Reports

Microbial Origin of Desert Varnish

Abstract. *Scanning electron microscopy and energy dispersive x-ray analyses of desert varnish reveal that microorganisms concentrate ambient manganese that becomes greatly enhanced in brown to black varnish. Specific characteristics of desert varnish and of varnish bacteria support a microbial origin for manganese-rich films. Varnish microbes can be cultured and produce laboratory manganese films. Accordingly, natural desert varnish and also manganese-rich rock varnishes in nondesert environments appear to be a product of microbial activity.*

Desert varnish is a natural coating dominated by clays and iron and manganese oxides that are derived from airborne dust and other sources external to the underlying rock (*1*). Black manganese-rich films are the most conspicuous form of desert varnish, but orange iron-rich varnish and chemically intermediate dusky-brown forms are common. A microbial origin of varnish has been suggested but with little verification (*2, 3*). Evidence presented here for a biological origin for black manganese-rich varnish includes environmental relationships, electron microscope observations, energy dispersive x-ray analysis, culturing, and laboratory replication of natural varnish. An understanding of the origin of varnish is of importance to archeologists and geomorphologists who use varnish in relative age determinations, and to paleo-environmental research, geochemical prospecting, and studies of the biogeochemical cycling of manganese (*4*).

Several general factors suggest a biological genesis of the black manganese-rich varnish. (i) Manganese-rich varnish is often conspicuous where water intermittently streams over rock surfaces; these moistened surfaces are favorable for microbial colonization and development. (ii) Varnish often reaches maximum development on porous, easily flushed surfaces such as talus and alluvial breccia deposits that are poor in the organic nutrients favored by fast-growing heterotrophic microbes, which in rich environments out-compete manganese-oxidizing mixotrophs (*5*). Paucity of organic matter encourages the oxidation of divalent manganese by mixotrophic microorganisms (*6*). (iii) Manganese is usually translocated in the divalent state (*7*), but manganese in rock varnish is in the tetravalent state (*8*). In environments that lack abundant organic acids, abiotic oxidation of manganese occurs only above a *p*H of 9 (*9*). Manganese-rich black varnish from 40 different sites in the western United States had near-neutral *p*H values, far below the value necessary for purely physicochemical precipitation. However, these near-neutral *p*H values are favorable to microorganisms that can oxidize and concentrate manganese (*10*). In contrast, manganese-poor orange rock varnishes have high *p*H values, which are unsuitable for manganese-concentrating bacteria. (iv) There are many interactions between clay, the major constituent of desert varnish, and bacteria: bacterial cells can adsorb onto large clay particles; montmorillonite and illite adsorb well onto bacterial surfaces; clays concentrate inorganic and organic nutrients; bacterial respiration is stimulated by the presence of montmorillonite-type clay minerals; clays can mediate chemical fluctuations; and a clay cover helps shield bacteria against dessication, high temperatures, and other harsh environmental conditions (*11*). (v) Organisms that oxidize and fix manganese are ubiquitous in the biosphere, and a biological origin for many manganese deposits is acknowledged (*12*). Similarities between the environments of these deposits and of rock varnish include near-neutral *p*H values, low concentrations of organic nutrients, fairly stable substrates, low ambient concentrations of manganese, aerobic conditions, and budding bacteria observed on the edges of the manganese deposits.

Manganese-concentrating bacteria were observed on desert varnish with scanning electron microscopy. We noted *Metallogenium*-like and other budding bacteria on many varnishes (Fig. 1, A and B) as well as unidentified cocci and rod bacteria. Comparisons of varnish chemistry within a few micrometers of microorganisms with the overall varnish by energy dispersive x-ray analysis indicates active manganese concentration by those organisms that are not entirely obscured by clays (Fig. 1C). The fungi observed on varnish have not noticeably concentrated manganese. This is consistent with the high clay content in varnishes that have lamellate micromorphologies, because clays inhibit fungal respiration (*13*).

