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Comparative Analysis of Microbial Community Composition Throughout Three Perennially Ice-Covered Lake Systems in the McMurdo Dry Valleys, Antarctica and its Relationship With Lake Geochemistry

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Comparative Analysis of Microbial Community Composition Throughout Three Perennially Ice-Covered Lake Systems in the McMurdo Dry Valleys, Antarctica and its Relationship With Lake Geochemistry

A Dissertation submitted in partial satisfaction of the requirements for the degree of

Doctor in Philosophy

in

Environmental Sciences

by

Wilson L. Foo

December 2009

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ABSTRACT OF THE DISSERTATION

Comparative Analysis of Microbial Community Composition Throughout Three Perennially Ice-Covered Lake Systems in the McMurdo Dry Valleys, Antarctica and its Relationship With Lake Geochemistry

by

Wilson L. Foo

Doctor of Philosophy, Graduate Program in Environmental Sciences
University of California, Riverside, December 2009
Dr. Brian Lanoil, Chairperson

The McMurdo Dry Valley lakes are the closest analog on Earth to extraterrestrial environments that may harbor life. Phylogenetic analysis of its bacterial communities offers insight into the origins of life and its evolution. In this study, we determined the bacterial community composition throughout the water column of the McMurdo Dry Valley lakes. While these bacterial communities were largely stable on an inter-annual basis, the spatial patterns in bacterial diversity were strongly linked with physical and chemical gradients in the lakes, indicating that bacterial activity modulates ecosystem function in extreme environments. Investigation of acetate and glucose utilizers in the McMurdo Dry Valley lakes imply that even minor components of the overall bacterial community may play important roles in the nutrient cycling of these lakes.
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Introduction

It has been suggested that the presence of liquid water is necessary for life to exist. With mounting evidence in support of the presence of paleolakes on Mars (Carr, 1983), it is feasible that Mars could once have harbored life. The ice-covered lakes and surrounding landscape of the McMurdo Dry Valleys, Antarctica provide the closest analog to both Mars and “snowball Earth.” Identification of the bacterial communities in the McMurdo Dry Valley lakes can provide insight on the evolutionary history of life on Earth and highlight the types of microorganisms we may discover in extraterrestrial locations.

The perennial ice cover of the McMurdo Dry Valley lakes enables long term vertical stratification of the water column. As there are clear differences between the oxygenated freshwater surface depths and anoxic, saline bottom waters of Lake Bonney and Lake Fryxell, we would assume a priori that the bacterial communities are distinct between the different regions of the lake and their physiologies would correspond with the geochemical profiles of each lake system. Determining the differences between these bacterial communities may give insight into how early forms of life are capable of evolving to thrive in cold environments. The identification of abundant microorganisms will also improve our understanding of ecosystem function.

The use of culture-independent techniques to identify bacteria involved in glucose and acetate utilization will improve our understanding of global carbon cycling and help us model the potential influence of primitive biological organisms on other planets. Thus,
a clear understanding of the microbial ecology in the McMurdo Dry Valleys will enhance future explorations elsewhere in our solar system.

**A Brief History of Microbial Ecology**

Derived from the Greek words, *oikos* and *logia* which mean household knowledge, ecology provides a fundamental understanding of the abundance and distribution of organisms, the interactions among them, and how these organisms affect ecosystem function. The major goals of ecology are to understand how the natural environment functions and to predict changes created by biological interactions with the physical environment. Ecology settles our curiosity about how the world functions and how our interactions may have an impact on our surroundings. Understanding how our actions affect the world will help improve our ability to minimize broad-scale detrimental impacts we have on the environment, such as the ozone hole (Farman et al., 1985) and global warming (Cox et al., 2000), as well as resolve accidents like the Exxon Valdez oil spill (Peterson et al., 2003). Ultimately, ecology is an interdisciplinary field that connects scientists in biology, chemistry, biochemistry, geology, hydrology, atmospheric sciences, and many other fields to develop a cohesive understanding of the world.

In its beginnings, the field of ecology was focused on determining the relationship between plants and animals and their impact on the physical environment. Changes in the abundance of these organisms have clear implications on the food chain and could directly impact human society. A lack of knowledge about the food web can lead to unintended consequences that are catastrophic to an ecosystem as highlighted in recent
history by the spread of invasive species including rabbits in Australia (McLeod, 2004) and zebra mussels in North America (MacIsaac, 1996). Though these plant/animal interactions remain prominent in the minds of many ecologists, the field of microbial ecology has entranced scientists who are interested in the environmental impact created by organisms that remain invisible to the naked eye.

The origins of microbial ecology are linked more with microbiology than ecology. Microbiology began in the 1670s when Anton van Leeuwenhoek first identified fast moving “animalcules” through a hand-ground lens (Dobell, 1933). Despite this early discovery of microorganisms, the field of microbiology remained inactive until the mid-1800s. Much of the scientific work during this intervening period concerned spontaneous generation, a theory that life can instantaneously arise from non-living matter. Spontaneous generation remained scientifically controversial until it was disproved in 1861 by Louis Pasteur (Pasteur, 1861). While his famous experiment primarily involved microbial fermentation, the majority of Pasteur’s research contributed to the germ theory of disease. The focus on medical microbiology continued through the work of Robert Koch, whose work on the etiology of anthrax (Koch, 1876) and tuberculosis (Koch, 1890) firmly established postulates for the germ theory of disease. Though early microbiology would continue to focus on human health, the work of Martinus Beijerinck and Sergei Winogradsky in the late 19th and early 20th centuries directly contributed to the advent of microbial ecology.

Considered co-founders of the “Delft School” of microbiology, Beijerinck and Winogradsky pioneered the use of general microbiology to investigate biochemical
changes in the environment. One of their greatest contributions was the development of the enrichment culture method to isolate bacteria involved in metabolic processes of interest. This method involves inoculating a nutrient medium selective for the enrichment of bacteria of a specific physiology with an environmental sample. Examples of their successes include the isolation of nitrifying bacteria (Winogradsky, 1890) and nitrogen-fixing bacteria (Beijerinck, 1901). Prior to the development of enrichment culturing, isolation largely relied upon the chance growth of the organism on non-selective media. By using the enrichment culture method, researchers could now identify bacteria capable of carrying out specific metabolic processes regardless of their abundance in the environment.

The work of Beijerinck and Winogradsky was followed by Albert Jan Kluyver, a student of Beijerinck (Singleton, 2000). His primary contribution to microbial ecology was the concept of biochemical unity (Kluyver, 1924). Although bacteria can have diverse physiologies, the biochemical pathways they utilize are limited and shared between them. This concept of biochemical unity and the common use of DNA by all life suggest that organisms are similar at the molecular level, therefore giving credence to the theory that all life on Earth may have arisen from a single ancestor (Berg et al., 2002). The essential biochemical processes common to all organisms likely appeared early in the evolution of life, and the current diversity of life may be attributable to evolutionary processes over millions of years. Comparative microbiology also led to the development of microbial physiology and the discovery that bacteria play an important role in biogeochemical cycling. The pioneering research led by these prominent microbial
ecologists built the foundation upon which we can now relate microorganisms with biogeochemical cycling and the origins of life on Earth.

**Origins of Life**

Though the origins of life remain controversial, many members of the scientific community believe that complex biochemistry resulting from simple chemical reactions in the “primordial soup” was the original source of life on Earth (Peretó, 2005). While proof of this theory remains elusive, it lays the groundwork on which we can relate the three domains of life with a common ancestor. In the late 1970s, Carl Woese and his colleagues at the University of Illinois at Urbana-Champaign were using 16s ribosomal RNA to analyze the phylogenetic relationships between multiple prokaryotes (Woese and Fox, 1977). The results of his research delineated prokaryotes into two separate groups. The group of organisms either involved in methane production or lived in extreme environments, such as hydrothermal vents, clustered away from previously identified bacteria and eukaryotes. With the discovery of the archaea, Woese would later propose the current three domain system based upon a phylogenetic tree of life (Woese et al., 1990). The phylogenetic tree of life shows the evolutionary distance among biological species with a common ancestor. One current hypothetical rooted tree indicates that the archaea and bacteria are evolutionary closer to the common ancestor, whereas eukarya likely evolved from the archaea branch. Therefore, studies focused on microorganisms will yield important information regarding the origins of life and its evolution.
The discovery of archaea also heralded theories that life on Earth may have an extraterrestrial origin (Haldane, 1980; Gogarten-Boekels et al., 1995). The unique metabolisms exhibited by archaea in extreme environments, such as hydrothermal vents (Jones et al., 1983) and hot springs (Barns et al., 1994), advocate their potential aptitude in surviving conditions found in outer space. Although the archaea were initially treated solely as extremophiles, their identification as abundant members of the bacterioplankton community in the oceans (DeLong, 1992) indicate their adaptability and capability to co-exist and compete with marine bacteria. Recent discoveries of archaea in hypersaline lakes (Cytryn et al., 2000) further highlight the potential for these organisms to persist and thrive in environments believed to have little life.

**Life in Cold Environments**

Recent hypotheses based upon carbon isotope studies of carbonate rocks dating to the Neoproterozoic period (1,000 to 544 million years before present) suggest that glaciation events during this period were so severe that ice sheets completely covered the Earth, thereby creating a state called “snowball Earth” (Kirschvink, 1992; Hoffman et al., 1998; Hoffman and Schrag, 2002). Modeling has indicated that the decline in temperature was so great that that the average temperature at the equator was equivalent to temperatures found now in Antarctica (Hyde et al., 2000). This sharp downturn in temperature resulted in a near collapse of all biological productivity on Earth. Life during this bleak period would likely have relied upon anaerobic processes and hydrothermal vents or associated extreme environments for their heating and nutrient
requirements. Research on psychrophilic microorganisms will yield important information regarding how life can persist in extremely low temperatures and how it evolves once conditions change.

Research concerning microorganisms in cold environments is also vital to the search for extraterrestrial life. While some researchers would argue that Mercury and Venus could potentially harbor life (Schulze-Makuch et al., 2005), the extreme temperatures and the lack of liquid water on both planets make the discovery of any biological organism improbable. Thus, the focus on extraterrestrial life in our solar system is largely focused on ice-covered habitats similar to those found on early Earth.

With its perpetually low temperatures throughout the year, present-day Antarctica may be the most similar to extraterrestrial environments within our solar system capable of harboring life. While Antarctica may appear unique when compared to the more prevalent temperate ecosystems on Earth, its physical features may be common with those found on Europa and Mars (Jakosky and Shock, 1998). In recent years, there has been increasing physical evidence of aqueous processes on Mars (Ming et al., 2006). Since the presence of liquid water is essential in creating a stable habitat for life, the existence of paleolakes on Mars may have supported early life. Carr et al. has suggested that these Martian lakes would have cooled over time to develop seasonal and perennial ice covers (Carr, 1983).

One of the more distinguishing features of Antarctica is the presence of perennally ice-covered lakes. Although both polar regions on Earth contain a wide variety of lakes, Antarctica possesses more freshwater lakes that sit directly above sea
water (Laybourn-Parry, 2003; Laybourn-Parry and Marshall, 2003). Furthermore, Lyons et al. has observed that the biogeochemical characteristics of Arctic lakes tend to be heavily influenced by their contact with terrestrial ecosystems (Lyons et al., 2001). These interactions greatly complicate our understanding of Arctic ecosystem, thereby making Antarctica potentially more suitable for developing a better understanding of the relationship between microbiology and geochemistry in cold environments.

The McMurdo Dry Valleys

The McMurdo Dry Valleys form the largest relatively ice-free region on the Antarctic continent (Moorhead et al., 1999). They cover an area of approximately 4800 km² in Southern Victoria Land, Antarctica. First discovered during Robert F. Scott’s Discovery Expedition in 1903, the region was dubbed the “Valley of the Dead” as it was believed to harbor very little life (Scott, 1905). The McMurdo Dry Valleys are a polar desert characterized by annual precipitation of less than 10 cm and an annual average temperature of –20°C (Keys, 1980; Clow et al., 1988). Even during the warmer summer months, the air temperature lingers near freezing. Strong katabatic winds strongly affect the climate in the McMurdo Dry Valleys by increasing the average temperature by 0.7°C to 2.2°C while decreasing the relative humidity by –1.8 to –8.5% (Nylen et al., 2004).

On account of their high latitude location, the McMurdo Dry Valleys only receive solar radiation between August and April each year (Dana et al., 1998). The McMurdo Dry Valleys provide the closest analog to Mars based on climate and temperature. Despite the harsh conditions, the McMurdo Dry Valleys are home to a variety of ecosystems,
including the pore spaces of rocks (de la Torre et al., 2003), transient glacier meltstreams (McKnight et al., 1999), the surfaces of perennially ice-covered lakes (Priscu, 1998) and within their water columns (Laybourn-Parry et al., 1996b; Takacs and Priscu, 1998). Studies of the geochemistry and microbiology of the McMurdo Dry Valleys will provide an accurate depiction of the region’s history over the last several thousand years.

The McMurdo Dry Valleys are comprised of three main valleys: Taylor Valley, Victoria Valley, and Wright Valley. Among these valleys, the Taylor Valley has been a major focal point of early researchers interested in the McMurdo Dry Valleys, thereby resulting in excellent characterization of its ecological history and past climate conditions. During the last glacial maximum (~18,000 years ago), the mouth of the Taylor Valley, located at the edge of the continental shelf, was sealed by the expansion of the Ross Ice Shelf. This effectively created the ice-dammed Glacial Lake Washburn, a proglacial saline lake reaching nearly 350 ft elevation above sea level in Taylor Valley (Denton and Marchant, 2000; Hall and Denton, 2000). Though lake levels fluctuated during the Holocene, the rise in average temperatures resulted in the evaporative reduction of Glacial Lake Washburn. The decline of Glacial Lake Washburn resulted in the creation of multiple smaller lakes in the Taylor Valley. Presently, the Taylor Valley can be divided into three major basins (each separated respectively by a threshold of increasing elevation): the Explorers Cove, Fryxell basin, and Bonney basin. The deepest parts of Fryxell and Bonney basins now house the ice-covered lakes we find today.

