

Isolation of Polyhydroxyalkanoate (PHA)-Producing *Azotobacter* spp. from Crop Rhizospheres Located in Lima, Peru

Selección de *Azotobacter* spp. productores de polihidroxialcanoatos (PHA) aislados de rizósfera de cultivos ubicados en la región de Lima - Perú

Lisset Tupa-Andrade, Junior Caro-Castro, Jorge León-Quispe *

Originales: Recepción: 07/09/2023 - Aceptación: 02/07/2025

ABSTRACT

PHAs are polyesters found as internal granules in several microorganisms. *Azotobacter* is known for its ability to produce PHA. This study aimed to isolate *Azotobacter* from the rhizosphere of selected crops located in Lima and evaluate their PHA-producing potential. Nile Red medium was used for PHA detection, and Sudan Black B staining allowed microscopic observation. Biopolymer production and quantification were carried out in Burk's medium, PHA minimal medium (PHAMM), and modified PHAMM. In Nile Red medium, 68.2% of strains produced PHA, with *Azotobacter* AzoLur20 exhibiting the highest production, 2.1 g/L of PHA at 96 hours in PHAMM. However, strain AzoLur19 showed higher productivity and stability, achieving 0.06 g/L*h of PHA. Additionally, Sudan Black B staining in Burk's medium revealed larger *Azotobacter* cells with more defined granules. AzoLur19 was classified as *Azotobacter chroococcum*. In conclusion, *Azotobacter* species isolated from crops located in Lima can produce PHA with high yields, with *A. chroococcum* as the predominant species.

Keywords

Azotobacter • polyhydroxyalkanoate • PHA production • bioplastics

Universidad Nacional Mayor de San Marcos. Facultad de Ciencias Biológicas.
Laboratorio de Ecología Microbiana. Lima-Perú. * jleonq@unmsm.edu.pe



Licenses Creative Commons
Attribution - Non Commercial - Share Alike

RESUMEN

Los PHA son poliésteres que se encuentran como gránulos internos en varios microorganismos. *Azotobacter* se caracteriza por producir PHA. El objetivo de este estudio fue aislar *Azotobacter* de la rizósfera de cultivos ubicados en la región de Lima y evaluar su potencial productor de PHA. Se utilizó el medio Rojo de Nilo para la detección de PHA y la tinción con Sudan Black B para la observación microscópica. La producción y cuantificación del biopolímero se realizó en medio Burk, medio mínimo PHA (PHAMM) y PHAMM modificado. El 68,2% de las cepas produjeron PHA en el medio Rojo de Nilo, siendo la cepa de *Azotobacter* AzoLur20 la más importante produciendo 2,1 g/L de PHA a las 96 horas en el PHAMM; sin embargo, la cepa AzoLur19 fue la más productiva y estable con 0,06 g/L*h de PHA. En cuanto a la tinción con Sudan Black B en medio Burk, se observaron células de *Azotobacter* de mayor tamaño y con gránulos más definidos. Además, el análisis molecular de la cepa AzoLur19 la identificó como *Azotobacter chroococcum*. En conclusión, *Azotobacter* sp. es capaz de producir PHA con un alto rendimiento, destacándose la especie predominante *A. chroococcum* en la rizósfera de varios cultivos.

Palabras clave

Azotobacter • polihidroxiclcanoato • producción de PHA • bioplásticos

INTRODUCTION

Petroleum-derived plastics significantly contribute to global pollution. Their slow degradation process can take several decades. As a result, the quest for eco-friendly alternatives has become urgent, and polyhydroxyalkanoates (PHAs) are one majorly investigated bioplastics. PHAs share similar properties with petrochemical plastics and have various applications, including the production of disposable everyday items, biomedical devices, pharmaceutical products, agricultural materials, textiles, and nanotechnology products (11, 19, 25, 27).

PHAs are polyesters produced and stored by various microorganisms as internal granules, which serve as carbon and energy sources (26). Although PHA-producing bacteria typically synthesize this compound under stress conditions like an excess of carbon sources or a nutrient limitation, certain exceptions, such as *Azotobacter*, can produce PHA without a stress inducer (23).

Azotobacter species, primarily isolated from the rhizosphere, synthesize PHA continuously during growth. They produce higher quantities when ammonium salts, like ammonium acetate, are supplied to the culture medium (16). Studies achieving high PHA production using *Azotobacter* mutant strains include Burk medium with 0.12% NH_4Cl (7) and PHA minimal medium (PHAMM) with urea (20). Both studies achieved high PHA production using *Azotobacter* mutant strains.

Although further research is still needed, *Azotobacter* is recognized as a good PHA producer (8), easy-to-handle, non-pathogenic, and versatile microorganism. Among *Azotobacter* species, *A. chroococcum* has recently received significant attention (1). Currently, efforts enhancing PHA production and recovery, particularly the most predominant type of PHA, polyhydroxybutyrate (PHB), focus on optimizing media formulation for production, extraction methods, and quantification techniques (17, 22).

This study evaluated the PHA-producing potential of *Azotobacter* isolated from crop rhizospheres in Lima, Peru, determining physicochemical parameters for optimizing PHA production.

MATERIALS AND METHODS

Collection of Rhizosphere Samples

Rhizosphere samples were collected from the districts of Lurin (latitude: -12.261063, longitude: -76.888297) and Pachacamac (latitude: -12.166853, longitude: -76.857761) in

Lima, Peru. Three crops from Lurin (onion, corn, and sweet potato) and five from Pachacamac (corn, strawberry, cucumber, chilli pepper, and sweet potato) were selected. The samples were transported to the Laboratorio de Ecología Microbiana at the Universidad Nacional Mayor de San Marcos.

***Azotobacter* Isolation and Selection**

Microbial isolation was performed according to Escobar *et al.* (2011). Initially, 10 g of each sample were diluted in 90 mL of saline (10^{-1}). Then, a subsequent dilution (1:10) in saline obtained a second dilution (10^{-2}). The resulting dilution was seeded on Ashby Mannitol Agar and Burk Agar solid media. Simultaneously, a selective enrichment was performed in Ashby Sucrose Broth, later seeded in the mentioned solid media. Isolated bacterial colonies were subcultured until pure strains were obtained and preserved at -20°C in glycerol-containing media.

