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Diversity of Microorganisms within Rock Varnish in the Whipple Mountains, California†

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Rock varnish from Arizona's Whipple Mountains harbors a microbial community containing about 10^8 microorganisms g^{-1} of varnish. Analyses of varnish phospholipid fatty acids and rRNA gene libraries reveal a community comprised of mostly *Proteobacteria* but also including *Actinobacteria*, eukaryota, and a few members of the *Archaea*. Rock varnish represents a significant niche for microbial colonization.

Rock varnish (also known as desert varnish) is a dark, thin (usually 5 to 500 μm thick), layered veneer composed of clay minerals cemented together by oxides and hydroxides of manganese and iron (11, 20, 56, 63, 64). Nineteenth century references to rock varnish include those of Humboldt (42) and Darwin (14). Modern observations of varnish were initiated with the studies of Laudermilk (49) and Engel and Sharp (25); however, despite decades of study, the nucleation and growth mechanisms of rock varnish remain a mystery (11, 18, 37, 44, 57, 58).

Mn(II) is the soluble form of manganese that is available to organisms. It is stable between pH 6 and 9. Mn(III) and Mn(IV) primarily form insoluble oxides and oxyhydroxides. Microbial Mn(II) oxidation could thus result in the formation of manganese oxides as mineral phases in varnishes, as occurs in other environments (23, 39). Like manganese oxidation, iron oxidation (95) occurs at the exterior of the cell surface. Iron hydroxides are often deposited on the remains of biogenic structures (24). The extracellular deposition of ferric hydroxides is a way for iron-oxidizing organisms to prevent encrustation in iron oxide precipitates (88). Such precipitates might be incorporated in a varnish matrix through the activities of iron-oxidizing bacteria.

Rock varnish may hold a record of the microclimate in which it is found (7, 10, 11, 30), a hypothesis that has been questioned previously (67, 68). Some investigators suggest that rock varnish may harbor a historical record of important environmental processes such as long-term climate change (51). Bao et al. (7) studied preservation of atmospheric signatures in rock varnish and concluded that rock varnishes or other surface deposits may provide a record of paleoclimatic information and sulfur biogeochemical cycles. As a deposit of submicrometer layering, rock varnish may record the activity of dust storms, moisture and temperature fluctuations, biological activity, and the occurrence of fires over thousands of years. Rock varnish forms very slowly at rates thought to be between <1 to about 40 μm per 1,000 years (50), thus archeologists have been interested in dating the age of varnishes to place petroglyphs etched into varnish by ancient cultures into their full historical context (22, 90). Unfortunately, radiocarbon dating of varnish has proven difficult, and results must be used with caution (7, 9, 17, 22, 62).

It has been suggested that varnish or varnish-like materials may exist on Mars (2, 36, 44, 65). If so, varnish may be a niche for colonization by extraterrestrial life forms such as bacteria. Microorganisms are ubiquitous within varnishes on Earth. Thus, the study of Earthly varnishes may lead to the proper design of experiments in coming decades for detection of life on other planets. For example, iron and manganese oxidation by microbes cultivated from varnish has been extensively investigated (1, 19, 26, 33, 43, 46, 47, 54, 56, 77, 78, 80, 83). Perry et al. (59) observed a variety of amino acids in rock varnish and suggested that this is evidence for an intimate association of bacteria with the varnish material. A large variety of bacterial genera have been cultivated from rock varnish. These include *Bacillus* (43, 56), *Geodermatophilus*, *Arthrobacter*, *Micrococcus*, *Curtobacterium*, *Cellulomonas* (43, 48), *Pedomicrobium*, and a *Metallogenium*-like strain (19, 20). Eppard et al. (26) isolated several actinomycete species including *Geodermatophilus*. Staley et al. (78, 79) observed microcolonial fungi on rock varnish. Taylor-George et al. (83), Gorbushina et al. (32), and Perry (55) have provided evidence that these fungi may be involved in the formation of varnish.

