

ISOLATION, CULTURE AND MOLECULAR IDENTIFICATION OF EXTREME LICHEN MYCOBIONTS AND PHOTOBIONT FROM THE PAMPAS DE LA JOYA, DESERT OF PERU

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RESUMEN

El desierto de La Joya es considerado un análogo de Marte por sus condiciones de hiper-aridez, por sus altos niveles de radiación, cambios bruscos de temperatura entre el día y la noche, una cantidad muy baja de precipitaciones y humedad, así como variaciones muy notorias en la composición del suelo, todo ello dificulta la supervivencia de material biológico en esta zona. No existen estudios en los cuales se haya reportado especies de cianobacterias extremófilas en el desierto hiper-árido de las Pampas de Joya. En este estudio se han encontrado líquenes endolíticos y epilíticos nunca antes estudiados, los cuales fueron recolectados del desierto en la región hiper árida de las Pampas de La Joya, para lo cual se realizó el aislamiento y cultivo de los fotobiontes y micobiontes. Luego de la purificación del cultivo se realizó la extracción y secuenciación de ADN de las especies del talo liquénico, identificándose dos especies de hongos, *Neocatenulostroma microsporum* y *Acarospora cf. Baullat*; ambas especies con un 95% de homología las cuales se reporta como nuevas especies. Se realizó el estudio filogenético de estas demostrando también la no existencia de un registro anterior, dando una idea del linaje que las relaciona. Se realizó una selección de medio de cultivo en el que se probaron tres medios de cultivo (BBM, BG11 y F/2 modificado), determinando que el mejor para su crecimiento fue el medio F/2 modificado. Este es uno de los primeros estudios en el que se reporta la presencia de estos microorganismos en este desierto hiper-árido.

ABSTRACT

The desert of “La Joya” is considered an analog of Mars due to its hyper-arid conditions, in which very high amounts of radiation are contemplated, sudden changes in temperature between day and night, a very low amount of precipitation and humidity, as well as very noticeable variations in the composition of the soil, which make it difficult for biological material to survive in this area. There are no studies in which extremophilic cyanobacteria species have been reported in the hyper-arid desert of the Pampas de la Joya. In this study, endolithic and epilithic lichens never before studied have been found, which were collected from the desert in the hyper arid region of the Pampas de Joya, for which the isolation and cultivation of the photobionts and mycobionts was carried out. After the purification of the culture, DNA extraction and sequencing of the lichen thallus species was carried out, identifying two species of fungi, *Neocatenulostroma microsporum* and *Acarospora cf. Bullata*; both species with 95% homology which is reported as new species. The phylogenetic study of these was carried out, also demonstrating the non-existence of a previous record, giving an idea of the lineage that relates them. A selection of culture medium was carried out in which three culture media (BBM, BG11, and F/2 modified) were tested, determining that the best for its growth was the modified F/2 medium. This is one of the first studies in which the presence of these microorganisms is reported in this hyper-arid desert.

Key Words: deserts — extremophiles — symbiosis

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1. INTRODUCTION

The Atacama Desert is located on the western slope of the central Andes 15° and 30°, with an elevation of 3,500 meters above sea level. Geographically is on the center of the dry diagonal of South America. The extension of the Desert is approximately 3500 km from La Serena (29° 55'S) in Chile, and towards the north close to Ecuador (5° 00'S). The Andes mountain range produces a barrier that affects the permanence of the Southeast Pacific anticyclone and the existence of the coastal Humboldt current, which explains why this region does not receive the humid air masses from the Atlantic, and the poor evaporated moisture from the Pacific is blocked, remaining in the form of “camanchaca” in the narrow strip at the base of the coastal mountain range (Valdivia-Silva 2016). Rain events are almost null in the mountain range, on the coast, and in the central depression to the west of the Andes Mountains (Valdivia-Silva 2016). Studies on lichens have been reported in the Chilean part of the Atacama Desert, and most of the research involves the botanical and floristic description and their habitat from south to north. There is a lack of studies from the lichens' ecological, population, distribution, and community structure on the Peruvian side. The current study proposes mycobionts and photobionts presence on hyperarid areas as an astrobiological model.

