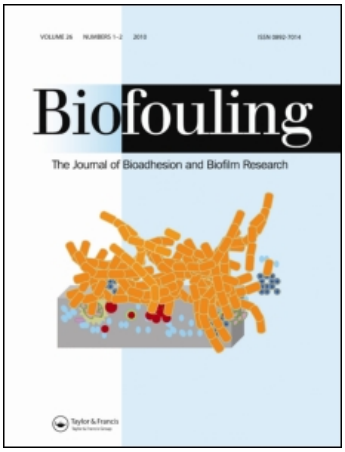

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Cyanobacteria-containing biofilms from a Mayan monument in Palenque, Mexico

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Surfaces of buildings at the archaeological site of Palenque, Mexico, are colonized by cyanobacteria that form biofilms, which in turn cause aesthetic and structural damage. The structural characterization and species composition of biofilms from the walls of one of these buildings, El Palacio, are reported. The distribution of photosynthetic microorganisms in the biofilms, their relationship with the colonized substratum, and the three-dimensional structure of the biofilms were studied by image analysis. The differences between local seasonal microenvironments at the Palenque site, the bioreceptivity of stone and the relationship between biofilms and their substrata are described. The implications for the development and permanence of species capable of withstanding temporal heterogeneity in and on El Palacio, mainly due to alternating wet and dry seasons, are discussed. Knowledge on how different biofilms contribute to biodegradation or bioprotection of the substratum can be used to develop maintenance and conservation protocols for cultural heritage.

Keywords: cyanobacteria; biofilm structure; Mayan monuments; biodeterioration; bioprotection

Introduction

The Mayan Empire spanned 350,000 km^2, encompassing modern day Mexico, Guatemala, Honduras, El Salvador, and Belize. The Palenque archaeological site, discovered in 1785 (Ruz 1997), is a prime example of a Mayan sanctuary from the classical period, reaching its height between 600 and 900 AD. Palenque was declared a National Park by the National Commission of Protected Natural Areas (CONANP) in 1981 and was added to UNESCO's World Heritage List in 1987. The region is humid tropical, with precipitation as the main hydro-climatological variable (Snow 1976), and alternating wet and dry seasons. The quarry stones of the building are mainly calcareous, and all Mayan structures were originally coated with stucco (Gaylarde et al. 2001).

Under suitable conditions, both natural and artificial substrata can become colonized by communities of microorganisms enclosed in exopolysaccharide matrices called biofilms (Warscheid and Braams 2000; Di Pippo et al. 2009). The composition and local distribution of biofilms are determined by the spatial and temporal variation of several biotic and physico-chemical factors, including microclimate. The capacity for a given stratum to be colonized is known as bioreceptivity, a term coined by Guillitte (1955). The bioreceptivity of building materials varies widely and is chiefly dictated by surface roughness, initial porosity, moisture, and mineralogical nature (Guillitte 1995). The biofilms that grow in areas exposed to light tend to contain photosynthetic organisms. Cultural heritage sites made of natural materials (eg stone) are highly susceptible to damage caused by biofilms, namely, through chemical and physical deterioration (Ortega-Calvo et al. 1991; Morton and Surman 1994; Wakefield and Jones 1998; Gorbushina 2007) as well as surface discoloration.

Many of the illuminated surfaces on Mayan monuments are covered by biofilms formed by subaerial phototrophic species that resist environmental changes, including extended droughts, high temperature, or prolonged solar exposure (Novelo and Ramírez 2006). Survival strategies related to desiccation are well known (Potts 1999; Wynn-Williams 2000), and include the use of water retained within the substrata and the formation of protective, drought-resistant compounds (Gorbushina and Krumbein 2000). It can be assumed that just as some photosynthetic microorganisms cope with high solar irradiance by synthesizing ultraviolet radiation (UVR) screens (Castenholz and Garcia-Pichel 2000; Fleming and Castenholz 2007), others, avoiding water stress inside rock substrata, will ultimately be limited by light penetration (Bell 1993; Walker and Pace 2007). The most abundant species are cyanobacteria and a few

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green algae; their presence is generally dictated by local humidity and desiccation (Saiz-Jiménez and Videla 2002; Crispim et al. 2003; Videla et al. 2003; Caneva et al. 2005; Ortega-Morales et al. 2005; Gaylarde et al. 2006; McNamara et al. 2006; Novelo and Ramírez 2006).

The organic matter produced by phototrophs is often exploited by non-photosynthetic microorganisms such as fungi or bacteria, which subsequently flourish. Reported examples include Proteobacteria and Actinobacteria, mainly among epilithic communities, and Acidobacteria, Actinobacteria and low GC Firmicutes, primarily in endolithic communities (Wakefield and Jones 1998; Caneva et al. 2005; McNamara and Mitchell 2005). The environmental conditions associated with these biofilms were reasonably well understood, but little was known about their structure or how they respond to seasonal changes.