To our knowledge, only two studies to date have investigated the microbial phylogenetics of varnish (26, 57). Eppard et al. (26) used 16S rRNA gene sequencing techniques to examine phylotypic characteristics of bacteria that had previously been cultured from various rock varnishes. Kuhlman et al. (48) employed 16S rRNA gene sequencing to identify several UV light-resistant bacteria isolated from rock varnish obtained in the Whipple Mountains of the U.S. Mojave Desert. These strains included representatives of the genera *Geodermatophilus*, *Arthrobacter*, *Curtobacterium*, and *Cellulomonas*. There are several reports of "microcolonial" fungi living within rock varnish or on the surfaces of rocks in hot, dry deserts (45, 55, 56,

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78, 79, 83). The first scanning electron microscope images of such microcolonial fungi were reported from samples collected in the Sonoran Desert in 1978 (55). The fungi observed were thought to be representatives of the ascomycetes. It has been suggested that these fungi are the predominant biological forms observed on rock varnish coatings (32, 55, 60, 79, 80) and may be involved in the formation of varnish (83).

Most organisms in nature are refractory to cultivation (4, 8, 85, 89); therefore, many organisms that occupy the varnish habitat remain to be discovered. We report here the characterization of microbial rock varnish communities from the Whipple Mountains of the American Sonoran Desert via preparation of 16S and 18S rRNA gene clone libraries. These analyses are leading us to a greater understanding of the microbial diversity within these communities and their relevance to the possible occurrence of microbial life on other planets, such as Mars, where life forms face exposure to high fluxes of damaging UV light (13) and extremes of temperature and desiccation, such as are seen in locations on Earth where rock varnishes form. Microorganisms such as those that live or survive in varnish may also be potential forward contaminants on spacecraft structures (73).

Varnish samples were collected from alluvial fan deposits surrounding the Whipple Mountains, California. The Whipple Mountains lie west of Parker, Arizona, along the eastern boundary of the Mojave Desert. Previous studies conducted at the site demonstrated relatively thick varnishes (5 to 100 μm thick) on various rock types (5). Varnished rocks and surrounding soil samples were collected in February 2003 when the ground was still wet from winter rains. Only clasts with thick coatings (greater than 50 μm) on relatively large, flat surfaces were collected. It was critical that the samples be collected as aseptically as possible. Sample purity can never be completely verified, since it is very difficult to characterize contamination associated with local wildlife. However, contaminant potential was monitored during phylogenetic investigations by preparing control DNA libraries from soil adjacent to the point of collection of varnished rock. Varnished rocks were approached from the downwind direction, photographed in situ, picked up at arm's length using sterile gloves, and placed within sterile Whirl-pak bags large enough to hold entire rocks, and bags were sealed. Loose dirt on the undersides of the varnished rocks was brushed off in the field. The bags were then wrapped in protective material to prevent damage and subsequent contamination. The varnish was harvested from the host rock in a laminar flow bench. A Dremel grinding tool with flame-sterilized coarse bit was used to grind the varnish from the host rock into a sterile container. This approach minimized possibilities for atmospheric contamination of the varnish and ensured that the majority of bacteria removed came from within the varnish matrix.

Microbial biomass for enumeration and phospholipid fatty acid (PLFA) analysis was obtained from powdered rock varnish (0.1 g) obtained by aseptically grinding the varnish from the rock surface and then adding the varnish to 1 ml of sterile, double-distilled water (18 MΩ MilliQ water) in a 1.5-ml sterile microcentrifuge tube. Serial 1:10 dilutions were made, giving a range of dilutions from 10^{-1} to 10^{-3}. The dilution samples were fixed with 2% ice-cold, high-performance liquid chromatography-grade methanol, vortexed until the sample appeared homogeneous, and incubated at room temperature for 30 min. The samples were stained with 60 μl ml^{-1} of a stock 4′,6′-diamidino-2-phenylindole (DAPI) or acridine orange (50 μg ml^{-1}) solution. DAPI-stained samples were incubated at room temperature for 30 min in the dark before filtration. The most appropriate volume for analysis was determined to be 500 μl of the 10^{-3} dilution. This dilution contained >25 but <250 cells per field and had a low enough mineral content to both count cells in about one microscopic plane and not have cells obscured by the varnish minerals. Samples were filtered onto 25-mm Millipore Isopore 0.22-μm pore-size black polycarbonate filters (Millipore, Billerica, Mass.) with Whatman 25-mm GF/F filters used for support. Fluorescing cells were counted on a Zeiss Research epifluorescence microscope equipped with an Osram xenon short arc photo optic lamp XBO 75W and Chroma no. 31000 filter set for DAPI/Hoechst/AMCA (Zeiss, Inc., Thornwood, N.Y.). The mean number of fields counted per sample ($n = 15$) was 57.16. The standard deviation per sample was 7.48 fields. The average DAPI direct count of the rock varnish was 9.0×10^7 cells g^{-1} (standard deviation = 1.2×10^7). There was no difference between DAPI and acridine orange direct counts.

