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Distribution and Diversity of Bacterial Chemolithotrophs in Marine and Freshwater Sediments

Lisa M. Nigro

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**DISTRIBUTION AND DIVERSITY OF BACTERIAL CHEMOLITHOTROPHS
IN MARINE AND FRESHWATER SEDIMENTS**

By

Lisa M. Nigro

B.S. Rutgers University, 2001

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

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(in Microbiology)

The Graduate School

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May, 2006

Advisory Committee:

Dr. Gary M. King, Professor of Microbiology and Oceanography, Advisor

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Thesis Advisor: Dr. Gary M. King

An Abstract of the Thesis Presented
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Bacterial chemolithotroph population structure has been investigated in Lowes Cove marine intertidal mudflat and Damariscotta Lake, Maine. A 492 to 495 fragment of the *cbbL* gene, coding for the large subunit of Form I ribulose-1,5- biphosphate carboxylase/oxygenase (rubisco) was amplified from lake surface (upper 2 mm) sediments and mudflat surface (upper 2 mm), subsurface (5-7 cm), and *Mya arenaria* burrow wall sediments, as well as sulfide-oxidizing bacterial mat samples. Amplified DNA was used to construct *cbbL* clone libraries. Phylogenetic analysis showed that Damariscotta Lake *cbbL* clones were mainly of the 1C type, indicating a facultative carbon monoxide/hydrogen-oxidizing community. Conversely, clones constructed from Lowes Cove sediments were dominated by Form 1A *cbbL*-containing chemolithotrophs that were most closely related to *cbbL* genes of sulfur-oxidizing bacteria. This suggested

that the chemolithotroph community structure in lake sediments differs greatly from marine sediments. Phylogenetic P-tests of Lowes Cove sediments indicated that surface, subsurface and burrow wall sediments contain significant phylogenetic differences. AMOVA and LIBSHUFF statistical analyses of Lowes Cove sediment *cbbL* libraries suggested that *Mya arenaria* burrow wall sediments did not harbor distinct communities when compared to mudflat surface and subsurface libraries. However, Lowes Cove surface and subsurface *cbbL* libraries displayed moderate genetic difference by AMOVA analyses and were observed to contain distinct chemolithotroph communities by LIBSHUFF analysis of homologous and heterologous coverages.

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CHAPTER 1: INTRODUCTION

Bacterial Chemolithotrophs

Prokaryotes display great metabolic diversity, utilizing phototrophic, lithotrophic, and heterotrophic pathways for energy production. They can even use a combination of these methods. Chemolithotrophy, the use of inorganic substrates for energy, is of particular interest because it is unique to prokaryotes. Bacterial chemolithotrophs are predominately members of the *Proteobacteria*, and can be subdivided into two major groups: obligate and facultative (e.g. Shively et al., 1998). All chemolithotrophs are capable of utilizing inorganic substrates for energy, and inorganic C (generally in the form of CO₂) for cell carbon. While obligate chemolithotrophs depend on this autotrophic metabolism, facultative chemolithotrophs also grow heterotrophically.

Obligate chemolithotrophs include sulfur, ammonium, and nitrite oxidizers (Madigan et al., 2003). Sulfur oxidizers have been observed to utilize sulfide, thiosulfate or elemental sulfur, and some sulfur oxidizers also utilize hydrogen (Nishihara et al., 1990; Nishihara et al., 1998; Shima and Suzuki, 1993; Huber et al., 1992) or ferrous iron (Rawlings and Kusano, 1994; Kusano et al., 1991). Facultative chemolithotrophs typically include aerobic hydrogen and carbon monoxide oxidizers, as well as some reduced-sulfur oxidizers (Madigan et al., 2003), and metal oxidizers (Francis et al., 2001; Holden, PJ, personal communication). Many known facultative chemolithotrophs can use both carbon monoxide and hydrogen as electron donors, and most grow mixotrophically, utilizing both inorganic and organic substrates. Both obligate and facultative chemolithotrophs are able to fix CO₂, and most function aerobically, using oxygen as a

terminal electron acceptor. However, some strains function anaerobically, including several sulfur-oxidizing strains (English et al., 1992; Fossing et al., 1995; McHatton, et al., 1996; Schulz et al., 1999; Otte et al. 1999) and an arsenite oxidizing bacterium (Oremland et al., 2002), which can use nitrate as an electron acceptor.

The ecological interactions of obligate and facultative chemolithotrophs are poorly understood. While facultative phototrophy has been investigated in phytoplankton (Stickney et al., 2000; Jones, 2000; Tittel et al., 2003; Cloern and Dufford, 2005; Troost et al., 2005), the ecological significance of facultative chemolithotrophs and their relation to obligate chemolithotrophs and heterotrophs has been considered by only a few studies. Fitness costs of facultative chemolithotroph metabolisms have been poorly researched, however, studies have observed that facultative sulfur oxidizers had a lower maximum specific growth rate than obligate sulfur oxidizers and specialized heterotrophs (Kuenen and Beudeker, 1982). This indicates that there may be a cost in maintaining two metabolic systems in at least some facultative chemolithotrophs (Gottschal et al., 1979).

It has been proposed that despite slower growth rates, mixotrophic growth by facultative chemolithotrophs may be a survival strategy in environments where both inorganic and organic substrates are present, but are limited (Kuenen and Beudeker, 1982). Chemostat experiments by Gottschal et al. (1979) showed that at intermediate levels of thiosulfate and acetate, a facultative sulfur oxidizer could dominate a mixed population that also contained a specialized sulfur oxidizer and a specialized heterotroph. In competitive interaction studies of a specialized heterotroph and a facultative sulfur-oxidizing strain, the heterotroph was only able to completely exclude the facultative

population when the inorganic substrate was absent. Furthermore, the facultative organism was able to dominate when the dilution rate of the chemostat was very low.

Chemolithotrophy in Sediments

Prokaryotes dominate the biomass and biological activity of sediments (Nealson, 1997). Overlying water limits oxygen diffusions to sediments, and respiration results in anoxia below the sediment surface and anaerobic breakdown of organic matter. In littoral sediments, where respiratory activity is often high, oxygen depletion can occur within a few millimeters depth. After oxygen is exhausted, organic matter decomposition occurs via anaerobic pathways. A series of vertical gradients normally forms within sediments, in which various electron acceptors are utilized commonly in the order of redox potential, e.g. the potential to generate energy by coupling the oxidation of organic matter to the reduction of varying available terminal electron acceptors (O_2 , Mn^{4+} , NO_3^- , Fe^{3+} , SO_4^{2-} and CO_2) (Figure 1.1). Reduced inorganic ions produced by this process can be re-oxidized by chemolithotrophs generating energy that can be coupled to CO_2 fixation (e.g. Nealson, 1997). Anaerobic fermentation processes and breakdown of organic matter also provide reduced inorganic substrates for chemolithotrophs (Barns and Nierzwicki-Bauer, 1997).

