Biochemical markers in rock coatings

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Abstract. Rock coatings are ubiquitous in arid regions of the world. We have measured amino acids in desert varnish coatings and considered other organic compounds as chemical biosignatures in coatings. Understanding the mechanisms of formation or rock coatings and identifying their active and fossil biosignatures will provide useful methods for contrasting biotic and abiotic systems on Earth and other planetary bodies.

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]. In addition, NASA is interested in furthering general knowledge of the chemical signatures of bacterial fossils, to facilitate observation of their possible presence in meteorites, especially those from Mars [5-6].

We first provide a brief background on various rock coatings, including desert varnish, and go on to discuss biofilms and microbial relationships to desert varnishes. Then we address the biosignature issue, including our most current results and our rationale for expansion of biosignatures to include saccharides.

Rocks on Earth weather and change through time and microbes play an important role in this process [7-9]. This role may be either an active one in forming minerals, as for example, the formation of magnetite in microfossils [5] 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 exopolymeric substances (EPS). Rocks also become coated with minerals (Fig.1-4) that may protect them from weathering, and microbes may form or contribute to the formation of the coatings. Bacterial, Archaean and fungal cells 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 [10].

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 (Fig. 4), composed principally of clays, oxides, hydroxides, manganese, and iron. The bulk inorganic chemistry has been well characterized [e.g. 3, 11-12]. Previously, organic compounds had been thought to be only a small component of varnishes [1, 13]. Most electron microprobe data has shown 80-90% weight percent oxides and it has been reported by Perry [14] that the water content is close to 10%. However, only a few samples of varnish were analyzed for their water content and they may have been composed of uncharacteristically hydrated clays. Organic compounds may comprise the material that was unaccounted for, as supported also by Nagy *et al*. [13] and Perry *et al*. [15] whose studies indicated that there is a measurable biogenic, organic component to rock varnish and that the organic components possibly can be used as chemical markers for coatings.

Biofilms are composed of EPS and microbes and are ubiquitous even in arid conditions. They can be highly hydrated in aqueous environments. However, biofilms on exposed rocks may have as little as 1% water [16]. Subaerial biofilms on natural surfaces collect detrital grains in their slime and complex metals [17]. EPS and cell components may contain many chelation sites, which are implicated in mineralization processes [17]. Individual microbes rarely come directly into contact with minerals but rather attach to surfaces with extracellular polymers [18, 19].

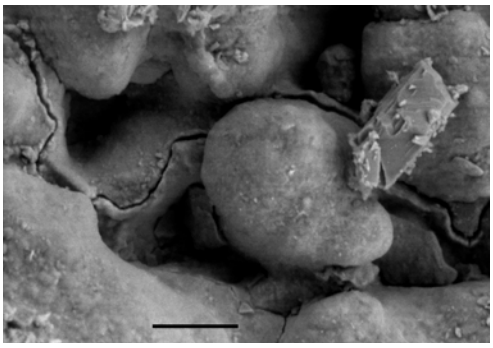

Fig. 1 Surface texture of varnish. Detrital grain in upper right quadrant. Scale bar is 2µm.

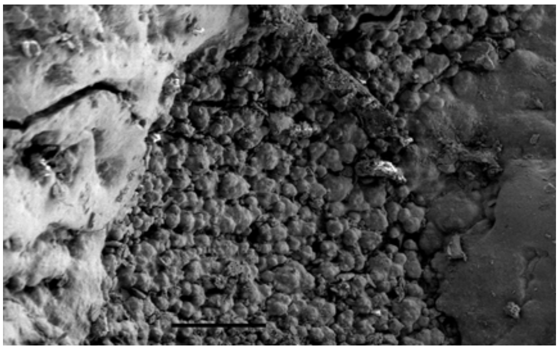

Fig 2. Surface texture of varnish x1000. Uncoated Rock substrate left and right. Scale bar is 20µm.

