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**André Pellerin**

-----  
AUTEUR DE LA THÈSE / AUTHOR OF THESIS

**M.Sc. (Earth Sciences)**

-----  
GRADE / DEGREE

**Department of Earth Sciences**

-----  
FACULTÉ, ÉCOLE, DÉPARTEMENT / FACULTY, SCHOOL, DEPARTMENT

**Endostromatolites: Life in Extreme Environments and Lessons for the Detection of Life on Mars**

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TITRE DE LA THÈSE / TITLE OF THESIS

**Dr. I. Clark**

-----  
DIRECTEUR (DIRECTRICE) DE LA THÈSE / THESIS SUPERVISOR

**Dr. D. Fortin**

-----  
CO-DIRECTEUR (CO-DIRECTRICE) DE LA THÈSE / THESIS CO-SUPERVISOR

**EXAMINATEURS (EXAMINATRICES) DE LA THÈSE / THESIS EXAMINERS**

**Dr. A. Desrochers**

**Dr. M. Smith**

**Gary W. Slater**

-----  
Le Doyen de la Faculté des études supérieures et postdoctorales / Dean of the Faculty of Graduate and Postdoctoral Studies

**Endostromatolites: Life in Extreme Environments and Lessons for the Detection of  
Life on Mars**

**André Pellerin**

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Faculté des études supérieures et postdoctorales  
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## Abstract

Finely laminated carbonate columns – endostromatolites, grow in carbonate rock fissures in permafrost regions. Their formation is thought to be microbially mediated but the processes of accretion are thought to be very slow and possibly intertwined with abiotic mineralization processes. The samples analyzed in this study are from the Haughton impact structure, located on Devon Island, Nunavut in the Canadian high Arctic. The carbon and nitrogen isotopic composition of the endostromatolites, along with the microbial community diversity were determined. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of the organic matter contained within the endostromatolites averaged around -30‰ and 0‰, respectively. Scanning electron microscopy observations revealed the presence of spheroidal calcite and filamentous structures reminiscent of biological activity. Molecular phylogenetic analysis of the endostromatolites and soil samples found in Haughton crater showed that the endostromatolite microbial community is mostly aerobic and chemoheterotrophic, belonging in large part to the Phylum *Actinobacteria* and the subphylum *Alphaproteobacteria*. *Rubrobacter radiotolerans* was the dominant species in the endostromatolites. Soil bacterial communities were more diverse, harboring all the phyla found in the endostromatolites as well as many others which were not encountered in endostromatolites. Understanding the variability of microbial life between specific environments might shed some light on the mechanisms responsible for endostromatolite formation and provide useful data for contrasting abiotic and biotic systems on earth and other planetary bodies, such as Mars.

## Résumé

Les endostromatolites sont des structures sédimentaires finement laminées se formant à l'intérieur de fissures dans les roches calcaires des régions nordiques. Leur origine est encore mal connue, mais certaines études ont émis l'hypothèse qu'ils pourraient être d'origine microbienne. Les échantillons analysés dans ce projet proviennent de l'arctique canadien, soit le cratère Haughton sur l'île Devon au Nunavut. La composition isotopique du carbone et de l'azote a été déterminée, ainsi que la composition de la communauté microbienne. Les valeurs de  $\delta^{13}\text{C}$  et  $\delta^{15}\text{N}$  de la matière organique présente dans les endostromatolites se situent respectivement autour de -30‰ et 0‰. Des observations par microscopie à balayage ont révélé la présence de calcite sphéroïdale et de structures filamenteuses qui ressemblent à des structures d'origine biologique. L'analyse de l'ADN des endostromatolites et des sols adjacents dans le cratère ont démontré que la communauté microbienne des endostromatolites est constituée en majeure partie de microorganismes aérobiques chimiohétérotrophiques. Ces derniers sont classés sous le phylum actinobactéries, ainsi que sous le subphylum alphaprotéobactéries. *Rubrobacter Radiotolerans* était l'espèce dominante des endostromatolites. Dans les sols, les communautés microbiennes étaient similaires à celles des endostromatolites, mais il y avait plus de diversité. En somme, il est important de comprendre la diversité microbienne des endostromatolites car les résultats peuvent nous aider à mieux comprendre les facteurs responsables de leur formation. De plus, cela nous permettra éventuellement de contraster des systèmes biotiques et abiotiques sur terre ainsi que sur d'autres planètes, tel Mars.

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## List of Acronyms

BLAST	Basic Local Alignment Search Tool
BP	Before Present
bp	Base pairs
BIM	Biologically Induced Mineralization
BCM	Biologically Controlled Mineralization
CFU	Coliform Forming Units
DIC	Dissolved Inorganic Carbon
DW	Dry Weight
DNA	Desoxy Nucleic Acid
EDS	Energy Dispersive Spectroscopy
EPS	ExoPolySaccharides
EGTA	Ethylene Glycol Tetraacetic Acid
EDTA	EthyleneDiamineTetraacetic Acid
ESEM	Environmental Scanning Electron Microscopy
LB	Luria-Bertani
LOI	Loss On Ignition
NASA	National Aeronautics and Space Agency
NCBI	National Center for Biotechnology Information
NMR	Nuclear Magnetic Resonance
OMC	Organic Matter Content
OTU	Operational Taxonomic Units
PCR	Polymerase Chain Reaction
RDP	Ribosomal Database Project
rpm	Rounds Per Minute
SD	Standard Deviation
SDS	Sodium Dodecyl Sulfate
SEM	Scanning Electron Microscopy
TBE	Tris Borate EDTA
UQAM	Université du Québec A Montréal
UV	UltraViolet

## 1. General introduction

Endostromatolites are finely laminated carbonate columns or crusts that grow in carbonate rock fissures in permafrost regions. Their formation is thought to be microbially mediated (Clark et al. 2004), just like the microbial laminated structures of marine stromatolites. There are a wide range of carbonate crusts found throughout the world but only a few are thought to be microbially mediated (Lacelle, 2007). Endostromatolites are among them. Studying these potentially biologically mediated crusts is therefore essential, but challenging at the same time. The contributions of bacteria to carbonate mineralization in natural environments are only beginning to be understood. Recently, a gene cluster in *Bacillus subtilis* was associated with biomineralization of carbonates (Barabesi et al., 2007) which may help understand how and why microbes mineralize carbonate. So far, in endostromatolites, the processes of accretion are thought to be very slow (thousands of years) and possibly intertwined with abiotic mineralization processes.