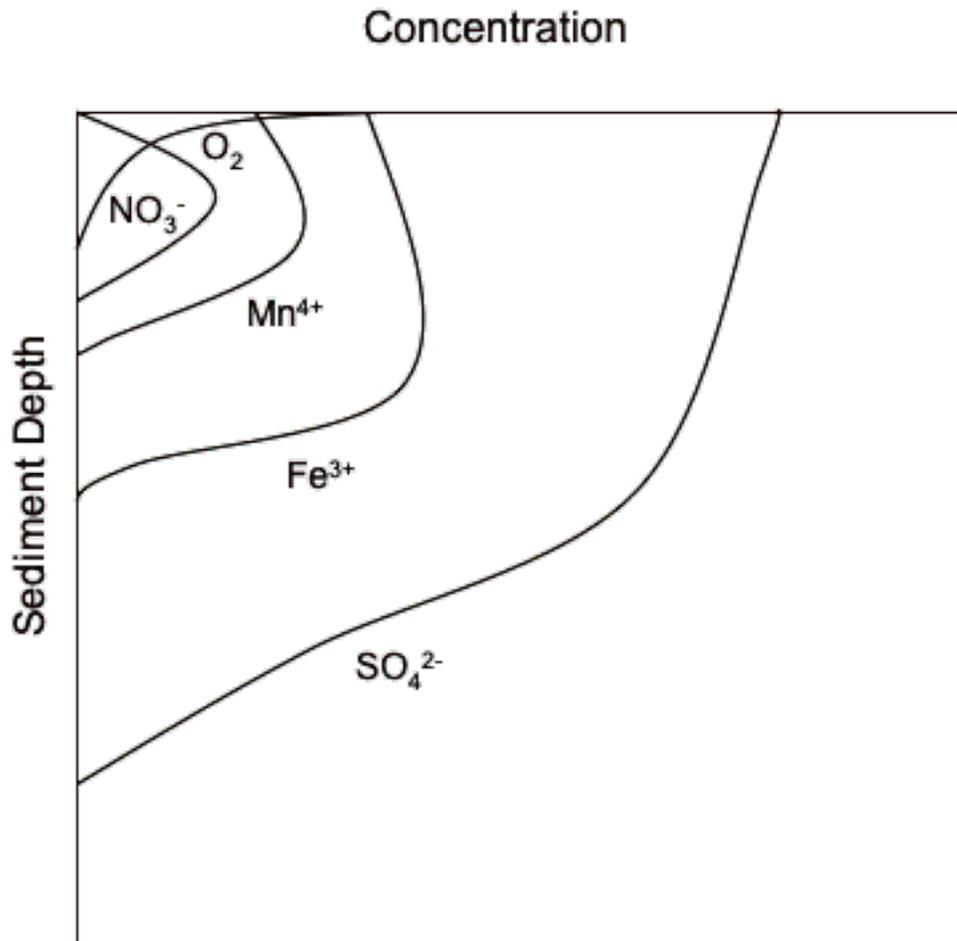


Figure 1.1 Idealized representation of a vertical profile of electron acceptors in marine sediment. Redrawn from Kristensen (2000).

Sulfur Oxidizers

The primary difference between marine and freshwater sediments is that sulfate concentration is normally much higher in the former (up to 25 mM in marine versus 100-200 μ M in freshwater) (Nealson, 1997). In fact, sulfate reduction has been reported to account for the majority of carbon turnover in marine sediments (Fenchel and Jorgensen, 1977), while aerobic respiration dominates carbon turnover in freshwater sediments (Jones, 1982). Sulfate reduction occurs biologically by sulfate reducing bacteria (SRB), producing reduced sulfur compounds that can be utilized by sulfur-oxidizing chemolithotrophs. SRB function anaerobically, and are concentrated near the top of the anoxic zone where electron donors and acceptors are at their highest concentration (Jorgensen and Postgate, 1982). In littoral lake sediments, putrefaction (anaerobic decomposition of amino acids) may be a more important source of reduced sulfur (Jones, et al. 1982b).

Sulfur-oxidizing bacteria are represented by several genera and are known to function as either obligate or facultative chemolithotrophs, and well-described examples include *Thiobacillus* and *Beggiatoa* spp. (Barns and Nierzwicki-Bauer, 1997; Madigan et al., 2003). While sulfate is chemically stable in both oxic and anoxic environments, reduced sulfur compounds, especially sulfide, are prone to chemical oxidation by O₂. Sulfide also readily reacts with iron, producing pyrite (FeS₂), which precipitates and is unavailable to most sulfide oxidizers. Sulfur oxidizers predominately use O₂ as an electron acceptor and, therefore, have developed strategies to obtain both electron donors and acceptors necessary for growth (Brune et al., 2000). These include movement between oxic and sulfidic layers (Schulz and Jorgensen, 2001), facultative nitrate

reduction (English et al., 1992; Fossing et al., 1995; McHatton, et al.1996; Schulz et al., 1996; Otte et al. 1999), and structures to increase oxygen flux (Fenchel and Glud, 1998).

Nitrifying Bacteria

Nitrification is a two-step process, involving the oxidation of ammonium to nitrite followed by the oxidation of nitrite to nitrate. Both processes occur biologically by obligate chemolithotrophs that use oxygen as an electron acceptor, and the energy gained is coupled to the fixation of CO₂. Examples of ammonium oxidizers include members of the genera *Nitrosomonas* and *Nitrospira*, while representative nitrite oxidizers include *Nitrobacter*, *Nitrospina*, and *Nitrococcus* species (Barns and Nierwicki-Bauer, 1997).

Ammonium is supplied to the sediments through several processes. The majority of ammonium is supplied via deamination of proteins, amino acids, and urea (Jones et al, 1982a). Nitrate can also be converted biologically to ammonium through dissimilatory reduction (DNRA), a fermentative process that competes with denitrification in sediments, and is thought to be of most important in environments with high organic carbon availability (e.g. Sorensen, 1978; Tiedje et al., 1982; Jones and Simon, 1981).

Metal Oxidizers

Iron and manganese are the dominant metals utilized by chemolithotrophs. One of the most studied groups of iron oxidizers is acidophilic species that include *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans* and *Thiobacillus thiooxidans*. *A. ferrooxidans* can be grown with pyrite and oxygen as electron donor and acceptor, respectively. Energy is obtained via oxidation of ferrous iron to ferric iron or reduced sulfur to sulfuric acid (Rawlings and Kusano, 1994). Iron oxidizers that live in

neutral pH environments have been less extensively studied due to difficulty in culturing these organisms. The most studied strain is *Gallionella*, which can grow mixotrophically (Barnes and Nierwicki-Bauer, 1997).

Manganese oxidizers have also been isolated from sediments, and these organisms obtain energy from the oxidation of Mn (II) to Mn(IV). Most function as lithoorganotrophs, while others function as facultative chemolithotrophs (Francis et al., 2001). Examples of manganese oxidizing genera include *Arthrobacter*, *Leptothrix*, *Hydromicrobium*, *Oceanospirillum*, *Vibrio* and *Metallogenium* (Barnes and Nierwicki-Bauer, 1997). Biological oxidation of other metals is considered to be minor. Very little is known about oxidation of metalloid compounds. However, oxidation of such trace metalloids as arsenite [As(III)] can be important in sediments where arsenic concentrations are locally high. Arsenite is primarily supplied to arsenic oxidizers by biological dissimilatory arsenate reduction (Oremland et al., 2003).

Hydrogen and Carbon Monoxide Oxidizers

Hydrogen and carbon monoxide oxidizers are taxonomically diverse and common in both soil and aquatic environments (Friedrich and Schwartz, 1993). Most CO oxidizers also possess the ability to utilize H₂ for energy, and almost all CO/ H₂ oxidizers are facultative chemolithotrophs that grow mixotrophically with organic substrates. Some also grow phototrophically (Bowien and Schlegel, 1981).

CO and H₂ are supplied to sediments as byproducts of anaerobic fermentation (e.g. Gray and Gest, 1965). Another important source of H₂ production includes N₂-fixing phototrophs, such as *Synechococcus* (Schmidt and Conrad, 1993). Photolysis of dissolved organic matter (DOM) is another possible source of CO to surface sediments (Zuo and

Jones, 1997). CO production predominately occurs in anoxic zones of sediment while CO consumption is thought to be favored in oxygenated surface sediments (King, submitted).

Carbon Dioxide Fixation

There are four pathways for carbon dioxide fixation in prokaryotes, including the Calvin-Benson-Bassham (CBB) cycle, the reductive TCA (rTCA) cycle, the reductive acetyl CoA pathway, and the 3-hydroxypropionate cycle. The predominant pathway in bacterial chemolithotrophs is the CBB cycle, in which ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) is the key enzyme for CO₂ fixation (e.g. Tabita, 1999; Shively et al., 1998). Rubisco also catalyzes the “wasteful” oxidation of ribulose bisphosphate (RuBP), and the efficiency of a particular rubisco enzyme is related to substrate discrimination between carboxylation and oxygenation functions.

There are three identified forms of rubisco, Forms I, II and III, which can be distinguished both structurally and phylogenetically (Figure 1.2). Most bacterial chemolithotrophs contain Form I, which has large and small subunits encoded by the *cbbL* and *cbbS* genes, respectively. The subunits form a hexadecameric L₈S₈ quaternary structure. Form I rubisco is further divided into four phylogenetic subgroups: 1A, 1B, 1C and 1D. The phylogeny of rubisco genes is incongruent with the phylogeny of 16S rRNA genes, possibly resulting from several horizontal gene transfer (HGT) events as well as gene duplication and loss (Delwiche and Palmer, 1996). However, particular chemolithotrophic functional groups have been associated with different *cbbL* subgroup types. Form 1A is found in cyanobacteria and chemolithotrophs of mainly the obligate type. Exceptions include *Hydrogenophaga pseudoflava*, a facultative hydrogen and

carbon monoxide oxidizer (Lee and Kim, 1998), and facultative sulfur oxidizers (Rawlings and Kusano, 1994). Form 1C has been found in red algae, most facultative hydrogen and carbon monoxide-oxidizing chemolithotrophs (e.g. Shively et al., 1998; Atomi, 2002) and facultative metal oxidizers (Holden and Brown, 1993; Caspi et al., 1996). A monophyletic clade of obligate chemolithotrophic *Nitrosospira* spp also occurs in Form 1C (Utaker et al., 2002). Plants, green algae and some cyanobacteria contain Form 1B, while red algae contain Form 1D.