The McMurdo Dry Valley lakes are extreme environments characterized by physical features not found in temperate lakes. The most significant of these features is
the presence of a perennial ice cover. Though many temperate lakes produce temporary ice covers during the winter season, the McMurdo Dry Valley lakes possess a permanent ice cover throughout the year. The presence of a perennial ice cover blocks wind-driven mixing, which forces molecular diffusion to become the primary mechanism of vertical transport within these lakes (Wharton et al., 1993). This condition strongly contrasts with the vast majority of other lakes, which tend to be holomictic; meaning physical mixing between the surface and bottom waters occur at least once a year. Deeper temperate lakes often undergo thermal stratification during the summer seasons, in which the sun-warmed surface waters do not mix with the colder bottom waters. The consequence of this stratification is the eventual depletion of nutrients in the surface waters and the formation of anoxic conditions in the bottom waters. However, as the surface water temperature declines in autumn to match the temperature of the deep water, wind blown along the lake surface will physically mix the water column in these lakes. This wind-driven mixing would thereby reestablish equilibrium and homogenize the lake by pushing oxygen-rich surface water down towards the sediment and bringing nutrient-rich bottom water up towards the surface. Conversely, the McMurdo Dry Valley lakes are amictic. The presence of a perennial ice cover prevents physical mixing of the water column and enables long-term vertical stratification of the water column in these lakes. While some dimictic lakes, such as Mono Lake, can also be density-stratified and allow metabolic processes such as sulfate reduction (Oremland et al., 2000) and anaerobic arsenite oxidation (Oremland et al., 2002) to occur at certain times, a single mixing event can
completely homogenize the lake making it difficult to relate the bacterial community with lake geochemistry on an inter-seasonal basis (Hollibaugh et al., 2001).

The perennial ice covers of amictic lakes also restrict direct atmospheric gas exchange and sediment deposition into the lakes (Doran et al., 1994). Furthermore, the ice covers decrease light penetration into the lakes and can thereby reduce primary productivity by phytoplankton within the lakes (Vincent, 1981; Laybourn-Parry et al., 1996a). The high-latitude position of the McMurdo Dry Valley lakes also results in seasonal patterns of light availability. As a result, Antarctica possesses a decoupled light-dark cycle, in which it only receives sunlight during the summer and remains completely dark during the winter. While there are studies on the carbon transformation in the McMurdo Dry Valley lakes during the summer months (Priscu et al., 1999), little is known about carbon cycling during the dark season.

As a result of the limited precipitation in the McMurdo Dry Valleys, the primary source of input into the lakes is glacial meltwater. For only 6 to 10 weeks a year, transient streams are formed via glacial melt and flow across the dry valley landscape into the lakes. Though these streams intermingle with the landscape prior to deposition into the lakes, this interaction is limited by the impoverished landscape itself. Though some of these meltwater streams are barren (Alger et al., 1997), others contain diverse microbial communities comprised of cyanobacteria, chlorophytes, and diatoms (McKnight et al., 1999). To enhance biomass production and prevent desiccation, the algae found in the streambeds form mats with different colorations based on their photosynthetic pigments (McKnight and Tate, 1997). Though most algal mats remain in
place throughout the year, some organisms may be flushed into the surface waters of each
lake during the summer months (McKnight et al., 1998). The ramifications from this
potential source of algal inoculation into the lakes have not been clearly determined. In
addition to replacing water loss through ablation of the ice cover, the streams remain
important sources of carbon and nutrients for the McMurdo Dry Valley lakes (Lyons et
al., 1998b)

Unlike the complex food webs seen in temperate environments, the McMurdo
Dry Valley lakes possess a simplified, primarily microbial food web (Laybourn-Parry et
al., 1997). The lakes contain no crustaceans; the top of the food web consists of rotifiers.
The lack of nutrient input from higher level organisms or from direct soil deposition
forces the surface waters of these lakes to be largely oligotrophic. Despite the low
nutrient availability in the surface waters, the lakes possesses phytoplankton communities
that produce a deep chlorophyll maxima at the bottom of the euphotic zone just above the
chemocline in two McMurdo Dry Valley lakes (Vincent, 1981). Primary production by
phytoplankton in the summer months provides a constant supply of dissolved organic
carbon (DOC) in the water column of these lakes (Takacs and Priscu, 1998; Takacs et al.,
2001). Furthermore, microbial communities capable of photosynthesis and nitrogen
fixation have been identified in aeolian-derived sediments on the ice covers of these lakes
(Priscu, 1998). The sporadic inoculation of the water column when these sediments melt
through the ice cover can be a major source of organic carbon in the lakes (Priscu et al.,
1999).
The McMurdo Dry Valley lakes are members of the LTER (Long Term Ecological Research) system. The LTER has collected geological, hydrological, and geochemical data from the entire ecosystem (e.g. soils, streams, and lakes) for the past 15 years and remains a continued source for this information. While the LTER has also surveyed Lake Vida in the Victoria Valley and Lake Vanda in the Wright Valley, their data is particularly rich for the three major lakes found in the Taylor Valley: Lake Bonney, Lake Hoare, and Lake Fryxell. The three major Taylor Valley lakes are geographically close to one another; the two furthest points have a maximal distance of 17 km. Despite their shared descendancy from Glacial Lake Washburn and close physical proximity, all three lakes possess distinct geochemical profiles (Spigel and Priscu, 1998). Geologic research has attributed some of these differences to the past histories of each lake system. Doran and colleagues (Doran et al., 1994) have suggested that the drying of Lake Vanda around 1,200 years ago (Wilson, 1964) may be the most interesting event in the more recent history of these lakes. It is believed that this warming event may have also affected Lake Bonney (Matsubaya et al., 1979) and Lake Fryxell (Hendy et al., 1977). Using stable isotope measurements of chlorine in Lake Fryxell and Lake Hoare along with previous data from Lake Bonney and Lake Vanda, Lyons et al. (1998a) described how differences in the evaporation rates of the McMurdo Dry Valley lakes during that time period may have substantially contributed to the overall salinity of each lake system. The warming event melted the ice covers of the Taylor Valley lakes and evaporated portions of their water column. These data suggest that the bottom waters of Lake Bonney possess salts derived from a marine source dating from
10,000 to 100,000 years ago. The evaporation of both east lobe Lake Bonney and west lobe Lake Bonney into saline ponds concentrated the salts from this ancient water in the bottom waters of both lake systems (approximately 5 times and 4 times the salinity of seawater, respectively); whereas, their surface waters are attributable to recent salt-free glacial meltwater (Matsubaya et al., 1979). Although Lake Hoare and Lake Fryxell also faced the same process of evaporative reduction as Lake Bonney, they experienced greater levels of desiccation. The lack of $^{37}$Cl in Lake Hoare suggests the complete desiccation of this lake; therefore, the oldest water in Lake Hoare is likely derived from glacial meltwater approximately 1,000 years ago. In contrast, the bottom waters of Lake Fryxell are saline but at a much lower concentration than either east lobe Lake Bonney or west lobe Lake Bonney. The desiccation of Lake Fryxell resulted the wind-blown loss of most of its ancient salt (Lyons et al., 1999). However, the retention of a small amount of its ancient saltwater likely seeded the saline bottom waters we find today. While much of the physical and chemical constraints found in the McMurdo Dry Valley lakes is attributable to their paleohistory, recent warming events may have greatly impacted the biogeochemistry of these lakes. Foreman and colleagues (Foreman et al., 2004) have determined that episodic warming events resulting in higher levels of glacial melt can greatly increase the nutrient levels found within the lakes. While the impact on primary productivity in the lakes varied, these types of modern flooding events will likely play an important role in maintaining the McMurdo Dry Valley lakes.
Lake Bonney

Lake Bonney is located in an enclosed basin at the head of the Taylor Valley at 77°43’S and 162°23’E. The lake possesses a 4-6 m thick ice cover and is approximately 7 km long and 40 m deep. Lake Bonney is divided into two lobes separated by a narrow channel (50 m wide) with a sill rising to 13 m depth (Hendy et al., 1977). Each lobe is vertically divided by a chemocline present at approximately 15 m in west lobe Lake Bonney (WLB) and 20 m in east lobe Lake Bonney (ELB). While the sill effectively separates the bottom waters of ELB and WLB, horizontal mixing of water above the sill occurs between the surface waters of both lobes. Above the chemocline, both ELB and WLB possess oxygen-rich but nutrient-poor freshwater. Water below the chemocline, however, is highly saline and becomes increasing anoxic with depth (Spigel and Priscu, 1998). Physical characterization of the Lake Bonney by Angino and colleagues
indicated that the temperature profile of both lobes of Lake Bonney were similar, however, WLB possessed lower temperatures than ELB (Angino et al., 1964). They speculated that this lower temperature may be the result of the proximity of WLB to the Taylor Glacier, which lies on the lake’s western shore. Based upon their measurements, heat flow between ELB and WLB occurred through the channel connecting them to warm the surface waters of WLB. The rate of flow between the two lobes is heavily influenced by the physical width and depth of the channel connecting them as well as the flow of glacial meltwater from the multiple tributaries that flow into Lake Bonney during the summer months.

Recent studies have also demonstrated that the Lake Bonney possesses several geochemical anomalies that cannot be explained by thermodynamics (Lee et al., 2004a). ELB possesses a high N$_2$O peak below the chemocline (Priscu et al., 1996). Since denitrification is thermodynamically unfavorable in the oxic region of ELB, the formation of N$_2$O was initially attributed to nitrification as evidence for denitrification was not initially detected in ELB. While the recovery of denitrifying *Marinobacter* sp. ELB17 from ELB (Ward and Priscu, 1997) is suggestive that the process may occur, attempts to measure their abundance or stimulate the rate of denitrification in ELB have remained inconclusive (Ward et al., 2003; Ward et al., 2005). A 16s rDNA study of the microbial assemblages a few meters above and below the chemocline of Lake Bonney was published on samples from the 2001 field season (Glatz et al., 2006). With the exception of their lowest depth in ELB, their clone libraries contained a high proportion of plastid sequences. Both ELB and WLB possessed relatively low levels of diversity.
Out of 438 bacterial clones analyzed, 55 distinct phylotypes were identified. The major bacteria phyla were the *Gammaproteobacteria*, *Actinobacteria*, and *Bacteroidetes*. Their results indicated differences in the microbial communities above and below the chemocline, and the dominance of a few select species. Phylotypes related to halophilic and halotolerant bacteria, such as *Halomonas* and *Virgibacillus*, were found only below the chemocline in ELB. Clone sequences related with other Antarctic bacteria, including *Rhodoferax antarcticus*, *Marinobacter sp.* strain ELB17 (Ward and Priscu, 1997), and Lake Bonney ice clone LB3-27 (Gordon et al., 2000), were also identified in this study. Archaea were detected in only two hypersaline samples analyzed and displayed extremely low diversity (only one halophile sequence between the two samples). While this study displayed a difference in bacterial diversity between the two lake systems, it did not provide a comprehensive analysis of the microbial communities throughout each lake system due to limitations in depths analyzed and clone library size.

ELB also has one of the highest levels of dimethyl sulfoxide (DMSO) found in an aquatic system (Voytek et al., 1999). Conversely, WLB possesses one of the highest levels of dimethyl sulfide (DMS) found in an aquatic system. While Lee and colleagues (Lee et al., 2004b) have proposed that differing redox conditions in ELB and WLB attribute to their distinct biogenic sulfur profiles, the role of the microbial assemblages remains unknown. As there are currently no clear explanations for many of these thermodynamically unfavorable features Lake Bonney, further analysis of the microbial communities in the bottom waters of ELB and WLB may uncover how recent biological activity in the lake may impact the concentrations of the biogenic sulfur compounds.
The chemical and biological processes found in WLB may also be significantly affected by input from Blood Falls, a saline discharge from the Taylor Glacier (Black et al., 1965). This iron-rich outflow located at the base of the Taylor Glacier periodically discharges into WLB, thereby injecting the lake with iron, organic carbon and viable microorganisms (Mikucki et al., 2004; Mikucki and Priscu, 2007), including clone related to *Thiomicropira arctica*, an autotrophic sulfur oxidizer. While their most recent study highlights how the microbial community in Blood Falls can impact the biological sulfur cycle (Mikucki et al., 2009), their influence on WLB is unaccounted for.

Based upon the evidence of horizontal mixing (Spigel and Priscu, 1998) and the geochemical profiles of Lake Bonney (Lee et al., 2004a), the surface waters of Lake Bonney chemically and physically appear to be one system, the waters below the ELB chemocline are a second, and the waters below the WLB chemocline are a third. Thus, *a priori* I would predict three distinct microbial communities in Lake Bonney—a shared surface water assemblage, most likely strongly influenced by stream inputs and photosynthetic activity; an ELB bottom water assemblage, most likely influenced by salinity and sub-oxic conditions; and a WLB bottom water assemblage, most likely influenced by salinity, suboxic conditions, and inputs from Blood Falls.

**Lake Fryxell**

Located at 77°37ʹS and 163°11ʹE, Lake Fryxell (FRX) is a large and shallow lake closest to the tail end of the Taylor Valley and McMurdo Sound. Found between the Canada Glacier and the Commonwealth Glacier, FRX is approximately 4.5 km long and
possesses a maximal depth of 18 m. Its proximity to McMurdo Sound leads to increased precipitation (Lyons et al., 1998a). The main source of water recharging FRX remains glacial meltwater from aforementioned glaciers, from which thirteen meltstreams have been previously identified (Conovitz et al., 1998). Like Lake Bonney, FRX is a chemically stratified lake system; in which the surface water is fresh and super-saturated with oxygen, while the bottom water is saline and fully anoxic. However, the bottom waters of FRX are considerably less saline (equivalent to 1/4 seawater salinity) than the bottom waters of ELB and WLB (Angino et al., 1962). Although FRX possesses the highest levels of bacterial productivity among the three major Taylor Valley lakes (Takacs and Priscu, 1998), its oxygen and nutrient gradients strictly dictate the position of the microbial community in its water column.

