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Fungal colonization and succession on newly painted buildings and the effect of biocide

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Abstract

This report describes the sequence of fungal colonization and the influence of biocide incorporation on paint films, determined using quantitative methods. Two buildings were painted with an acrylic paint, with and without an experimental biocide formulation containing a carbamate (carbendazin), *N*-octyl-2H-isothiazolin-3-one and *N*-(3,4-dichlorophenyl)*N*,*N*-dimethyl urea (total biocide concentration 0.25% w/w). One week after painting, the major groups of organisms detected were yeasts and *Cladosporium*. The yeast population fell to undetectable levels after the third week and this microbial group was not detected again until the 31st week, after which they increased to high levels on the 42nd week. *Aureobasidium* showed a pattern similar to the yeasts. The main fungal genera detected over the 42-week period were *Alternaria, Curvularia, Epicoccum, Helminthosporium*, Coelomycetes (mainly *Pestalotia*/*Pestalotiopsis*), *Monascus, Nigrospora, Aureobasidium* and *Cladosporium*. The latter was the main fungal genus detected at all times. The physiological factors controlling colonization are discussed. *Cladosporium, Aureobasidium, Tripospermum* and yeasts on the painted surfaces were all able to grow on mineral salts agar containing 10% sodium chloride. This is the first time that the genus *Tripospermum* has been reported on painted buildings. The fungal population on biocide-containing surfaces was significantly lower than on non-biocide-containing paint after 13 weeks and continued so to 42 weeks after painting, but there was no statistically significant difference in the level of fungal biodiversity.

Keywords: Biocide; Fungal colonization; Osmolyte; Paint film; Salt-tolerance; *Aureobasidium*; *Cladosporium*

1. Introduction

The surface of painted walls supports a very diverse microbial flora; bacteria (including actinomycetes and cyanobacteria), algae (Bacillariophyceae, Xanthophyceae, Chlorophyceae, Dinophyceae and Rhodophyceae), animals (protozoa, rotifers and nematodes) and fungi (slime-molds, chytrids, hyphochytrids, oomycetes, zygomycetes and dikaryomycetes) [1]. This microbial colonization of painted buildings causes aesthetic problems and can lead to degradation and spalling of the coating. The literature suggests that phototrophic microorganisms are the primary colonizers [2]. There is at least one publication proposing bacteria for this role [3], but the importance of phototrophs was not considered. Fungi are, however, generally considered to be the major deteriogens of painted surfaces [4,5]. Statistical analysis of data gathered from painted buildings in Latin America suggests that algae precede cyanobacteria in the colonization sequence [6]. More practical evidence comes from the results of a biocide trial. A copper-containing biocide was able to retard discoloration of a painted wall for 6 years and was shown to reduce the bacterial and fungal biomass, as well as the algal biomass substantially [7]. Since copper at the concentration used acts mainly against algae, this suggests that primary production by phototrophs is required for the sustained growth of heterotrophic organisms. Fungal di-

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versity in the above situation was high even though the biomass remained small [7].

Apart from restricted studies some years ago [3,8], the colonization sequence of microorganisms on painted surfaces has not been empirically established. This study aims to establish the natural colonization sequence of phototrophs and fungi on newly painted surfaces, with and without a broad-spectrum biocide formulation, by experimental observation. Since the numbers of phototrophs detected to date are insufficient to describe the colonization sequence accurately, these data will be presented in a subsequent paper.

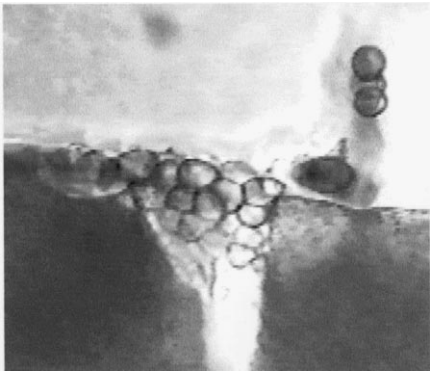

Fig. 2. *Aureobasidium* growing within a crack on deteriorating plastic, seen after 2h rehydration of tape strip on MKM.

