Bacterially-Mediated Formation of Rock Coatings in Kärkevagge, Swedish Lapland: A Mineralogical and Micro-Environmental Analog for Mars

Cassandra L. Marnocha

University of Arkansas, Fayetteville

Follow this and additional works at: https://scholarworks.uark.edu/etd

Part of the Environmental Microbiology and Microbial Ecology Commons, Physical Processes Commons, and the The Sun and the Solar System Commons

Citation
Retrieved from https://scholarworks.uark.edu/etd/1001

This Dissertation is brought to you for free and open access by ScholarWorks@UARK. It has been accepted for inclusion in Graduate Theses and Dissertations by an authorized administrator of ScholarWorks@UARK. For more information, please contact scholar@uark.edu.
Bacterially-Mediated Formation of Rock Coatings in Kärkevagge, Swedish Lapland: A Mineralological and Micro-Environmental Analog for Mars
Bacterially-Mediated Formation of Rock Coatings in Kärkevagge, Swedish Lapland: A Mineralogical and Micro-Environmental Analog for Mars

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Space and Planetary Sciences

by

Cassandra L. Marnocha
University of Wisconsin – Green Bay
Bachelor of Science in Biology, 2009

December 2013
University of Arkansas

This dissertation is approved for recommendation to the Graduate Council.

____________________________
Dr. John Dixon
Dissertation Director

____________________________
Dr. Timothy Kral
Committee Member

____________________________
Dr. D. Mack Ivey
Committee Member

____________________________
Dr. Vincent Chevrier
Committee Member

____________________________
Dr. Penelope Boston
Committee Member
ABSTRACT

The search for past or present life on Mars is, for now, limited to surface environments. An often neglected surface environment that could have served as an abode for life and could presently preserve evidence of that life is that of rock coatings. Rock coatings are mineral accretions on rock surfaces. On Earth, they are widespread and occur with considerable chemical diversity. There is growing evidence for a biotic role in their formation on Earth, particularly with respect to rock varnish. As a result, rock varnish has become a target of astrobiological interest on Mars, where varnish-like coatings have been observed. However, a number of coating types compatible with martian mineralogy exist but have yet to be investigated thoroughly. In this dissertation, I present a study of three principle rock coating types from a glacially eroded valley, Kärkevagge, in northern Sweden. The coatings consist of iron films, sulfate crusts, and aluminum glazes, all with primary mineralogies that are compatible with those minerals that have been identified on Mars.

To examine the role of microbiology in these terrestrial rock coatings and what the biotic formation of coatings might tell us about observed coatings on Mars, we asked three basic questions: 1) What microbes inhabit the coatings, 2) What are those microbes contributing to the geochemistry of the coatings, and 3) How are the microbes contributing to the overall formation of the rock coating? To answer these questions, we undertook two bacterial diversity surveys – Sanger sequencing and 454 pyrosequencing. Using the results of these surveys, we were able to assess diversity, richness, and metabolic potential of the communities. Microscopy and spectroscopy were used in order to visualize microbial communities inhabiting the coatings and to observe evidence of biomineralization. Using the answers to those questions – who, what, and how – a conceptual model of coating formation was developed to relate the terrestrial process of biological rock-coating formation to what may have occurred in the martian past.
ACKNOWLEDGEMENTS

Special thanks are given to my advisor, Dr. John Dixon, for taking a chance on a microbiologist that wanted to play in the dirt. Thank you for your support, guidance, and mentoring. I also gratefully acknowledge the members of my committee, Drs. Tim Kral, Mack Ivey, Vincent Chevrier, and Penelope Boston, for their support, helpful conversations, and use of lab space. I also thank Ryan Sheehan and Jeremey Jacobs, students whom I mentored as part of the National Science Foundation’s Research Experience for Undergraduates program.

This work was funded in part by the Royal Swedish Academy of Sciences, Abisko Naturvetenskapliga Station, the American Philosophical Society, and Arkansas Space Grant Consortium and the support from these institutions is greatly appreciated.

I thank my husband and family for their support and encouragement. A special thanks to good friend and talented artist Rasmus Johansson for his assistance with figures throughout this work. And a heartfelt thanks to the lifelong friends I made as a graduate student for their help, personal and professional. Thanks for getting me through it.
DEDICATION

To Nick: For your love, patience, sacrifice, and encouragement through this journey and those to come. You are my refuge.

To Lexie: I hope you're half as proud of me as I am of you.

To J. Michael: For always inspiring me to better myself.

To Pam and Kim: You're both my favorite aunt.

To Kathy: For your patience, wisdom, and for being my home away from home.

To Dr. Yingst: For paying it forward.

To Freya: For being my loyal canine best friend. For always being happy to see me.

In loving memory of Gretchen Marnocha. In life, you were the personification of love, home, and comfort. You remind me that there is always love and laughter in my life, and that home need not be a place. I miss you every day.
TABLE OF CONTENTS

1 Introduction ........................................................................................................................................ 1
   1.1 Dissertation overview and goals .................................................................................................. 1
   1.2 Study area .................................................................................................................................... 5
   1.3 Rock coatings ............................................................................................................................... 9
      1.3.1 Definition, classification, and distribution ........................................................................... 9
      1.3.2 Proposed formation mechanisms ......................................................................................... 10
   1.4 Bacterial biomineralization ........................................................................................................ 11
      1.4.1 Microbial communities ........................................................................................................ 12
      1.4.2 Acid-mine drainage ................................................................................................................ 14
      1.4.3 Endoliths, rock varnish, and other coatings ....................................................................... 15
   1.5 Astrobiology on Mars ................................................................................................................ 15
      1.5.1 Mars analogs – environmental and material biosignatures ............................................. 15
      1.5.2 Biosignatures ...................................................................................................................... 17
         1.5.2.1 Chemical and morphological biosignatures .............................................................. 18
   1.6 Dissertation outline ................................................................................................................... 19
   1.7 References .................................................................................................................................... 20

2 Bacterial communities in Fe/Mn films, sulfate crusts, and aluminum glazes from Swedish Lapland: Implications for astrobiology on Mars................................................................. 28
   2.1 Abstract ...................................................................................................................................... 28
   2.2 Introduction .................................................................................................................................. 29
   2.3 Methods ...................................................................................................................................... 34
      2.3.1 Sample collection .................................................................................................................. 34
      2.3.2 16S rRNA gene amplification and sequencing .................................................................... 35
      2.3.3 Scanning electron microscopy ............................................................................................ 36
   2.4 Results ........................................................................................................................................ 37
      2.4.1 Aluminum glaze ................................................................................................................... 38
      2.4.2 Sulfate crust .......................................................................................................................... 40
      2.4.3 Fe/Mn film ............................................................................................................................ 41
      2.4.4 Scanning electron microscopy ............................................................................................ 42
   2.5 Discussion .................................................................................................................................... 44
      2.5.1 Environmental influences on bacterial diversity ............................................................... 44
      2.5.2 Relationship to acid-mine drainage (AMD) ..................................................................... 47
      2.5.3 Implications for astrobiology on Mars .............................................................................. 48
   2.6 Conclusions .................................................................................................................................. 51
   2.7 Acknowledgements ..................................................................................................................... 52
   2.8 References .................................................................................................................................... 53
   2.9 Appendix ...................................................................................................................................... 60

3 Pyrosequencing of endolithic bacterial communities in rock coatings from Kärkevagge, Swedish Lapland ............................................................................................................................... 61
   3.1 Abstract ....................................................................................................................................... 61
   3.2 Introduction .................................................................................................................................. 61
   3.3 Methods ...................................................................................................................................... 65
4 Bacterially-facilitated rock-coating formation as a component of the geochemical budget of cold climates: An example from Kärkevagge, Swedish Lapland ........82
4.1 Abstract ..................82
4.2 Introduction ..................82
4.3 Methods ..................................87
4.4 Results ..................................88
  4.4.1 Aluminum glazes ..................88
  4.4.2 Fe/Mn films ..................89
  4.4.3 Sulfate crusts ..................89
  4.4.4 Sequencing ..................90
4.5 Discussion ..................93
  4.5.1 Aluminum glazes ..................93
  4.5.2 Fe/Mn films ..................94
  4.5.3 Sulfate crusts ..................96
  4.5.4 Bacterial scavenging ..................96
4.6 Conclusions ..................97
4.7 Acknowledgements ..................98
4.8 References ..................99
4.9 Appendix ..................107

5 A conceptual model of microbially-mediated Fe-film and sulfate crust formation in Kärkevagge, Sweden ..................................108
5.1 Abstract ..................................108
5.2 Introduction ..................108
5.3 Methods ..................................111
  5.3.1 Study site ..............................111
  5.3.2 Sampling ..................................113
  5.3.3 Microscopy ..................................114
5.4 Results and Discussion ..................114
  5.4.1 Fe-films ..................................114
  5.4.2 Sulfate crusts ..................118
  5.4.3 Biotic model for formation ..................121
  5.4.4 Rates of formation ..................124
  5.4.5 Astrobiology on Mars ..................126
5.5 Conclusions ................................................................................................................. 127
5.6 Acknowledgements ................................................................................................. 127
5.7 References ............................................................................................................... 129

6 Conclusions .................................................................................................................. 135
6.1 Summary of results ................................................................................................. 135
6.2 Directions for future work ...................................................................................... 138
6.3 References ............................................................................................................... 140
LIST OF PAPERS

Chapter 2:

Chapter 4:
1 Introduction

1.1 Dissertation overview and goals

To avoid pure speculation in the search for life elsewhere in the solar system, we define the life we look for as life that closely resembles that of terrestrial organisms: that is, organisms that are cellular, capable of growth, reproduction, with genetic information stored within DNA or RNA, and proteins carrying out the essential functions the cell. With this assumption in mind, most environments outside of the Earth are simply too inhospitable for life. With respect to the traditional notion of a habitable zone and the apparent universal requirement for water, extreme temperatures, pressures, and radiation can make even the development of the simplest and most extremophilic life-forms physically impossible (Fig. 1). At face value, the outlook for “life as we know it” elsewhere in our solar system is bleak. In this dissertation research, we investigate rock coatings for their potential as abodes for past or present life on Mars. The rock coatings in this research are strong mineralogical analogs to Mars and thus represent an astrobiological target that has been largely ignored outside of rock varnish.

However, one must consider the aspects of space and time when investigating the potential for life elsewhere in the solar system. The Earth today is much different than it was 4.3 billion years ago when life is thought to have first developed (Mojzsis et al., 1996; Schopf and Hazen, 1999) Likewise, there is evidence that our celestial neighbors, Venus and Mars, were not the toxic greenhouse and frozen desert, respectively, that they are today (Kasting, 1988; Kulikov et al., 2006; Kulikov et al., 2007; Pollack et al., 1987; Squyres and Kasting, 1994). As the research presented in this dissertation is focused on applications to astrobiology on Mars, analog examples in this introduction will focus on Mars. The objective of this research is to present an investigation
into the role of bacteria in rock coatings from Kärkevagge as an appropriate geochemical analog for the formation of similar coatings on Mars and their potential as astrobiology signatures.

Figure 1. Illustration of habitable zones based on mass of star. The solar system is shown at the top, with Earth and Mars fully within the habitable region. Gliese 581, a less massive star around which several exoplanets have been detected, is shown below. Two of the identified exoplanets of Gliese 581 are mostly within the proposed habitable zone, depending on various circumstances. Image credit: European Southern Observatory.

Perhaps more important are the spatial considerations. The biosphere on the Earth does not only include the surface, but also the atmosphere and subsurface. When discussing the potential for life on Mars, subsurface habitats for microbes such as caves and lava tubes cannot be overlooked. These environments on the earth are home not only to prokaryotes, but also higher eukaryotes that have developed to the low-light, low-nutrient environment of the subsurface.
Microbial communities have likewise adapted to these conditions, and as a result, communities in caves and lava tubes tend to be significantly distinguished from other microbial communities, such as soils, marine environments, and so on (Boston et al., 2001; Northup et al., 2003; Northup et al., 2011).

In addition to temporal and spatial considerations, the range of environments in which life can survive and thrive has expanded considerably with the advent of molecular biology. In many hostile environments, relatively speaking, cultivation yields but 1% of microbes in the community (Glausiusz, 2007). The exponential advances in genetic sequencing technology have allowed for the characterization of microbial communities in virtually every environment in which they are present, and representation of the communities is increased tremendously. The discovery of diverse and robust microbial communities at extreme pH, salinity, aridity, and temperatures has expanded the possibilities in which life might thrive (Amaral-Zettler et al., 2011; Cavicchioli, 2006; Marnocha et al., 2011; Morgan-Kiss et al., 2006; Rothschild and Mancinelli, 2002; Sorokin et al., 2010; Zablen et al., 1975). The environmental extent of known terrestrial organisms is shown in Table 1.

**Table 1.** Range of environmental conditions for which life has been conclusively identified to thrive within. Modified from Cavicchioli (2006).

<table>
<thead>
<tr>
<th>Threshold</th>
<th>Location</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High pH (alkaline)</strong></td>
<td>pH\textsubscript{opt} &gt;10</td>
<td>Soda lakes</td>
</tr>
<tr>
<td><strong>Low pH (acidic)</strong></td>
<td>pH\textsubscript{opt} 0.7</td>
<td>Dry, solfatartic soil</td>
</tr>
<tr>
<td><strong>High temperatures</strong></td>
<td>113°C T\textsubscript{max} (growth)</td>
<td>Submarine vent</td>
</tr>
<tr>
<td><strong>Low temperatures</strong></td>
<td>-15°C</td>
<td>Snow, sediment, ice</td>
</tr>
<tr>
<td><strong>Radiation</strong></td>
<td>&gt;5,000 Gy</td>
<td>Soil, nuclear reactor water core</td>
</tr>
<tr>
<td>High pressure</td>
<td>120 MPa</td>
<td>Deep sea; diamond anvils</td>
</tr>
<tr>
<td>--------------</td>
<td>---------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>High salt content</td>
<td>5.2M saturated salt</td>
<td>Saline lakes, evaporation ponds</td>
</tr>
<tr>
<td>Low water activity</td>
<td>0.6-0.75 aH2O</td>
<td>Hypersaline organic fluids</td>
</tr>
</tbody>
</table>

With the considerations of time, space, and the pervasiveness of microbial life, terrestrial analogs are often used to identify the potential for habitability on other worlds. Analogs are selected based on environmental, mineralogical, climatic, and geochemical compatibility, as well as those environments that offer energy sources for putative microbial communities and protection from conditions such as irradiation, extreme temperature swings, and other disturbances.

One such way that analogs are used is to understand the environment’s microbial ecology. The term *ecology* is generally associated with the interactions between plants and animals, predators and prey, and eukaryotic organisms and their environment. Microbial ecology addresses similar questions, but with a focus on microscopic life and its interactions with the environment, micro or macro. In microbial ecology, relationships between the abiotic and biotic world are still analyzed and general ecosystem models of energy acquisition and consumption can be developed. In this way, the microbial ecology and community structure of terrestrial analogs for Mars (environments or materials) can expand the traditional notions of habitability and habitable zones into ‘real world’ case studies. The microbiology of caves, hydrothermal vents, and acid-mine drainage represent a few analogs that are used to understand the dynamics of microbes in extreme environments (Baker and Banfield, 2003; Blumenberg et al., 2011; Bond et al., 2000; Boston et al., 2001; Fleming et al., 2013; Macalady et al., 2007; Northup et al., 2003; Spilde et al., 2002). Materials can likewise be analogous to those on Mars and include rocks/minerals, rock coatings and weathering rinds, stromatolites and similar formations, and ices. While no analog,
environmental or material, is a perfect substitute, each can offer insights through its own microbial ecosystem as to how such an ecosystem might function on Mars.

This dissertation focuses on bacterial communities in rock coatings from the deglaciated valley, Kärkevagge, in Swedish Lapland. Rock coatings from Kärkevagge are potential material analogs for coatings that have been observed on Mars, and the valley itself is a micro-environmental analog. The bacterial communities, their functional metabolic capacity, and associated micro-structures and minerals can serve as a resource for comparison when targeting samples for analysis by rover. The goals of this research are to garner a comprehensive representation of bacteria taxa present in rock coatings and to analyze them in the spatial context of the coating material. From bacterial taxonomy, physiology can be inferred, and thus it is possible to approximate the dynamics of rock coating formation in association with microbial metabolisms. Spatial associations with minerals and micro-structures provide further evidence of bacterially-facilitated rock coating formation.

1.2 Study area

This dissertation research focuses on rock coatings from a field site in Swedish Lapland – Kärkevagge. Kärkevagge is a glacially eroded, classically U-shaped valley located in the Northern Caledonide Mountains of northern Sweden near the Norwegian border (68°26’N and 18°18’E, Fig. 2). The valley stretches approximately 5 km in length, with elevation ranging from 600 meters above sea level (masl) at the mouth, to 800 masl at the head. The mountains to its east and west reach elevations between approximately 1350 and 1500 masl. The valley is drained by Kärkejokk, which flows out of Lake Rissajaure at the southern head of the valley.
The meteorological record at Kärkevagge is brief and comes from a recently (1992) installed weather station. The mean annual air temperature is -2°C and precipitation measures approximately 1100 mm annually (Strömquist and Rehn, 1981). Of this, upwards of 75% of the precipitation comes in the form of snow over a period of 220-240 days and ranges in depth from 0.75-1.5m. Ground surface temperatures can reach as low as -15°C during the coldest parts of the year. West of Kärkevagge, the Katterjack station has a much longer record and records at -1.7°C mean annual temperature and 1750mm mean annual precipitation at 515 masl (Eriksson, 1982).

Figure 2. Location of Kärkevagge in greater Scandinavia. Valley is outlined in dashed lines.
Kärkevagge is bounded by steep walls, made up of metamorphic rock. The upper valley walls are dominated by garnet mica schist, while the lower valley walls are dominated by quartz mica schist (Atlas and Bartha, 1992). The two units are separated by thinly bedded marble and finely disseminated pyrite occurs throughout the valley (Fig. 3). The name Kärkevagge means “valley of boulders”, earning its name from giant rock-fall deposits that cover much of the valley floor, as well as form the dam that impounds Lake Rissajaure (Fig. 4).
In 1960, the site was the focus of a study on hillslope processes in periglacial environments by Anders Rapp. In his thesis, Rapp identified the significance of geochemical processes in landscape denudation. To support this claim, he cited the widespread occurrence of Fe- and Ca-rich weathering rinds and crusts found on detached boulders and the valley walls, respectively. Building upon Rapp’s work, Dixon and colleagues (Darmody et al., 2007; Darmody et al., 2000; Dixon et al., 1995; Dixon et al., 2004; Dixon et al., 2002) demonstrated that chemical weathering played a significant role in Kärkevagge, whereas the conventional wisdom has often been that physical processes dominate the weathering regime in cold climates. One particular focus of their
geochemical studies has been that of the widespread rock coatings that were first identified by Rapp (1960).

