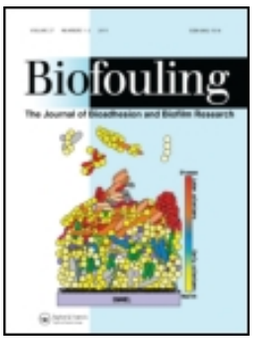

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Short Communication

Biodeterioration of Mayan Buildings at Uxmal and Tulum, Mexico

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Uxmal and Tulum are two important Mayan sites in the Yucatan peninsula. The buildings are mainly composed of limestone and grey/black discoloration is seen on exposed walls and copious greenish biofilms on inner walls. The principal microorganisms detected on interior walls at both Uxmal and Tulum were cyanobacteria; heterotrophic bacteria and filamentous fungi were also present. A dark-pigmented mitosporic fungus and *Bacillus cereus*, both isolated from Uxmal, were shown to be acidogenic in laboratory cultures. Cyanobacteria belonging to rock-degrading genera *Synechocystis* and *Gloeocapsa* were identified at both sites. Surface analysis previously showed that calcium ions were present in the biofilms on buildings at Uxmal and Tulum, suggesting the deposition of biosolubilized stone. Apart from their potential to degrade the substrate, the coccoid cyanobacteria supply organic nutrients for bacteria and fungi, which can produce organic acids, further increasing stone degradation.

Keywords: biodeterioration; biofilms; Cyanobacteria; fungi; limestone; Mayan heritage

INTRODUCTION

Mayan archaeological sites are numerous in the Yucatan Peninsula of Mexico. Uxmal, founded in 800 BC, is one of the most important sites and an outstanding example of the Puuc style of architecture. Most of the surviving buildings date from around 800–1000 AD. The site is in the central-west of the Yucatan Peninsula and is surrounded by relatively undeveloped countryside. Tulum, on the other hand, is situated in the North Eastern part of the state of Quintana Roo, in a coastal environment by the Caribbean Sea. This site consists of late postclassic style Mayan buildings from the 12th and 13th centuries. The temples, pyramids and other structures at these two sites are built predominantly of limestone. Thick green and black biofilms are commonly

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seen on interior walls, while external walls are usually less heavily colonized and the biofilms are generally grey or black.

Mechanisms of biodeterioration of limestone have been considered to be acid attack by bacteria, fungi and algae (Grant, 1982; Koestler *et al.*, 1985; Krumbein, 1988; May *et al.*, 1993) and direct boring activity by fungi and phototrophs (Hoffmann, 1989; Ortega-Calvo *et al.*, 1991). Only certain genera of phototrophs are considered to possess this activity. It is thus important to determine the types of organisms present on buildings of historic and cultural interest in order to predict risk factors and implement appropriate treatment strategies. The aim was to detect stone-biodegrading microorganisms on buildings at the cultural heritage sites of Uxmal and Tulum, Mexico.

MATERIALS AND METHODS

Samples were taken aseptically from biofilms or black crusts on surfaces of an external wall behind the Magician's pyramid (El Adivino), the internal walls of the Nunnery (Casa de las Monjas) and the Governor's House in Uxmal and from a cubicle close to the Castle (Structure 1), from internal walls of the Temple of the Wind (Miniature Temples Group) and two external walls near the House of the Cenote in Tulum. Maps of the sites, showing the position of the buildings, can be found in Kelly (1993). The samples were collected non-destructively with a sterile wooden spatula, or using the adhesive tape technique (Gaylarde & Gaylarde, 1998).

Microbiological Analyses

The major biomass in the samples was identified by microscopy following rehydration. For algae and cyanobacteria, samples were incubated at 28°C, under standard, low-light conditions, on solid medium for algae (Modified Knop's medium; MKM) (Gaylarde & Gaylarde, 1998). *In situ* microscopy was performed daily for 4–5 d, and thereafter weekly for up to 4 weeks. Identification was by morphology (light and fluorescence microscopy). Cyanobacteria were principally classified according to Bergey's Manual (Holt *et al.*, 1994) and algae according to Prescott (1964) and Smith (1950).

For bacterial and fungal analyses, small grains of samples were cultured directly, or 1 g was suspended in 10 ml sterile saline and the liquid cultured on nutrient agar, yeast extract/glucose/chloramphenicol agar (YGC) and Postgate's Medium B. Bacterial colonies growing on the solid media after 24 h were identified by Gram staining and growth on selective media for bacteria. Fungi were identified by their morphology.

Acid production by two isolates, *Bacillus cereus* and a dark-pigmented mitosporic fungus, was tested after 24 h growth at 30°C in 100 ml simple mineral medium, pH 7.0 (Salvarezza *et al.*, 1983) containing glucose as sole carbon source at 1% for the bacterium and 2% for the fungus. The organisms were inoculated using a platinum loop and pH was measured at the end of incubation.

Electron Microscopy

Samples were examined with the scanning electron microscope (SEM) and environmental SEM (ESEM). SEM samples were fixed in buffered 2.5% glutaraldehyde, washed in phosphate buffer, dehydrated through an acetone series to 100%, and finally critical point dried and gold sputtered. Samples for ESEM required no previous preparation.

