

# ACCUMULATION AND DEPOSITION OF INORGANIC AND ORGANIC COMPOUNDS BY MICROCOLONIAL FUNGI

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## Abstract

A seemingly unique feature of desert varnish is its worldwide association with microcolonial fungi (MCF). The surface environments on rock coatings are some of the most hostile on Earth. High temperatures, low humidity, high incidence of UV light and low nutrients require microorganisms that have evolved special survival skills. MCF contain melanin, microsporines, carotinoids and probably other as yet unidentified pigments that help protect them from UV light. During the hottest and driest months, few bacteria are observed on specimens we have examined from several desert regions of the world. The survivability in these extreme conditions sets MCF apart from bacteria. Sporulating bacteria have developed survival mechanisms but must expend energy to create spores. MCF in contrast survive and flourish where only few bacteria are present and lichens are unable to survive.

## Introduction

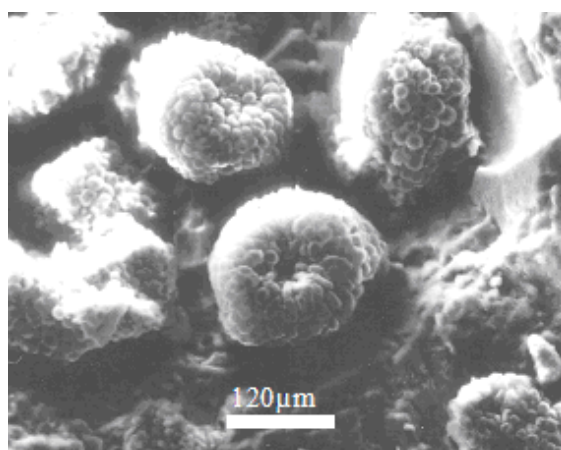
Hot and dry deserts are considered to be one of the most hostile habitats for life on Earth. Drastic temperature changes, high UV levels, long periods of dryness punctuated with rain and snow and low nutrient availability require specially adapted organisms. Also found in deserts is a rock coating commonly called desert varnish<sup>2-5</sup>. A unique association exists between the usually black varnished rocks and black microcolonial fungi (MCF). The rock coatings are black because of high concentrations of manganese and the MCF due to copious amounts of melanin pigments.

## Desert Varnish

Desert varnish, also called rock varnish, is found in deserts and semi-arid regions throughout the world. They are coatings, not weathering products of the substrate, composed principally of clays, oxides, hydroxides, manganese, and iron. The bulk inorganic chemistry has been well characterized<sup>2,4,6-9</sup>. Desert varnish coatings are usually characterized by hardness (typically ~5-6 on the Mohs scale), unique chemistry,

micro-laminations, and botryoidal structures.<sup>4</sup> Varnish coatings are typically black, when rich in both iron and manganese, but they may also be red coatings deficient in manganese. Previously, organic compounds had been thought to be only a small component of varnishes<sup>4,10</sup>.

It has often been suggested that the process of varnish formation may be microbially mediated<sup>3,4,10-16</sup>. Several bacteria, predominately Gram-negative bacteria have been isolated from varnish surfaces that are able to oxidize manganese, however plausible mechanisms for inorganically oxidizing manganese exist<sup>9</sup>. Varnishes form in open environments where there are both microbes and inorganic compounds. Even if inorganic processes can enrich elements and or minerals in coatings, microbes have the ability to enhance this process. However, a third alternative exists: the presence of (bio)organic compounds derived from microbes not living<sup>17</sup>. The pervasive presence of fungi could provide many organic compounds as well as



**Figure 1.** SEM image of the first known reporting of microcolonial fungi from the Sonoran Desert. They were identified as ascomycetes a black yeast and thought to be related to *Torula*. Imaged in 1978 after critical-point drying<sup>1</sup>.

possibly altering the mineralogy of coatings.

### Microcolonial Fungi (MCF)

MCF have a unique ability to survive where other microorganisms are rarely observed<sup>17,18</sup>. The survivability in these extreme conditions sets MCF apart



**Figure 2.** Fungal microcolonies on a quartzite pebble from a Namibia Desert pavement. New colonies are formed by hyphae. The hyphae detach from the colonies as shown in lower right.

from bacteria. Sporulating bacteria have developed survival mechanisms but must expend energy to create spores. MCF in contrast survive and flourish where only few bacteria are present and lichens are unable to survive.