1.3  Rock coatings

1.3.1  Definition, classification, and distribution

Rock coatings are defined as accretions on rock surfaces made up of material transported to that surface (Dorn, 1998). Coatings are chemically and environmentally diverse, found in virtually every environment on earth, and perhaps the most well recognized of coatings is rock varnish (traditionally, desert varnish – a misnomer). Coatings can occur on cobbles, boulder, volcanic rock, cliff faces, and more. Carbonates, nitrates, sulfates, heavy metals, iron oxides, halite, silica, and clays can all form a significant or secondary proportion of coating material. Even within a coating type, such as iron films, chemical and textural differences can further subcategorize the film. The diversity in rock coatings comes in chemical and mineral composition, texture, layering and stratification, formation mechanisms and timescales for formation, and microbial diversity.

The three primary coating types investigated in this research are Fe-films, sulfate crusts, and aluminum glazes (Figure 5). Fe-films are predominately iron oxyhydroxides composed primarily of goethite and hematite. Sulfate crusts are dominated by jarosite and minor amounts of gypsum. Aluminum glazes are basaluminite, an aluminum sulfate, with lesser amounts of alunite (Darmody et al., 2007). The coatings are found widely distributed throughout the valley, with characteristic textures and spatial associations, and sometimes are strongly associated with one another, as in the case of the Fe-films and jarosite crusts. These mineralogies occur extensively on the surface of Mars, thus making Kärkevagge a particularly useful geochemical analog.

1.3.2 Proposed formation mechanisms

A number of abiotic and biotic mechanisms for the formation of rock coatings has been suggested. Studies of rock varnish, perhaps best studied for its potential to be formed through microbial action, supply significant evidence for a microbial influence. Of course, the minerals observed the coatings can be produced abiotically in the right geochemical conditions. However, the presence of microbes capable of producing those same minerals via metabolic actions cannot be ignored. The driving force behind understanding microbial influences on rock coating formation begin at understanding community structure and lead into thermodynamic models and culturing
experiments in attempt to replicate the observed coating mineralogy and structure. Chapter 5 of this dissertation focuses on a conceptual model for rock coating formation in Kärkevagge.

1.4 Bacterial biomineralization

Biomineralization is defined as the production of minerals by living organisms. In higher eukaryotes, carbonates in shells and skeletons are the most easily recognizable biominerals. Silica biomineralization is also common in diatoms and sponges. Microbes have a wide range of minerals they are able to produce, including magnetite, sulfates, carbonates, Fe oxides, and elemental Au, S, and Cu, as some examples. While the extent of mineral capable of being produced is obviously large in eukaryotes, where virtually entire skeletons are biomineralized, the ability of microbes to biomineralize in large quantities has been underestimated (Emerson and Revsbech, 1994a; Emerson and Revsbech, 1994b; Hawes et al., 2011; Hawes et al., 2013; Konhauser, 2007; Van Veen et al., 1978).

Biomineralization can be facilitated in two general ways: biologically-controlled or biologically-induced. Biologically-controlled biomineralization results from a biochemical reaction within the cell that produces minerals for a physiological purpose (Konhauser, 1997). In the case of eukaryotes, calcite biomineralization by marine animals to produce shells is an example of biologically-controlled biomineralization. In prokaryotes, biologically-controlled biomineralization comes in the form of magnetotactic bacteria. These bacteria biomineralize magnetite and greigite in structures called magnetosomes that can be used for navigation using the earth's magnetic field (Abreu et al., 2011; Bazylnski et al., 1995; Komeili, 2012).

Biologically-induced mineralization occurs through a more indirect process, either through using a byproduct of cellular metabolism as a precursor, or through microbe-environment
interactions (Konhauser, 1997). In many cases these processes can be interconnected, and a group of organisms in a community are necessary to see the mineralization process through to completion. Extracellular polymeric substance (EPS) or microbial “slime”, for example, can attract metals electrostatically (Chan et al., 2009; Gehrke et al., 1998; Miot et al., 2009; Sand and Gehrke, 2006). Metals attached to cell surfaces then allow the cells to serve as nucleation sites for mineral growth, which can be facilitated by the metabolisms of other microbes (e.g. iron-reducing bacteria). Sheathed bacteria can non-enzymatically scavenge chemical species, contributing to the availability of metabolites for organisms – the former, another example of biologically-induced biomineralization (Beveridge, 1989; Ehrenreich and Widdel, 1994; Fleming et al., 2013; Ghiorse, 1984; Konhauser, 2007).

1.4.1 Microbial communities

An important component of understanding biomineralization processes in a system is understanding the direct processes of biologically-controlled biomineralization. While many microbes, regardless of taxonomy or metabolic function, can serve as nucleation sites for mineral growth, biologically-controlled biomineralization requires metabolism suited to the production of minerals directly. In this sense, it is essential understand the makeup of the microbial community and in effect, the metabolic capabilities of that community.

Microbial community surveys are commonly undertaken to accomplish just this. While there are more direct routes to identifying active metabolic processes (qPCR, transcriptomics, etc.), general surveys of microbial diversity allow for the identification of a large set of microbial species found in a given environment and therefore allow for the inference of a greater number of metabolic pathways. Prior to the advent of molecular biology, culture-based isolation of microbes was the only way to garner an understanding of community makeup. However, molecular biology
techniques, and in particular, sequencing technologies, allow for a substantially improved means of elucidating community structure. It is estimated that 99% of environmental microbes are “unculturable” – that is, using conventional culturing techniques, a vast majority of microbes inoculated simply will not proliferate to the point where they can be identified on growth media. Considerable alterations to growth media and conditions can be made to improve the odds of isolation; however, an enormous number of variables must be considered when attempting to accommodate (e.g. nutrient and ion levels, atmosphere, temperature, diurnal cycles, concomitant species) (Glausiusz, 2007). These considerations make diversity studies via culture-dependent techniques vastly inferior to molecular techniques in terms of capturing a higher percentage of the community’s overall structure.

Essential to microbial community surveys is the 16S ribosomal RNA gene. It is less the function of this gene that lends itself to molecular biology, but the nature of its genetic conservation through evolutionary time. Highly conserved sites serve as primer binding sites and these regions are highly conserved between many different species of bacteria and archaea. Primers designed for these highly conserved sites can be used with polymerase chain reactions (PCR) to amplify fragments of the 16S gene from a mixed pool of genomic DNA. That is, given a mix of genetic information from both prokaryotes and eukaryotes, specially designed primers can amplify the 16S gene from prokaryotes specifically, and these amplicons can be used in downstream applications. Interestingly, mitochondrial and chloroplastic rRNA is also amplified in these reactions, further supporting the theory of endosymbiosis (Zablen et al., 1975).

The 16S rRNA gene also contains variable regions that are conserved within species. This means that once the 16S gene fragment has been isolated, it can then be sequenced and used for microbial identification. Depending on the length of the fragment sequenced, comparing the
isolated sequence to existing databases of 16S sequences can determine taxonomy down to the species level. At the species level, metabolic functions for characterized microbes can be easily inferred. However, even at higher levels of taxonomy, some basic metabolic functions or classifications can be made if the isolated sequence does not have a high percent match or is shorter in length than desirable.

1.4.2 Acid-mine drainage

Acid-mine drainage is an anthropogenic phenomenon produced when earthmoving equipment exposes oxidizing minerals to the surface during mining excavations. The result can be ecologically devastating – rapid mineralization and acidification of nearby waters can drastically change the local environment. The phenomenon has been observed since humans began mining, as far back as 4,500 years ago and one of the best examples of acid-mine drainage in the world: Rio Tinto, Spain (Leblanc et al., 2000). Acid-mine drainage is generally associated with ore and coal mines, particularly those extracting Fe- and S-rich minerals from the earth. As a result, the acid-rock produced is usually oxidized forms of Fe and S minerals, such as pyrite.

Sites of acid-mine drainage, Rio Tinto in particular, have become increasingly prevalent in the literature as examples of mineralogical analogs to Mars (Amils et al., 2007; Banfield et al., 2001; Elwood Madden et al., 2004; Fernández-Remolar and Knoll, 2008; Fernández-Remolar et al., 2005; Navarrete et al., 2012). Molecular biology has made a solid case for microbial action playing a significant role in the generation of acid-mine drainage and the rate at which it is generated (Baker and Banfield, 2003; Bond et al., 2000; Edwards et al., 2000).

1.4.3 Endoliths, rock varnish, and other coatings

This dissertation focuses on a particular class of organisms: lithobionts. As its roots suggest, lithobionts are organisms that subsist on and co-exist with rock or rock material. While
this term can often refer to lichens, fungus, and mold, it is becoming increasingly apparent that bacteria and archaea play a significant role in rock weathering, pedogenesis, and secondary mineralization (Cary et al., 2010; DiRuggiero et al., 2012; Dorn, 1998; José et al., 2003; Wang et al., 2011; Wierzchos et al., 2006). We define several classes of lithobionts: endoliths, cryptoendoliths, chasmoloths, and hypoliths. Endoliths refers to organisms that survive within rocks. Cryptoendoliths are organisms that colonize cavities in porous rock. Chasmoloths inhabit cracks or fissures in rocks, and hypoliths survive on the surfaces of rocks.

One of the best studied endolithic habitats is that of rock varnish. Sometimes called ‘desert varnish’ (though found in humid environments as well as deserts), rock varnish is a thin, shiny coating of iron and manganese oxides with associated clay minerals (Dorn, 1998). Rock varnish studies have a heated history that extends into the modern literature regarding its formation mechanisms, rates of growth, and significance for astrobiology on Mars.

1.5 Astrobiology on Mars

1.5.1 Mars analogs – environmental and material

Both geology and biology necessitate field work, and while rovers, landers, and satellites focused on Mars are useful, there are limits to the types of work they are able to perform. To combat these limitations, analogs to Mars are sought out on Earth and consist of both environmental and material analogs. Environmental analogs are generally cold, acidic, and dry environments that can approximate (with some margin of error) conditions on Mars. Both hot and cold deserts, like the Atacama in Chile or the Antarctic Dry Valleys are common analogs for these purposes. The Rio Tinto in Spain is an extremely acidic, sulfate-rich environment often used as a Mars analog, as is the Canadian High Arctic, as a temperature analog. No single locale is a perfect
approximation for Mars, but they do allow scientists to investigate how life survives, thrives, and interacts with its environment in those extreme conditions.

Material analogs are those that are either natural or synthetic and can generally be independent of an analog environment – though, material analogs in an environmental analog are of course preferred to best approximate conditions on Mars. Synthetic material analogs include martian regolith simulants, such as JSC Mars-1. Natural simulants for regolith are often primarily basalts. Clays, sulfates, and iron oxides are also useful material analogs for Mars, as they are some of the most common minerals observed on the planet.

Kärkevagge is a relatively cold, dry, and acidic environment. Though it is not as cold as Antarctica or as dry as the Atacama Desert, the valley displays many of the environmental conditions simultaneously. This set of environmental stresses combined offers a look into how microbes might survive when faced with multiple stresses from a number of conditions. It is for these reasons that we classify Kärkevagge as a micro-environmental analog. More importantly, however, the rock coatings of Kärkevagge are excellent material analogs. Fe-films are made up predominately of goethite and hematite, both of which have been identified on Mars via rover (Klingelhöfer et al., 2004) and satellite (Christensen et al., 2000; Klingelhöfer et al., 2007). Sulfates such as the jarosite and gypsum in found in crusts in the valley, have also been observed via satellite and rover (Bibring et al., 2005; Bibring et al., 2006; Gendrin et al., 2005; Klingelhöfer et al., 2004; Squyres et al., 2004; Wang et al., 2006). The secondary mineral in aluminum glazes of Kärkevagge, alunite, has also been identified by satellite on Mars (Swayze et al., 2008)

1.5.2 Biosignatures

Biosignatures or biomarkers can be defined in one of two ways. In clinical biology, biomarkers are typically related to immunology, and less often referred to as biosignatures. In
astrobiology, biosignatures refer to both the definitive and suggestive signatures of life that are, for the moment, only observed on the Earth. The analogs described above provide accessible sample material to determine what components can be deemed biosignatures and how they might be detected. Definitive biosignatures, such as nucleic acids, photosynthetic pigments, chirality in amino acids, and actively metabolizing cells serve as a starting point for identifying the more elusive suggestive biosignatures. A suggestive biosignature on its own is not enough to demonstrate the presence of extinct or extant life. For example, the ALH84001 martian meteorite, discovered in Alan Hills, Antarctica, contained what appeared to be fossilized remains of “nanobacteria” and were considered strong morphological evidence of life on Mars (McKay et al., 1996). It was soon shown that these structures were not organic in nature, and the morphology itself was in question due to the unconfirmed potential for existence of terrestrial “nanobacteria” (McKay, 1997; Sears and Kral, 1998).

Nevertheless, some suggestive biosignatures make stronger cases than others. Atmospheric methane and O\textsubscript{2} in measureable quantities are largely associated with microbial metabolism and photosynthesis, respectively. The identification of seasonal methane in the martian atmosphere in recent years has further demonstrated the significance of methane as a biomarker (Formisano et al., 2004; Mumma et al., 2009). Abiogenic sources of the gas exist, however, but the seasonality and what appears to be a geologically recent source for the gas provide further evidence for methane as a biosignature (Mumma et al., 2009). However, the abundance and even existence of methane in the martian atmosphere has come under scrutiny most recently, with the Mars Science Lab, Curiosity rover failing to identify methane in any appreciable quantity on Mars (Webster et al., 2013).
In the case of molecular oxygen, the abundance of the gas in a planetary atmosphere can theoretically determine its strength as a biosignature. The Earth’s atmosphere is approximately 20% O\(_2\), or 209,460 ppmv and can be largely attributed to photosynthesis. Europa, one of the Galilean satellites of Jupiter and another intriguing target for astrobiology, has an atmosphere composed mostly of O\(_2\). However, the surface pressure of the moon is only 0.1\(\mu\)Pa, and thus, the total volume of O\(_2\) in the Europan atmosphere is but a small fraction of the Earth’s atmospheric oxygen. Additionally, the source O\(_2\) in the Europan atmosphere can be explained by abiotic sources, namely radiolysis and sputtering interactions between radiation, charged particles, and the water-ice of the moon’s surface (Hall et al., 1995).

Despite the questionable nature of some biosignatures, or at least, the reliability of a suggestive biosignature in an investigation, they remain the only viable option for astrobiology and planetary exploration. As a result, robotic missions target both chemical and morphological biosignatures, with more recently a focus on suggestive signatures, as to cover scientific ground that is both of astrobiological and geological or abiotic scientific importance.

1.5.2.1 Chemical and morphological biosignatures

For the purposes of this dissertation, biosignatures will be divided into two categories: chemical or morphological. Chemical biosignatures encompass a wide swath of chemistry. Elemental abundances, characteristic gases (e.g. methane, O\(_2\)), isotopic ratios, mineralogy, biological molecules (proteins, DNA, etc.), and byproducts of the breakdown of biological molecules (e.g. hopanes and steranes from cell membrane lipids) (Brocks et al., 1999; Parnell et al., 2004; Summons and Walter, 1990). As a result of the breadth of chemical signatures available, the instrumentation needed to detect and/or identify any one of these chemicals is typically quite specific to the sub-divisions described.
Morphological biosignatures are unilaterally less concrete. As in the case of the ALH84001 martian meteorite and others, many ambiguous forms can be mistakenly interpreted as cellular or organic morphologies (McKay et al., 1996; Sears and Kral, 1998). Thus, it is imperative to be clear when a morphological interpretation stands alone, and ideally, to support the morphological evidence with other evidence of biogenicity.

1.6 Dissertation outline

The chapters 2-5 are arranged as manuscripts that are either in preparation, under review or published. Chapter 2 discusses the preliminary findings of Sanger sequencing of rock coating materials, associated micro-structures, and the implications of those findings to astrobiology on Mars. Chapter 2 is published in the International Journal of Astrobiology in 2013. Chapter 3 expands upon the limited community representation afforded by Sanger sequencing, and uses pyrosequencing to gain a much more representative picture of community structure in the rock coatings. Chapter 3 has been submitted to FEMS Microbiology Ecology. In Chapter 4, the data collected from the work presented in Chapters 1 and 2 are applied in a broader sense to geochemical budgets in cold climates, where bacterially-mediated rock coating formation may play an important role. This chapter is published in Geomorphology. Chapter 5 examines evidence of bacterial colonization and biomineralization at the micron level using scanning electron microscopy. Chapter 5 is currently in preparation for publication and will be submitted to Earth and Planetary Sciences.
1.7 References


Sand, W., Gehrke, T., 2006. Extracellular polymeric substances mediate bioleaching/biocorrosion via interfacial processes involving iron (III) ions and acidophilic bacteria. Research in Microbiology, 157(1), 49-56.


2 Bacterial communities in Fe/Mn films, sulfate crusts, and aluminum glazes from Swedish Lapland: Implications for astrobiology on Mars

2.1 Abstract

Rock coatings have been observed on Mars by Mars Pathfinder, Viking, and the Mars Exploration Rovers. Though rock varnish has been studied for its potential as a biosignature, other types of rock coating have been largely ignored. In Kärkevagge, Swedish Lapland, sulfate crusts, aluminum glazes, and Fe/Mn films occur with mineralogies mimicking those observed on the surface of Mars. Molecular analysis and scanning electron microscopy (SEM) were used to investigate the bacterial communities associated with these rock coatings. Molecular techniques revealed differences in community structure and metabolisms associated with the production of secondary minerals between the three coating types. SEM analysis showed evidence of encrustation in mineral coatings in the Fe/Mn films and aluminum glazes, and evidence of abundant microbial communities in all three coating types. These observations provide evidence for bacterial participation in the genesis of rock coatings. For astrobiology on Mars, rock coatings are an attractive biosignature target scientifically and logistically: they are surface environments easily accessible by rovers, endoliths are afforded protection from surface conditions, and evidence of life could potentially be preserved through biomineralization and lithification. This study describes the bacterial communities from rock coatings compatible with martian mineralogy, explores the potential for biologically-facilitated rock coating formation, and supports rock coatings as targets of astrobiological interest on Mars.
2.2 Introduction

Rock coatings of considerable chemical diversity occur extensively on the Earth’s surface as accumulations of materials on rock surfaces brought from external sources (Dorn, 1998). Chemical coatings and the mechanisms of their genesis are of particular interest in astrobiology, as they have been observed on the surface of Mars and have the potential to serve as biomarkers (Strickland, 1979). Using the rock coating classification described by Dorn (1998), this study investigates the role of bacteria in the formation of sulfate crusts, aluminum glazes, and iron-manganese films on boulder surfaces (Fig. 1). Additionally, it promotes rock coatings as a high-priority astrobiology target for current and future missions on Mars.

