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Potential Growth Stimulant of *Azotobacter* Strains Isolated from Cuban Agroecosystems

Yoania Rios Rocafull[1], Beatriz Ramos García[2] & Marisel Ortega García[3]

[1]ORCID https://orcid.org/0000-0003-1774-0868, INIFAT, Department of Microbial Genetic Resources and Bioactive Products, Havana, Cuba, [2]ORCID https://orcid.org/0000-0003-1317-3835, INIFAT, Department of Microbial Genetic Resources and Bioactive Products, Havana, Cuba, [3]ORCID https://orcid.org/0000-0002-8076-2675, INIFAT, Scientific Office, Havana, Cuba.

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Email: dpagrobiotec@inifat.co.cu, yrrocafull@gmail.com

Abstract

Context: The genus *Azotobacter* is used to stimulate the growth of economically important crops. Knowing the potential of native strains will allow for better use of the bacteria as active ingredients in bioproducts for Cuban agriculture.
Aim: To characterize three strains of *Azotobacter* isolated from Cuban agroecosystems as plant growth promoters.
Methods: The *Azotobacter* strains INIFAT-12, INIFAT-20, and INIFAT-21, conserved in the INIFAT Bacteria Collection, were characterized for their tolerance to abiotic stress conditions, nitrogen-fixing potential, nutrient solubilization, production of lytic enzymes, action against pathogenic fungi, and the effect of their application on beans, wheat, and tomatoes under controlled conditions.
Results: The growth of the three *Azotobacter* strains decreased under abiotic stress conditions, although a positive result was always observed, suggesting the presence of some tolerance mechanism. All strains fixed nitrogen and released protease and lipase enzymes; however, none of them solubilized nutrients or released cellulase enzymes. Only the INIFAT-20 strain produced amylase enzymes. Antagonistic activity was similar against *Curvularia palense*, while for *Fusarium chlamydosporum*, the INIFAT-20 strain stood out. The application of bacteria had a positive effect on the growth of bean, wheat, and tomato seedlings.
Conclusions: *Azotobacter* strains residing in Cuban agroecosystems have a potential as plant growth promoters, making this a promising genus for obtaining new agricultural bioproducts in Cuba.

Keywords: *management, microorganisms, products.*

Introduction

Different microorganisms can be used to develop agricultural bioproducts. Among them is the genus Azotobacter, which stands out for its potential to fix atmospheric nitrogen and produce phytohormones, though there are strains that also solubilize phosphates (Zavala et al., 2020). In recent years, its scope of action has expanded, demonstrating antagonistic effects against pathogens such as *Alternaria*, *Fusarium*, *Rhizoctonia*, *Macrophomina*, *Curvularia*, *Helminthosporium*, and *Aspergillus* (Cesa-Luna et al., 2020). It also excels in bioremediation and plant tolerance to abiotic stress conditions (Sumbul et al., 2020).

Internationally, numerous studies support the effectiveness of species like *A. chroococcum* and *A. vinelandii* in stimulating the growth of green vegetables, legumes, grass, pastures, and other crops (Alcarraz et al., 2020; Pilatuña et al., 2021). This research led to the development of several products, with this genus as the active ingredient or part of it.

Despite these positive results, exploiting the growth-promoting potential of new native strains can be a tool to obtain more efficient and competitive products that can be part of the agronomic management of economically important crops. Therefore, this study aimed to characterize three strains of Azotobacter isolated from Cuban agroecosystems as plant growth promoters. All strains demonstrated the presence of

stimulation mechanisms, making them promising materials for obtaining biofertilizers.

Materials and methods

***Azotobacter* strains used in the study**: Three strains of *Azotobacter* spp from the INIFAT Beneficial Bacteria Collection (INIFAT-12, INIFAT-20, and INIFAT-21) were used, preserved in 30% glycerol as a cryoprotective agent (Table 1).