PLFA analyses were carried out by Microbial Insights, Inc. (Rockford, Tenn.). Total lipids were extracted (91) and the polar lipids separated by column chromatography (35). The polar lipid fatty acids were derivatized to fatty acid methyl esters, which were quantified using gas chromatography (69). Fatty acid chemical structures were verified by chromatography/mass spectrometry and equivalent chain length analysis. PLFA are essential components of the membranes of all cells except those of the *Archaea*, so their profiles allow for examination of most of the important members of many microbial communities. Since phospholipids break down rapidly upon cell death in environments examined thus far (91, 92), PLFA analysis should be also an accurate method for determining the amount of viable microbial biomass in an environment (92). The sum of the PLFA expressed in pmol is proportional to the number of cells, sensu lato. The proportion used here was 20,000 cells $pmol^{-1}$ total PLFA (Microbial Insights, Inc., Rockford, TN). The number of cells within the microbial community from a sample of rock varnish was thus determined from its PLFA content (31.253 pmol g^{-1} [dry weight]). The microbial content of the varnish was $\sim 10^8$ cells g^{-1} (dry weight), in excellent agreement with the direct microscopic counts of $9.0 \times 10^7 \pm 1.2 \times 10^7$ cells g^{-1}. The PLFA profile of biomass within the varnish indicated a relatively simple community structure primarily composed of monoenoic PLFA, indicative of *Proteobacteria* with smaller but significant populations of *Actinobacteria* and *Eukarya* (Table 1).

Three rRNA gene libraries from the Whipple Mountains rock varnish community DNA and two control libraries from soil lacking visible varnish adjacent to the varnished rock were generated using DNA isolated directly from 0.5 g ground varnish and 0.5 g adjacent, unvarnished soils with an Ultraclean soil DNA kit (Mo Bio, Solana Beach, Calif.). We were careful to avoid collecting rock fragments with our soil samples. Varnish 16S rRNA gene libraries were prepared for *Bacteria* and *Archaea*, and an 18S rRNA gene library for was prepared for *Eukarya*. Genes for 16S and 18S rRNA genes were PCR amplified from purified DNA using primers specific for *Bacteria*

TABLE 1. PLFA analysis of Whipple Mountain rock varnish[a]

Parameter (unit)	Value
Biomass (pmol PLFA g^{-1} dry weight)	31,253
Cells (g^{-1} dry weight)	6.25×10^8
% Gram negatives and firmicutes (terminally branched, saturated PLFA)	2.4
% *Proteobacteria* (monoenoic PLFA)	81.8
% Actinomycetes and sulfate-reducing bacteria (mid-chain branched, saturated PLFA)	2.3
% General (normal saturated PLFA)	13.0
% Eukaryotes (polyenoic PLFA)	0.5

[a] PLFA signatures of anaerobic metal reducers were not observed (branched monosaturated PLFA); physiological status is nonstressed (cyclopropyl acid/*cis* acid ratios and *trans*/*cis* ratios were both 0.00).

(338f/907r) (84), *Archaea* (A21f/A958r) (29), and *Eukarya* (NS3/NS8) (94). Clone libraries were prepared using a TA cloning kit (Invitrogen, Carlsbad, Calif.); 100 to 200 clones were prepared for each library. To better assess the diversity of the varnish community with the available resources, analysis of more partial-length sequences was favored over analysis of fewer, full-length sequences. Partial sequences are a reasonable compromise for the assessment of diversity which does not require precise taxonomic placement (12, 76). Each clone library was further classified by placing clones into restriction fragment length polymorphism (RFLP) restriction group patterns by digesting the DNA of individual clones with HhaI followed by electrophoresis on a 2% SeaPlaque agarose gel. Banding patterns were compared (34) by visual inspection of restriction digests of each clone, and a representative clone from each distinct RFLP group was sequenced. Sequencing was performed using an ABI Prism 3100 automated DNA sequencer (Applied Biosystems, Foster City, Calif.). Individual sequencing reactions were performed using the T7 and T3 primers, and a contiguous sequence was generated using ContigExpress software (Vector NTI-Informax; Invitrogen Corp., Carlsbad, Calif.). Clone sequences were evaluated using the Chimera Check program implemented in the Ribosomal Database Project (52). Two chimeras were found in both the *Bacteria* and *Eukarya* sequence libraries, while one chimera was found in the surrounding soil bacterial library as well as the varnish-associated *Archaea* library. These chimeras were excluded from further analysis. No chimeras were found in the surrounding soil *Archaea* library. Nonchimeric sequences were matched by a standard BLAST search (3) within the NCBI GenBank database to determine closest matches. Sequences were submitted to GenBank for rock varnish *Bacteria*, *Archaea*, and *Eukarya* and nonvarnished soil *Bacteria* and *Archaea* (see "Nucleotide sequence accession numbers" below).