Biochemical Characterization of *Azotobacter* Strains

Strains exhibiting presumptive morphological features of *Azotobacter* were evaluated through biochemical tests, including sugar fermentation, denitrification, urease testing, and cyst formation.

PHA Detection in Nile Red Medium

Azotobacter strains were inoculated into Nile Red medium. PHA detection was conducted using a 365 nm UV transilluminator (JUNYI brand, model JY02S), reading every 24 hours for 4 days. Presence or absence of intense pink to fuchsia fluorescence classified strains as PHA positive or negative, respectively (5).

PHA Production in Liquid Culture Media

The top five PHA-positive strains from Nile Red medium were subjected to quantitative tests for PHA production in the following liquid media: A) Burk medium with 2% glucose and 0.12% ammonium chloride (7); B) PHA minimal medium (PHAMM), with 0.54 g/L urea and 2% sucrose (20); and C) a modified PHAMM containing 2% glucose and 0.12% ammonium acetate. Aliquots were taken at 24-hour intervals, up to 96 hours, determining dry cell weight, Sudan Black B cell staining, and medium absorbance. Retains were also processed for polymer extraction using sodium hypochlorite and chloroform. The polymer was quantified using sulfuric acid.

Analytical Methods for the Evaluation of PHA Production

Biopolymer accumulation and productivity were evaluated according to Becerra (2013):

$$\text{A- Percentage of PHA accumulation: } \%PHA = \frac{g_{PHA}}{g_X} \times 100$$

$$\text{B- PHA Productivity: } P_{PHA} = \frac{g_{PHA}}{L \times h}$$

where:

gPHA = grams of PHA

gX = grams of biomass

L = liters

h = hours.

In addition, ANOVA, Tukey's test and correlation analysis ($p < 0.05$) were performed using InfoStat v. 2020.

Molecular Characterization of *Azotobacter*

Bacterial DNA extraction was performed using the GeneJET Genomic DNA Purification Kit (Thermo Fisher, USA). Subsequently, the 16S rRNA gene was amplified by PCR with the primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTACGACTT-3'). The PCR products were shipped to Macrogen Inc. (Seoul, Korea) for Sanger sequencing.

The obtained sequences were assembled and aligned with other 16S rRNA gene sequences from different *Azotobacter* species recovered from the GenBank database (2). Phylogenetic inference was conducted using MEGA11 (2021), employing the Neighbor-Joining method with 1000 bootstrap replications.

RESULTS AND DISCUSSION

Isolation and Characterization of *Azotobacter* sp.

Twenty-two *Azotobacter* strains were isolated. Eighteen (82%) strains were recovered from Lurin (table 1). Although there are no previous studies from this area, Lurin offers more suitable edaphic conditions for *Azotobacter* than Pachacamac. Additionally, most isolates were obtained from corn crops, as typically seen (3). Malynovska *et al.* (2021) emphasized that the highest prevalence of *Azotobacter* occurs in extensive crops, particularly in soils with fewer contaminants.

Table 1. Source and Origin of *Azotobacter* Strains.
Tabla 1. Fuentes y origen de las cepas de *Azotobacter*.

Geographic location	Crop	Total number of isolated strains	Strains confirmed as <i>Azotobacter</i>
Pachacamac	Cucumber (<i>Cucumis sativus</i>)	AzoMan1, AzoMan2, AzoMan25, AzoMan26, AzoMan27, AzoMan28	
	Corn (<i>Zea mays</i>)	AzoMan3, AzoMan4, AzoMan5, AzoMan24	
	Sweet potato (<i>Ipomoea batatas</i>)	AzoMan6, AzoMan7, AzoMan8, AzoMan9, AzoMan19, AzoMan20, AzoMan21, AzoMan22, AzoMan23	
	Strawberry (<i>Fragaria × ananassa</i>)	AzoMan10, AzoMan11, AzoMan12, AzoMan13	AzoMan12
	Yellow chili (<i>Capsicum baccatum</i>)	AzoMan14, AzoMan15, AzoMan16, AzoMan17, AzoMan18	AzoMan14, AzoMan16, AzoMan18
Lurin	Onion (<i>Allium cepa</i>)	AzoLur1, AzoLur2, AzoLur11, AzoLur12, AzoLur13, AzoLur14	AzoLur11, AzoLur12, AzoLur13, AzoLur14
	Corn (<i>Zea mays</i>)	AzoLur4, AzoLur5, AzoLur6, AzoLur7, AzoLur8, AzoLur9, AzoLur10, AzoLur15, AzoLur16, AzoLur17, AzoLur18, AzoLur19, AzoLur20, AzoLur21	AzoLur4, AzoLur5, AzoLur6, AzoLur7, AzoLur8, AzoLur10, AzoLur15, AzoLur17, AzoLur18, AzoLur19, AzoLur20, AzoLur21
	Sweet potato (<i>Ipomoea batatas</i>)	AzoLur3, AzoLur22, AzoLur23	AzoLur22, AzoLur23

Qualitative Selection of PHA in Nile Red Medium

Fifteen of the 22 strains (68%) tested positive for PHA detection using Nile Red medium. The highest fluorescence was observed between 72 and 96 hours of analysis (figure 1, page 49). The best results were obtained when acetone was used as a solvent for the lipophilic dye Nile Red, as suggested by Giraldo *et al.* (2020). When using agar medium as suggested by Carballo (2003), strong fluorescence was observed in the positive strains.