Form II rubisco, found in dinoflagellates and *Proteobacteria*, is comprised of only large subunits (L_2) and is considered a more ancient form of the gene, as it has poor discrimination for carboxylation and is thought to have evolved before an oxygenated atmosphere. Form III rubisco has been discovered in several thermophilic *Archaea* and is also composed of large subunits, having a $(L_2)_5$ structure (Watson et al., 1999; Atomi, 2002; Maeda et al., 2002).

Rubisco has a sluggish catalytic rate constant and organisms normally compensate by producing the enzyme in large quantities (Shively et al., 1998). While obligate chemolithotrophs must continuously make rubisco, facultative chemolithotrophs often regulate its production. Transcription of rubisco-encoding genes in carbon monoxide and hydrogen oxidizers has been observed to be strictly controlled, being induced during autotrophic growth and repressed to different degrees during heterotrophic or mixotrophic growth (Gibson and Tabita, 1997; Vichivanives et al., 2000; Bowien and Kusian, 2002).

Molecular Diversity and Distribution of Chemolithotrophs

The *cbbL* gene has been successfully used to determine the diversity of Form 1A-containing chemolithotrophs in several aquatic environments. Sequences from hydrothermal vents and deep sea sediments contained diverse *cbbL* genes that were phylogenetically related to sulfur-oxidizing thiobacilli (Elsaied and Naganuma, 2001). Form 1A *cbbL* has also been amplified from low oxygen groundwater and aquifer environments that have been polluted with BTEX (Benzene, Toluene, Ethylbenzene, and Xylene) and chlorobenzene, respectively. Sequences were diverse and phylogenetic analysis revealed strains closely related to *Acidithiobacillus ferrooxidans* and *Hydrogenophaga pseudoflava* (Alfreider et al., 2003). Conversely, Form 1A and 1B *cbbL* sequences isolated from a redox gradient in Mono Lake showed little diversity. Most sequences were related to *Hydrogenovibrio marinus cbbL* genes. Other sequences were related to *cbbL* genes from sulfur, sulfide, nitrogen and arsenic oxidizers, methanogens, and cyanobacteria (Giri et al., 2004).

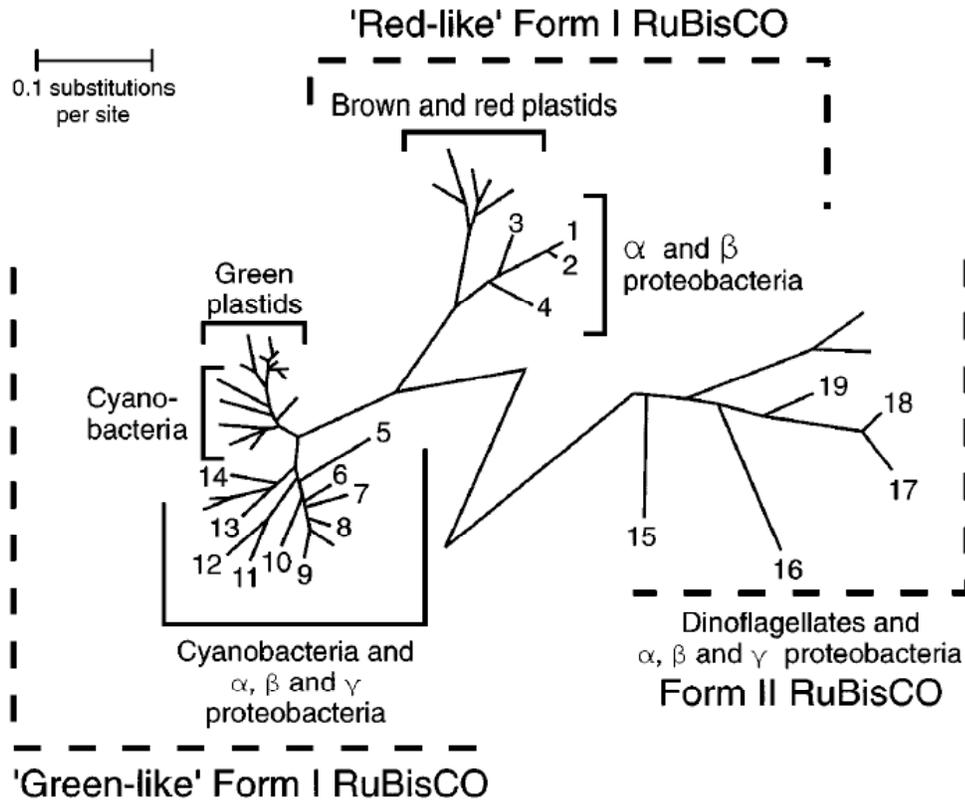


Figure 1.2 Phylogenetic organization of Form I and Form II rubeisco.

1, *Ralstonia eutropha* (chromosomal); 2, *R. eutropha* (plasmid); 3, *Rhodobacter sphaeroides*; 4, *Xanthobacter flavus*; 5, *Hydrogenovibrio marinus* 1; 6, *Thiobacillus neapolitanus*; 7, *Nitrobacter vulgaris*; 8, *Thiobacillus denitrificans*; 9, *Chromatium vinosum* 1; 10, *Thiobacillus ferrooxidans*; 11, *Pseudomonas hydrogenthermophila*; 12, *Rhodobacter capsulatus*; 13, *C. vinosum* 2; 14, *H. marinus* 2; 15, *T. denitrificans*; 16, *H. marinus*; 17, *R. sphaeroides*; 18, *R. capsulatus*; 19, *Rhodospirillum rubrum*.

From Shively et al., 1998

The diversity of both 1A and 1C forms was considered in studies of *cbbL* in differently managed agricultural soils. The form 1C *cbbL* genes were phylogenetically diverse while form 1A *cbbL* sequences were much less diverse and most closely related to *Nitrobacter* spp *cbbL* genes (Selesi et al., 2005). In another study, a single primer set was utilized to amplify 1A and 1C *cbbL* genes from agricultural plots containing different plants. A large percentage of clones were of the facultative 1C type, supporting results from Selesi et al. (2005). Furthermore, clones were genetically diverse and plots harbored statistically different *cbbL* populations (Tolli and King, 2005) The same primer set utilized in Tolli and King (2005) was used to determine chemolithotroph diversity in several Hawaiian volcanic deposit sites that varied in stages of succession after lava flow. All clones were of the 1C type, suggesting a facultative community. A statistical analysis of the clone libraries showed that they differed significantly among sites (Nanba et al., 2004).

Study Objectives

The aim of this study was to investigate the distribution and diversity of bacterial chemolithotrophs in littoral sediments of a freshwater lake and an intertidal estuary by utilizing the *cbbL* gene as a genetic biomarker. The first objective addressed in this study was to determine which bacterial chemolithotrophs dominate marine and freshwater sediments. The diversity of Form 1A *cbbL*-containing chemolithotrophs in sediments has been investigated by few studies (Elsaied and Naganuma, 2001; Alfreider et al., 2003) and to date, no study has addressed the importance of Form 1C-containing

chemolithotrophs in sediments. The importance of the sulfur cycle in marine sediments has been well documented (e.g Fenchel and Jorgensen, 1977; Nedwell, 1982). We hypothesized that Lowes Cove intertidal sediment would, therefore, be dominated by Form 1A *cbbL*-containing sulfur oxidizing chemolithotrophs. Conversely, NH_4^+ , metal, H_2 and CO-oxidizing chemolithotrophs may play a more important role in freshwater sediments (Jones et al., 1982a; Conrad et al., 1983; Zuo and Jones, 1997; Rich and King, 1999), where sulfur compound concentrations are often low in comparison to marine sediments (e.g. Jones, 1982).