Nine distinct RFLP groups obtained from 107 bacterial clones, 13 groups from 57 *Eukarya* clones, and two groups from 76 *Archaea* clones were analyzed. Rarefaction analysis (see Fig. S1 in the supplemental material) of the *Archaea* and the surrounding soil *Archaea* libraries reached saturation, indicating these two communities were well sampled and of low diversity. In contrast, rarefaction curves for the *Bacteria*, *Eukarya*, and surrounding soil *Bacteria* did not yet reach a plateau, indicating these populations were even more diverse than our initial phylogenetic analyses suggest.

The phylogenetic relationships of bacterial and eukaryotic rRNA gene sequences are shown in Fig. 1 and 2, respectively. The closest database relatives of all sequences were chosen as reference sequences for phylogenetic analysis based on BLAST (3) comparisons. The 16S and 18S rRNA gene sequences were aligned using MegAlign (DNASTAR, Inc., Madison, Wis.). The model for the 16S data set (GTR+I+Γ) was selected based on relative goodness of fit. The relative goodness of fit of 16 alternative models of sequence evolution was compared using the likelihood ratio test (81). The model for the 18S data set (TrNef+I+Γ) was selected using DT-ModSel (53). Maximum likelihood searches were conducted using PAUP* (version 4.0b10) (82) with stepwise addition (10 random sequence additions) and tree bisection-reconnection branch swapping. Nodal support was estimated using bootstrap analysis (100 replicates) under the appropriate model. Posterior probabilities (10^7 generations, sampling every 100 generations) were determined under the GTR+I+Γ model of sequence evolution using MrBayes (MRBAYES 3.0b4) (40). Multiple independent runs were performed, and the parameter values were visually inspected to ensure convergence. The diversity of the ribotypes in each clone library were compared by rarefaction analysis calculated in DOTUR (72).

PLFA analysis (Table 1) provides indirect evidence that these bacteria are physiologically active (see below). It is possible that products produced by varnish bacteria (e.g., pigments) may play important roles in varnish morphogenesis. We did not observe organisms known to be involved in metal oxidation. Since rarefaction analysis (see Fig. S1 in the supplemental material) for the bacterial varnish clone libraries failed to reach saturation, it is possible that ribotypes related to metal oxidizers were simply not sampled from the larger, diverse varnish community. It is possible that ribotypes related to known metal oxidizers may have been found if the total diversity within the varnish had been assessed. Within the 16S rRNA gene libraries of the uncultivated microbial rock varnish community, we observed a number of sequences related to known environmental strains but not to known metal oxidizers. rRNA gene analyses are not sufficient in themselves to determine which organisms are an integral part of the rock varnish community over geologic time scales but do give an indication of the phylogenetic groups that can occupy this niche at a particular moment in time. We previously confirmed by pure culture techniques that viable radiation-tolerant bacteria are present in Whipple Mountains rock varnish. We isolated five UV-resistant bacterial cultures on tryptic soy agar plates after exposure to UV irradiation treatment. All of these strains were found to be related to known strains of *Actinobacteria* lineages (48). However, since most members of the varnish microbial community observed by molecular techniques were not cultivated (Fig. 1 and 2; see Tables S2 and S3 in the supplemental material), it is possible that these organisms are indeed able to oxidize iron and/or manganese. We cannot know for certain unless the observed strains are eventually cultivated for physiological studies or direct in situ techniques are developed to observe microbe-associated metal oxidation within the intact varnish matrix.