Table 1 Origin of *Azotobacter* strains used in the study

Strain	Origin
INIFAT-12	Red Ferrallitic Soil from Güira de Melena
INIFAT-20	Red Ferrallitic Soil from Güira de Melena
INIFAT-21	Rhizosphere of pepper (organoponic) (cv Verano 1). Boyeros

***Evaluation of tolerance to abiotic stress conditions*:** The strains were characterized according to their tolerance to drought, salinity, different pH values, and temperature. Different concentrations of PEG 6000 (0, 5, and 10%) were used to simulate drought, while three concentrations of NaCl (0, 5, and 10%) were used for salinity. For the pH assay, the medium was adjusted to 4.5, 6.8, and 8 before sterilization, whereas for the temperature study, incubation was performed at 28°C, 5°C, and 40°C. In all cases, Nutrient Broth (BIOCEN) was used as the base medium, inoculated with 1 mL of a pre-inoculum obtained in the same medium through submerged fermentation in a Labolan orbital shaker (24 h at 150 r.p.m and 28°C). Except for the temperature assay, all others were conducted with test tubes containing 5 mL of inoculated medium placed under agitation (150 r.p.m). Bacterial growth was measured by absorbance at 600 nm in a UV-Visible spectrophotometer (JENWAY 6850) at 24, 48, and 72 hours.

Evaluation of the presence of plant growth stimulation mechanisms: The strains were evaluated for their nitrogen-fixing potential, nutrient solubilization, production of lytic enzymes, and antagonistic activity against agricultural pathogens. Biological nitrogen fixation (BNF) was qualitatively determined by microbial growth in nitrogen-free Asbhy medium (Pérez-Cordero et al., 2014). For solubilization, microorganisms were inoculated on solid NBRIP medium (Nautiyal et al., 1999) with calcium, iron, and aluminum phosphates. A potassium solubilization medium (Cruz Cárdenas et al., 2021) was also used. A positive response was indicated by the formation of a halo around the bacterial colony in every case.

To determine the presence of amylase, protease, and lipase enzymes, Nutrient Agar medium (BIOCEN) with 1% soluble starch, 10% skim milk, and 1 mL of Tween 80 were used respectively, whereas a specific medium proposed for this determination (Cruz Cárdenas et al., 2021) with the addition of 10 g of crystalline cellulose was used for detecting cellulase and glucanase enzymes. The detection of amylase and glucanase was performed according to the methods described by Harrigan & McCance (1968) and Bertini et al. (2016), respectively. In all cases, the halo around the bacterial colonies was measured with a caliper (0.05 mm error).

Antagonistic activity of *Azotobacter* strains: Antagonistic activity was determined by the Dual Culture method (Vera Loor et al., 2020). Pathogenic fungi *Curvularia palense* (3579) and *Fusarium chlamydosporum* (2022) from the INIFAT Pure Fungi Collection were used. Potato Dextrose Agar (PDA) medium (BIOCEN) was inoculated with a 5 mm punch of the fungus at 4 cm from the edge of the Petri dish. The bacterium was inoculated at the opposite edge. The fungal mycelium diameter was measured at 10 days, and the mycelial inhibition percentage (IM) was calculated: IM=((Dt-Di)/Dt)*100, where Dt is the mycelium diameter in control plates, and Di is the mycelium diameter in inoculated plates. Controls included plates with only the pathogen inoculated.

Effect of microorganism application on green vegetables and grains: The application effect was evaluated *in vitro* (Ortiz et al., 2021). Seeds of bean (*Phaseolus vulgaris* L.) cv F 248-1, wheat (*Triticum aestivum* L) cv M-04, and tomato (*Solanum lycopersicum* L.) cv T-60, all from the INIFAT Germplasm Bank, were used. The experiment was conducted in 150 mm Petri dishes lined with filter paper before sterilization. Seeds were disinfected with 4% hypochlorite for 10 minutes and distilled water. Subsequently, they were soaked for 10 min in a sterile distilled water cell suspension of each strain at a concentration of 10^8 CFU mL^-1 (tube No. 4 on the McFarland scale). For the control treatment, the same protocol was followed, but only sterile distilled water was used. Twenty seeds per plate (inoculated and control) were used. The number of germinated seeds was evaluated during the first three days after inoculation. At six days, the seedlings were extracted and measured for radicle and hypocotyl length with a graduated ruler (cm) while stem diameter was measured with a caliper gauge (0.05 mm error). Fresh mass was also quantified with a semi-analytical balance (Nahita, 0.01 g error). For beans and wheat, dry mass (g) was added after maintaining the plants in an oven (MLW) at 70°C.