Culturing of microorganisms in media rich in organic nutrients and in media poor in organic nutrients revealed that manganese-oxidizing microorganisms are present at all sites tested for biota

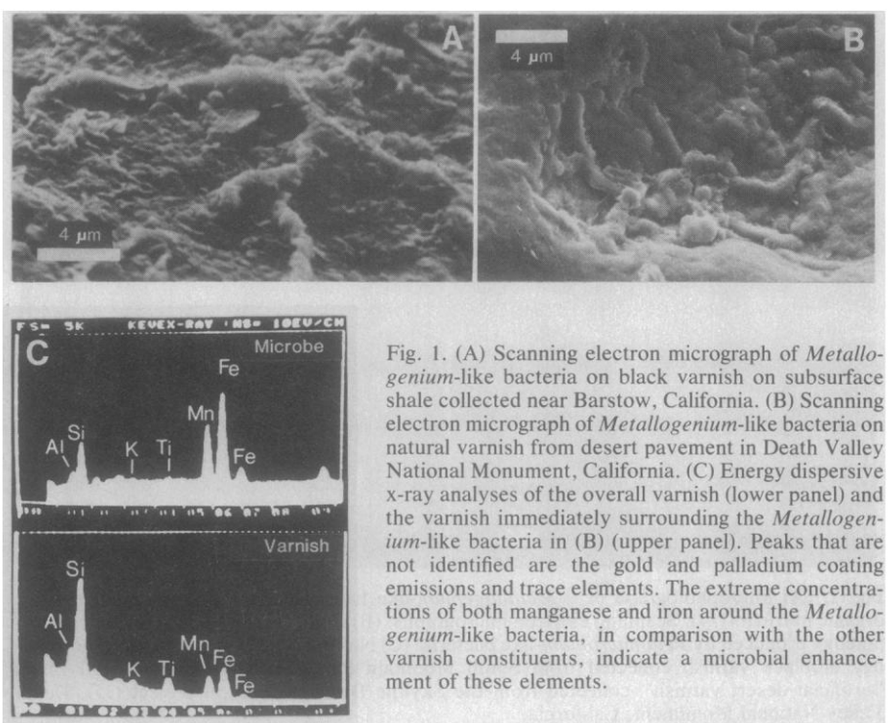

Fig. 1. (A) Scanning electron micrograph of *Metallogenium*-like bacteria on black varnish on subsurface shale collected near Barstow, California. (B) Scanning electron micrograph of *Metallogenium*-like bacteria on natural varnish from desert pavement in Death Valley National Monument, California. (C) Energy dispersive x-ray analyses of the overall varnish (lower panel) and the varnish immediately surrounding the *Metallogenium*-like bacteria in (B) (upper panel). Peaks that are not identified are the gold and palladium coating emissions and trace elements. The extreme concentrations of both manganese and iron around the *Metallogenium*-like bacteria, in comparison with the other varnish constituents, indicate a microbial enhancement of these elements.

0036-8075/81/0911-1245$01.00/0

Table 1. Results of culturing manganese-oxidizing microorganisms from varying environments in media poor in organic nutrients (P) or media rich in organic nutrients (R) (*14*).

Substrate	Number of localities from which the following organisms were cultured:							
	Metallogenium-like		*Pedomicrobium*-like		Unidentified bacteria		Unidentified fungi	
	P	R	P	R	P	R	P	R
Arid black surface varnish	13 (13)*	0 (8)	13 (13)	7 (8)	13 (13)	8 (8)	13 (13)	8 (8)
Nonarid black surface varnish	3 (4)	0 (2)	2 (4)	1 (2)	2 (4)	2 (2)	4 (4)	2 (2)
Arid dusky-brown surface varnish	0 (2)		2 (2)		1 (2)		2 (2)	
Arid black crack varnish	3 (3)	1 (1)	3 (3)	1 (1)	2 (3)	1 (1)	3 (3)	1 (1)
Arid unvarnished rock surface	2 (10)	0 (8)	4 (10)	2 (8)	1 (10)	3 (8)	7 (10)	7 (8)
Nonarid unvarnished rock surface	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	1 (1)	1 (1)
Arid soil surface	0 (2)	0 (1)	1 (2)	1 (1)	0 (2)	1 (1)	2 (2)	1 (1)
Arid airborne fallout	3 (8)	0 (8)	6 (8)	3 (8)	2 (8)	8 (8)	8 (8)	7 (8)

*Numbers in parentheses are the number of localities sampled.

(Table 1). The dominant recognizable manganese-rich varnish bacteria capable of surviving in nutrient-poor media are *Metallogenium*- and *Pedomicrobium*-like microbes (Fig. 2A).

We produced varnish in the laboratory by first isolating *Metallogenium*- and *Pedomicrobium*-like bacteria from natural varnish sites on a medium poor in organic nutrients (*14*). Then the organisms were inoculated in a liquid medium; Wyoming bentonite was added at *p*H values of 6.8, 7.5, and 8.5. After several months, *Metallogenium*-like bacteria produced nodules with a lamellate micromorphology. *Pedomicrobium*-like bacteria produced smaller accretions with a semi-botryoidal micromorphology in the neutral culture. In a third stage, the same microorganisms were inoculated in a similar neutral medium, and sterilized gneiss chips were added. Rock varnishes developed in about 6 months. A control, consisting of the same medium but lacking transplanted microorganisms, failed to produce oxide concentrations. Scanning electron microscopic observation indicates little morphological difference between cultured varnish and natural rock varnish on outcrops in desert regions (Fig. 2, B and C).