The second objective explored by this study was to determine chemolithotroph distributions within Lowes Cove microhabitats. Lowes Cove is highly bioturbated by macrobenthos. Burrows span the oxic and anoxic layers of sediments, and macrofauna therefore provide oxygen to both the burrow and the surrounding anoxic sediment. In turn, this can change the microbiological composition of the burrow sediments, fostering distinct communities of bacteria and increasing chemolithotroph activity (Aller and Yingst, 1978; Aller and Aller, 1986; Kristensen, 2000). Conversely, increased mixing of sediments by burrowing animals provides a means of active transport for bacteria between the surface and subsurface, which may prevent formation of distinct bacterial communities.

To investigate chemolithotroph distribution and diversity, DNA was extracted from lake and marine surface sediments (upper 2 mm), marine subsurface sediments and burrow walls of the soft shell clam *Mya arenaria*. In addition, DNA was also extracted from sulfide-oxidizing bacterial mats on the fringe of Lowes Cove. A 492 to 495 bp fragment of the *cbbL* gene was amplified by PCR with primers designed to target both

Form 1A and Form 1C *cbbL* genes. Amplicons were used to construct clone libraries. DNA inserts of the correct size were sequenced and analyzed phylogenetically to assess chemolithotroph distribution. Clone libraries were evaluated statistically to calculate sequence diversity and to determine if different sediment environments harbor distinct chemolithotroph communities. Maximum potential biological oxidation rates of ammonium, thiosulfate and CO were also calculated from each sediment type to obtain a basic understanding of which chemolithotroph activities may dominate.

CHAPTER 2: DIVERSITY AND DISTRIBUTION OF THE LARGE SUBUNIT GENE OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE/OXYGENASE IN MARINE AND FRESHWATER SEDIMENTS

Introduction

Bacterial chemolithotrophs are a metabolically diverse group of prokaryotes that utilize reduced inorganic substrates for energy and CO₂ for cell carbon. Obligate chemolithotrophs include nitrifying bacteria and many sulfur oxidizers (Madigan et al., 2003). Facultative chemolithotrophs grow mixotrophically, utilizing both organic and inorganic substrates simultaneously, and include hydrogen- and carbon monoxide-oxidizing aerobes (Friedrich and Schwartz, 1993), some sulfur oxidizers (e.g. Jorgensen and Postgate, 1982) and metal oxidizers (Francis et al., 2001; Holden, PJ, personal communication).

The relative distributions of bacterial chemolithotrophs have been largely unexplored. While 16S rRNA gene cloning and sequencing techniques are often used to gain insights into microbial population structure, chemolithotrophy occurs in a wide range of phylogenetically dissimilar bacteria. However, bacterial chemolithotrophs are united in their ability to fix CO₂ for cell carbon, in which the Calvin-Benson-Bassham (CBB) cycle is the dominant pathway (e.g. Shively, 1998; Tabita, 1999). Ribulose-1,5- bisphosphate carboxylase/oxygenase (rubisco) catalyzes the first reaction of the CBB cycle, the carboxylation of ribulose bisphosphate to two molecules of 3-phosphoglycerate. Form I rubisco is composed of large and small subunits (L₈S₈) and is found in most phototrophs and bacterial chemolithotrophs. Form II and III rubisco have only large subunits. Form II

is found in some chemolithotrophs and dinoflagellates, while Form III rubisco has been reported in *Archea* (Watson et al., 1999; Atomi et al., 2002, Maeda et al., 2002).

Analysis of the *cbbL* gene (also *rbcL*), coding for the large subunit of Form I rubisco, has been useful in determining bacterial chemolithotroph community composition (Alfreider et al., 2003; Elsaied and Naganuma, 2001; Giri et al., 2004; Nanba et al., 2004; Selesi et al., 2005; Tolli and King, 2005). The *cbbL* gene contains four phylogenetically distinct subgroups, 1A through 1D. Nitrifying bacteria and sulfur oxidizers typically contain Form 1A *cbbL*, while hydrogen-, carbon monoxide- and metal oxidizing facultative chemolithotrophs contain Form 1C. Exceptions include *Hydrogenophaga pseudoflava*, a facultative hydrogen and carbon monoxide oxidizer containing a Form 1A *cbbL* (Lee and Kim, 1998), and some obligate chemolithotrophic *Nitrosospira* spp containing a monophyletic group of *cbbL* genes within the Form 1C clade (Utaker et al., 2002). Also, several photochemolithotrophic H₂ and CO oxidizers have both Form 1A and Form 1C (Uchino and Yokota, 2003).

Few studies have investigated chemolithotroph *cbbL* diversity in aquatic ecosystems, and none have targeted Form 1C. Phylogenetically diverse Form 1A *cbbL* genes were reported for deep-sea seep and hydrothermal vent environments (Elsaied and Naganuma, 2001) as well as BTEX-contaminated groundwater and chlorobenzene-contaminated aquifers (Alfreider et al., 2003). Conversely, little phylogenetic diversity was observed in a redox gradient of Mono Lake (Giri et al., 2004). Analyses of agricultural soil *cbbL* genes revealed large phylogenetic diversity of Form 1C but not in Form 1A (Selesi et al., 2005). Furthermore, Form 1C was found to be the dominant type

in both agricultural soils (Tolli and King, 2005) and volcanic deposit sites (Nanba et al., 2004).

Oxygen limitation in sediments results in anaerobic breakdown of organic matter and production of reduced inorganic compounds, some of which can be used by bacterial chemolithotrophs (e.g. NH_4^+ , NO_2^- , H_2S , $\text{S}_2\text{O}_3^{2-}$, S^0 , Fe^{2+} , Mn^{2+} , H_2 , CO) (e.g. Malcom and Stanely, 1982). Sulfate concentrations in marine sediments are usually much higher than in freshwater sediments, and sulfide production is typically an important process in the former (e.g. Nedwell, 1982). Marine sediments are, therefore, expected to have larger populations of sulfur-oxidizing chemolithotrophs. Conversely, H_2 and CO are thought to play major roles in terrestrial and freshwater environments (Friedrich and Schwartz, 1993) and may be particularly important in lake sediments, where concentrations of sulfur compounds are comparatively low (e.g. Jones, 1982; Nedwell, 1982).

Sediments are also heterogeneous, containing microhabitats that may support distinct populations of bacteria. For example, burrowing macrofauna open deeper sediments to overlying water, providing oxygen to both the burrow and the surrounding anoxic sediment. In turn, this can change the microbiological composition of burrow sediments, both quantitatively and qualitatively, fostering distinct communities of bacteria and increasing biogeochemical activity (Aller and Yingst, 1978; Aller and Aller, 1986; Hansen et al., 1996; Chung and King, 1999; Kristensen, 2000). Conversely, active transport of bacteria by burrowing animals may prevent the formation of distinct bacterial communities, instead promoting bacterial homogeneity among the surface, subsurface and burrow microhabitats.

In this study, we report chemolithotroph distribution and diversity in sediments of an intertidal saltmarsh and the littoral zone of a freshwater lake in Maine. DNA was extracted from surface sediments (upper 2 mm) of Damariscotta Lake, and surface, subsurface (5-7 cm) and *Mya arenaria* burrow wall sediments of Lowes Cove intertidal saltmarsh. DNA was also extracted from sulfide-oxidizing bacterial mats located on the fringe of Lowes Cove. A 492 to 495 base pair fragment of the *cbbL* gene was amplified by polymerase chain reaction (PCR) with primers that target both Form 1A and 1C *cbbL* (Nanba et al., 2004).

Phylogenetic and statistical analyses of *cbbL* clone libraries indicate that marine and freshwater sediments greatly differ in bacterial chemolithotroph community structure, with Form 1A dominating marine sediments and Form 1C dominating lake sediments. Meanwhile, statistical analyses of Lowes Cove surface, subsurface and *Mya arenaria* burrow wall sediments indicated different degrees of overlapping community structure based on phylogenetic variation, genetic diversity and evolutionary distance measurements. ANOVA analyses of maximum potential biological oxidation rates were consistent with molecular data Lowes Cove sediments but not Damariscotta Lake sediments.