FRX shares many similarities with eutrophic lake systems. It possesses sulfidic waters that correspond with the depletion of sulfate at 15 m. The depletion of sulfate in FRX is clearly driven by the biological activity of sulfate reducers, which have been identified using 16s rRNA gene sequences from DGGE, the \textit{dsrAB} gene (Karr et al., 2005) and enrichment culturing (Sattley and Madigan, 2006). Methane is also present below the chemocline in FRX and has been attributed to methanogenic archaea near the sediment (Karr et al., 2006). Surprisingly, the majority of methanogenic activity has been found in the water column and not in the sediments of Lake Fryxell, although methanogenic archaea were found in the surface sediments as well. Based upon redox chemistry, the lack of nitrate in FRX likely corresponds to its complete consumption through denitrification.
FRX also supports the growth of microbial mats near the lake sediment. These microbial mats are composed largely of cyanobacteria, diatoms, and heterotrophs that are also present in the water column of FRX. A 16s rDNA study of cultured and uncultured prokaryotes revealed isolates highly related to *Flavobacterium hibernum*, *Janthinobacterium lividum*, and *Arthrobacter flavus* (Brambilla et al., 2001). High bacterial diversity was found within the mats but archaean diversity was low (only two clone sequences recovered). This study was later revisited through the use of denaturing gradient gel electrophoresis (DGGE), and the majority of 16s rDNA sequences acquired were classified as *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* (Stackebrandt et al., 2004). The cyanobacterial diversity in these microbial mats were also analyzed via microscopy and 16s rDNA studies (Taton et al., 2003; Taton et al., 2006). Anoxygentic photosynthetic bacteria were also identified through the use of primers targeting the *pufM* gene (Achenbach et al., 2001). Other work involving the microbial mats include: the isolation of *Rhodoferax antarcticus* (Jung et al., 2004) and isolates related to anoxygenic photosynthesis (Karr et al., 2003).

**Lake Hoare**

Lake Hoare is located in the Fryxell basin at 77°38’S and 162°51’E. Measuring approximately 4.2 km in length, the lake lies 5 km northeast of Lake Bonney and is dammed by the Canada Glacier from otherwise draining into Lake Fryxell, which is located across the glacier 3 km further northeast. Like Lake Bonney, the major source of water input into Lake Hoare is glacial meltwater either directly from the Canada Glacier.
or from ephemeral streams during the summer season. Among the three major Taylor Valley lakes, Lake Hoare possesses the most rugged ice cover (Clocksin et al., 2007). The lake possesses a maximal depth of 34 m and remains oxic to 28 m depth; making it the most oxygenated of the Taylor Valley lakes (Wharton et al., 1993). In certain cases, the oxygen can penetrate all the way to the bottom of the water column and even into the sediments. It is the least saline and most oligotrophic lake among the three major Taylor Valley lakes. Because the lake is predominantly freshwater, variations in seasonal meltwater can greatly impact the solute concentration of the lake (Welch et al., 2000). With primary productivity in Lake Hoare being extremely low, it has been suggested that the microbial mats in Lake Hoare may contribute more than the pelagic microbial community (Hawes et al., 2004; Moorhead et al., 2005). With cryptophytes being the dominant phytoflagellates in Lake Hoare (Roberts and Laybourn-Parry, 1999), it is assumed that these mixotrophic organisms survive the winters by consuming bacteria.

Despite the extreme conditions attributable to Lake Hoare’s high latitude position, its relative youth, freshwater content, and oligotrophic conditions make it comparable to lower latitude lake systems. Its lack of a chemocline and segregated ancient water distinguish it from both Lake Bonney and Lake Fryxell, and thereby preventing comparison to either early Earth or extraterrestrial environments. Therefore, I decided to exclude the analysis of the microbial community of Lake Hoare from this dissertation.
Objectives of this Dissertation

Prior studies by Lizotte and Priscu focused on bulk level measurements of chlorophyll a, light fluorescence, and thymidine uptake to determine primary and secondary productivity in the McMurdo Dry Valley lakes (Lizotte and Priscu, 1992, 1994, 1998). Recent advances in molecular biology have enabled others to further improve our understanding of the phytoplankton community composition and distribution by taxonomically identifying these phytoplankton in related Antarctic lake systems (Pearce, 2003; Pearce et al., 2003; Pearce, 2005). Despite the focus on phytoplankton in these Antarctic lake systems, significantly less work has been conducted to determine the role of bacteria in the carbon cycle of the McMurdo Dry Valley lakes. As sensitive systems to global warming, analysis of the bacterial communities in the McMurdo Dry Valley lakes may provide important information regarding the role of bacteria in the carbon cycling occurring in cold environments.

The goal of this dissertation is to determine whether the lake geochemistry in ELB, WLB, and FRX is attributable to recent activity by the pelagic bacteria community or if it is merely a legacy effect of each lake’s evolutionary history. By using 16s rRNA genes to determine the bacterial community composition and correlating the presence of specific members to geochemical parameters, we can develop a preliminary understanding of how this ecosystem functions. This information will improve our understanding of how life can adapt to cold environment and be used in future research to target the isolation of novel bacterial species and the discovery of extraterrestrial life. Since the geochemical profile of the McMurdo Dry Valley lakes have been stable over a
long period of time, an *a priori* assumption has been that the biological component of this ecosystem also remains largely unchanged. To test this hypothesis and reduce the bias associated with single time point sampling, it is necessary to determine if the bacterial community composition is stable on an inter-annual basis. Furthermore, a culture-independent analysis of the bacterial community involved in acetate and glucose consumption may clarify how carbon cycling functions in Antarctic lake systems.
References


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Chapter 1: Vertical Patterns in the Bacterial Diversity of Three Perennially Ice-Covered Lakes in the McMurdo Dry Valleys, Antarctica

Summary

Lakes Bonney and Fryxell are chemically-stratified, perennially ice-covered lakes in the McMurdo Dry Valleys, Antarctica. Vertical stratification in these lakes allows major geochemical differences to persist over thousands of years between the fresh surface waters and the saline bottom waters in each lake system. 16S rRNA gene analysis was used to characterize the diversity and distribution of bacteria in twelve samples throughout the water columns of east lobe Lake Bonney (ELB), west lobe Lake Bonney (WLB), and Lake Fryxell (FRX). DGGE banding patterns indicated that while the lake systems share similarities above the chemocline, they were highly different below the chemocline. Sequence-confirmed RFLP analysis of 1117 non-plastid clones resulted in 204 distinct operational taxonomic units (OTUs). FRX possessed the highest OTU richness (117 OTUs), followed by WLB (90 OTUs), and ELB (83 OTUs). Of the twenty-five most abundant OTUs in our clone libraries, 40% most closely matched previously identified clones in a study of Lake Bonney from the 2001 field season (Glatz et al., 2006), suggesting inter-annual biological stability in these lake systems. While samples located in the same region either below or above the chemocline in a single lake system clustered together, they shared less than 50% similarity based upon UPGMA cluster analysis of clone library results. Shannon diversity indexes indicated that each lake system possessed different diversity patterns with increasing depth. With depth, the diversity increased in FRX, was nearly stable in WLB, and decreased in ELB. The
majority of sequences were closely related to previously cultured isolates or environmental clones; however, several major OTUs were highly unusual and were only distantly related to previously characterized sequences. Overall, the lakes were dominated by six major phyla: *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria*. Clone library data revealed significant differences not only between lake systems but also within each lake system.

**Introduction**

The McMurdo Dry Valleys (MCM) are the closest analog on Earth to extraterrestrial environments found on Mars and Europa based upon climate and temperature. As the only known habitats within the Dry Valleys to possess liquid water capable of supporting life throughout the year, the MCM lakes can represent similar extraterrestrial ecosystems in our solar system that may capable of harboring life. The MCM lakes are characterized by the presence of perennial ice covers that prevent wind-driven mixing, gas exchange with the atmosphere, direct sediment input, and direct light penetration (Wharton et al., 1993; Priscu et al., 1996; Priscu, 1997; Spigel and Priscu, 1998). The lack of wind-driven mixing in these amictic lakes has enabled vertical stratification of the water column; thus, molecular diffusion is the primary mechanism of vertical transport in these lakes. Unlike the complex food webs seen in temperate environments, the MCM lakes possess a simplified, primarily microbial food web (Laybourn-Parry et al., 1997).
These lakes have been the focus of extensive limnological study due to their unique physical features and geochemical anomalies that cannot be explained by basic thermodynamics (Lee et al., 2004). For example, ELB possesses a methane peak in the oxygenated zone of the water column. It also possesses a high nitrous oxide peak below the chemocline (Voytek et al., 1999). ELB also possesses one of the highest levels of DMSO found in an aquatic system. Conversely, WLB possesses one of the highest levels of DMS found in an aquatic system. It is unclear whether this unusual geochemistry is the result of modern biological activity or a “legacy” of ancient activity that remains in the lakes as a result of low biological activity or thermodynamic constraints (Priscu et al., 1996; Priscu, 1997).

Despite the extensive work on broad ecosystem parameters and paleohistory of LB and FRX, there has been comparatively little work characterizing the microbial community composition and the majority of those have been cultivation based. Since cultivation introduces significant biases (Staley and Konopka, 1985), these studies have likely vastly underestimated the total microbial diversity of the lakes and may not have obtained the organisms that most strongly interact with the geochemistry of the lakes. The few studies utilizing the less biased molecular biology based methods are quite recent and have been limited in scope; nonetheless, they have provided significant insights into the microbial communities in the MCM lakes not provided by previous, cultivation based approaches. FRX was shown to have sulfate-reducing bacteria (Karr et al., 2005) and archaea (Karr, 2006), neither of which were abundant in LB (Glatz et al.,
2006). However, because these studies were highly limited in scope, it is difficult to draw more general conclusions regarding the composition of the microbial communities.

Due to the diverse geochemical parameters present in these lake systems, the high inter-annual geochemical and physical stability of the lakes, and the presence of a primarily microbial food web, these sites present an optimal area to study microbial diversity and its relationship to lake geochemistry. In this study, we thoroughly compare the microbial diversity across the full water columns of Lake Bonney and Fryxell. We analyzed twelve water samples collected during the 2003-2004 field season from ELB, WLB, and FRX by using denaturing gel electrophoresis (DGGE) and 16S rRNA gene analyses. This study clarifies whether the general trends observed in previous studies hold true throughout the water columns of all three lakes. Direct comparisons between the bacterial communities between ELB, WLB, and FRX provide a more robust data set from which we can enhance our understanding about the relationship between microbiology and lake geochemistry. This is a baseline study to characterize the diversity of the MCM lakes and to examine relationships between the microbial diversity and the chemistry of the lakes.

Materials and Methods

Field Sites

Lake Bonney (LB) is located at the head of the Taylor Valley at 77.43°S, 162.20°E. The lake possesses two glacially scoured basins that are separated by a sill at 13 meters depth. The lake is 38 meters deep, with a chemocline at approximately 15
meters depth. The presence of Blood Falls at the terminus of the Taylor Glacier results in periodic discharges of iron salts into WLB. This subglacial discharge may influence the geochemistry of LB. Nevertheless, East Lake Bonney (ELB) and West Lake Bonney (WLB) are more chemically similar in their surface waters; however, they are distinct below the chemocline and thus are treated as separate systems in this and previous studies. During the warmer summer months, LB is fed by meltwater primarily from the Taylor Glacier but also from other mountain glaciers in the surrounding valleys. There are no surface outflows from LB, and the only known water loss occurs from the ablation of ice from the surface of the lake.

Lake Fryxell (FRX) is located at the tail end of the Taylor Valley at 77.37°S, 163.09°E, between the Commonwealth and Canada Glaciers. The lake is 18 m deep with a weak chemocline at approximately 10 m, below which the water is brackish and sulfidic. Meltwater sources include the Commonwealth and Canada Glaciers as well as surrounding mountain glaciers. Like LB, FRX has no surface outflows, and the only source of water loss is through ablation of surface ice.

Both LB and FRX possess chemoclines separating oxygen-rich, fresh surface waters from oxygen-depleted, saline deep waters. Both lobes of LB possess hypersaline, sub-oxic deep waters. FRX has a much weaker chemocline located at ~10 m depth and possesses highly sulfidic, brackish deep waters.
Sample Collection and Processing

Twelve water samples were collected November 2003 from ELB (at 6 m, 13 m, 25 m, 37 m), WLB (at 6 m, 13 m, 18 m, 38 m), and FRX (at 6 m, 9 m, 12 m, 18 m). These depths were chosen based upon their representation of the distinct geochemical zones present in the water column of each lake system. The uppermost depth was immediately below the ice, the second was just above the chemocline, the third was below the chemocline, and the last was just above the sediments. The top two depths represent distinct regions of the oxygenated, fresh surface water, while the lower two depths represent the sub-oxic, saline deep waters. Samples were collected using a 5-liter acid-washed Niskin bottle through a sampling hole directly drilled through the ice cover of each lake system. All samples were transferred to McMurdo Station in acid-washed, sterile containers and stored in the dark at 4°C. All samples were processed within 24 hours of retrieval. 3 liters from each sample were vacuum filtered through 0.2 µm pore size 90 mm polysulfone filters (Pall Corporation, East Hills, NY). All filters were stored in sealed plastic bags with 3 ml of sucrose lysis buffer (20 mM EDTA, 400 mM NaCl, 0.75 M sucrose, and 50 mM Tris-HCl [pH = 8.0]) and frozen at -80°C. Total DNA was extracted from the filters through chemical treatment, following procedures described by Gordon and Giovannoni (1996).