![Figure 1. Rock coating types used in this study. (A) Rust-colored Fe/Mn film with possible green/yellow jarosite crust residue. (B) Sulfate crust, jarosite depicted, often found on the unexposed underside of boulders. (C) White, aluminum glazes commonly found along stream beds and in the form of streaks on the eastern valley wall. Photo credit: Cassandra Marnocha, Sweden, 2010.](image)

The coatings investigated in this study were collected from Kärkevagge, in Swedish Lapland. Kärkevagge is a glacially-eroded U-shaped valley in Swedish Lapland, adjacent to the Norwegian border (68° 26’ N and 18° 18’ E) (Fig. 2). The valley is bounded by steep bedrock walls, with upper valley walls dominated by beds of resistant garnet mica schist (Dixon et al., 1995). Lower valley walls are predominately quartz mica schist dominated, and separating the two schist
units is thinly bedded marble. Finely disseminated pyrite is found throughout the valley and thought to be a primary source of sulfur that is incorporated into the rock coatings, along with sulfate ions found in streams (Darmody et al., 2007). The valley floor is approximately 600 meters above sea level (masl) at its mouth and rises to 800 masl at its head (Dixon et al., 2008). While mean annual air temperature in Kärkevagge is in the vicinity of -2°C (Thorn et al., 1999), investigation of shallow soil and bedrock/soil interface temperatures reveal thermal regimes on daily and hourly scales that approximate -15°C (Thorn et al., 2001). The majority of the precipitation in the valley is in the form of snow (50-75%) with depths ranging from 0.75-1.5m over much of the year. Total annual precipitation is approximately 800 mm, as measured at the Riksgränsen-Katterjäkk station to the west of Kärkevagge (Eriksson, 1982).

An early study of geomorphological processes operating in the valley was undertaken by Rapp (1960) who identified what he originally described as “lime crusts” and “rust coatings” on bedrock surfaces. Recent studies have determined these to be alumina glazes (composed of

Figure 2. Left: Location of Kärkevagge in greater Swedish Lapland. Right: Geological map of Kärkevagge with sampling areas shown.
basaluminite and gypsum) and Fe/Mn films respectively (Darmody et al., 2007; Dixon et al., 2008; Dixon et al., 1995; Dixon et al., 2002). We focus on three coating types in this study classified using nomenclature from Dorn (1998): sulfate crusts are dominated by jarosite \([\text{KFe}_3(\text{SO}_4)_2(\text{OH})_6]\) composed of approximately 70% O, 1% Si, 10% S, 4% K, and 15% Fe and minor amounts of gypsum \([\text{CaSO}_4\cdot\text{H}_2\text{O}]\) composed of approximately 69% O, 2% Al, 1% Si, 12% S, 14% Ca, 2% Fe (Darmody et al., 2007). The aluminum glazes are predominantly basaluminite, an amorphous aluminum oxyhydroxide sulfate \([\text{Al}_4(\text{SO}_4)(\text{OH})_{10}\cdot\text{H}_2\text{O}]\) consisting of approximately 52% \(\text{Al}_2\text{O}_3\), 10% \(\text{SiO}_2\), 11% \(\text{SO}_3\), 1% K and Ca, and 0.1% Na, Mn, and Mg (Darmody et al., 2007). Iron films are predominantly iron oxyhydroxides with compositions of 44% Fe, 9% Al, 6% Mg, 17% Si, 1.4% S, 0.3% K, 0.08% Ca, 0.05% Mn. Iron films are composed of primarily goethite and hematite. The Fe in the coatings is thought to be sourced from pyrite oxidation, subsequent bedrock weathering and release of Fe into the hydrologic system (Dixon et al., 2002).

Kärkevagge represents a potential martian micro-environmental analog: the valley is cold, relatively dry, and has generally acidic water chemistry. Geochemically and mineralogically, the valley is a strong analog with abundant sulfates and iron dominated mineralogies. As rock coatings by definition are composed of materials transported to the rock surface, parent rock lithology is insignificant in the development of the coating, and thus is not considered in the argument for Kärkevagge as a geochemical and mineralogical analog to Mars.

While pure water is unstable on the surface of Mars, theoretical and experimental approaches have demonstrated that sulfate-rich brines can remain stable over extensive longitudinal ranges between 0° and 30° latitude because of lowered freezing points and evaporation rates (Chevrier and Altheide, 2008; Altheide et al., 2009). Models show that sulfate brines derived from acid-sulfate weathering similar to that operating in Kärkevagge have the
potential to deposit hematite, jarosite, and gypsum at the martian surface and shallow subsurface (Chevrier and Altheide, 2008; Chevrier and Rivera-Valentin, 2012). Similarly, Chevrier and Rivera-Valentin (2012) have demonstrated that some sulfates and chlorides may remain fluid at and near the martian surface under temperature conditions similar to those of Kärkevagge on south-facing crater walls between 30°-50°. Likewise, sulfate-reducing bacteria have been shown to survive in many of these sulfate brines at concentrations as high as the eutectic (Marnocha et al., 2011). Rock coatings have been observed on the surface of Mars since the Viking landers (Strickland, 1979) and by the Mars Exploration Rovers (MER) (Krinsley et al., 2009). Because of the distinct appearance of rock varnish and its strong association with biomineralizing microbes, rock varnish has frequently been suggested as a potential biosignature on Mars (Krinsley et al., 2009; Dorn, 1998; Bishop et al., 2002; Murchie et al., 2004; Barnouin-Jha et al., 2000; Allen et al., 2004). However, despite the widespread occurrence of gypsum, jarosite, and iron oxides (Bibring et al., 2005; Bibring et al., 2006; Gendrin et al., 2005; Squyres et al., 2004; Klingelhöfer et al., 2004; Klingelhöfer et al., 2007; Christensen et al., 2000; Swayze et al., 2008), other rock coatings compatible with known martian mineralogies have been largely ignored as potential locations for life on Mars.

A number of mechanisms for bacterial biomineralization have been suggested (Petrush et al., 2012; Konhauser, 1997; Dorn, 1998; Ghiorse, 1984; Ghiorse and Ehrlich, 1992; Beveridge and Fyfe, 1985; Beveridge, 1989; Konhauser et al., 2008; Konhauser et al., 2011; Benzerara and Miot, 2011; Benzerara et al., 2008; Kleinmann et al., 1981; Kappler et al., 2006). Many of these mechanisms involve the scavenging and concentration of relevant chemical species (Fe, Mn, S, etc.) from the surrounding environment within the extracellular polymeric substances (EPS). These trapped ions can then serve as nucleation points for reactions that lead to the precipitation of
minerals. While this occurs, microbial metabolisms (e.g. iron and sulfide oxidation) can simultaneously affect local pH, thus affecting the overall chemistry and sometimes leading to additional precipitation by promoting stable conditions for those minerals (e.g. jarosite) (Petrash et al., 2012; Konhauser, 1997).

Iron oxides and hydroxides can form through bacterially induced processes (e.g. bacterial EPS as nucleation sites), or bacterial controlled processes, in which bacterial oxidation of Fe(II) produces large quantities of precipitated iron (Konhauser, 1997). Microbial oxidation of Fe(II) can occur and compete with abiotic oxidation in acidic and neutral conditions (Kappler et al., 2005; Williams et al.; Varnali and Edwards). Concentration of Fe(II) and co-reacted ions, pH, and other environmental factors contribute to determination of which mineral is produced and remains stable (Zachara et al., 2002; Roh et al., 2003). Biomineralization products can include goethite, hematite, magnetite, and siderite (Zachara et al., 2002; Banfield et al., 2000; Larese-Casanova et al., 2010; Roh et al., 2003), where goethite and hematite are the dominant minerals in Fe/Mn films in Kärkevagge.

Precipitation of sulfates occurs through a series of biological and abiotic reactions, with biological influence from Fe$^{2+}$ oxidation and hydrolysis (Kleinmann et al., 1981; Konhauser, 1997; Clarke et al., 1997). This process has been described as follows (Kleinmann et al., 1981):

\[
\begin{align*}
\text{FeS}_2 + 3.5\text{O}_2 + \text{H}_2\text{O} & \rightarrow \text{Fe}^{2+} + 2\text{H}^+ + 2\text{SO}_4^{2-} & (1) \\
\text{Fe}^{2+} + 0.25\text{O}_2 + \text{H}^+ & \rightarrow \text{Fe}^{3+} + 0.5\text{H}_2\text{O} & (2) \\
\text{FeS}_2 + 14\text{Fe}^{3+} + 8\text{H}_2\text{O} & \rightarrow 15\text{Fe}^{2+} + 2\text{SO}_4^{2-} + 16\text{H}^+ & (3) \\
\text{Fe}^{2+} + 2.5\text{H}_2\text{O} + 0.25\text{O}_2 & \rightarrow \text{Fe(OH)}_3 + 2\text{H}^+ & (4)
\end{align*}
\]

Where initial oxidation of sulfides occurs through biotic or abiotic means (reaction 1), Fe$^{2+}$ is oxidized through microbial oxidation (reaction 2). This is followed by accelerated oxidation of
sulfides (reaction 3) and finally the production of a precipitate from oxidation and hydrolysis of Fe$^{2+}$ (reaction 4) (Kleinmann et al., 1981). Ferric hydroxysulfate and jarosite may precipitate in acidic environments (Lazaroff et al., 1982; Brady et al., 1986), while ferric hydroxides precipitate at higher pH (Brady et al., 1986; Carlson and Schwertmann, 1980).

It is through these mechanisms of biologically controlled and biologically induced biomineralization that we suggest bacteria may play a role in the genesis of rock coatings in Kärkevagge. Studies of rock coatings in Kärkevagge until now have been from an abiotic, geochemical perspective. However, microbes and other organic materials have been reported in previous work (Dixon et al., 1995). Thus, it has suggested that they may play a role in rock coating genesis.

This work represents the first study to investigate the bacterial communities inhabiting the rock coatings from Kärkevagge and the communities’ metabolic potential for biomineralization processes and rock coating formation. Given the geochemical and mineralogical analog that Kärkevagge represents for Mars, and the observations of rock coatings on Mars, we propose rock coatings as high priority targets for astrobiology on Mars and present the results of our initial investigations of the bacterial communities of rock coatings in Kärkevagge.

2.3 Methods

2.3.1 Sample collection

Rock debris displaying accumulations of the principal rock coating types was collected from field sites associated with sampling transects established along the length of the valley’s east side. These transects are subsequently identified by the letters V, L, K, J, and H. Samples were subsampled from each of the following rock coating types: Fe/Mn films, aluminum glazes, and
sulfate crusts. Two Fe/Mn films, two sulfate crusts, and one aluminum glaze were selected for subsequent analysis. Bulk rock samples were then subdivided into sterile tubes for storage at both 3°C and -20°C. Collections stored at -20°C were used in this study. Genomic DNA was extracted from crushed rock samples using a PowerSoil® DNA Isolation Kit from MoBio (Carlsbad, CA) according to manufacturer’s protocols after transport. Extracted DNA was stored at -20°C for downstream analysis.

2.3.2 16S rRNA gene amplification and sequencing

Bacterial small subunit ribosomal RNA genes were amplified via polymerase chain reaction (PCR) using universal 533-forward (5’- GTG CCA GCC GCC GCG GTA A -3’) and 1392-reverse primers (5’-GGT TAC CTT GTT ACG ACT T-3’) on two Fe/Mn films, two sulfate crusts, and one aluminum glaze. PCR was carried out in 25µl reaction, consisting of 12.5µl GoTaq Green Master Mix (Promega Corporation), 2µl each of primer, 5µl of the extracted rock coating DNA, and nuclease-free water to bring the reaction volume to 25µl according to manufacturer protocols. Thermocycler parameters, modified from Macalady et al. (2007), were as follows: 5’ at 94°C initial denaturing, followed by 30 cycles of 1’ at 94°C, 45” at 47°C and 1’ at 72°C, concluding with a 7’ final extension at 72°C. Amplifications using two archaeal primer sets, 21-forward/958-reverse (DeLong, 1992) and 340F/1000R from (Gantner et al., 2011), were also attempted, but were ultimately unsuccessful. After successful confirmation of PCR amplification from the universal primers using gel electrophoresis, products were purified with an UltraClean® PCR Clean-up Kit (MoBio Laboratories, Inc.).

Purified PCR amplicons were then cloned into pSC-A cloning vectors using a StrataClone PCR Cloning Kit (Agilent Technologies). Purified PCR products were not diluted, though the procedure was performed otherwise according to manufacturer instructions. Following
transformation, *Escherichia coli* cells were plated on Luria Burtani-ampicillin plates with 2% x-gal and incubated no more than 24 hours. Positive, white colonies were then randomly selected and further PCR-amplified using M13 forward reverse primers with binding sites on the pSC-A vector. PCR thermocycler parameters used were the same as those used for universal 533-forward and 1392-reverse reactions described above.

Following successful M13 PCR confirmed on a gel electrophoresis, PCR products were shipped on dry ice to Functional Biosciences (Madison, WI) for Exo/SAP clean-up and sequencing using the T7 primer. Sequences were checked for chimeras using Bellerophon (Huber et al., 2004) and putative chimeras were removed from subsequent analyses. The final set of sequences was then aligned using the Basic Local Alignment Search Tool (BLAST) and matched to nearest neighbors for use in phylum-based analysis.

Sequences for each sample were then divided by phyla, or more specific taxonomy, when available through BLAST and greengenes (DeSantis et al., 2006) best matches. Rarefaction curves were calculated for all samples using the *mothur* software program (Schloss et al., 2009). Diversity indices were also calculated using *mothur*, after alignment via *greengenes*. Phylogenetic trees for each sample type were created using MEGA5 (Tamura et al., 2011) and ClustalW alignment followed by the construction of a maximum-likelihood phylogenetic tree for each coating type, with 1000 bootstraps using the Jukes-Cantor model (Jukes and Cantor, 1969). Nearest neighbors included in the phylogenetic trees were selected from the BLAST database. 16S rRNA gene sequence data generated in this study is cataloged in GenBank under accession numbers JQ677813-JQ677911.
2.3.3 **Scanning electron microscopy**

Coating samples were examined using a Nova Nanolab FEG scanning electron microscope (SEM), coupled with energy-dispersive X-ray spectroscopic (EDX) analysis. Rock chips of each representative coating type were mounted on carbon tape sample mounts and observed without the addition of a metal coating. Samples were analyzed under 15.00 kV for both SEM and EDX. FEI software was used to capture images on a PC for subsequent interpretation. Chemical analyses were obtained via mapping and point analysis of bacteria and mineralogical materials using EDX.

2.4 **Results**

Bacterial 16S rDNA was amplified for samples from sites K, L, V, and H. The non-chimeric sequences from the five coating samples underwent further analysis. Rarefaction curves (Fig. 3) show the expected number of operational taxonomic units (OTUs) observed per number of clones sampled, generating a curve to show the trend of representativeness in terms of diversity each clone library was for each sample. In all, both the community richness estimates and rarefaction curves suggest that communities may be more diverse than found in this initial study.
2.4.1 Aluminum glaze

Phylogenetic analyses (Fig. 4) show that the aluminum glaze clones are related primarily to soil bacteria, with relationships to other endoliths, such as those found in tufa and dolomite, and are associated with cold climate representatives in Antarctic soils, tundra, and alpine environments. The aluminum glaze contained sequences with nearest neighbors from acid-mine drainage (AMD) environments, including Actinobacteria, Acidisphaera sp., and Acidocella sp. clones. Clones also had nearest neighbors from sites of appreciable uranium concentration, both in mine waste piles and contaminated soil.
Figure 4. Inferred phylogeny for the aluminum glaze (H1) clones with nearest neighbors. Evolutionary histories for Fig. 4 and 5 were inferred using the maximum-likelihood method based on the Jukes-Cantor model (Jukes and Cantor, 1969). The percentage of trees in which the associated taxa clustered together is shown next to the branches as a bootstrap value. The trees are drawn to scale, with branch lengths measured as the number of substitutions per site. Accession numbers are shown after each isolate.
2.4.2 Sulfate crust

The sulfate crust clones (Fig. 5) display soil, alpine, endolith, and AMD relatives. BLAST database searches show a stronger relationship between sulfate crust clones and those found in AMD settings, including sulfur and Pb/Zn mine waste. Sequences from the *Actinobacteria* and *Acidobacteria* phyla were found in the K2 site sample, along with *Acidiphilum* sp. In one jarosite
sample, 8 out of 15 clones had nearest BLAST neighbors from coal mine and sulfur-rich mine tailing sites.

Unlike the other coating types, sulfate crust sequences also have unique nearest neighbors isolated from glacial environments, deep sea sediments and stony corals, and hot springs when compared to the greengenes database.

2.4.3 Fe/Mn film

The Fe/Mn films display the lowest bacterial diversity and exhibit Bacillus spp. as nearest neighbors from a limited number of environments. Because of the low diversity and close relationships of the Fe/Mn film clones, no phylogenetic tree was generated. Fe/Mn films were sampled from both the eastern and western side of the valley in areas of low-to-neutral pH (4.5-7.9) (Campbell et al., 2001). One of the coatings was sampled from a site with mixed bicarbonate/sulfate ion water chemistry and contained predominantly clones with nearest neighbors from acidic environments. It should also be noted that SEM analyses have shown the presence of cocci-type bacteria in the Fe/Mn films, both in this study and as previously reported from Kärkevagge by Dixon et al. (1995), whereas only Bacillus sp. (and thus, bacillus morphologies) were identified through 16S rRNA analysis.

Table 1. Taxonomy and postulated physiology for those clones with high percent matches within the database and genus-level taxonomy available for nearest neighbors.

<table>
<thead>
<tr>
<th>Coating type</th>
<th>Nearest neighbor taxonomy</th>
<th>Inferred physiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfate</td>
<td>Acidiphilum rubrum</td>
<td>Fe-reducer, S-oxidizer</td>
</tr>
<tr>
<td>Aluminum</td>
<td>Acidocella sp.</td>
<td>Fe-reducer</td>
</tr>
<tr>
<td>Aluminum</td>
<td>Acidisphaera rubrifaciens</td>
<td>Fe-reducer</td>
</tr>
<tr>
<td>Aluminum</td>
<td>Tetrasphaera sp.</td>
<td>Mn-oxidizer</td>
</tr>
<tr>
<td>Fe/Mn</td>
<td>Bacillus subtilis</td>
<td>Fe-reducer/oxidizer, Mn-reducer/oxidizer</td>
</tr>
</tbody>
</table>
Physiologies of rock coating bacteria that can be inferred with confidence are shown in Table 1. Other dominant phyla include *Actinobacteria*, *Chloroflexi*, *Acidobacteria*, and *Firmicutes* for the sulfate crusts, *Actinobacteria*, *Firmicutes*, α-, β-proteobacteria for the aluminum glazes, and *Firmicutes* for the Fe/Mn films.

Community structure, when assessed through the number of shared OTUs (Fig. 6), appears to be tied to coating mineralogy. Very little diversity exists between clones from the Fe/Mn films, compared to the aluminum glazes and sulfate crusts. There is also 1 OTU overlap between the aluminum glaze and sulfate crust at a pairwise distance of 0.05, and no overlap for any group assuming all sequences represent unique OTUs.

Figure 6. Venn diagram of shared OTUs between rock coating types at a pairwise distance of 0.05.
2.4.4 Scanning electron microscopy

SEM analysis revealed bacterial and fungal morphologies in the three rock coating types sampled in this study. Fe/Mn films showed strong evidence of iron coating of putative biological forms (Fig. 7). EDX spectra show strong signals for Fe and O, with lesser amounts of Al and S, suggesting a possible iron oxide coating over the biological forms. The aluminum glaze exhibited similar encrustation to the Fe/Mn films. Aluminum glazes also contained multiple dark streaks across the encrusted area (Fig. 8), with similarly sized filaments observed nearby.
Figure 7. SEM micrographs of a Fe/Mn film. (A) Putative cocci-form bacteria and ringed (fungal?) filaments (arrows). (B) Cocci and bacilli-form bacteria covered in a coating highly concentrated in Fe. (C) EDX spectra of the region shown in 7A.

