

Ascomycete fungi on dimension stone of the “Burg Gleichen”, Thuringia

Christine Hallmann · Diana Fritzlar ·
Lorena Stannek · Michael Hoppert

Received: 7 January 2011 / Accepted: 22 April 2011 / Published online: 13 May 2011
© The Author(s) 2011. This article is published with open access at Springerlink.com

Abstract In the present study, the diversity of ascomycete fungi was investigated on two wall areas of the “Burg Gleichen”, Thuringia (Germany), made of various types of sandstones, travertine and Grenz dolomit. From a W-exposed, shaded wall area, free-living ascomycetes (mainly “black fungi”) and green algae could be retrieved from sandstone lithologies. Sandstone from an ESE-exposed area was mainly colonized by lichen ascomycetes and the lichen alga *Trebouxia*. Both areas share a small number of generalist species, related to the ascomycete black fungi *Sarcinomyces petricola*, *Phaeococcomyces chersonesos* and *Stichococcus mirabilis*. Free-living black fungi were isolated and characterized with respect to cell wall morphology and melanin content. A remarkably rigid melanin layer, incorporated in the cell wall of a *Cladosporium* isolate is presented in detail.

Keywords Dimension stone · Biofilm · Ascomycete fungi · Green algae · Cell wall · Melanin

Introduction

Ascomycete fungal organisms are most successful colonizers of all terrestrial habitats, such as rock and soil (e.g. Gorbushina and Krumbein 2000; Anderson and Cairney 2004). They interact in different ways with other organisms, as symbionts (in mycorrhiza or lichen symbiosis), as pathogens or as important destructors of most organic compounds, especially of plant litter (Hattenschwiler et al. 2005). Although they are heterotrophic, i.e. they depend on organic substrates produced by other organisms, many of them are adapted to low availability of nutrients (Wainright et al. 1993). Moreover, fungal spores are resistant to desiccation and radiation. Owing to their small size, the spores are dispersed by a variety of vectors, in particular by wind, but also by animals and by plant diaspores.

Fungal hyphae penetrate surfaces and grow inside soil, but also in various types of clastic rocks (e.g. sandstone) and homogeneous material, e.g. dolomitic limestone (Gorbushina et al. 1993, Hoppert et al. 2004, Gorbushina 2007). The fungal hypha is among the fastest growing cell in nature: hyphae grow with a velocity between 10 and 40 $\mu\text{m}/\text{min}$ (Trinci and Saunders 1977). An active growth zone of 10–15 μm extends behind the apex (Steinberg 2007). The active growth zone is also important for surface adhesion, the excretion of lytic enzymes (e.g. for penetration of wood) and the exertion of mechanical forces on the surface. For endolithic growth, active dissolution of calcitic matrices by organic acids (e.g. Ascaso et al. 1998) is one important mechanism for active penetration. Fungal hyphae find a path in sedimentary material (cf. Fig. 1) by growing around small particles. This feature is brought about by fast changes in the growth direction of the hyphal tip and could also be observed in organisms of other phyla, such as plant root hairs or streptomyces (Geitman and Emons 2000; Flärldh 2003).

C. Hallmann
Abt. Experimentelle Phykologie und Sammlung für
Algenkulturen, Albrecht-von-Haller-Institut für
Pflanzenwissenschaften, Georg-August-Universität Göttingen,
Untere Karspüle 2, 37073 Göttingen, Germany

D. Fritzlar · L. Stannek · M. Hoppert (✉)
Institut für Mikrobiologie und Genetik,
Georg-August-Universität Göttingen,
Grisebachstraße 8, 37077 Göttingen, Germany
e-mail: mhopper@gwdg.de

M. Hoppert
Courant Research Centre Geobiology,
Georg-August-Universität Göttingen,
37077 Göttingen, Germany

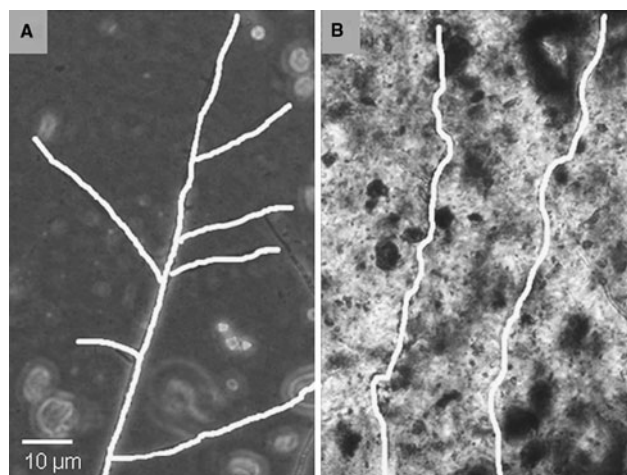


Fig. 1 Growth experiment with a *Cladosporium* isolate. **a** Growth in agar medium without solid particles. **b** Growth in agar medium mixed with sandstone clasts. Hyphae are traced (white lines). Note the rapid changes of growth directions as compared with **a**

Ascomycete fungi on building stone are well known and have been frequently described, mostly in context to bio-deterioration (e.g. Gorbushina et al. 1993; Diakumaku et al. 1995; Sterflinger et al. 1997; Gorbushina and Krumbein 2000; Simonovicova et al. 2004). One important group of colonizers, the Dematiaceae, produce melanin in vegetative hyphae (Cooke 1961; Wollenzien et al. 1995). Though all fungi produce various types of pigments, melanin is predominant in cell walls of reproductive structures, such as conidia or fruiting bodies, including all types of spores (Bell and Wheeler 1986). The melanisation of vegetative cells in Dematiaceae leads to a heavy black pigmentation of all colonized surfaces (Saiz-Jimenez 1995). The frequently described meristematic growth form of Dematiaceae with spherical instead of filamentous cells may be an additional adaptation to adverse growth conditions in rock and stone habitats, e.g. desiccation stress (Wollenzien et al. 1995).

The presented study is part of a comparative analysis of microbial communities from two natural stone walls from the Burg Gleichen (cf. Stück et al. 2011 [this issue]). Certain differences in the abundance of cyanobacteria on wall sections have already been described (Hoppert et al. 2010). Here, we show the occurrence of ascomycete fungi on two wall sections. Typically, the fungal isolates produce melanin in vegetative cells. These melanin deposits form distinct layers, extractable from cell walls of one *Cladosporium* isolate.

