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Chlorophyceae from Biofilms on Monuments of a Mexican Archaeological Area

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ABSTRACT

The microbial community interacts in different ways with the environment. Regarding mineral materials particularly, the microbial community may be present either on the surface, the divisions or in the fissures. This microbial interaction with the materials and their environment can lead to biodeterioration, a common problem in monuments and archaeological sites. Microorganisms form biofilms, which are communities structured by bacteria, algae, cyanobacteria, fungi and protozoa that are embedded in a polymeric matrix. The aim of this work was the morphological identification of Chlorophyceae microorganisms from biofilm mounds of the Yohualichan, an archaeological zone in Puebla, Mexico. Biofilm samples were collected from archaeological monuments in the area known as "Juego de Pelota" (Ballgame), as well as in the East Building, the West Building and "Las Grecas" (Fretworks). The strains were isolated by using BG-11 medium, solidified with 1.3% bacteriological agar, until obtaining monoalgal cultures and the propagation of isolates in liquid BG-11. Twenty one monoalgal cultures were obtained, grouped into five different orders and nine genera: *Chlorella*, *Chlorococcum*, *Chlorokybus*, *Desmodesmus*, *Elakatothrix*, *Fernandinella*, *Fottea*, *Klebsormidium* and *Oocystis*. To conclude it can be said that microalgae primarily make biofilms present in this archaeological site.

Introduction

The use of stone as a medium for artistic expression has gone from the construction of ancient monuments and historical buildings to small statues. While the erosion of rocks during the formation of soil is undoubtedly essential for the evolution of life on earth, the decline of stone artifacts of cultural importance represents an irretrievable loss of cultural and historical heritage (Warscheid and Braams, 2000).

Most of the elements of the cultural and artistic heritage of the country, which have been built in stone, are at risk, not only for the damages caused by the passage of time and exposure to different abiotic conditions, but also for the alterations produced by the colonization of organisms and microorganisms. The stone, since it is extracted from the quarry, comes into contact with various physical, chemical and biological agents that act on it. The combination of all these factors makes the material that constitutes the monument become the habitat of different microorganisms (heterotrophic bacteria, cyanobacteria, algae and fungi), lichens, mosses and vascular plants, which induce changes that affect the aesthetics of the monument and they produce deeper mechanical and chemical alterations. The set of all these processes of alteration of the monument, generated by the presence and biological activity, is what is called biodeterioration and its contribution to the total deterioration of the monument must be taken into account to protect and preserve the artistic and cultural heritage (De Los Ríos, 2008). One of the most important problems affecting archaeological zones today is the appearance of biofilms that generate damage to the structure of the remains (De Belie, 2010; De Felice et al., 2010; Romaní et al., 2008). Therefore, it is important to determine which photosynthetic microorganisms constitute the biofilms collected in the mounds of this archaeological zone, and to evaluate some of their metabolic characteristics in order to propose some management to stop the deterioration caused by these communities. In Mexico there have been few studies in this regard, however, and none of them has been done in the archaeological zone of Yohualichan, Puebla, this site is part of the Totonac culture and is of particular interest because its vestiges prove the presence of groups of the coast in this region of Puebla, Mexico (Guevara, 1991; Ruiz, 1996) (Fig. 1).

The objective of this work was to isolate and identify the Chlorophyceae microorganisms found in the samples of the biofilms collected in the mounds of the archaeological zone of Yohualichan, Puebla.

Materials and methods

Study area

The archaeological zone is in 20 ° 03 '42.79 "N and 97 ° 30' 11.31" W. It is located 189 km from the capital of the state of Puebla, and 9 km from the town of Cuetzalan. The climate of this region is tropical rainy with a temperature of 8 ° to 32 ° C and an average relative humidity of 58% throughout the year (Juan, 2002).

Samples collection

The samples were obtained from the buildings where the presence of biofilms was observed: Juego de Pelota (Ball Game, JP), Edificio Este (East Building, EE), Edificio Oeste (West Building, EO) and Edificio Las Grecas (Las Grecas Building, EG). Samples were taken with tweezers or spatula and placed in plastic containers (all material was previously sterilized), then moved to the Plant Physiology Laboratory of the Botany Department of the National School of Biological Sciences where they were placed in freezing at -20°C to preserve them.