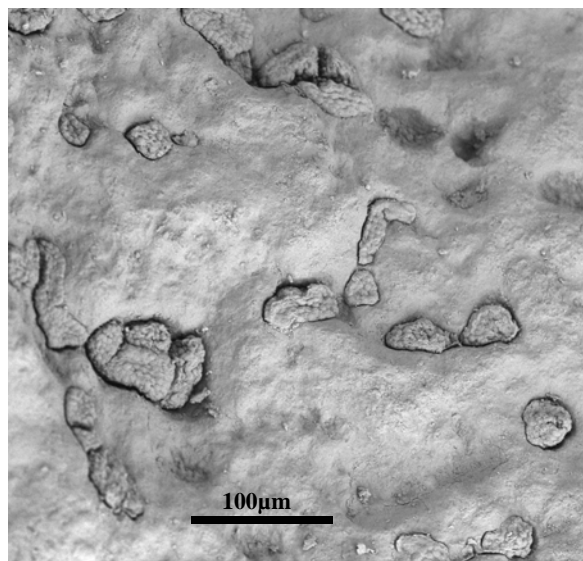
They were first identified in the Sonoran Desert by Perry and Adams in 1977 and subsequently imaged<sup>1</sup> with a SEM (Figure 1). Morphological analysis placed them in the class Ascomycetes. For years they were referred to as blackberries and black globular units until they were more appropriately named microcolonial fungi by J. Staley<sup>19</sup>, and placed in the class Ascomycetes, family *Dematiaceae*.

MCF contain melanin, mycosporines, carotenoids and possibly other unidentified pigments that help protect them from UV light. MCF produce spheroidal clusters ca 100 $\mu$ m (Figure 1-4) on rock and rock coatings in arid and semi-arid deserts. Found worldwide, they have been observed in many of the most arid deserts including the Sonoran, Mojave, Gobi, and Namibian (Figures 1-4). The growth form of MCF is characterized by the formation of restricted microcolonies, which are visible on rock surfaces (Figure 1-3). Vegetative cells have complex intracellular division patterns or a yeast-like budding pattern. Vegetative cells within these microcolonies are highly stress tolerant and extremely long living. They are chemoorganotrophs deriving their nutrients from aeolian-supplied organics such as pollen. They are the predominant biological form observed on desert varnish rock coatings<sup>1,17,19-21</sup>. This unique association has caused some researchers to investigate them as the causative agent for desert varnish<sup>18</sup>.

MCF express a restricted growth pattern of yeast-like development both *in situ* and *in vitro*. Individual microcolonies appear to be size limited, rarely forming clusters exceeding several hundred spheres. It is possible that this size limitation is also a protective mechanism and that an equilibrium between exposure, nutrient uptake and size is reached. New colonies are formed with hyphae runners (Figure 2).

### Rock weathering and (bio)chemicals

Rocks on Earth weather and change through time and microbes play an critical role in this process. This role may be either an active one in forming minerals, as for example, the possible formation of magnetite in microfossils<sup>22</sup> or it may be a passive role such as change in redox conditions, the by-products of metabolic processes, pH changes, or the complexing of ions by extracellular polymeric substances (EPS). Bacterial, Archaeal and fungal cell walls and their associated EPS and spores, interact with mineral surfaces and ions; microbes eventually die, and all of their substances, composed of both living and dead cells are reprocessed and may become part of rock coatings and biominerals such as forsterite or opal<sup>23</sup>.

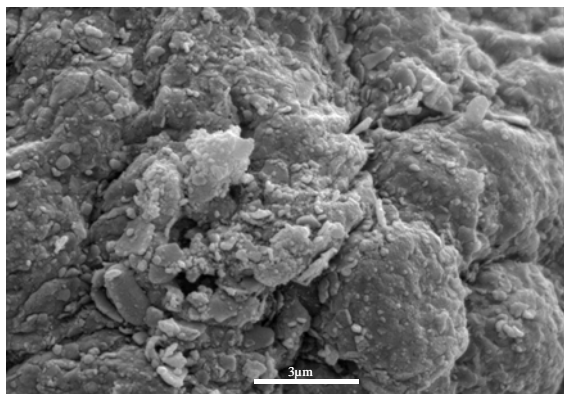


**Figure 3.** MCF lodged in low areas of rock from a stone playa in the Gobi Desert. The image employs backscatter to show that the MCF are enriched in heavier elements (light) as is the varnish coating.

### Organic compounds and desert varnish coatings

Biofilms are composed of EPS and may form during wet periods. Biofilms on exposed rocks may have as little as 1% water<sup>24</sup>. Subaerial biofilms on natural surfaces collect detrital grains in their slime and complex metals. EPS and cell components may contain many chelation sites, which are implicated in mineralization processes<sup>25</sup>. Similarly, polymers from MCF could contribute to weathering and alteration of minerals. Recently, several organic compounds have

been found in desert varnish coatings. Amino acids<sup>26</sup> and DNA<sup>27</sup>. Differing from culture techniques, sequencing of 16S rRNA in coatings shows bacterial fragments not only from Gram-negative but *Alpha* and *Beta Proteobacteria* and others.