Endostromatolites develop typically in cold and dark environments, understanding their mode of formation might shed some light on the origin of life under extreme conditions and provide useful tools for the search of past biological activity in the Earth's rock record. In addition, the type of cold and dry environments where endostromatolites form occurs on other planetary bodies in the solar system, including Mars. The search for past life on Mars and other habitable planets has been the focus of the National Aeronautics and Space Administration (NASA) for more than a decade now. The NASA astrobiology roadmap (<http://astrobiology.arc.nasa.gov/roadmap/>) clearly indicates that unequivocal biosignatures must be identified in order to fully ascertain the presence of life on other planets. Finding true biosignatures is however not an easy task.

## 2. Literature Review

### 2.1 *Early work on calcareous crusts*

Calcareous environments in permafrost regions are subject to the formation of secondary carbonate precipitates known as calcretes. Most authors have attributed the formation of these precipitates to an intense evaporation of calcite saturated waters (Swett, 1974; Bunting and Christensen, 1978) or to calcite supersaturation provoked by closed system freezing and cryodesiccation of Ca-HCO<sub>3</sub> porewaters (Marlin et al., 1993, Marlin and Dever, 1995). Crusts up to 2 cm in thickness have been studied on Ella Island and Albert Heims Bjoerje in Greenland by Swett (1974), who suggested the possible contribution of freezing to the formation of these crusts as well as the biological activity of algal mats living on the crusts. In the first case, permafrost would prevent percolation of carbonate waters deeper into the ground, enabling precipitation of carbonate crusts while the water evaporated. A thermal gradient was also suggested as a contributing factor along the active layer of the ground where calcite, which is soluble at low temperature, would precipitate on the cobbles on the ground surface where it is warmer than at depth. Bunting and Christensen (1978) added further knowledge of these crusts by affirming that microlaminations were not always present on the crusts and that they could form with or without the presence of algal mats. This led them to conclude that it was the intense evaporation in the summer that supersaturated the waters, allowing the carbonate crusts to precipitate out of solution.

Marlin and Dever (1995) and Marlin et al. (1993) studied secondary carbonate crusts on the peninsula of Brogger in Spitsberg, Greenland). They emphasized the importance of permafrost in the formation of these crusts within an active layer, but also suggested a combination of water evaporation in the summer coupled with freezing up from the permafrost and downward from the atmosphere (in the winter). This scenario creates supersaturated porewaters locked in the middle between the two waves of freezing where carbonate can precipitate out of solution onto the cobbles. With changes in climate, the layer of supersaturated water where precipitation occurs would shift up or



down inside the active layer, accounting for the fact that the carbonate precipitates can be found all over the active layer.

Concretions in fissures of limestone were also studied and characterized near Lake Centrum in northeastern Greenland by Adolphe and Loubière (1987) and Loubière (1987). The authors noticed the presence of calcification in the fissures of bedrock as well as on buried cobbles. The cauliflower shaped concretions were of regular morphology in between samples and showed microlaminations. A brief description of the microstructures, as well as paleoclimatic interpretations followed, but no formation models were suggested. The authors were however able to grow cultures of bacteria in the laboratory from calcrete samples.

A recent review by Lacelle (2007), provides a classification of the various types of cold-climate carbonate precipitates as well as a summary of the isotopic composition of these different deposits. Abiotically precipitated carbonate precipitates are compared to potentially microbially-mediated precipitates (endostromatolites) from an isotopic point of view showing the distinct isotopic signatures of microbially-mediated carbonates precipitates.

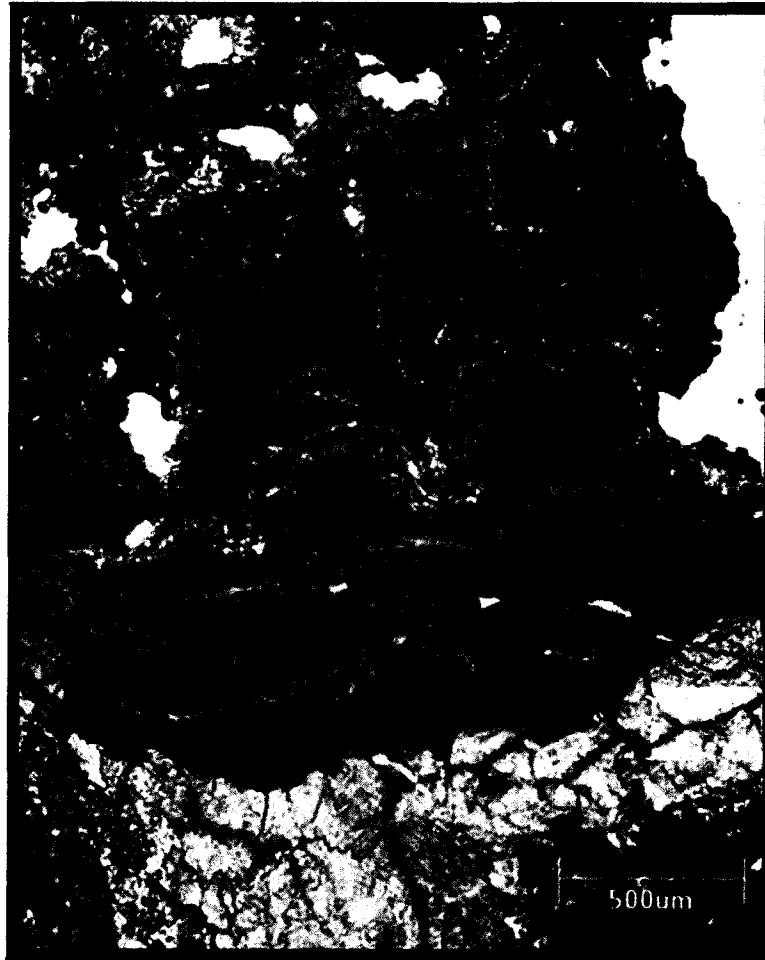
## 2.2 *Endostromatolites and their formation*

Endostromatolites, (also referred to as fissure calcrete by Lauriol and Clark, 1999; Chauret, 1999)) have been extensively studied in the northern Yukon Territory. They are one of many types of secondary carbonate precipitates found in permafrost regions but are the only one with a possible microbially mediated origin (Lacelle 2007). Macroscopically, they consist of finely laminated columns of ~1 mm in diameter and vary from <1mm to greater than 2-3 cm in length (Lauriol and Clark, 1999).