Desert varnish presents botryoidal (Fig. 1,2) or in ultra-thin sections of ~10µm [12]] wavy laminae (Fig. 4). In thin section, biofilms also consist of finely repetitive layers that are wavy and discontinuous. Even with severe conditions in deserts, where temperatures reach over 60ºC and frequently reach 80ºC on the dark varnish surfaces; EPS and biofilms, along with complexed metals might be preserved or incorporated into varnish coats. Biofilms are almost ubiquitous where there is a substrate interface and a liquid, and since water is available even in extreme arid conditions, desert varnish can be realistically described as a subaerial biofilm. There is ample evidence for associations of microbes with varnish-coated rocks. Many have suggested that varnish formation may be microbially mediated [2, 15, 20-26]. Hungate *et al.* [25] isolated 79 strains of bacteria from varnish coatings from the Negev Desert. Seventy-four of the bacteria could oxidize manganese and all but one were gram-positive. As noted by Staley *et al.* [2], most of the bacteria isolated from varnish are gram-positive organisms. *Bacillus subtilis* was cultured from varnish from the Deem Hills area north of Phoenix, AZ [12]. A study by Palmer *et al*. [27] found that most of the isolates from the Sonoran and Mojave Deserts capable of manganese oxidation were gram-positive bacteria including *Micrococcus, Planococcus, Arthrobacter, Geodermatophilus,* and *Bacillus.* Eppard *et al.* [28] found only members of the order actinomycetes, including *Geodermatophilus* species, on rocks and monuments with the exception of one *Bacillus.* In addition to bacteria, fungus may play a role in varnish formation. Rock varnishes frequently have associated colonies (Fig.1) of micro colonial fungus (MCF) [14]. Some evidence implicates the involvement of MCFs in desert varnish formation [24, 29, 30].

As bacteria on desert varnishes expire, their representative amino acids could become part of the varnish coating, and then possibly be used as biosignatures. Perry *et al.* [15] found D-alanine and D-glutamic acid in the hydrolyzates of desert varnish from the Sonoran and Mojave deserts. Two other non-protein amino acids that were also found are β-alanine and γ-amino butyric acid (ABA). D-aspartic acid was also present. The discovery of this D-amino acid is consistent with the report by Nagy *et al.* [13] who found this compound as the sole D-enantiomer in their investigation of varnish coatings. The finding of the D-enantiomers of glutamic acid and alanine suggests that peptidoglycan is a component of desert varnish. Peptidoglycans are present in large quantities in gram-positive bacteria and only in very small quantities in gram-negative bacteria. In addition lysine, found in gram-positive bacterial peptidoglycans was present and diaminopimelic found in gram-negative bacterial peptidoglycans was absent. The amino acid evidence is suggestive of a bacterial biosignatures presence in varnish coatings and possibly gram-positive bacteria similar to those that have been cultured from the surface [2, 20-26] of varnishes. Another possible candidate for a biosignature within peptidoglycans would be the peptide inter-bridge composed of five glycines, which is found in some gram-positive bacteria. DNA studies of 16S rRNA and 18S rRNA have produced as yet unquantified but measurable amounts of DNA in varnishes [31]. Use of melanin as biomarker from MCF colonies from desert varnishes is planned.

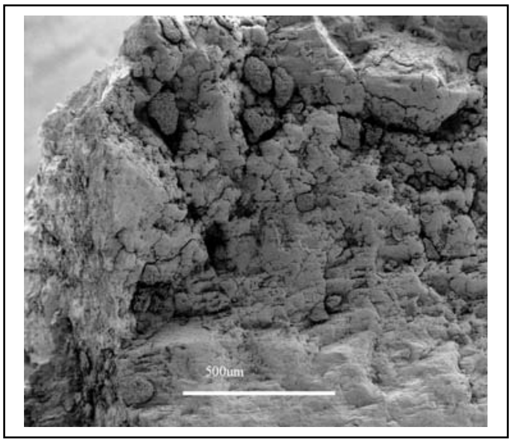

Fig. 3. Microcolonial fungus on varnished rock.