Direct microscopic cell counts

Aliquots of 9 ml of water were added to 15 ml Falcon tubes containing 1 ml of filtered 37% formaldehyde in the field and stored at 4°C for transport back to the lab.
Microbial abundance was determined by DAPI direct cell counts following procedures described by Porter and Feig (1980). All counts were done in blind triplicates and in parallel with sterile Nanopure water blanks. No cells were detected on negative control blanks.

**Denaturing Gradient Gel Electrophoresis (DGGE)**

Partial 16S rRNA genes were amplified with primers 341F with a 40 base pair GC clamp and 518R (Muyzer et al., 1993) as described by Kulp et al. (2006). For each sample, five replicate PCR products were pooled to minimize well-to-well PCR bias. Amplification products were analyzed by electrophoresis on a 1.5% low melting point agarose gel in TAE buffer for proper size. The intensity of the band was calculated to equalize the loading concentration of DNA. The equalized PCR products for all 12 samples were separated by DGGE. DGGE banding data was analyzed in GelCompar II using Dice correlation coefficients. A cladogram was generated by UPGMA cluster analysis.

**Clone Library Construction, Restriction Fragment Length Polymorphism (RFLP), and Sequencing**

Nearly full-length 16S rRNA genes were amplified with primers 27F and 1492R (Giovannoni, 1991) and cloned into the pCR2.1 vector using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA) as described elsewhere (Skidmore et al., 2005). Grouping into operational taxonomic units (OTUs) by RFLP analysis were carried out as described
by Lanoil et al. (2001) using HhaI and an additional restriction enzyme, MspI, for improved detection of individual OTUs. Comparative analysis of RFLP patterns was conducted in GelCompar II and clustered using Dice correlation coefficient with an optimization factor of 2%. Representatives of each OTU were selected for partial sequencing using the primer 907R (Lane et al., 1985). For OTUs with 5 or greater clones, multiple representatives were partially sequenced. All partial 16S rRNA sequences were compared with known Genbank sequences using the BLASTn search tool. Chimera detection was performed using Pintail (Ashelford et al., 2005). 380 clones were identified as plastid and 40 clones were identified as chimeric and removed from further analysis. Clones that were >97% similar based upon partial sequencing results were grouped together as a single OTU. For all OTUs possessing greater than 10 members, the full-length sequence of a single representative clone was obtained.

To compare the relative diversity between samples, the Shannon diversity index (Shannon and Weaver, 1949) and Simpson diversity index (Simpson, 1949) were calculated. The estimated diversity covered based upon rarefaction analysis, Chao-1 (Chao, 1984, 1987) and abundance-based coverage (ACE) estimator (Chao et al., 1993) was computed using EstimateS version 7.5 (http://viceroy.eeb.uconn.edu/EstimateS). Based upon OTU identification from clone library results, the distribution of OTUs throughout the 12 samples was compared using the agglomerative hierarchical clustering function in XLSTAT 2006 (Addinsoft, New York, NY). A distance matrix was generated among the 12 samples, and a cladogram was generated by a UPGMA cluster analysis of Pearson correlation coefficients.
Results

Cluster analysis of DGGE banding patterns for all 12 samples indicates that the bacterial communities found just below the ice cover in all three lake systems were highly similar (Figure 1.1a). ELB and WLB 6 m samples shared the highest level of similarity at 90%, followed by FRX 6 m and 9 m samples that were 82% similar. At 13 m depth, ELB and WLB samples were 72% similar and most closely related with one another. Though the 6 m and 13 m samples are both found in the freshwater oxygenated regions of both ELB and WLB, they are distinct from each other; sharing merely 50% similarity. Higher similarity was found throughout the water column of FRX (59% similarity) than within the surface water regions of LB. The bottom water communities of LB show little similarity with the surface water communities or with each other.

To better resolve the bacterial diversity of these lakes, 16S rRNA gene clone libraries were constructed for all 12 samples (Table 1.1). Out of 1117 clones, a total of 204 operational taxonomic units (OTUs) were identified by RFLP analysis and confirmed by partial sequencing of representative clones. Cluster analysis of the clone library composition indicates that though the levels of similarity between samples were much lower than those exhibited by DGGE, some relationships remained the same (Figure 1.1b). At both 6 m and 13 m depth, respectively, ELB and WLB samples continued to cluster together. The same occurred in FRX clone libraries, which continue to exhibit the same clustering patterns seen in DGGE results. However, the clone libraries highlighted a closer relationship between the bottom water samples within each lobe of LB. ELB 25 m and 37 m samples were 44% similar, while WLB 18 m and 38 m samples shared 25%
similarity. Though the cluster analysis of the clone library data provided substantially lower similarity values, this data supported the substantial differences between the surface water communities and bottom water communities in each individual lake system. The low similarity values in both DGGE and clone library results also indicated that the bottom water communities in all three lake systems were completely distinct.

Overall, the majority of OTUs are unique to each individual system; however, several are shared between systems as well (Figure 1.2a). For the total system, 74% of OTUs are found in only one lake system. 9% of OTUs are shared amongst all three systems; however, these OTUs have some of the most abundant in the clone libraries. For the remaining OTUs, 10% are shared only between ELB and WLB, whereas a much smaller proportion is shared between either and FRX (4% and 3%, respectively). A closer look at the distribution of these OTUs reveals that in the depths above the chemocline, 32% of OTUs are found in more than one system, with 15% found in all three systems (Figure 1.2b). However, in the depths below the chemocline, no OTUs were shared between all three systems, and >87% of OTUs are found in only a single system (Figure 1.2c), further confirming our observation that the surface communities share members, whereas the deep water communities are more biologically isolated.

The Shannon diversity index (SDI) is a widely utilized measure of biodiversity and can therefore be used to compare different environmental systems. Typical SDI values range between 2 and 3.5; however, in complex environments such as temperate soils, SDI values of up to 7.1 have been previously reported (Dunbar et al., 1999). To determine how overall diversity varied spatially in our samples, SDI was calculated for
lake systems as a whole and for depth specific samples based on clone libraries (Table 1.1). All three lake systems possessed moderate levels of diversity comparable to other cold environments (Mikucki and Priscu, 2007). FRX possessed the highest average SDI (3.28) of the three lake systems followed by WLB (2.89) and ELB (2.36). Bacterial diversity in FRX slightly increased with depth from a value of 3.10 at the surface to 3.57 at the bottom. This minor increase in diversity corresponded with an increase in cell abundance once below the chemocline (Figure 1.3). Bacterial diversity in the WLB was largely stable throughout the water column with a total range of only 0.32 (from 2.75 to 3.07). While ELB began with a higher initial diversity value than WLB, its overall average is much lower due to the drastic decrease in diversity with increasing depth from a value of 3.40 at the surface to 0.95 at the bottom. Though the decline in bacterial diversity corresponded with decreased cell abundance in the bottom waters of ELB, there was no clear relationship between cell abundance and bacterial diversity in WLB. To verify these diversity patterns, the Simpson diversity index was utilized as an alternate approach. Whereas the Shannon index emphasizes the number of unique species, the Simpson index gives more weight to the number of dominant species in a sample (Hughes et al., 2001). The use of both indices established that the overall diversity in both FRX and WLB remained fairly stable throughout the water column with relatively small ranges (Table 1.1). ELB also displayed a distinct decline in diversity with depth (Simpson index, -37.82), reflecting the different origin and chemistry of the bottom waters of the lakes and the more extreme conditions found in ELB.
Two non-parametric estimators, ACE and Chao-1, were used to approximate the total OTU richness in each individual sample and all samples together, based on the distribution of OTUs in each clone library. Each individual clone library attained greater than 30% coverage of Chao-1 estimated richness, and the combined clone libraries covered 70% of the Chao-1 estimated total richness (Table 1.1). The lower values in total diversity covered in the samples above the chemocline were attributed to the lower number of clones analyzed due to higher proportion of excluded sequences (i.e. plastids) in those samples. The difference in Chao-1 values for individual libraries relative to combined libraries was a result of OTU sharing across multiple samples. At 30% individual library coverage, these libraries are comparable to values observed in other aquatic 16s studies (Kemp and Aller, 2004). On the other hand, the total combined coverage observed in this study is significantly higher than average, which is suggestive that LB and FRX possess less overall diversity than observed in marine or temperate freshwater systems.

The clone libraries were dominated by six major phylum level groups: 
*Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria* (Table 1.2). Two of these phyla were evenly represented in clone libraries from each of the three systems: *Betaproteobacteria* and *Actinobacteria*, where each varied less than 5% in their relative proportions in the clone libraries. This lack of variation may indicate these bacterial phyla do not vary between the Taylor Valley lakes due to their shared origins and responses to similar environmental conditions in Antarctica. However, other phyla were distributed asymmetrically in clone libraries.
from the three systems. For example, *Gammaproteobacteria* ranged from 2% to nearly 25% of clones in FRX and WLB libraries, respectively, whereas *Deltaproteobacteria* clones represented less than 1.5% of the ELB and WLB libraries but were 31% of the FRX libraries. While the presence of *Deltaproteobacteria* is largely attributable to sulfate reduction found solely in FRX (Karr et al., 2005), the relationship between *Gammaproteobacteria* and lake chemistry remains unclear. *Firmicutes*-related clones were also highly abundant; however, this was due to the dominance of the *Virgibacillus* spp. found only in the lower two depths of ELB. If this OTU was removed from analysis, the proportion of *Firmicutes* was below 5% in all lake systems.

Aside from indicating the distribution of major phyla within single systems, table 2 also highlights the community composition differences between the surface waters and the bottom waters of each lake system. The most visible difference occurs in the presence of *Firmicutes* in the bottom waters and its absence in the surface waters of all three lake systems. The highest contrast occurs in ELB where *Firmicutes* constituted 62.2% of the clone libraries representing the bottom water communities. The relative proportion of *Bacteroidetes* in the surface waters and bottom waters of ELB also varied respectively varied from 8.2% to 41.2%. Differences in the proportion of major phyla in the surface waters and bottom waters of WLB were more muted. In WLB, the surface waters had a higher proportion of *Betaproteobacteria*, and the bottom waters had a higher proportion of *Gammaproteobacteria*. FRX, which possessed the greatest number of bacterial phyla, also exhibited significant difference between its surface water and bottom water communities, which may relate to differences in lake geochemistry. Its surface
waters were dominated by *Betaproteobacteria* (15.1%), *Actinobacteria* (23.8%), *Bacteroidetes* (19.8%), and *Planctomycetes* (23%). Identification of these bacterial phyla has often been associated with freshwater aquatic systems (Zwart et al., 2002). The bottom waters of FRX, on the other hand, were dominated by *Deltaproteobacteria* (48%); the majority of which are associated with sulfate reducing bacteria.

For highly represented OTUs possessing 10 or greater members (11.8% of all OTUs, 56.8% of all libraries), a nearly full-length 16S rRNA gene sequence of a representative member was determined and compared to the GenBank database (Table 1.3). The most abundant OTU in the clone libraries (12.1% of clones) was 97% similar to *Virgibacillus carmonensis*, a moderately halophilic bacterium that has been found in biofilms on deteriorating tomb murals (Heyrman et al., 2003). Found only below the chemocline in ELB, it is the dominant OTU in both the ELB25 and ELB37 libraries and is the sole member of the *Firmicutes* phylum with greater than 10 clones in our libraries. The high level of similarity between *Virgibacillus carmonensis* and the OTU identified in ELB indicated that both are members of the same genus, cultivated members of which are adapted to living in cold, halophilic environments. The second most abundant OTU in our libraries was not closely related to any organism in the database. Its closest relative is *Aequorivita lipolytica* (<90% similarity), an isolate associated with terrestrial and marine Antarctic systems. This low similarity indicated that our OTU may come from an entirely different bacterial family but share common ancestry with other microorganisms found in Antarctica. Both of these most dominant groups were observed in a previous more limited 16S rRNA gene clone study of LB (Glatz et al., 2006),
possibly indicating that they are permanent, stable members of the microbial assemblage. Of the twenty-four most abundant OTUs in our clone libraries, ten were most closely related to LB clones previously identified by Glatz et al. However, the remaining OTUs were identified only in our samples. While the presence of abundant OTUs in multiple lake systems may support the concept of shared origin, nearly half were found in only a single system. This may be attributable to the significant geochemical differences between ELB, WLB, and FRX.

**Discussion**

The aim of this study was to provide a baseline characterization of bacterial diversity in the highly studied MCM Dry Valley lakes ELB, WLB, and FRX. Though these lake systems are geographically close, each system possesses a distinct geochemical profile based upon the distribution of nitrogen and sulfur compounds (Lee et al., 2004) and geologic history based upon Holocene desiccation rates (Lyons et al., 2000). We determined that the bacterial communities within each lake system were distinct as evidenced by both DGGE band profiles and 16S rDNA clone libraries. In comparing the bacterial diversity between and throughout the water columns of ELB, WLB, and FRX, our results seem to support three general conclusions. First, the variation in bacterial community composition within each lake mirrors differences observed in lake geochemistry. Lyons and his collaborators have previously indicated that the landscape position of the MCM lakes impacted their past evaporation rates resulting in the overall salinity of each lake we observe now (Lyons et al., 1998; Lyons et al., 2000). The impact
on the lake chemistry of FRX and LB based upon the different evaporation rates forced
the divergence between these lakes and created different ecological legacies for each.
Therefore, it is not surprising that the biological component of these lakes reflect the
same differences. Second, within any single lake system, there is also clear distinction
between the surface water bacterial communities and the bottom water bacterial
communities. Previous studies of LB and FRX have suggested the bottom waters of
these lakes are more ancient than their surface waters (Doran et al., 1994; Lyons et al.,
1999). The age difference, low bacterial turnover rate, and chemical differences between
the two water layers (Lee et al., 2004) are likely factors affecting the present spatial
differences in bacterial community composition. Third, the bacterial communities in the
surface waters of LB and FRX share much higher similarity with each other than was
observed among the bottom water bacterial communities in these lake systems. The
geochemistry of the upper portion of the water column is primarily determined by the
annual water inputs from overland flow, whereas the bottom portion of the water column
remains chemically and physically isolated. Thus, it is predictable that the bacterial
communities in waters above the chemocline in these lakes share many of the same
members, whereas the bottom water bacterial communities remain strongly distinct due
to their physical and chemical isolation.