**Figure 2.1:**Endostromatolite forming within a fissure of the dolomitic bedrock of Haughton Crater, Devon Island, Nunavut. The carbonate columns are approximately 5mm in length.

Those structures occur in closed fissures within carbonate rocks located on the south facing side of mountains. The precipitates are always found anchored to the external surface of the fissure, suggesting a thermal process of accretion and nucleation (Lauriol and Clark, 1999). Microscopically, they are finely ( $< \text{mm}$ ) laminated stromatolite-looking structures. They can be less than a millimeter to 3-4 centimeters in height. They have a basal layer  $< 1\text{mm}$  in thickness which quickly segregates into evenly spaced columns. Both the basal layer and the columns have dark bands possibly caused by organic matter staining (Lauriol and Clark, 1999).



**Figure 2.2:** Photomicrograph of Haughton Crater endostromatolite thin section. The bedrock at the bottom is covered with a basal layer from which laminated columns branch out.

The formation of endostromatolites is a slow ongoing process, which can halt for some periods of time. Current  $^{14}\text{C}$  dating of endostromatolites from the Yukon Territory suggests that carbonate accretion occurred between 1 300 and 14 000 BP (Clark et al., 2004). The results coincide with the Holocene epithermal July insolation maximum (Berger and Loutre, 1991) for  $65^{\circ}\text{N}$ , a time when the climate was wetter and warmer than it is today in the Arctic.

The isotopic composition of endostromatolites is distinct from other secondary carbonate features of the Arctic (Lauriol and Clark 1999). They show strong enrichments in  $\delta^{13}\text{C}$  which in some samples, increases towards the summit of the column (Clark et al.

2004). There are only a few processes capable of generating carbonate precipitates with  $\delta^{13}\text{C}$  values  $\geq 0\text{‰}$ . These include:

- i) Cryogenic formation of secondary calcite by rapidly freezing Ca-HCO<sub>3</sub> water (Clark and Lauriol, 1992)
- ii) Equilibrium with atmospheric CO<sub>2</sub> during inorganic precipitation of calcite from water at low temperature (Bottinga, 1968)
- iii) Carbonate precipitation associated with methanogenesis (Clark et al. 2004; Whiticar et al. 1986; Dimitrakopoulos and Muehlenbachs, 1987; Aravena et al., 1995; Lansdown et al. 1992).

Endostromatolites form within the rock mass where thermal fluctuations occur on a scale of days to months and not seconds (Clark et al. 2004). The process of cryogenic freezing (possibility i, above) is therefore not a reliable explanation of the  $\delta^{13}\text{C}$  enrichment.

The second possibility (ii) requires diffusion of CO<sub>2</sub> into wet fissures, and necessitates an evaporative process to concentrate cations to catalyze carbonate precipitation. This process is plausible in polar desert environments, especially since it is known that in these dry permafrost environments, desiccation and evaporative water loss are significant and have even been documented to be a limiting factor for algal growth in Sandstones of Antarctica (Kennedy, 1993). Further evidence for this theory is proposed by Ghaleb et al., (1997) who determined U/Th ratios of many endostromatolite samples. They discovered that important uranium concentrations were present, suggesting a non-reducing environment of formation, evidence corroborated by the work performed by Chauret, (1999) who compared the Fe/Mn ratios of the calcites. The Fe/Mn ratio can provide information regarding the oxidation conditions during precipitation of calcite because Mn<sup>2+</sup> and Fe<sup>2+</sup> ions tend to precipitate as substitute ions of Ca<sup>2+</sup> in calcite. The proportion of each is controlled by the oxidation potential of the deposition environment (McLane, 1995, Barnaby and Rimstidt, 1989) In fact, since Fe and Mn have different solubilities at different pH, their concentrations in calcite provides information about the precipitation environment. Increasing substitution for Ca<sup>2+</sup> in calcite by Mn<sup>2+</sup> increases

cathodo luminescence and occurs progressively as more soluble ions are put in solution by the increasing reducing conditions. This phenomenon is however overshadowed by Fe solubilization which also replaces Ca ions in calcites and causes dark luminescence. Chauret (1999) investigated the luminescent properties of calcites in endostromatolites from the Yukon Territory and concluded that the minerals had either no luminescence which would indicate oxic conditions of formation or dark luminescence meaning that the luminescence was repressed by the amount of Fe substitutions in the calcite which would indicate more reducing environments of formation. Based on the above, the second theory remains a plausible cause for the formation of endostromatolites.

The final process capable of enriching  $\delta^{13}\text{C}$  requires an anoxic environment and methanogenic bacteria to enrich the  $\delta^{13}\text{C}$  of the inorganic DIC reservoir. The environmental conditions necessary for this to take place may have been present in the Arctic during the Holocene where the climate in the high Arctic was warmer and wetter than it is today. These conditions in fact would provide the necessary amounts of water and temperature for methanogenic or acetogenic microorganisms to grow. This would engender the subsequent calcite precipitation as a byproduct of the biological processes taking place in the fissures. Two very different processes are therefore capable of generating the isotopic signatures observed in endostromatolites. Some studies argue for an aerobic (possibly abiotic or biotic) process (LeDruillenec, 2003) while others suggest an anaerobic, fully aqueous and biotic formation (Clark et al., 2004, Marschner, 2007). While some evidence points towards the second process, the debate remains open.

## 2.3 *Tools to investigate for evidence of past and present life*

### 2.3.1 The link between carbonates and the evidence for life on Mars

The environmental conditions necessary to fully simulate a Martian surface are inexistent on earth. Mars is and has been for quite some time a cold, dry place with no ozone layer to protect its surface from life-threatening UV and cosmic rays. Furthermore, the lack of significant greenhouse gases in the Martian atmosphere causes extreme temperature fluctuations between day and night, not to mention a series of other chemical

and physical life-limiting parameters (ex: water boils at 10°C). When trying to compare and find the closest possible match on Earth to what Mars would resemble, no one site can successfully replicate conditions on the red planet. Some aspects from different earthly environments can however accurately resemble what can be expected of Mars. Geological materials and their environments of formation that are known to have been investigated for their analogue potential include (but are not limited to) cemented lava Breccias (Treiman et al., 2002), microbialites (Laval et al., 2000), cave environments (Leveille et al. 2007, Barton et al. 2006) and Martian meteorites containing carbonate nodules found on earth (McKay et al., 1996). The reason for using carbonates as a focal point of research for extraterrestrial life/exobiology is primarily that carbonate deposits are often associated with water since they can be the product of precipitation from an aqueous environment. Since there is little to no water on the Martian surface, the residual carbonates are a good place to start looking for past (or present) life.