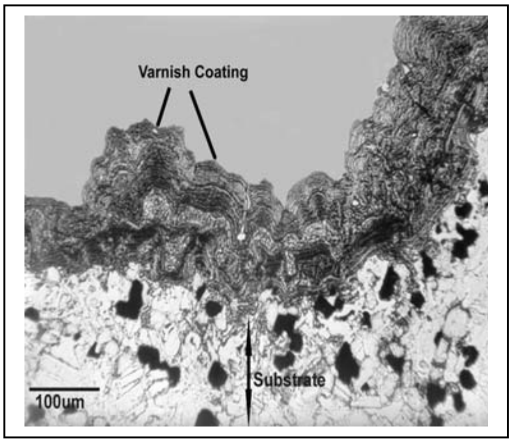

Fig. 4. Thin section (10µm) of desert varnish.

Since fragments of peptidoglycans have been recovered from desert varnishes, we posed a question: could other peptidoglycan components be recovered as well, and could they be used as additional biosignatures? Peptidoglycans are complex polysaccharides found in bacterial cell walls. They contain linear polymers of two alternating sugars, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), which are cross-linked with the short peptides. These peptides are composed of some common amino acids, as well as some unusual ones, such as D-glutamic acid, diaminopimelic acid (DAP), and D-alanine [15]. The peptide cross-links in the peptidoglycans may protect the sugars from decomposition, enabling them to serve as biomarkers.

It is currently unknown if polysaccharides or their transforms exist in varnish coatings. In general, sugars have not been studied as a biosignature. There is ample reason for this. Common sugars, such as glucose, ribose, arabinose, or fructose, contain aldehyde or keto groups in conjunction with hydroxyl groups that make them very chemically sensitive. Such sugars are rapidly destroyed under the basic conditions. They isomerize under both acidic and basic conditions. Isomerization causes racemization of the optically active centers, and an eventual destruction of the molecules [32] thus preventing their use as biomarkers. However, some sugar derivatives that are devoid of aldehyde and keto groups, notably sugar-related acids and alcohols, are more stable and have been isolated from the Murchison meteorite [33]. Sugars may be more stable under arid conditions where most common rock varnishes are formed. Sugars in general make a variety of stable complexes with metals, such as calcium, aluminum, iron, manganese [34] that are commonly found on natural surfaces and soils. Such complexes may further protect sugars against destruction.

The most likely part of bacteria to interact with rocks is the outer part of the cell wall that contains oligosaccharides from peptidoglycans, and also other saccharides, such as teichoic acids and related sugar-lipids. Bacteria, upon attachment to the surfaces also produce adhesive substances that are predominantly polysaccharides. It is likely that bacterial polysaccharides will interact with the natural surfaces in a process that is probably facilitated by their initial complexation with metals that are found on these surfaces. The complexation may be followed in some cases by a redox-type reaction. It is known that peptidoglycans are highly interactive with dissolved metal ions [35]. An additional pathway could be via complexation with silicates [36].

Many chemicals have been investigated as possible biomarkers for microbes such as hopanoids and chiral amino acids [e.g.37]. We hope to add additional biomarkers in our study of rock coatings, specifically polysaccharide components from peptidoglycans, from which we have already isolated unusual amino acids as biomarkers. A simultaneous finding of these unusual amino acids, and the peptidoglycan polysaccharides or their transforms, could indicate the remnants of the cell walls of bacteria. This would be of importance in the identification of bacterial fossils. Another possible biomarker is dipicolinic acid (DPA) (pyridine-2,6-dicarboxylic acid), which is specific to bacterial spore coats [38]. Spores concentrate ions such as those of Mn and Ca in their coats. These may become incorporated in varnishes and DPA might remain stable in coating.

With new technologies for better microanalyses, the possibilities for identifying additional biosignatures seem imminently achievable. Perhaps also, new questions need to be asked. Because desert varnish is a coating that can form in extreme environments, is a chemical process, and may be microbially mediated, it provides a unique opportunity for testing our ability to understand and interpret biochemical signatures on earth before attempting to understand their possible existence on other planetary bodies [39-41].

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