Materials and methods

Sampling

The sampling sites were several wall sections of the Burg Gleichen, near Gotha, Thuringia (50°52'49" N, 10°50'20" E).

Samples (approx. 100 µl dry volume per sample) were collected in May 2009 from ESE (wall area A) and W-exposed (wall area B) walls with different lithologies (Fig. 2). The samples were scraped off with a sterile scalpel and collected in sterile 2-ml reaction tubes. Samples for clone libraries were randomly taken from sites, where sufficient material could be removed with a scalpel. All samples in area A were taken from the wall base (Fig. 2) made of either Gleichenberger Rätsandstein or Semionotussandstein. Wall joints were mostly closed, i.e. filled with mortar. Area B exhibited a variety of lithologies (travertine, Grenzdolomit, Rätsandstein). Most wall joints were open. Samples from Rätsandstein from the wall base were taken into consideration for this study. Each sample was labeled with either A or B (according to the wall section) and with a respective running code number. The subset of samples from sandstone lithologies was processed for the detection of fungal clones.

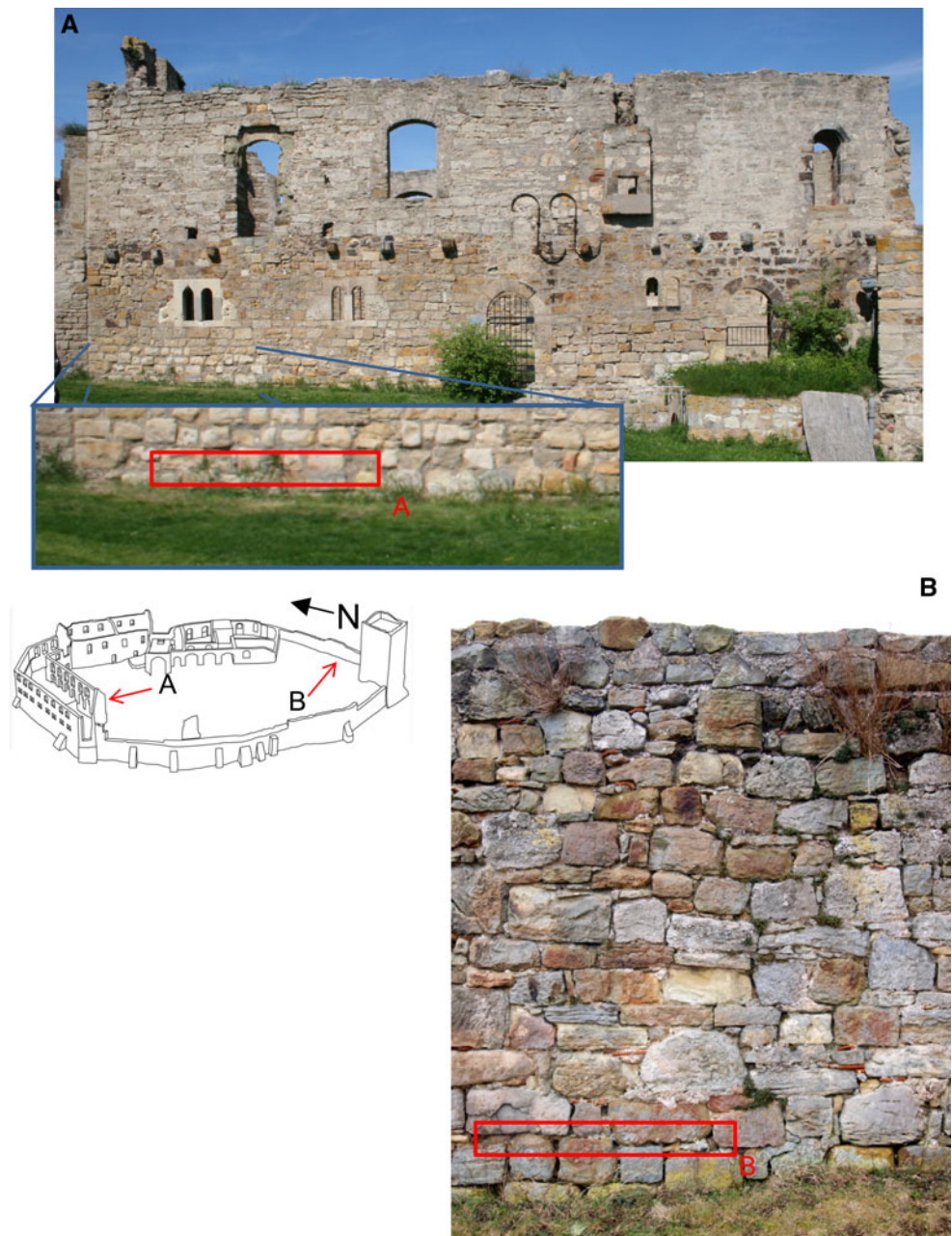
Cultivation and isolation

For the enrichment and isolation of filamentous fungi, small amounts of the biofilm samples were used to inoculate culture media prepared according to Staley 1968, with modifications (peptone/yeast extract/glucose medium, PYG): Bacto-peptone 0.25 g/l, yeast extract 0.25 g/l, glucose 0.25 g/l, basal salt solution 19 ml/l, trace element solution 1 ml/l (basal salt solution in 500 ml: nitrilotriacetic acid 3.0 g, $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ 7.2 g, $\text{CaCl}_2 \times 2\text{H}_2\text{O}$ 1.7 g, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \times 4\text{H}_2\text{O}$ 3.0 mg, $\text{FeSO}_4 \times 7\text{H}_2\text{O}$ 49.5 mg). Trace element solution in 100 ml: EDTA 250.0 mg, $\text{ZnSO}_4 \times 7\text{H}_2\text{O}$ 1.1 mg, $\text{MnSO}_4 \times \text{H}_2\text{O}$ 154.0 mg, $\text{FeSO}_4 \times 7\text{H}_2\text{O}$ 500.0 mg, $\text{CuSO}_4 \times 5\text{H}_2\text{O}$ 39.2 mg, $\text{CoSO}_3 \times 7\text{H}_2\text{O}$ 19.6 mg, $\text{Na}_2\text{B}_4\text{O}_7$ 9.34 mg). For solid media, 1.5% (w/v) Bacto-Agar was added. Isolation of representative strains was performed by repeated streaking either single colonies or conidia on agar plates until apparent macroscopic and microscopic homogeneity of colonies and conidia. Isolates were incubated about 2 weeks at room temperature under ambient light. Liquid cultures were incubated in a shaking water bath in the dark. For some growth experiments, a piece of Seeberger Sandstein was grounded in a mortar and sterilized by autoclaving. Equal volumes of isolated strains were mixed with PYG agar medium (kept liquid at 95°C). The mixture was applied on a sterile microscopic slide and inoculated after solidification. The samples were inspected by light microscopy (Axioscope 40, including AxioCam MRm, Carl Zeiss Micro-imaging, Göttingen).