Mineral crusts are strong candidates in the search for evidence of life, both in terrestrial extreme environments and during planetary exploration (Parnell et al. 2006). The major reasons enumerated by the same authors are the following:

- (1) The growth of any mineral precipitate implies an active flow of water to transport the solutes for mineral growth. Water is a vector for life.
- (2) The precipitation of mineral from water also implies that the water has a high load of dissolved ions, which should include nutrients for living matter.
- (3) As the mineral precipitates, the growing crystals can entrap, and thereby preserve, organic matter. This can range from different types of biomolecules to whole cells with morphological form, i.e. the evidence entrapped can be both chemical and physical.
- (4) Mineral precipitates can also incorporate an inorganic record of life, including microbially mediated crystal growths, and isotopic evidence for microbial metabolism.
- (5) In addition to entrapment of ambient life, mineral precipitation may create a new microenvironment (microporosity) for an active biota.

In the particular case of Mars:

(6) The philosophy of searching for evidence of life on Mars has been 'follow the water'. Any surface crusts on Mars are likely to represent the most recent mineral precipitation from water.

(7) We already have evidence of crust formation in Martian soil through precipitation of sulphate salts.

(8) Observations of fog/frost in several contexts on Mars suggest that mineral dissolution and re-precipitation on a micro-scale could be still occurring in some surface crusts.

The impact crater environment, widespread on Mars, is an especially favorable case for crust development:

(9) Active circulation of water follows the impact event due to hydrothermal activity.

(10) Ponding of water may occur temporarily in the crater depression.

(11) The impact detritus presents a substantial surface area for mineral dissolution, to create saturated ground waters which can re-precipitate as crusts.

(12) The anomalous heating in a crater allows chemical reactions (including dissolution, re-precipitation), to proceed at a faster rate than ambient, as well as favoring any organic metabolism.

Organic matter is not evidence of life, but heterotrophs require it for their metabolism. Therefore if we are ever to find life in other contexts than the earth itself, as has been demonstrated by close analysis of the Martian surface, it will be very small. Since it is possible that microorganisms could survive long interstellar trips (Mastrapa et al., 2001), it may be that the universe is teeming with microscopic life just waiting for the correct conditions to flourish. Life on barren worlds is most likely opportunistic. An opportunist usually requires such adaptative abilities as quick growth. Given the now much recognized abundance of abiotically derived organic carbon available throughout the universe (e.g. Carbonaceous chondrites), it is all the more likely that opportunistic, quick growing organisms utilizing heterotrophic metabolism be the first "extraterrestrial" life discovered. The endostromatolites growing within carbonate fissures in the arctic are a good analogue of this because, as this research shows, most bacteria are heterotrophic opportunist microorganisms that have a readily available reservoir of organic matter at

their disposal (the hydrocarbon inclusions within the rock). In order to access them they need to dissolve the rock.

### 2.3.2 Biomarkers

The subsurface of other planets and rocky bodies will be a biological and geological target of future research and missions in the search for life beyond Earth (Boston et al., 1992; Boston 2000a; Hofmann, 2007) Byproducts of life (e.g. biominerals, biologically fractionated isotopic signatures and anomalous concentrations of elements) are difficult to interpret (Boston et al. 2001) when varying degrees of preservation and post-fossilization alteration processes interfere with the analytical techniques. The detection of biomarkers is at the forefront of the search for extraterrestrial life. Endostromatolites offer a novel testing ground for the detection and interpretation of these since they are believed to be biogenic in origin, but direct evidence of this has yet to be supported. The investigation of biomarkers can be grouped in two categories I) detection of known organic compounds such as lipids, nucleic acids, amino acids etc. and II) inorganic signs of life, which includes biogenic minerals and their spatial arrangements, distribution of trace metals and mineral dissolution patterns related to microbial presence as well as microfossils and isotopic signatures (Douglas, 2004).

A wide range of research is currently being undertaken in order to develop techniques and knowledge of biomarkers and how to interpret them. Boston et al. (2001) suggested that when considering whether a given lithic or mineral structure or texture is the result of biological activity, three questions must be addressed: (1) Is the material “alive” at present? That is, does it contain active organisms interacting with their environment or other evidence of biological activity? (2) Is the preserved material the actual or merely perceived successor to the previously living (or in some cases, still living) microbial community? (3) How is the living material and its products and effects altered to produce the observed structural, geochemical and isotopic biosignatures? A wide range of tools are available to researchers in order to answer these three questions. Visual interpretation with the use of environmental scanning electron microscopy

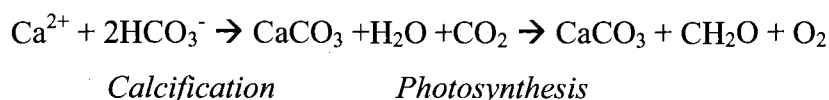


(ESEM) coupled with Energy dispersive spectroscopy (EDS) for the detection of biominerals, and changes in mineral patterns associated with biological activity have been useful in detecting biominerals on mineral precipitates such as evaporitic gypsum crusts (Douglas, 2004), hot spring precipitates (Walker et al. 2005), endostromatolites (Clark et al. 2004, Chauret 1999,) and a wide range of other materials. A panoply of other methods employed by researchers for detecting arcane life are used but it is beyond the scope of this study to enumerate them all. One may find very useful literature and references on this subject in Douglas et al., (2004) and L veill  et al., (2007). Both of these authors have made significant contributions in the search for detecting life in unexpected places, the first focusing on biomineral formation using ESEM and working on raw materials sampled from hot desert environments and the second focusing on the isotopic signatures of the detection for life in Arctic climates.