Experimental design and statistical processing: All experiments were conducted with a Completely Randomized Design. Statistical processing first tested

for normality and variance homogeneity with Cochran C, Hartley, and Bartlett tests, followed by Analysis of Variance. Means were compared with Duncan's test (5% error probability). STATGRAPHICS Plus version 5.0 was used.

Results and Discussion

Tolerance of strains to abiotic stress conditions: Abiotic stress conditions affected the growth of *Azotobacter* strains (Table 2). The lowest absorbance values were reached for salinity and temperature stress, indicating that these factors markedly affect the growth of these microorganisms. Other studies have shown significant temperature and pH values for *Azotobacter* multiplication (Zavala et al., 2020), highlighting the need to consider these elements in working with this bacterial genus.

Remarkably, microorganisms multiplied under all stress conditions, which was observed through medium turbidity. This is important as it supports the possibility of using these strains to stimulate plant growth under stress conditions, where applying plant growth-promoting bacteria has improved productive outcomes for different plant species (Beleño-Carrillo et al., 2022).

Table 2. Response to different abiotic stress conditions of three *Azotobacter* strains isolated from Cuban agroecosystems

	Salinity (NaCl)			
Strain	0%	5 %	10%	*Esx*
INIFAT-12	1.45 a	1.14 b	0.04 c	*0.0014*
INIFAT-20	1.69 a	1.39 b	0.58 c	*0.0024*
INIFAT-21	1.53 a	0.57 b	0.15 c	*0.0010*
		pH		
	4.5	6.8	8	
INIFAT-12	1.72 b	1.72 b	1.76 a	*0.0030*
INIFAT-20	1.74 a	1.70b	1.66 c	*0.0033*
INIFAT-21	1.84 b	1.89 a	1.83 c	*0.0009*
	Drought (PEG 6000)			
	0 %	5 %	10%	
INIFAT-12	1.61 a	0.76 b	0.19 c	*0.0027*
INIFAT-20	1.37 a	1.03 b	0.98 c	*0.0120*
INIFAT-21	1.64 a	1.31 b	1.03 c	*0.0075*
	Temperature (°C)			
	5	28	40	
INIFAT-12	0.23 c	0.95 a	0.62 b	*0.0042*
INIFAT-20	0.31 c	0.89 a	0.65 b	*0.0021*
INIFAT-21	0.26 c	1.05 a	0.41 b	*0.0043*

Means with different letters for the same strain differ according to ANOVA with Duncan's test at 5% probability of error. *Data corresponds to the measurement taken at 72 hours of incubation.*

The growth of *Azotobacter* strains under abiotic stress suggests that these microorganisms have some tolerance mechanism. Various authors highlight the genus's ability to form cysts, resistant structures that allow them to survive freezing, salinity, drought, and UV radiation (Pavone, 2022; Sánchez-Yánez et al., 2022). Additionally, the bacteria's ability to form biofilms and produce polysaccharides contributes to their survival (Huamán-Castilla et al., 2021), acting as a resistance barrier.

Presence of plant growth stimulation mechanisms in the three *Azotobacter* strains: The three *Azotobacter* strains grew in nitrogen-free Asbhy medium, indicating their potential for fixation of nitrogen from the atmosphere. However, none solubilized phosphates or potassium (Table 3).

Table 3. Direct growth stimulation mechanisms present in three *Azotobacter* strains isolated from Cuban agroecosystems

	BNF	Nutrient Solubilization			
Strain		Ca	Fe	Al	K
INIFAT-12	+	-	-	-	-
INIFAT-20	+	-	-	-	-
INIFAT-21	+	-	-	-	-

Note: BFN: presumptive biological nitrogen fixation. Ca: calcium phosphate, Fe: iron phosphate, Al: aluminum phosphate, K: potassium.

The genus *Azotobacter* is widely recognized for its ability to fix atmospheric nitrogen, determined by various methods, some more accurate than the one presented here (Zavala et al., 2020). It is significant to know qualitatively that the three studied strains might have this metabolic attribute, enhancing their potential as future active ingredients for biofertilizers.

Although other authors have achieved positive results for phosphate solubilization with *Azotobacter* strains (Zavala et al., 2020), this trait is not typically associated with the genus. A strategy could involve using these non-solubilizing strains in microbial consortia with genera known for this function, such as *Bacillus*, to leverage the positive attributes of both microorganisms for a more efficient final product.