DNA extraction, PCR, cloning and sequencing

For isolation of environmental DNA as well as DNA from fungal isolates, cloning and sequencing steps were performed as already described (Hallmann et al. 2010 [this issue]) with following modifications: For two samples (A1-4 and B18) cell disruption for 30 s and 50 s of beat

Fig. 2 Wall areas of the Burg Gleichen selected for sampling. **a** Wall area A (Romanesque Hall). **b** Wall area B (section of the ring wall). Sampling sites are marked by *red squares*. The schematic drawing (original figure: Wanja Wedekind) shows the location of the walls in the castle complex



beating was applied (if not especially mentioned, results of 30 s beat beating were shown). For identification of ascomycetes, the primer pair ITS1 (5'-TCCGTAGGTGAACCTGCGG) and ITS4 (5'-TCCTCCGCTTATTGATATGC) for the ITS (internal transcribed spacer) were used (Anderson and Cairney 2004). Accession numbers of sequences related to this study are deposited online (GenBank™: <http://www.ncbi.nlm.nih.gov/genbank>) with this publication as a reference.

Electron microscopy

For electron microscopy, vegetative filaments were either taken from solid or liquid cultures, concentrated by filtering

over a 0.45-µm size pore filter and embedded in resin according to Spurr (1969). Agar blocks were chemically fixed overnight in 2.5% (v/v) glutaraldehyde (grade I for electron microscopy, Sigma–Aldrich, St. Louis, MO, USA) and for 2 h in osmium tetroxide (1%, w/v, aqueous solution, Science Services, Munich). Samples were dehydrated in a graded ethanol series and infiltrated with Spurr resin over 48 h before polymerization at 70°C for 12 h. Ultrathin sections of 80-nm thickness were cut with glass knives. Post-staining of sections was performed with 1.5% (w/v) phosphotungstic acid for 5 min (Hoppert 2003). Electron microscopy was performed with a Zeiss EM 902 transmission electron microscope, equipped with a 1 K digital camera (Carl Zeiss NTS, Oberkochen).

For immunogold labeling with the lectin concanavalin A (Con A), the protocol according to Hallmann et al. 2010 (this issue) was applied. Gold markers in images were digitally enhanced as described (Hoppert and Holzenburg 1998).

Melanin preparation for electron microscopy was performed according to Rosas et al. 2000, with modifications. A melanised *Cladosporium* isolate was cultured on PYG-medium and then centrifuged for 30 min at $3,000\times g$. The cells were washed in 100 mM potassium-phosphate buffer, supplemented with 0.9% (w/v) sodium chloride (phosphate-buffered saline, PBS). Then, cells were resuspended in 1.0 M sorbitol/0.1 M sodium citrate solution (pH 5.5), containing 10 mg/ml lysis enzyme from *Trichoderma harzianum* (Sigma–Aldrich) and incubated at room temperature overnight. Cells were again centrifuged, resuspended in PBS and incubated in 4 M guanidinium thiocyanate solution for 12 h at room temperature. After washing the cells in PBS by centrifugation and resuspension in reaction buffer (10 mM Tris, 1 mM CaCl_2 , 0.5%, w/v SDS), 1 mg/ml final concentration proteinase K was added. The suspension was incubated for 4 h at 65°C. Cells were again washed in PBS and boiled for 6 h in 6 M aqueous HCl solution. The remaining melanin preparation was dialyzed for 2 days against distilled water (Visking dialysis tubing, molecular weight cutoff 12,000–14,000, Serva, Heidelberg). The prepared melanin was then subjected to embedding in Spurr resin as described above.

Results

Both areas sampled (Fig. 2) exhibited obvious colonization by green algae (in addition, cyanobacteria on area A) and epilithic or endolithic lichens (cf. Fig. 3). Approximately, one-third of the collected samples was useful for cloning of fungal genera. For generation of fungal clone libraries, sequencing with ITS primers turned out to be most appropriate (Anderson and Cairney 2004). From areas A and B, 65 (48) and 30 (75) fungal (algal) clones were retrieved, respectively. It has to be kept in mind that the organisms were identified by their sequences of closest known relative by BLASTn analysis (National Center for Biotechnology Information, Bethesda, MD, USA). The genus and species names given in Tables 1 and 2 represent the closest known relative according to these sequence similarities.

Table 1 lists the fungi identified from the clone libraries, along with the algal clones. Mostly, the apparent colonization correlates with clone bank data. All sites from area A were apparently colonized by endolithic lichens. Accordingly, a major part of algae and fungi retrieved from the clone banks were, in fact, lichen associated. Samples



Fig. 3 Macroscopic appearance of (microbially) colonized sandstone surfaces. **a** Endolithic lichens in conjunction with scaling and backweathering of the surface. **b** Layer of green algae

B13, B14 and B18 were taken from sites apparently colonized by green algae. The (non-lichen associated) algae *Stichococcus* and *Pseudochlorella* could also be retrieved from the clone banks. In addition, numerous *Sarcinomyces* and *Phaeococcomyces*-related fungal clones were present.

Because the cell disruption method may have detrimental effects on the quality of clone libraries (i.e. with respect to species richness), different intensities of bead beating were applied on two samples. The data in Table 1 (sample B18 30 s and 50 s beat beating time; sample A1-4 30 s and 50 s) document a minor variation in the retrieved clones, but do not show the occurrence of essentially different species groups. Thus, both pairs of samples could be assigned to either wall area A or wall area B-typical groups of clones.