The aim of this work was to obtain information on the distribution, 3D structure, and adaptation to seasonal changes of Palenque phototrophic biofilms, particularly, to determine the conditions under which each morphospecies thrives. A multistep approach was employed, including light microscopy (LM), scanning electron microscopy in back-scattered electron mode (SEM-BSE), energy dispersive X-ray microanalysis (EDX), X-ray diffraction (XRD), and the non-destructive technique of confocal laser scanning microscopy (CLSM). In CLSM, samples are observed without the need to remove fluorescently or otherwise-labeled items from the substratum. Fluorescence of photosynthetic pigments was used to compare the architecture of the biofilms and determine morphospecies, the depth at which they thrive, and whethers they were alive (Roldán et al. 2004; De los Ríos and Ascaso 2005; Horat et al. 2006). Phenotypic information on morphospecies, spatiotemporal variability, and the physiological status of the cells were also determined (Neu et al. 2004; Roldán et al. 2006).

Methods

Site description and sample collection

Palenque is located in the state of Chiapas, in southeast Mexico (17°29′00″ N, 92°03′00″ W). The region is tropical with rainfall fluctuating between heavy rains, from August to November, and a dry season, from December to April (http://smn.cna.gob.mx/productos/emas/#). Samples of epilithic biofilms were collected from an archaeological site, in a monument known as El Palacio (Figure 1). Taxonomic studies were begun in 2003 (Novelo and Ramírez 2006) and the samples were collected in August 2007 and January 2008. The samples were taken from four sites (Figure 2) on the building walls, each of which faces

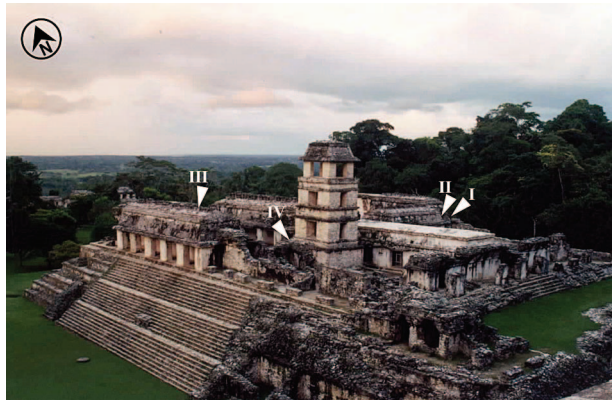

Figure 1. Location of sampling sites (I, II, III and IV) at El Palacio (Palenque Archaeological Site).

different abiotic conditions: Site I (exposed to light, alternately wet and dry); Site II (protected from both light and direct rainfall); Site III (partially exposed to light, with persistent moisture); and Site IV (partially exposed to light, alternately wet and dry).

The moisture and temperature of the biofilm surfaces were measured with a Surveymaster BLD 5360 protimeter (General Electric, USA).

Small flakes and splinters of substrata were collected from all four sites, whose biofilms varied in shape, color, and texture (Figure 2). The color of each biofilm was dictated by its constituent algae and cyanobacteria and by its substratum. The samples were divided into aliquots that were either cultured, observed directly, or fixed for CLSM and SEM studies.

Microscopy

Light microscopy

An Axioplan microscope (Carl Zeiss, Oberkochen, Germany) was used, and images were recorded with an AxioCam MRc5 digital camera. The constituent algae, cyanobacteria and their extracellular polymeric substances (EPS) were identified through direct observation of fresh, cultured and preserved samples, based on specialized literature (Geitler 1932; Ettl and Gärtner 1995; Komárek and Anagnostidis 1999).

CLSM

Samples for CLSM were observed either live or fixed (with 3% paraformaldehyde in 0.1 M PBS buffer and 60 mM sucrose). Images were captured with a Leica TCS-SP5 AOBS CLSM (Leica Microsystems Heidelberg GmbH, Mannheim, Germany) using Plan-Apochromatic 40 × (NA 1.25, oil) and 63 × (NA 1.4, oil) objectives. The biofilms were observed with multi-channel detection. Con A-Alexa 488 (0.8 mM)

