"III Congreso Latinoamericano de Astrobiología (2021)"
Revista Mexicana de Astronomía y Astrofísica Serie de Conferencias (RMxAC), **55**, 65–67 (2023)

https://doi.org/10.22201/ia.14052059p.2023.55.17

CHARACTERIZATION OF CULTIVABLE PSYCHROPHILIC BACTERIA WITH PHOSPHATE-SOLUBLE ACTIVITY AND NITROGEN FIXATION CAPACITY, PRESENT IN SEDIMENTS OF THE NEVADO DEL RUIZ (CALDAS, COLOMBIA)

J. Bolaños[1], J. Buitrago[1], M. Leal[2,3], D. Tovar[2,3], E. Ruíz[2,4], and J. Sánchez[2,5]

RESUMEN

Se efectuó muestreo de suelo en seis puntos georreferenciados del Nevado del Ruiz (Caldas, Colombia) para aislamiento y evaluación cualitativa de bacterias nativas fijadoras biológicas de nitrógeno y solubilizadoras de fosfatos, para explorar su potencial como promotores de crecimiento vegetal y potencial uso como biofertilizantes en climas fríos, para lo cual se realizó curva de crecimiento a diferentes temperaturas (4°C, 15°C, 25°C, 37°C). Se obtuvieron 56 aislamientos de las muestras de suelo de las cuales 38 arrojaron resultados positivos para el grupo funcional solubilizador de fosfato y 39 para fijación de nitrógeno. Las curvas de crecimiento se registraron hasta fase estacionaria con resultados positivos de crecimiento en todas las temperaturas en un rango de tiempo máximo de 36 horas, con un óptimo de temperatura en todos los aislamientos de 37°C.

ABSTRACT

A soil sampling was carried out in six georeferenced points of Nevado del Ruiz (Caldas, Colombia) for the isolation and qualitative evaluation of native biological fixative bacteria and phosphate-soluble capacity, to explore their potential as promoters of plant growth and potential as a biofertilizer in cold climates, for which a growth curve was made at different temperatures (4°C, 15°C, 25°C, 37°C). 56 isolations were obtained from the soil samples, of which 38 were positive for dissolve the phosphate functional group and 39 for nitrogen fixation. The growth curves are recorded up to the stationary phase with growth results at all temperatures in a maximum time range of 36 hours, with an optimum temperature in all isolates of 37°C.

Key Words: bacterial growth — functional groups — growth curve — psychrophilic organisms

1. INTRODUCTION

Psychrophilic microorganisms are of great importance in the field of biotechnology due to their particular metabolism (Margesin & Feller 2010). They are potential sources of pigments, enzymes active at lower temperatures, antifreeze compounds and have agricultural potential as inoculants and biocontrol agents in extreme habitats (Sharma et al. 2011).

The ability to grow in low temperatures implies adaptations inherent to cold climates, among them, the enzymes of these bacteria are active at low temperatures and this is the basis of the physiological adaptation to these environments. Molecularly they have been reported as they are sensitive to heat and have been observed to be inactivated at temperatures that are not harmful to their mesophilic counterparts, their enzymatic activity can be up to ten times higher at low and medium temperatures compared to that of mesophilic bacteria, for which they are said to have advantages in the biotechnological field due to their high metabolic activity, less concentration of catalyst is required to achieve the desired activity, the costs of enzymatic preparation are reduced. As a result of their activity in the cold, they are able to remain efficient at room temperature, thus avoiding the increase in temperature control costs in industrial-scale processes. Being sensitive to heat, they can be selectively inactivated after some process by means of moderate heat inputs to the system (Margesin & Feller 2010).

The objective of this study was to evaluate the presence of cultivable psychrophilic bacteria in sediment samples from Nevado del Ruz (Colombia) and their potential qualitative activity to dissolve phosphate and biological nitrogen fixation, as well as to evaluate their growth capacity at different temperatures.

