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Succession of fungi colonizing porous and compact limestone exposed to subtropical environments

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ABSTRACT

Little is known about the dynamics of succession of fungi on limestone exposed in subtropical environments. In this study, the colonization of experimental blocks of compact and porous limestone by a fungal community derived from natural biofilms occurring on Structure X from the archaeological site of Becán (México), was studied using a cultivation-dependent approach after short-term (9 m) exposure in order to provide a preliminary insight of the colonization process under seminatural conditions. Microbial growth seen as the change of colour of stone surfaces to black/dark green was more abundant on the porous limestone. There was a fairly clear difference in microbial colonization between the onset of the experiment and the 6th month for both limestone types, but no significant increase in the colonization of coupons occurred between months 6 and 9. This could be related to the low rainfall expected for this period, corresponding to the dry season. A total of 977 isolates were obtained. From these, 138 sterile fungi were unidentified, 380 could only be assigned to the order *Sphaeropsidales*; the remaining isolates (459) were grouped into 27 genera and 99 different species. Nearly all detected fungal species belonged to the *Ascomycota* (90 %). Rare taxa (species represented by one to three isolates) included the recently described genus *Elasticomyces*, several species of genera *Hyalodendron*, *Monodyctis*, *Papulospora*, *Curvularia*, and *Septoria*. Other taxa were *Minimedusa* and *Gliomastix luzulae*, which have not been previously described for stone environments. Abundant fungi included several species of the common genera *Cladosporium*, *Alternaria*, and *Taeniolella* typical for a range of habitats. Succession of populations was observed for certain taxa, this shift in the composition of fungal communities was more evident in porous limestone. After 6 m of exposure, species of the genera *Scolecobasidium*, *Hyalodendron*, and *Taeniolella* were predominant, while after 9 m, the predominant species belonged to the genera *Curvularia* and *Alternaria*, particularly on porous stone. These results suggest that *Curvularia* and *Alternaria* replaced other fungi, due to a higher tolerance towards low levels of available water during the dry season. Higher levels of water within the porous stone, keep longer periods

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of microbial activity, minimizing the impact of desiccation. This study contributes to understand the diversity of fungal communities in stone surfaces in subtropical settings and the dynamics of colonization on limestone.

Introduction

The composition and distribution of microorganisms colonizing stone monuments depends on environmental conditions, building architecture, and orientation, along with substrate properties (Scheerer *et al.* 2009). The susceptibility of substrata to biological colonization, known as bioreceptivity, is determined by surface roughness, porosity, and mineralogical composition (Guillitte 1995; Miller *et al.* 2010). Porosity is perhaps the most influential property among substratum features. In general, highly porous substrata support higher levels of microbial biomass, presumably by holding more water within the pore system to allow sustained microbial growth (Ortega-Morales *et al.* 1999; Miller *et al.* 2006). This higher water-holding capacity of porous stone may also influence microbial community structure, however, this has been the object of fewer studies (Miller *et al.* 2006, 2010).

A diverse array of microorganisms colonizes limestone monuments and includes phototrophs (cyanobacteria and microalgae) and heterotrophs (lichens, fungi, bacteria, and protozoa) (Mansch & Bock 1998; Burford *et al.* 2003; Ortega-Morales 2006); of these, phototrophs are often the biomass-dominating organisms (Gaylarde *et al.* 2007). In general, microbial colonization initiates with the colonization of stone by phototrophs, which provide organic matter to sustain heterotrophic growth, through heterotrophic metabolism, bacteria, and fungi then alter stone. Fungi are generally regarded as more active in stone weathering by a combined chemical and physical mechanisms (Sterflinger 2000; Burford *et al.* 2003; Gadd 2007). Under extreme degradation stages, the physical matrix of highly weathered stone substrata is further disaggregated by fungal hyphae producing a protosoil (Sterflinger 2000; Miller *et al.* 2006). The weathering potential displayed by fungi is generally explained in terms of their abundance and the occurrence of specific taxa (Sterflinger 2000; Sterflinger & Prillinger 2001; Gadd 2007).

Most studies undertaken to assess fungal colonization of building stone have been conducted in temperate settings (Sterflinger & Prillinger 2001; Gorbushina *et al.* 2002; Moroni & Pitzurra 2008). Fewer studies have been carried out to characterize colonization in tropical environments (Gaylarde *et al.* 2007). In addition, the temporal variation of the microbial colonizers is seldom considered. In this study, we investigated the influence of season and porosity of two contrasting limestone types on fungal composition during short-term colonization experiments.

Materials and methods

Limestone types

The Península de Yucatán is a plain with only a few elevations above sea level, which are constituted by calcareous rocks belonging to the Cenozoic. The Mayan people used this type of rock to build monuments (Espinosa *et al.* 1996; Ortega-Morales *et al.* 1999). Two limestone types were used in this work, one was obtained from a quarry from which materials are derived for restoration of the archaeological site of Chichén-Itzá (compact stone), while the second limestone (porous) is used in modern finishing and decoration in the local building industry.

Both limestone types were characterized by petrographic and geotechnical studies following standard procedures (ASTM D2216-98 2007). The compact limestone constituted by microfossils and fragments thereof (foraminifera and ooids) with little micrite matrix, most have microfossils from micritic, on the edge and oxides and hydroxides of Fe, which can also be seen associated to the matrix. While porous limestone consisting of bioclasts (fragments of bivalves, bryozoans, ostracods, and molluscs), most of their structures formed by micritic calcite and few sparite. The physical properties of both limestone types are summarized in Table 1.

The stone samples were cut into coupons 4 × 4 × 1.5 cm employing a low-speed diamond saw. The coupon surfaces were left unpolished in order to increase surface roughness and thus facilitate microbial colonization (Guillitte 1995). To reduce the microbial burden on the types of limestone, coupons were sterilized using UV light exposure for 48 h, after autoclaving at 121 °C for 6 h and drying in an oven at 115 °C for 24 h (Miller *et al.* 2008).

Preparation of inoculum

Samples of natural black biofilm were taken from Becán, a Mayan archaeological site (18°31′00″N, 89°20′00″W). This type of

Table 1 – Average values and standard deviation (±) of absorption by capillarity and open porosity of the studied limestone types.

Physical properties	Compact limestone	Porous limestone	NORM
Water absorption (%)	0.53 ± 0.20	6.47 ± 2.1	UNE-EN 13755:2001, NORMAL 7/81
Effective porosity (%)	1.22 ± 0.64	12.61 ± 3.77	ISRM 1981, TSE 1987