Regarding lytic enzyme production, the best results were obtained for proteases and lipases, as no strain released cellulases or glucanases, and only INIFAT-20 produced amylase enzymes (Table 4).

Lytic enzymes play a significant role in the biocontrol effect exerted by plant growth-promoting bacteria due to their action on the cell walls of pathogenic fungi (Blanco & Castro, 2021). Detecting their presence in the *Azotobacter* strains under study is intriguing, as it adds value by suggesting their application could not only directly promote plant growth but also indirectly counteract pathogen attacks.

Table 4. Production of lytic enzymes by three *Azotobacter* strains isolated from Cuban agroecosystems

Strain	Ami	Prot	Lip	Cel	Gluca
INIFAT-12	-	2.91 a	3.0 b	-	-
INIFAT-20	0.58	1.63 b	3.3 b	-	-
INIFAT-21	-	0.38 c	4.25 a	-	-
Esx		*0.1666*	*0.1787*		

Note: Ami: amylases, Prot: proteases, Lip: lipases, Cel: cellulases, Gluca: glucanases. Means with different letters for the same pathogen differ according to ANOVA with Duncan's test at 5% probability of error.

Regarding the antagonistic activity, interesting results were also achieved. Although the mycelial inhibition percentage was below 50%, the *Azotobacter* strains demonstrated to negatively affect the growth of *C. palense* and *F. chlamydosporum* (Fig. 1).

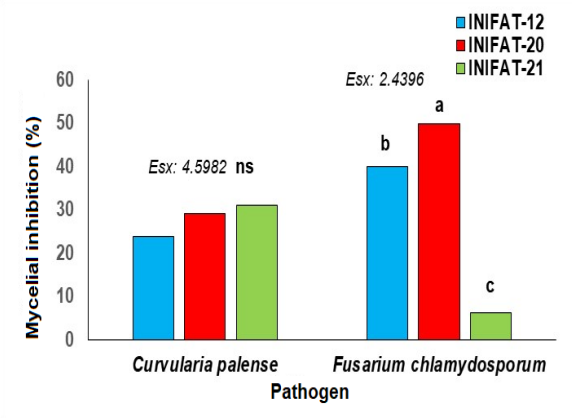

Fig.1. Antagonistic activity of three *Azotobacter* strains isolated from Cuban agroecosystems against *Curvularia palense* and *Fusarium chlamydosporum*.Means with different letters for the same pathogen differ according to ANOVA with Duncan's test at 5% probability of error.

In this biocontrol effect, differences among the strains were observed, with better results for INIFAT-12 and INIFAT-20. On this topic, Pavone (2022) highlighted the action of *A. chroococcum* against *Alternaria*, *Fusarium*, *Rhizoctonia*, *Macrophomina*, *Curvularia*, *Helminthosporium*, and *Aspergillus*, related to the production of antimicrobial substances, toxins, and plant growth hormones.

Overall, the three *Azotobacter* strains under study exhibited growth-stimulating mechanisms, making them promising candidates for developing new products based on this bacterial genus. In Cuba, for example, only one biofertilizer with *Azotobacter* as the active ingredient is registered (Soil and Fertilizer Office, 2021), so these findings are quite novel.

Effect of the application of the three *Azotobacter* strains: When applying the three *Azotobacter* strains to bean, wheat, and tomato seeds, no differences were observed in germination rates compared to the control treatment without microorganisms. This was attributed to the seeds' good quality and sanitary condition, leading to 100% germination in all cases.

However, inoculating the microorganisms had a positive effect on seedling growth, with the action depending on the strain and crop. Fig. 2 shows that for beans, the INIFAT-20 and INIFAT-21 strains stood out, while for wheat, INIFAT-12 excelled, and for tomatoes, both INIFAT-20 and INIFAT-12 showed the best results. The positive effect of the strains could be linked to the characteristics determined in this study and the production of phytohormones, a well-studied growth-promotion mechanism for *Azotobacter*, with many strains showing relevant results (Zavala et al., 2020).

The plant-microorganism interaction is a complex process mediated by various factors, involving both the crop and the plant growth-promoting bacteria. Plant root exudates, for example, regulate the dynamics of rhizospheric microbial populations and the attraction of the inoculated bacteria (Chávez-Díaz et al., 2020). Additionally, the greater the growth-promotion potential of the microorganism, the more significant its effect on the crop may be. Therefore, it is essential to study potential candidate strains for bioproduct active ingredients, determining not only their *in vitro* potential but also their direct effect on plant species.