Within the 16S rRNA gene libraries of the uncultivated microbial community of Whipple Mountains rock varnish, we observed a number of sequences related to known environmental strains. Among the most common sequences observed

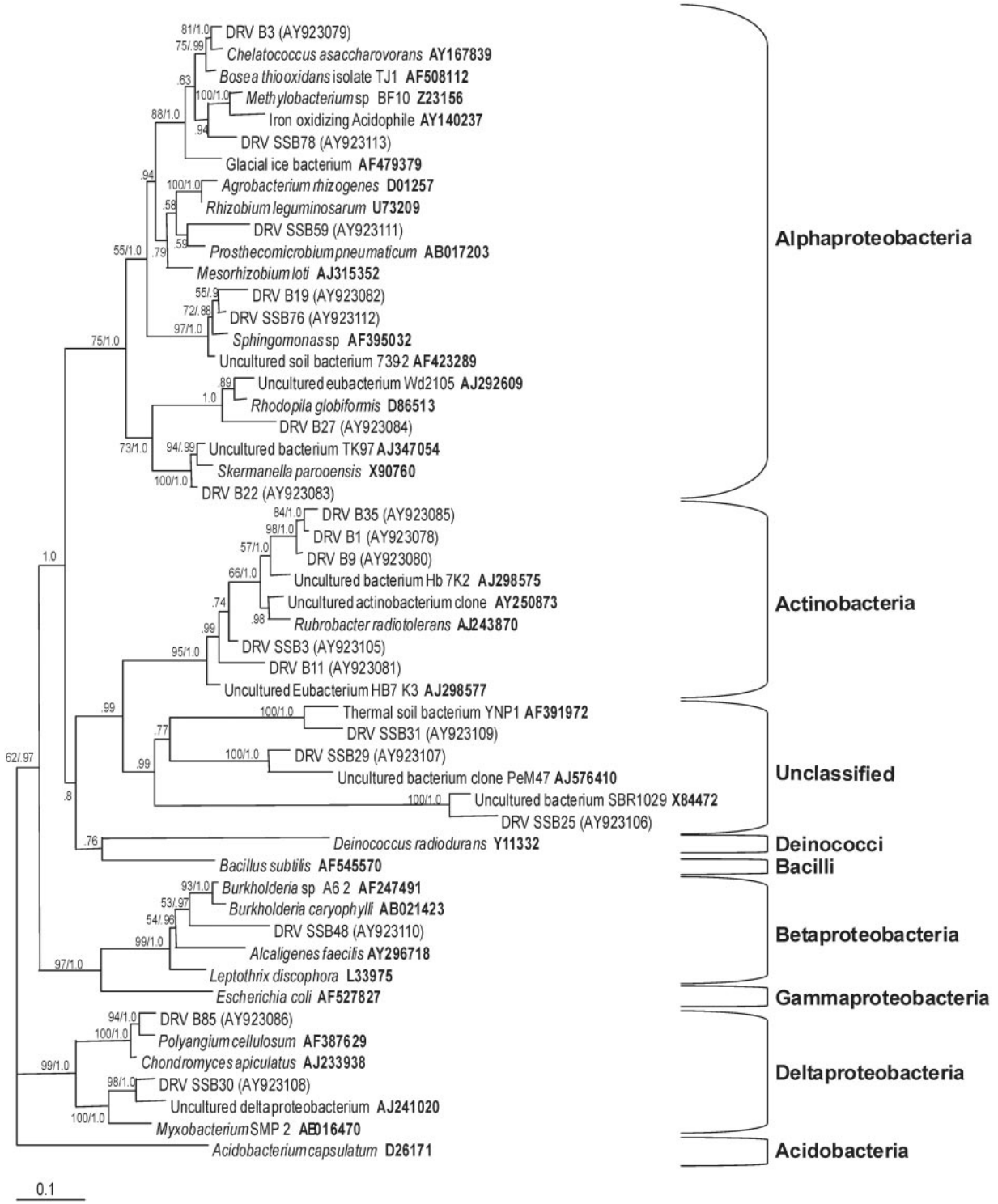

FIG. 1. Phylogenetic tree showing the relationships of bacterial 16S rRNA gene sequences to one another and to known microbial groups using 1,573 aligned characters. Nodal support values with bootstrap support (100 replicates) and posterior probabilities (10^7 generations), respectively, are listed next to the branches of the unrooted maximum likelihood tree (ln L = −4,404.18186 [TrNef+I+Γ model]; p_{inv} = 0.512289; α = 0.599910). Line length, 0.1 substitutions/site; DRV, desert rock varnish isolate. Accession numbers are shown in boldface type or in parentheses.

were those closely related to the genus *Rubrobacter* (clones DRV B1, DRV B9, and DRV B35) (see Table S2 in the supplemental material). Van de Kamp et al. (87) reported in an abstract that actinobacterial 16S rRNA gene sequences were amplified by PCR from rock varnish obtained near Socorro, New Mexico. *Rubrobacter* species are of actinobacterial lineage and are known to inhabit masonry and lime wall paintings, where they cause a rosy discoloration (71). Diverse, yet-