### 2.3.3 Biominerals

Among bacterially precipitated minerals, carbonates, and in particular calcium carbonate ( $\text{CaCO}_3$ ), are probably the most important (Ferris et al. 1988). Synthesis of minerals from bacteria can be grouped in two modes: Biologically induced mineralization (BIM) and biologically controlled mineralization (BCM), (Lowenstam and Weiner, 1989). In BCM, the organism controls the process to a high degree and it is carried out predominantly by tissue-forming multicellular eukaryotes and leads to the production of complex and specialized structures, such as shells, teeth, and skeletons (Barabesi et al. 2007). Minerals that result from BIM processes generally nucleate and grow extracellularly as a result of metabolic activity of the organism and subsequent chemical reactions involving metabolic byproducts (Bazylnski and Frankel 2003). BIM is presumably an unintended and uncontrolled consequence of metabolic activity. The nucleation of a mineral, “results initially from the neutralization of chemically reactive sites on the cell, and proceeds via nucleation of additional metal ions with the initially sorbed metals” (Southam 2000). S layers, which are very common in Archaea, are paracrystalline cell surface assemblages composed of protein or glycoprotein that self assemble and associate with the underlying wall through non-covalent interaction (Koval

1988). pH increase can lead to the precipitation of carbonate minerals on the S layer of bacteria (Fortin and Beveridge 2000). This is of particular interest to our study of endostromatolites because calcite precipitates make up the matrix of those structures. Microbiologically-induced  $\text{CaCO}_3$  precipitation is recognized as a far more complicated process than chemically-induced precipitation (Stocks-Fischer et al., 1999). The bacterial cell surface can nonspecifically induce mineral deposition by providing nucleation sites (Ferris et al. 1986, 1987). Given the fact that  $\text{Ca}^{2+}$  is not utilized by bacteria through metabolic activity, it can therefore accumulate outside the cell (Silver, 1975). Precipitation of calcite on the S layer has however mostly been observed in light harnessing Cyanobacteria (Fortin and Beveridge 2000). The general reactions thought to be taking place are (Erez 2003):



By consuming  $\text{CO}_2$  for photosynthesis, the Cyanobacteria create a disequilibrium which permits the precipitation of calcium carbonate from the seawater. Aerobic and anaerobic oxidation of carbon compounds consisting of carbon and hydrogen without oxygen has also been demonstrated to occur in a well-buffered neutral or alkaline environment containing adequate amounts of calcium or other appropriate cations (Krumbein, 1977). The same author indicated that at least some of the  $\text{CO}_2$  that is generated can be transformed into carbonate, which can then precipitate with appropriate cations. This was illustrated by the formation of aragonite and other calcium carbonates by bacteria and fungi in seawater media containing organic matter at concentrations of 0.01 and 0.1% (Krumbein, 1977). Microorganisms have been observed to accelerate the mineral dissolution rate through production of simple acids (Bennett et al., 2001). Additionally extracellular polysaccharides (EPS) in microbial biofilms can accelerate or retard dissolution rates. These polymers may also act to stabilize secondary mineral precipitation with contaminant metal species (McNamara et al., 2006)

In terrestrial hypogean environments, Braissant et al. (2004) stressed that relationships between morphologies and mineralogies of  $\text{CaCO}_3$  encountered in soils and surficial sediments can reveal a bacterial influence related to microbial biofilms, especially when taking into account the role of a specific exopolysaccharide (xanthan EPS) and amino acids on calcium carbonate crystallization. They concluded that spherulitic calcite and/or vaterite crystals may indicate the presence of mucilaginous bacteria or biofilms at the time of formation, a phenomenon corroborated by Sanchez-Moral et al. (2003) who observed spheroidal bodies associated with heterotrophic bacterial biofilms inside Saint Callixtus Catacombs, a hypogean environment. Sanchez-Moral et al. (2003) also went on to conclude that small quantities of  $\text{CO}_2$  must be consumed by heterotrophic microorganisms in order to explain the physicochemical modification of intergranular fluids that lead to the creation of supersaturation conditions suitable for neoformation of metastable minerals.

#### 2.3.4 Molecular phylogenetic analysis

Molecular phylogenetic analysis can be useful in determining the presence of life in a given sample if the material in question has allowed preservation of the DNA. Today, the accepted maximum ages for DNA survival are between 100 Kyr and 1 Myr on the basis of both theoretical and empirical data (Hebsgaard et al. 2005).

Like rocks found on other planets, target DNA in secondary carbonate precipitates will be extremely low to non-detectable. Especially in high calcium environments, the low DNA values makes conventional DNA extraction methods unsuccessful due to the interactions of  $\text{Ca}^{2+}$  cations with the slightly negative charge of DNA (Barton et al., 2006). However, the same authors have developed an extraction protocol for low-biomass calcareous cave environments. In order to reduce the binding of calcium ions, Barton et al. (2006) found that using a phenol:chloroform extraction helped reduce the binding of calcium. They use a mix of EGTA and EDTA to chelate the calcium, followed by the addition of a DNA carrier (Poly dIdC) and a dialysis step to remove calcium. This important step is crucial because calcium not only binds strongly to DNA but it also

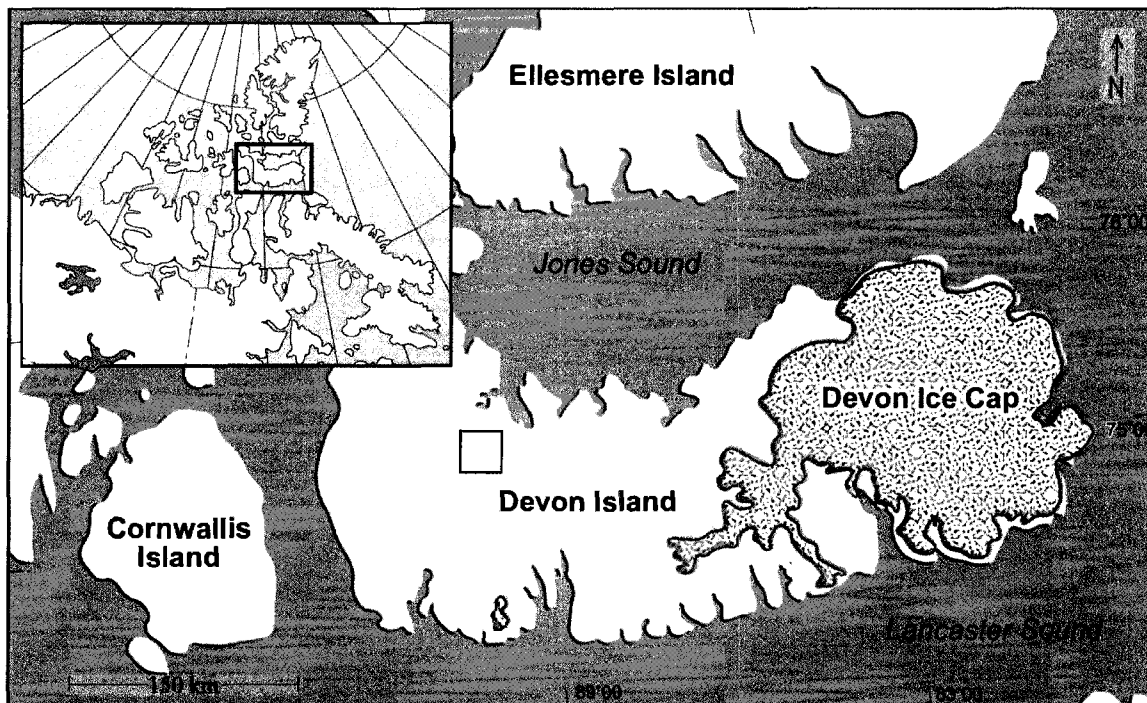
inhibits subsequent Polymerase Chain Reaction (PCR), because it is a known (PCR) inhibitor (Powell et al., 1994, Bickley et al., 1996). These innovations however come at a price in terms of cost of extraction, extraction time and additional extraction steps. Every additional step increases the likelihood of contamination. For instance, the addition of poly dIdC increases the probability of contamination by bacteria since the raw material cannot be completely purified by the manufacturer and must be UV irradiated prior to use and negative controls must be followed to ensure the UV irradiation was successful in destroying all the microbes.