The extent of bacteria diversity in our samples is slightly lower than levels found
in other aquatic systems (Kemp and Aller, 2004). The extreme physical and chemical
conditions in the McMurdo Dry Valley lakes likely contribute to the decreased levels of
bacterial diversity we found in comparison with temperate lake systems, including Crater
Lake, an oligotrophic freshwater lake in Oregon (Urbach et al., 2001; Page et al., 2004), and Mono Lake, a saline meromictic lake in California (Humayoun et al., 2003) based upon library size and the number of unique clones identified. The permanently cold temperatures and limited nutrient inputs into the McMurdo Dry Valley lakes are possible reasons for limitations on bacterial abundance, productivity, and diversity observed in both Lake Bonney and Lake Fryxell. The McMurdo Dry Valley lakes also appear to have lower bacterial diversity than the coastal (Crump et al., 1999) and deep oceans (Suzuki et al., 1997; Sogin et al., 2006). Despite the cold temperatures associated with deep sea environments, the oceans likely harbor greater bacterial diversity than the Dry Valley lakes based upon their wide expanse and circulation of material through ocean currents. Among the McMurdo Dry Valley lakes, it is clear that FRX possesses much higher levels of OTU richness than LB. This difference between OTU richness may be the result of the more extreme lake geochemistry found in LB relative to FRX.

Unlike other extreme environments such as hydrothermal vents (Muyzer et al., 1995) or highly acidic environments (Bond et al., 2000; Edwards et al., 2000), 75% of the highly abundant OTUs identified in the MCM lakes are >95% similar to those found in other, more temperate environments. This suggests that it may be easier for bacteria to adapt to low temperatures rather than high temperatures (Zecchinon et al., 2001). However, the remaining 25% of highly abundant OTUs present in these lake systems are highly divergent from any known isolate or environmental gene clone, specifically the OTUs distantly related to *Aequorivita antarctica* (91% similarity), uncultured Crater Lake clone CL500-3 (90% similarity), uncultured clone Hyd 24-32 (92% similarity),
Cryomorphaceae clone CML50 (92% similarity), uncultured clone nmsp VI20 (93% similarity), and uncultured Gammaproteobacterium HAL40b (91% similarity). These OTUs may be specifically adapted to the extreme environment conditions found in the MCM lakes.

In comparing our 24 most abundant OTUs with the Genbank database, we discovered that ~42% of these OTUs were most closely similar related to clones previously identified from LB samples from the 2001 field season by Glatz et al. (2006) despite their use of samples taken at different depths two years earlier. This suggests that the bacterial community in LB may be highly stable. Fuhrman et al. (2006) demonstrated that the distribution and abundance of bacterial taxa may be predictable based upon relationships with environmental variables in an analysis of marine bacterial communities. Crump et al. (2003) also indicated that the bacterioplankton community in an Arctic lake shifted seasonally based upon organic matter in the system. Based upon these studies, it is likely that the physical and chemical stability of the MCM lakes will contribute to the maintenance of the abundant members of the bacterial communities in these lakes.

This idea of inter-annual stability of the microbial community is particularly evident in the bottom waters, which are less directly affected by glacial melt inflow or modern climate patterns. This was best exemplified by the continued presence of OTUs related to Virgibacillus spp. in ELB and Aequorivita lipolytica in WLB, which were found only below the chemoclines of their respective lake systems and observed as major components of the clone libraries in both this study and the Glatz et al. study.
While the presence of sulfate reducing bacteria in FRX (Karr et al., 2005) clearly suggests that the bacteria can drive the geochemistry of the MCM lakes, the geochemistry in other cases clearly drives the microbiology of the lakes, as evidenced by the dominance of OTUS related to *Halomonas* and *Virgibacillus* in the saline bottom waters of ELB. Aside from salinity and sulfate, the relationship between lake geochemistry and the bacterial community remains unclear. Future research will focus on more quantitative approaches to ascertain a better understanding of the relationship between the role of specific bacteria species in the MCM lakes and corresponding chemical parameters.
References


Table 1.1. Summary of clone library analysis results concerning the number of OTUs found in each library, the estimated number of total OTUs in each library, and diversity indices.

<table>
<thead>
<tr>
<th></th>
<th># of Clones</th>
<th># of OTU</th>
<th>Ace Estimate</th>
<th>Chao1 Estimate</th>
<th>Total Diversity Covered</th>
<th>Shannon Diversity Index</th>
<th>Simpson Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELB 6m</td>
<td>62</td>
<td>36</td>
<td>69</td>
<td>76</td>
<td>47.4%</td>
<td>3.40</td>
<td>39.40</td>
</tr>
<tr>
<td>ELB 13m</td>
<td>103</td>
<td>33</td>
<td>45</td>
<td>47</td>
<td>70.2%</td>
<td>3.04</td>
<td>16.89</td>
</tr>
<tr>
<td>ELB 25m</td>
<td>122</td>
<td>20</td>
<td>24</td>
<td>25</td>
<td>80.0%</td>
<td>2.03</td>
<td>4.48</td>
</tr>
<tr>
<td>ELB 37m</td>
<td>111</td>
<td>12</td>
<td>23</td>
<td>37</td>
<td>32.4%</td>
<td>0.95</td>
<td>1.58</td>
</tr>
<tr>
<td>WLB 6m</td>
<td>61</td>
<td>27</td>
<td>47</td>
<td>53</td>
<td>50.9%</td>
<td>2.90</td>
<td>15.12</td>
</tr>
<tr>
<td>WLB 13m</td>
<td>60</td>
<td>25</td>
<td>43</td>
<td>48</td>
<td>52.1%</td>
<td>2.82</td>
<td>15.00</td>
</tr>
<tr>
<td>WLB 18m</td>
<td>120</td>
<td>32</td>
<td>40</td>
<td>42</td>
<td>76.2%</td>
<td>2.75</td>
<td>9.51</td>
</tr>
<tr>
<td>WLB 38m</td>
<td>127</td>
<td>39</td>
<td>81</td>
<td>92</td>
<td>42.4%</td>
<td>3.07</td>
<td>15.97</td>
</tr>
<tr>
<td>Fryx 6m</td>
<td>61</td>
<td>35</td>
<td>85</td>
<td>96</td>
<td>36.5%</td>
<td>3.10</td>
<td>14.64</td>
</tr>
<tr>
<td>Fryx 9m</td>
<td>65</td>
<td>33</td>
<td>66</td>
<td>73</td>
<td>45.2%</td>
<td>3.12</td>
<td>18.57</td>
</tr>
<tr>
<td>Fryx 12m</td>
<td>112</td>
<td>42</td>
<td>59</td>
<td>62</td>
<td>67.7%</td>
<td>3.33</td>
<td>22.69</td>
</tr>
<tr>
<td>Fryx 18m</td>
<td>113</td>
<td>54</td>
<td>105</td>
<td>112</td>
<td>48.2%</td>
<td>3.57</td>
<td>25.83</td>
</tr>
<tr>
<td>All libraries</td>
<td>1117</td>
<td>204</td>
<td>287</td>
<td>291</td>
<td>70.1%</td>
<td>4.44</td>
<td>37.24</td>
</tr>
</tbody>
</table>

Table 1.2. Phylum Level Distribution for Clone Library Results. Community composition differences highlighted when comparing above chemocline communities with below chemocline communities in all three lake systems.

<table>
<thead>
<tr>
<th>Total Lake System</th>
<th>Above the Chemocline Only</th>
<th>Below the Chemocline Only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ELB</td>
<td>WLB</td>
</tr>
<tr>
<td>Alpha Proteobacteria</td>
<td>3.8%</td>
<td>4.6%</td>
</tr>
<tr>
<td>Beta Proteobacteria</td>
<td>6.0%</td>
<td>10.3%</td>
</tr>
<tr>
<td>Gamma Proteobacteria</td>
<td>13.8%</td>
<td>24.7%</td>
</tr>
<tr>
<td>Delta Proteobacteria</td>
<td>0.8%</td>
<td>1.4%</td>
</tr>
<tr>
<td>Epsilon Proteobacteria</td>
<td>0.3%</td>
<td>0.3%</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>11.1%</td>
<td>11.1%</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>36.4%</td>
<td>7.9%</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>21.9%</td>
<td>35.6%</td>
</tr>
<tr>
<td>Planctomycetes</td>
<td>4.0%</td>
<td>1.1%</td>
</tr>
<tr>
<td>Verrucomicrobia</td>
<td>1.5%</td>
<td>1.4%</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acidobacteria</td>
<td>-</td>
<td>0.3%</td>
</tr>
<tr>
<td>Chloroflexi</td>
<td>-</td>
<td>0.3%</td>
</tr>
<tr>
<td>Green Nonsulfur Bacteria</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>JS1</td>
<td>-</td>
<td>1.4%</td>
</tr>
<tr>
<td>OP9</td>
<td>-</td>
<td>0.5%</td>
</tr>
<tr>
<td>OP11</td>
<td>-</td>
<td>0.8%</td>
</tr>
<tr>
<td>TM6</td>
<td>0.5%</td>
<td>-</td>
</tr>
<tr>
<td>WS6</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 1.3. Taxonomic affiliation for OTUs with greater than 10 members as determined by BlastN search results against the Genbank public database.

<table>
<thead>
<tr>
<th>Nearest Neighbor</th>
<th>Group</th>
<th>% Similarity</th>
<th>Location</th>
<th># of Clones</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Virgibacillus carmonensis</em> (AJ316302)</td>
<td>Firmicutes</td>
<td>97%</td>
<td>E25, E37</td>
<td>140</td>
</tr>
<tr>
<td><em>Aequorivita lipolytica</em> (AY027805)</td>
<td>Bacteroidetes</td>
<td>91%</td>
<td>W18, W38</td>
<td>47</td>
</tr>
<tr>
<td>Uncultured bacterium clone WLB13-222 (DQ015847)</td>
<td>Actinobacteria</td>
<td>99%</td>
<td>E6, E13, F6, W6, W13, W18, W38</td>
<td>44</td>
</tr>
<tr>
<td>Uncultured bacterium clone ELB16-036 (DQ015793)</td>
<td>Bacteroidetes</td>
<td>99%</td>
<td>E6, E13, E25, F6, W6, W13, W18, W38</td>
<td>32</td>
</tr>
<tr>
<td>Uncultured bacterium clone CL500-3 (AF316767)</td>
<td>Planctomycetes</td>
<td>90%</td>
<td>F6, F9, F12</td>
<td>28</td>
</tr>
<tr>
<td>Uncultured bacterium clone Hyd 24-32 (AJ535231)</td>
<td>Delta Proteobacteria</td>
<td>92%</td>
<td>F12, F18</td>
<td>27</td>
</tr>
<tr>
<td>Uncultured bacterium clone ELB19-198 (DQ015829)</td>
<td>Gamma Proteobacteria</td>
<td>99%</td>
<td>E6, E13, E25, W6, W13, W18</td>
<td>25</td>
</tr>
<tr>
<td>Sulfitobacter sp. SC10 (AY690684)</td>
<td>Alpha Proteobacteria</td>
<td>98%</td>
<td>E13, E25, W13, W18, W38</td>
<td>25</td>
</tr>
<tr>
<td><em>Cellulophaga pacifica</em> (AB100841)</td>
<td>Bacteroidetes</td>
<td>96%</td>
<td>E6, E13, E37, F9, F12, F18, W6</td>
<td>24</td>
</tr>
<tr>
<td>Uncultured bacterium clone WLB13-202 (DQ015842)</td>
<td>Bacteroidetes</td>
<td>99%</td>
<td>E13, F6, F12, W13</td>
<td>23</td>
</tr>
<tr>
<td>Uncultured actinobacterium clone S4 (AJ575506)</td>
<td>Actinobacteria</td>
<td>97%</td>
<td>E6, F6, F9, F12</td>
<td>22</td>
</tr>
<tr>
<td>Uncultured bacterium clone WLB16-007 (DQ015849)</td>
<td>Alpha Proteobacteria</td>
<td>99%</td>
<td>E25, W18, W38</td>
<td>20</td>
</tr>
<tr>
<td>Uncultured bacterium clone nmspVI20 (AB212894)</td>
<td>Gamma Proteobacteria</td>
<td>93%</td>
<td>W18, W38</td>
<td>20</td>
</tr>
<tr>
<td><em>Algoriphagus antarcticus</em> (AJ577142)</td>
<td>Bacteroidetes</td>
<td>96%</td>
<td>E6, E13, E25, F6, W6, W13, W18, W38</td>
<td>19</td>
</tr>
<tr>
<td><em>Desulfbacterium phenolicum</em> (AJ237606)</td>
<td>Delta Proteobacteria</td>
<td>96%</td>
<td>F9, F12, F18</td>
<td>18</td>
</tr>
<tr>
<td><em>Carnobacterium pleistocenium</em> strain FTR1 (AF450136)</td>
<td>Firmicutes</td>
<td>95%</td>
<td>W38</td>
<td>17</td>
</tr>
<tr>
<td>Uncultured bacterium clone ELB19-210 (DQ015832)</td>
<td>Gamma Proteobacteria</td>
<td>99%</td>
<td>E25</td>
<td>15</td>
</tr>
<tr>
<td>Uncultured Alcaligenaceae bacterium clone LA1-B29N (AF513937)</td>
<td>Beta Proteobacteria</td>
<td>98%</td>
<td>E6, E13, F6, W6, W13, W38</td>
<td>14</td>
</tr>
<tr>
<td>Uncultured bacterium clone R4b16 (AF482441)</td>
<td>Delta Proteobacteria</td>
<td>96%</td>
<td>F12, F18</td>
<td>14</td>
</tr>
<tr>
<td>Uncultured eubacterium AB16 (AF273926)</td>
<td>Delta Proteobacteria</td>
<td>95%</td>
<td>F12, F18</td>
<td>14</td>
</tr>
<tr>
<td><em>Rhodoferax antarcticus</em> strain Fryx1 (AY609198)</td>
<td>Beta Proteobacteria</td>
<td>100%</td>
<td>F12</td>
<td>13</td>
</tr>
<tr>
<td>Uncultured bacterium clone ELB16-113 (DQ015810)</td>
<td>Actinobacteria</td>
<td>99%</td>
<td>E6, E13, F6, F9, W6, W13</td>
<td>12</td>
</tr>
<tr>
<td><em>Clostridium bowmanii</em> (AJ506120)</td>
<td>Firmicutes</td>
<td>98%</td>
<td>W38</td>
<td>11</td>
</tr>
<tr>
<td>Uncultured bacterium clone ELB19-048 (DQ015788)</td>
<td>Gamma Proteobacteria</td>
<td>99%</td>
<td>W18, W38</td>
<td>10</td>
</tr>
</tbody>
</table>
Figure 1.1. UPGMA cluster analysis of DGGE banding patterns based on Dice correlation coefficients and UPGMA cluster analysis of clone libraries based on Pearson correlation coefficients.