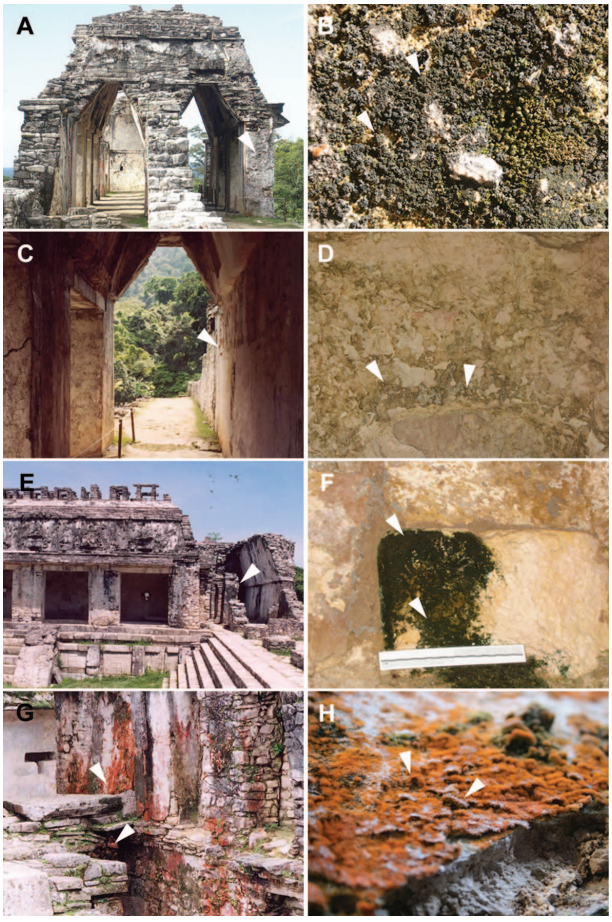

Figure 2. Detail of the sampling sites. (A) Sampling site I. General view of an exposed pilaster. (B) Detail of the black patchy biofilm in I. (C) Sampling site II. Ornamented stucco protected from both strong light and direct rainfall. (D) Detail of the green flakes in II. (E) Sampling site III. Partly protected niche, with persistent moisture. (F) Detail of the patchy black crust in III. (G) Sampling site IV. General view of the partly exposed mortar. (H) Detail of the orange patchy biofilm in IV.

targets EPS and was recorded in the green channel (excitation, 488 nm; emission, 495–540 nm).

The autofluorescence of photosynthetic pigments in different regions of the visible spectrum was observed. The color differentiation stems from the specific pigment composition of cyanobacteria and green algae. Chlorophylls in the photosynthetic microorganisms (algae and cyanobacteria) autofluoresce at 670–790 nm (excitation, 594 nm) and were recorded in the blue channel. Phycobilins in cyanobacteria autofluoresce at 570–615 nm (excitation, 561 nm) and were registered in the red channel. Some cyanobacteria appear magenta because they were visualized in both the blue and red channels.

The fluorescence of chlorophylls and phycobiliprotein pigments was assessed to trace the internal distribution of microalgae and cyanobacteria inside the biofilm and substratum. The presence or absence of pigment fluorescence inside sheaths was used as the basis for discriminating between live and dead cells, respectively, and therefore was a direct method for monitoring the temporal dynamic in field material. The fluorescence intensity observed by CLSM indicates the relative activity of pigments (either chlorophylls or phycobilins) and therefore, for a given morphospecies, enabled comparison and discrimination of different physiological states for images captured under equivalent conditions. Physiological changes are often accompanied by changes in morphology, internal structure of cells (Roldán et al. 2006), different phases of the life cycle, and sheaths or external deposits. Samples in which most of the cells were dividing, and whose cells had compact cytoplasm (eukaryotic cells) or centroplasm (prokaryotic cells) with small or no inclusions, were considered to be in the exponential growth phase. In contrast, samples in which only a few cells were dividing, and whose cells had large inclusions related to cell shape, were considered either to grow slowly or to be in a stationary phase. Dry-season biofilms were characterized as having empty sheaths and some colorless or collapsed cells (Figure 5). Samples observed by CLSM were subsequently broken, and then studied by LM. The results were contrasted with those from previous samples.

The substrata and other solid inorganic material were visualized by reflection and were recorded in the grey channel (excitation, 488 nm; emission, 480–495 nm). Three-dimensional data were collected from XY images every 0.5 μm in Z (depth), with a 1 Airy unit confocal pinhole. To characterize the biofilms, different projections (Roldán et al. 2004) were generated from the XYZ series using Imaris v. 6.1.0 software (Bitplane, Zürich, Switzerland).

SEM-BSE and EDX

Samples for SEM were fixed in 0.2% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer, dehydrated in a gradient of ethanol, critical-point dried, and then sputter-coated with carbon. They were examined by SEM-BSE and by EDX (Quanta 200, FEI + EDAX). Selected samples were subsequently sputter-coated with gold, which improved the photographs for assessing the relationships among species and between species and substratum.

X-ray diffraction

Stone samples scratched off from underneath the biolfims on El Palacio walls were analyzed using an X'Pert PRO MPD AlphaI PAN analytical geometric Bragg-Brentano diffractometer (Almelo, The

Netherlands) (radius = 240 mm, Cu radiation). The crystallographic structure and chemical composition were determined.

Results

Environmental conditions and substrata

The climatic conditions of the sampling sites vary by season and are characteristic of a tropical rainforest climate (http://smn.cna.gob.mx/productos/emas/#). The conditions for each biofilm at the sampling time are shown in Table 1. The temperature at the biofilm surface ranged from 25 to 31°C. The decrease in humidity during the dry season was also confirmed by measurements taken at the biofilm surfaces. The El Palacio substratum is complex, containing different morphotypes of calcium carbonate characterized by pure calcite, dolomite, small amounts of aragonite, and traces of quartz. Several building elements (eg mortar, stucco and limestone) were found in the samples examined. Those substrata would comprise the same primary materials (lime and sand), although the elemental distribution in each site varied slightly as a function of local levels of magnesium, silicates, and aluminum (Figure 3).

Organisms and biofilms

At the sampling sites the photosynthetic microorganisms formed patches that were light green to orange-green or blackish. Examples of building colonization are shown in Figure 2. Cyanobacteria comprised the largest proportion of the photosynthetic community, except for the green alga *Trentepohlia aurea* (Linneaus) Martius. The cyanobacteria and algae identified by LM and SEM are summarized in Table 2 in the supplementary material [Supplementary material is available *via* a multimedia link on the online article webpage].