Isolation of the strains

To perform the isolation of the Chlorophyceae from the samples of the biofilms an inoculum was taken and streaked on plates with BG-11 medium solidified with 1.3% bacteriological agar (Castenholz, 1988), and incubated in white light illumination (1755 luxes) with photoperiod of 16: 8 h (light: dark), at 25 ± 1°C for one to four weeks. The strains were isolated by micromanipulation, selecting them under the microscope, and they were placed in 8 mL glass vials with 3 mL of liquid BG-11 medium Olvera-Ramírez et al. (2003). Once the growth was visible, they were isolated by cross streaks in plates of the same medium, until obtaining monoalgal cultures. Isolates were cultured in liquid medium BG-11 and maintained with constant aeration, illumination with white light (1755 luxes) with photoperiod of 16: 8 h (light: dark), at 25 ± 1°C, in Erlenmeyer flasks of 500 mL.

Fig. 1: Study area, Yohualichan, Puebla, Mexico.

Taxonomic identification

To achieve the taxonomic identification of the isolated photosynthetic microorganisms, fragments were taken from the isolates that were stained with lugol and observed with a Nikon Alphaphot optical microscope 2 YS2 with 40x and 100x lens. The taxonomic keys of Wehr and Sheath (2003), John et al. (2005) and Bicudo and Menezes (2006) were used. The morphological characteristics of the isolated photosynthetic microorganisms that were considered to carry out their identification were: shape and size of the cells, shape of the plastids, and absence, presence and number of pyrenoids. The measurements were recorded using a Nikon Alphaphot 2 YS2 optical microscope.

Results and discussion

Taxonomic identification of the strains

From a total of 21 strains that were obtained, five taxa could be identified based on their morphological characteristics up to genus and species, and four up genus only; the strains were grouped in two orders. Table 1 shows the taxa identified and location and biofilm from they were sampled.

The macroscopic and microscopic images of the species of Chlorophyceae identified are shown on Figs. 2, 3, 4, 5 and 6.

A wide variety of photosynthetic microorganisms were identified in the different monuments of the archaeological zone of Yohualichan, Puebla; most of the isolates belong to the Chlorophylaceae, the representative orders within are Chlorellales and Klebsormidiales, both of them obtained in this work. In Crispim et al. (2003) a comparison was made of the phototrophic microorganisms that can colonize monuments in which the base is cement or limestone, they sampled 14 historical places in Europe and Latin America.

Table 1. Chlorophyceae from biofilms of Yohualichan.

Order	Sample source	Name of the algae	No. of times isolated
Chlorellales	JP, EO; soft and sticky biofilms	*Chlorella vulgaris* Beijerinck	5
	JP; soft and sticky biofilms	*Chlorella* sp.	2
	JP; soft and sticky biofilms	*Oocystis parva* West et G. S. West 1898	2
Chlorococcales	JP, EG, EO; soft and sticky biofilms	*Chlorococcum minutum* R. C. Starr 1955	3
	JP, EE, EO; soft and sticky biofilms	*Chlorococcum* sp.	4
Klebsormidiales	JP; soft and sticky biofilms	*Klebsormidium subtile* (Kûtzing) Tracanna ex Tell	1
	JP; soft and sticky biofilms	*Chlorokybus* sp.	1
	JP; soft and sticky biofilms	*Elakatothrix* sp.	1
Sphaeropleales	JP; soft and sticky biofilms	*Desmodemus* sp.	2
	JP; soft and sticky biofilms	*Fernandinella* sp.	1
Ulotrichales	EO; soft and sticky biofilms	*Fottea cylindrica* Hindák	1

Fig. 2: *Chlorella vulgaris*, colonies and microscopic view (100x).

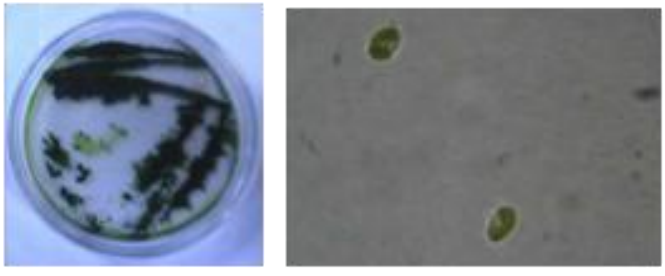

Fig. 3: *Oocystis parva*, colonies and microscopic view (100x).