Although the retrieved clone banks also differ from sample to sample, two groups of species typical for either of the wall areas can be clearly distinguished. It is obvious that the fungal diversity on area A is higher when compared with area B, where more algal than fungal species could be identified. Just a small number of identical species (represented by the respective clones) were present in both

Table 1 Distribution of fungal and algal clones on wall sections

Closest relative species	A2-1	A1-4	A1-4 (50 s)	A1-8	B13	B14	B17	B18	B18 (50 s)	B22	% sequence similarity to closest relative species
<i>Sarcinomyces petricola</i> *	1		1	1	6	5		2	5		97-98
<i>Phaeococcomyces chersonesos</i> *		2	2	3	1			4	1	1	96-97
Uncultured <i>Cladosporium</i> clone*		1							1		99-100
<i>Caloplaca decipiens</i>				1		2					99
<i>Capnobotryella</i> sp.*		6	3								99
<i>Cladosporium cladosporioides</i> *	1	1									99
<i>Phaeobotryphaeria citrigena</i>		1									90
Uncultured <i>Dothideomycetes</i>		3	10								82-83
<i>Anisomeridium polypori</i>	16			1							86-88
<i>Dolichousnea longissima</i>	1										96
<i>Phaeophyscia ciliata</i>	8										89-90
<i>Pseudocyphellaria fimbriatoides</i>			1								83
<i>Umbilicaria arctica</i>				1							85
<i>Erysiphe alphitoides</i>								1			99
<i>Pleospora herbarum</i>						1					100
<i>Stichococcus mirabilis</i>	1	7	4	16	7	5		15	8	7	89-96
<i>Trebouxia</i> sp.	1					1					98-99
Uncultured <i>Trebouxia</i> photobiont	4	1	11								93-100
<i>Trebouxia arboricola</i>			1								97
<i>Chlorella</i> sp.	1										92
<i>Stichococcus</i> related					1	2	18	5	1		79-82
<i>Pseudochlorella</i> sp.				1	1			2	2		86-89

Black fungi are marked by an (*), lichen algae and lichen fungi are marked in red
 Boxed areas indicate species present in both wall areas (green), exclusively present in area A (blue) or in area B (beige)
 The predominance of *Sarcinomyces petricola* in area A is indicated by a dark green color

Table 2 BLAST search results of fungal isolates

Sample no.	Closest relative species, accession no.	Percentage sequence similarity to closest relative species
B 1-2	<i>Beauveria bassiana</i> , GQ302680	100
A 1-2, B1-3, B12, B 2-5, B10	<i>Cladosporium cladosporioides</i> strain F12, HQ380766	99–100
B 1-8, B14	<i>Phaeococcomyces chersonesos</i> , AJ507323	96

areas: representatives of two ascomycete fungi, *Sarcinomyces petricola*- and *Phaeococcomyces chersonesos*- as well as *Stichococcus mirabilis*-clones (green algae) were retrieved with high clone numbers. One lichen ascomycete (from *Caloplaca decipiens*) and a *Trebouxia* sp. lichen alga were found in samples from both walls, but just in low numbers. In particular, the fungal clones in both groups of samples are different (Fig. 4). On area A, most ascomycetes are related to genera known from lichen symbiosis (cf. Wirth 1995). Also the presence of several lichen alga

clones (*Trebouxia*) reflects this feature. This is also in accordance with the observation that mainly at the wall base, the sandstone was intensively colonized by endolithic lichens.

Although also area B exhibited colonization by endolithic lichens at the wall base, mainly non-lichenized organisms could be retrieved: Here, just one clone of the typical lichen alga and two lichen fungi clones could be found. Among the fungal organisms, especially *Sarcinomyces* and *Phaeococcomyces* clones were retrieved,

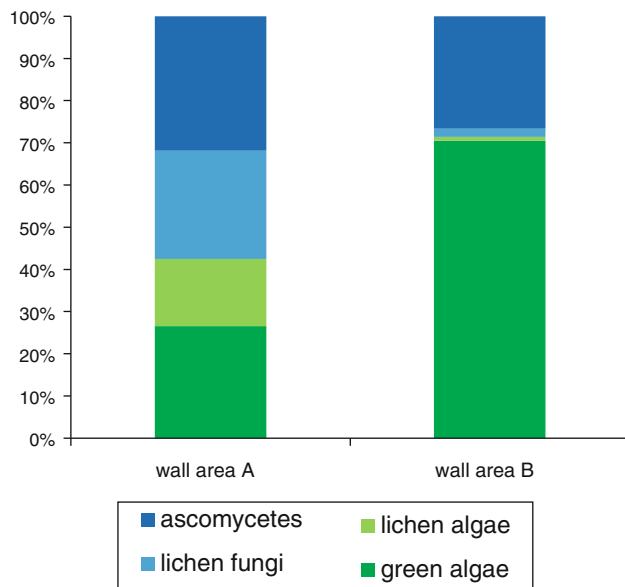


Fig. 4 Relative amounts of clone numbers of large phylogenetic groups in wall areas A and B

i.e. representatives of free-living, non-lichenized black fungi. The overall diversity was considerably lower (10 different blast responses) than on area A (19 different blast responses).

Several ascomycetous black fungi could be isolated from the samples, especially from area B, as listed in Table 2. Although the diversity of ascomycete clones was higher on area A, just one fungal isolate could be obtained from this area. Since the abundant lichen ascomycetes from area A are rather difficult to culture in standard media, they could not be retrieved as pure cultures. Several *Cladosporium* and *Phaeococcomyces*-related isolates could be obtained from area B. Though just one *Cladosporium*-related clone could be retrieved from clone banks, several *Cladosporium* strains could be isolated, accounting for a high abundance of diaspores, but a low number of actively growing organisms at the time of sampling.