Figure 8. SEM micrographs of an aluminum glaze. (A) Dark streaks and a deep trench (arrow) indicating possible remnants of fungal filaments. (B) Additional dark streaks (arrow) observed in an area of heavy encrustation. Botryoidal cocci bacteria are visible beneath the smooth alumina coating.

2.5 Discussion

2.5.1 Environmental influences on bacterial diversity

All coating type sequences contained nearest neighbors from cold climates, ranging from glacial ice, deglaciated and tundra soils, Antarctic and Arctic environments, and other environments simply categorized in the databases as “cool” or “cold”. Rock coatings are present in other cold environments; in particular, Al-, Si-, SO₄-, and Fe-rich rock varnishes have been observed in coastal (Victoria Land), inland Antarctica, and Tibet (Dorn et al., 1992; Giorgetti and

Summer air temperatures in Kärkevagge range from 6-17°C (Thorn et al., 1999), moderate enough for mesophilic bacteria to thrive. During the remainder of the year, when air temperatures drop to 1-2°C and ground surface temperatures to as low as -15°C, psychrotolerant and psychrophilic bacteria could thrive in the rock coatings at these temperatures (Deming, 2002; Junge et al., 2004; Price, 2007; Rothschild and Mancinelli, 2002). Alternatively, spore-forming bacteria would withstand the environmental conditions until temperatures were above freezing. Many of the sequences from rock coatings, including the dominant *Bacillus sp.* in the Fe/Mn films, are related to spore-forming bacteria, capable of producing endospores that could survive dormant in a number of conditions until environmental conditions (e.g. temperature, nutrient availability) were once again conducive to survival and growth (Roszak and Colwell, 1987; Vreeland et al., 2000). Though spore-forming bacteria can be found in any number of environments where conditions do not necessarily promote endospore generation, spore-forming bacteria are often found in cold climates such as Arctic and Antarctic sediments and soils (Vorobyova et al., 1997; Yergeau et al., 2007), glacial ice and glacial outflow (Miteva and Brenchley, 2005; Mikucki and Priscu, 2007), and permafrost (Vorobyova et al., 1997; Hinsa-Leasure et al., 2010).

While arctic and arid environments typically present lower diversity and richness for plants and other higher order organisms, bacterial diversity and community richness tend not to follow these biogeographic trends (Fierer and Jackson, 2006). Desert soils, for example, have shown significantly higher bacterial diversity and species richness than soils in the tropics (Fierer and Jackson, 2006). In this case, soil pH plays a pivotal role as an indicator of microbial diversity. Kärkevagge water pH ranges from 4.4-8.0, with most values closer to neutral. Soils maintain
similar, though more neutral pH values. Comparing local soil and water pH from the rock coating sample sites shows a relationship between neutral pH and increased bacterial diversity.

Fe(II) oxidation to Fe(III) can occur through abiotic, chemical reactions in neutral or alkaline conditions, while Fe(II) remains stable in acidic conditions (Kappler et al., 2005; Bae and Lee, 2013). Fe/Mn films were sampled from areas with local water chemistry pH ranging from acidic to neutral (Campbell et al., 2001). In more acidic conditions, where Fe(II) would be stable, microbes could be responsible for the oxidation and conversion of pyrite, which is abundant throughout the valley, to the goethite observed in the Fe/Mn films from those sites. In areas of more neutral pH, microbial oxidation of Fe(II) is able to compete with abiotic oxidation (Kappler et al., 2005; Varnali and Edwards; Williams et al.; Williamson et al., 2013).

Lithic microbial communities are found above and below ground, on rock surfaces, and within rocks themselves. Desert rock varnish, which differs from Fe/Mn films because of the presence of clay minerals, has been a focus of several of phylogenetic studies. Rock varnish from Black Canyon, New Mexico shares some phyla with those found in the rock coatings in this study, namely Actinobacteria, Ktedobacter, Chloroflexi, and Firmicutes (Northup et al., 2010). Despite the similarities in mineralogy between rock varnish and Fe/Mn films, very few of the isolates from the Black Canyon varnish were from the Firmicutes phylum, whereas the Fe/Mn sequences from Kärkevagge were entirely Firmicutes bacteria, namely Bacillus spp. It should be noted, however, that Bacillus spp. sequences represented some of the only known manganese oxidizers isolated from the varnish samples (Northup et al., 2010). Inferred physiology, microscopy, culturing, and mineralogical analyses of rock varnish suggest a bacterial influence on its generation (Northup et al., 2010; Krinsley et al., 2009; Wang et al., 2011; Dorn, 1998). Sulfate crusts consisting of gypsum and halite also show distinct endolithic bacterial communities (Stivaletta et al., 2010; Wierczhos
et al., 2006; Wong et al., 2010). These coating types are unique in that they are translucent enough to allow for photosynthesis, and thus, many of the analyses of these coating types either directly or indirectly suggest a strong presence of photosynthetic bacteria. Sequences in this study contained a small representation of photosynthetic bacteria from the Chloroflexi phylum.

2.5.2 Relationship to acid-mine drainage (AMD)

Given its association with acid-mine drainage, jarosite has frequently been proposed as a possible byproduct of microbial metabolism in these environmental settings (Sharp et al., 1999; Verplanck, 2008; Baker and Banfield, 2003). The similar geochemistry between AMD sites and that found in Kärkevagge, along with some shared bacterial community compositions, suggests that the rock coatings from Kärkevagge may similarly be generated through bacterial metabolism. The deglaciation of the valley represents the anthropogenic earth-moving processes associated with the development of AMD, which exposes the finely disseminated pyrite found throughout the valley to oxidation (Darmody et al., 2007; Darmody et al., 2001). In AMD, acidic waters generated by microbial metabolisms promote the precipitation of sulfate minerals into the environment (Johnson, 1998; Schippers et al., 2010; Meier et al., 2012). Microbes have also been shown to affect the rates of sulfur oxidation (Edwards et al., 2000). Because base metal mining is often associated with pyrite-rich deposits, microbial metabolism is thought to play a role in the acidification of mining sites and the generation of AMD (Baker and Banfield, 2003). Communities of bacteria found in AMD environments that have physiologies compatible with reactions producing sulfates and other AMD-related minerals. Such taxa include the phyla Nitrospira, Actinobacteria, Firmicutes, and Acidobacteria, and the genera Acidocella, Leptospirillum, Acidithiobacillus, Acidisphaera, Leptospirillum, Thiobacillus, Sulfobacillus, Ferroplasma, Sulfobacillus, and Acidiphilum (Baker and Banfield, 2003; Bond et al., 2000; Schippers et al.,
The Actinobacteria, Firmicutes, and Acidobacteria phyla and the genera Acidocella, Acidisphaera, and Acidiphilium are common sequences isolated from the rock coatings in this study. Similarly, the acidic, sulfur-rich aspects of martian geochemistry have been related to AMD on Earth (Elwood Madden et al., 2004). This type of geochemical regime is shared between Mars, Kärkevagge, and some acid-mine drainage environments.

The bacterially facilitated formation of Al-rich sulfate minerals in AMD settings has recently been examined in a study by Meier et al. (2012). They identify white-grey aluminum precipitates produced in acidic enrichments by sulfate-reducing bacteria (SRB). Equilibrium calculations predict these precipitates to be alunite and gypsum, though this was not supported by experimental data (Meier et al., 2012). Further, Al-sorption to cells has been observed in SRB, with higher absorbance during freezing and thawing of cells and at low pH (Hard et al., 1999). Though no sulfate-reducing bacteria were identified in the aluminum glaze, it is conceivable that SRB are still present in the coatings but were not identified in the small clone sample size. A relatively novel species, Acidocella aluminiidurans, has been identified as being Al-tolerant with optimum growth at low-to-neutral (3-7) pH in media containing aluminum sulfate or aluminum chloride (Kimoto et al., 2010). Significantly, the Al-rich crusts in Kärkevagge are dominated by basaluminite (an aluminum oxyhydroxide sulfate), with minor abundances of gypsum and alunite.

2.5.3 Implications for astrobiology on Mars

Kärkevagge represents a strong mineralogical and geochemical, but more limited climatic, analog for Mars. Goethite and hematite, the primary minerals of the Fe/Mn films in Kärkevagge, have been detected using Mössbauer spectroscopy onboard the Mars Exploration Rover (MER) Spirit at Columbia Hills (Klingelhöfer et al., 2007). Crystalline hematite mineralization has been observed in Meridiani Planum by the Mars Global Surveyor Thermal Emission Spectrometer, with
surface coatings cited as a possible mechanism for the observed coarse-grained hematite (Christensen et al., 2000). Jarosite and gypsum are present in Kärkevagge as dominant minerals in sulfate crusts, and have also been detected on Mars through both observational satellites and MER rovers (Klingelhöfer et al., 2004; Squyres et al., 2004; Wang et al., 2006; Bibring et al., 2005; Bibring et al., 2006; Gendrin et al., 2005). The aluminum glazes in Kärkevagge are predominately basaluminite, and while basaluminite itself has not been detected on Mars, alunite has been detected by the CRISM instrument on board the Mars Reconnaissance Orbiter (Swayze et al., 2008). Alunite has been inferred as a subordinate mineral in the aluminum glazes in Kärkevagge (Darmody et al., 2007). Goethite, hematite, jarosite, and gypsum have all been identified as minerals that can be directly and indirectly formed in association with bacteria (Roh et al., 2003; Zachara et al., 2002; Konhauser, 1997; Kleinmann et al., 1981; Larese-Casanova et al., 2010; Dorn, 1998; Banfield et al., 2000; Abreu et al., 2011; Clarke et al., 1997; Kappler et al., 2006).

As an environmental analog, Kärkevagge is a moderately cold, acidic, and dry environment, and may represent environmental conditions that existed in the putative early warm and wet Mars (Manga et al., 2012; Pollack et al., 1987; Baker et al., 1991; Phillips et al., 2001; Sagan and Mullen, 1972; Fairén et al., 2011). Rock coatings are present in extreme Earth environments that are similar to martian conditions, including the Atacama Desert (Wierzchos et al., 2006; DiRuggiero et al., 2012), Antarctica (Wierzchos et al., 2003; Weed and Ackert, 1986; Weed and Norton, 1991; Matsuoka, 1995), and numerous acidic and sulfur-rich environments (Fernández-Remolar et al., 2005; Fernández-Remolar and Knoll, 2008; Nordstrom and Alpers, 1999; Keith et al., 2001; Jamieson et al., 2005).

Terrestrial rock coatings have been proposed as possible analogs for life on the martian surface because of reduced irradiation levels (McKay, 1993; Wynn-Williams and Edwards, 2000;
Cockell et al., 2000). Viking demonstrated that surface radiation on Mars can apparently destroy any detectable organics, even those expected from meteoritic delivery (Dartnell et al., 2007; Doi, 1973). However, the reduced radiation levels afforded by rock layers could protect organics preserved in the coatings themselves (Wynn-Williams et al., 1999; Wynn-Williams and Edwards, 2000). Microbes colonizing rock coatings need only be at a depth of 1-2mm for DNA-affecting radiation to be reduced to levels that the surface of present-day Earth experiences (Cockell et al., 2000; Friedmann, 1982; Nienow and Friedmann, 1993; Nienow et al., 1988).

The ChemCam instrument onboard Mars Science Laboratory (MSL) provides a means of micron-scale depth profiling of rock surfaces and subsequent elemental analysis using laser-induced breakdown spectroscopy (LIBS). A logistical concern described by the ChemCam team was discerning between regolith dust coatings on the surfaces of rocks and the target parent rock. However, this same analysis can be used to collect data on putative rock coatings. Backscatter electron microscopy has shown μm-scale layer sub-structure of rock coatings from Kärkevagge, with especially apparent layers in iron films (Dixon et al., 2002). ChemCam’s LIBS instrument provides a means of identifying rock coatings through elemental analysis at depth, thus providing targets for analysis by other MSL instrumentation, such as the Mars Hand Lens Imager (MAHLI) and the Alpha-Particle X-ray Spectrometer (APXS).

Thin rock coatings rich in goethite and hematite have been observed by the Viking Lander on Mars (Clark et al., 1976). These minerals are the primary minerals in the Fe/Mn films from Kärkevagge, and thus, the Fe/Mn films represent terrestrial analogs to the martian coatings observed by Viking. Molecular analyses in this study have identified the presence of Fe- and Mn-concentrating bacteria in the films and SEM has shown evidence of microbe-mineral interactions.
and possible biomineralization. This preservation of these features could be applied to future Mars missions in the search for evidence of extinct or extant life on the planet.

### 2.6 Conclusions

Rock coatings on Mars are biologically intriguing targets for potential sites of past or present life. Though the subsurface of the planet may represent environments most hospitable for life as we know it, rock coatings provide a more readily accessible rover and satellite target. As a surface environment, coatings have the added benefit of affording protection from radiation and other stresses that might be harmful for life and its biosignatures. Further, the coating itself can potentially preserve evidence of past life in the form of mineralized filaments and other morphological characteristics. In this study, we have confirmed bacterial colonization of rock coatings in a terrestrial setting and identified taxa that have the capacity to participate in the biomineralization of iron oxides and aluminum and iron sulfates, thus setting the framework for the potential of rock coatings to serve as biosignatures on Mars.

Establishing the biogenicity of formations that cannot immediately be identified as biological, such as rock coatings, is a persistent challenge in astrobiology. While biofilms and stromatolites have textures and morphologies characteristic of life, biologically-mediated mineral accretions such as rock coatings, rock varnish, and some cave formations have obfuscated indicators of the presence of microorganisms. Understanding the community structure of the rock coatings is an essential first step toward the end goal of developing a catalog of biosignatures that are detectable by the instrumentation aboard current and future Mars missions.

Rock coatings are a unique habitat for microbes with distinct communities and subsequently, a range of metabolic capabilities. Sequences from rock coatings in Kärkevagge
possess the capability to participate in the genesis of rock coating accretions, and possibly are essential to that process. Rarefaction curves and low diversity in the Fe/Mn films suggest that even greater bacterial diversity may be found in these coatings. Our results represent an initial investigation at the bacterial communities present in rock coating types that have been largely ignored as targets of astrobiological interest, despite their compatibility with martian mineralogy. Thus, we suggest that other rock coatings, in addition to varnish, warrant further study for their potential to form biosignatures.

2.7 Acknowledgements

This paper was published by Cambridge University Press in the *International Journal of Astrobiology* in 2013, and reprinted here with permission. The authors would like to acknowledge Jack Denson, D. Mack Ivey, Tim Kral, and Ryan Sheehan for their invaluable assistance in the laboratory. Field work was carried out through funding and logistical support from the Royal Swedish Academy of Sciences and the Abisko Naturvetenskapliga Station (ANS), which the authors gratefully acknowledge. The authors also thank Dr. Mourad Benamara for access to the University of Arkansas imaging laboratory and his expertise. We additionally thank Rasmus Johansson for graphical assistance and an anonymous reviewer for helping improve the quality of this paper.
2.8 References


Baker, B.J., Banfield, J.F. 2003, FEMS Microbiology Ecology, 44(2), pp. 139-152.


Glasby, G., Macpherson, J. 1981 Desert varnish in southern Victoria land, Antarctica, Department of Scientific and Industrial Research.


Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. 2011, Molecular biology and evolution, 28(10), pp. 2731-2739.


Williams, A.J., Buck, B.J., Soukup, D.A., Merkler, D.J. Geomorphology, (0).


November 22, 2013

To Whom it May Concern:

I hereby certify that the first author of the two papers included in this dissertation is my doctoral student Cassandra Marnocha and that she completed at least 51% of the work reported in each paper. The two relevant papers are as follows:


Sincerely

John C. Dixon

Professor.
3 Pyrosequencing of endolithic bacterial communities in rock coatings from Kärkevagge, Swedish Lapland

3.1 Abstract

Rock coatings in Kärkevagge, Swedish Lapland are widespread and mineralogically diverse. A preliminary study of the rock coatings revealed higher than expected bacterial diversity for an endolithic environment in the arctic. Using 454 Roche pyrosequencing of the 16S rDNA gene, nine rock coating samples from three different coating mineralogies were sequenced. The three coating types include Fe films of goethite and hematite, sulfate crusts of jarosite and gypsum, and aluminum glazes of basaluminite and alunite. Over 20,000 quality sequences were analyzed, and over 2,800 operational taxonomic units (OTUs) were identified. Diversity indices and richness estimates confirmed high levels of diversity, particularly in the sulfate crusts with diversity indices at the level of complex soils. Inferred physiology shows the presence of both heterotrophs and phototrophs, with genera of autotrophic Fe- and S- metabolisms present in at least 2% of the total for each coating type. The most common phyla included Proteobacteria, Acidobacteria, and Actinobacteria – all common soil taxa. Coatings also showed distinct community structure between coating type/mineralogy. Given the diversity in coating types found in areas receiving the same chemical and environmental inputs, the distinct microbial communities suggest a biological role in coating development.

3.2 Introduction

The relationship between microbial communities and the lithic substrates they inhabit has been well established (Amy et al., 1992; Dorn, 1998; Friedmann, 1982; Krinsley et al., 2009;
Kuhlman et al., 2006; Tang et al., 2012; Wierzchos et al., 2006). The biogeochemical cycling and microstructures necessary to form rock coatings necessitates high microbial diversity. These diverse communities participate in Fe-, S-, and Mn-cycles, for example, and have been shown to play a significant role in the development of cave speleothems (Boston et al., 2001; Melim et al., 2009; Melim et al., 2008; Northup et al., 2003), acid-mine drainage deposits (Baker and Banfield, 2003; Bond et al., 2000; Chan et al., 2001; Chen et al., 2013; Hao et al., 2012; Tan et al., 2007), sulfate deposit formation (Glamoclija et al., 2012; Meier et al., 2012; Parnell et al., 2004; Wilhelm et al., 2012), and rock varnish development (Dorn, 1998; Krinsley et al., 2009; Kuhlman et al., 2006; Northup et al., 2010; Perry et al., 2004; Schelble et al., 2005). Despite the interest in rock varnish, the microbial diversity associated with other rock coatings types has not been investigated in as much detail. Rock coatings are defined as mineral accretions on the surfaces of rocks that form from material transported to the parent rock (Dorn, 1998). The Fe-films, sulfate crusts, and aluminum glazes occurring in Kärkevagge, Swedish Lapland, are examples of such under-investigated rock coating types that have not been well-studied. The objective of this study is to investigate the pyrosequencing of bacterial assemblages associated with specific types of rock coatings.