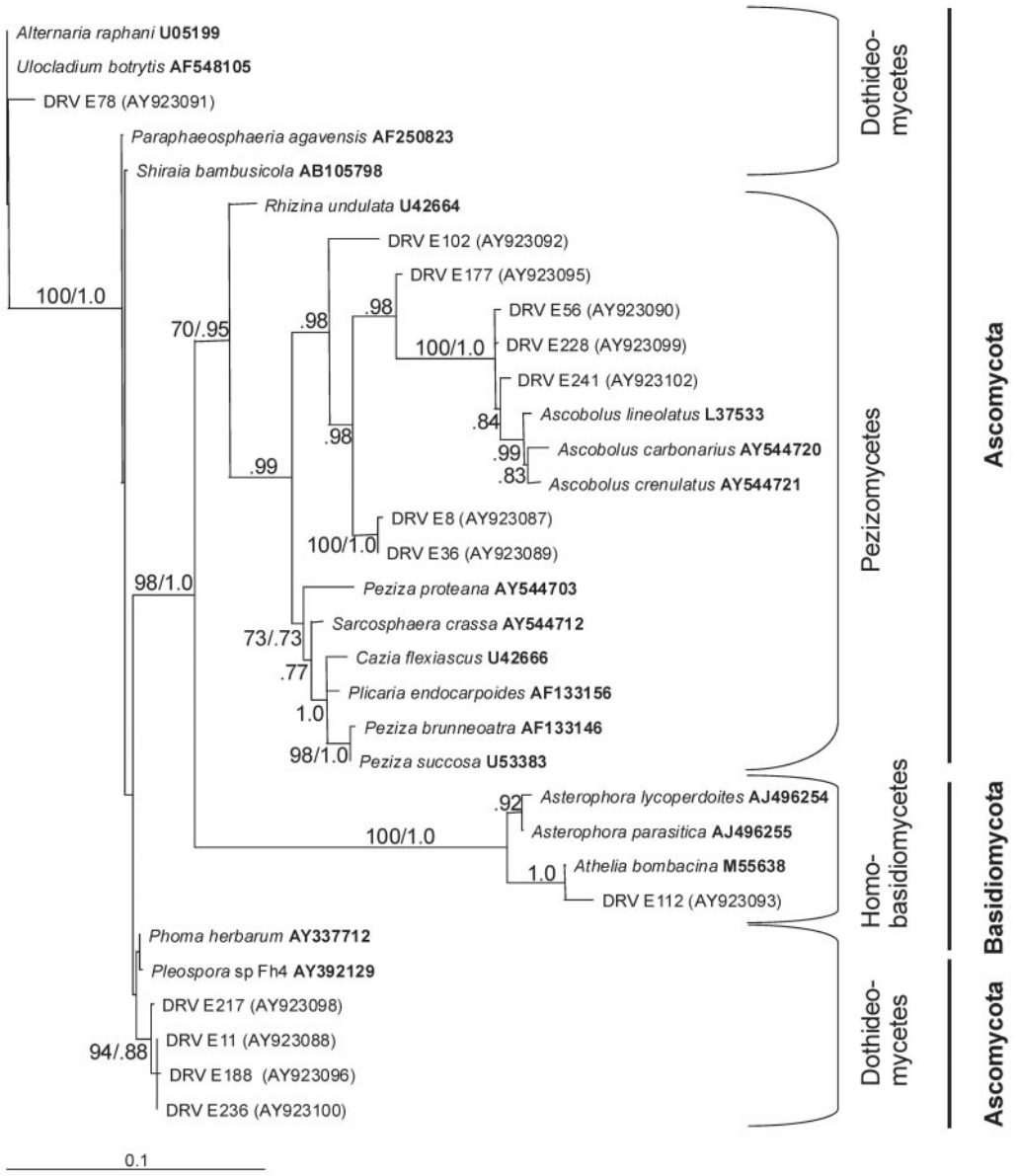

FIG. 2. Phylogenetic tree showing the relationships of eukaryotic 18S rRNA gene sequences to one another and to known microbial groups using 1,201 aligned characters. Nodal support values with bootstrap support (100 replicates) and posterior probabilities (10^7 generations), respectively, are listed next to the branches of the unrooted maximum likelihood tree (ln L = −4,404.18186 [TrNef+I+Γ model]; p_{inv} = 0.512289; α = 0.599910). Line length, 0.1 substitutions/site; DRV, desert rock varnish isolate. Accession numbers are shown in boldface type or in parentheses.