All isolates belong to the black (melanised) fungi and served as model organisms for further studies like observation of melanin production and growth experiments. The

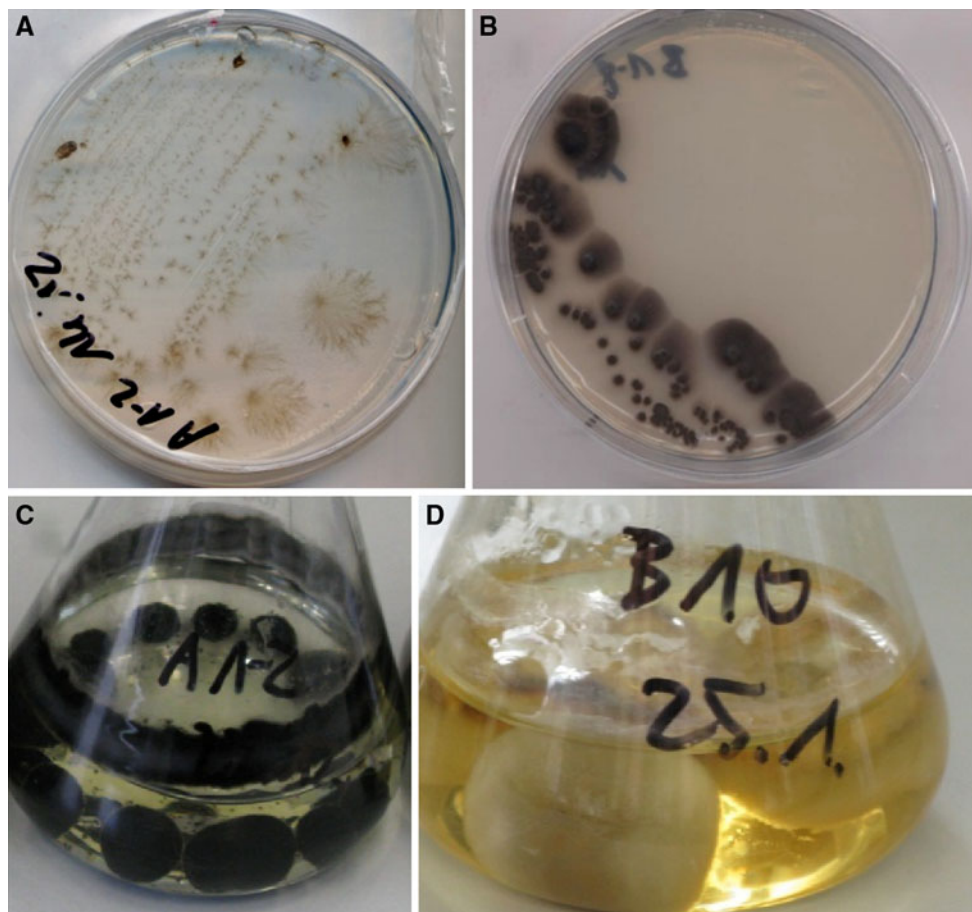


Fig. 5 Melanin production in *Cladosporium* and *Phaeococcomyces* strains. *Cladosporium* (a) and *Phaeococcomyces* (b) on solid medium. c *Cladosporium* strain in liquid culture; vegetative hyphae

form globules due to culturing conditions and show a dark stain. d Growth of *Cladosporium* without formation of melanin after several culture passages

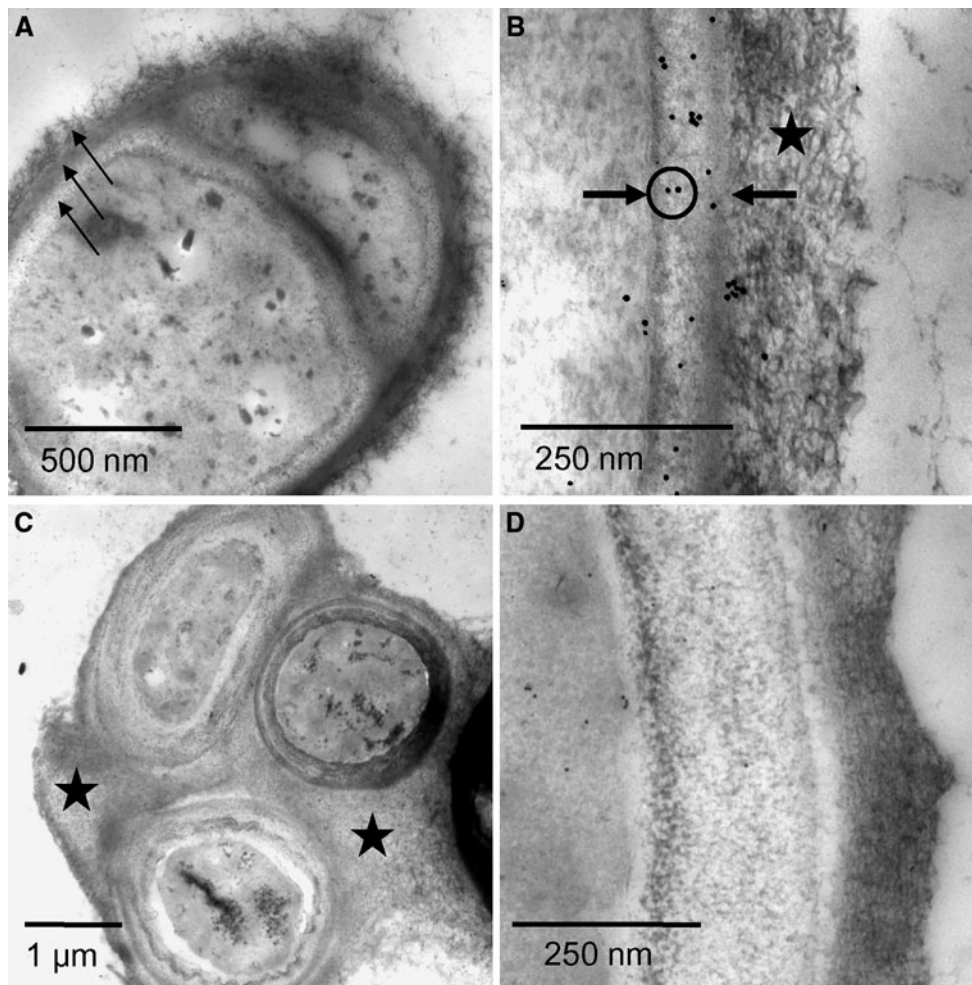


Fig. 6 *Phaeococcomyces* and *Cladosporium* cell walls; transmission electron microscopy of ultrathin sections. **a, b** *Phaeococcomyces* showing a multilayered cell wall (arrows) with an outermost exopolymer layer. **b** Detail with a Con A-gold labeled wall (arrows;

gold label encircled) and an unlabeled outermost exopolymer layer (asterisk). *Cladosporium* strain: cross sections of three cells (c), embedded in an extracellular matrix (asterisks). Multilayered cell wall of a single cell (d)