For *Azotobacter*, several references support its application effect on various plant species. Examples include vegetables like radish (Ibarra et al., 2021), lettuce, and tomatoes (Pilatuña et al., 2021), and grains such as corn (Sule et al., 2023) and cowpea (Alcarraz et al., 2020), to name just a few examples. In all cases, the microorganism stimulated growth and yield indicators, and demonstrated its great potential for use in agriculture.

In Cuba, relevant results have also been achieved in various vegetables, grains, fruit trees, and ornamental plants (Martínez & Dibut, 2012), demonstrating the impact that *Azotobacter*-based products can have in the country. However, the results obtained in this study could be interesting for developing new biofertilizers based on the bacterial genus, seeking products with a broad spectrum of action and multiple functions, based on one or more strains.

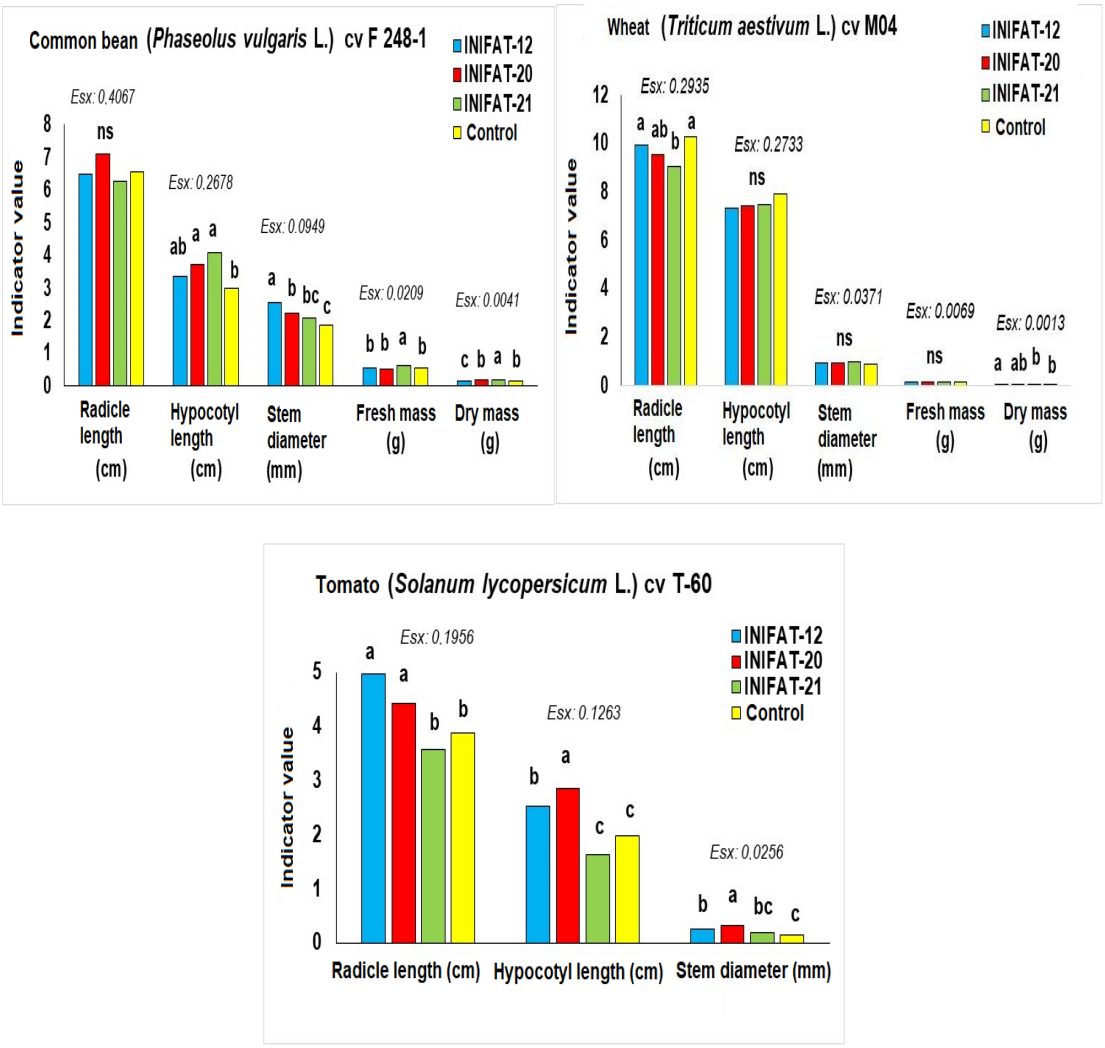

Fig. 2. Effect of applying three *Azotobacter* strains isolated from Cuban agroecosystems on grains and vegetables under controlled conditions. ns: not significant differences. Means with different letters for the same indicator differ according to ANOVA with Duncan's test at 5% probability of error.

Conclusions

Azotobacter strains residing in Cuban agroecosystems have potential as plant growth promoters, making this a promising genus for obtaining new agricultural bioproducts in Cuba.

Author contribution statement

Yoania Ríos Rocafull: Research planning, literature review, experiment execution, data analysis, article writing, final review.

Beatriz Ramos García: Research planning, final review.

Marisel Ortega García: Research planning, data analysis, final review.

Conflict of interest statement

The authors declare the absence of conflicts of interest

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References

Alcarraz, M., Gonzales, E., & Heredia, V. (2020). *Azotobacter* y *Rhizobium* como biofertilizantes naturales en semillas y plantas de frijol caupí. *Avances*, *22*(2), 239-251. http://www.ciget.pinar.cu/ojs/index.php/publicaciones/article/view/538/1610.