The varnish we produced contains about 80 percent clay, derived from the bentonite in the medium, along with unidentified manganese and iron oxides and hydroxides. The hardness of the cultured varnish is under 2.5 on the Mohs scale instead of the 4.5 to 5 that is typical of black desert varnish, because wetting and drying cycles, important in fixing manganese in natural varnishes, were omitted to maintain aseptic conditions. The biologically produced laboratory varnish does not contain the manganese-rich and manganese-poor laminations seen in some natural varnishes (*3*, *4*), because the experimental conditions were not alternately favorable and unfavorable to manganese enhancement.

Although the microbial origin of desert varnish does not exclude the possibility that natural varnish films can form without biological assistance, a purely physicochemical origin seems unlikely, and the field observations support a microbial origin. Manganese-rich varnishes, which begin as microscopic black flecks that grow both laterally and vertically into a uniform dark coating, are found in environments as dry as the Sinai peninsula and as moist as the Canadian Rockies. Their irregular development on rock surfaces resembles biogeochemical deposits, not physicochemical coatings. Physicochemical manganese enhancement requires high *p*H values, but the measured *p*H values of the classic black varnishes are near neutral, and neither varnish films nor manganese nodules formed at alkaline *p*H values (*p*H 7.5 and 8.5) in the laboratory replication experiment.

Elvidge and Moore (*15*) proposed a purely chemical model of varnish formation, supported by "artificial desert varnish" formed by precipitating iron and manganese oxides and hydroxides onto rock surfaces. This artificial varnish contains little clay, a dominant constituent of natural varnish (*1*), and under the scanning electron microscope its crystalline structure bears no resemblance to natural varnish (Fig. 2, C and D). To

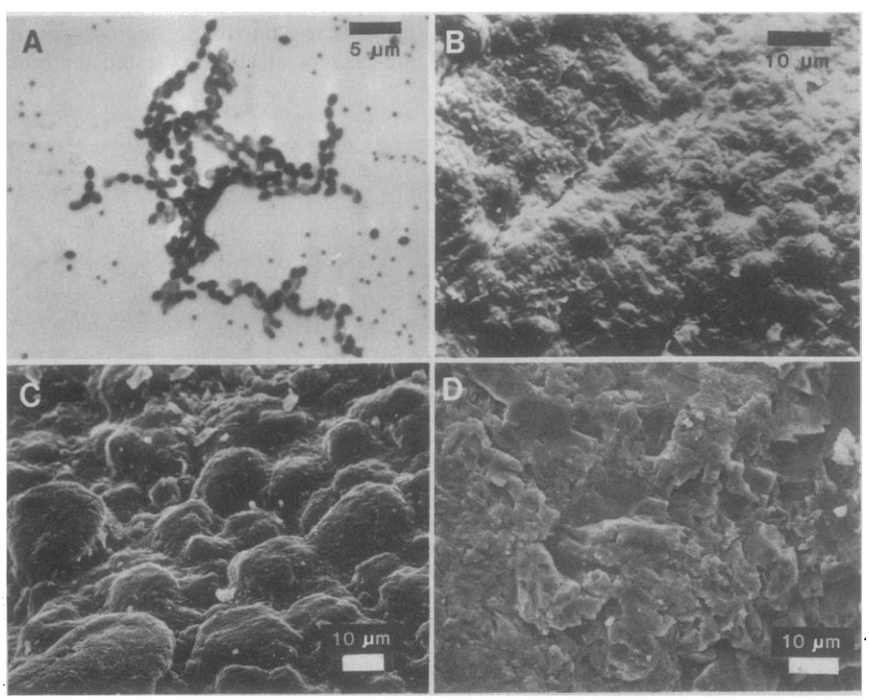

Fig. 2. (A) Microphotograph of a *Metallogenium*-like bacteria cultured on medium poor in organic nutrients (*14*). Scanning electron micrographs: (B) Cultured semi-botryoidal micromorphology produced by *Pedomicrobium*-like bacteria. (C) Natural semi-botryoidal micromorphology of black varnish collected from South Mountain Park, Phoenix, Arizona. (D) Black "artificial desert varnish" collected from the Skyline Drive restoration project (*15*), Death Valley National Monument, California.