2. METHODOLOGY

In the isolation of the mycobiont, the sample was inoculated in BBM culture medium in tubes and allowed to grow at room temperature. Subsequently, the rinsed sample was placed on PDA agar and Sabouraud Dextrose in a laminar flow chamber, allowing them to grow at approximately 25 °C. After this, different rings were made in tubes with PDA agar to isolate the different fungal components that made it up. The isolation of the photobiont followed the technique described by Gasulla & Barreno (2010) and was modified, where the photobiont was detached from the lichen thallus from the rock, then washing was completed with tap water and sterile distilled water. After this treatment, a soft crushing was performed in a mortar, avoiding physical damage to the bionts. The samples were placed in Eppendorf tubes with sterile distilled water and placed in the centrifuge (K PLC-05, Gemmy Industrial Corp.) at 4 thousand rpm for 5 minutes. After centrifugation, a green layer was observed in the upper part and a few particles in the gray background. The green layer was recovered, avoiding other interfaces. This portion was transferred to new tubes containing

sterile distilled water and this process was repeated five times. Afterward, the sample was seeded in tubes and flasks with 3NBBM medium (Three times more nitrogen as NaNO₃), allowing it to grow at room temperature with a photoperiod of 12 h. The molecular identification of the mycobiont was performed at the Uchumayo DNA Molecular Analysis Laboratory, in which DNA extraction was made using the mechanical method with liquid nitrogen and molecular sequencing with the ITS primer described by Buitrago et al. (2007). Identification of the photobiont, DNA extraction, and molecular identification was performed in the molecular biology area of the University of La Laguna in Spain. Two molecular primers were used for identification, ITS1, and ITS4. The sequences were compared and matched on the GENE BANK web platform. The evaluation of growing photobiont species required an aerobic evaluation where reactors with 200 mL were placed with enough BBM culture and other anaerobic environments hermetically closed. Subsequently, the culture media was selected (BBM, BG-11, F/2 modified, exchanging seawater for distilled water) at two different photoperiods (12h/24h).

3. RESULTS

It was possible to isolate the mycobiont using the rinse method, obtaining the fungal part of the lichen free of bacteria and photobionts. Two species were distinguished, a white strain corresponding to *Acarospora cf. Bullata* and a black colored strain identified as *Neocatenulostroma microsporum*. Two potential species of mycobionts for each of the samples were obtained. After performing the BLAST of the DNA sequences, it was determined that each of the samples has a percentage of homology no greater than 95%, which tells us that they are sequences of microorganisms not yet discovered, since it leaves us 5% for each of the sequences that have not been identified before. The growth of the photobiont from lichens from the La Joya dessert was achieved in the 3NBBM medium, which served for the molecular identification of the species, resulting in the species *Chloridium sp.*, also described as *Chlorella angustoeilipsoidea*. The BLAST of the two markers ITS1 and ITS4, was performed, where it was found that the highest percentage of homology is 99.3%. The growing experiment indicates that the best microalgae growth was obtained in the F/2 medium, reaching a final concentration of 18669.778 cells/mL. In comparison with the other culture media in which lower concentrations were obtained as shown in figure 1.

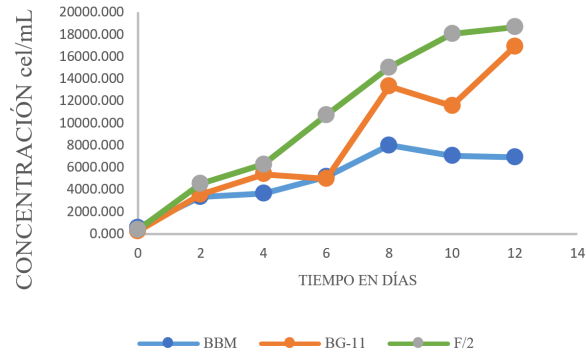


Fig. 1. Chlorella growth curve during 12 hours in artificial media

4. CONCLUSIONS

We isolated the species that make up the lichen thallus from the hyper-arid desert of La Joya finding two species of fungi and one species of photobiont. We managed to cultivate each of them in different cultures media. Two new species of fungi with indications of lichen formations were molecularly identified, one corresponding to *Neocatenulostroma microsporium*, and the other to *Acarospora cf. Bullata*. The molecular analysis of the photobiont gave us the species *Chloridium sp.* with a maximum percentage of homology of 99.36%, which would also put us in front of a new species. This would indicate the existence of a new species of lichen discovered in the desert of La Joya, which is clearly native to the locality. The reproduction of the photobiont in culture medium was successful, obtaining a quantity of algal biomass, artificially cultivated in liquid media; Thus, the culture medium F/2 in the 12-hour photoperiod resulted the best conditions for the growth of the *Chloridium* species, with which the best results were obtained based on a more significant amount of biomass according to this study.

TABLE 1

GROWTH AVERAGES IN *CELLS/ML*, AT TWO PHOTOPERIODS OF LIGHT AND THREE DIFFERENT CULTURE MEDIA OF THE *CHLORIDIUM DE LA JOYA* SPECIES.

| Photoperiod | Cultivation medium | Chloridium |
|-------------|--------------------|------------------|
| 12 | BBM | 8399.83 ± 0,899 |
| | BG-11 | 10190.56 ± 0,051 |
| | F/2 | 25964.44 ± 0,031 |
| 24 | BBM | 16469.17 ± 0,851 |
| | BG-11 | 11197.11 ± 0,310 |
| | F/2 | 20920.00 ± 0,561 |

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