2. Materials and methods

Two buildings, **1** and **2**, on the campus of the University of São Paulo (USP), in the South-East of Brazil (23°33′S; 46°44′W), were used in this study. One had been painted many years ago, was surrounded by trees and had a heavy black discoloration **1**; the second had been painted more recently, showed little obvious biofilm and had few trees in the surrounding area **2**. The external walls of the buildings were prepared for painting. They were treated with hypochlorite (approximately 2%) for 15 min and then washed with a high-pressure (11 MNm^{-2}) water jet. Samples were taken for microbiological analysis before and after cleaning and fungi and phototrophs identified.

The walls were then professionally painted with a white acrylic paint with an experimental film biocide formulation containing carbendazin, *N*-octyl-2H-isothiazolin-3-one and *N*-(3,4-dichlorophenyl)*N*,*N*-dimethyl urea (total biocide concentration 0.25% w/w). Control paint contained no film biocide. The biocide-containing and control paints were applied in alternating vertical strips to randomize the effects of local environmental factors, such as windows, which modify the run-off of water. Four samples were taken at approximately 5 m above ground level from each biocide-containing and non-biocide-containing site on each of the buildings at intervals (see Section 3). Sampling was by the adhesive tape method [9] and by the carpet replica technique for fungi [10]. Carpet samples were cultured onto Sabouraud dextrose agar and fungi identified by cell and colony morphology [11,12]. Tape strip samples taken for phycological analysis were incubated on solid modified Knop's medium (MKM) and phototrophs identified as described in Gaylarde and Gaylarde [1] after up to 3 months incubation. The latter sampling and culture technique was also used, with up to 2 weeks incubation, to identify and count fungi directly on the plate by transmitted light microscopy with objectives up to ×25. The tape used was unbranded plastic tape; standard cellulose products (Sellotape, Scotch Tape, Durex brands) are unsuitable, as they are rapidly degraded by cellulose utilizing fungi. 3M produces a suitable product, but there are substantial variations in the formulation of the adhesive, and in some batches the adhesive takes up water and becomes unsuitable for microscopy. The tape used is tested prior to use and we find that cheap brands (10 small rolls for approximately 1$ US in Brazil) retain good optical characteristics for up to 5 months incubation on agar. Figs. 1, 2 and 5 show oil immersion photomicrographs taken directly through a tape sample in the present study after 2 h incubation on agar. Samples were placed on sheets of glossy paper from self-adhesive labels after cleaning with ethanol and were sent by post from São

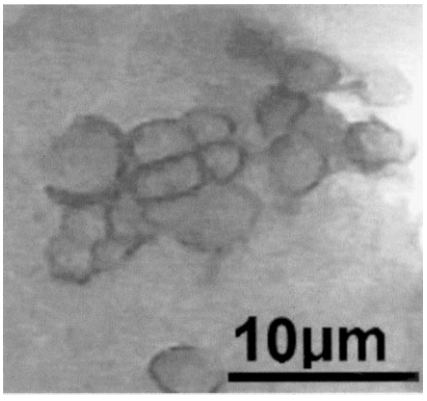

Fig. 1. *Aureobasidium* microcolony, seen after 2 h rehydration of tape strip on MKM.

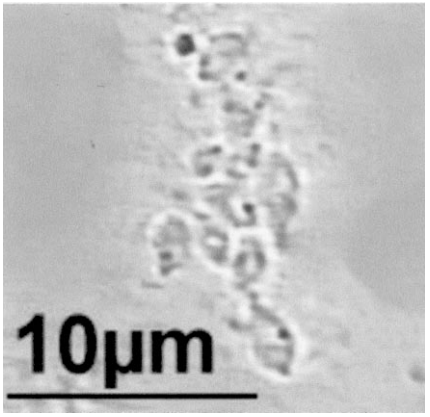

Fig. 3. *Aureobasidium* oidial form in culture on MKM.

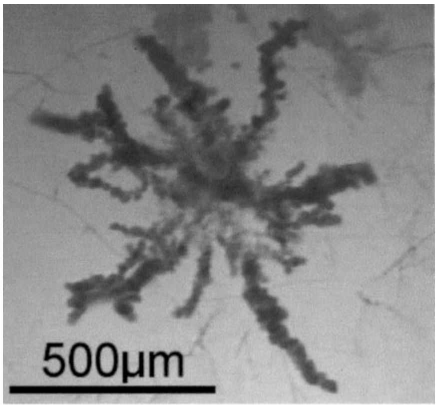

Fig. 4. *Aureobasidium* microcolony after 1 month's growth of sample on MKM.