Kärkevagge is a glacially eroded valley in Swedish Lapland, initially the site of a comprehensive study of slope processes in Arctic/Alpine environments (Rapp, 1960). Significantly, Rapp elucidated the importance of chemical weathering processes in this environment from the presence of white streaks on the valley walls and rust-colored coatings on boulder surfaces. Subsequent studies by Dixon and colleagues identified these deposits as rock coatings (aluminum glazes and Fe-films, respectively), along with sulfate crusts and a variety of heavy metal skins (Dixon et al., 2002).
Despite apparent environmental similarities, coating types of different chemical composition occur in close proximity to each other. Sulfate crusts and Fe films can be within meters of one another, a phenomena recently described in detail from Yosemite Valley by Larson and Dorn (2012). These host rocks are presumably fed by the same moisture sources with similar chemical content for the building materials of rock coating minerals. With the same or similar parent rock and environmental exposure, this suggests a non-environmental component in the determination of which coating is accreted on rock surfaces. Microbes have been shown to participate in biomineralization processes, through both controlled and inductive processes, to produce the coatings observed in Kärkevagge (Marnocha and Dixon, 2013a, 2013b). However, the compositions of the discrete assemblages of microbes previously reported has received little attention.

Biologically-controlled mineralization is associated with the direct metabolic reactions of the microbe (Konhauser, 1997). A bacterium may produce a mineral precipitate, even when not thermodynamically favorable abiotically. Likewise, organisms can produce chemical byproducts from their metabolisms that are later transformed abiotically into mineralized precipitates. In the case of biologically-controlled mineralization, high microbial diversity provides a micro-environment more conducive to biomineralization, with more taxonomic diversity generally meaning more metabolic diversity. Through microbial diversity surveys, taxonomic assignments can be made, and from those assignments, the metabolic potential of the community can be inferred. The metabolic capacity of a microbial community derived from diversity studies is valuable in assessing what precipitates may be formed through biologically-controlled mineralization (Abreu et al., 2011; Benzerara et al., 2008; Macalady et al., 2007; Miot et al., 2009; Zachara et al., 2002).
Biologically-induced mineralization is the interaction between microbial cells and the surrounding environment (Konhauser, 1997). Microbes need not contain any specific metabolisms or structure to participate in biologically-induced mineralization. The charged nature of the cell, extracellular polymeric substances (EPS) associated with bacteria, and the release of cations from the cell during slight chemical perturbations can occur with virtually all microbes. Thus, biologically-induced mineralization is the predominant biomineralization mechanism for bacteria, if only for the lack of a need for any specialization. Biologically-induced mineralization can work in tandem with biologically-controlled mineralization. For example, sulfate-reducing bacteria (SRB) produce \( \text{H}_2\text{S} \) as a byproduct of their primary metabolism. \( \text{H}_2\text{S} \) can react with Fe to produce iron sulfide precipitates, including pyrite and mackinawite, the latter exclusively produced by bacteria in surface reducing environments (Herbert Jr et al., 1998; Langumier et al., 2009; Pósfai et al., 1998).

To understand the mineralogical diversity of rock coatings in Kärkevagge, we have undertaken a study of bacteria diversity using 454 Roche pyrosequencing. We have used these results to assess community structure, compare diversity, and assess the metabolic capacity of the incorporated communities. From taxonomic assignment, metabolic potential of the community can be inferred and associations between mineralizing metabolisms and rock coating mineralogy can be made.

There are frequent cases in Kärkevagge where a Fe film on a boulder may exist within a meter or two of another boulder with a sulfate crust. With the same bedrock mineralogy and the assumption that inputs to the system and environmental conditions (e.g. pH, temperature, etc.) are the roughly the same, the question remains as to how two boulders in close proximity may accommodate rock coatings with different mineralogies, textures, and structures.
We suggest that the composition of bacterial communities that facilitate or inhabit the rock coatings are responsible for determining what coatings are produced.

3.3 Methods

3.3.1 Study site and sampling

Rock coating material was sampled from Kärkevagge, Swedish Lapland (68°26′N, 18°18′E). Kärkevagge is a classically U-shaped, deglaciated valley with a mean annual air temperature of -2°C (Thorn et al., 1999) and bedrock/soil interface temperatures that approximate -15°C (Thorn et al., 2001). The valley receives approximately 800 mm of precipitation annually, 50-75% in the form of snow (Eriksson, 1982). Snow is present on the ground for approximately 240 days per year and has an average depth of 1.5m. The region was deglaciated some 10,000 years ago (André, 2002). A cosmogenic exposure date from the boulder field on the valley floor dates at 13,000 +/-1638. The oldest buried soils developed in colluvial deposits on the valley floor from radiocarbon dated arbuscular mycorrhizae spores date at 5589 years before present (Thorn et al., 2009).
Three primary rock coating types were selected for study. Fe-films are ubiquitous in the valley and range in texture and appearance from rust-colored, paint-chip-like coatings, to thicker, smoother, and darker coatings (Fig. 1a). They are composed predominantly iron oxhydroxides (e.g. goethite \([\alpha-\text{FeO(OH)}]\), hematite \([\text{Fe}_2\text{O}_3]\)) with up to 44% Fe content and 0.05% Mn (Darmody et al., 2007). Sulfate crusts (Fig. 1b) examined in this study are dominated by jarosite \([\text{KFe}^{3+}\text{Fe}^{3+}(\text{OH})_6(\text{SO}_4)_2]\) with minor abundances of gypsum \([\text{CaSO}_4\cdot2\text{H}_2\text{O}]\) (Darmody et al., 2007). Aluminum glazes are thin, soft, white coatings found in or near streambeds (Fig. 1c). They are composed of basaluminite \([\text{Al}_4(\text{SO}_4)(\text{OH})_{10}\cdot\text{H}_2\text{O}]\) and alunite \([\text{KAl}_3(\text{SO}_4)_2(\text{OH})_6]\) (Darmody et al., 2007).
Rock coating material was aseptically collected on sampling transects established by Rapp (1960) along the valley walls. Samples were stored in sterile, screwtop tubes, transported, and kept at -20°C until submission to the Research and Testing Lab (Lubbock, TX) for 16S rRNA 454 Roche pyrosequencing using a 28F-519R assay.

3.3.2 Pyrosequencing

Representative samples of each coating type were submitted to the Research and Testing Lab (Lubbock, TX) for DNA extraction and subsequent 454 pyrosequencing of a 28F-519R 16S rRNA assay, quality control and taxonomic identification. Reads were trimmed and issued quality scores, clustered using USEARCH, checked for chimeras using UCHIIME, and denoised. The remaining denoised sequences were then clustered into operational taxonomic units (OTUs) with 100% identity using USEARCH and taxonomically identified with BLASTn+ and assigned taxonomic classification based on identity scores to well characterized database sequences. Sequences with percent identity below 77% were not used in subsequent analyses.

3.3.3 Statistical analysis

Partial 16S rRNA sequences were obtained from nine rock coating samples—three each of Fe-films, sulfate crusts, and aluminum glazes—following the quality and denoising protocols described above. The software package, mothur (Schloss et al., 2009), was used to generate distance matrices, rarefaction curves, richness indices, and diversity indices. It should be noted that values for Simpson’s index can range from 0 to 1, with 0 representing infinite diversity and 1 representing no diversity. The UniFrac P test was used to test whether any of the coating communities had structures significantly different from one another (Doi, 1973). Operational taxonomic unit (OTU) analyses were constructed at a genetic distance of 0.03 unless otherwise
stated. Preliminary 16S Sanger sequencing data referenced in this study underwent similar quality control and statistical analyses (Marnocha and Dixon, 2013a)

3.4 Results

After quality and chimera checks, 21,371 unique sequences from 9 sample were analyzed (Table 1). Quality sequences ranged from 105-537 basepairs, and averaged 408 basepairs. Fe and Al coatings returned approximately 8500 quality sequences each, while sulfate crusts returned about half as many – likely the result of a sequencing artifact. Rarefaction curves indicate good coverage at a genetic distance of 0.05, though it is likely that additional sequencing of sulfate crusts would yield significantly greater diversity (Fig. 2). ACE and Chao1 values estimate that 29-67% of the community was represented by sequencing for Fe-films, 35-50% for sulfate crusts, and 46-57% for aluminum glazes.

**Table 1.** Sequences, OTUs, richness estimates, and diversity indices. Number of sequences are based on those that passed quality and chimera checks and OTUs calculated at a genetic distance of 0.03. Richness estimates and diversity indices were likewise calculated at a genetic distance of 0.03. For the Simpson’s index of diversity, a number closer to zero indicates higher diversity.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Sequences</th>
<th>OTUs</th>
<th>ACE</th>
<th>Chao1</th>
<th>Shannon</th>
<th>Simpson’s</th>
<th>I-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe-films</td>
<td>8799</td>
<td>795</td>
<td>2752.081</td>
<td>1776.4</td>
<td>3.8955</td>
<td>0.132894</td>
<td>0.867</td>
</tr>
<tr>
<td>Sulfate</td>
<td>4226</td>
<td>1055</td>
<td>3035.931</td>
<td>2091.769</td>
<td>5.5934</td>
<td>0.014044</td>
<td>0.986</td>
</tr>
<tr>
<td>Aluminum</td>
<td>8346</td>
<td>1014</td>
<td>2196.534</td>
<td>1761.763</td>
<td>4.1313</td>
<td>0.119759</td>
<td>0.880</td>
</tr>
</tbody>
</table>

Fifteen phyla were present across the nine samples, with ten of those phyla present in all three coating types. *Proteobacteria* (α-, β-, γ-, and ε-proteobacteria) made up 67.8% of all sequences in Al glazes, 68.3% in Fe-films, and 41.5% in sulfate crusts (Fig. 3). Photosynthetic
Cyanobacteria were common in sulfate crusts, contributing to 13.3% of all sequences. Acidobacter (19.0% SO₄, 12.0% Fe-films) and Actinobacter (13.4% Fe-films) were also well-represented.

Figure 2. Rarefaction curves for combined sequences for each coating type. Top: rarefaction curves calculated from a genetic distance of 0.03; Bottom: Rarefaction curves at a genetic distance of 0.05.
When sequences were grouped by coating type and communities are compared against one another, there appears to be no significant correlation between communities based on coating mineralogy ($P < 0.01$, Fig. 4). The most diverse sequences came from the sulfate crusts, followed closely by the Al glazes, and then the Fe films.

Figure 3. Distribution of phyla across all 9 rock coating samples as classified by BLASTn+. Proteobacteria have been separated on the class level.

Fe(III), Cr(VI), Mn(IV), sulfate, sulfite reduction; Fe(II), S, U, arsenic oxidation; photosynthesis, nitrogen-fixation and methylotrophy were among the most common metabolisms.
throughout the coatings. These metabolisms were inferred at the genera level, and represented 2% or more of the total community in at least one coating type.

Figure 4. Venn diagram of shared OTUs between rock coating groups at distance of 0.03.

3.5 Discussion

3.5.1 General characteristics of rock coating bacterial communities

Heterotrophic bacteria were common in the coatings and heterotrophism was the most common energy-acquiring mechanism for the 32 OTUs shared between all three coating types. The widespread distribution may be a result of generic nutrient requirements readily available from sources throughout the valley. Photoautotrophs would need to be either hypoliths (rock surface-dwelling microbes), or shallow endoliths with some transparency available in the coating matrix for the acquisition of photons. Chemoautotrophs may require more specific compounds or minerals
in order to be competitive in the microbial community. In this case, sulfate-reducing bacteria populations may only thrive on sulfate crusts or aluminum glazes, where sulfates are a primary component.

The high diversity in the sulfate crusts could be explained by the typical distribution of crusts in the valley. Crusts often occur on the underside or un-/under-exposed surface of a boulder, with mats of lichens inhabiting the top and more exposed surfaces of the boulder. The lichens could serve as a source of organic nutrients that would provide a stable and regular source of organic compounds to support large heterotrophic communities.

With respect to metabolisms relevant to the formation of rock coatings, Fe- and S-metabolisms are by far the most common. These oxidation and reduction pathways/cycles have the capacity to directly precipitate relevant minerals, or produce chemical species as waste products that may combine with ions in the environment to produce minerals.

3.5.2 Comparison to other microbiomes

The bacterial communities in Kärkevagge perhaps best mirror those of soil communities, where Protoebacteria, Acidobacteria, and Actinobacteria make up the dominant phyla (Janssen, 2006). Protoebacteria, Acidobacteria, Actinobacteria, Verrucomicrobia, Bacteroidetes, Chloroflexi, Planctomycetes, Gammatimonadetes, and Firmicutes make up a normalized average of over 90% of clone libraries found in an analysis of 16S rRNA studies in soils. Like Kärkevagge, of the Proteobacteria, Alphaproteobacteria make up the largest contribution in 21 clone libraries analyzed (Janssen, 2006). Studies suggest that phyla abundance can be influenced by soil moisture (Buckley and Schmidt, 2001) and pH (Verrucomicrobia and Acidobacteria, respectively). These influences may in part explain the dominance of Acidobacteria in rock coatings from Kärkevagge (near neutral-to-acidic pH), and the lesser abundance of Verrucomicrobia (driest area in Sweden).
In a bacterial diversity study of basaltic glass and obsidian, *Actinobacteria*, *Acidobacteria*, *α-proteobacteria*, and *Cyanobacteria* were the most dominant phyla (Kelly et al., 2010). This study found that OTUs were specific to substrate. Other work by Kelly et al. (2011) shows that volcanic glasses have comparable diversity levels to volcanic soils, in which, *Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, and *Cyanobacteria* are the most abundant phyla. There is significant overlap to the communities present in the basaltic glass and those in volcanic soils. This relationship may be evidence for the source of the original colonization of the rocks. In Kärkevagge, for example, the “building material” for coatings as well as the keystone microbial species may be sourced from the nearby soil and explain the abundance of soil microbes in the rock coatings. Sourcing of microbes from soil to rock surfaces could occur through hydrological processes (rain, meltwater, streams, snow and snowmelt) or from transport of soil microbes made airborne.

Bacterial communities in endolithic soil gypsum varied with sample site. *Cyanobacteria* dominated clone libraries from Jordan (~80%) and the Atacama Desert (36%), with *α-proteobacteria* making up 30% of the Atacama Desert library and 40% of the Mojave Desert library (Dong et al., 2007). The differences in community structure appear to be determined by the micro-structure of the gypsum crusts, with heterotrophs more common in fine-grained gypsum from the Atacama and Mojave (Dong et al., 2007). The optical properties of the fibrous gypsum sampled from the Jordan Desert allow for deeper colonization below the surface and likely influence the abundance of *Cyanobacteria* (Dong et al., 2007).

Large heterotrophic populations were an unexpected constituent of the microbial communities. While phototrophs and chemolithoautotrophs can utilize the abundant light and inorganic compounds to metabolize and grow, heterotrophs require organics. It is conceivable that
heterotrophs, at least in the sulfate crusts, are fed the necessary organic material from eukaryotes. Sulfate crusts often appear on the underside of boulders, with mats of lichens on the topside. These lichens could provide the organic acids necessary to sustain a diverse heterotrophic population. In fact, a common symbiont of lichens, the photosynthetic Nostoc spp., made up over 6.5% of all sulfate crust sequences.

The Shannon diversity index takes both evenness and abundance into consideration and generally ranges between 1.5 and 4.5. In the most diverse and complex environments, such as soils and lakes (Magurran, 1988; Mikucki and Priscu, 2007), values can exceed 4.5 and in some cases be as high as 7 or more. Given the number of soil microbes present in the rock coatings, it is not surprising that all coating types exceeded 3.5, with sulfate crusts reaching over 5.5. In arctic and Antarctic environments (permafrost, ice wedges, brines) Shannon index values typically lie on the lower end of the expected range (Hinsa-Leasure et al., 2010; Mikucki and Priscu, 2007; Perreault et al., 2007; Wilhelm et al., 2012). Cold environments do not necessarily indicate lower diversity, as actual microenvironment plays a significant role. Lake sediments from West Lake Bonney in Antarctica, for example, had several Shannon values of 4.5-4.7 (Tang et al., 2013).

However, substrate or environmental conditions appear to serve as possible limiting factors in microbial diversity. A study of endolithic microbes in volcanic deposits showed Shannon values ranging between approximately 2.5 and 3.75 (Gomez-Alvarez et al., 2007), and endolithic communities in dolomite and limestone also had values in this range (Tang et al., 2012). Yet, hypoendoliths in crusted soils averaged Shannon values around 1.1, but were comparably higher than that of the surrounding sandy, barren soils (Bhatnagar et al., 2008). In acid-mine drainage environments, Shannon values appear to correspond directly with pH and decreasing Fe concentration (Mendez et al., 2008). High Fe concentration may explain the lower diversity values
for the Fe-films in Kärkevagge. When compared to an earlier study in Kärkevagge by the authors, Al glazes, not sulfate crusts, were most diverse, though Fe films were also found to be the least diverse (Marnocha and Dixon, 2013a). Simpson’s diversity indices (D), where lower values for D mean higher diversity, match well with the Shannon index values.

3.5.3 What influences coating formation?

A question that remains in the understanding of rock coating development in Kärkevagge is what influences one particular coating type to form over another. In some cases, two boulders may be separated by a couple of meters, though one is covered in an iron film, and the other a sulfate crust. The environmental exposures and inputs to these coatings are likely the same, so what determines the coating mineralogy that is formed?

16S sequences from chloroplasts were present on many of the samples. These chloroplast sequences may represent microscopic plant material transferred to the rock surface. The appearance of chloroplast DNA across all coating types, however, provides further evidence that the inputs to the rock coatings are the same or similar. It should be noted, however, that the distribution of aluminum glazes is more limited than the other coating types. The Al-glazes are found almost exclusively along streambeds or near small springs along the valley wall. This may provide a more consistent source of Al ions. Consequently, as mean average annual temperatures increase in the valley over time, increased meltwater may also contribute in some part to the observed decline in the abundance of aluminum coatings over the last two decades.

3.6 Conclusions

Pyrosequencing yielded a significant increase in diversity coverage when compared to the Sanger sequencing of our previous investigations (Marnocha and Dixon, 2013a). In comparison
with similar environments, the bacterial diversity of rock coatings observed in the valley is relatively high. Bacterial communities also appear to be relatively diverse when compared to other rock coating communities, including rock varnish from both desert and non-desert environments. The diversity of the rock coatings in Kärkevagge is similar to what is observed in complex microbial ecosystems like soils, and indeed, many of the taxonomic groups present in the rock coatings are known soil inhabitants. Community makeup also appears to be distinct between rock coating mineralogy and offers evidence that microbes may play a key role in the determination of what rock coating develops on which host rock.

3.7 Acknowledgements

The authors thank the American Philosophical Society and the Lewis and Clark Fund for Exploration and Field Research in Astrobiology for grant funding for the 2012 field season and portions of the data analysis. We gratefully acknowledge Abiskonaturvetenskapliga and the Polar Sekretariat of Sweden for logistical support in the Abisko area during fieldwork. We additionally thank Rasmus Johansson for assistance with figures and Dr. Tim Kral for the use of lab space and facilities.
3.8 References


4 Bacterially-facilitated rock-coating formation as a component of the geochemical budget of cold climates: An example from Kärkevagge, Swedish Lapland

4.1 Abstract

Environmental microbiology and advances in molecular techniques have been a driving force in advancing the understanding of microbial communities in previously understudied environments. Though it is widely accepted that biological and geological processes are closely linked, the importance of microbes in geomorphological processes has been understated. Microbes interact with the environment, playing a significant role in nutrient cycling, ion mobilization, and metal scavenging and concentration. Although in some of these areas understanding is expanding, the role of microbes in geochemical budgets in cold climates has been largely ignored. To investigate one such case of microbial influence, we focus on rock-coating development in the glacially eroded valley, Kärkevagge, in arctic-alpine Sweden. This bacterial diversity study shows evidence of a link between microbe-mineral interactions and key processes in the formation of diverse geochemical rock coatings. Here, we present a study of the bacterial role in metal scavenging and coating formation as a component of the geochemical budget of the valley.