A. Cluster analysis of DGGE results

B. Cluster analysis of clone library results
Figure 1.2. Venn diagrams indicating shared OTUs between ELB, WLB, and FRX. Diagrams indicate overlapping OTUs between (a) all depths, (b) only the upper two depths, and (c) only the lower two depths from each lake.

A. Total Lake

B. Top Depths

C. Bottom Depths
Figure 1.3. Variation with depth of bacterial cell abundance and Shannon Diversity Index based on both DGGE and clone libraries.
Chapter 2: Inter-annual Stability of the Bacterial Community in Lake Bonney and Lake Fryxell, Antarctica

Introduction

Lake Bonney and Lake Fryxell are perennially ice-covered lakes located in the McMurdo Dry Valleys, Antarctica. Formed by glacial activity and the last marine incursion into the Dry Valleys during the Pliocene (Elston and Bressler, 1981), these lakes are characterized by perennial ice covers, ranging from 4 to 6 m thick, which enable chemical stratification of the water column by blocking wind-driven mixing (Priscu, 1998; Priscu et al., 1999; Roberts et al., 2000). As primarily microbial ecosystems, these lakes have mixotrophic protozoa and heterotrophic nanoflagellates as the top level grazers (Roberts and Laybourn-Parry, 1999; Roberts et al., 2000). This simplified food web also facilitates the vertical stratification of bacterial populations as indicated by Glatz et al. (2006) and described in Chapter 1 of this dissertation.

Recent studies of meromictic lakes have indicated that bacterial community structure and differences in the physiology of its members contribute to temporal variability in these lakes (Dimitriu et al., 2008). Disruption of the chemocline in meromictic lakes can significantly change the bacteria community composition, thereby affecting the future stability of an ecosystem for years (Tonolla et al., 2005). While limnology studies of the McMurdo Dry Valley lakes suggest long-term chemical stability of the water column (Spigel and Priscu, 1998; Lyons et al., 2001), there has also been clear evidence of episodic warming events capable of injecting nutrients and organic carbon into these lakes (Foreman et al., 2004). Using bulk measurements of biomass and
thymidine uptake, Takacs and Priscu (1998) established that the bacterioplankton populations in these lakes fluctuate not only inter-seasonally but also inter-annually. While these fluxes were correlated with changes in light availability and grazing effects, the identity of bacterial community and its changes were not identified. Furhman et al. (2006) has demonstrated in marine environments that temporal patterning of the marine bacterial community may modulate ecosystem function. Analysis of the inter-annual variability of bacterial communities in the McMurdo Dry Valley lakes is necessary to ascertain if this concept also holds true in ice-covered lakes. In this study, we examined the bacterial communities of ELB, WLB, and FRX across multiple depths and though 5 field seasons to determine what changes occur in bacterial community composition over time and if there is a relationship between these changes and specific physical or chemical gradients in the water column of these lakes.

**Materials and Methods**

**Sample collection**

Twelve water samples were collected each year between November and December 2003 to 2007 from ELB (at 6 m, 13 m, 25 m, 37 m), FRX (at 6 m, 9 m, 12 m, 18 m), and WLB (at 6 m, 13 m, 18 m, 38 m). Samples were collected using 5 liter acid-washed Niskin bottles through sampling holes drilled directly through the ice cover of each lake system and transferred to acid-washed sterile polypropylene carboys. All samples were transported within 8 h to Crary Laboratories at McMurdo Station and
stored in the dark at 4°C until processing. Within 48 hours of retrieval, 3 to 5 liters of water from each sample were vacuum filtered through multiple 0.22 µm pore size 47 mm polysulfone filters (Pall Corporation, East Hills, NY). All filters were stored in heat-sealed plastic bags with between 3 to 5 ml of sucrose lysis buffer (20 mM EDTA, 400 mM NaCl, 0.75 M sucrose, and 50 mM Tris-HCl [pH = 8.0]) and kept frozen at -80°C.

DNA extraction, PCR, DGGE, Cloning, and Sequencing

Total DNA was extracted from the filters through chemical treatment, as previously described (Gordon and Giovannoni, 1996). DNAs were amplified with bacterial primers (341f with a 40 bp GC clamp and 518r (Muyzer et al., 1993) in a PTC-200 thermal cycler (Bio-Rad, Hercules, CA) as previously described (Gaidos et al., 2004). Amplification products were analyzed by electrophoresis on a 1.5% agarose gel in SB buffer to check for proper size and to determine concentration. Band intensity was used to normalize the DNA loading concentration for DGGE. The samples were electrophoresed on 8% polyacrylamide gels with a 40% to 60% denaturant range at 50V for 825 minutes using a Bio-Rad D Code System as described previously (Kulp et al., 2006). The gels were digitally photographed and band positions were automatically marked and manually corrected using GelCompar II (Applied Maths, Austin, TX). Cladograms were generated by UPGMA cluster analysis using Dice correlation coefficients (Vauterin and Vauterin, 1992) with a 1% optimization factor. Targeted DGGE bands were cut directly from the gels, re-amplified with bacterial primers 357f
and 518r, cloned into the pBluescript vector, and sent for sequencing. The band position for each clone was confirmed via DGGE prior to sequencing.

**Statistical Analysis.**

Multidimensional scaling (MDS) analysis was performed with XLStat 7.5.2 for Windows (Addinsoft, New York City, NY). DGGE band position and intensity data was exported from GelCompar II and converted into natural log values. Physical and chemical environmental data for ELB, WLB, and FRX are publicly available on the McMurdo Dry Valleys Long-Term Ecological Research webpage (http://www.mcmlter.org). The combination of our DGGE band intensity data and the geochemical data acquired from the LTER was used to conduct canonical correspondence analysis (CCA) in Canoco (Plant Research International, Wageningen, Netherlands) to determine which environmental features best-explained community variation. Based upon CCA results, regressions of individual DGGE bands and individual environmental features were examined in XLStat using Pearson correlation coefficients. Correlations supported by a p-value <0.01 were saved and re-analyzed using logit and regress functions in Stata 8 (StataCorp, College Station, TX). Stata 8 was also used to determine p-values when comparing individual DGGE bands with multiple environmental factors to test for co-variation.
Results

Cluster analysis of DGGE banding patterns indicates that spatial variability in bacteria diversity exceeds inter-annual variability in each lake system. Among the three lake systems analyzed, WLB exhibited the clearest indication of an inter-annually, vertically stratified bacterial community (figure 2.1), where the bacterial community in each depth clustered together and were distinct from communities in the other three depths. In WLB, the bacterial communities in the surface waters (6 m and 13 m) were less than 45% similar to those in the bottom waters (18 m and 38 m). With the exception of 2003 sample of WLB 13 m, the surface waters of WLB were 65% similar with each other. A higher similarity value of 72% could be obtained when individually comparing WLB 6 m and WLB 13 m samples. Correspondingly, the bottom water communities in WLB also possess 63% similarity among themselves. WLB 18 m and 38 m samples, however, are distinguished with inter-annual samples of each obtaining 73% and 75% similarity, respectively.

Though ELB exhibits separation between surface water communities and bottom water communities, it possesses only three distinguishable clusters and a greater number of anomalies (figure 2.2). Unlike WLB samples, the inter-annual variability in ELB 25 m and ELB 37 m make it difficult to distinguish these two samples from one another, thereby creating a cluster that shares nearly 70% similarity. The surface water communities of ELB also remain 63% similar, however, closer similarity values could be attained for the majority of ELB 6 m samples (78%) and ELB 13 m samples (72%). The 2003 samples for ELB 25 m and 37 m are clustered together, whereas samples from those
two depths are clustered further apart in later years. The 2007 sample for ELB 37 m is an anomaly in our results, because it groups closer with surface water samples than bottom water samples. As the interaction between surface and bottom waters of ELB are unlikely based upon the strong density difference between them and the results for the four years prior, this anomaly may be the result of sampling errors.

The cluster results for FRX suggests that the lake really possesses merely two unique clusters, which correspond with either the surface water communities or the bottom water communities (figure 2.3). Being merely 52% similar, the surface water communities of FRX show far greater inter-annual variability than those of ELB or WLB. In contrast, the bottom waters are highly similar (73%) despite an inability to separate FRX 12 m from FRX 18 m.

Based on presence/absence data from the DGGE banding patterns, MDS indicated the presence of six broad clusters, in which partitioning occurred based upon sample location rather than the field season in which the sample was retrieved. With the exception of ELB 37 m 2007 and FRX 9 m 2006, each cluster represented either the surface or bottom water bacterial community within a single lake system (figure 2.4).

By incorporating chemical data with the biological profiles of each sample location, we could determine which environmental factor influenced variations in bacteria diversity (figure 2.5). CCA analysis indicated a positive correlation between the bottom waters of FRX and the presence of SRP and Si. Likewise, the bottom waters of ELB could be correlated with nitrate, nitrite, and DOC, which may be attributable to denitrifying bacteria in the lake (Ward and Priscu, 1997). The surface water communities
of all three lake systems were less distinguishable and displayed only minor correlations
with primary and secondary productivity.

By comparing band presence with environmental variables made available by the
LTER, 29 DGGE bands were identified as having a correlation with specific variables
with p-values <0.01 (table 1). Among these 29 bands, only 6 bands were found
exclusively in a single lake system. The other 23 bands were identified in multiple lake
systems, and most were detectable in both surface and bottom water samples. Although
the DGGE cladograms and MDS analysis indicate significant differences within and
between lake systems, those bands found in multiple lake systems seem to share the
greatest amount on inter-annual fluctuation and therefore could be linked to specific
environmental factors.

**Discussion**

The goals of this research were to access how the bacterial community
composition in the MCM lakes varies over time and to determine if these changes
correspond with lake geochemistry. While chapter 1 of this dissertation identified
significant differences in the bacterial community based upon their location in the water
column, it was uncertain whether these results may be subject to single time point bias or
if the MCM bacterial communities would reoccur annually as described in the open
oceans (Fuhrman et al., 2006). Along with studies of inter-seasonal phytoplankton
dynamics indicating that microorganisms are capable of surviving through the cold, dark
winter months in Antarctica (Takacs and Priscu, 1998), it has also been argued that the
mixotrophic cryptophytes likely harness their energy by the consumption of bacteria during the winter (Roberts and Laybourn-Parry, 1999; Roberts et al., 2000). While cultivation-based studies of FRX (Sattley and Madigan, 2006; Sattley et al., 2008) and LB (Ward and Priscu, 1997) provide clear evidence that bacteria survive the Antarctic winter, the impacts of grazing on the abundance and diversity of bacteria species in the MCM lakes remained unclear. It is also unclear how periodic flooding events (Foreman et al., 2004) and differential amounts of chemical and biological input from Blood Falls (Mikucki et al., 2004; Mikucki et al., 2009) would impact the MCM lakes.

Among the three MCM lake systems, WLB shows the clearest evidence of vertical stratification of the water column. It was the only lake to contain four distinguishable clusters that represent of each of the four sample locations in this study. Though the variation in bacterial diversity of WLB is attributable to spatial location based upon both DGGE cluster analysis and MDS analysis, identification of specific physical or chemical gradients that correspond to these shifts in bacteria community structure remains largely unclear. CCA suggests that a negative correlation with the presence of NH$_4$ may be the primary factor that distinguishes the WLB bacteria communities. This may corroborate with previous studies that suggest the importance of ammonia oxidation (Voytek et al., 1999) and denitrification in WLB (Ward and Priscu, 1997; Ward et al., 2005). Despite variations in the discharge of iron-rich saline water from Blood Falls (Mikucki et al., 2004; Mikucki and Priscu, 2007), no detectable changes in the pelagic community of WLB could be attributed, because the spatial variability of the bacteria community remains higher than the temporal variability.
This concept of long-term stability of the bacterial community structure also holds true in ELB. The surface water and bottom water communities of ELB remain distinct as shown in both DGGE cluster analysis and MDS analysis. The differences between these communities are clearly related to correlations with salinity, DOC, NO$_2$, and NO$_3$.

Although proof of denitrification in ELB remains slim, the presence of clones related to denitrifying bacteria in ELB in the Glatz et al. (2006) study and chapter 1 of this dissertation may suggest that processes related to the nitrogen cycle likely play an important role in bottom waters of ELB. The anomaly of finding the 2007 sample of ELB 37 clustered among the surface water samples can be attributable to aeolian sediments from the ice cover that sunk through the water column and landed in the bottom of the lake (Priscu, 1998; Lancaster, 2002). Since the ELB 37 m sample is taken near the sediments, it is possible that in just that single year due to sampling errors, we acquired an aeolian sediment-based bacterial community that bears greater resemblance to the surface water communities.