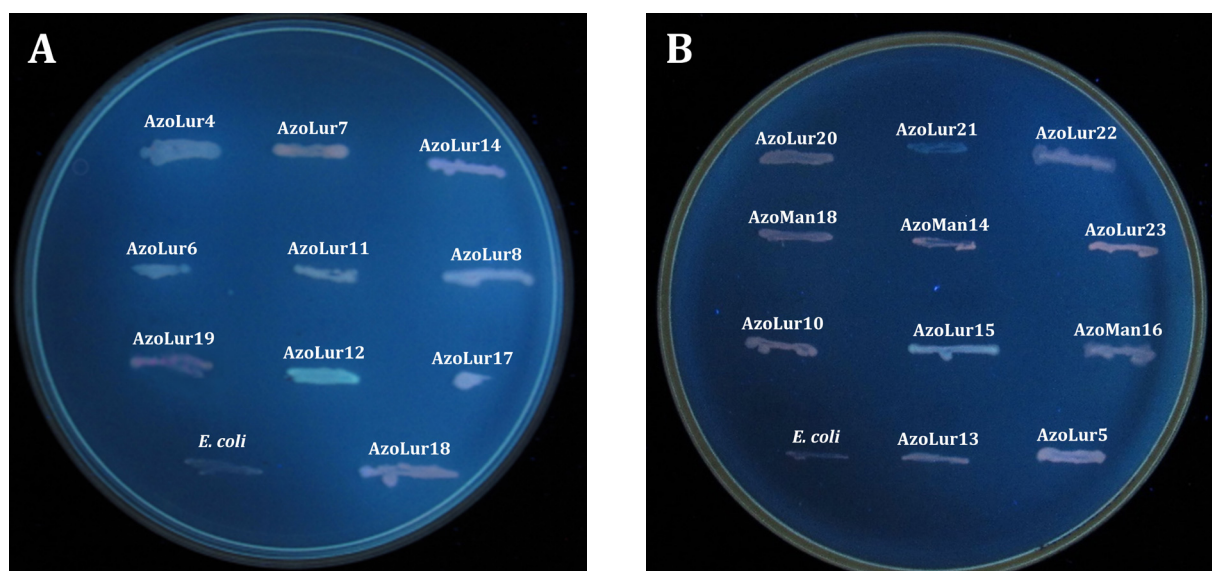


Figure 1. PHA production by *Azotobacter* strains in Nile Red medium observed under a 365 nm transilluminator at A) 72 hours, and B) 96 hours.

Figura 1. Producción de PHA por cepas de *Azotobacter* en medio Rojo de Nilo visto a través de un transiluminador (365nm) a A) 72 horas y B) 96 horas.

Quantitative Evaluation of PHA in Different Media

The highest PHA producer strain in Burk medium was AzoLur23, generating 1.25 g PHA/L at 48 hours from a biomass of 1.58 g/L (figure 2, page 50). In contrast, Cerrone (2011) reported 2.3 g PHA/L at 72 hours for a hyperproducing mutant strain using the same medium. Our result is notably high.

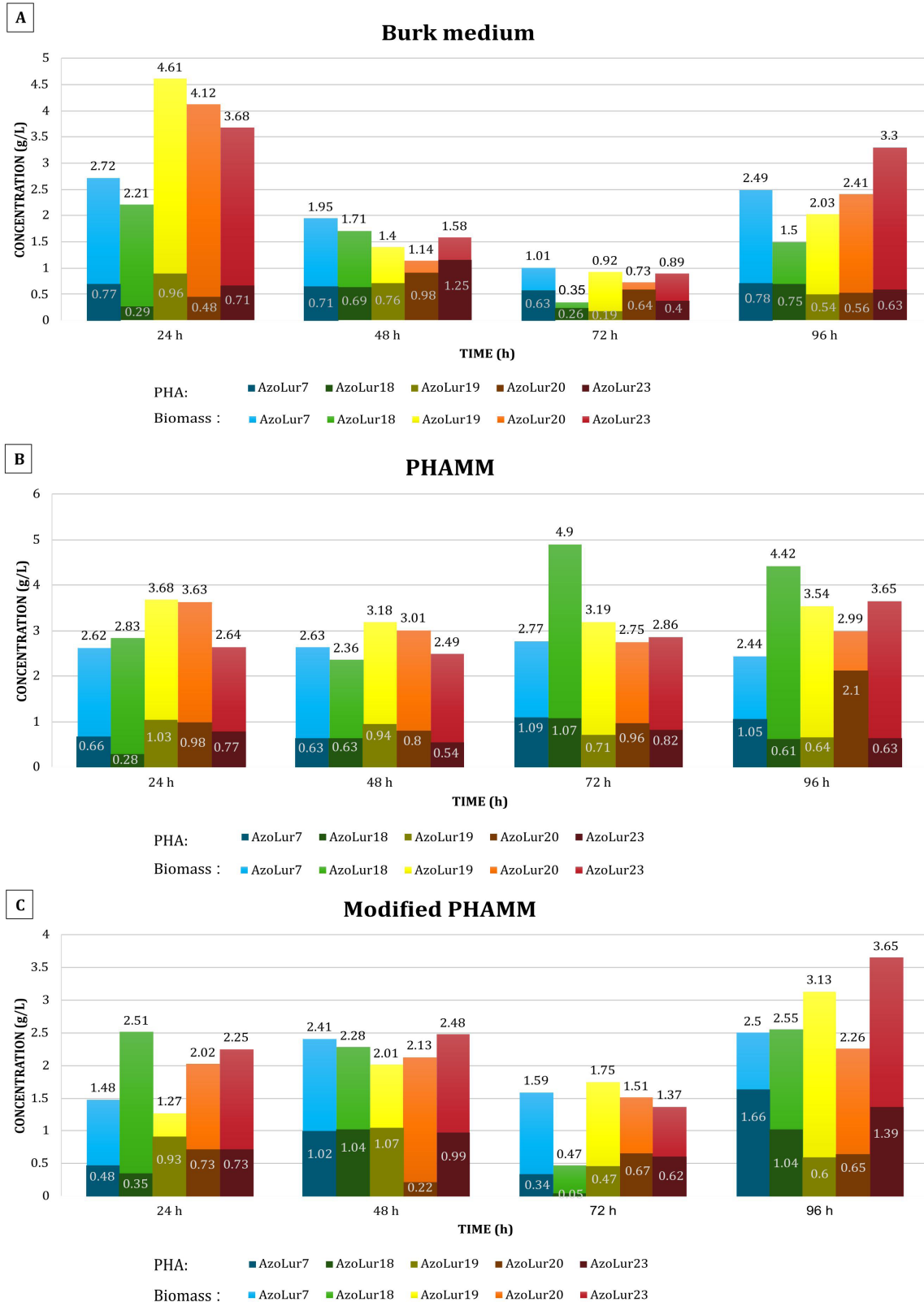
The highest PHA producer in PHAMM was AzoLur20, generating 2.1 g PHA/L at 96 hours from a biomass of 2.99 g/L (figure 2, page 50). Pei *et al.* (2017) obtained 1.5 g PHA/L from one of their evaluated mutant strains. Our higher result indicates that the AzoLur20 strain has potential for future biopolymer biosynthesis.

