XK and Chen YG. 2008. Chemistry and weak antimicrobial activities of phomopsins produced by mangrove endophytic fungus *Phomopsis* sp. ZSU-H76. Phytochemistry 69:1604–1608. https://doi.org/10.1016/j.phytochem.2008.02.002

Ikeda CA, Bassani LL, Adamoski D, Stringari D, Cordeiro VK, Glienke C, Steffens MBR, Hungria M and Galli-Terasawa LV. 2013. Morphological and genetic characterization of endophytic bacteria isolated from roots of different maize genotypes. Microbial Ecology 65: 154-160. https://doi.org/10.1007/s00248-012-0104-0

Kloepper JW, Ryu CM and Zhang S. 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathology 94: 1259-1266. https://doi.org/10.1094/phyto.2004.94.11.1259

Martínez S, Barboza U, Hernández G and Bideshi D. 2020. Chitinases of *Bacillus thuringiensis*: Phylogeny, modular structure, and applied potentials. Frontiers in microbiology 10(1): 30-32. http://dx.doi.org/10.3389/fmicb.2019.03032

Pérez-Cordero AF, Rojas-Sierra JN y Fuentes-Cuello JR. 2010. Diversidad de bacterias endófitas asociadas a raíces del pasto colosuana (*Bothriochloa pertusa*) en tres localidades del departamento de Sucre, Colombia. Acta Biológica Colombiana 15:219-228. http://dx.doi.org/10.24188/recia.v2.n1.2010.331

Pereira P, Ibáñez F, Rosenblueth M, Etcheverry M and Martínez-Romero E. 2011. Analysis of the bacterial diversity associated with the roots of maize (*Zea mays* L.) through culture-dependent and culture-independent methods. International Scholarly Research Network. 1-10. https://doi.org/10.5402/2011/938546

Pikosvkaya R and Pikosvkaya RI. 1948. Mobilization of phosphorus in soil connection with the vital activity of some microbial species. Microbiology 17: 362-370.

Rocha N, Claros M, Calisaya JJ y Ortuño N. 2019. Selección de bacterias endófitas tipo *Bacillus* como promotoras de crecimiento en el cultivo de papa variedad Huaycha (*Solanum tuberosum* subsp. *andigena*). Revista Latinoamericana de la Papa 23(1): 14–34. http://ojs.papaslatinas.org/index.php/rev-alap/index

Rosenblueth M and Martínez-Romero E. 2006. Bacterial endophytes and their interactions with hosts. Molecular Plant-Microbe-Interaction 19:827-837. https://doi.org/10.1094/mpmi-19-0827

Secretaría Agricultura y Desarrollo Rural (SADER). https://www.agricultura.gob.mx

Sánchez-Bautista A, León-García de A, Carlos D, Aranda-Ocampo S, Zavaleta-Mejía E, Nava-Díaz C, Goodwin PH y Leyva-Mir SG. 2018. Bacterias endófitas de la raíz en líneas de maíces tolerantes y susceptibles a sequía. Revista Mexicana de Fitopatología 36(1): 35-55. https://doi.org/10.18781/r.mex.fit.1710-3

Santoyo G, Moreno-Hagelsieb G, Orozco-Mosqueda MC and Glick BR. 2016. Plant growth-promoting bacterial endophytes. Microbiological Research 183:92-99. https://doi.org/10.1016/j.micres.2015.11.008

Schwyn B and Neilands JB. 1987. Universal chemical assay for the detection and determination of siderophores. Analytical Biochemistry 160:47-56. https://doi.org/10.1016/0003-2697(87)90612-9

Shanmugaiah V, Mathivanan N, Balasubramanian N and Manoharan PT. 2008. Optimization of cultural conditions for production of chitinase by *Bacillus laterosporus* MML2270 isolated from rice rhizosphere soil. African Journal of Biotechnology 7(15): 2562-2568. http://www.academicjournals.org/AJB

Shi T, Reeves RH, Gilichinsky DA and Friedmann EI. 1997. Characterization of viable bacteria from Siberian permafrost by 16S rDNA sequencing. Microbial Ecology 33:169-179. https://doi.org/10.1007/s002489900019

Strobel G; Daisy B. 2003. Bioprospecting for microbial endophytes and their natural products. Microbiology and Molecular Biology Reviews 67(4):491-502. https://doi.org/10.1128/mmbr.67.4.491-502.2003

Tejera-Hernández B, Rojas-Badía MM y Heydrich-Pérez M. 2011. Potencialidades del género *Bacillus* en la promoción del crecimiento y el control biológico de hongos fitopatógenos. Revista del Centro Internacional de Investigaciones CNIC 42(3): 131-138. https://doi.org/10.15517/am.v24i2.12535

Yang CJ, Zhang XG, Shi GY, Zhao HY, Chen L, Tao KY and Hou TP. 2011. Isolation and identification of endophytic bacterium W4 against tomato *Botrytis cinerea* and antagonistic activity stability. African Journal of Microbiology 5(2):131-136 http://dx.doi.org/10.5897/AJMR10.815

Wang C, Rong L, Zhongqi H, Yongchun Z, Yeni W, Xiaohan H.Z. 2019. Cadmium-resistant rhizobacterium *Bacillus cereus* M4 promotes the growth and reduces cadmium accumulation in rice (*Oryza sativa* L.). Environmental Toxicology and Pharmacology 7(2):103-111. http://dx.doi.org/10.1016/j.etap.2019.103265

Wang M, Geng L, Xiaoxiao S, Changlong S, Fuping S and Jie Z. 2020. Screening of *Bacillus thuringiensis* strains to identify new potential biocontrol agents against *Sclerotinia sclerotiorum* and *Plutella xylostella* in *Brassica campestris* L. Biological Control 279 (1): 104-262. https://doi.org/10.1016/j.biocontrol.2020.104262

Zgadzaj R, James EK, Kelly S, Kawaharada Y, Jonge N and Jensen DB. 2015. A legume genetic framework controls infection of nodules by symbiotic and endophytic bacteria. PLOS Genetics 11:e1005280. https://doi.org/10.1371/journal.pgen.1005280