Paulo to Porto Alegre. Microorganisms collected in this manner may be recovered after storage under ambient conditions for several years (unpublished observations). Strips of tape of standard length may be placed on these glossy sheets and sterilized in situ by UV irradiation if the samples are to be incubated on nutrient-rich media. In general, this step is unnecessary for samples incubated on mineral salts media.

The tape samples were examined after 2 h, before growth began, but after the cells had been rehydrated. Sequential observations were then made daily for 4 days and intermittently up to 2 weeks. Microcolonies (colonies not visible to the naked eye, Figs. 1, 3 and 4) could be counted directly beneath the tape, but sporulation, necessary for identification, usually occurred only beyond the tape periphery or after removal of the tape following 1-week prior incubation. The tape removed was reincubated on fresh agar. Where sporulation occurred beneath the tape, the spore-forming structures were not generally of a form consistent with definite identification. Examination of the biofilm at higher magnifications while retaining spatial integrity was possible only by destructive techniques, involving removing the tape from the agar and placing it on a microscope slide, or cutting out a block of agar which had been incubated in contact with the tape and viewing either directly through the tape or after removal and using a glass coverslip. Counts performed on tape samples were possible only at low colonization density (up to 4 weeks after painting) and were not included in the final analysis. However, comparison of the numerical results showed that there was excellent rank order correlation for the two methods during the first 4 weeks, even though the absolute numbers differed. The clear advantage of the tape technique is that samples may be repeatedly observed in situ, transient growth of some organisms, especially phototrophs and protozoa, which may be

Table 1
Major fungal genera found on the two buildings at different sampling times after painting

Month	July				Aug.				Aug.				Aug.				Sept		Oct.				Nov.		Dec.		Jan.		Feb.		March		May			
Week	1				2				3				4				9		13				18		22		26		31		35		42			
Building	1		2		1		2		1		2		1		2		2		1		2		2		2		2		2		2		1		2	
Treatment	C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B
Fungal genus																																				
Alternaria			+		+		+	+		+	+		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
Arthrinium											+						+												+		+					
Ascomycetes						*									*						+					+	*		+	+			+		+	
Aspergillus	N	N			+					+	+																									
Aureobasidium			+	+	+	+	+	+	+	+	+	+	+			+			+	+									+	+			+	+	+	+
Cladosporium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+			+	+	+	+
Coelomycetes			+				#	+		+	+#		+#				#	#	+#	+	#	#	#	#	#	#	#	+#	+#	+#	#	#	+#	#	+#	+#
Curvularia								+				+				+	+			+						+	+	+	+	+	+	+	+		+	+
Epicoccum					+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+			+	+	+	+	+	+	+	+
Fusarium					+										+																					+
Helminthosporium				+	+	+	+	+	+	+	+				+	+		+		+								+	+	+	+		+			
Monascus			+					+	+		+	+	+	+	+	+	+	+			+	+	+		+		+		+	+		+	+	+		+
Nigrospora			+				+			+	+	+	+	+	+		+	+	+	+		+	+	+	+		+	+	+	+	+	+	+	+	+	+
Peyronellaea											+		+							+																
Pithomyces																	+												+	+	+	+	+			+
Tripospermum				+					+	+																			+				+			+
Trichoderma																					+	+	+				+		+	+			+		+	+
Ulocladium										+																					+	+				
Yeasts	+	+	+	+	+	+		+			+	+																	+	+		+	+	+	+	+

C = Control (No biocide); B = Biocide; N = *Aspergillus niger* * = *Chaetomium* # = *Pestalotia* or *Pestalotiopsis*

lost after prolonged incubation, may be detected, and intermediate morphological forms may be seen during the development of the colony. Some samples were incubated on MKM containing 5, 7, 10 or 20% sodium chloride to assess the salt-tolerance of the paint population.

Numerical results (number of fungal colony-forming units on Sabouraud dextrose agar) were analyzed statistically by non-parametric methods (see Section 3).

3. Results and discussion

Before cleaning, *Cladosporium* was the major fungal biomass on both buildings, followed by *Aureobasidium*, and the fungal biodiversity was greater on building **2**. The major biomass of the biofilm on building **1** consisted of phototrophs and the abundance of cyanobacteria and the presence of Pleurocapsales suggested that it was a long-standing biofilm [6], in agreement with the visual appearance. The major biomass on building **2** was fungal, and the diversity of phototrophs on this relatively clean building was less than on building **1**, except in a surface crack, where it was greater. The majority of the phototrophs detected on both buildings were coccoid, rather than filamentous, agreeing with results previously obtained on painted surfaces in Brazil [1]. After cleaning, almost no phototrophs were detected and no actinomycetes or protozoa were seen, although they were present on the original surfaces.