Materials and Methods

Sampling and Site Information

Marine sediments were obtained from Lowes Cove, an intertidal mudflat of the Damariscotta River estuary in Walpole, ME, which has been described previously (King et al., 1983; Hansen et al., 1996). The mudflat is highly bioturbated by a large

macrobenthic population, and sulfide-oxidizing bacterial mats were present in some areas along the edge of the cove. Freshwater sediments were collected from Damariscotta Lake Park in Jefferson, Maine. Damariscotta Lake is mesotrophic and has an area of 19 km², and three hydrologically distinct basins. Damariscotta Lake Park is located off the northernmost and largest basin, which has an area of 8 km² and a maximum depth of 33.5m (Damariscotta Lake Watershed Association). The sediment at the collection site was composed of clay overlain by several centimeters of sand.

Lowes Cove sediments were collected at low tide, when the mudflat had no overlying water. For CO, ammonium and thiosulfate biological oxidation rate assays, Lowes Cove surface (upper 2 mm) and subsurface (5-7 cm) sediments were collected by coring with 6.5 cm diameter acrylic tubes that were washed in a bleach solution and liberally rinsed with tap water. For DNA extraction, surface and subsurface sediments were collected by coring with sterile 50 cm³ syringes with bottoms cut off. For both DNA extraction and biological oxidation rate assays, sulfide-oxidizing bacterial mats were collected with 50 cm³ syringes, and burrow wall sediments of *Mya arenaria* were sampled *in situ* with a sterile spatula and transferred to Whirlpak bags. Cores were processed with a 75% ethanol rinsed spatula or knife.

Damariscotta Lake sediments were collected by coring with 6.5 cm acrylic tubes that were washed in a bleach solution and liberally rinsed with tap water. Sediment was collected within 2 meters of the shore, and overlying water was approximately 0.5 m deep. Cores were equilibrated for one minute before removal and taken to the lab for processing. Overlying water was removed with a sterile syringe. Surface sediment was

removed with a sterile spatula. All sediment samples were processed immediately after collection.

Carbon monoxide oxidation

One gram fresh weight (gfw) of each triplicate sediment sample was transferred to 60 ml serum bottles. One ml sterile artificial sea water (ASW) or filter sterilized lake water was added to the Lowes Cove and Damariscotta Lake samples, respectively. Serum bottles were sealed with gas-tight stoppers and CO was added to the headspaces via needle and syringe to a final concentration of 200 ppm. Gas samples were removed at intervals for a total of 5 days by syringe and needle. Samples were analyzed based on the HgO to Hg vapor conversion technique by a RGA3 gas chromatographer (GC) (Trace Analytical) equipped with a mercury vapor detector (see Rich and King, 1997). The GC was standardized with a 1000 ppm CO standard.

Ammonium Oxidation

Slurries were prepared by placing 2 gfw of each triplicate sediment sample in 50 mL sterile disposable centrifuge tubes containing 10 ml sterile deionized water with 1 mM ammonium chloride and 10 mM sodium chlorate to prevent oxidation of produced nitrite (Belser and Mays, 1980). Subsamples of 1 ml were taken at intervals for a total of 2 weeks and placed in microcentrifuge tubes. Nitrite concentrations were determined colorimetrically based on an azo dye reaction of nitrite with acid sulfanilamide and n-(1-naphthyl-) ethylenediamine dihydrochloride. Subsamples were centrifuged to pellet sediment. An 800 μ l liquid volume from each subsample was then aliquoted into 2 ml disposable cuvettes. Absorbance at 543 nm was determined using a Beckman DU640

spectrophotometer before and after 16 μ l of 1% acid sulfanilamide (buffered in 10% sulfuric acid) and 16 μ l of 0.1% n-(1-naphthyl-) ethylenediamine dihydrochloride were added.

Thiosulfate Oxidation

One gfw of triplicate sediment samples was added to 120 ml sterile bottles. Bottles were amended with 10 ml sterile ASW or filter sterilized lake water, for marine and freshwater sediments, respectively, containing 10 mM thiosulfate. Bottles were capped with sterile rubber stoppers. Subsamples of 300 μ L were taken at intervals for 2 days for marine samples and 6 days for Damariscotta Lake samples. Subsamples were placed in 1.5 ml microcentrifuge tubes. After subsampling, microcentrifuge tubes were centrifuged to pellet sediment. Supernatants were then placed in 15 mL falcon tubes with 2.5 mL reaction buffer (0.1 M sodium phosphate and 1mM EDTA, pH 8.0) and 50 μ l of 4mg ml⁻¹ 5,5' – dithio-bis-(2-nitrobenzoic acid) (DNTB). DNTB reacts with thiosulfate to produce a quantifiable colored product (Ellman, 1959). Samples were analyzed on a Beckman DU640 spectrophotometer at a wavelength of 412 nm. In addition to the above protocol, two control reactions were simultaneously set up, including a background control, with no thiosulfate addition, and autoclaved killed controls to determine the potential for chemical thiosulfate oxidation.

DNA extraction and amplification of *cbbL* genes

DNA was extracted from triplicate samples using a MoBio UltraClean Soil DNA Kit (MoBio Labs, Carlsbad, California), according to the manufacturer's instructions.

DNA was amplified with primers K2f and V2r from Nanba et al. (2004). PCR reactions were processed with Master*Taq* DNA polymerase (Brinkmann Inc.) using the manufacturer's recommendations for buffers, magnesium, dNTPs and Master*Taq*. Reactions were amplified on an Eppendorf Mastercycler thermocycler (Brinkmann Inc.) with the following conditions: initial 3 minute 94°C denaturation, 30 cycles of 94°C for 45 s, 62°C for 60 s and 72°C for 60 s, and a final extension of 72°C for 7 minutes. PCR products were electrophoresed on a 1% agarose gel and visualized with UV after staining with ethidium bromide. Products of the correct size were immediately processed for cloning or stored at -20°C for no longer than 24 hours before processing.

Clone library construction

Triplicate PCR products from lake surface sediment were pooled and purified with a MoBio PCR cleanup kit (MoBio Labs, Carlsbad, California) or Qiagen Gel Extraction Kit. Clone libraries were constructed using the Invitrogen TOPO TA cloning kit and *Escherichia coli* TOP10 competent cells according to the manufacturer's instructions (Invitrogen Life Technologies, Carlsbad, California). Clones were arbitrarily picked and grown with shaking overnight at 37°C in agitated Luria-Bertani broth containing 50 µg ml⁻¹ kanamycin. Cultures were centrifuged and pelleted cells were washed and resuspended in 10 mM Tris buffer. Resuspended cells were amplified by PCR with TOPO cloning vectors T3 and T7 supplied by the manufacturer. PCR products indicating inserts of the correct size were purified with a MoBio PCR purification kit and sequenced by the University of Maine's DNA Sequencing Facility using the vector primer T7. Sequences were screened by BLAST (Altschul et al., 1997) to confirm assignment as *cbbL* genes, and to determine their similarity to published sequences.

Phylogenetic Analysis

DNA sequences were aligned using Clustal X version 1.01 (Thompson et al., 1997). The *cbbL* phylogeny was analyzed with a neighbor-joining algorithm with the PAUP* phylogeny analysis program (Swofford, 1998). Confidence in phylogenetic tree topology was assessed with bootstrapping by the NJ method in PAUP*.

Statistical Analyses

Substrate oxidation rates were analyzed with a two-tailed t-test ($\alpha = 0.05$), to determine the significance of association between dependent and independent variables. Maximum potential oxidation rates were determined by calculating the slopes of the linear regressions for each replicate at what appeared to be the maximum rate of oxidation. Significant differences in maximum potential biological oxidation of the sites were determined by performing a one-way analysis of variance with the R statistical software package (www.r-project.org). Transformations were applied when necessary to obtain normality and constant variance. Normality was measured with the Lilliefors (Kilmorov-Smirnov) test, and the null hypothesis of normality was accepted at a probability level ≥ 0.15 . Constant variance was measured with the Levine test, and the null hypothesis of constant variance was accepted at a probability level of ≥ 0.05 . When significant differences in means were present, as measured by a F-statistic ($P \leq 0.05$), a Tukey HSD pothoc test was performed with 95% familywise confidence.

Clone libraries obtained from Damariscotta Lake and Lowes Cove sediments were analyzed by Analysis of Molecular Variance (AMOVA) with Arlequin (Schneider et al., 2000) to estimate the significance of differences in population pairwise fixation indices (F_{ST} values) among *cbbL* libraries. Arlequin was also used to estimate nucleotide

diversity and average pairwise differences of aligned *cbbL* sequences. Nucleotide diversity estimates the probability that two randomly chosen homologous nucleotides will differ, while average pairwise difference estimates the number of nucleotide differences observed when each clone sequence is compared with all other clone sequences.