PCR-based community fingerprinting also has its share of biases, as with any other methods. This includes inefficient cell lysis during DNA extraction (Krsek and Wellington, 1999) and preferential and selective amplification of 16S rDNA fragments (Polz and Cavanaugh, 1998). Furthermore, the use of general bacterial primers does not fully guarantee the amplification of every single 16S rDNA strand, leading to an underestimation of the bacterial diversity, although primers are designed to anneal at stable points along the DNA molecules.

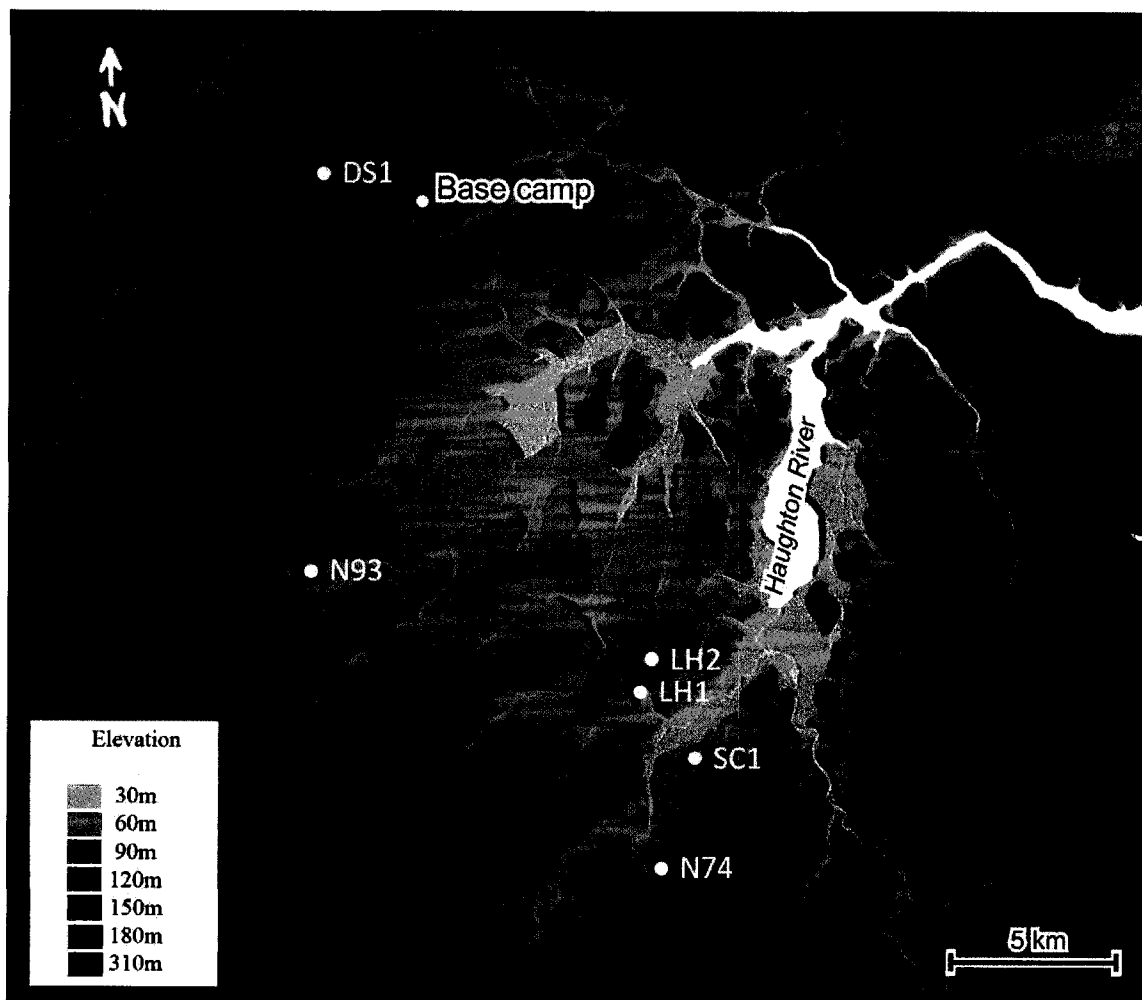
## 2.4 *Study sites*

### 2.4.1 Haughton Crater

The site for this research is the Haughton impact structure, located on Devon Island, Nunavut in the Canadian high arctic. The Haughton impact structure is located at 75°22' N, 89° 41'W on Devon Island