Table 1 shows the fungi detected after painting. After 1 week, two filamentous fungal genera (*Aspergillus* and *Cladosporium*) were detected on building **1** and eight (*Alternaria, Aureobasidium*, *Helminthosporium*, *Monascus, Nigrospora, Phoma, Tripospermum* and *Cladosporium*) on **2**. Pink and white yeasts were detected in all samples and the actinomycete genus *Geodermatophilus* in one of the eight samples. Two weeks after painting, the number of yeasts decreased and they were not detected between the 3rd and 31st week, after which they increased in number. The initial colonization could have been the result of their presence in the paint in-can, since yeasts are common contaminants of liquid paints [5]. However, as is discussed later, the complete fungal flora detected on the painted surfaces closely resembles that found in the phylloplane, and it has been reported in a number of studies that yeasts are early colonizers, which are an initial transient population on leaves, returning again as late colonizers (see [13] for a review). Thus it is probable that the yeast population on new paint is a real colonization of the film.

Alternaria, Curvularia, Epicoccum, Helminthosporium, Coelomycetes (mainly *Pestalotia*/*Pestalotiopsis*), *Monascus, Nigrospora* and *Aureobasidium* became common isolates as time progressed. *Cladosporium* was at all times the most common filamentous fungus isolated, as it is on internal painted surfaces in São Paulo [10] and in the air of various countries [14,15]. *Aureobasidium* was present early on the surfaces, but numbers fell from week 4, recovering after week 26. Webb et al. [16] showed that the adhesion of *Aureobasidium* to plastic was enhanced by increased hydrophobic interaction in the presence of plasticizers; similarly, the surface of fresh paint may initially be more hydrophobic until it has weathered, encouraging initial attachment of *Aureobasidium*. This fungus appears to be excluded by changes occurring in the paint, which presumably encourage other fungi preferentially, but is able to recover as a member of the population when further weathering occurs. Springle [17] also notes the preponderance of *Aureobasidium* on aged surface coatings.

Table 2
Numbers of filamentous fungi isolated at various times after painting (colony-forming units on Sabouraud glucose agar)

Week	Building	Without biocide (Control, C)	With biocide (B+)	Odds ratio (B+ < C)	Logits (Log odds ratio)	Cumulative logits
1	2	0, 1, 4, 5	4, 4, 4, 5	1:3.12	0.492	0.492
2	1	1, 3, 6	1, 2, 2, 2	4:1	−0.602	−0.110
	2	3, 4, 4, 7	4, 5, 6, 8	1:4.83	0.684	0.574
3	1	1, 3, 10, 17	3, 4, 9, 9	1.26:1	−0.100	0.474
	2	12, 14, 16, 20	4, 9, 10, 12	68.9:1	−1.838	−1.364
4	1	3, 4, 7, 8	4, 4, 7, 10	1:1.92	0.283	−1.080
	2	11, 13, 20, 25	6, 9, 10, 15	16.5:1	−1.217	−2.298
9	2	11, 34, 41, 72	6, 10, 15, 26	16.5:1	−1.217	−3.515
13	1	11, 14, 18, 18	2, 6, 7, 13	34.0:1	−1.531	−5.046
	2	12, 16, 19, 23	5, 11, 12, 13	34.0:1	−1.531	−6.577
18	2	17, 12, 17, 8	9, 9, 13, 7	4.83:1	−0.684	−7.261
22	2	19, 37,19, 19	28,20,16,32	1:1.26	0.100	−7.161
26	2	24, 28, 21, 33	15, 20, 24,33	3.12:1	−0.492	−7.653
31	2	42, 23, 10, 15	22, 22, 20, 18	1:1	0	−7.653
35	2	24, 16, 11, 11	10, 14, 12, 21	1.26:1	−0.492	−8.145
42	1	22, 36, 25, 6	20, 8, 6, 14	9:1	−0.954	−9.099
	2	84, 48, 34, 51	32, 44, 48, 9	16.5:1	−1.217	−10.316

Odds ratios were calculated using the Rank Sum test. For building **2**, the final cumulative probability value, P, for B+ < B− at week 42 is 3.9×10^{-8}. For both buildings together at week 42, $P=4.8\times10^{-11}$.