Considering the modified PHAMM, AzoLur7 was the highest PHA producer, generating 1.66 g PHA/L at 96 hours from a biomass of 2.5 g/L (figure 2, page 50). Although the result was lower than that obtained with the unmodified PHAMM, it is still higher than the values reported by Pei *et al.* (2017).

Biomass production and PHA concentration followed a normal distribution across all media ($p > 0.05$). Significant differences ($p < 0.05$) were observed when comparing the biomass obtained in the Burk and PHAMM media with the biomass in the modified PHAMM medium (figure 3A, page 51). In contrast, no significant differences in PHA concentration were found among the three media evaluated (figure 3B, page 51).

Staining with Sudan Black B

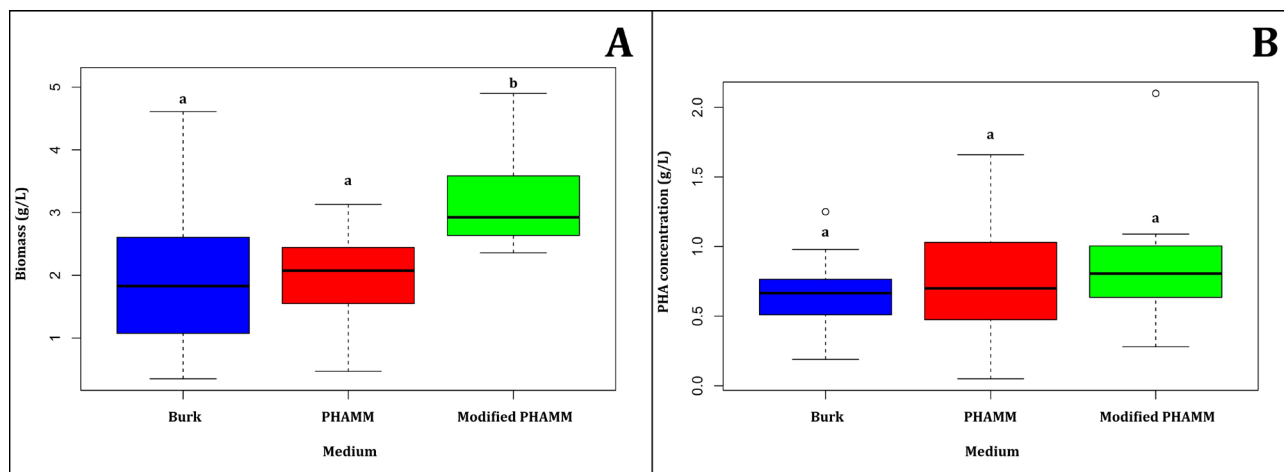
Cell staining with Sudan Black B dye was observed under a light microscope, allowing the dark internal granules to be differentiated from the pink-colored cytosol by the counterstain safranin, as described by Mohammed *et al.* (2019). Cells from Burk medium were larger, and the granules were more prominent compared to the cells recovered from PHAMM and modified PHAMM media (figure 4, page 51). Metals present in the composition of the latter two media may have affected cell growth and development, as previously noted by Lara *et al.* (2010).



A) Burk medium, B) PHAMM, and C) Modified PHAMM. / A) Medio Burk, B) MMPHA y C) MMPHA modificado.

Figure 2. Determination of biomass and PHA production at 24, 48, 72, and 96 hours.

Figura 2. Evaluación de la biomasa y producción de PHA a las 24, 48, 72 y 96 horas de evaluación.