to-be-cultured members of the *Rubrobacter* subdivision are also widespread in Australian arid soils (38). The sequences of uncultivated bacteria we observed are related to *Rubrobacter radiotolerans* and *Rubrobacter taiwanensis*, bacteria known for their exceptional resistance to gamma radiation (28). One rock varnish-inhabiting genus observed in clone DRV B85 was related to *Chondromyces* or *Polyangium* (both 96% similarities). These genera are in the lineage of *Myxococcales* (75), which contains many members known to inhabit extreme environments (15) and to make bioactive substances (66).

A likely representative of the genus *Sphingomonas* (clone DRV B19) was observed in the uncultivated rock varnish community. This genus inhabits environments such as the deep subsurface (6) and has recently been observed within an endolithic community in Antarctica where it was found within translucent gypsum crusts on the surface of ice-free sandstone boulders (41). This, like rock varnish, represents an environment exposed to high levels of UV irradiation. The genus *Sphingomonas* contains many representatives that are able to degrade compounds such as polynuclear aromatic or halogenated molecules that might be present at low concentrations in the atmosphere (93).

Clone DRV B27 is a potential member of the genus *Rhodopila* (93% similarity), an obligatory aerobic, bacteriochloro-

phyll *a*-containing bacterium genus. While 93% similarity is not sufficient for unequivocal identification at the genus level, where 95% is the commonly accepted, albeit contentious, standard (4, 27, 70, 72), this GenBank match hints at a possible mechanism of bacterial survival in rock varnish. The environment of rock varnish would obviously be conducive to the development of photosynthetic life forms such as *Rhodopila*.

As is normal for this type of investigation, several sequences (Fig. 1) (clones DRV B9, DRV B11, DRV B19, DRV B27, and DRV B35) showed similarities to uncultured bacteria. This is confirmation that, as in other environments, many or perhaps most of the bacteria in rock varnish have never been cultured.

To our knowledge, PLFA analyses of rock varnish microbial communities have not been performed previously. Lipid analyses are a very useful supplement to DNA analyses when characterizing a complex microbial community such as the one studied here (21, 61). PLFA analysis of the rock varnish microbial community (Table 1) supports the conclusions made from our rRNA gene analyses. The PLFA profiles of biomass within the varnish indicate a relatively simple community structure primarily composed of monoenoic PLFA, indicative of *Proteobacteria*. The PLFA profiles also show the presence of a smaller but significant population of *Actinobacteria* (mid-chain branched saturated PLFA). *Proteobacteria* and *Actinobacteria* are of particular interest in that they represent organisms that have the ability to utilize a wide range of carbon sources and adapt quickly to environmental change, such as would be the situation in the rock varnish environment. PLFA also indicated the presence of eukaryota (e.g., fungi), and this was confirmed by 18S rRNA gene fingerprinting (Fig. 2; see Table S2 in the supplemental material).

The membranes of microorganisms adapt to the changing conditions of an environment, particularly under stressful conditions. These changes are reflected in the profiles of PLFA, with a change of *cis* fatty acids toward more *trans*-configured acids (35). Also, the *Proteobacteria* respond to starvation by making cyclopropyl (35) or mid-chain branched fatty acids (86). The "physiological status" of a microbial community can thus be assessed by dividing the amount of the stress-induced fatty acids by the amount of their precursors (Microbial Insights, Rockford, Tenn.). Biomarker ratios calculated for the rock varnish community harvested during the winter (February 2003) season (wet at the time of sampling) were indicative of a community not experiencing starvation or unusual stress. Cyclopropyl acid/*cis* acid ratios and *trans*/*cis* ratios were both 0.00.

Other microbial populations inhabit the surface environment of rocks in what is termed an "endolithic" lifestyle. Endoliths are found within the rock matrix just below the surface and occur in both hot and cold deserts (31). Endolithic communities are invariably colonized by cyanobacterial representatives of the *Bacteria* that, through photosynthesis, provide the fundamental source of carbon and energy that supports the endolithic community (16, 74). It is important while sampling rock varnish to be certain that endolithic communities are not also collected inadvertently. We observed no 16S rRNA gene sequences indicative of cyanobacteria in our samples of varnish, even though we employed conventional primers targeting conserved regions of 16S rRNA genes that should have detected cyanobacterial sequences. This is support that we sampled true varnish communities and not endolithic communities and also implies that other photosynthetic forms (e.g., bacteriochlorophyll *a*-containing bacteria such as *Rhodopila*, as represented by clone DRV 27) might be found in varnish-based ecosystems. This hypothesis merits testing at a variety of varnish sites within both hot and cold deserts.