Table 2 shows the quantitative data. The data were analyzed using the one-tailed rank sum test and the individual probabilities combined using the logistic method of analysis explicitly shown in the table. The results show clearly that the biocide treatment is effective ($P < 10^{-10}$), and that the biocide remains effective throughout the period of study, since the cumulative probability of the biocide effect continues to increase. Statistical analysis of fungal numbers indicated that the overall numbers increased with time ($P < 0.001$, Spearman rank order correlation). Biodiversity (total number of genera detected on Sabouraud dextrose agar and MKM) on these two surfaces was not significantly different ($P = 0.07$) according to Fisher's Exact test, suggesting that the various types of fungi are affected more or less equally by this broad-range biocide. An earlier study showed that phototroph biodiversity was also not affected by biocide treatment [7]. A copper-based biocide was applied to a wall in the southern Brazilian town of Porto Alegre. The wall had been painted 2 years before biocide application and was moderately heavily colonized by fungi and phototrophs. Following treatment, the discoloration of the wall faded and microbial populations were reduced to virtually zero after 4 months. Numbers remained low for 12 months, after which the biodiversity gradually increased although the total biomass (and wall discoloration) remained low. After 6 years, the phototroph biodiversity on the treated wall was equal to that on an adjoining untreated area, although there was a much greater biomass and visual discoloration at the second site.

Previous studies on the fungi associated with external painted surfaces have reported the common species described in this study. The earliest study to report *Cladosporium* is that of Galloway [18] and Goll and Coffey [19] first report *Aureobasidium*. Goll et al. [20] studied discolored paint on 600 house exteriors in the USA and detected a fungal flora similar to that reported here. They found fungi in all discolored painted areas. Rothwell [21] and Drechsler [22], in other large studies, reported *Cladosporium*, *Alternaria*, Coelomycetes (*Phoma*) and *Aureobasidium* as the major biomass. *Epicoccum* and *Helminthosporium* have seldom been reported on paint, probably because their identification requires spore production, which is stimulated by light [23]. However, they are normal members of the phylloplane and of the aerospora; *Nigrospora* and *Curvularia* are also normal members of the aerospora and phylloplane in the tropics, but are uncommon in temperate climates [13]. It is not surprising to find these fungi on painted surfaces in Brazil. *Aspergillus* and *Penicillium*, along with *Cladosporium* and *Aureobasidium*, are the major biomass reported on indoor painted surfaces in temperate climes and are found with *Stemphyllium* and *Stachybotris* [5]. These first four fungal genera are common aerospora in São Paulo [24] and were all found on our external walls. However, *Aspergillus* and *Penicillium*, along with *Fusarium*, were transient and infrequent visitors to the surfaces and cannot be considered as pioneer colonizing species. It is interesting to note that fungal numbers fell in the summer months of November, December and January. Genera particularly affected were *Aureobasidium*, *Epicoccum* and *Helminthosporium*.

In general, the fungal genera detected on MKM and Sabouraud dextrose agar were in good agreement, but yeasts, *Aureobasidium* and *Tripospermum* were detected with greater sensitivity on the tape samples. *Tripospermum* was found only on MKM. This genus has not previously been recorded in paint biofilms and our results emphasize the importance of both the growth medium and the sequential observations used in this study. It is obvious that data acquired by different research groups, using different detection methods, cannot be directly compared. This situation may soon be changed by the development of improved methods and data banks for the analysis of fungal DNA. Molecular techniques for the analysis of fungal biofilms on paint are currently under development [25], but as yet are limited by the availability of basic information on fungal genes.

The advantages of culturing on a mineral salts medium are that the developing colonies may be directly observed at intervals, and the propagules on the tape may be observed before growth. The use of cellulose adhesive tape for bacterial culture has been described previously [9], and the tape lift method for the study of fungal spores is used routinely by many workers [26], but we are not aware of the use of culture techniques in combination with the tape lift method for fungi. On a mineral salts medium, the fungal colonies may be examined by transmitted light microscopy, since their total optical density is low. We routinely use objectives up to $\times 25$ to identify colonies in situ on the plate beneath the tape and the oil immersion objective for more detailed examination. Figs. 1, 2 and 5 were all photographed under the tape after its removal to a glass slide, while Figs. 3, 4, 6–10 were taken on agar squares removed from the plate and viewed through a glass coverslip.