4.2 Introduction

Bacteria have long been associated with mineral weathering and accretionary processes (Beveridge et al., 1985, 1989; Krumbein and Dyer, 1985; Ferris et al., 1987; Adams et al., 1992; Konhauser et al., 1993; Urrutia and Beveridge, 1994; Dorn, 1998;). Microbes are ubiquitous in nature, found in virtually every environment. The nature of microbial metabolisms enables even a single bacterium to metabolize a variety of chemical species. In addition to physiology, cellular
structure and metabolism-independent processes associated with the cell also interact with the surrounding environment. As such, bacteria are able to scavenge metals, break down minerals, concentrate elements, and precipitate a wide variety of minerals (Konhauser, 1997; Benzerara et al., 2004; López-García et al., 2005; Abreu et al., 2011; Williams et al., 2013). The functional capabilities of bacteria and other microorganisms make them an integral component of geochemical weathering processes.

One such process is that of geochemical rock-coating formation. Rock coatings are ubiquitous, occurring in great mineralogical diversity in a range of environments. Coatings are the mineral accumulations of materials brought from external sources through weathering (Dorn, 1998). The genesis of coatings, in particular rock varnish, is strongly associated with bacteria and their physiologies (Perry et al., 2004; Krinsley et al., 2009; Northup et al., 2010; Wang et al., 2011). As weathering agents, bacteria can dissolve and mobilize ions, subsequently incorporating them into rock coatings. Bacteria can also function as concentrating and mineralizing agents, hence serving both ends of the rock-coating formation process. While rock varnish is well-studied, other coating types are underrepresented in the literature. In this study, we focus on aluminum glazes, sulfate crusts, and Fe/Mn films from the Kärkevagge valley.

Kärkevagge (68° 26’ N, 18° 18’ E) is a glacially-eroded, U-shaped trough located in the Northern Caledonide Mountains of Swedish Lapland (Fig. 1). The valley is approximately 5 km in length, ranging from 600 meters above sea level (masl) in elevation at the mouth of the valley to 800 masl at its head. The mean annual air temperature is -1°C and precipitation measures approximately 1100 mm annually (Strömquist and Rehn, 1981), of which 50-75% comes in the form of snow over a period of 220-240 days.
Figure 1. Location of Kärkevagge in Swedish Lapland.

The lower valley walls are composed of quartz mica schist and upper valley walls of garnet mica schist. The two layers are divided by thin beds of calcite marble, and pyrite is finely disseminated throughout the valley. Kärkevagge, or “Boulder Valley”, gets its name from the large rockfall debris covering much of the valley floor (Fig. 2).
Figure 2. Geological map of Kärkevagge.

Kärkevagge has been the site of investigations of landscape evolution and landscape geochemistry for many decades (Rapp, 1960; Darmody et al., 2000a, b; Thorn et al., 2001; Campbell et al., 2002; Dixon et al., 2002). These investigations have revealed the importance of chemical weathering in cold climates. Rock coatings are ubiquitous in the valley and represent some of the strongest evidence for an active geochemical weathering system in the valley. Preliminary investigations have identified the presence of bacteria in rock coatings through scanning electron microscopy (SEM) and a small-scale diversity study (Marnocha and Dixon, 2011, 2012a, b; Sheehan et al., 2012).

Biomineralization is one mechanism by which bacteria can concentrate chemical species and facilitate rock-coating formation. Bacteria participate in both biologically-controlled and biologically-induced biomineralization. Biologically-controlled biomineralization results from a
biochemical reaction within the cell that produces minerals for a physiological purpose (Konhauser, 1997). In the case of eukaryotes, calcite biomineralization is by far the most common form of biomineralization, where many marine animals precipitate the mineral to serve as the primary constituent in shells. With prokaryotes, biologically-controlled biomineralization comes in the form of magnetotactic bacteria. These bacteria biomineralize magnetite and greigite in structures called magnetosomes, with which they utilize the earth’s magnetic field for navigation (Bazylinski et al., 1995; Abreu et al., 2011; Komeili, 2012).

Biologically-induced biomineralization occurs as either a byproduct of the cell’s metabolism or as through microbe-environment interactions (Konhauser, 1997). In many cases, these processes are interconnected and a number of species in a bacterial community participate in mineralization. For example, the extracellular polymeric substance (EPS) or “slime” of a cell can attract metals electrostatically (Gehrke et al., 1998; Comte et al., 2006; Sand and Gehrke, 2006; Chan et al., 2009; Miot et al., 2009; Smith et al., 2013). These cells then serve as nucleation sites for mineral growth by the metabolism of other bacteria (e.g. Fe(II) reducing bacteria). Similarly, sheathed bacteria are an important component of non-enzymatic scavenging (Ghiorse, 1984; Beveridge, 1989; Ehrenreich and Widdel, 1994; Konhauser, 2007).

Bacteria primarily participate in the scavenging of chemical species in three ways. The first involves direct bacterial action, in the form of bioaccumulation, concentration, and uptake of chemical species for use in metabolic reactions, such as the aforementioned biomineralization. Additionally, some metabolic precipitates themselves can scavenge chemical species. Scavenging can also occur indirectly, through a bacterially-mediated reaction and subsequent abiotic scavenging. Bacterially-facilitated precipitation reactions, following changes in the oxidation state of ions in solution, can be effective at scavenging reactive aqueous species (Banfield et al., 2005).
For example, manganese oxides, which can be produced by manganese oxidizers, are well known scavengers of metallic cations and frequently associated with ferric iron (Geloso, 1927; Goldberg, 1954).

The role of bacteria in geochemical rock-coating formation, both directly and indirectly, should not be understated. While the cycling of C, N, and P in soils is well-studied, microbial cycling of Fe, Mn, Al, and other elements as components of geochemical budgets is not well understood, especially in cold climates. Here, we present the results of sequencing of bacteria in the rock coatings from Kärkevagge and examine the metabolic capacity of bacterial communities as potential cation sinks in the valley.

4.3 Methods

Rock debris displaying accumulations of the principal rock coating types was collected from field sites associated with sampling transects established along the length of the east side of the valley. These transects are subsequently identified by the letters V, L, K, J, and H following Rapp’s (1960) designations. Samples were subsampled from each of the following rock coating types: aluminum glazes, sulfate crusts, and Fe/Mn films. Bulk rock samples were collected aseptically in sterile screwtop tubes, transported, and stored at -20°C.

Three representatives from each coating type (Fe/Mn, sulfate, aluminum) were subsampled and sent to Research and Testing Laboratory (Lubbock, TX) for 454 pyrosequencing of the 16S gene region, 28F-519R primer set, subsequent quality check, and preliminary assignment of taxonomic groups to sequences using BLAST. Sequences were clustered into groups with 100% identity. For further clustering into operational taxonomic units (OTUs) and statistical analysis, the software package mothur was used (Schloss et al., 2009).
4.4 Results

4.4.1 Aluminum glazes

Aluminum glazes present as very thin white coatings that are strongly adhered to rock surfaces (Fig. 3). The glazes are almost exclusively found on cobbles within or very near streams draining from the sides of the valley. Aluminum glazes are composed primarily of basaluminite, an amorphous aluminum oxyhydroxide sulfate \([\text{Al}_4(\text{SO}_4)(\text{OH})_{10}\cdot\text{H}_2\text{O}]\), and minor abundances of gypsum and alunite (Darmody et al., 2007). Although made up primarily of sulfates, the aluminum glazes are distinct from the K-Fe-sulfate crusts in texture, appearance, and chemical composition. The glazes consist of approximately 52% \(\text{Al}_2\text{O}_3\), 10% \(\text{SiO}_2\), 11% \(\text{SO}_3\), 1% K and Ca, and 0.1% Na, Mn, and Mg (Darmody et al., 2007).

![Figure 3. Thin, white aluminum glaze coatings found in a stream. Photo credit: Cassandra Marnocha, Sweden, 2010.](image-url)
The most common genera of bacteria identified in aluminum glaze samples include *Methylobacterium, Staphylococcus, Acidisphaera*, and *Acinetobacter*, making up just over 50% of total aluminum glaze sequences.

### 4.4.2 Fe/Mn films

Fe/Mn films vary in texture and appearance throughout the valley, ranging from a thin, flaky chip-like texture on boulders throughout the valley, to thick, smooth-textured darker coatings found primarily along the western valley wall near streambeds (Fig. 4). The Fe/Mn films are predominately iron oxyhydroxides (primarily hematite and goethite), with compositions of 44% Fe, 9% Al, 6% Mg, 17% Si, 1.4% S, 0.3% K, 0.08% Ca, 0.05% Mn (Darmody et al., 2007).

![Fe/Mn films](image)

**Figure 4.** Left: Lighter colored, flaky form of Fe/Mn films found in the valley. Right: Darker colored and smooth-textured Fe/Mn film. Photo credit: Cassandra Marnocha, Sweden, 2012.

The most common bacterial genera identified in the Fe/Mn films included *Methylobacterium, Acidisphaera*, and *Acidobacterium*, making up approximately 50% of total Fe/Mn film sequences.

### 4.4.3 Sulfate crusts

Sulfate crusts are found throughout the valley, sometimes in association with Fe/Mn films. Crusts are thick and flaky, whitish-yellow/green in color, and found most commonly on the unexposed side of boulders (Fig 5). Sulfate crusts are also strongly associated with “rotten rock”
in Kärkevagge – boulders disintegrating where they stand as outer layers chip and flake off. The sulfate crusts examined in this study are dominated by jarosite \(\text{KFe}_3\text{(SO}_4\text{)}_2\text{(OH)}_6\), composed of approximately 70% O, 1% Si, 10% S, 4% K, and 15% Fe, and lesser amounts of gypsum \([\text{CaSO}_4\cdot\text{H}_2\text{O}]\), composed of approximately 69% O, 2% Al, 1% Si, 12% S, 14% Ca, 2% Fe (Darmody et al., 2007).

\[\text{Figure 5.} \quad \text{A common occurrence of sulfate (jarosite) crusts. The yellow-green crust in this picture appears on a lesser exposed surface on the boulder, while the top of the boulder displays a Fe/Mn film. Photo credit: Cassandra Marnocha, Sweden, 2012.}\]

As the most bacterially diverse coating group, the sulfate crusts did not contain a dominant genus. Approximately 50% of the total sequences were made up of the \textit{Acidobacterium}, \textit{Acidiphilium}, \textit{Salinibacter}, \textit{Nostoc}, \textit{Thiomonas}, \textit{Flavobacterium}, and \textit{Acinetobacter} genera.

\subsection*{4.4.4 Sequencing}

Sequencing yielded \(~8800\) sequences for the Fe/Mn films, \(~4200\) sequences for the sulfate crusts, and \(~8400\) sequences for the aluminum glazes. The greatest diversity was observed in the sulfate crusts, though all three coating groups had high diversity. Comparing diversity between coating types revealed communities that are significantly different from one another, despite some coating samples coming from the same sampling transect. The differences in community structure
therefore suggest that coating mineralogy plays a key role in the composition of the bacterial communities (Fig. 6).

![Venn diagram of shared operational taxonomic units (OTUs) between coating types.](image)

**Figure 6.** Venn diagram of shared operational taxonomic units (OTUs) between coating types. OTUs are groups clustered together based on genetic distance. The OTUs in this figure were generated at a genetic distance of 0.03, that is, sequences were grouped together if their genetic divergence was >3%.

The most common genera in the three major coating types include *Acidiphilium, Acidisphaera, Acidobacteria, Acinetobacter,* and *Methylobacterium.* Collectively, these five genera alone contribute to a metabolic capacity that includes Fe, S, Cr redox metabolisms,
metabolisms associated with the nitrogen cycle, and methylotrophy. Photosynthesis was similarly common, though across a greater number of genera.

**Table 1.** Selected bacterial genera and physiologies. Genera were selected based on their ability to metabolize relevant elements/minerals. * denotes genera that were represented at 2% or greater in at least one coating type.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Physiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidimicrobium</td>
<td>Fe(II) oxidation, Fe(III) reduction</td>
</tr>
<tr>
<td>Acidiphilium</td>
<td>Fe(III), Cr(VI) reduction</td>
</tr>
<tr>
<td>Acidisphaera</td>
<td>Fe(III) reduction</td>
</tr>
<tr>
<td>Acidithiobacillus</td>
<td>S reduction; Fe(II), S, sulfide oxidation</td>
</tr>
<tr>
<td>Acidobacterium</td>
<td>Fe(III) reduction, Fe(II) oxidation</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>Cr(VI) reduction, Mn(IV) reduction</td>
</tr>
<tr>
<td>Aquabacterium</td>
<td>Fe(II) oxidation</td>
</tr>
<tr>
<td>Arcobacter</td>
<td>Sulfide oxidation</td>
</tr>
<tr>
<td>Arthrobacter</td>
<td>Mn and Fe(II) oxidation, Mn reduction</td>
</tr>
<tr>
<td>Bacillus</td>
<td>Fe/Mn oxidation and reduction</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>Fe(III) reduction</td>
</tr>
<tr>
<td>Carnobacterium</td>
<td>Mn(IV) reduction</td>
</tr>
<tr>
<td>Dechloromonas</td>
<td>Perchlorate reduction</td>
</tr>
<tr>
<td>Deferribacter</td>
<td>Fe(III) reduction</td>
</tr>
<tr>
<td>Dehalococcoides</td>
<td>Hydrogen oxidation</td>
</tr>
<tr>
<td>Desulfomicrobium</td>
<td>Sulfate, arsenate reduction; Mn oxidation</td>
</tr>
<tr>
<td>Desulfortomaculum</td>
<td>Sulfate reduction</td>
</tr>
<tr>
<td>Desulfuromonas</td>
<td>Elemental S, sulfate, Fe(III) reduction</td>
</tr>
<tr>
<td>Ferrimicrobium</td>
<td>Fe(III) reduction</td>
</tr>
<tr>
<td>Ferrithrix</td>
<td>Fe(III) reduction</td>
</tr>
<tr>
<td>Geopsychrobacter</td>
<td>Fe(III) reduction</td>
</tr>
<tr>
<td>Hyphomicrobium</td>
<td>Mn oxidation</td>
</tr>
<tr>
<td>Leptothrix</td>
<td>Fe(II), Mn oxidation</td>
</tr>
<tr>
<td>Polaromonas</td>
<td>Sulfur oxidation</td>
</tr>
<tr>
<td>Pseudomonocardia</td>
<td>Sulfide oxidation</td>
</tr>
<tr>
<td>Ralstonia</td>
<td>Se reduction</td>
</tr>
<tr>
<td>Sarcina</td>
<td>Cr(VI) reduction</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>Hg, Fe(III) reduction</td>
</tr>
<tr>
<td>Thiobacillus</td>
<td>Fe(II), S, U oxidation; V reduction</td>
</tr>
<tr>
<td>Thiomonas</td>
<td>Sulfur oxidation, arsenic oxidation</td>
</tr>
<tr>
<td>Variovorax</td>
<td>Sulfite reduction</td>
</tr>
</tbody>
</table>
Fe and S metabolisms are by far the most strongly represented in the rock coatings of Kärkevagge (Table 1). Photosynthesis and methylotrophy are also common, and in some cases can be coupled with the oxidation of other elements and minerals.

4.5 Discussion

4.5.1 Aluminum glazes

Aluminum, even moderate concentrations, can be toxic to many species of bacteria (Keyser and Munns, 1979; Wood, 1995). However, in environments where Al is more concentrated, microbes have developed tolerances to Al and other typically toxic metals. A novel species of Acidocella was recently isolated from extremely acidic, sulfate-rich soils in Vietnam (Kimoto et al., 2010). The organism, Acidocella aluminiidurans, displayed optimum growth at pH 3-7 in culturing media containing aluminum sulfate or aluminum chloride (Kimoto et al., 2010). In addition to Acidocella sp., other acidophilic bacteria including Acidiphilium sp. and Acidobacterium sp. have been shown not only to be tolerant to aluminum, but also exhibited increased growth in its presence (Wakao et al., 2002). Aluminum phosphate and aluminum sulfate provided the greatest enhancement in growth at a pH of 3.5 and lower (Wakao et al., 2002). Acidocella sp. were previously isolated from aluminum glazes in Kärkevagge through a small-scale diversity study (Marnocha and Dixon, 2012a, b). In this study, Acidocella sp. were identified in all three rock coating types and most abundantly in the aluminum glazes. Acidiphilium and Acidobacterium were among the most common genera across all coating types. Additionally, botryoidal cocci bacteria have been observed beneath smooth aluminum coatings (Dixon and Marnocha, 2012).
A study investigating the ability of sulfate-reducing bacteria (SRB) to bioremediate acid mine drainage (AMD)-contaminated waters showed that SRB were able to sorb aluminum in uranium mine tailings (Hard et al., 1999). Aluminum sorption was greater when SRB were exposed to freeze/thaw cycles and acidic pH values (Hard et al., 1999). In a recent study by Meier et al. (2012), SRB have been shown to facilitate the formation of Al-rich sulfate minerals in AMD settings. This example of aluminum fixation is passive – that is, the bacteria are not actively metabolizing Al, but rather their cell walls are serving as binding sites for metals like Al and Mg. Because aluminum is not utilized as an electron donor or acceptor in bacteria, Al scavenging will present as either aluminum fixation to cell walls as described above, or geochemically following the bacterial precipitation of other minerals.

4.5.2 Fe/Mn films

Iron is an essential element to prokaryotic and eukaryotic life alike, and as such, iron metabolisms are perhaps one of the best studied physiologies in bacteria. Iron in its various forms can be oxidized, reduced, concentrated, and deposited – sometimes by the same species or small group of species. Since it can be used as an electron acceptor (ferric iron) or donor (ferrous iron), bacteria scavenging can be in the form of biologically-induced biomineralization. In AMD environments, ground water, marine sediments, and rock surfaces, bacteria are able to precipitate ferrihydrite (Ferris et al., 1989; Konhauser, 1993, 1994, 1996, 1997; Clarke et al., 1997). Iron(II) oxidation generates little energy, and because of this, iron oxidizing bacteria must oxidize substantial amounts of iron in order to maintain growth. As a result, iron oxidizing bacteria can precipitate large amounts of iron (Madigan, 2005).