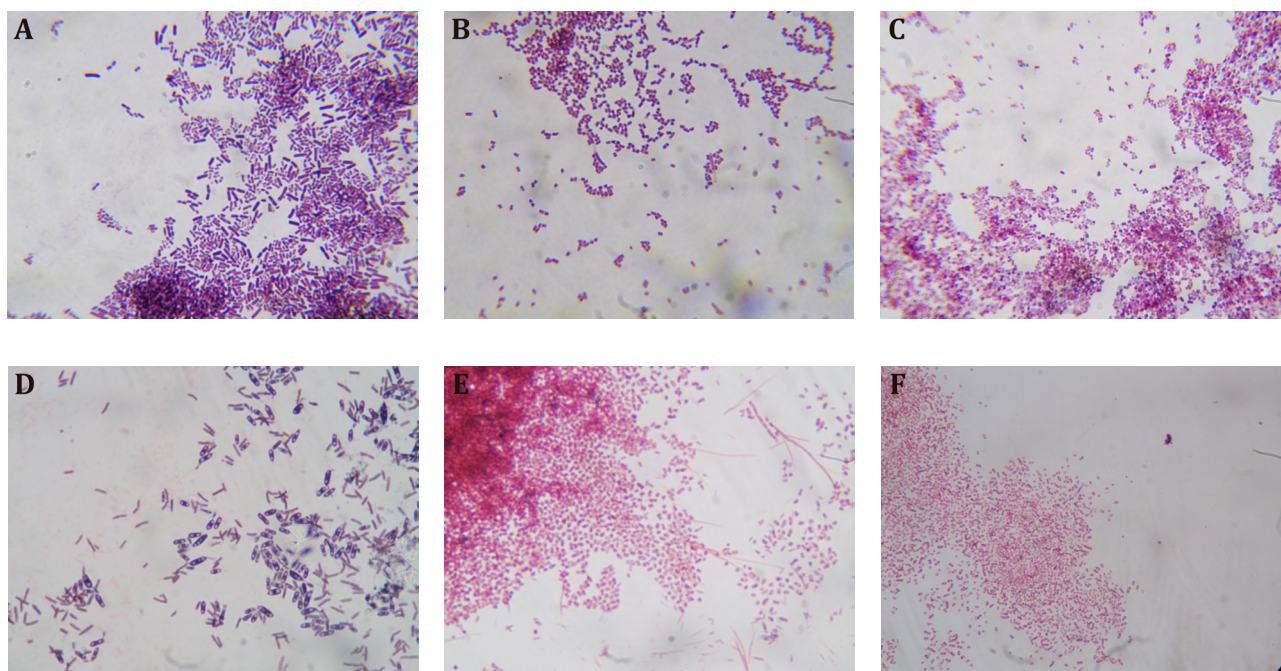


A) biomass and B) PHA concentration across different production media. Lowercase letters indicate significant differences ($p < 0.05$) among the production media.

A) biomasa y B) concentración de PHA en diferentes medios de producción. Las letras minúsculas indican diferencias significativas ($p < 0,05$) entre los medios de producción.

Figure 3. Statistical analysis of biomass and PHA concentration across different production media.

Figura 3. Análisis estadístico de la biomasa y concentración de PHA en diferentes medios de producción.



A) AzoLur19 in Burk medium, B) AzoLur23 in PHAMM, C) AzoLur19 in modified PHAMM, D) AzoLur19 in Burk medium, E) AzoLur19 in PHAMM, F) AzoLur23 in modified PHAMM.

A) AzoLur19 en medio Burk, B) AzoLur23 en MMPHA, C) AzoLur19 en MMPHA modificado, D) AzoLur19 en medio Burk, E) AzoLur19 en MMPHA, F) AzoLur23 en MMPHA modificado.

Figure 4. Cell staining with Sudan Black B dye, observed under an optical microscope at 24 hours (A, B, and C) and 96 hours (D, E, and F).

Figura 4. Tinción de células con Negro de Sudán B vistas al microscopio óptico a las 24 horas (A, B y C) y a las 96 horas (D, E y F).

PHA Organic Extraction

As previously mentioned (9), sodium hypochlorite and chloroform effectively extracted the PHA biopolymer.

PHA Accumulation and Productivity

According to strain evaluation, the highest biopolymer accumulation in PHAMM was 70.23% at 96 hours for the AzoLur20 strain (table 2). In contrast, the highest accumulation in the modified PHAMM medium was 74.33% at 96 hours for the AzoLur23 strain (table 3). Additionally, the highest PHA accumulation across all strains was observed in Burk medium with the AzoLur20 strain, reaching 87.67% at 72 hours (table 4). El-Nahrawy *et al.* (2018) reported similar results, using ammonium sulfate $(\text{NH}_4)_2\text{SO}_4$ with a final concentration of 0.2%. In contrast, Castillo *et al.* (2017) utilized the same culture medium but achieved 80% PHA accumulation, lower than that observed in our study.

Table 2. Percentage of PHA accumulation in selected strains in Burk medium.

Tabla 2. Porcentaje de acumulación de PHA de las cepas seleccionadas en medio Burk.

	24 h	48 h	72 h	96 h
AzoLur7	28.31%	36.41%	62.38%	31.33%
AzoLur18	13.12%	40.35%	74.29%	50.00%
AzoLur19	20.82%	54.29%	20.65%	26.60%
AzoLur20	11.65%	85.96%	87.67%	23.24%
AzoLur23	19.29%	79.11%	44.94%	19.09%

Table 3. Percentage of PHA accumulation in selected strains in PHAMM.

Tabla 3. Porcentaje de acumulación de PHA de las cepas seleccionadas en MMPHA.

	24 h	48 h	72 h	96 h
AzoLur7	25.19%	23.95%	39.35%	43.03%
AzoLur18	9.89%	26.69%	21.84%	13.80%
AzoLur19	27.99%	29.56%	22.26%	18.08%
AzoLur20	27.00%	26.58%	34.91%	70.23%
AzoLur23	29.17%	21.69%	28.67%	22.19%

Table 4. Percentage of PHA accumulation in selected strains in the modified PHAMM.

Tabla 4. Porcentaje de acumulación de PHA de las cepas seleccionadas en PHAMM modificado.

	24 h	48 h	72 h	96 h
AzoLur7	32.43%	42.32%	21.38%	66.40%
AzoLur18	13.94%	45.61%	10.64%	40.78%
AzoLur19	73.23%	53.23%	26.86%	19.17%
AzoLur20	36.14%	10.33%	44.37%	28.76%
AzoLur23	32.44%	39.92%	45.26%	74.33%

Considering productivity, AzoLur19 strain achieved the highest value of 0.04 g/L·h across all three tested media. Upon repeating the evaluation in triplicate, productivity values reached up to 0.06 g/L·h in both PHAMM and modified PHAMM. These results were comparable to those reported by Ramirez *et al.* (2011), who reported a productivity of 0.04 g/L·h in *Azotobacter* OPN mutant strain using Burk medium with ammonium acetate.

No significant differences were observed in AzoLur19 productivity between 24 and 48 hours. However, at 96 hours, significant differences in productivity were noticed in Burk medium compared to PHAMM and modified PHAMM. This disparity arose because, at 96 h, the culture in Burk medium was in exponential phase, whereas cultures growing in both PHAMM and modified PHAMM were in stationary phase (figure 5). Although values obtained by AzoLur19 strain did not exceed the ones reported by Cerrone (2011) and Pei *et al.* (2017), the analysis provided insight about strain behavior in different culture media and PHA production over time. Additionally, it supports the proposal of modified PHAMM for potential PHA production.