Beleño-Carrillo, J., Gómez-Gómez, L., & Valero-Valero, N.O. (2022). *Bacillus mycoides* y ácidos húmicos como bioestimulantes de fríjol caupí bajo estrés por salinidad. *Rev. U.D.C.A Act. & Div. Cient*, *25*(2), e1974. http://doi.org/10.31910/rudca.v25.n2.2022.1974

Bertini, E.V., Leguina, A. C. V., Castellanos, L. I., & Nieto, C.G. (2016). Caracterización de bacterias endofíticas de caña de azúcar productoras de N-acil homoserina lactonas. *Archivos de Bioquímica Química y Farmacia, 15*(1), 5-19.

Blanco, E.L., & Castro, Y. (2021). Antagonismo de rizobacterias sobre hongos fitopatógenos, y su actividad microbiana en el potencial biofertilizante, bioestimulante y biocontrolador. *Revista Colombiana de Biotecnología*, *23* (1), 6-16. https://doi.org/10.15446/rev.colomb.biote.v23n1.84808

Cesa-Luna C, Baez A, Quintero-Hernández V, Cruz-Enríquez J. D. L, Castañeda-Antonio M. D., & Muñoz-Rojas, J. (2020). The importance of antimicrobial compounds produced by beneficial bacteria on the biocontrol of phytopathogens. *Acta Biológica Colombiana*, *25* (1), 140-154. https://doi.org/10.15446/abc.v25n1.76867

Chávez-Díaz, I.F., Zelaya, L.X., Cruz, C.I., Rojas, E., Ruíz, S., & de los Santos, S. (2020). Consideraciones sobre el uso de biofertilizantes como alternativa agro-biotecnológica sostenible para la seguridad alimentaria en México. *Revista Mexicana Ciencias Agrícolas*, *11* (6), 1423-1436. https://doi.org/10.29312/remexca.V9i4.1389

Cruz Cárdenas, C.I., Zelaya-Molina, L. X., Chávez-Díaz. I.F., Rojas-Anaya, E., & Arteaga-Garibay, R.I. (2021). *Conservación de cepas microbianas para biofertilizantes. Libro teórico.* (No. 02). Centro Nacional de Recursos Genéticos. Tepatitlán de Morelos. Jal. México.

Dirección de Suelos y Fertilizantes (2021). *Listado Oficial de Fertilizantes Autorizados.* Registro Central de Fertilizantes.

Harrigan, W.F., & M. Mc. Cance. (1968). *Métodos de Laboratorio de Microbiología*. Ed. Academia, España.