In Kärkevagge, Fe redox reactions are by and large the most common metabolisms in rock coating bacterial communities. Bacteria that utilize Fe-oxidation coupled with nitrate reduction
form ferric iron precipitates and in some cases can be encrusted with those minerals (Kappler et al., 2006; Benzerara et al., 2008). Preliminary studies from Kärkevagge revealed the presence of both putative cocci- and bacilli-form bacteria, as well as ringed fungal structures beneath a smooth Fe-rich coating (Dixon and Marnocha, 2012). The smooth coating morphology with associated bacterial forms supports bacterially-facilitated Fe coating formation (Dorn, 1998). Conversely, photosynthetic Fe-oxidizing bacteria have been shown to not become encrusted by its precipitates (Ehrenreich and Widdel, 1994). Mn redox reactions can similarly produce mineral precipitates such as rhodochrosite, alabandite, birnessite, todorokite, and buserite (Lewis and Landing, 1991; Roden and Lovley, 1993; Tebo et al., 2004, 2005; Ehrlich and Newman, 2008).

The other mechanisms of metal scavenging are likewise applicable with Fe/Mn oxidizing and reducing bacteria. Metabolic byproducts precipitated by the bacteria can scavenge metals from the environment. Scavenging of Fe, Mn, and other metals has been well documented in marine and hydrothermal systems (Cowen et al., 1985; 1986, 1990; Mandernack and Tebo, 1993; Moran and Buesseler, 1993), as well as in acid mine drainage (AMD) (Ferris et al., 1989; Edwards et al., 2006) and as manganese nodules in both terrestrial and aquatic environments (Bonatti and Nayudu, 1965; He et al., 2008; Mero, 1962; Robbins et al., 1992). Extracellular processes such as encrusted bacterial sheaths and electrostatic interactions with chemical species and the EPS are common with Fe and Mn (Ghiorse and Ehrlich, 1992; Konhauser, 2007; Ehrlich and Newman, 2008). Though sheaths are limited to only some species, they are a permanent structure of those cells that have them, and therefore are especially efficient at metal scavenging.

4.5.3 Sulfate crusts
Sulfur is a necessity for all life, and as such, sulfur metabolisms likely evolved early on in Earth’s history (Canfield et al., 1999, 2006; Wagner et al., 1998). Sulfur metabolisms are diverse, and sulfur utilizing bacteria can carry out redox reactions of elemental sulfur, sulfides, thiosulfates, and sulfates. Like iron metabolisms, sulfur metabolisms are common in the bacterial communities of rock coatings in Kärkevagge.

Kärkevagge has been described as a natural analog for acid mine drainage, such as that of coal mines or sulfur-rich mine tailings. Geologically recent deglaciation in the valley exposed pyrite to oxidation, where in acid mine drainage, anthropogenic processes expose sulfidic materials (Darmody et al., 2001). This comparison was initially described in a strictly geochemical sense; however the analog is valid in terms of the impact on microbial processes. Jarosite, a primary mineral in the sulfate crusts from Kärkevagge, is a common constituent of sulfidic mine waste, and there is a strong association between jarosite precipitation and microbial metabolism (Johnson, 1998; Sharp et al., 1999; Baker and Banfield, 2003; Verplanck, 2008; Breuker et al., 2009; Schippers et al., 2010; Meier et al., 2012). Several of the genera found in AMD-settings are shared with common genera found in the rock coatings of Kärkevagge. These genera include Acidocella, Acidithiobacillus, Acidisphaera, Thiobacillus, and Acidiphilium (Bond et al., 2000; Baker and Banfield, 2003; Schippers et al., 2010).

4.5.4 Bacterial scavenging

The ability of bacteria to scavenge, concentrate, and deposit metals and other cations is well documented (Ghiorse, et al., 1984, 1992; Beveridge et al., 1985, 1989; Ferris et al., 1989; Konhauser, et al., 1993, 2007, 2011; Urrutia and Beveridge, 1994; Dorn, 1998; Ehrlich and Newman, 2008). Endolithic bacteria can create sinks for Mn, Fe, Ca, S, and other materials through mobilization, fixation, and metabolic reactions carried out during rock-coating formation (Uren
and Leeper, 1978; Krumbein, 1983; Nealson, 1983; Gerdes and Krumbein, 1987; Ghiorse and Ehrlich, 1992; Konhauser et al., 1994; Russ et al., 1996). It is likely that a consortium of bacteria and their respective metabolisms work in tandem to facilitate coating formation.

There are three primary means by which rock coating bacteria are able to serve as cation sinks. The first is the uptake of chemical species for use in metabolic reactions. The second scavenging mechanism is geochemical scavenging by minerals precipitated by bacteria. Last, bacteria are able to passively scavenge chemical species through extracellular processes (metal binding and sorption to sheaths, EPS, etc.) In this process, bacterial cells need not be actively metabolizing or even alive, as the organic material remaining from the cell is still able to serve as a binding site for chemical species. In some cases, binding ability can be increased by cellular damage (Hard et al., 1999).

Bacteria colonizing rock coatings from Kärkevagge are able to scavenge and store chemical species in some abundance during coating formation; in this respect, we suggest they represent an appreciable cation sink in the drainage basin.

4.6 Conclusions

Biologically facilitated accumulation of coating-related elements is strongly suggested by the distinct OTUs in each coating type, the bacterial morphologies, and the metabolic pathways of the dominant species. Rock coatings in Kärkevagge are home to diverse microbial communities. The structure of these communities appears to be strongly linked to coating mineralogy. These communities also have diverse metabolic capacities, with chemotrophic metabolisms capable of redox reactions of Fe, Mn, Al, S, and other elements/compounds, and associated K and Ca. These metabolisms can also facilitate bacterial-mediated biomineralization and genesis of rock coatings.
Rock coatings thus serve as cation sinks in Kärkevagge, and are an important and often neglected component of the geochemical budget of cold climate environments.

4.7 Acknowledgements

This paper was published by Elsevier in the journal Geomorphology in 2013, and reprinted here with permission. The authors thank the American Philosophical Society and the Lewis and Clark Fund for Exploration and Field Research in Astrobiology for funding the 2012 field season. We acknowledge Dr. Tim Kral for assistance in and utilization of lab space. We gratefully acknowledge Abiskonaturvetenskapliga for logistical support in the Abisko area. We additionally thank Rasmus Johansson for assistance with the figures presented in this paper.
4.8 References


November 22, 2013

To Whom it May Concern:

I hereby certify that the first author of the two papers included in this dissertation is my doctoral student Cassandra Marnocha and that she completed at least 51% of the work reported in each paper. The two relevant papers are as follows:


Sincerely,

John C. Dixon

Professor.
5 A conceptual model of microbially-mediated Fe-film and sulfate crust formation in Kärkevagge, Sweden

5.1 Abstract

Microbial biomineralization is a well-documented process that occurs in diverse environments and on diverse substrates. Microbially-mediated generation of microbial mats, stromatolites, acid-mine drainage deposits, and cave speleothems are well-described. In Kärkevagge, Swedish Lapland, Fe-films and sulfate crusts are the dominant type of rock coating. Bacterial communities in these coatings have been recently described and include microbial taxa compatible with the biomineralization of observed minerals in the coatings. Using scanning electron microscopy (SEM), we observe microbial colonization of the coatings, an abundance of microbial structures including filaments, chains, and stalks, and evidence of biomineralization and layering of microbe-mineral materials. Comparing environment and community structure of rock coatings from Kärkevagge to other environments with documented microbial activity, we estimate possible timescales for coating formation. In addition, we assert the application of terrestrial rock coatings as analogs to those observed on Mars and their relevance to astrobiology. Here, we present the results of our observations and develop a conceptual, biotic-based model for the formation of sulfate crusts and Fe-films.

5.2 Introduction

Rock coatings, mineral accretions on rock surfaces, form from materials transported to the host rock. While coating development has traditionally been associated with abiotic processes (e.g. mineral precipitation, Fe-rich mineral oxidation), there is abundant evidence for a significant
biological component to the formation process. Organisms, microbes in particular, have the ability to participate in mineralization processes both directly and indirectly, and in some cases, can generate coatings at a rate comparable to or exceeding abiotic processes (Emerson and Revsbech, 1994b; Kappler et al., 2006; Konhauser, 2007). Biomineralization processes can be separated into two categories: biologically-induced biomineralization and biologically-controlled biomineralization.

Biologically-induced mineralization occurs through indirect processes, either using a byproduct of cellular metabolism as a precursor, or interactions between microbe and environment (Konhauser, 1997). In many cases these processes can be interconnected, and a group of organisms in a community are necessary to see the mineralization process through to completion. Extracellular polymeric substance (EPS) or microbial “slime”, for example, can attract metals electrostatically (Chan et al., 2009; Gehrke et al., 1998; Miot et al., 2009; Sand and Gehrke, 2006). Metals attached to cell surfaces then allow the cells to serve as nucleation sites for mineral growth, which can be facilitated by the metabolisms of other microbes (e.g. iron-reducing bacteria). Sheathed bacteria can non-enzymatically scavenge chemical species, contributing to the availability of metabolites for organisms – the former, another example of biologically-induced biomineralization (Beveridge, 1989; Ehrenreich and Widdel, 1994; Fleming et al., 2013; Ghiorse, 1984; Konhauser, 2007).

Biologically-controlled biomineralization results from a biochemical reaction within the cell that produces minerals for a physiological purpose (Konhauser, 2007). In eukaryotes, calcite biomineralization by marine animals to produce shells is an example of biologically-controlled biomineralization. In prokaryotes, biologically-controlled biomineralization comes in the form of magnetotactic bacteria. These bacteria biomineralize magnetite and greigite in structures called
magnetosomes that can be used for navigation using the earth's magnetic field (Abreu et al., 2011; Bazylinski et al., 1995; Komeili, 2012).

Rock coatings from Kärkevagge, a glacially eroded valley in Swedish Lapland, were first identified by Rapp (1960) as “lime crusts” and “rust coatings”. Later studies showed these coatings to be basaluminate, an aluminum sulfate, and Fe oxy/hydroxide films, respectively. More recently, coatings from the valley were studied from an abiotic geochemical perspective by Dixon and colleagues (Darmody et al., 2001; Darmody et al., 2007; Dixon et al., 1995; Dixon et al., 2002). However, microbes were observed in the Fe-films in association with iron platelets, suggesting a possible link between coating development and microbial activity (Dixon et al., 1995).

Recent studies by the authors show significant microbial diversity found within the rock coatings, with communities of bacteria specific to each coating mineralogy. Metabolisms compatible with rock coating development (e.g. Fe-, S-, and Mn-oxidation and reduction) were present in several of the most commonly observed taxa in the rock coatings. Understanding community make-up has supplied further evidence that microbes play a role in coating development, however, understanding how exactly this process occurs in Kärkevagge is not well understood. In this study, present a conceptual model of rock coating formation in Kärkevagge as it relates to microbially-mediated rock coating development.

Kärkevagge represents a potential martian micro-environmental analog: the valley is cold, relatively dry, and has generally acidic water chemistry. Geochemically and mineralogically, the valley is a strong analog with abundant sulfates and iron dominated mineralogies. As rock coatings by definition are composed of materials transported to the rock surface, parent rock lithology is insignificant in the development of the coating, and thus is not considered in the argument for Kärkevagge as a geochemical and mineralogical analog to Mars.
Using scanning electron microscopy techniques, coupled with EDX analyses to build on our previous molecular biology techniques, we here present a conceptual model of coating formation.

5.3 Methods

5.3.1 Study Site

Samples for this study were collected from Kärkevagge, Swedish Lapland, a glacially-eroded U-shaped valley located at (68° 26’ N and 18° 18’ E, (Fig. 1). The valley is bounded by steep bedrock walls, with upper valley walls dominated by beds of resistant garnet mica schist (Dixon et al., 1995). Lower valley walls are predominately quartz mica schist, and separating the two schist units is thinly bedded marble. Finely disseminated pyrite is found throughout the valley and thought to be a primary source of sulfur that is incorporated into the rock coatings, along with sulfate ions present in streams (Darmody et al., 2007). The valley floor is approximately 600 meters above sea level (masl) at its mouth and rises to 800 masl at its head (Dixon et al., 2008). While mean annual air temperature in Kärkevagge is in the vicinity of -2°C (Thorn et al., 1999), investigation of shallow soil and bedrock/soil interface temperatures reveal thermal regimes on daily and hourly scales that approximate -15°C (Thorn et al., 2001). The majority of the precipitation in the valley is in the form of snow (50-75%) with depths ranging from 0.75-1.5m over much of the year. Total annual precipitation is approximately 800 mm, as measured at the Riksgränsen-Katterjäkk station to the west of Kärkevagge (Eriksson, 1982).
Figure 1. Location of Kärkevagge in Swedish Lapland.

In his study of denudation processes operating in the valley, Rapp (1960) identified what he originally described as “lime crusts” and “rust coatings” on bedrock surfaces. Recent studies have determined these to be alumina glazes (composed of basaluminite and gypsum) and Fe/Mn films respectively (Darmody et al., 2007; Dixon et al., 1995; Dixon et al., 2008; Dixon et al., 2002). We focus on two of the dominant rock coating types found in the valley (Fig. 2), classified using nomenclature from Dorn (1998). Sulfate crusts are dominated by jarosite [KFe$_3$(SO$_4$)$_2$(OH)$_6$] composed of approximately 70% O, 1% Si, 10% S, 4% K, and 15% Fe and minor amounts of gypsum [CaSO$_4$.H$_2$O] composed of approximately 69% O, 2% Al, 1% Si, 12% S, 14% Ca, 2% Fe (Darmody et al., 2007). Iron films are predominantly iron oxyhydroxides with compositions of 44% Fe, 9% Al, 6% Mg, 17% Si, 1.4% S, 0.3% K, 0.08% Ca, 0.05% Mn. Iron films are composed
of primarily goethite and hematite. The Fe in the coatings is thought to be derived from pyrite oxidation, subsequent bedrock weathering by weak sulfuric acid and release of Fe into the hydrologic system (Dixon et al., 2002).

Figure 2. Rock coatings used in this study: a) sulfate crusts (jarosite), and b) Fe-films. Photo credit: Cassandra Marnocha, Sweden, 2012.

5.3.2 Sampling

Rock debris displaying accumulations of the principal rock coating types was collected from field sites associated with sampling transects along the length of the valley’s east side. These transects are identified by the letters V, L, K, J, and H, as established by Rapp (1960). Two field
seasons, one each in summer of 2010 and 2012, were undertaken in order to collect samples. Bulk rock samples were then subdivided into sterile tubes for storage at both 3°C and -20°C.

5.3.3 Microscopy

Coating samples were examined using a Nova Nanolab FEG scanning electron microscope (SEM) at the University of Arkansas, coupled with energy-dispersive X-ray spectroscopic (EDX) analysis. Where indicated, several of the SEM images were taken at the Rochester Institute of Technology. Rock chips of each representative coating type were mounted on carbon tape sample mounts and sprayed with a thin Au-Pb coating. Samples were analyzed under 15.00 kV for both SEM and EDX. FEI software was used to capture images on a PC for subsequent interpretation. Chemical analyses were obtained via mapping and point analysis of bacteria and mineralogical materials using EDX.

5.4 Results and Discussion

5.4.1 Fe-films

Fe-films vary in texture and morphology throughout the valley, ranging from a thin, flaky chip-like texture on boulders, to thick, smooth-textured darker coatings found primarily along the western valley wall near streambeds. Micromorphologically, filaments, sheathes and stalk-like formations are common in the Fe films (Fig. 3, 4). Many of these filaments appear to be mineralized, as they are often exhibit cracks corresponding to cracks in the underlying substrate, and under EDX, are often enhanced in Fe and S (Fig. 4, 5).
Figure 3. Putative mineralized stalks in Fe-films. On left, stalk appears to be connected via EPS to underlying material and bridges over gaps in the rock material. On right, fine grained crystals occur in close proximity and on the surface of stalks.

Freshwater and marine Fe-oxidizing bacteria such as *Gallionella feruginea* and *Mariprofundus ferrooxydans* are able to form twisting, ribbon-like formations know as stalks (Chan et al., 2010). Stalk-like formations similar to those formed by *G. feruginea* and *M. ferrooxydans* were observed in the Fe films (Fig. 3). The morphology and size of these putative stalks is variable in the coatings and different textures and morphologies may represent differences in mineralization state, or age-.
Figure 4. Long filaments, possibly fungal, which “break” along cracks in the underlying rock material, suggesting mineralization of the biological material.

Ferrous iron [Fe(II)] is oxidized to Fe(III) at neutral or alkaline pH through abiotic oxidation by molecular oxygen. Under acidic conditions, Fe(II) remains stable. However, microbes can catalyze Fe(II) oxidation at acidic and neutral pH (Blake et al., 1993; Blake et al., 1992; Emerson, 2000; Emerson and Revsbech, 1994a; Hanert, 2006). The result of microbial Fe(II) oxidation includes the formation of ferric hydroxide particles that can transform via internal rearrangement to hematite, or undergo decomposition and transformation to goethite (Kappler et al., 2006). In low-pH environments, microbes have been shown to facilitate the release of dissolved iron and rapid transformation of ferrihydrite to goethite (Kappler et al., 2005; Roden et al., 2004). These precipitates readily encrust the cells involved in their formation.
Figure 5. Putative stages of mineralized bacterial filaments. Lowest layer is slightly elevated from the rock matrix and cracks along with the matrix, suggesting mineralization. Filaments above the lower layer bridge over the gap/crack and have more defined margins.

Mineralization can sometimes be demonstrated by EDX spectra. Areas enhanced in Fe, S, or Mn will show strong peaks in the spectra and suggest concentration if not encrustation or mineralization. Carbon (C) peaks are uncommon in the coatings from Kärkevagge. Strong C signals can indicate the presence of free-living cells when they occur on cell-like morphologies, and can also show areas of unmineralized EPS material. When imaging alone proves difficult to discern between mineralized and non-mineralized material, C signals can sometimes differentiate (Fig. 6). However, C signals are not always reliable sources for identifying living cellular material, as the C peak can become obscured by Au, Ca, and K – all of which will be present in some quantity in the Au-Pb coated samples. Thus, it is important to take the context of both the structures and their location relative to other materials when interpreting EDX spectra.
Figure 6. Branching filament from a Fe-film with corresponding EDX spectra for C, S, and Fe. Filament is mostly enriched in Fe and S, with arrows highlighting areas of C-enhancement. Areas of high C occur at the node branch of the filament, a large rod/bud, and an individual rod-shaped cell. Carbon with relatively minimal enhancement in Fe and S suggests these areas are not mineralized.

5.4.2 Sulfate crusts

Sulfate crusts are found throughout the valley, sometimes in association with Fe/Mn films. Crusts are thick and flaky, whitish-yellow/green in color, and found most commonly on the underside of boulders. Sulfate crusts are also strongly associated with “rotten rock” in Kärkevagge
– boulders disintegrating where they stand as outer layers chip and flake off. Coccoidal cells were observed on sulfate crusts (Fig. 7). These cells appeared as individual units, chains with slight narrowing at cell contact, and as fused chains. Single chains and fused chains occur in parallel lines, with some chains jointed at nearly 90 degree angles. Mineral lineaments appear to serve as the guide upon which cells grow. When nearby chains of cells fuse to one another, they appear as rectangular sheets of amorphous material that parallel the underlying mineral crystal structure. On sulfide minerals, bacteria have been observed to grow along parallel crystallographic directions (Edwards et al., 2000; Edwards and Rutenberg, 2001). A similar phenomenon has been previously observed in hematite from banded iron formations (Kolo et al., 2009), however, the samples were of ancient origin and appeared to be mineralized remnants of cells and cellular material, rather than extant cells. Kolo et al. (2009) suggest that when these structures were active, they were likely Fe(III)-reducing bacteria that exploited the crystal structure of the hematite.