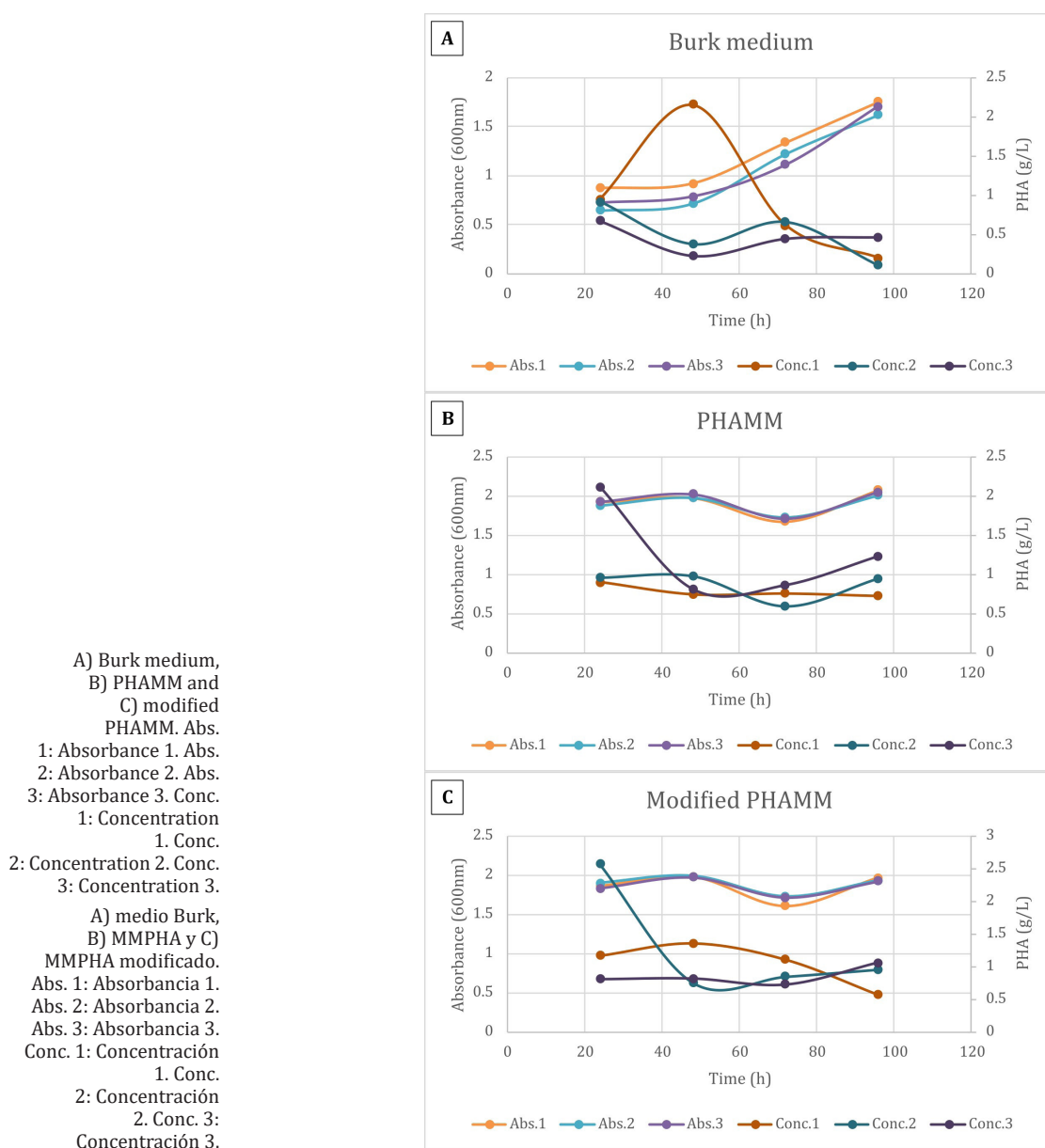


Figure 5. Comparison between cell growth and PHA production of AzoLur19 strain.

Figura 5. Comparación entre el crecimiento celular y la producción de PHA de la cepa AzoLur19.

Molecular Analysis

Based on BLASTN and phylogenetic analysis, the AzoLur19 strain was identified as *Azotobacter chroococcum* (GenBank accession number: MZ570428.1) (figure 6). Previous biochemical tests are consistent with this analysis. Aasfar *et al.* (2021) noted that *A. chroococcum* is predominant in the rhizosphere of several crops. Before this, Kim and Chan (1998) had demonstrated its high capacity to produce PHA.