Sediment *cbbL* clone libraries were also analyzed with the webLIBSHUFF computer program (<http://libshuff.mib.uga.edu>), which calculates homologous and heterologous coverages using a Cramer-von Mises statistic with a Monte Carlo test procedure (Singleton et al., 2001). The distance matrix used in the LIBSHUFF analysis was obtained using DNADIST of the PHYLIP program (v. 3.65; J. Felsenstein; (evolution.genetics.washington.edu/phylip.htm)). Libraries were considered significantly different at $P \leq 0.05$. A phylogenetic P-test was performed to test if the distribution of unique sequences between different clone libraries displays significant covariance with phylogeny (Martin, 2002). One thousand random trees were constructed in PAUP* from combined clone libraries. The tree length of the combined library was determined by constructing a parsimony tree using the heuristic search algorithm in PAUP*. Clone libraries were considered significantly different if the actual tree length was less than the 95% lower confidence limit of the random trees. Bonferroni corrections for multiple comparisons were applied for LIBSHUFF and P-test analyses.

Accession Numbers

Chromatium vinosum, D90204; *Synechococcus* strain CcmK U46156; *Synechococcus* T6SY9, AY157474; *Nitrosomonas* ENI11, AB061373; *Nitrosospira* TCH716, AF459718; *Hydrogenephaga pseudoflava*, U55037; *Thiobacillus* sp., M34536; *Bradyrhizobium japonicum* USDA 110, AF041820; *Nitrosospira* sp. strain III2,

AF426416; *Nitrosospira* sp. strain 40K1, AF426428; *Nitrosospira* sp. strain AF, AF426415; *Nitrosospira multiformis* ATCC 25196, AY157474; *Ralstonia eutropha*, U20585; *Rhodobacter blasticus*, AB082959; *Solemya velum* sulfur-oxidizing symbiont AY531637. Terrestrial clones: HM34, AY422874 ; PN5.81, DQ149802; PN5.58, DQ149798; GP0.95, DQ149762; Sulfide-oxidizing mat clones: CM4, AY422060. Hydrothermal vent clones: 1a04, AY431011; clone Suiyo (IC)-3, AB181164. Manganese-oxidizing bacterium SI85-9A1, L32182.

Results

Biological Oxidation Assays

Measurable rates of ammonium oxidation were observed for Lowes Cove surface and burrow sediments, but not for Damariscotta Lake surface sediments (Table 2.1). Thiosulfate and carbon monoxide oxidation were observed in samples from all sediment environments (Table 2.1). Example plots of CO, ammonium and thiosulfate from Lowes Cove surface sediments are displayed in Figure 2.1. Transformations of the data in both ammonium and thiosulfate oxidation models greatly improved normality and homogeneity of variances (Table 2.2). Significant differences between site means ($P < 0.05$) were observed in ammonium and thiosulfate biological oxidation assays, but not for CO uptake (Table 2.2). A TukeyHSD (95% confidence) was performed on the ammonium and thiosulfate rate models. Ammonium oxidation in Lowes Cove surface and burrow wall sediments were not significantly different ($P = 0.31$), but were significantly higher than subsurface sediments ($P < 0.0008$). Biological thiosulfate

oxidation rates of Damariscotta Lake surface and Lowes Cove subsurface sediments were not significantly different ($P = 0.62$). Lowes Cove surface and burrow sediments had comparable thiosulfate oxidation rates ($P = 0.78$) and were significantly higher ($P < 0.05$) than Damariscotta Lake and Lowes Cove subsurface sediments. All sediment types had significantly less biological thiosulfate oxidation than Lowes Cove sulfide-oxidizing bacterial mats ($P < 0.05$).

Sample	Assay	AVG (pmol / g h⁻¹)
LC surface	Ammonium	15.4 ± 53.2
LC burrow	Ammonium	47.5 ± 16.9
LC subsurface	Ammonium	3.4 ± 2.4
D lake	Ammonium	0.0
Sample	Assay	AVG (nmol / g h⁻¹)
LC surface	CO	4.2 ± 7.0
LC burrow	CO	4.1 ± 1.0
LC subsurface	CO	3.4 ± 2.4
D lake	CO	6.6 ± 2.2
Sample	Assay	AVG (umol / g h⁻¹)
LC surface	Thiosulfate	72.5 ± 30.8
LC burrow	Thiosulfate	110.0 ± 53.2
LC mat	Thiosulfate	363.6 ± 51.1
LC subsurface	Thiosulfate	23.1 ± 2.8
D lake	Thiosulfate	17.9 ± 11.1

Table 2.1 Potential maximum oxidation (with standard deviations) of ammonium, carbon monoxide, and thiosulfate. Abbreviations: Lowes Cove sediment (LC), Damariscotta Lake surface sediment (D Lake). All averages are of triplicate samples.

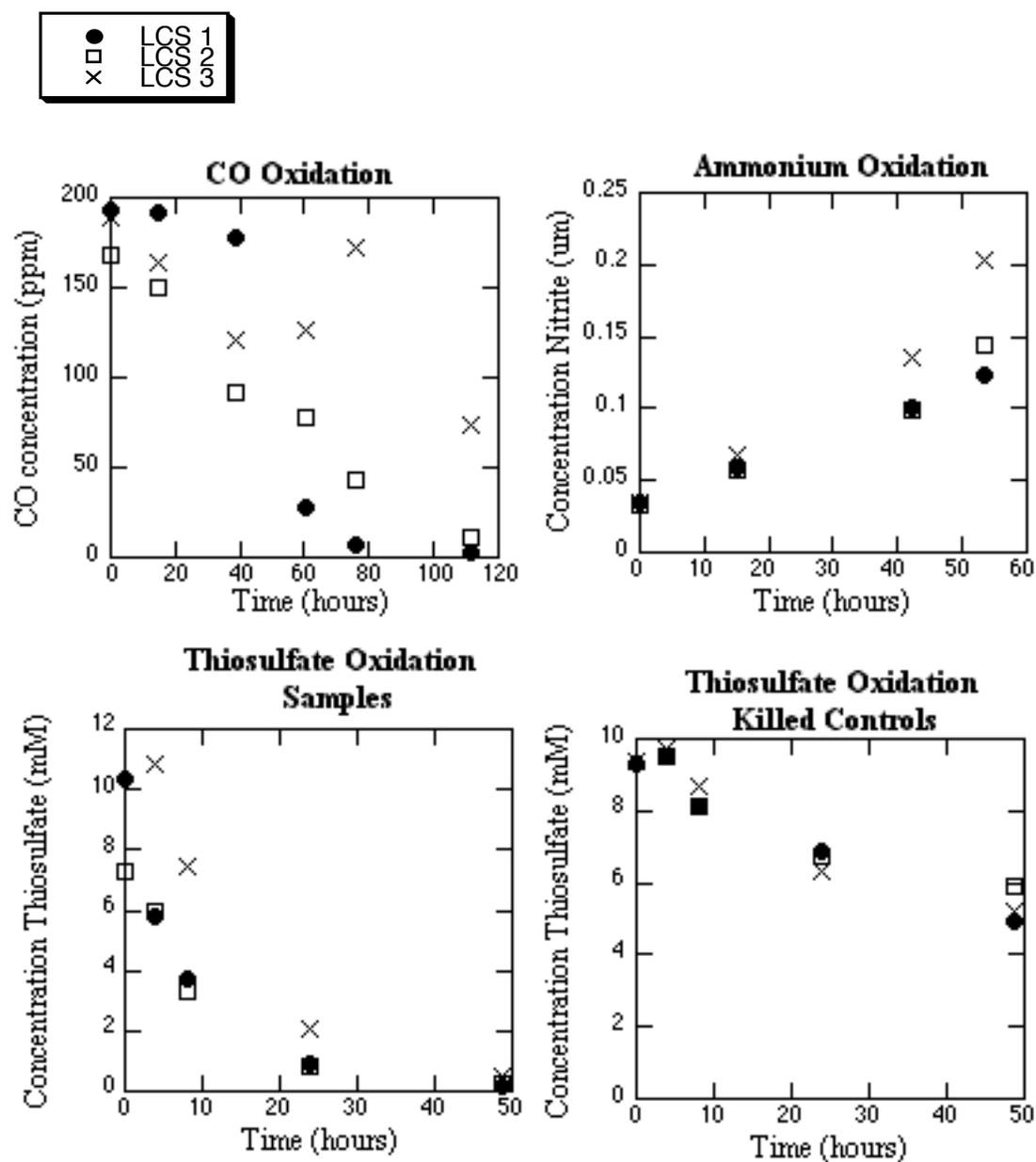


Figure 2.1 Plots of CO, ammonium and thiosulfate potential biological oxidation for Lowes Cove triplicate (LCS 1, 2 and 3) surface samples.