Huamán-Castilla, N. L., Allcca-Alca, E. E., Allcca-Alca, G. J., & Quispe-Pérez, M. L. (2021). Biopolímeros producidos por Azotobacter: síntesis y producción, propiedades físico-mecánicas, y potenciales aplicaciones industriales. *Scientia Agropecuaria, 12*(3), 369-377. https://dx.doi.org/10.17268/sci.agropecu.2021.040

Ibarra, J.A., Llica, W.R., & Lazo, R.S. (2021). Determinación de la influencia de Azotobacter nativos en cultivos de Raphanus sativus como biofertilizante. *Ingeniería investiga, 3*(1), 579-590. https://doi.org/10.47796/ing.v3i1.482

Martínez, R. & Dibut, B. (2012). *Biofertilizantes Bacterianos.* Editorial Científico-Técnica. Instituto Cubano del Libro.

Martínez, V. R., López, M., Brossard, F. M., Tejeda, G. G., Pereira, A. H., Parra, Z. C., Rodríguez, S. J., & Alba, A. (2006). *Procedimientos para el estudio y fabricación de Biofertilizantes Bacterianos.* (Serie B, No. 11). Ed. INIA - Maracay.

Nautiyal, S. C. (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganism. *FEMS Microbiology Letters*, *170*, 265-275.

Ortiz, Y., Ríos, Y., Aguado, Y., Rodríguez, L.C., Lorenzo, Y., Deliz, L., Álvarez, M.E., Rodríguez, J., Zulueta, I., & Fresneda, J.A. (2021). Selección de cepas bacterianas con potencial estimulador del crecimiento vegetal en *Phaseolus vulgaris* L. (cv. 'Lewa'). *Agrotecnia de Cuba*, *45*(1), 42 – 58.

Pavone, D. (2022). *Azotobacter en la agricultura: Una bacteria biofertilizante que protege a las plantas.* TecnoVita. https://tecnovitaca.com/wp-content/uploads/2022/12/Azotobacter.pdf

Pérez-Cordero, A., Tuberquia-Sierra, A., & Amell-Jiménez, D. (2014). Actividad *in vitro* de bacterias endófitas fijadores de nitrógeno y solubilizadores de fosfato. *Agronomía Mesoamericana, 25*(2), 213-223. https://www.scielo.sa.cr/pdf/am/v25n2/a01v25n2.pdf

Pilatuña, M.F., González, M.M., Mero, M.E. & Risco, D. (2021). Evaluación agronómica de bacterias fijadoras de nitrógeno aisladas de suelos andinos en plántulas de lechuga y tomate. *Investig. Agrar*, *23*(1), 47-52. http://dx.doi.org/10.18004/investig.agrar.2021.junio.2301680.

Sánchez-Yánez, J. M., Velázquez-Medina, A., Cabrera-Reinaldo, I., Amador-Vargas, W.L.., & Vela-Muzquiz, G. R. (2022). Supervivencia de *Azotobacter* y otros grupos microbianos en suelo seco almacenado. *Journal of the Selva Andina Research Society*, *13*(1), 3-15. https://doi.org/10.36610/j.jsars.2022.13010000 3

Sule, I.O., Agbabiaka, T.O., Saliu B.K., Ajijolakewu K.A., & Zakariyah R.F. (2023). Assessment of the potentials of Azotobacter spp. as bioinoculants on the growth of potted maize plants. *Science World Journal*, *18*(2), 276-

282. https://dx.doi.org/10.4314/swj.v18i2.16.

Sumbul, A., Ali Ansari, R., Rizvi, R., & Mahmood, I. (2020). Azotobacter: A potential bio-fertilizer for soil and plant health management. *Saudi Journal of Biological Sciences*, *27*, 3634-3640. https://doi.org/10.1016/j.sjbs.2020.08.004

Vera Loor, M., Bernal, A., Vera, D., Leiva, M., Rivero, A., & Agustín, A. (2020). Antagonismo *in vitro* de bacterias endófitas formadoras de endosporas frente a *Moniliophthora roreri* H.C Evans. *Revista de Protección Vegetal*, *35*(2), 8.

Zavala, J., Alcarraz, M., & Julian, J. (2020). Evaluación para la producción de *Azotobacter sp.* promotor de crecimiento para cultivos de *Coffea arabica. Ciencia e Investigación*, *23*(1), 45-50. http://dx.doi.org/10.15381/ci.v23i1.18751