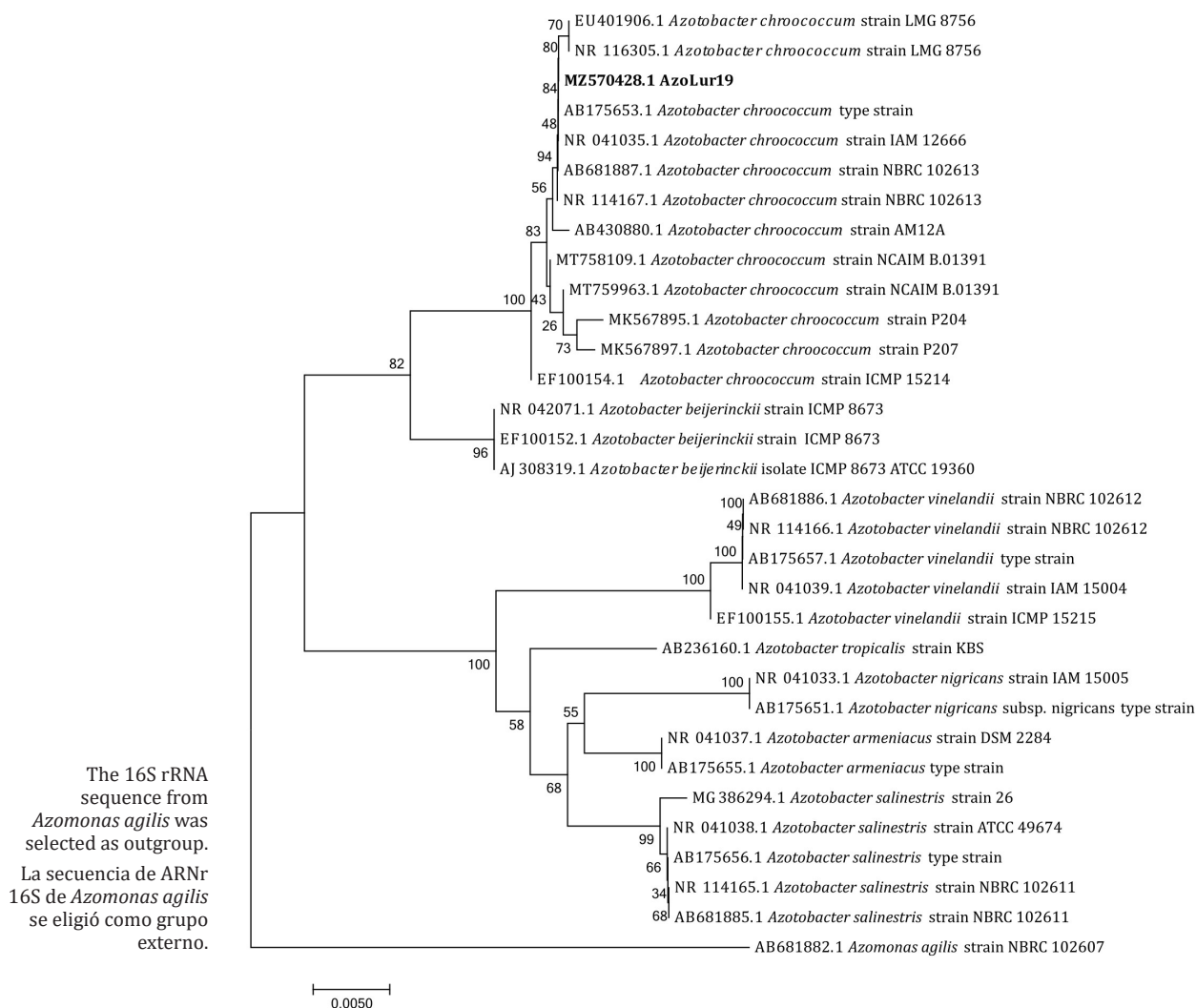


Figure 6. Phylogenetic tree of the 16S rRNA gene of the AzoLur19 strain and other sequences of *Azotobacter* sp., inferred using the Neighbor-Joining method with 1000 bootstrap replicates.

Figura 6. Árbol filogenético del gen 16S rRNA de la cepa AzoLur19 y otras secuencias de *Azotobacter* sp. inferido utilizando el método de unión de vecinos y 1000 de *bootstrap*.

CONCLUSIONS

Azotobacter strains are predominant in the rhizospheres of several crops. *A. chroococcum* is easily isolated and characterized, while being an effective PHA producer, especially after 24 and 48 hours of incubation. In this study, PHA detection was effectively achieved using the Nile Red medium, facilitating the selection of top-producing strains and allowing for production and quantitative evaluation. Additionally, staining with Sudan Black B enabled the observation of PHA intracellular granules. Finally, the AzoLur19 strain exhibited high productivity in both PHAMM and modified PHAMM, highlighting its potential for PHA synthesis.