While the surface water and bottom water communities of FRX remain distinct from each other, they displayed the least effects of vertical stratification within the same region either above or below the chemocline. This may be caused by the relative shallowness of FRX in comparison to LB. The bottom water bacterial communities of FRX were positively correlated with Si and SRP. Both Si and SRP are generally associated with phytoplankton, in which Si stimulates the growth of diatoms and the availability of phosphorus can be a limiting factor. However, previous studies have indicated that FRX is limited by the availability of nitrogen not phosphorus (Priscu,
Likewise, dinoflagellates and diatoms do not appear to be not abundant in FRX (Seaburg et al., 1979; Roberts et al., 2000). Among the 9 bands found to positively correlate with Si ion concentration, all were found in FRX samples. All 5 bands that correlated with SRP were also found among FRX samples. As the relationship between these clones and Si and SRP is unclear, targeted isolation of these bacteria in future research may yield an answer to their relationships.

Electrical conductivity (EC) also appears to have strong correlations with multiple DGGE bands in our samples. In all three lake systems, changes in EC can be directly related to ions involved with salinity. 41% of DGGE bands showing significant correlation to an environmental parameter involve EC. Though cluster analysis has indicated overall separation between the freshwater surface and the saline bottom water, this does not apply to all DGGE bands identified in this study. Since a great proportion of the DGGE bands could be found throughout all samples, there is greater likelihood that we can draw a relationship between these bands and their surrounding levels of salinity. In most cases, this would simply indicate that those bands positively correlated with EC are more likely to be found in the saline bottom waters, whereas bands with negative correlation are found in the surface waters.

Chapter 1 has emphasized that stratification of microbial populations can be based upon chemical stratification of the water column in these McMurdo Dry Valley lakes. This chapter indicates that vertical variation in bacterial communities is far greater than temporal variation. Therefore, the bulk of the bacteria community is likely stable on an inter-annual basis and closely linked with the stable geochemistry of the lakes. However,
the abundance of certain bacterial species is subject to change based upon fluctuation in temperature, salinity, the ionic concentration of Si, and availability of SRP. Thus, the recent activities of the bacteria community in the MCM lakes are linked with geochemical profiles. Thus, analysis of biological activity in nutrient cycles can help us understand how recent activity can modulate nutrient cycling, despite the legacy conditions found in the MCM lakes.
References


Figure 2.1. UPGMA cluster analysis of DGGE banding patterns from WLB based upon Pearson correlation coefficients.

Figure 2.2. UPGMA cluster analysis of DGGE banding patterns from ELB based upon Pearson correlation coefficients.
Figure 2.3. UPGMA cluster analysis of DGGE banding patterns from FRX based upon Pearson correlation coefficients.
Figure 2.4. Multidimensional Scaling Analysis to Compare the Spatial and Temporal Variation in WLB, ELB, and FRX throughout all 5 field seasons.
Figure 2.5. Canonical correspondence analysis incorporates environmental data and DGGE banding data to help explain the variation between samples.
Table 2.1. Taxonomic affiliation for DGGE bands correlated with environmental data

<table>
<thead>
<tr>
<th>DggeBand</th>
<th>Locations Found</th>
<th>Closest Relative</th>
<th>Percent Similarity</th>
<th>Correlated With (p &lt; 0.01)</th>
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<tr>
<td>9</td>
<td>F12, F18, W13</td>
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<tr>
<td>13</td>
<td>F12, F18</td>
<td>Uncultured bacterium clone ZB13</td>
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<td>Si, SRP</td>
</tr>
<tr>
<td>18</td>
<td>E6, F6, F12, F18, W6, W13</td>
<td>Uncultured bacterium clone e13</td>
<td>91 (179/195)</td>
<td>Conductivity</td>
</tr>
<tr>
<td>20</td>
<td>F9, F12, F18</td>
<td>Uncultured bacterium clone e13</td>
<td>91 (179/195)</td>
<td>Si, TDR, POC, PON</td>
</tr>
<tr>
<td>23</td>
<td>F6, F12, F18</td>
<td>Uncultured bacterium clone 23J40</td>
<td>94 (161/171)</td>
<td>Si, SRP</td>
</tr>
<tr>
<td>24</td>
<td>E13, E25, E37, W13, W18, W38</td>
<td>Uncultured bacterium clone ELB19-095</td>
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<td>Conductivity, pH</td>
</tr>
<tr>
<td>27</td>
<td>F6, F9, W6, W13, W38</td>
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<td>CHL, Temp (-)</td>
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<tr>
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<td>NO3</td>
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<td>Uncultured bacterium clone FGL12</td>
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<td>Conductivity</td>
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<td>Uncultured bacterium clone ELB19-095</td>
<td>98 (169/171)</td>
<td>Conductivity</td>
</tr>
<tr>
<td>35</td>
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<td>Uncultured bacterium clone Zplanct86</td>
<td>88 (160/180)</td>
<td>Conductivity(-), Temp</td>
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<td>Temp (-)</td>
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<td>Conductivity, CHL(-)</td>
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<td>Conductivity(-), CHL, pH, DIC(-), DOC(-), SO4(-)</td>
</tr>
<tr>
<td>48</td>
<td>E25, E37</td>
<td>Uncultured Salinisphaera sp. clone</td>
<td>100 (195/195)</td>
<td>Conductivity</td>
</tr>
<tr>
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<td>E25, F6, F9, F12, F18</td>
<td>Uncultured bacterium clone 104B417</td>
<td>92 (181/195)</td>
<td>Si, SRP, POC, PON</td>
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<tr>
<td>52</td>
<td>E13, F9, F18</td>
<td>Uncultured Comamonadaceae bacterium clone Gap-3-83</td>
<td>100 (194/194)</td>
<td>CHL</td>
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<tr>
<td>53</td>
<td>E6, E13, E25, W6, W13, W18</td>
<td>Uncultured bacterium clone YU201</td>
<td>100 (174/174)</td>
<td>Si(-), DIC(-), DOC(-)</td>
</tr>
<tr>
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<td>E25, E37, F6, F9, F12, F18, W38</td>
<td>Pontibacillus sp. TB238</td>
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<td>Si</td>
</tr>
<tr>
<td>55</td>
<td>E6, F9</td>
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<td>100 (171/171)</td>
<td>CHL, TDR</td>
</tr>
<tr>
<td>59</td>
<td>F12, F18, W18, W38</td>
<td>Uncultured bacterium clone Chun-w-49</td>
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<td>Conductivity(-), SO4, Temp(-), pH(-)</td>
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<td>F6, F12, F18, WLB all</td>
<td>Uncultured Algoriphagus sp. isolate DGGE gel band DL35-1</td>
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<td>76</td>
<td>F6, W18, W38</td>
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<td>Temp (+), Ca, pH(-), SO4, DIC</td>
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Chapter 3: Identification of Bacterial Glucose and Acetate Utilizers in Two Geochemically Distinct Regions of the Water Column of Lake Fryxell, Antarctica

Introduction

With the continued increase in atmospheric CO$_2$ concentrations linked to global climate change, research concerning the global carbon cycle has intensified in recent years. Despite its geographic isolation, Antarctica has played a pivotal role in discussions concerning global environmental change. Though much of the physical evidence regarding past atmospheric conditions are derived from Antarctic samples (Petit et al., 1999), less emphasis has been placed on determining how the biological component of Antarctica is involved in global carbon cycling.

Carbon cycling in polar environments is of particular interest due to the increased sensitivity of these regions to environmental change. Like other ecosystems throughout the world, Antarctica’s involvement with the carbon cycle revolves around the biological absorption of CO$_2$ by photosynthesis and production of CO$_2$ via respiration. While some of the most compelling research has largely focused on marine phytoplankton in the Southern Oceans (Watson et al., 2000; Coale et al., 2004), there have also been several studies describing the carbon dynamics of Antarctic ice-covered lakes (Wharton et al., 1993; Priscu et al., 1999; Laybourn-Parry et al., 2006).

Lake Fryxell (FRX) is a perennially ice-covered lake located in the McMurdo Dry Valleys, Antarctica. The constant presence of a 4 to 6 m thick ice cover coupled with the minimal flux in and out of the lake has enabled stable chemical stratification of the water column (Angino et al., 1962; Lawrence and Hendy, 1985). Located at approximately 10
m depth in FRX, the chemocline effectively divides the water column into two compartments. The limnology and geologic history of FRX indicate significant differences in the age and chemical composition between the surface waters and bottom waters of this lake (Spigel and Priscu, 1998; Lyons et al., 1999). While the surface waters contain freshwater supersaturated with oxygen, the bottom waters are anoxic, saline and sulfidic. Since FRX is a primarily microbial lake system (Laybourn-Parry et al., 1996), it provides us the opportunity to correlate microbial community structure with lake geochemistry. FRX possesses a chlorophyll maxima at 9 m depth in the water column (Roberts et al., 2000). The presence of the corresponding phytoplankton and oxygen in these surface waters suggests that the carbon cycling in the region above the chemocline is largely attributable to primary productivity followed by aerobic respiration.

Though the phytoplankton communities in Antarctic lakes and the microbial food web have been previously characterized (Laybourn-Parry et al., 1996; Lizotte and Priscu, 1998; Roberts et al., 2000), less is known about the heterotrophic microorganisms responsible for the consumption of dissolved organic carbon (DOC) in these lakes.

While the activities of heterotrophs have been accessed through bulk measurements of DOC and thymidine uptake (Takacs and Priscu, 1998; Takacs et al., 2001), the identities of these microorganisms have not been determined. The use of 16s rRNA gene analysis of the bacteria community in Chapters 1 and 2 of this dissertation have identified microorganisms related to previously identified heterotrophs and indicated that the abundant members of the bacteria community in FRX possess temporal stability. Though the phylogeny of these organisms was determined, proof of their direct
involvement in ecosystem function was not shown. Stable isotope probing provides a culture-independent means to identify bacteria responsible for specific biological activity (Radajewski et al., 2000). The goal of our research is to determine which bacteria are responsible for the consumption of DOC in Lake Fryxell, whether the surface water communities differ from the bottom water communities due to different physiological response to the presence of specific carbon substrate, and how these bacteria impact carbon cycling in Antarctic lakes overall.

**Materials and Methods**

**Sample Collection and Processing.**

Two water samples were collected November 2007 from the 6 m and 12 m depths in Lake Fryxell. These depths were chosen based upon their representation of the two distinct geochemical zones present in the water column. The 6 m depth sample represents the oxygenated region of the water column, whereas the 12 m depth sample represents the anoxic, saline portion of the lake that is directly below the chemocline. Samples were collected using a 5-liter acid-washed Niskin bottle through a sampling hole directly drilled through the ice cover. All samples were transferred to McMurdo Station in acid-washed, sterile containers and stored in the dark at 4°C. Both samples were processed within 24 hours of retrieval. 1 liter of each sample was transferred into sterile containers and incubated aerobically in the dark at 4°C for two weeks with the addition of 1 mM $^{13}$C-labelled acetate. A separate set of these two samples was simultaneously
incubated with the addition of 1 mM $^{13}$C-labelled glucose. After incubation, samples were vacuum filtered through 0.2 µm pore size 47 mm polysulfone filters (Pall Corporation, East Hills, NY). Both filters were stored in sealed plastic bags with 3 ml of sucrose lysis buffer (20 mM EDTA, 400 mM NaCl, 0.75 M sucrose, and 50 mM Tris-HCl [pH = 8.0]) and frozen at -80°C.

**DNA Extraction, Denaturing Gradient Gel Electrophoresis and Clone Libraries.**

Total DNA was extracted from the filters as previously described in Chapter 1 of this dissertation. Isopycnic centrifugation of extracted DNA from the filters enabled separation of the $^{13}$C-labelled DNA of glucose or acetate utilizers from the $^{12}$C DNA representing the rest of the bacterial community (Radajewski et al., 2000). $^{12}$C DNA was not further analyzed in this study. $^{13}$C-labelled DNA (the denser band) was isolated and purified as previously described (Radajewski et al., 2000) $^{13}$C-labelled DNA were amplified with primers 341F with a 40 base pair GC clamp and 518R (Muyzer et al., 1993) as previously described (Kulp et al., 2006). For each sample, five replicate PCR products were pooled to minimize well-to-well PCR bias. Amplification products were analyzed by electrophoresis on a 0.8% agarose gel in SB buffer for proper size. Samples were separated by DGGE (Muyzer, et al. 1993) on a 40% polyacrylamide gel. A cladogram was generated in GelCompar II by UPGMA cluster analysis based on Dice correlation coefficients (based on band presence/absence) as previously described (Kulp et al., 2006).
All $^{13}$C-labelled DNA were amplified with primers 27F and 1492R (Giovannoni, 1991) and cloned into the StrataClone PCR cloning vector using the StrataClone cloning kit (Stratagene, Garden Grove, CA) as described elsewhere (Skidmore et al., 2005). Grouping into operational taxonomic units (OTUs) by RFLP analysis was carried out as previously described (Lanoil et al., 2001) using the restriction endonuclease HhaI (Liu et al., 1997). Comparative analysis of RFLP patterns was conducted in GelCompar II and clustered using Dice correlation coefficient with an optimization factor of 2%. Representatives of each OTU were selected for partial sequencing using the primer 907R (Lane et al., 1985). All partial 16S rRNA sequences were compared with known Genbank sequences using the BLASTn search tool. To compare the relative diversity between samples, the Shannon diversity index (Shannon and Weaver, 1949) and Simpson diversity index (Simpson, 1949) were calculated. Non-parametric estimation based upon the Chao-1 (Chao, 1984, 1987) and abundance-based coverage (ACE) estimator (Chao et al., 1993) was computed using EstimateS version 8.2 (http://viceroy.eeb.uconn.edu/EstimateS).