Sequence analyses of representative clones of RFLP groups within an *Archaea* rRNA gene library (see Table S1 in the supplemental material) revealed only two RFLP types with 76 clones analyzed. Both types were related to previously observed uncultivated *Archaea*. Rarefaction curves obtained from the archaeal clone library reached saturation (see Fig. S1 in the supplemental material). Thus, *Archaea* appear to be present in low diversity in Whipple Mountains rock varnish.

Eukaryota observed by preparation of an 18S rRNA gene library from rock varnish (Fig. 2; see Table S2 in the supplemental material) were dominated by RFLP groups of fungal representatives of the ascomycota and included representatives of the genera *Phoma*, *Plicaria*, *Ascobolus*, *Alternaria*, *Sarcosphaera*, and *Rhizina*. One RFLP group representative most closely aligned with the basidiomycota (the genus *Athelia* in the family *Corticiaceae*; clone DRV E112). The dominance of ascomycota in our library is in accord with the microscopic observations of Perry (55), who characterized by scanning electron microscopy a microcolonial fungus from varnish also collected in the Sonoran Desert. Staley and colleagues identified this organism as an ascomycete within the family *Dematiaceae* (78, 79). The 18S rRNA gene sequences we observed were not of this family but fell into families such as *Pleosporaceae* (clone DRV E78), *Rhizinaceae* (clone DRV E102), *Pezizaceae* (clones DRV E8 and DRV E36), and *Ascobolaceae* (clone DRV E56).

Sequence analyses of representative clones of bacterial and archaeal rRNA gene libraries of nonvarnished soil (see Table S3 in the supplemental material) revealed mostly lineages different from those seen in rock varnish libraries. Only two nonvarnish clones overlapped. These were a *Rubrobacter radiotolerans*-like sequence and a *Sphingomonas*-like sequence. Both of these groups could reasonably be expected to inhabit both soil and varnish habitats.

The analyses reported here are not quantitative, so little can be said of the overall importance of individual RFLP groups or phylogenetic taxa observed to the overall community composition. In this regard, it is of interest that sequences similar to those of UV-resistant pure cultures isolated from the same Whipple Mountains rock varnish used in work reported here (e.g., *Geodermatophilus*, *Arthrobacter*, *Curtobacterium*, and *Cellulomonas*) (48) were not observed in varnish 16S rRNA gene libraries. This indicates that these strains, though present, probably do not dominate the community, a result that is supported by rarefaction analysis that revealed that the varnish contains a higher diversity of both *Bacteria* and *Eukarya* than was sampled in this study. It should be possible in future work to design PCR primers targeting specific RFLP groups (e.g., the *Rubrobacter* or ascomycota groups) and employ these using quantitative PCR techniques, such as real-time PCR, to examine the quantitative compositions of rock varnish microbial communities. In addition, quantitative real-time PCR using primers specific for enzymes known to be involved in metal oxidation may shed light on the roles that the microbial com-

munities found within rock varnish play with respect to the formation and maintenance of the varnish.

Nucleotide sequence accession numbers. Sequences were submitted to GenBank under the following accession numbers: rock varnish *Bacteria*, AY923078 to AY923086; rock varnish *Archaea*, AY923076 and AY923077; rock varnish *Eukarya*, AY923087 to AY923102; nonvarnished soil *Bacteria* and *Archaea*, AY923105 and AY92310.

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ADDENDUM IN PROOF

Since we submitted our paper another article that is relevant to work reported here was published (R. T. Schelble, G. D. McDonald, J. A. Hall, and K. H. Nealson, Geomicrobiol. J. **22:**353–360, 2005). In that paper, the authors report on their analyses of fatty acid methyl esters of samples collected from the Mojave Desert and compare the microbial community structure of desert varnish with that of adjacent desert soil. Their analyses indicated that prokaryotic and fungal communities are present in both desert varnish and soil samples. Fatty acid methyl esters specific to gram-positive bacteria were found more often and in greater abundance in varnish samples than in adjacent soils.

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