REFERENCES

1. Aasfar, A.; Bargaz, A.; Yaakoubi, K.; Hilali, A.; Bennis, I.; Zeroual, Y.; Kadmiri, I. 2021. Nitrogen Fixing *Azotobacter* Species as Potential Soil Biological Enhancers for Crop Nutrition and Yield Stability. *Frontiers in Microbiology*. 12: 1-19.
2. Altschul, S.; Madden, T.; Schaffer, A.; Zhang, J.; Zhang, Z.; Miller, W.; Lipman, D. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*. 2(17): 3389-3402.
3. Arsita, R.; Karim, H.; Hala, Y.; Iriany, N.; Jumadi, O. 2020. Isolation and identification of nitrogen-fixing bacteria in the corn rhizosphere (*Zea mays* L.) originating from Jeneponto Regency, South Sulawesi. *IOP Conf. Series: Earth and Environmental Science*. 484: 012051.
4. Becerra, M. 2013. Producción de un polímero tipo polihidroxialcanoato (PHA) empleando residuos de la producción de biodiesel. Tesis de Magister en Ciencias, Microbiología. Facultad de Ciencias. Universidad Nacional de Colombia. Bogotá. Colombia. 129 p.
5. Carballo, M.; Iglesias, Y.; Martínez, J.; Solano, R.; Fernández, A.; Villaverde, M. 2003. Evaluación de la producción de polihidroxialcanoatos por cepas bacterianas marinas. *Revista Biología*. 17(1): 52-57.
6. Castillo, C.; Flores, C.; Segura, D.; Espín, G.; Sanguino, J.; Cabrera, E.; Barreto, J.; Díaz, A.; Peña, C. 2017. Production of polyhydroxybutyrate (PHB) of high and ultra-high molecular weight by *Azotobacter vinelandii* in batch and fed-batch cultures. *Journal of Chemical Technology & Biotechnology*. 92: 1809-1816.
7. Cerrone, F. 2011. Producción de poliésteres biopoliméricos (PHAs) desde alpeorujó por medio de bacterias fijadoras de nitrógeno. Tesis doctoral, Doctorado europeo. Instituto del agua. Universidad de Granada. Granada. España. 152 p.
8. Chávez, M. 2021. Metabolitos de *Azotobacter* spp. con posible aplicación biotecnológica. Tesis de grado en Biología. Facultad de Ciencias Biológicas. Benemérita Universidad Autónoma de Puebla. Puebla. México. 108 p.
9. El-Nahrawy, S.; El-Kodoos, R.; El-Sayed, B.; El-Shouny, W. 2018. Production of Poly- β -hydroxybutyrate (PHB) by *Azotobacter* sp. Isolated from Different Sources. *Environmental Biodiversity and Soil Security*. 2: 183-192.
10. Escobar, C.; Horna, Y.; Carreño, C.; Mendoza, G. 2011. Caracterización de cepas nativas de *Azotobacter* spp. y su efecto en el desarrollo de *Lycopersicon esculentum* Mill. "tomate" en Lambayeque. *Scientia Agropecuaria*. 2(1): 39-49.
11. Gatea, I.; Sabr, A.; Abdul, E.; Abbas, A.; Halob, A.; Mahmood, M. 2019. Isolation and characterization of local *Azotobacter* isolate producing bio-plastics and consuming waste vegetable oils. *IOP Conf. Series: Earth and Environmental Science*. 388: 012082.
12. Giraldo, J.; Castaño, G.; Rivera, F. 2020. Bacteria from industrial waste: potencial producers of polyhydroxyalkanoates (PHA's) in Manzales, Colombia. *Environmental Monitoring and Assessment*. 192(7): 480.
13. Kim, B.; Chang, H. 1998. Production of poly(3-hydroxybutyrate) from starch by *Azotobacter chroococcum*. *Biotechnology Letters*. 20(2): 109-112.
14. Lara, C.; García, L.; Oviedo, L. 2010. Medio de cultivo utilizando residuos-sólidos para el crecimiento de una bacteria nativa con potencial biofertilizante. *Revista Colombiana de Biotecnología*. 12(1): 103-112.
15. Malynovska, I.; Yula, V.; Asanishvili, N.; Ptashnik, M.; Lyubchich, A. 2021. Influence of crop species on quantity and physiological activity of rhizosphere microorganisms. *Ukrainian Journal of Ecology*. 11(1): 286-290.
16. Martinez, M.; Gonzalez, J.; Rodelas, B.; Pozo, C.; Salmeron, V. 1995. Production of poly- β -hydroxybutyrate by *Azotobacter chroococcum* H23 in chemically defined medium and alpechin médium. *Journal of Applied Bacteriology*. 78: 413-418.
17. McAdam, B.; Brennan, M.; Mcdonal, P.; Mojicevic, M. 2020. Producción de polihidroxibutirato (PHB) y factores que afectan sus características químicas y mecánicas. *Polímeros*. 12(12): 2908.
18. Mohammed, S.; Panda, A.; Ray, L. 2019. An investigation for recovery of polyhydroxyalkanoates (PHA) from *Bacillus* sp. BPPI-14 and *Bacillus* sp. BPPI-19 isolated from plastic waste landfill. *International Journal of Biological Macromolecules*. 134: 1085-1096.

19. Navia Porras, D.P.; Poveda Perdomo, L.G.; Cuervo Mulet, R.A.; Esparza Estrada, J.; Hernández Umaña, J. 2024. Antibacterial activity and physicochemical characterization of bioplastic films based on cassava (*Manihot esculenta* Crantz) starch and rosemary (*Salvia rosmarinus*) essential oil. *Revista de la Facultad de Ciencias Agrarias*. Universidad Nacional de Cuyo. Mendoza. Argentina. 56(2): 126-136. DOI: <https://doi.org/10.48162/rev.39.136>
20. Pei, M.; Kumar, S.; Woan, L.; Bor, J.; Najimudin, N. 2017. Characterization of polyhydroxyalkanoate production by mutant *Azotobacter vinelandii*. *Malaysian Applied Biology*. 46(1): 1-8.
21. Ramírez, M. E.; Herrera, S. L.; Domínguez, M.; Romo, Á.; Segura, D.; Peña, C. 2011. Evaluación de la producción y peso molecular del polihidroxibutirato (PHB) sintetizado por diversas cepas mutantes de *A. vinelandii*. Sociedad Mexicana de Bioquímica, Congreso Nacional 2011. Querétaro. Cartel CV-68.
22. Ramos, A. 2019. Extracción, purificación y modificación de un biopolímero del tipo poli (3-hidroxibutirato) obtenido de la fermentación de ácidos grasos con *B. cepacia*. Tesis de Maestría en Ingeniería Química. Facultad de Ingeniería. Universidad Nacional de Colombia. Bogotá. Colombia. 192 p.
23. Sharma, V.; Sehgal, R.; Gupta, R. 2021. Polyhydroxyalkanoate (PHA): Properties and Modifications. *Polymer*, 212: 123161.
24. Tamura, K.; Stecher, G.; Kumar, S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11, *Molecular Biology and Evolution*. 38(7): 3022-3027.
25. Vega, O. 2016. Extracción y caracterización estructural de un PHA, obtenido de residuos de cáscaras de yuca y piña mediante procesos de fermentación; y su aplicación en la fabricación de fibras por electrospinning. Tesis de doctor en Ingeniería. Facultad de Ingeniería. Universidad de Antioquia. Medellín. Colombia. 163 p.
26. Villota-Calvachi, G.; González, K.; Marulanda, S.; Galeano, N.; Velasco, D.; Ocampo, L.; Castañeda, L.; Giraldo, C.; Rodríguez, N. 2022. Aislamiento y caracterización de bacterias productoras de biopolímeros a partir de efluentes industriales. *Revista Colombiana de Biotecnología*. 24(1): 27-45.
27. Zambrano, H.; Riera, M. 2021. Desafío de los polihidroxialcanoatos como solución al problema de los plásticos de un solo uso. *Publicaciones en Ciencia y Tecnología*. 15(1): 15-26.

ACKNOWLEDGMENT

This research was funded by the Universidad Nacional Mayor de San Marcos - RR N° 05969-R-18 (code B18100214) of the Vicerrectorado de Investigación y Posgrado - VRIP- Undergraduate Thesis Promotion Program -2018. Our special thanks to Juan José Aponte and Liliana Salazar for their support during the manuscript review.