**Results**

DGGE gel images of FRX samples indicate that only a subset of the total bacteria community in FRX 6 m and FRX 12 m were acquired after incubation with acetate and glucose (Figure 3.1). Cluster analysis of the DGGE banding patterns for the 4 samples in this study indicated that the Fryxell 6 m samples were completely distinct from the Fryxell 12 m samples, regardless of which substrate was added (Figure 3.2). No DGGE
bands were shared between the two separate depths. The bacterial diversity of both glucose and acetate utilizers appears much higher in Fryxell 6 m as compared to Fryxell 12 m. Although the added carbon substrates were different, Fryxell 6 m samples were 90% similar, perhaps indicating overlap amongst organisms that utilize these substrates. This high level of similarity, however, was not exhibited among the Fryxell 12 m samples, which were only 45% similar.

To better resolve the differences in the bacterial diversity between the acetate and glucose incubations, 16s rRNA gene clone libraries were constructed for each of the four samples (Table 3.1). Roughly 180 clones were picked for each library and grouped into OTUs by RFLP analysis. Out of 731 clones, a total of 54 operational taxonomic units (OTUs) were identified. As with the DGGE banding patterns, the Fryxell 6 m samples exhibited higher bacterial diversity than their Fryxell 12 m counterparts based on the Shannon and Simpson diversity indices. The difference in bacterial diversity between the acetate-treated and glucose-treated Fryxell 6 m samples was negligible. Conversely, the Fryxell 12 m samples exhibited a significant difference in bacterial richness, wherein the acetate-consuming community possessed twice as many OTUs as the glucose-consuming community. This difference was also reflected in the Shannon and Simpson diversity indices, where the acetate-incubated sample displayed higher values than the glucose-incubated sample. Non-parametric estimators, Ace and Chao-1, indicated that the Fryxell 6 m samples were well covered with >50% total potential diversity covered. Due to the lower total number of OTU and the correspondingly higher proportion of singlets, both estimators assigned relatively higher values to the possible diversity in the Fryxell 12 m
samples. Nonetheless, the libraries representing both samples were represented with 34-36% coverage based on the Ace1 estimator.

Representative clones from each of the 54 OTUs were sent for partial sequencing using the 907r bacterial primer to determine their relationships with previously identified 16S rRNA gene sequences. The majority of the nearest neighbors based upon BLAST results were related to uncultured bacteria clones (83.3%). 13 of the 54 OTUs were related to previously identified bacteria found in the McMurdo Dry Valleys (Table 3.2). The most abundant OTU found in all 4 libraries was 98.5% related to an uncultured Arcobacter clone found in mangroves sediments (Liao et al., 2007). Though this OTU was present in both Fryxell 6 m libraries, it was dominant in the Fryxell 12 m libraries. Though several abundant OTUs could be found in all 4 samples, the relative abundance differed greatly depending upon the sample location (Table 3.2). Aside from the Arcobacter-related OTU, this could also be seen for OTUs related to Bacterium HTCC4086 and Psychromonas antarcticus. Whereas the OTU related to Bacterium HTCC4086 comprised greater than 20% of the Fryxell 6 m libraries and only 0.6% of the Fryxell 12 m libraries, the OTU related to Psychromonas antarcticus exhibited the opposite trend in which it occupied a higher proportion of the Fryxell 12 m libraries in comparison to the Fryxell 6 m libraries. Most OTUs were found exclusively in a single depth. Based upon their relative abundance, two notable OTUs in the clone libraries were related to Thiomicrospira sp. Ch-1 found only in Fryxell 12 m or one related to an uncultured bacterium clone first identified in Lake Vida, Antarctica, found only in Fryxell 6 m.
Discussion

Sample incubation with acetate and glucose successfully enriched the bacterial community involved in carbon utilization in FRX. As acetate is an ester potentially involved in fatty acid synthesis and glucose is a simple sugar easily metabolized in bacterial respiration, the use of both substrates was used to target a broader range of bacterial species capable of consuming either carbon compound. There was enrichment in specific bacterial OTUs related to uncultured *Arcobacter* sp. DS172, Bacterium HTCC4086, and *Hydrogenophaga* sp. BAC57, which were not abundant in the whole community analyses of FRX in Chapters 1 and 2 of this dissertation. Abundant OTUs (with greater than 5 clone representatives) were found exclusive to either the Fryxell 6 m acetate or glucose incubated samples, which suggests that the bacteria species found in these incubations are relying on either substrate to build biomass.

The Fryxell 12 m samples, on the other hand, were merely 45% similar with each other based on DGGE cluster analysis and had markedly different levels of bacterial diversity. Though the DGGE profiles had originally suggested that the acetate-incubated sample may have fewer bands than the glucose-incubated sample, the clone libraries, which offer a higher resolution analysis of the bacterial community, indicated the opposite trend. The Fryxell 12 m acetate-incubated sample had twice the number of OTUs as the Fryxell 12 m glucose-incubated sample. Unlike the situation found in Fryxell 6 m, substrate specificity along with the 2 week incubation period sufficiently distinguished the two Fryxell 12 m samples. The Fryxell 12 m glucose-incubated sample possessed merely 2 OTUs not found in the counterpart acetate-incubated sample.
Conversely, the Fryxell 12 m acetate-incubated sample possessed 13 OTUs not found in corresponding glucose-incubated sample.

Though present in all four samples, the two most abundant OTUs in our clone libraries represented very different proportions of each library based upon its vertical location in the water column. While relative abundance of a clone library is a value subject to PCR bias and is thus semi-quantitative, all four clone libraries were amplified in the same reaction with identical primers in a master mix. Therefore, it is likely that PCR bias associated with 16s rDNA clone libraries would be distributed throughout all four clone libraries, and thus major differences in the proportion of abundant OTUs may accurately reflect real differences in the original samples. In this study, the OTU related to uncultured *Arcobacter* sp. DS172 comprised ~10% of the FRX 6 m libraries and roughly 50% of the FRX 12 m libraries. One possible explanation for this difference in relative abundance is that this OTU has a physiological preference for the environmental conditions found below the chemocline in FRX. Another possibility is that the limited diversity of acetate and glucose utilizers in FRX 12 m may also contribute to higher proportion of this OTU among those libraries. Alternatively, the OTU related to *Bacterium HTCC4086* comprised >20% of the FRX 6 m libraries but less than 1% of the FRX 12 m samples. This indicates that while *Bacterium HTCC4086* is involved in the carbon dynamics of FRX 6 m, it was not enriched in either FRX 12 m incubations and likely plays a minimal role below the chemocline of FRX.

Another intriguing result was the identification of an OTU related to *Thiobacillus chilensis*, an obligately chemolithotrophic sulfur-oxidizing bacterium (Brinkhoff et al.,
1999). Though this OTU was not previously identified previously in Lake Fryxell (Chapter 1 and 2 of this dissertation), it was the second most abundant OTU found in both Fryxell 12 m acetate-incubated and glucose-incubated samples. Due to Fryxell 12 m micro-aerophilic conditions and hydrogen sulfide concentration of ~400 nM (Karr et al., 2005), it was not be surprising that this organism would be found exclusively at this depth, especially since psychrophilic and halophilic species of *Thiobacillus* have been previously found (Knittel et al., 2005; Sorokin et al., 2006). This OTU may possess an ability to supplement autotrophy with acetate assimilation, which has been previously suggested for *Sulfurimonas denitrificans* (Sievert et al., 2008). An alternative explanation is that labeled organic carbon could be converted to CO₂ by other bacteria and this CO₂ would then be utilized by this *Thiobacillus*-related OTU. Furthermore, as phylogeny does not necessitate insight into an organism’s physiology, it is entirely possible that this OTU is merely a heterotrophic relative to *Thiobacillus chilensis*. Nonetheless, identification and future cultivation of this organism may yield benefits in bioreactors and other applications.

The bacterial community differences based upon vertical location in the water column were more significant than the divergence between the acetate-incubated samples and the glucose-incubated samples. This is attributable to a wide variety of factors, such as oxygen levels, nutrient levels, photic levels, and sulfide content. The fact that these differences continue to persist after incubation clearly delineate the differences in the bacterial community composition prior to incubation. This study has identified the subset of the bacterial community responsible for carbon utilization in both FRX 6 m and FRX
12 m. Most OTUs identified in this study were not abundant in prior clone libraries (Chapter 1), which suggests that while these organisms may comprise only a minor component of the overall bacterial community, they are responsible for most of bacterial carbon cycling activity in Lake Fryxell. Some of the organisms enriched in this study may also possess unique physiologies that can be valuable for biotechnological application. Future work may involve not only optimization of the incubation times and conditions to minimize bottle effects but also the targeted isolation of these organisms.
Reference


Figure 3.1. DGGE banding patterns for FRX 6 m and 12 m samples from $^{13}$C-acetate and $^{13}$C-glucose incubated samples and for the whole bacterial community from 2006 samples.

Figure 3.2. UPGMA cluster analysis of DGGE banding patterns for all $^{13}$C-acetate and $^{13}$C-glucose incubated samples based on Dice correlation coefficients.

Table 3.1. Summary of clone library analysis results concerning the number of OTUs found in each library, the estimated number of total OTUs in each library, and diversity indices.

<table>
<thead>
<tr>
<th></th>
<th># of Clones</th>
<th># of OTU</th>
<th>Ace Estimate</th>
<th>Chao-1 Estimate</th>
<th>Shannon Diversity</th>
<th>Simpson Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRX 6 m Acetate</td>
<td>184</td>
<td>35</td>
<td>55.56</td>
<td>69</td>
<td>2.68</td>
<td>8.95</td>
</tr>
<tr>
<td>FRX 6 m Glucose</td>
<td>186</td>
<td>34</td>
<td>45.17</td>
<td>38.58</td>
<td>2.70</td>
<td>14.89</td>
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<td>FRX 12 m Acetate</td>
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<td>22</td>
<td>61</td>
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Table 3.2. Taxonomic affiliation for OTUs with greater than 5 members as determined by BlastN search results against the Genbank public database.

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<th>F6a</th>
<th>% of Library</th>
<th>F6g</th>
<th>F12a</th>
<th>F12g</th>
<th>Closest Relative</th>
<th>% Similar</th>
<th>Accession</th>
<th>Source</th>
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<td>11.4</td>
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</table>
Conclusion

As one of the closest analogs to extraterrestrial environments in our solar system that may harbor life (Mikucki et al., 2009), the McMurdo Dry Valley lakes are a valuable source for improving our understanding of how ancient life is capable of thriving in cold environments. With theories concerning the origins of life and “snowball Earth” remaining controversial (Vincent and Howard-Williams, 2000; Peretó, 2005), research into the phylogeny of psychrophilic microorganisms will be essential to our understanding how life evolved on Earth and how it may begin in other worlds. The goals of this dissertation were to characterize the bacterial community in the McMurdo Dry Valley lakes, relate the bacterial community with lake geochemistry, and determine how recent biological activity may contribute to ecosystem function.

Chapter 1 of this dissertation was devoted to the use of culture-independent molecular methods to identify the diversity and distribution of bacterial species throughout the water columns of WLB, ELB, and FRX. While similar studies of each individual lake had been previously conducted (Karr et al., 2003; Karr et al., 2005; Glatz et al., 2006), their limited scope make it difficult to draw conclusions about the overall composition of the microbial communities throughout these lakes. By covering the entire range of the water column in all three lake systems, our study determined that the bacterial communities within the water column of a single lake system were distinct. Furthermore, these bacterial communities also diverge greatly between the lake systems, which correlate with the distinct lake geochemistries and past geologic histories of each lake system. Chapter 1 creates a baseline analysis of the bacterial communities in these
three lake systems and also provides phylogenetic information that will aid in the isolation of novel bacterial species.

Analysis of the temporal variability in the bacterial community of the MCM lakes in Chapter 2 of this dissertation further reinforce the relationship between the microbiology and the geochemistry of these lakes. Though the physical limnology of the MCM lakes suggests the long term stability of these lakes (Spigel and Priscu, 1998), biological stability was not previously determined. Our analysis of the bacterial community over five field seasons suggest that the majority of the bacterial community in the MCM lakes are stable on an inter-annual basis, while potential fluctuation in the abundance of bacterial species may be attributable to specific environmental variables, including salinity, DOC, NO₂, NO₃, Si, and SRP.

Stable isotope probing provides a culture-independent means to directly link bacterial activity with specific nutrient cycles in the environment. In chapter 3 of this dissertation, we utilized this method to determine the acetate and glucose utilizers in FRX. Though we had previously acquired phylogenetic data in chapter 1 suggesting that abundant OTUs may be related to heterotrophic microorganisms, this is insufficient for directly identifying the bacteria involved in the consumption of organic carbon in these lakes. Thus, this chapter rectifies this situation and allows us to directly link current bacterial activity with carbon dynamics in the MCM lakes.

In summation, this dissertation has successfully accomplished its goals of identifying the bacterial community composition of the MCM lakes, determining the spatial and temporal variation of this community, and linking bacterial activity with
carbon cycling in the lakes. This provides important insight into the types of bacterial species found in Antarctic lakes and what roles they may have in regulating ecosystem function. Though bacterial species in the McMurdo Dry Valley lakes share phylogenetic similarity with organisms found in temperate freshwater systems and marine environments, the overall bacteria community structure in these lakes are distinct. The McMurdo Dry Valley lakes exhibit lower levels of bacterial abundance and diversity, which is likely attributable to their geographic isolation, permanently cold temperatures, and low nutrient input. This is particularly evident in the bottom waters of the Dry Valley lakes, where we find OTUs that share little similarity with cultured bacterial species. Therefore, the phylogenetic data obtained from this research both expands publicly available information about bacteria found in cold environments and can also be used to direct the isolation of these organisms (Stingl et al., 2008).
References