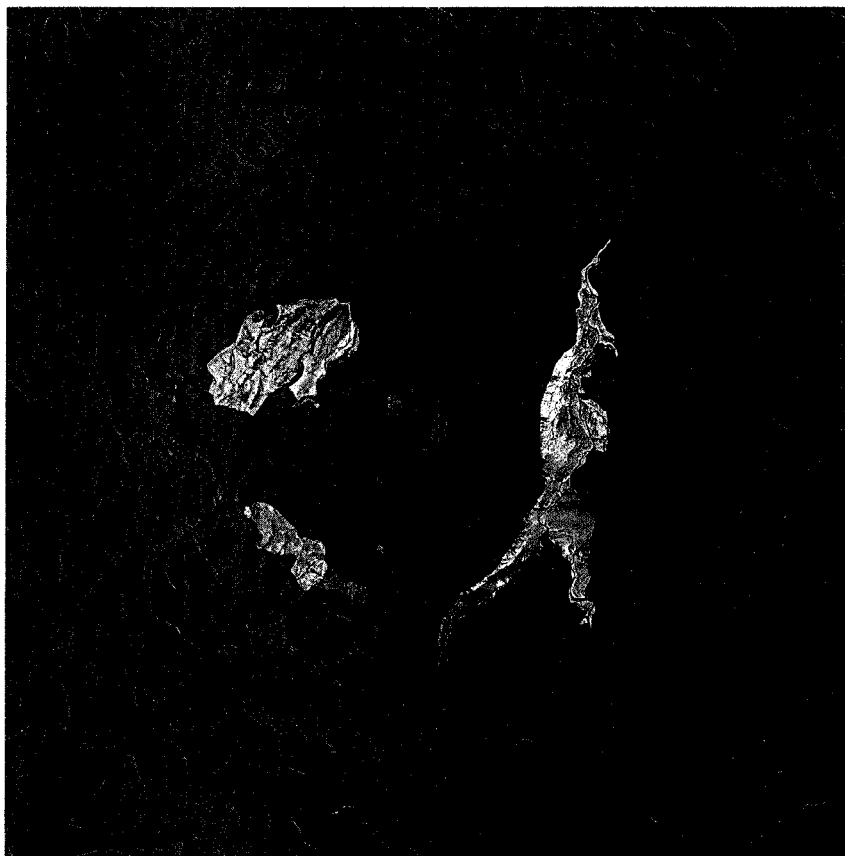


**Figure 2.3:** Map of Devon Island in the Canadian arctic. The square indicates the location of Houghton Crater. The higher resolution of the square in northwestern Devon Island is available in figure 1.4.



**Figure 2.4:** Map of Haughton Crater showing the topography. Base camp is located to the North of the crater while the crater itself is shown by lighter colors due to lower elevation and is cut by the Haughton River. Locations where samples were taken are also shown. Exact GPS locations are available in Appendix 11.

**GEOLOGICAL UNITS**  
*after: Frisch and Thorsteinsson (1978);  
 Ferling (1988); with modifications*  
 ON  
**AERIAL PHOTOMOSAIC**



**Figure 2.5** : Geological map of Haughton Crater on aerial photomosaic showing the principal geological units. White units are recent alluvial deposits. Image produced by V. L. Sharpton.

[http://www.lpi.usra.edu/publications/slidesets/craters/images/20\\_HAUGHTON.JPG](http://www.lpi.usra.edu/publications/slidesets/craters/images/20_HAUGHTON.JPG)

It was created by an asteroid impact 39 +/- 2 Ma ago (Sherlock et al. 2005). The geology of the impacted area consists of lower Paleozoic sedimentary rocks overlain by Mesozoic and Tertiary sediments (Frisch & Thorsteinsson, 1978). The impact at Haughton occurred in dolomitized carbonate rocks of the Allen Bay formation (Ordovician and Silurian age) containing intergranular solid bitumen, and liquid hydrocarbons within fluid inclusions (Parnell et al., 2004). Oil inclusions have also been recorded in similar-aged rocks located 180 km to the west on Truro Island (Stasiuk and Fowler, 1994). The hydrocarbon generation probably occurred from Lower Palaeozoic source rocks during the Late Paleozoic (Gentzis et al., 1996), before the Eocene impact event. The fossil component (preserved in the dolomites) represents conventional oil that was generated from Lower palaeozoic marine source material (Parnell et al. 2007). Inside the crater, exposed are mostly carbonate breccias but are also found Precambrian metamorphic

shocked gneiss likely brought up from lower units by the impact event. The impact structure is approximately 24km in diameter (Grieve, 1988).

Haughton Crater and its surroundings, resembles the surface of Mars. Its annual mean precipitation is (139.6 mm) (Lim and Douglas, 2003), (considered a polar desert), its mean annual temperature is -17°C (although it can go down to -58°C in the winter and +20°C in the summer) and it is subject to nearly 3 months of constant UV irradiation by the sun. The crater lies in an arid polar desert with less than 5% vegetation cover (Cockell et al. 2001). The summer growing season is short, i.e., 188 degree-days in July (Gold, 1988). Biologically, the rocks have been known to support, on the edges of fissures, mosses and lichens (Cockell et al. 2001) most likely deriving humidity from the fissures. Additional observations have shown that the rocks also contain endolithic and cryptoendolithic bacteria (Cockell et al. 2005). Shocked gneiss and breccia from the deposits inside the crater have been studied by Fike et al. (2003) through culturing and 16S rDNA isolation. The authors showed that the rocks harbour many types of bacteria, notably *Arthrobacter*, *Pseudomonas*, *Bacillus*, *Stenotrophomonas*, *Planococcus*, *Caulobacter* and *Janthinobacter*. Soils are primarily dolomitic and oligotrophic (Bliss et al. 1994) and bacterial abundance in the soil is 3-4 x 10<sup>6</sup> colony-forming units (CFU) per gram of dry weight of soil (Cockell et al. 2001) very low if compared to a normal temperate soil.