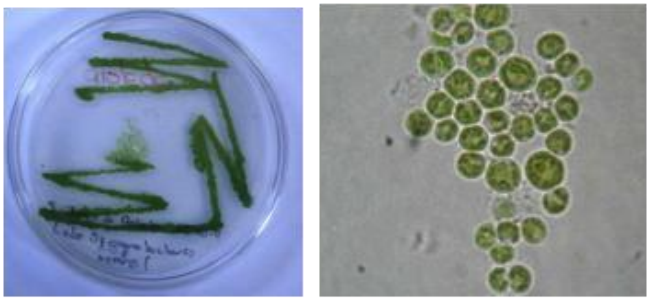

Fig. 4: *Chlorococcum minutum*, colonies and microscopic view (100x).

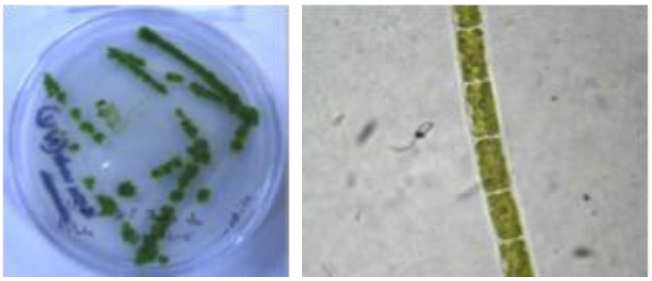

Fig. 5: *Klebsormidium subtile*, colonies and microscopic view (100x).

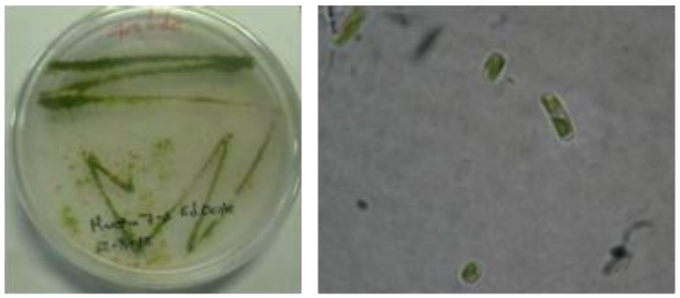

Fig. 6: *Fottea cylindrica*, colonies and microscopic view (100x).

In general, the best represented microorganisms were cyanobacteria called *Synechocystis* and several coccoid Chlorophyceae in all the monuments, and among the least frequent were representatives of the Stigonematales in Blaye, France, and diatoms in Dorset, United Kingdom, and Porto Alegre, Brazil. When comparing these results with what was identified in the archaeological zone of Yohualichan, it is observed that a large number of coccoid Chlorophyceae were also obtained as in the limestone monuments studied by Crispim et al. (2003), and although these authors only reported *Klebsormidium* on cement and mortar monuments, it is worth mentioning that *Klebsormidium subtile* was identified as part of the photosynthetic microorganisms that make up the biofilms found in the Ball Game, which is made of limestone. Videla et al. (2003) also found *Chlorella* in Mayan buildings in Tulum, Mexico.

Crispim et al. (2003) mention that all the substrates sampled in their study were of calcareous nature, the most important difference between limestone (a natural rock) and the other artificial substrates, is probably the porosity, which is usually lower in limestone, also they mention that the cement is mainly formed by calcium silicate hydrate, which is very porous and retains a large amount of water in its fine pores. The porosity of the substrate is related to the penetration and retention of water, which, in turn, affects microbial colonization. Therefore, since the archaeological site of Yohualichan is built of limestone (Molina, 1980), a calcareous rock that can retain a large amount of water and there are environmental conditions (high humidity) that favor growth, *Klebsormidium* can colonize the surfaces.

Conclusion

The biofilms in the form of soft and sticky deposits present in the archaeological zone of Yohualichan are mainly formed by microalgae of the Chlorellales and Ulotrichales orders. The predominant morphology of the Chlorophyceae present was unicellular with the exception of *Klebsormidium subtile*, the only filamentous Chlorophyceae. *Chlorococcum* is the genus of microalgae that is widely distributed in the archaeological zone since it was isolated from most of the samples collected.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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