growth experiment in Fig. 1 illustrates that *Cladosporium*, like all other isolates, is able to grow invasive in agar media containing a suspended fraction of crushed sandstone. One ascomycete (*Beauveria bassiana*) could be obtained in pure culture, but not from the clone banks. The formation of melanin in solid and liquid cultures is shown in Fig. 5a–c. The melanised isolates exhibit thick, multilayered cell walls (Fig. 6). In *Phaeococcomyces*, two chemically distinct cell wall layers could be observed: the inner layer is labeled by the lectin marker concanavalin A (cf. Hallmann et al. 2010 [this issue]), i.e. the Con A-gold particles bind to this feature. An outermost layer consists of less densely packed extracellular polymers (exopolymers; Fig. 6a, b). In *Cladosporium* isolates, thick layers of exopolymers between adjacent cells could be observed (Fig. 6c). The walls are approximately 400 nm in thickness (Fig. 6d). A liquid culture shown in Fig. 5d illustrates

that melanin production may also get lost after several passages of culturing in the laboratory. There is a striking difference in cell wall thickness between melanised and non-melanised *Cladosporium* isolates (Fig. 7). The non-melanized cell wall appears by a factor of 10 thinner, without any sign of exopolymer formation (Fig. 7b).

Although the melanised cell wall appears thick, the melanin itself represents just a small, but very rigid proportion of the cell. While it was not possible to extract melanin from the *Cladosporium* isolates, distinct melanin cell wall fragments (“melanin ghosts”) from *Phaeococcomyces* could be retrieved. Here, the melanin represents one distinct layer of the cell wall that could be isolated after harsh treatment with proteolytic and glycolytic enzymes as well as boiling in hydrochloric acid. Even after this treatment, the original shape of the cell wall was still preserved (Fig. 8).

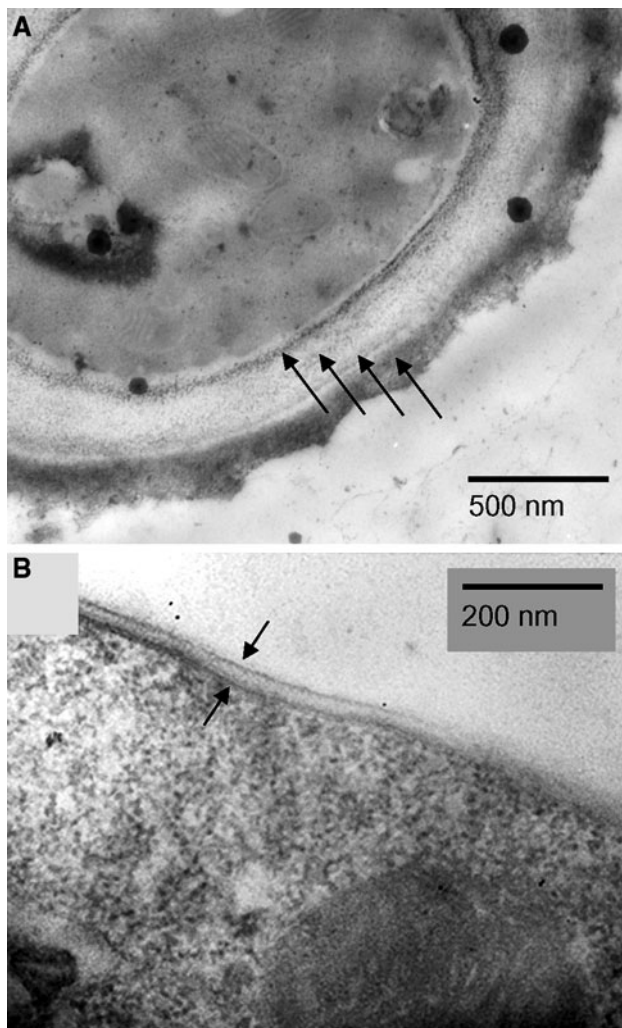


Fig. 7 Cell walls with and without melanin in comparison; transmission electron microscopy of ultrathin sections. *Cladosporium* strain, liquid culture. **a** Melanin-producing cell in cross section with a multilayered cell wall (arrows) of several 100 nm in thickness (cf. Fig. 5c). **b** Thin cell wall (between both arrows) of some tens of nm (non-melanised variant, cf. Fig. 5d)

Discussion

This study is focused on ascomycete fungi, but the clone libraries revealed also green algal clones. The selected primer pair exhibits a broad specificity for ITS sequences of fungal (as well as algal) organisms and has already been successfully applied for a similar study (Berdoulay and Salvado 2009). However, a phylogenetic analysis comprising all phylotypes within fungi (and other eukaryotic microorganisms) would require a whole set of primers (Anderson and Cairney 2004 and references therein). Thus, though we could state clear differences between the wall areas, the whole diversity of present phylotypes, as e.g. analyzed in a study of rock-inhabiting fungi related to Dothideomycetes, could not be retrieved (Ruibal et al.

2009). It should be noted that also the lack of sequence information from well-characterized isolates in the public databases limits the “taxonomic resolution” in our approach (cf. Anderson and Cairney 2004). Hence, sometimes just low sequence similarities of the clones with already known relative species could be retrieved (cf. Table 1). Especially, the data on lichen ascomycete genera must be interpreted carefully and shall be taken as an indicator for the presence of ascomycetes involved in lichen symbiosis, but not necessarily for the occurrence of a defined lichen species.

Generally, the significant differences between ascomycete fungi on different wall sections of the “Burg Gleichen” are obvious. Though all samples in this study were taken from sandstones, some impact from surrounding limestone lithologies (in area B), fine soil in wall joints and mortars may influence directly or indirectly the situation (pH, available nutrients or ions) for colonizing microorganisms. Particularly, gypsum-containing mortar in wall area A (cf. Hoppert et al. 2010) may have influenced species diversity on this site. Moreover, open wall joints in area B provide numerous small niches for depositions of soil, moisture, bird droppings and other nutrient sources that may influence microbial growth. These effects cannot be completely excluded in most “field” situations, and are difficult to quantify. There is, however, no direct evidence for the influence of the limestone in area B. Otherwise, more lichen fungal clones deriving from calcicolous and or nitrophilous lichens should be expected (cf. Arino et al. 1997), which is, in fact, not the case. Generally, wall area A is directly exposed to sunlight, and is therefore more subjected to desiccation stress than the W-exposed wall area B, directly located in the shadow of an adjacent tower (cf. Fig. 2).