[1]Departamento de Biología. Universidad Nacional de Colombia.
[2]Grupo de Ciencias Planetarias y Astrobiología GCPA. Universidad Nacional de Colombia.
[3]Facultad de Educación. Universidad de La Sabana.
[4]Laboratorio microbiología del suelo. Departamento de Biología. Universidad Nacional de Colombia.
[5]Departamento de Biología. Universidad Nacional de Colombia.

III Latin American Congress of Astrobiology (3CLA)(Virtual-Modality, August 3-6, 2021)
Editors: Leticia Carigi, Sandra I. Ramírez Jiménez, Miguel Chávez Dagostino, and Millarca Valenzuela - DOI: https://doi.org/10.22201/ia.14052059p.2023.55.17

2. METHODS AND RESULTS

2.1. *Microbial Cultures*

Six sediment samples were taken from the planetary sciences and astrobiology research group (GCPA) of the National University of Colombia in Bogot, in georeferenced areas of Nevado del Ruz (Colombia). For the isolation of the bacteria, a stock solution containing 10 g of sediment from each of the sampling points was started in 90 mL of sterile saline at 0.85%, incubated at 15°C, five times until growth was observed and produced massive cultures in selective medium R2A (Yadav et al. 2015) for isolation of oligotrophic bacteria, to verify their purity. From this, 56 cultivable isolates of bacteria capable of growing at 15°C in culture medium were obtained for oligotrophic organisms in R2A culture medium.

2.2. *Qualitative Evaluation of Potential Phosphate Soluble Activity*

For the evaluation of the potential soluble activity of inorganic phosphates, culturing was carried out in the SRS medium (Gupta et al. 1994) incubating between 24 and 48 hours at 15°C according to the IGAC protocol (IGAC 2006), all tests were carried out in triplicate. Those that grew by acidifying the culture medium, visualized by transparent halos around the colonies or by turning the medium from purple to yellow, were taken as a positive result for the isolates. It was determined that 38 isolates had the capacity to dissolve phosphates.

2.3. *Qualitative Evaluation of Potential Nitrogen Fixation Activity*

Selective medium NFB (Nitrogen free broth) was used for isolation of biological nitrogen fixing bacteria (Rennie & Rennie 1984), and it was incubated at 15°C for 7 days in triplicate. The observation of growth was recorded as a positive result. It was determined that 39 isolates with the ability to fix nitrogen were obtained.

2.4. *Evaluation of Optimal Growth Temperatures*

Bacteria with the ability to dissolve phosphates, fix nitrogen, and grow at 15°C were evaluated at temperatures of 4°C, 10°C, 25°C, and 37°C. Growth curves were made for isolates capable of growing at the four temperatures in a period of 24 hours. Growth was measured as changes in optical density (OD) at 660 nm using a spectrophotometer (Azzota SV-1100, Digital 4NM Visible Spectrophotometer), taking aliquots of the culture broth at regular time intervals during growth for the respective measurements. In order to estimate the total viable cell count, plating was performed after each optical density (OD) measurement in R2A culture medium, for the count of colony forming units (CFU) per milliliter (between 30 and 300 CFU/g). For the building of the growth curves the RStudio software was used. From this evaluation, two isolates that accomplish the conditions of being able to dissolve phosphates, fixate nitrogen and grow at the evaluated temperatures were selected: 43-1 corresponding to endosporated Gram negative bacilli and the 2-3 isolate consisting of endosporated short Gram negative bacilli. The curves were made with the help of a control organism from Antarctica with nitrogen fixation capacity, phosphate-soluble activity, and growing at the temperatures studied in a range of 36 hours. The viability tests were carried out once the optical density values of 0.20 were exceeded, which were read two days after their cultivation. The results obtained from these tests were above the maximum standard reading range (300 CFU/mL) in all the treatments performed.