Assay	Model (response)	Lilliefors p-value	Levine p-value	ANOVA p-value
Ammonium	-1/rate	0.1618	0.1371	0.00074
CO	rate	0.3187	0.05098	0.2424
Thiosulfate	LN(rate)	0.6104	0.05346	1.529e-05

Table 2.2 Models used in ANOVA and P-values. Sites were considered significantly different at a probability level of ≤ 0.05 .

Phylogenetic Analysis

Lowes Cove sediments were dominated by Form 1A *cbbL* while Damariscotta Lake sediments predominately contained Form 1C (Figure 2.2 and Figure 2.3). All seven *cbbL* clones from Lowes Cove sulfide-oxidizing bacterial mats were of the 1A form that is typical of sulfur oxidizers. All but one subsurface clone contained Form 1A *cbbL*, and a majority of surface (91%) and *Mya arenaria* burrow wall (86%) clones were of the 1A type (See Table 2.4 for number of clones obtained from each site). Lowes Cove 1A *cbbL* clones were phylogenetically similar to each other, although some were also closely related to hydrothermal vent and sulfide-oxidizing mat clones reported in the NCBI database (Table 2.3 and Figure 2.2). Twenty-three Damariscotta Lake sediment *cbbL* clones were analyzed and 21 (91%) were Form 1C. The Form 1A clones were most closely related to *Rhodobacter blasticus*, a carbon monoxide and hydrogen-oxidizing phototroph. All other 1C sequences were diverse and most closely related to terrestrial strains (Table 2.3 and Figure 2.3).

Clone Name	<i>cbbL</i> Form	Accession Number	Description	Identity
LCB90, LCB33, LCB57, LCB170, LCM138, LCM225, LCS91, LCS139, LCS66, LCS42, LCS256, LCS27, LCS269, LCS222	1A	AY422061	Sulfide-oxidizing bacterial mat clone CM6R	93-98%
LCS120	1A	AY422060	Sulfide-oxidizing bacterial mat clone CM4	91%
LCS147	1A	AB175812	Hydrothermal vent clone Suiyo (I)-8	90%
LCM8	1C	DQ149792	Soil bacterium clone PN5.12	99%
LCS224	1C	DQ149802	Soil bacterium clone PN5.81	98%
DL72	1C	DQ149762	Soil bacterium clone GP0.95	99%
DL279	1C	DQ149786	Soil bacterium clone GP5.183	95%
DL434, DL469	1C	DQ149777	Soil bacterium clone GP5.105	94%

Table 2.3 Results from BLAST searches. Clones that had $\geq 90\%$ identity to a sequence in the NCBI nucleotide database are reported.

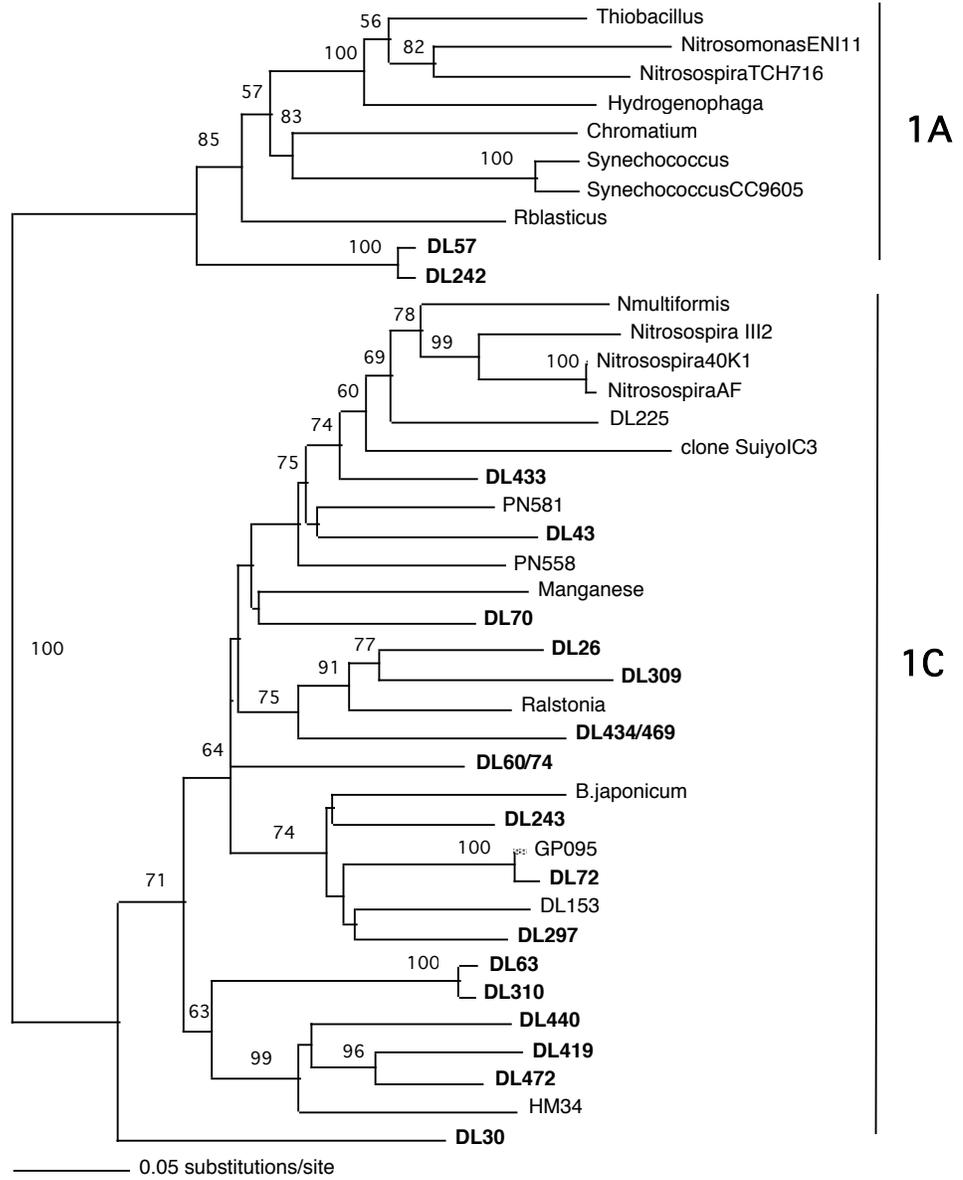


Figure 2.3 Neighbor-joining tree of Damariscotta Lake (DL) partial *cbbL* sequences.

Values of 1000 bootstrap replicates are displayed above nodes.

Clone library statistical analyses

Nucleotide diversity was similar among all sediment environments, as were within-site average pairwise differences (Table 2.4). Among site average pairwise differences of Lowes Cove microhabitats were nearly identical, while differences between Lowes Cove samples and Damariscotta Lake sediments were higher (Table 2.5).

Values of Wright's fixation index (F_{ST}) (Table 2.5) indicated varying levels of genetic differentiation among clone libraries (see Hartl and Clark, 1997). The highest F_{ST} values were observed between Lowes Cove *cbbL* clone libraries and the Damariscotta lake *cbbL* sequences, supporting the hypothesis that marine and freshwater sediments harbor different chemolithotroph communities. Lowes Cove surface and subsurface sediment sequences showed moderate genetic differentiation, while little genetic diversity was observed among burrow wall clones and marine surface and subsurface environments (Table 2.5).

Site	No. of clones	ND	$\theta[\pi]$
LC Surface	45	0.22(0.11)	109.8 (48.0)
LC Subsurface	33	0.17(0.08)	85.4(37.7)
LC Burrow	20	0.26(0.13)	126.1(56.6)
DL Surface	23	0.28(0.14)	137.8(61.3)

Table 2.4 Values for nucleotide diversity (ND) and within site average pairwise difference ($\theta[\pi]$) for Lowes Cove (LC) and Damariscotta Lake (DL) samples. Standard deviations are in parentheses.