Table 1. Particular conditions for each biofilm.

Sampling site	Material	BT (°C)	BRH (%)
I	Limestone and mortar	(w) 30	100
		(d) 25	18
II	Stucco	(w) 31	81
		(d) 25	17
III	Stucco and limestone	(w) 30	89
		(d) 27	65
IV	Mortar	(w) 30	96
		(d) 27	20

w, wet season, 06.08.2007; d, dry season, 14.01.2007; BT, biofilm temperature; BRH, biofilm relative humidity.

Sampling site I (exposed to light; alternately wet and dry)

The sample was taken from a southwest-facing exterior wall. The substratum was a porous, heterogeneous repaired mortar that contained limestone as well as non-lime materials such as sand. It has been heavily restored and showed formation of shrinkage cracks (Table 1, Figures 2C,D and 4A).

Wet season

The biofilm comprised two layers: an external crust formed of abundance of filaments of *Scytonema guyanense* (Mont.) Bornet et Flahaut (Table 2, supplementary material [Supplementary material is available *via* a multimedia link on the online article webpage] and Figure 4A and B) and a few mosses, and a lower layer chiefly comprising coccoid cyanobacteria, dead cells, and inorganic material (Figure 5A). In August 2007, 2 months after the start of the wet season, heavy rain and strong runoff cleaned part of the dry aerial biofilm. Abundant hormogonia of *S. guyanense* started to develop, mixed with the remains of the previous dry season. The aerial biofilm was shiny and dark green due to its hydrated sheaths, which remained expanded throughout the wet season. The surface of this biofilm was easily removed from the base, which remained attached to the substratum. At the base, under the canopy of filamentous forms, CLSM revealed colonies of the coccoid cyanobacteria *Gloeocapsa calcicola* Gardner and *Gloeocapsa quaternata* Kützing (Table 2, supplementary material [Supplementary material is available *via* a multimedia link on the online article webpage]), sheath remnants, and

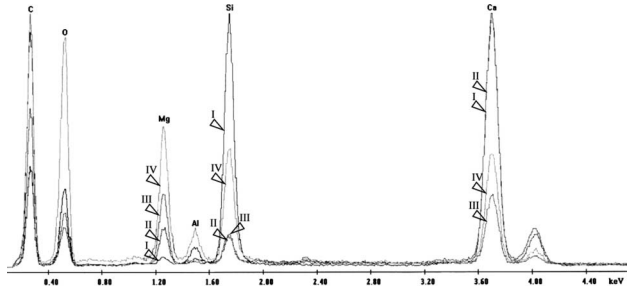

Figure 3. Overlap of individual EDX analyses from the four sampling sites at El Palacio. Note the same elements in different proportions. Sampling site I showed the highest concentration of silicon. Sites II and III still retained some of the original stucco and the substratum material primarily contained calcium carbonate and small amounts of magnesium and silicon. Sampling site IV, with the highest magnesium concentration, was on calcite dolomite rock, representing the original building material.

cells that had little fluorescence mixed with strongly fluorescent colonies of *Asterocapsa divina* Komárek and thin *Leptolyngbya* cf. *compacta* (Kützing ex Hansgirg) Komárek et Anagnostidis filaments (Table 2, supplementary material [Supplementary material is available *via* a multimedia link on the online article webpage]). SEM-BSE showed inorganic material attached to the soft base of the biofilm, which appeared to be limestone with irregular "rice-grains" or crystals of calcite, and a few wart-like colonies of *A. divina*.

Dry season

At the beginning of the dry season the sheaths of *S. guyanense* dried out and shrank, carrying away small substratum particles with them. Under shelter of the remains, inside the desiccated sheaths, some filaments of *S. guyanense* produced hormogonia. The protected biofilm base comprised remains and colonies of fluorescent *A. divina* (Figure 4C), which had thin, colorless envelopes (Table 2, supplementary material [Supplementary material is available *via* a multimedia link on the online article webpage]); *Gloeocapsa* spp.; other coccoid cyanobacteria; and the filamentous *L.* cf. *compacta*, which was also abundant and fluorescent. The base varied little between the start of the wet and dry seasons, except for the mucilage covering the coccoid cyanobacteria, which was soft and watery at the beginning of the dry season (only 2 months after the last rainfall). Throughout this season, more inorganic material and dead cells were observed, although there was little difference in the total thickness of the fluorescent portion of the base (data not shown). Fluorescence from chlorophylls and from phycobiliproteins was detected inside the substratum at depths up to approximately 50 μm. This depth was considered the maximum at which the phototrophs were alive.

Sampling site II (protected from both strong light and direct rainfall)

The sample was collected from a southeast-facing interior wall with bas-reliefs, close to Site I (Figure 2C and D). The substratum was stucco with a high calcium matrix, and characterized by its strength and hard surface (Table 1), although some laminations were observed.

Wet season

Irregular pale green stains formed mainly of *G. calcicola* were distributed on and in the substratum (Figure 5B). Chlorophyll and phycobiliproteins were present.