**Figure 4.** Close up of Gobi Desert MCF. Note minerals on the surface of the individual MCF cells.

#### Mycosporins Melanins and other Pigments

It has been suggested that spectral changes of rock surfaces that are usually attributed to metal oxidation of iron and manganese might also be staining by microbially produced organic and inorganic pigment absorbed polymers<sup>28</sup> on rock surfaces or in coatings. As the colonies become part of rock coatings the organic and inorganic constituents might also be preserved. Mycosporine-like amino acids (MAAs), frequently possessed by cyanobacteria are present in MCF. However, MAAs are not present in fungibut mycosporins are and have been isolated. Fungal polysaccharides may form stable organomineral complexes with some clays. EPS substances from MCF might also bind with clays, metal oxides or amorphous silica. This accumulation of inorganic substances mixed with organic compounds may be preserved in rock coatings and provide unique chemical markers.

#### Extracellular polymeric substances (EPS)

Large amounts of exopolymeric substances are produced by the colonies. MCF becomes coated with detritus and perhaps the encrustation of these grains and mineral plates is also a protective mechanism. Small wind-blown clay platelets can be oriented by polysaccharides and may provide additional protection from solar energy. It is also possible that the physical nature of the spherical clusters provide a protective mechanism. Fungal polysaccharides may form stable organomineral complexes with some clays. EPS substances from MCF might also bind with clays, metal oxides or amorphous silica. This accumulation of inorganic substances mixed with organic compounds may be preserved in rock coatings and provide unique chemical markers.

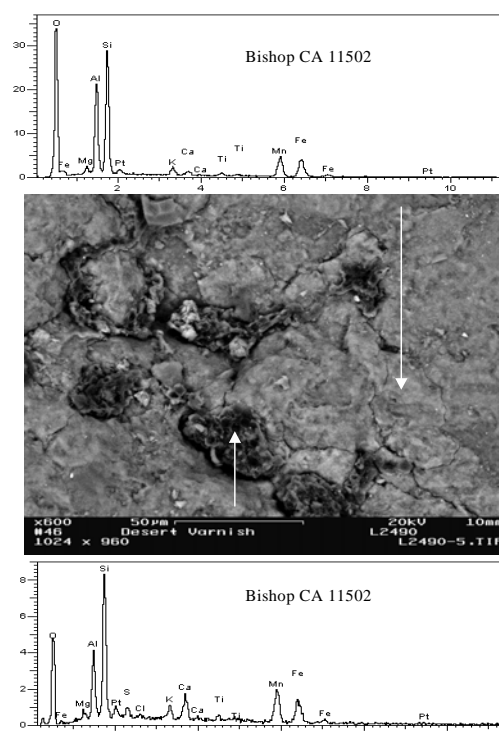
#### Mineralization

The fungal colonies grow very slowly over dozens to hundreds of years. While the colonies are slow growing, they eventually die and subsequently degrade. Younger colonies have less mineral concentrations and were analyzed using scanning electron microscopy

(SEM) with x-ray mapping and EDS. Generally, the elemental composition other than carbon and nitrogen, of young colonies is O > Si > Al > Ca and lesser amounts of Mg > K > Na > Fe > Mn (Figure 5). The idea that MCF and black yeast microcolonies degrade and become part of rock coatings is supported in earlier investigations<sup>1,18,29</sup>. While the elemental chemistry resembles varnish substrates, older colonies also appear to become more mineral rich. As the colonies become part of rock coatings the organic and inorganic constituents might also be preserved.

#### Mineralization of MCF

Our investigations have shown that MCF mineralize over time and as they degrade<sup>17,29</sup>, their appearance and chemistry becomes indistinguishable from varnish coatings (Figure 5). Large amounts of exopolymeric substances are produced by the colonies. MCF become coated with detritus over time (Figure 4). Small wind-



**Figure 5.** SEM with EDS e-ray analysis of a degrading MCF and its desert varnish substrate.

blown clay platelets can be oriented by exopolymeric substances (polysaccharides) and perhaps the encrustation of these grains and mineral plates provides additional protection from light and heat.

## Relevance to Mars

Rock coatings are ubiquitous in arid regions of the world [e.g. 1-3]. It is suspected that rock coatings may also exist on Mars, as suggested by observations on both Viking and Mars Pathfinder landing sites [4]. It is one of NASA's goals to look for the biosignatures of those coatings [4]. Rock varnish coatings may exist on Mars and might sequester evidence of past or present life. Understanding how fungi such as MCF protect themselves from UV, dehydration, and temperature extremes and subsequently become part of mineral coatings, provides an interesting possible analogue for detection of past or present life on Mars.

## Methods- Scanning Electron Microscopy (SEM) and Energy Dispersive Microanalysis System (EDAX)

SEM analysis (Figure 1) was done at the University of Washington using a Joel U-23. Figures 2-4 were imaged at ICBM, University of Oldenburg using a Hitachi S3200N SEM. Figure 5 was imaged at Pacific Northwest National Laboratories (PNNL), a Department of Energy facility in Richland Washington. SEM imaging was performed using a LEO 982 Field Emission Scanning Electron Microscope with EDAX.

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