The only organisms detected in established biofilms in situ by direct microscopy of tape lift samples are *Aureobasidium* (Figs. 1–3; also shown as a typical microcolony after incubation of tape strip on agar for 1 month in Fig. 4), *Cladosporium* (Fig. 5), *Epicoccum* spores and picnidia of coelomycetes. Other spore forms are occasionally seen, mostly *Alternaria* spores, but although *Fusarium* is very frequently detected in long-standing paint biofilms and the macrospores of *Fusarium* are distinct, no macrospores have ever been observed in samples taken from walls. *Aspergillus* and *Penicillium* spores are sufficiently distinctive to be recognized by microscopy using air samplers or the tape lift method and these genera are common in buildings [14,15,27], whereas *Cladosporium* spores are always the predominant spores in outdoor air samples [14,27]. The flora observed on our paint closely matches that found in the outdoor air. *Acremonium* and *Verticillium* are the

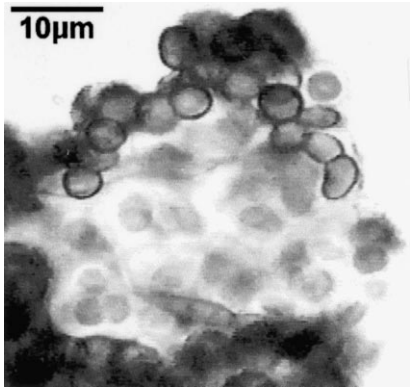

Fig. 5. *Cladosporium* filament growing around a *Synechocystis* microcolony, seen after 2 h rehydration of tape strip on MKM.

only species common in the air not detected in this study; the former occurs in older biofilms and the latter sporulates after at least 2 weeks on MKM and was not included in the current analysis. Other late-sporulating organisms seen, but not included, are *Cephalosporium, Sporotrichum*, *Stemphylium* and *Trichothecium*. Very infrequently detected fungi were also not included in Table 1; these included *Chloridium*, *Oidodendron*, *Paecilomyces*, *Staphylotrichium* and *Torula*. The principle source of fungal spores of the aerospora is the phylloplane [13] and the close match between the species observed on paint and the phylloplane shows that the same conditions for growth are present on paint and leaves. The paint flora does not match that found in the soil and does not closely resemble the fungal flora associated with damp, painted walls within buildings, apart from the prevalence of *Cladosporium* and *Aureobasidium* [5,10].

The algal medium used in this study, Knop's medium, was modified by increasing its tonicity with a higher concentration of magnesium sulfate ($MgSO_4 \cdot 7H_2O$, 2.50 g l^{-1}) to inhibit the growth of *Mucor, Rhizopus, Thamnidium* and agarolytic gliding bacteria, which can kill or mask other microorganisms present. *Rhizopus* is especially damaging in this context. All the above organisms are, however, part of the normal flora of painted surfaces. Further manipulation of Knop's medium by the addition of NaCl showed that many of the components of the biofilm on paint are very osmotolerant. Some of the unexpected members of this osmotolerant group, detected in samples from painted mortar in the necropolis of Carmona, Spain, were the myxomycetes, which grew more actively on up to at least 7% NaCl agar, while other organisms from the same site growing under these conditions were apparently obligate halophiles, such as the Chlorophyte, *Trochiscia*. Some of the fungi in the paint community of the current study showed no marked osmotolerance and *Epicoccum* did not grow in the presence of 5% NaCl. We believe that this organism must only grow either when free water is available or during periods of high humidity and thus must complete its life cycle in a brief spell of time. On the other hand, *Cladosporium, Aureobasidium, Tripospermum* and other yeast-like forms were seen to develop from resting propagules on paint and all were able to grow at NaCl concentrations of at least 10%. Figs. 1 and 2 show *Aureobasidium* microcolonies on tape samples. The microcolony in Fig. 1 resembles the growth form on 15% NaCl illustrated by Urzi et al. [28] who demonstrated growth on potato dextrose agar containing 15% saline in 4 of 10 samples isolated from marble (6 samples) and other sources. All grew on agar containing 5% saline. Fig. 3 shows the pleiomorphic, oidial form of *Aureobasidium*; this resembles the form of *Neurospora crassa* induced by osmotic inhibition of cell wall synthesis [29]. Fig. 5 shows *Cladosporium* growing around a coccoid cyanobacterial colony; such close physical associations between various microbial groups is common on these surfaces. Both *Cladosporium* and *Aureobasidium* may be isolated from natural waters containing 30% NaCl [30] and from salted fish [31] and clearly fall into the class of species that are extremely halotolerant. This ability to survive osmotic stress provides them with a clear advantage on the painted surface, which is frequently dry. These organisms, along with some of the phototrophs on paint, are able to resist desiccation by retaining viable vegetative cells. Many of the paint community form spores as an alternative survival strategy, and include actinomycetes, gliding bacteria, aerobic spore-forming bacteria and chlorophytes such as *Trentepohlia*.