Clearly, lichen fungi and lichen algae represent a major part of the microbial flora on the sun (ESE)-exposed wall surface. At the W-exposed wall surface (area B), the non-lichenized black fungi *Sarcinomyces petricola* and *Phaeoococcomyces chersonesos* as well as *Stichococcus mirabilis* and other *Stichococcus*-related algal clones could be retrieved in high abundance, but clones of non-lichenized ascomycetes as well as lichen-associated genera were rare. *Sarcinomyces* and *Phaeoococcomyces* were also present in area A accounting for broad ecological amplitudes of these organisms (cf. Wollenzien et al. 1997; Bogomolova and Minter 2003; Michailiuk 2008; Hallmann et al. 2010 [this issue]). *Sarcinomyces*, however, could only be retrieved in low abundance from area A. It has to be discriminated between the *Stichococcus mirabilis*-related clones (abundant in both areas), and clones, more distantly related to the genus *Stichococcus* (“*Stichococcus*-related” in Table 1). The latter group could be clearly assigned exclusively to wall area B (cf. Table 1).

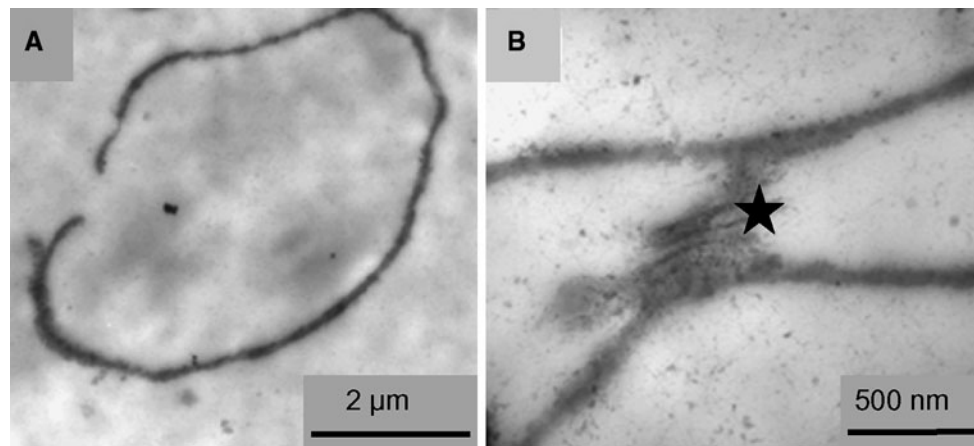


Fig. 8 “Melanin ghosts” of *Phaeococcomyces*, transmission electron microscopy of ultrathin sections. **a** Cross section. **b** Sagittal section, depicting the area of a former septum (*asterisk*)

These data imply that lichenized organisms are successful competitors on area A, compared with some non-lichenized green algae, but also compared with the black fungus *Sarcinomyces*. The equal distribution of *Phaeococcomyces chersonesos*- and *Stichococcus mirabilis*-related clones in both areas indicate that some species remain obviously completely unaffected. In wall area B, although lichen colonization was observable, non-lichenized fungi and algae dominated. It may be possible that the rather moist and nutrient-rich conditions are more favorable for non-lichenized generalists (cf. Hoppert and König 2006).

Most of the ascomycetes, either identified by their sequences or isolated from the stone surface are known as melanised strains. Although melanisation is also known from lichen ascomycetes, the non-lichenized genera have been intensively studied with respect to their pigmentation (e.g. Diakumaku et al. 1995). Besides endolithic growth (in particular, the formation of micropits), pigmentation is an obvious hazard on stone surfaces caused by these organisms on stone surfaces. This effect is rather important for the appearance of smooth sculptured surfaces (especially marble) than for the natural building stone as presented here. However, it has to be kept in mind that any surface color of a building stone is darkened by the melanised organisms. In contrast to the green color of algae (and some bright colors of crustose lichens), this darkening is not perceived as a “biogenic” stain, but rather as a natural color of the stone or as a successive darkening by other factors, e.g. soot deposits.

Melanization is an essential feature for protection against high light intensities, ultraviolet and even ionizing radiation (Bell and Wheeler 1986; Dadachova and Casadevall 2008). However, melanin is not essential for growth in the dark and may be not expressed in isolates grown under laboratory conditions (Fig. 5). Accordingly, in laboratory

cultures of *Cladosporium* isolates, just very thin cell walls could be observed.

The isolation procedure of melanins from cell walls of *Phaeococcomyces* illustrates the rigidity of the melanised cell wall. Even boiling in diluted hydrochloride solution over several hours did neither destroy the melanised cell wall layer nor the molecule itself. All other features of the cell, including all structures of the cell envelope were destroyed (Fig. 8). On building stone, after cell death and decay of all other organic compounds, the melanised cell wall fragments remain on the site for relatively long times. Since the filamentous fungi are endolithic, these particles are not just attached to the building surface, but are deposited in deeper layers of the material.

Conclusion

Although colonization of building surfaces by microorganisms and their contribution to biogenic weathering is a well known fact, differences in the species composition or in species diversity has been rarely addressed. The presented study shows a clear distinction between certain specialists, just present in either of the both wall areas under investigation and a low number of generalists. Organisms from both groups may affect the building material. Some lichens may take part in the formation of large scales (cf. Fig. 3a) on sandstone substrata. Algae and particularly black fungi contribute surface stains. It is likely that exposition and moisture regime strongly influence the dominance of either of these groups, but does not necessarily reduce or even exclude algal or fungal growth. Thus, also intervention in moisture regimes on building surfaces may change, but not necessarily reduce microbial growth on building material.