explain juxtaposed areas of manganese-poor orange varnish (from rock undersides and closed cracks) and manganese-rich black varnish (from rock surfaces and open cracks), the physicochemical model requires that water saturation and mobilization of manganese from the orange to the black varnish alternate with oxidizing conditions to fix the manganese. Under these conditions, swelling minerals would be modified to chlorite (*16*), but varnish clay minerals are composed of illite and montmorillonite (*1*), not chlorite. Our biological model associates orange varnishes with microenvironments where alkaline dust or soil is in contact with rock surfaces and causes them to be inhospitable to the manganese-concentrating microbes that are active in near-neutral *p*H environments, where black varnish forms. Other difficulties with the physicochemical model of varnish formation include the widely documented presence of iron-manganese films in nonarid environments and the noticeable absence of manganese-rich varnish around lichens on desert rocks, where the lichens generate the required *E*h-*p*H changes.

It appears that the phenomenon of desert varnish may be added to the expanding list of manganese concentrations that have been found to have a microbial origin. The irregular onset of bacterial colonization accounts for the puzzling inconsistency in varnish development from stone to stone on desert pavements. Thus it is hazardous for archeologists to use the degree of varnish development as the sole criterion to estimate the relative ages of individual stone artifacts in lithic assemblages.

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19 May 1981

Oxygen-18 Enrichment of Planktonic Foraminifera Due to Gametogenic Calcification Below the Euphotic Zone

Abstract. *Empty shells of spinose planktonic foraminifera on the seabed are significantly enriched in oxygen-18 as compared with the shells of their living counterparts in surface waters. This enrichment is due to gametogenic calcification, which extracts calcium carbonate from deeper and colder waters as the shell sinks below the euphotic zone.*

Emiliani and subsequent investigators have amply demonstrated the usefulness of the oxygen isotopic composition ($\delta^{18}O$) of planktonic foraminifera as a tool for deciphering Cenozoic paleoenvironmental conditions (*1*). Yet the fundamental problem of whether these organisms secrete $CaCO_3$ in isotopic equilibrium with ambient water is still a matter of debate. Two different approaches have been used to estimate the extent to which isotopic equilibrium is achieved in planktonic foraminifera. The results obtained from the use of these two procedures have been contradictory.

The first approach, based on the study of well-dated Holocene surface sediments, yielded $\delta^{18}O$ values that were interpreted as indicating that the shell $CaCO_3$ was in isotopic equilibrium or close to isotopic equilibrium with surface-water conditions (*2–5*). These investigators assumed that the relationship between foraminiferal shell $\delta^{18}O$, seawater $\delta^{18}O$, and water temperature was the same as that for mollusks (*6*). The second approach is based on the isotopic analysis of plankton-tow specimens (*7, 8*). A plot of isotopic values versus temperature for most spinose planktonic foraminifera shows that the $CaCO_3$ of their shells is not in isotopic equilibrium with surface-water conditions but is slightly impoverished in ^{18}O when examined in light of the paleotemperature scale of Epstein *et al.* (*6*).

We recently studied the $\delta^{18}O$ variations in two common tropical spinose planktonic foraminiferal species, *Globigerinoides ruber* and *G. sacculifer*, in the upper 200 m of water of the subtropical and tropical Indian Ocean (*9*). With these measurements it was possible to evaluate the ^{18}O fractionation between foraminiferal carbonate and seawater as a function of temperature, because seawater temperature variations in this region are small and the life-span of *G. sacculifer* is on the order of a few weeks (*10*). The results (Fig. 1) show an excellent correlation between the calcite isotopic enrichment ($\delta^{18}_{foram} - \delta^{18}_{water}$) and the temperature of the surficial mixed layer (*11*). These data indicate that most of the calcite secretion occurred in this mixed layer (*12*). This result, which is consistent with the model of Fairbanks and Wiebe (*8*), suggests that foraminifera grow in the chlorophyll maximum since its depth is governed by the noticeable density increase which occurs at the base of the surficial mixed layer. Our results indicate that *G. ruber* and *G. sacculifer* could preferentially develop in the upper part of the chlorophyll maximum. Moreover, the $\delta^{18}O$ values of *G. ruber* and *G. sacculifer* shells are both isotopically lighter than equilibrium values by about 0.6 per mil (*6*), and the departures from equilibrium remain constant in the temperature range from 20° to 31°C.

Globigerinoides ruber shells with a diameter smaller than 250 μm have the

0036-8075/81/0911-1247$01.00/0