2.5. *Statistic Analysis*

With the purpose of comparing between replicas and treatments of the data obtained when making the growth curve, ANOVA was carried out. The RStudio software was used to calculate the homoscedasticity values, normality tests and other conditions necessary to perform the normal analysis of variance (P less than 0.05). The treatment of the data corresponding to the growth curves presented normal distribution and in general did not present significant homoscedasticity values, therefore it was not taken into account for the normal analysis of variance. The ANOVAs performed for the curves corresponding to the repetitions of the temperatures confirmed the fulfillment of the null hypothesis, while those that were performed between treatments confirmed the alternative hypothesis.

3. DISCUSSION

Based on the results obtained, it can be stated that most of the isolates met at least one characteristic of functional groups of microorganisms (biological nitrogen fixers, phosphate-soluble agent) and 20 isolates met with both characteristics, so it can be assumed that there is possibly a selection pressure

III Latin American Congress of Astrobiology (3CLA)(Virtual-Modality, August 3-6, 2021)
Editors: Leticia Carigi, Sandra I. Ramírez Jiménez, Miguel Chávez Dagostino, and Millarca Valenzuela - DOI: https://doi.org/10.22201/ia.14052059p.2023.55.17

that favors these adaptations in the soils of the sampling areas, (Boyd & Peters 2013). The evaluation of growth at different temperatures showed interesting data. Regarding the curves, the isolates studied grew successfully at the four proposed temperatures, which defines them in the group of psychrotolerant bacteria (Cavicchioli et al. 2002), the temperature at which they had a shorter time to reach to the stationary phase was 37°C, this acquires greater validity when contrasting the curves with the ANOVAs performed, since the population means of the repetitions are equal, which confirms that the repetitions were done properly under the same conditions, while when contrasting the treatments, a confirmation of difference of means, which solidifies the validity of the experimental phase of the realization of the curves. Values from feasibility tests were uncountable (CFU/ml), a possible reason that can explain these outliers is the use of R2A broth and media for conducting this test, since there are multiple reports of sodium pyruvate as recovery promoter of cells under stress (Gurtler & Beuchat 2005), reducing the amount of cells lost during the building of the curve, for which it is recommended the use of culture media without sodium pyruvate to rule out results biased by the effect of the compound. In addition to this, the culture medium R2A presents a better performance when sowing using the surface dissemination technique but if it is done in this way, a smaller volume of sample should be sown than for other techniques, this was not taken into account for the realization of the phase experimental and it is recommended to adapt the sample volumes when performing the seeding to reduce bias in counts.

4. CONCLUSION

This study reveals the potential existing of bacteria native to high mountain Colombian soils, particularly from selected isolates (2-3 and 43-1) of bacteria that can performed as biological nitrogen fixers with phosphate-soluble activity growing at 4°C, 15°C, 25°C, and 37°C and growth records in 36 hours and optical density of 0.235. They can be evaluated for use with agricultural and biotechnological purposes. It is recommended for further studies to carry out the taxonomic identification of the selected isolates and testing under greenhouse conditions to evaluate its potential activity in promoting plant growth, previously corroborating that they do not represent a potential biological risk.

REFERENCES

Boyd, E. S., & Peters, J. W. 2013, Frontiers in Microbiology, 4

Cavicchioli, R., Siddiqui, K. S., Andrews, D. et al. 2002, Current Opinion in Biotechnology, 13, 253

Gupta, R., Singal, R., Shankar, A., et al. 1994, PSM. J. Gen. Appl. Microbiol., 260, 255

Gurtler, J. B. & Beuchat, L. R. 2005, ApEnM, 71, 7661

Margesin, R. & Feller, G. 2010, Environmental Technology, 31, 835

Rennie, R. J. & Rennie, D. A. 2010, Canadian Journal of Microbiology, 30, 519

Sharma, S., Kumar, V., & Tripathi, R. B. 2011, J. Microbiol.Biotech. Res, 1, 90

Yadav, A. N., Ghosh Sachan, S., Priyanka, V., et al. 2015, World Journal of Microbiology and Biotechnology, 31, 1