Some bacteria commonly observed on walls are not known to form spores and may have other methods to resist desiccation, such as the production of osmolytes, substances produced to protect cell materials against such adverse conditions as desiccation and extreme temperatures. Some members of the paint community, such as rotifers, some cyanobacteria and algae, protozoa and some species of *Cladosporium* grow at temperatures below 0°C. *Cladosporium* has long been known to be a spoilage organism in freezers, and will grow at −6°C or less [32–34], whilst the Saccharomycete, *Oospora lactis* (*Geotrichum candidum*), can grow at −23°C [35]. Rotifers are well-known components of the cryoconite holes on permanent snowfields; a recent example is the description of at least 15 species of bdelloid rotifers at a single location in Greenland [36]. Numerous other small animals remain active well below zero, and the champion, an arthropod, remains active below −35°C using only the osmolyte, glycerol, as an antifreeze [37]. Mites, tardigrades and nematodes are all components of the paint fauna, and, in the case of the latter two, osmotic protectants are essential for survival. These organisms may rely on their diet for the bulk of their osmolyte requirements.

The evidence that a large number of groups commonly present on paint are active at temperatures below 0°C shows that they must be dependent on osmolytes, and can in extreme cases function with a cytoplasmic aqueous phase containing less than 30% water. The activity and survival of many organisms found on painted surfaces depends on their retention of very large amounts of osmo-

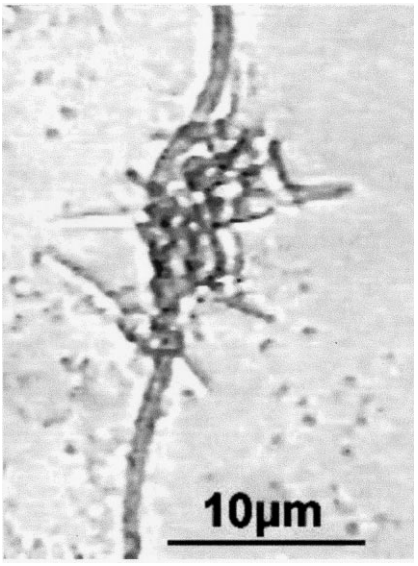

Fig. 6. *Tripospermum* in culture on MKM; tetrahedral form growing on a filament.

lytes. The slow rate of growth of the most successful components of the fungal flora on painted walls is a consequence of their internal economy. Those that are unable to adopt this way of life must be able to complete their life cycle from germination to spore formation in a single wet period. Some members, such as the aerobic spore-forming bacteria commonly found on paint, adopt a mix of both strategies [38].

The main function of osmolytes is probably protection of protein conformation, preventing the denaturation of proteins [39], but they may also prevent the increase in conformational stability of nucleic acids induced by high salt concentrations [40]. Osmolytes also help prevent the effects of cold shock [41], but whether this is by inhibiting protein denaturation or by preventing the production of shock factors is uncertain. The presence of high concentrations of any of a wide range of osmolytes is indicative that the organism is an extremophile. Extremes of heat, cold, pH or low water activity all denature proteins, and the prime function of all osmolytes is to stabilize protein conformations; they also provide other benefits and maintain turgor in vascular plants, prevent damage to cells by the inhibition of ice crystal formation and aid the uptake of water from the air by microorganisms in humid climates. The population on paint must be able to cope with high radiation levels and rapid changes in temperature and humidity; it is therefore dominated by extremophiles of almost every known type of microorganism, including halophilic archaea [42].