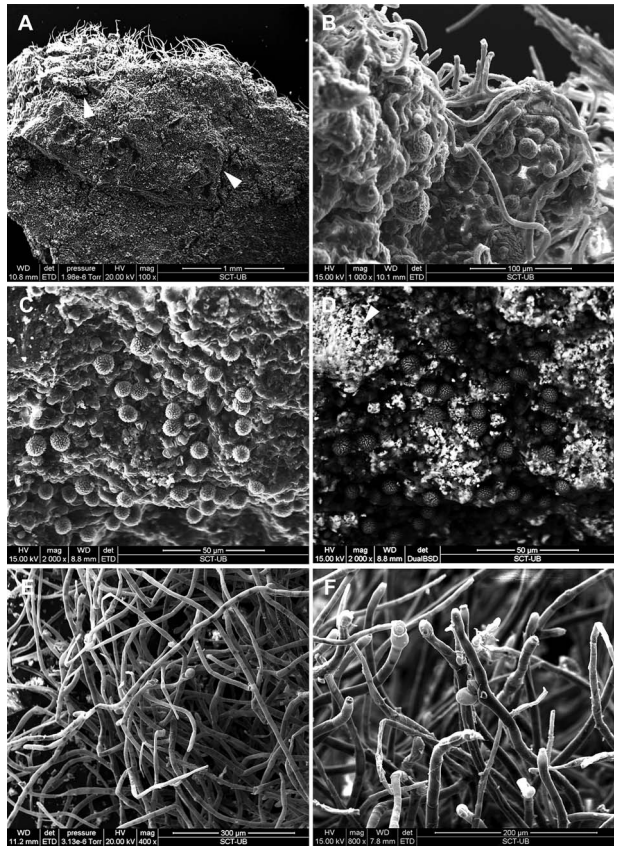

Figure 4. SEM-BSE images. (A) Low magnification overview of biofilms colonizing the surface of the sampling site I in the wet season. White arrows show shrinkage cracks. (B) Sampling site I showing filamentous *S. guyanense* and coccoid cyanobacteria in the dry season. (C) *A. divina* surface community at sampling site III, the cells were covered by wart-like ornamentation in the dry season. (D) BSE image of C showing *A. divina* with entrapped inorganic granules (in white). (E) Creeping filaments of *T. aurea* at the sampling site IV in the wet season. (F) Detail of *T. aurea* in E showing apical sporangia in the dry season.

Dry season

At the beginning of the dry season, stucco flakes or dust particles regularly fell off the carved decoration. Viable fluorescent cells were observed at depths up to nearly 100 μm. Fluorescent spots were all established just inside the substratum and were fewer in number and weaker in intensity than those present during the wet season. Coccoid cyanobacteria were not assembled in continuous layers, and it was not clear to what extent they contributed to the observed spalling.

Sampling site III (partially exposed to light, with persistent moisture)

The sample was obtained from a northeast-facing protected niche that did not receive direct insolation