Bacterial “nanowires” have also been suggested as a means of extracellular electron transfer (Childers et al., 2002; Gorby et al., 2006; Kolo et al., 2009; Lovley, 2008; Reguera et al., 2005). These structures allow non-motile cells to participate in reactions with chemical species, when chemotaxis is not an option. Nanowires may also help motile species in the identification of compatible chemical species, steer chemotaxis, and speed up reaction rates simply by allowing for easier contact between microbe and mineral.
Figure 7. Left: Coccoidal chains of cells growing in parallel lines, exhibiting necking at cell-to-cell contacts, and merging of chains into sheets. Right: Inset from left image. White arrows show evidence of mineral lineament along which chains are growing. Black arrows show examples of putative bacterial nanowires. White ‘V’ shows triangular contact of cell chains.

Other evidence for biomineralization in the sulfate crusts was observed in the form of micron-scale layering of microbial material (Fig. 8). Distinct changes in cell morphology and visibility of margins occurs radiating out from an arbitrary center, where cells occur mostly as individuals with well-defined membranes to their neighbors.
Figure 8. Progressive sulfate crust mineralization. Central portion of image shows individual cells, some in the process of dividing. White dashed lines outline layers of increasingly more mineralized and homogenous material.

5.4.3 Biotic model for formation

While microbes are not the only driving force in coating formation, evidence in the form of community structure data (Marnocha and Dixon, 2013a and b) and imaging suggest that they are a necessary component. Other biotic models of formation for material such as rock varnish, acid-mine drainage and mining deposits, cave speleothems, and other rocky material (Baker and Banfield, 2003; Boston et al., 2001; Clarke et al., 1997; Dorn, 1998; Krinsley et al., 2009; Mahaney et al., 2012; Matlakowska et al., 2012; Melim et al., 2009; Northup et al., 2003; Wang et al., 2011).

Dorn (1998) describes a hierarchical model for rock coating development. First-order processes deal with the exposure of rock to the land surface as the initial step in coating development. Second-order processes involve what happens to a coating that is exposed to erosion, and can include things such as growth of lithobionts on coatings, the deposition of a different coating on top of the original, or the accretion of the same coating. Third-order processes determine
whether a coating will become habitable by lithobionts. This includes not only microbes, but fungi, lichens, and some plants. Fourth-order processes are transportation pathways that allow for the continual supply for raw materials for growth, or determine which factor limits growth. Finally, fifth-order processes are biogeochemical barriers; that is, processes that fix the constituents of a coating in place, such as lithobionts, rock lithology, positioning, pH, exposure time, and disturbances. These processes describe a general model of the growth process for coatings, and while they do acknowledge the significant role of biology, the focus of this hierarchical model is not microbial processes.

Viles (2012) describes a model for microbially-influenced geomorphological processes that includes endolithic environments. This model defines two sets of microbial colonists on exposed rock surfaces: ‘r’ selected and ‘k’ selected (Hoppert and König, 2006). Microbial communities that contribute to rapid weathering of rock surfaces and can adapt to potential unstable conditions are referred to as ‘r’ selected species, while communities that are better adapted to stable conditions are designated ‘k’ selected species and often have a positive role in preserving and stabilizing the rock surface through mineralization and crust formation. Viles (2012) hypothesizes that following ice cover removal, such as in deglaciation processes, ‘r’ selected microbes will initially colonize a rock surface, promoting weathering, while ‘k’ selected microbial communities will become dominant at later stages. The ‘k’ selected communities thus promote stability during paraglaciation.

In Kärkevagge, deglaciation exposed much of the valley’s pyrite not only to oxidation by abiotic means, but also biological processes. The massive rock falls for which Kärkevagge, “valley of boulders” is named, results from the strongly jointed bedrock in the valley wall facilitating the movement of water into the rock mass. Boulder falls further expose rock surfaces for weathering
and biological inhabitation. Deglaciation was probably shortly followed by the colonization of microbes and fungi that contributed to the overall geochemistry and weathering regime of the valley. Exposed bedrock colonized by microbes would essentially follow a “first-come-first-serve” model, where a small group of keystone microbial species would become dominant and as a result, their metabolic means of survival would also dominate. Continued weathering and exposure of fresh rock surfaces allows for further colonization and the formation of new coatings. This model of dominant species colonization can in part explain why different coating mineralogies can be found in close proximity upon the same rock substrate. Biological processes have been documented to play a role in the variety of coatings distributed in the same environmental setting (Larson and Dorn, 2012). Microbial metabolisms and abiotic redox reactions in rock coatings likely follow a similar model to that of microbial mats. Coatings are accreted and gain thickness as each layer of microbe is mineralized over itself and serves as the substrate for the new layer. Our model follows three primarily steps for coating development: 1. Exposure, 2. Colonization, and 3. Accretion (Fig. 9).
Figure 9. Conceptual model for rock coating formation in Kärkevagge. 1. Processes involved in exposing rock surfaces to weathering and coating development: a. deglaciation of the valley and b. paraglacial release contributing to the jointing and eventual fall of boulders from the valley wall. 2. Colonization by microbial communities: c. weathering-promoting species that are adapted to shifting conditions and contribute to flaking, pitting, and other surface deformations that increase surface area for microbial colonization, and d. later colonization by stability-promoting microbes that contribute to mineralization. 3. Accretion of rock coatings over time at the: e. micro-scale, and f. macro-scale. Scale bars are approximate.

5.4.4 Rates of formation

Microbially-mediated mineralization and pre-curursors to mineralization can occur rapidly. At cell densities of $10^8$-$10^9$ cells/cm$^3$, rates of Fe(III) accumulation can be as high as 3 mm/day (Emerson and Revsbech, 1994a). At similar cell densities, iron can be precipitated by *G. ferruginea* at a rate of up to 1200 nmol Fe(II)/hour, which approximates an oxidation rate of approximately
1.1x10^{-11} \text{ mol Fe(III) per cell per year} (Emerson and Revsbech, 1994b). In the case of some \textit{Leptothrix spp.}, shed sheathes can accumulate at a rate of 1-2 \text{ um/min}, with abandoned sheathes continuing to deposit ferric hydroxide (Van Veen et al., 1978). Stable structures such as microbial mats can accumulate at a rate of 1 \text{ mm per year} (Konhauser, 2007).

Rock varnish, however, has rates of accumulation much lower than the aforementioned. Varnish growth rates have been estimated to be as slow as 1 \text{ um/ky} (Liu and Broecker, 2000). More recent estimates based on historical time markers place values over 500um/ky and suggest that the rate may be a reflection of the sum of both growth and weathering, rather than a pure growth rate (Dorn and Meek, 1995; Krinsley et al., 2011; Spilde et al., 2013). These rates, however, are based off time markers and while a microbial influence is inferred, metabolic dynamics are not taken into consideration for the calculations.

Kärkevagge was deglaciated approximately 10,000 years before present (André, 2002). The oldest buried soils developed in colluvial deposits on the valley floor from radiocarbon dated arbuscular mycorrhizae spores date at 5,589 yrs. before present (Thorn et al., 2009). Assuming coating thickness between 1-5mm (based on coating and immediately adjacent altered material that is difficult to discern from the coating itself), rates of accretion must be between 100-890um/ky. This rate assumes the rate of growth less weathering and exceeds the conservative rock varnish estimates. The rate is a minimal rate assuming that coatings observed today began their formation immediately or shortly after deglaciation. The case of rock coatings in Kärkevagge is more likely to occur on a far shorter time scale. Assuming a 100 year generation, rates range from 10-50um/year. While this rate is significantly faster than varnish estimates, it is substantially slower than high cell-density mineralizing environments, such as microbial mats. On a 10-year generation period, rates can reach up to half that of microbial mats.
Using another approach, we estimate rate of formation based on assumed rates. As Kärkevagge is snow covered as much as 50% of the year, we assume that microbial activity during this period is dormant or slow enough to be negligible in the following estimates. Microbial mats grow at a rate of 1 mm/year and assuming an average cell density of \(10^8\) – \(10^9\) cells per cm\(^3\) (Emerson and Revsbech, 1994a), while in deep subsurface endolith habitats, cell density ranges from \(10^2\) – \(10^4\) cells per g (dry weight) (Amy et al., 1992). We equate the rate in rock coatings from Kärkevagge to a conservative 5 um/ky, based on a 35% of the year active period and a cell count of \(10^4\). This rate is close to the lower, conservative rates of rock varnish formation. At cell densities of \(10^7\) cells/g dry weight, which has been observed in varnish (Kuhlman et al., 2006), rates increase to 50um/year or 50mm/ky.

5.4.5 Astrobiosocial Implications

Rock coatings have been observed on Mars by nearly every lander and rover mission since the Viking landers (Krinsley et al., 2009; Murchie et al., 2004; Strickland, 1979). These observations have sparked an interest in the astrobiological potential of rock varnish, often found in analog environments on earth, as a material analog to the martian coatings (Allen et al., 2004; DiGregorio, 2002; Krinsley et al., 2009; Mancinelli and Bishop, 2002; Wang et al., 2011). Other coatings compatible with martian mineralogy have been largely ignored, however. Fe-films and sulfate crusts are composed of minerals that have been identified by rover and satellite across the surface of Mars (Bibring et al., 2005; Bibring et al., 2006; Christensen et al., 2000; Gendrin et al., 2005; Klingelhöfer et al., 2007; Klingelhöfer et al., 2004; Squyres et al., 2004; Wang et al., 2006). Evidence of biomineralization in the rock coatings Kärkevagge offers a means of preserving biosignatures in the matrix of the rock coating. Filaments, stalks, and other structures are
commonly formed in the Kärkevagge coatings and many occur hardened and mineralized with visible cell morphologies.

5.5 Conclusions

Several lines of evidence point to a strong association between microbes and rock coatings in Kärkevagge. Fe-films and sulfate crusts occur sometimes within meters of each other on the same parent rock substrate, and yet have developed unique coating mineralogies. Previous studies by Marnocha and Dixon (2013b) demonstrated unique bacterial community structures between the Fe-films and sulfate crusts, offering one variable that may account for the differences in coating type developed. Deglaciation in the valley exposed finely disseminated pyrite found in much of the bedrock and likely made conditions favorable for microbes with biomineralization-capable metabolisms. Parent rock exposure from deglaciation or more recent weathering or spring snowmelt allows for the colonization of coatings from soil and water microbes. Upon exposure, be it following deglaciation or fresh exposure through weathering or snowmelt, key species able to metabolize relevant species (e.g. S metabolisms in sulfate crusts) outcompete the species that might produce other coatings (e.g. Fe-oxide metabolisms in sulfate crusts). Rates of formation are difficult to estimate, with little in the way of time markers available to give a frame of reference. However, based on known parameters, timescales for formation fall between those higher estimates for rock varnish to half the rate of high-cell density microbial mats.

5.6 Acknowledgements

Field work was carried out through funding and logistical support from the Royal Swedish Academy of Sciences and the Abisko Naturvetenskapliga Station (ANS), whom the authors
gratefully acknowledge. The authors also thank Dr. Mourad Benamara for access to the University of Arkansas imaging laboratory and his expertise, as well as Dr. Rich Hailstone at Rochester Institute of Technology. We additionally thank Rasmus Johansson for graphical assistance.
5.7 References


Sand, W., Gehrke, T., 2006. Extracellular polymeric substances mediate bioleaching/biocorrosion via interfacial processes involving iron (III) ions and acidophilic bacteria. Research in Microbiology, 157(1), 49-56.


6 Conclusions

6.1 Summary of results

The studies presented in this dissertation represent an investigation into the role of bacteria in the formation of rock coatings in Kärkevagge and how rock coatings may serve as biosignatures. These investigations have been multidimensional, focusing on microbial ecology, microbial metabolisms, geomicrobiology, terrestrial analogs in planetary science, and applications to astrobiology on Mars. Our results show diverse microbial communities using two approaches to molecular microbiology community surveys – Sanger sequencing and pyrosequencing. Each sequencing method has its merits and weaknesses, and each helped elucidate one of the many questions related bacterial community structure in the rock coatings of Kärkevagge. Understanding microbial ecology in the valley was also important. Though the valley is well-studied geochemically, the biological work in the valley is generally limited to eukaryotes. Since the valley was studied by Rapp (1960), Kärkevagge has been an example of a complex, cold-climate environment. With the addition of microbial influences, the dynamics of weathering and geochemistry become even more complex.

*Acidobacteria*, *Proteobacteria*, and *Actinobacteria* are the most common bacterial species identified across the three rock coating types. Pyrosequencing significantly increased the coverage of operational taxonomic units (OTUs) found per estimated richness in the coatings. Rarefaction curves and diversity indices suggest that the greatest diversity is found in the sulfate crusts. Fe-films had the lowest diversity, but when compared to other environments, had diversity indices comparable with complex environments such as soils.

Heterotrophs are common in the coatings, though a number of biomineralizing-capable taxa are present in at least 2% of each coating community. Fe-, S-, and Mn-oxidizing and reducing
metabolisms were among the most common, along with a number of S-related metabolisms, and Cr(VI) reduction. Methylytrophs were also a very common metabolic type, and may explain the lack of methane produced from anaerobic cultures of the coatings (Mickol and Marnocha, 2013), despite coming from an environment where methanogenic archaea should be present and active. Photosynthetic cyanobacteria were most common in the sulfate crusts. Less common metabolisms included U and arsenic oxidation, and Se, V, and perchlorate reduction.

The coatings from Kärkevagge also play a significant role as a cation sink in the valley. Microbes are capable of metal and cation scavenging, concentration, and deposition in a number of environments, and such is the case in the coatings from Kärkevagge. Sinks for Mn, Fe, Ca, and S are created through the mobilization, fixation, and metabolic reactions that are carried out during rock-coating formation. The process of rock-coating formation is an important, though often neglected, component of the total geochemical budget in the valley and in other cold climate environments.

Cells and their extracellular polymeric substance (EPS) alone can serve as nucleation sites for mineral formation and can also electrostatically interact with chemical species in the vicinity. Microbes can also become involved in mineral formation through directly precipitating out minerals or mineral precursors that will later be transformed. Microbial participation in rock-coating formation in this respect is best demonstrated through microscopy. For this research, scanning electron microscopy (SEM) showed evidence of microbial colonization, EPS or bacterial “slime”, bacterial nanowires and other microstructures, as well as larger structures such as filaments and stalks. These longer filaments sometimes extended across gaps in the underlying rock, suggesting it had either not yet been mineralized or was mineralized after the rock cracked.
However, more often a crack in the filament or cell itself was shown, explicitly demonstrating that the cell or structure was presently mineralized and had been at the time of the rock fracture.

When comparing taxa found in Kärkevagge to nearest neighbors, many were closely related to soil bacteria. A significant amount of sequences identified were closely related to extremophiles, with acidophiles and halophiles most common. Psychrophiles, and unexpectedly, thermophiles were also common. The extremophilic nature of the microbes identified, along with the cold, dry, and acidic environment of Kärkevagge suggest communities of hardy bacteria capable of withstanding multiple extremes simultaneously. This finding is especially important when making the case for Kärkevagge as a micro-environmental analog to Mars and solidifying the relevance of coatings and coating bacteria to the search for past or present life on Mars.

Coatings have been observed on the surface of Mars since the Viking landers (Strickland, 1979) and are especially intriguing astrobiological targets. Rock coatings are a surface environment easily accessible by rover, they provide a stable micro-environment with some protection from radiation, and they have the potential to preserve evidence of past life through biomineralization and associated features. The findings in this dissertation research affirm rock coatings as suggestive biosignatures. Sulfates and Fe-oxides are common to both Mars and the rock coatings in Kärkevagge, making a strong material and mineralogical analog. Extremophiles, including psychrophiles and radiation-resistant bacteria were observed in the coatings, along with preserved cells and microbial structures. Coatings also appear to have unique microbial communities that suggest a significant role in community structure in coating formation. Rock varnish has been established as a potential biosignature on Mars (Allen et al., 2004; DiGregorio, 2002; Krinsley et al., 2009; Mancinelli and Bishop, 2002); however, other rock coatings
compatible with martian mineralogy, such as those found in Kärkevagge, have been largely ignored with respect to astrobiology on Mars.

6.2 Directions for future work

In understanding microbial mechanics in a complex system, we ask, “Who is there, what are they doing, and how are they doing it?” Though all three of these questions have been addressed in this dissertation, the latter perhaps would benefit from further exploration. Geochemical kinetics of a process like biomineralization, are complex and multifaceted. A thorough geochemical model of the rock coating system in Kärkevagge, including microbial dynamics, would greatly benefit the overall understanding of the formation process.

Geochemical kinetics and modeling work could be greatly supplemented by additional experimental work. Though preliminary culturing studies were undertaken, the environment the coatings exist in represents a long-term culturing study in both identifying the appropriate media and conditions, as well as allowing for sufficient growth by microbes who are often in cold and/or freezing temperatures. Such modeling can also help determine which force dominates in the accumulation process of rock coating formation: biology or chemistry.

Beyond Kärkevagge, this work has demonstrated the relative lack of research being performed on coating types other than rock varnish. The rock varnish community itself is not especially large, but the community focused on coatings as a whole, particularly with respect to microbes, is even smaller. Coatings are found in virtually every environment and are extremely chemical diverse. Kärkevagge is an excellent example of the kind of diversity in coating types that can be found in a single environmental setting. However, there are certainly a number of other environments, arctic or otherwise, that hold the promise of diverse rock coatings with strong microbial associations. Understanding the microbial dynamics of coatings in these other
environments would only serve to solidify the understanding of the coatings in Kärkevagge and the microbial influence on their genesis.

With applications to planetary science, a collaboration with scientists at Los Alamos National Lab (LANL) was begun toward the end of the Ph.D. Mars Science Lab Curiosity (MSL) carries a laser-induced breakdown spectrometer (LIBS) instrument on its payload called ChemCam. This instrument fires a laser pulse at material and uses a telescope to obtain spectra from the resulting plasma. The instrument package allows for elemental analysis with imaging for context, as well as chemical analysis at depth by repeated pulses on the same location. The instrument is highly efficient, can access areas inaccessible by the rover arm, and is able to fire a pulse and get accurate data up to 7m away from the rover. Though the instrument was initially designed to remove dust layers and regolith coatings, Lanza et al. (2013) suggest that these surface layers may be reminiscent of terrestrial rock coatings and surface alteration. This collaboration with Lanza and colleagues intends to look at rock coatings from Kärkevagge as an analog to the material being targeted by ChemCam on Mars. Thin sections of rock coatings were made and preliminary scanning electron microscopy (SEM) has been performed on these thin sections. Work will continue to use SEM and thin sections and solid rock samples will eventually be sent to LANL. LANL will perform analyses using a ChemCam engineering mock-up to identify chemical species at depth, as well as to examine the amount of vaporization that occurs on different rock types.
6.3 References