RESULTS AND DISCUSSION

The major microbial biomass seen in all the samples was composed of cyanobacteria. A wide

variety of phototrophs was detected and coccoid forms were preponderant among both the algae and the cyanobacteria (Table I). Garcia de Miguel *et al.* (1995) found that cyanobacteria, as well as mosses, were the most abundant organisms associated with the Pyramid of the Great Jaguar at Tikal, Guatemala, which is located in an intensely forested, tropical area. These authors recorded the filamentous *Phormidium* (classified by Bergey as *Lyngbya*), *Plectonema*, *Scytonema*, and *Chlorogloeopsis* and the coccoid *Gloeocapsa* as the most representative cyanobacterial genera. They also noted that, except for *Chlorella*, eucaryotic algae were absent from most samples. The predominance of coccoid over filamentous cyanobacteria in the present samples may reflect the difference in environment, since Tikal is much more heavily forested and humid than the seasonal dry forest of the Yucatan. On the other hand, the difference may be explained by alternative detection methods used. No attempt was made in the present study to quantify or to isolate phototrophs, since detection in mixed culture biofilms after short-term incubation has been found to yield much better data in terms of biodiversity. Most coccoid cyanobacteria cannot be cultured except in mixed cultures which retain the initial spatial relationships of the organisms in the samples. Isolation of pure cyanobacteria in culture prior to identification frequently leads to the loss of coccoid forms and a major diminution in their detection rate (Gaylarde & Gaylarde, 1999). It is also noted that filamentous organisms of the Nostocaceae, Trentepohliaceae and *Eustigmatos* are frequently present in the biofilms of xerotic surfaces in a coccoid growth form and that Oscillatoriales seldom form long filaments in dry habitats. SEM of the present Uxmal and Tulum samples confirmed that there were very few filamentous forms present in the biofilms.

Heterotrophic bacteria (10^5–10^7 cfu g^{-1} on nutrient agar), including actinomycetes (detected in MKM cultures), were present in the samples (Table II), but made a low contribution to the biomass because of their small individual size. Few protozoa and fungi were noted by direct microscopic examination of samples; plating on YGC indicated that the fungal populations were between 10^2 and 10^5 cfu g^{-1}. The numbers of bacteria and fungi are, of course, artificially modified by the medium used for enumeration. Many fungi isolated from stone buildings have been shown to produce acids capable of degrading the structures (Resende *et al.*, 1996) and algae can contribute to fungal stone decay by providing organic substrates for the production of such

TABLE I Major phototrophs detected on walls of Mayan buildings in Uxmal and Tulum

Phototrophic group	Uxmal	Tulum
Cyanobacteria		
Subgroup 1 (coccoid and colonial)	*Gloeocapsa* *Gloeothece* *Synechococcus* *Synechocystis*	*Gloeocapsa** *Gloeothece* *Synechococcus*
Subgroup 2 (coccoid and colonial)	*Xenococcus** *Myxosarcina*	*Xenococcus** *Dermocarpa** *Pleurocapsa* *Chroococcidiopsis*
Subgroup 5 (filamentous)		*Chlorogloeopsis*
Algae		
Coccoid and colonial	*Chlorella* *Chlorococcum* *Dimorphococcus* *Coccomyxa*	*Chlorella*
Filamentous	*Eustigmatos* *Stichococcus* *Trentepohlia*	*Gongrosira*

* = major biomass

TABLE II Bacterial and fungal isolates from Uxmal and Tulum

	Uxmal	Tulum
Bacteria	*Pseudomonas* sp. *Bacillus cereus*	*Pseudomonas* sp. *Bacillus circulans* Sulphate-reducing bacteria
Fungi	Sterile mycelium *Monilia* sp. Dark-pigmented mitosporic fungus	*Aspergillus niger*

acidic metabolites (Koestler *et al.*, 1985; Gómez-Alarcón *et al.*, 1994). A dark-pigmented mitosporic fungus and *Bacillus cereus*, both isolated from Uxmal samples during this investigation, reduced the initial pH of 7.0 in the glucose/mineral salts medium to values between 3.0 and 4.0 pH units. Dark pigmented mitosporic fungi have also been shown to actively penetrate limestone, causing "biopitting" (Sterflinger & Krumbein, 1997), as have certain genera of cyanobacteria, including *Gloeocapsa*, *Chroococcus* and *Aphanocapsa* (Hoffmann, 1989). The latter two genera are classified in the Bergey's Manual system as *Gloeocapsa* and *Synechocystis*, respectively. This confirms the potential of the microbial flora on the present buildings for degradation of the structures and EDAX results previously obtained from Uxmal samples confirmed the presence of Ca (with minor peaks of Cl and Al) in the biofilms (Videla *et al.*, 2000), suggesting the deposition of biosolubilized calcareous material (McCormack *et al.*, 1996).

The presence of melanin-forming fungi and actinomycetes, and of cyanobacteria with dark-pigmented cells or capsules, frequently found in the present samples, indicates the role of the microbial flora in the aesthetic biodeterioration of these stone buildings, as previously discussed by Urzi *et al.* (1992). It has been claimed that only certain fungi, such as the dark-pigmented mitosporic types, and a few actinomycetes, are able to form melanins. Such microorganisms have been detected on other stone and marble surfaces (Letznicka *et al.*, 1988), as well as on the limestone buildings sampled here, and are doubtless important biodeteriogens of cultural and historic property.

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