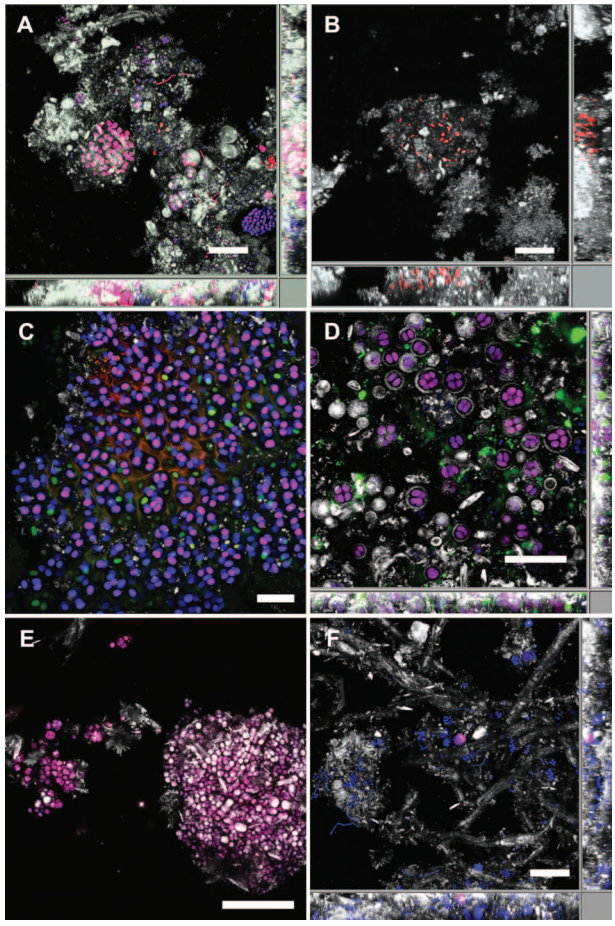

Figure 5. CLSM images from phototrophic biofilms. Color allocation: white = reflection from minerals; blue = autofluorescence of chlorophylls; red = autofluorescence of phycobilins; magenta = colocalized autofluorescence of cyanobacteria in the red (phycobilins) and blue channels (chlorophylls); green = extracellular polymeric substances dyed with Con A-Alexa 488. Scale bar = 50 μm. (A) A few filaments of *S. guyanense* and a basal layer formed by coccal cyanobacteria, a mass of dead cells and inorganic materials, in the dry season. Thickness biofilm = 42 μm. (B) *G. calcicola* distributed on and inside the substratum in the wet season. The low autofluorescence of chlorophyll (blue) is related to unhealthy cells. Biofilm thickness = 64.5 μm. (C) *A. divina* in the wet season with mucilaginous envelopes. Biofilm thickness = 62.3 μm. (D) *A. divina* biofilm attached to the substrata in the dry season. Celled groups covered with wart-like ornamentation and calcium carbonate. Biofilm thickness = 21.8 μm. (E) Several stages of *A. divina* at the beginning of the dry season. Biofilm thickness = 51 μm. (F) Filaments of *T. aurea* with low autofluorescence and desiccated sheaths in the upper part of the biofilm. Coccoid and colonial cyanobacteria at the bottom layer of the biofilm in the dry season. Biofilm thickness = 38.5 μm.

(Figure 2E and F). The substratum appeared to be a mixture of stucco remains and limestone with a fine texture; the site receives water from the back and retains humidity throughout most of the dry season (Table 1).

Wet season

Small patches of colonized stucco were examined by EDX: the material primarily contained calcium carbonate with small amounts of magnesium and silicate (Figure 3). In August (at the beginning of the wet season) the area was covered by a continuous, thin, compact dark-green layer, formed by *A. divina* growing as a firmly surface-bound layer. Various stages of *A. divina* coexisted simultaneously: some had small, abundant cells with little EPS (status nanocytosus) (Komárek 1993); some had large cells with abundant soft sheaths (Figure 5C); and, finally, others consisted of masses with little fluorescence, mixed with calcified sheaths and inorganic remains. Short filaments of *S. guyanense*, colonial cyanobacteria, *L.* cf. *compacta, Schizothrix* sp. and *T. aurea* were present, but scarcely developed. The mucilaginous biofilm was easily removed from the substratum, with no concomitant detachment of substratum particles.

Dry season

The layer formed by *A. divina* was lighter and less shiny than in the wet season, despite the fact that the inner walls had remained damp for extended periods of time. The colonies formed a thin biofilm tightly attached to the substrata in which CLSM revealed groups containing few cells that were enveloped by firm sheaths of mucilage (Figure 5D), sometimes mixed with calcium carbonate, and covered by wart-like ornamentation (Figure 4C and D). The living fluorescent colonies were hidden inside the substratum at a depth of approximately 50 μm, surrounded by clumps of calcite needles and other small, irregular inorganic substances (Figure 5E). When the biofilm was scratched off, it carried along particles of the substratum.

Sampling site IV (partially exposed to light; alternately wet and dry)

The sampling site was protected by lateral walls, but lacked a roof. The mortar was porous and reflected restorations covering the original dolomite containing calcium and magnesium carbonates (Table 1).

Wet season

The biofilm produced extensive, orange, felt-like patches on mortar or calcareous rock surfaces in the internal, southwest-facing and partially protected

lower walls (Figure 2G and H). The upper external layer comprised entangled filaments formed by elongated cylindrical *T. aurea* cells (Figure 4E). The lower layer comprised *T. aurea*, colonies of *Nostoc commune* Vaucher ex Bornet et Flahaut and other coccoid and colonial cyanobacteria (Table 2, supplementary material [Supplementary material is available *via* a multimedia link on the online article webpage]). In contrast, the lower cells were short and green (Table 2, supplementary material [Supplementary material is available *via* a multimedia link on the online article webpage]). *T. aurea* at the lower part had developed round masses as well as short, irregularly-branched creeping filaments, in which sporangia and swarmers were being produced (Figure 4F). Heavy rains at the beginning of the wet season washed away the desiccated filaments that had remained from the dry season, while the lower layer became soft, gelatinous, and embedded in the stone cracks.

Dry season

In January, at the beginning of the dry season, the outer filaments of *T. aurea* had grayish desiccated sheaths whose cells lacked fluorescence (Figure 5F) and which, by LM, appeared hyaline or empty. However, under the canopy, it formed gametangia or sporangia (Figure 4F), in creeping or erect filaments, respectively. Remains of organic matter, and fragments of calcareous material, were intermixed with fluorescent multicellular aggregates of *A. divina, Gloeocapsa* spp., and other cyanobacteria (Table 2, supplementary material [Supplementary material is available *via* a multimedia link on the online article webpage]). The prostrate cells of *T. aurea* penetrated this soft base and adhered to the rock surface; as such, removal entailed damaging the substratum and dragging along small inorganic fragments. The filaments that penetrated the substratum maintained their pigment fluorescence up to a depth of approximately 30 μm (Figure 5F). Lastly, SEM and EDX of the underside of 1 mm flakes did not reveal any cyanobacteria or *T. aurea*.