Site	F_{ST} or $(\theta[\pi])$ for Site			
	LCS	LCB	LCM	DL
LCS		120.0	103.8	216.9
LCB	0.019		105.3	213.1
LCM	0.059	0.001		222.2
DL	0.440	0.381	0.511	

Table 2.5 Among site average pairwise differences and fixation index (F_{ST}) values.

Average pairwise differences ($\theta[\pi]$) between populations are given above diagonal and F_{ST} values are given below diagonal. Values were all significant at ($P < 0.05$). Site abbreviations: LCS, Lowes Cove Surface; LCB, Lowes Cove Burrow; DL, Damariscotta Lake; LCM, Lowes Cove subsurface (5-7 cm).

LIBSHUFF analyses of clone library homologous and heterologous coverages (Table 2.6) indicated that Lowes Cove clone libraries were all significantly different than the Damariscotta Lake sediment *cbbL* library ($P = 0.001$). Among Lowes Cove microhabitats, surface and subsurface sediments contained distinct chemolithotroph populations ($P = 0.001$) while neither of these sites was significantly different than *Mya arenaria* burrow wall sediments ($P > 0.0085$).

The tree length of a parsimony tree containing Lowes Cove clone libraries (surface, subsurface, and *Mya arenaria* burrow wall sediments) was significantly less than the 95% lower confidence limit of 1000 random trees. P-tests of individual Lowes Cove libraries similarly indicated significant phylogenetic divergence of marine microhabitat populations. Removing Form 1C *cbbL* sequences from Lowes Cove libraries did not change the outcome of AMOVA, LIBSHUFF or P-test analyses.

X \ Y	LIBSHUFF P-values			
	LCS	LCB	LCM	DL
LCS		0.094	0.001	0.001
LCB	0.678		0.567	0.001
LCM	0.001	0.196		0.001
DL	0.001	0.001	0.001	

Table 2.6 LIBSHUFF probability values of XY and YX comparisons. Sites listed in the left column represent library X, while sites displayed in the top row represent library Y. Libraries were considered significantly different at 95% confidence if the XY or YX comparison had a probability value of ≤ 0.0085 , a Bonferroni correction for multiple comparisons. Site abbreviations LCS, Lowes Cove Surface; LCB, Lowes Cove Burrow; LCM, Lowes Cove subsurface (5-7 cm); DL, Damariscotta Lake.

Discussion

Few studies have addressed chemolithotroph diversity in sediments, and this was the first study to consider both 1A and 1C *cbbL*-containing chemolithotrophs. Elsaied and Naganuma (2001) observed phylogenetically diverse Form 1A *cbbL* genes in marine sediments and hydrothermal vent environments. Phylogenetic results from this study support a diverse 1A *cbbL*-containing community in marine intertidal sediments (Figure 2.2). A large majority of *cbbL* clones were of the 1A-type, and all sequences closely matched known sulfur-oxidizers or clone sequences from sulfide-oxidizing bacterial mats (Table 2.3 and Figure 2.2). These data support the strong influence of sulfur dynamics on bacterial populations of marine sediments. Form 1C *cbbL* sequences were also obtained from marine sediments, indicating that CO/ H₂ oxidizers also contribute to the marine chemolithotroph population.

Conversely, Damariscotta Lake surface (upper 2mm) sediment clones mainly contained Form 1C *cbbL* sequences, suggesting a chemolithotroph population of mainly H₂/CO oxidizers (Figure 2.3). The importance of soils in the H₂ cycle has been well documented (Novelli et al., 1999). However, few studies have addressed the population structure of chemolithotrophs in terrestrial environments. Nanba et al. (2004) reported a community strongly dominated by Form 1C *cbbL*-containing chemolithotrophs in volcanic deposit environments. Furthermore, Tolli and King (2005) observed that agricultural soils were dominated by Form 1C *cbbL*-containing chemolithotrophs. Few Form 1A *cbbL* sequences were obtained from the soils, and sequences were most closely related to *cbbL* genes of obligate nitrifying bacteria. Selesi et al. (2005) used two sets of primers to target Form 1A and Form 1C *cbbL* sequences separately. They reported large phylogenetic diversity of Form 1C-containing clones and small diversity of Form 1A *cbbL* clones, which were all closely related to nitrifying bacteria.

Some Form 1C *cbbL* clones of Damariscotta Lake closely matched *cbbL* sequences from terrestrial environments (Table 2.3 and Figure 2.3), with up to 99% identity for some sequences. Two Form 1A *cbbL* genes were also sequenced from Damariscotta Lake. However, they were not phylogenetically related to Form 1A *cbbL* sequences obtained from Lowes Cove sediments, known sulfur oxidizers, or nitrifying bacteria. The closest match in the NCBI database was to *Rhodobacter blasticus*, a H₂/CO-oxidizing chemolithotroph. This indicates that littoral mesotrophic lake sediments contain facultative chemolithotrophs similar to terrestrial environments, but may contain different Form 1A *cbbL* communities.

Statistical analyses of clone libraries indicated similar nucleotide diversity in all populations, suggesting that both intertidal and littoral lake sediments contain diverse chemolithotroph communities. This was supported by average pairwise difference values (Table 2.4). Among site pairwise differences indicated that marine and freshwater sediments were different from each other, however, among site pairwise difference of marine environments indicated similar population structure (Table 2.5).

Wright's fixation index (F_{ST}) indicated that genetic diversity within the Damariscotta Lake *cbbL* clone library was significantly less than the marine and lake library combined (Table 2.5). LIBSHUFF analysis supported distinct chemolithotroph communities among the lake and intertidal sediments (Table 2.6). However, F_{ST} values within Lowes Cove sediment *cbbL* libraries displayed less genetic differentiation. Surface (upper 2mm) and subsurface (5-7 cm) clone libraries showed only moderate genetic differentiation, and *Mya arenaria* burrow wall clones showed little within group genetic variation in comparison to genetic diversity of the groups combined. LIBSHUFF analysis of homologous and heterologous coverages as a function of evolutionary distance ($D=0.0$ to 0.5) indicated similar results for Lowes Cove *cbbL* clone libraries (Table 2.6). Burrow wall *cbbL* clones were not significantly different than surface and subsurface clone libraries. LIBSHUFF results also indicated that marine surface and subsurface environments contained distinct chemolithotroph communities ($P \leq 0.001$). This suggests that while within population marine surface and subsurface *cbbL* genetic diversity is only moderately different than the sediment microhabitats combined, the number of unique sequences as a function of evolutionary distance is greater within libraries than among

them. Furthermore, phylogenetic P-tests indicate that all Lowes Cove marine libraries represent different phylogenetic lineages.

Maximum potential biological oxidation rate assays supported molecular data from Lowes Cove, with thiosulfate oxidation having the highest rate, followed by CO consumption and ammonium oxidation. Interestingly, CO consumption was not significantly different from any other sediment environment (Table 2.2) and was comparable to oxidation rates observed in soils (Tolli and King, 2005). Potential maximum biological uptake rates in Damariscotta Lake, however, were not consistent with molecular data. While *cbbL* sequences indicated dominance of a facultative CO/ H₂-oxidizing community, potential biological consumption rates indicated that thiosulfate oxidation dominated. No sulfur-oxidizing *cbbL* type sequences were obtained from Damariscotta Lake sediments. Possible explanations to this discrepancy include thiosulfate consumption by chemoorganotrophic bacteria or other biological sulfur transferase activity (Alexander and Volini, 1987; Saidu, 2004).

Conclusion

Lowes Cove marine and Damariscotta Lake sediments harbored considerably different chemolithotroph communities. Lowes Cove sediment clone libraries were dominated by Form 1A *cbbL* while the Damariscotta lake clone library contained mainly Form 1C *cbbL*. AMOVA and LIBSHUFF statistical analyses further supported that Lowes Cove and Damariscotta Lake contained distinct chemolithotroph communities.

Chemolithotroph community structure and dynamics within Lowes Cove microhabitats were less clear. Phylogenetic P-tests indicated significant covariation

between Lowes Cove *cbbL* phylogeny and microhabitat community. Meanwhile, among site average pairwise difference values and fixation indices indicated high levels of sequence diversity among clone libraries relative to the diversity of the combined libraries. LIBSHUFF, which analyzes homologous and heterologous coverages as a function of evolutionary distance, suggested that Lowes Cove surface and subsurface libraries differed, while *Mya arenaria* burrow wall clone libraries were not distinct from the other microhabitat clone libraries.

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