Acknowledgments Funding of this project by the DBU (Deutsche Bundesstiftung Umwelt) is gratefully acknowledged.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

- Anderson IC, Cairney JWG (2004) Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. *Environ Microbiol* 6:769–779
- Arino X, Gomez-Bolea A, Saiz-Jimenez C (1997) Lichens on ancient mortars. *Intl Biodeterior Biodegrad* 40:217–224
- Ascaso C, Wierzychos J, Castello R (1998) Study of the biogenic weathering of calcareous litharenite stones caused by lichen and endolithic microorganisms. *Intl Biodeterior Biodegrad* 42:29–38
- Bell AA, Wheeler MH (1986) Biosynthesis and functions of fungal melanins. *Annu Rev Phytopathol* 24:411–451
- Berdoulay M, Salvado JC (2009) Genetic characterization of microbial communities living at the surface of building stones. *Lett Appl Microbiol* 49:311–316
- Bogomolova EV, Minter DW (2003) *Phaeococcomyces chersonesus*, a new microcolonial lithobiontic fungus from marble in Chersonesus (Crimea, Ukraine). *Mycotaxon* 86:195–204
- Cooke WB (1961) A taxonomic study in the “black yeasts”. *Mycopathologia* 17:1–43
- Dadachova K, Casadevall A (2008) Ionizing radiation. How to cope, adapt, and exploit with the help of melanin. *Curr Opin Microbiol* 11:525–531
- Diakumaku E, Gorbushina AA, Krumbein WE, Panina L, Soukharjevski S (1995) Black fungi in marble and limestones- an aesthetical, chemical and physical problem for the conservation of monuments. *Sci Total Environ* 167:295–304
- Flårdh K (2003) Growth polarity and cell division in *Streptomyces*. *Curr Opin Microbiol* 6:564–571
- Geitman A, Emons AMC (2000) The cytoskeleton in plant and fungal cell tip growth. *J. Microscopy* 198:218–245
- Gorbushina AA (2007) Life on the rocks. *Environ Microbiol* 9:613–631
- Gorbushina AA, Krumbein WE (2000) Rock dwelling fungal communities: diversity of life styles and colony structure. In: Seckbach J (ed) *Journey to diverse microbial worlds*. Kluwer, Amsterdam, pp 317–334
- Gorbushina AA, Krumbein WE, Hamman CH, Panina L, Soukharjevski S, Wollenzien U (1993) Role of black fungi in colour change and biodeterioration of antique marbles. *Geomicrobiol J* 11:205–221
- Hallmann C, Rüdrieh J, Enseleit M, Friedl T, Hoppert M (2010) Microbial diversity on a marble monument—a case study. *Environ Earth Sci* (this issue). doi:10.1007/s12665-010-0772-3
- Hattenschwiler S, Tiunov AV, Scheu S (2005) Biodiversity and litter decomposition systems in terrestrial ecosystems. *Ann Rev Ecol Evol Syst* 36:191–218
- Hoppert M (2003) *Microscopic techniques in biotechnology*. Wiley-VCH, Weinheim
- Hoppert M, Holzenburg A (1998) *Electron microscopy in microbiology*. Springer Publications, Hongkong
- Hoppert M, König S (2006) The succession of biofilms on building stone and its possible impact on biogenic weathering. In: Fort R, Álvarez de Buergo M, Gomez-Heras M, Vazquez-Calvo C (eds) *Heritage, weathering and conservation*. Taylor & Francis, London, pp 311–315
- Hoppert M, Flies C, Pohl W, Günzl B, Schneider J (2004) Colonization strategies of lithobiontic organisms on carbonate rocks. *Environ Geol* 46:4212–4428
- Hoppert M, Hallmann C, Stannek L, Fritzlär D, Enseleit M (2010) Makro- und Mikroflora in Fuge und Gestein. In: Siegesmund S, Hoppert M (eds) *Die Drei Gleichen—Baudenkmäler und Naturraum*. Edition Leipzig, Leipzig, pp 254–263
- Michailiuk TI (2008) Terrestrial lithophilic algae in a granite canyon of the Teteriv River (Ukraine). *Biologia* 63:824–830
- Rosas AL, Nosanchuk JD, Gomez BL, Edens WA, Henson JM, Casadevall A (2000) Isolation and serological analyses of fungal melanins. *J Immunol Methods* 244:69–80
- Ruibal C, Gueidan C, Selbman L, Gorbushina AA, Crous PW, Groenewald JZ, Muggia L, Grube M, Isola D, Schoch CL, Staley JT, Lutzoni F, De Hoog GS (2009) Phylogeny of rock-inhabiting fungi related to *Dothideomycetes*. *Stud Mycol* 64:123–133
- Saiz-Jimenez C (1995) Microbial melanins in stone monuments. *Sci Total Environ* 167:273–286
- Simonovicova A, Godyova M, Svec J (2004) Airborne and soil microfungi as contaminants of stone in a hypogean cemetery. *Int Biodeter Biodegrad* 54:7–11
- Spurr AR (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. *J Ultrastruct Res* 26:31–43
- Staley JT (1968) *Prosthecomicrobium* and *Ancalomicrobium*: new prosthecate freshwater bacteria. *J Bacteriol* 95:1921–1942
- Steinberg G (2007) Hyphal growth: a tale of motors, lipids, and the Spitzenkörper. *Eukaryot Cell* 6:351–360
- Sterflinger K, De Baere R, de Hoog GS, De Wachter R, Krumbein WE, Haase G (1997) *Coniosporium perforans* and *C. apollinis*, two new rock-inhabiting fungi isolated from marble in the Sanctuary of Delos (Cyclades, Greece). *Antonie van Leeuwenhoek* 72:249–263
- Stück H, Rüdrieh J, Siegesmund S (2011) Weathering behaviour and construction suitability of dimension stones from the Drei Gleichen area (Thuringia, Germany) (this issue)
- Trinci APJ, Saunders PT (1977) Tip growth of fungal hyphae. *J Gen Microbiol* 103:243–248
- Wainright M, Ali TA, Barakh F (1993) A review of the role of oligotrophic microorganisms in biodeterioration. *Int Biodeter Biodegrad* 31:1–13
- Wirth V (1995) *Die Flechten Baden-Württembergs*, 2nd edn. Ulmer, Stuttgart
- Wollenzien U, de Hoog GS, Krumbein WE, Urzi C (1995) On isolation of microcolonial fungi occurring on and in marble and other calcareous rocks. *Sci Total Environ* 167:287–294
- Wollenzien U, de Hoog GS, Krumbein W, Uijthof JMJ (1997) *Sarcinomyces petricola*, a new microcolonial fungus from marble in the Mediterranean basin. *Antonie van Leeuwenhoek* 71:281–288