The humidity at the biofilm surfaces varied considerably between the wet and dry seasons, except in Site III, which was protected. During the dry season, in which conditions were unfavorable, phototrophic microorganisms were hidden under the remains from the previous wet season and were protected by their respective resilience strategies (Potts 1999). During the wet season the dominant species grew quickly and developed structured biofilms that differed in thickness and coverage and that were generally stratified. The lower portions of the biofilms contained coccoid and colonial cyanobacteria, whereas the upper portions encompassed different taxa (Figure 6): filamentous cyanobacteria such as *S. guyanense* (A), coccoid cyanobacteria (B), and *A. divina* (C), or the green alga *T. aurea* (D).

Discussion

The biofilms that develop on the stone, stucco and mortar of El Palacio walls not only detract from aesthetics, but can also compromise conservation efforts. Strong seasonal changes in rainfall and relative humidity define a strict growth sequence and determine the shape and composition of the biofilms. Other factors that are important in the development of organisms and of biofilms include bioreceptivity and the relative orientation of each site to the sun.

All the biofilms contained the same species, although the relative amounts of these differed by site and by season. In Sites I and IV, which were exposed to heavy rains in the wet season, the filamentous *S. guyanense* and *T. aurea* dominated. However, in Sites II and III, which were relatively protected from the elements, colonial cyanobacteria, mainly *A. divina*, dominated and produced a mucilaginous

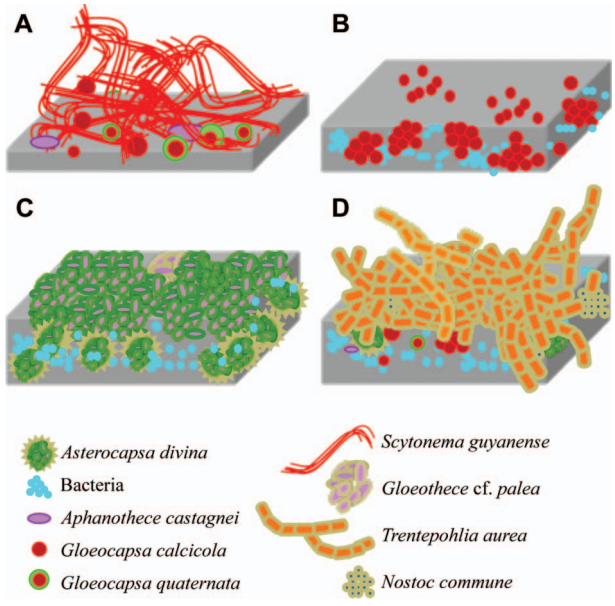

Figure 6. Schematic representation of biofilm structure, main species and distribution at each sampling site at El Palacio. (A) Sampling site I. Biofilm formed by *S. guyanense, Leptolyngbya* sp., *Chroococcus* sp., *Aphanothece castagnei, Gloeothece palea, G. quaternata*. (B) Sampling site II. Biofilm formed by coccal cyanobacteria; *G. calcicola, G. quaternata* and *Chroococcus* sp. (C) Sampling site III. Biofilm mainly formed by *A. divina* and some coccal cyanobacteria. (D) Sampling site IV. *T. aurea*, intermixed with aggregates of *A. divina, G. calcicola, G. quaternata* and the filamentous *S. guyanense* and *Nostoc commune*.

film associated with inorganic particles. These three species are tolerant to desiccation and also contribute strongly to biofilm growth in habitats similar to the Palenque site. However, their strategies to overcome the dry season appear to be different (Büdel 1999; Rindi and Guiry 2002).

A. divina, described from Mexico (Komárek 1993) and reported from caves (Aboal et al. 2003), presented two morphological stages in its life cycle (Komárek 1993) related to micro-environmental conditions (Montejano et al. 2008). A stage characterized by solitary cells or small colonies that divided generally by regular binary fission was present at all sites throughout the year. However, the other stage, in which cells in colonies undergo multiple fission, marked by a quick increase in cell number (Komárek and Anagnostidis 1999; Šmarda and Hindák 2006), was only recorded at Site II at the beginning of the wet season. The best local growth conditions for *A. divina* comprised the calcareous substratum and protective conditions at Site III, plus the residual moisture during the dry season. Moreover, thick walled stages facilitated survival in the dried state, due to the carbohydrate- and calcite-enriched sheaths. These resistant forms were less fluorescent and therefore, were deemed inactive (Grilli-Caiola et al. 1996; Häubner et al. 2006). Signs of decay in the *A. divina* samples observed by CLSM, namely, inorganic spots mixed with living cells and EPS, appeared to be associated with desiccation-rewetting cycles (Beech et al. 2005).

S. guyanense has been reported as being cosmopolitan and well adapted to tropical climates and strong light (Sant'Anna 1988; Büdel 1999; Novelo and Ramírez 2006). In Sampling Site I at El Palacio, it showed a defined succession pattern, reflected by strong variations in coverage. Extensive growth during the first heavy rains produced blackish, mucilaginous biofilms, which fell off easily when completely hydrated. The hot and dry conditions of the dry season were probably lethal for surface communities of *S. guyanense*, which lost fluorescence and eventually dried out. Once dry, the biofilm adhered tightly to the rock and was difficult to remove. Only hormogonia and short filaments maintained fluorescence; these remained viable and ultimately recovered quickly at the beginning of the wet season. These had been sheltered from intense solar radiation and desiccation by their own remains or were mixed with the lower mucilaginous communities, which retained humidity (Wynn-Williams 2000). Furthermore, the porous mortar surface would have facilitated water circulation, and consequently, their rapid development. The population reappeared at the same location and with the same appearance every year (Novelo and Ramirez 2006).