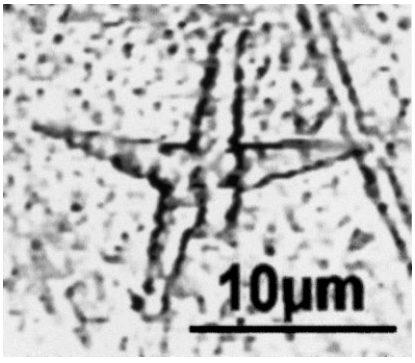

Fig. 7. *Tripospermum* in culture on MKM; isolated tetrahedral spore form.

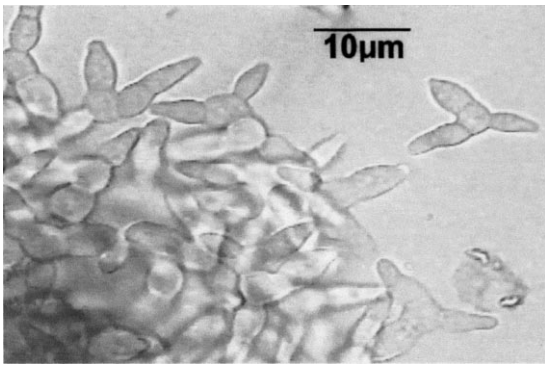

Fig. 8. *Tripospermum* in culture on MKM; planar triradial spores.

Many fungi are known not to sporulate on rich media, and tap water agar and Petri's agar (a salt solution similar to that used by phycologists), have often been used in the past. We have used a mineral salts agar primarily to culture phototrophs and we have generally good agreement between the fungi growing on Sabouraud dextrose agar and on MKM. One of the exceptions is *Tripospermum*, which is detected only on MKM, where it occurs as two morphotypes. Figs. 6 and 7 show the better-known type forming tetrahedral spores, while Fig. 8 shows a form with planar triradial spores. Both of these forms produce yeast-like colonies and develop *Aureobasidium*-like filaments with simple blastic spores (Fig. 9). Figs. 8 and 9 were taken from the same microcolony. *Tripospermum* has been highjacked by the aquatic mycologists, who appear to believe that all staurosporous forms are aquatic. However, *Tripospermum* species are typical members of the phylloplane community of trees and cause important economic losses on coffee and mango. It has also been reported to be pathogenic in many other trees. This genus is found in the 'sooty molds', the mixture of melanotic fungi that cover plastic garden furniture placed beneath trees, as well as the leaves of plants growing under trees. *Tripospermum* is halotolerant, growing on 10% saline agar, and has even been reported to be a pathogen in man [43].

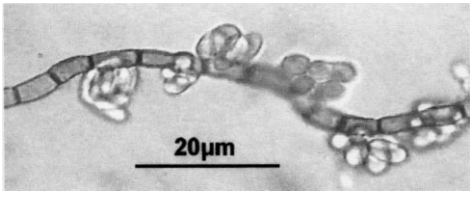

Fig. 9. *Tripospermum* in culture on MKM; filament with simple blastic spores resembling *Aureobasidium*.

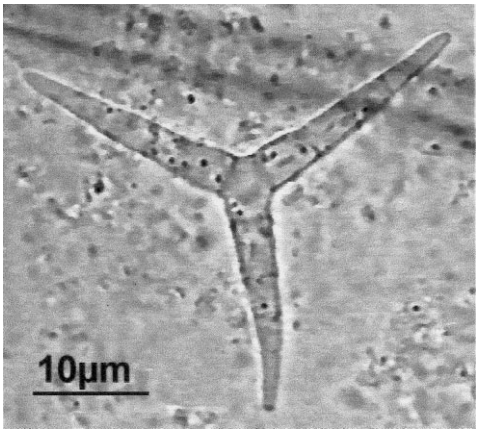

Fig. 10. Unidentified planar triradial spore, growing in culture on MKM.

Fig. 10 shows another, unclassified planar, triradial staurospore from paint. To our knowledge, this is the first time that these organisms have been detected on painted surfaces.

Salt efflorescences are common on masonry and it has been established that such deposits are associated with halotolerant bacteria [44]. However, the existence of a community of halotolerant fungi on painted walls in areas where rainfall and humidity are high throughout the year was unsuspected. We recently calculated that there are four million phototrophs yet to be identified on painted walls worldwide [6]. This present study shows that a wide variety of extremophiles are waiting to be found in everyday domestic environments.

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