T. aurea, like other species of this genus, is considered to have high tolerance and adaptability to severe conditions such as strong light (Abe et al. 1998) or long, dry periods, after which it rapidly reabsorbs water (Howland 1929). At El Palacio, *T. aurea* grew on stable rock and cement, comprising the upper layer located in the cyanobacterial lower cover, although it only slightly penetrated the rock substratum. In western Ireland *T. aurea* grows on cement (Rindi and Guiry 2004), and in India, on rock surfaces and tree bark (Gupta and Agrawal 2004). Moreover, it has previously been identified on limestone Mayan monuments (Novelo and Ramírez 2006). *T. aurea* grown in culture develops reproductive structures only after a drastic change in culture conditions (Rindi and Guiry 2004). The same behavior was observed in the field: the alga formed sporangia and gametangia according to the seasonal changes in the subaerial environment studied. Cells in these life cycle stages were protected by remains from the previous season, which helped *T. aurea* overcome the dry season.

The stratification of biofilms is dictated by several factors, including the quantity of light. The stress of low light reduces biofilm stratification, as well as thickness and species diversity (Albertano and Kovacik 1996; Hernández-Mariné et al. 2001); consequently, only certain species can survive under these conditions (Horat et al. 2006; Walker and Pace 2007). Unicellular cyanobacteria that are rich in the accessory pigment phycoerythrin (the magenta in Figure 5) are able to colonize shaded environments. The commonality of this pattern in Sites II and III, and at the lower of biofilms in Sites I and IV, could be rationalized by the fact that excess of light is one of the limiting factors at these locations. However, other factors define the tops of biofilms. Species diversity is higher for the surfaces that receive less sunlight (whether due to shading or orientation) and remain wet for a long time (Barberousse et al. 2006; Häubner et al. 2006). As reflected by their clear stratification, Sites I and IV, which had the greatest moisture levels in the wet season, had more species than Sites II and III. These results are comparable with those of extreme habitats ranging from Antarctic communities (Büdel et al. 2008) to deserts (Garcia-Pichel and Belnap 1996; Wynn-Williams 2000). In all cases the survival rate of each species, and the extent to which they enabled other, more vulnerable species to grow in the same biofilm, depends on their particular strategies for adapting to environmental changes and to their relative position in the biofilm. Generation of protective pigments by dark sheathed filamentous cyanobacteria allows them to be preponderant over algae in Latin America (Crispim et al. 2003). In the case of El Palacio, persistence of the photosynthetic

microorganisms at the biofilm base apparently depended on their ability to tolerate an annual desiccation period whereas the upper portion depended on the rate of growth when favorable conditions were established.

The spatial patterns of photosynthetic biofilms on the walls of El Palacio mainly derive from abiotic factors, chiefly, light, humidity and bioreceptivity, although the microorganisms can alter their environment and create an additional level of structural heterogeneity. Nevertheless, the influence of environmental conditions on the location of these microorganisms inside biofilms is difficult to gauge, as is the role played by the microorganisms, which, by changing the surrounding conditions, enable other microorganisms to become established in specific positions inside the biofilm (Kumar and Kumar 1999). Substratum bioreceptivity (Guillette 1995) may have influenced biofilm development at Sites I and IV, whose high mortar porosity would facilitate growth (Crispim et al. 2003). Indeed, at these sites, *S. guyanense* and *T. aurea* formed massive biofilms and exhibited more morphospecies (six each) than did the less porous Sites II and III (four and five, respectively).

The consequences of photosynthetic biofilms on biodeterioration and bioprotection are difficult to ascertain. Biofilms can be both detrimental and beneficial, depending on the substratum and microorganisms involved (Hoppert et al. 2004). Thin superficial biofilms can cause discoloration of stone surfaces (Hernández-Mariné et al. 2003) and mechanical and biochemical deterioration (Ortega-Calvo et al. 1991; Kumar and Kumar 1999), or favor the acceleration of weathering processes (Warscheid and Braams 2000) as observed in Sites II and III of El Palacio. Negative effects reported for *Trentepohlia* spp. include pitting (Gaylarde et al. 2006) and progressive mechanical degradation of buildings (Wakefield et al 1996; Noguerol-Seoane and Rifón-Lastra 1997). In contrast, *S. guyanense* protected the rock surface at Site I from direct solar exposure and, to a certain extent, reduced erosion from rain and wind and prevented the development of other organisms. This bioprotection could be related to the shield provided by the dark color of the sheaths. Indeed, no endolithic layers were observed under their canopies. This effect has already been reported in lichens (Ariño et al. 1995; Hoppert et al. 2004).

Further work on how biofilms induce biodegradation or bioprotection of their respective substrata will enable the development of maintenance and conservation protocols for cultural heritage sites.

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