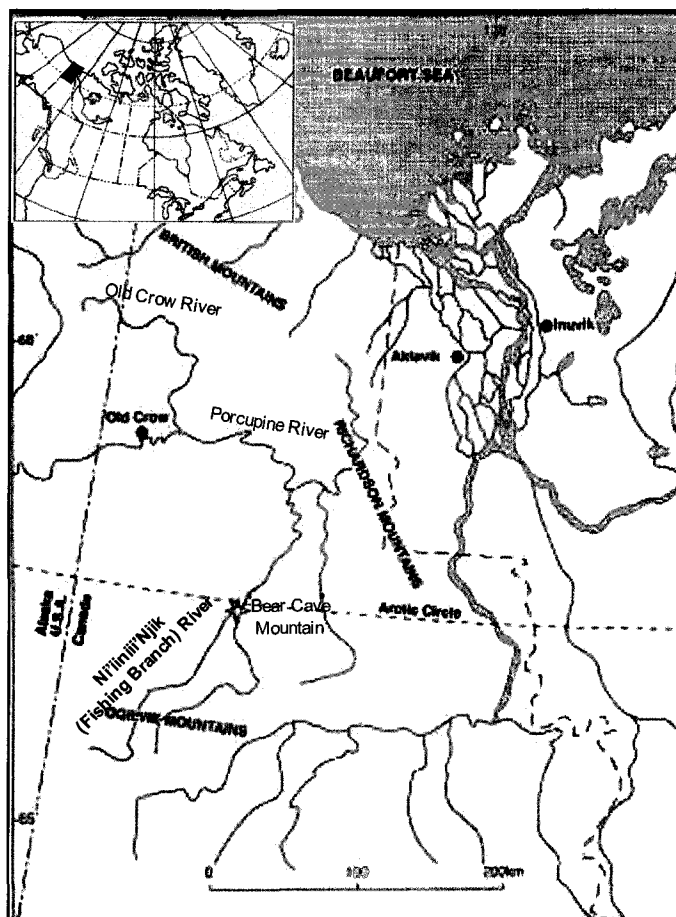
Haughton Crater is currently used in the spring and summer as a Mars surface analogue site by various researchers from different organizations including NASA, the Mars Institute, the Canadian Space Agency and a number of Canadian, American and European universities because of its intrinsic resemblance to the Martian surface. Over the years, Haughton Crater has been extensively studied from a geological point of view. The bedrock is completely mapped and the pre-impact and post-impact geological processes that have occurred are well documented (Osinski and Spray, 2005, Osinski et al., 2005 and others). Erosion processes are also well documented (Osinski and Spray, 2001, Parnell et al. 2004).



The most interesting aspect of Haughton Crater is that it hosts secondary carbonate precipitates in cryogenic crusts, which represent active layer crusts and endostromatolites. The origin and formation of these endostromatolites are the focus of this study.

#### 2.4.2 Yukon Territory

Previous publications on endostromatolites have focused on study sites in the Yukon Territory of Canada (Ghaleb et al., 1997, Clark et al., 2004, Lauriol and Clark, 1999). Endostromatolite samples were collected at these sites in order to provide additional samples for comparison purposes. The site where endostromatolites were sampled was Bear Cave Mountain which is geographically located at 66°25'N and 139°20'W. The closest weather station is located at Old Crow roughly 120 km to the North West and has average temperatures of -8.9°C and receives on average 265 mm of precipitation (Calculated from Environment Canada, 2003). The area is lined with continuous permafrost (90-100% permafrost). The area geology is dominated by folded and faulted Cambrian and Devonian carbonates and Cretaceous siliciclastics (Norris, 1984). The Bear Cave mountain where the samples were taken consists of a tightly folded anticline.



**Figure 2.6:** Location of Bear Cave mountain within northern Yukon, Canada. (Modified from Lauriol and Clark, 1998, with inset map from Norris, 1984)

There are some sharp differences between the climate in the Yukon Territory and Haughton Crater, namely that Haughton Crater is much colder and drier. There is also a significant amount of overburden vegetation in the Yukon Territory that is absent from the site in Haughton Crater which could significantly affect the mechanisms of formation of such precipitates.

## 2.5 Objectives and hypotheses

The first objective of this study is to determine the microbial communities living within endostromatolites. This will be done by means of molecular phylogenetic analysis of the microbial community. The finding of an aerobic-dominated community will suggest that endostromatolites are formed in an oxygen-rich environment. If the

community is anaerobic-dominated, then most likely, the living conditions were anaerobic. It is also hypothesized that if the microbial community is dominated by aerobic microorganisms, the microbial community should resemble that of other aerobic endolithic environments found in close proximity throughout the study site, such as adjacent soils and sands. If on the other hand an oscillatory system is present, producing seasonally anoxic and aerobic conditions by the saturation of the fissures followed by draining and prevalence of oxic conditions, it could be expected to find both aerobic and anaerobic microorganisms within the fissures.

The second objective of the study is to determine if the formation of endostromatolites results from abiotic or biotic processes. This will be achieved by reconciling the isotopic and chemical analyses with the microbial community results. The presence of aerobic microbial communities will not rule out abiotic precipitation of carbonates because abiotically-controlled carbonate precipitation can compete with the microbially-mediated processes or be provoked by it through the consumption of  $\text{CO}_2$ . The presence of a mainly anaerobic microbial community would on the other hand, indicate anaerobic conditions within the fissures and strongly suggest that the microbial communities contributed to the formation of biogenic carbonates, as hypothesized by Clark et al. (2004) and Marschner (2007) because no known abiotic processes can produce the isotopic signatures corresponding to the ones observed in endostromatolite calcite under an anoxic water-saturated environment.

### 3. Methods

#### 3.1 *Field sampling methods*

Endostromatolites and soils were sampled in Houghton Crater, Devon Island, Nunavut at strategic locations on the outer rim and inside the impact structure at sites where they were available and accessible. Yukon samples were selected in the same manner. Each sample that was selected for DNA phylogenetic analysis was sectioned using clean instruments (70% ethanol-washed) and sterilized gloves were used to handle the rocks. The samples were wrapped in sterile foil paper, labeled and enclosed in sterile plastic bags to prevent contamination during transport. Although keeping the rocks frozen for transportation was not possible, they were frozen as soon as they arrived at the University of Ottawa. Other endostromatolite samples selected for stable isotope analysis, loss on ignition, nuclear magnetic resonance and thin sectioning were simply sectioned, labeled and put in plastic bags for transportation to the laboratory.

#### 3.2 *Organic matter content*

Organic matter content (OMC) was determined by Loss On Ignition (LOI). Approximately 3.0g of sample were sectioned using a hammer and a chisel. The sectioned sample was pulverized in a mortar and pestle and transferred to an acid washed glass vial which was placed at 105°C overnight to evaporate all moisture. The samples were allowed to cool in a desiccator to prevent re-hydration. Approximately two grams of the samples were put in an acid-washed, dry, ceramic crucible of known dry weight. The exact weight of the ceramic crucible and sample was then recorded and transferred to an oven at 550°C for 4 hours. The crucibles were covered with ceramic covers to prevent loss of mass by explosive combustion. After 4 hours, the samples were taken out of the oven and allowed to cool in a desiccator and care was taken not to handle the crucibles with bare hands to avoid contamination of the surface with oils and contaminants from hand contact which could introduce a significant error. The combined exact weight of the

crucible and sample was recorded again. The loss of mass, which corresponds to the loss of organic matter by oxidation at 550°C was then calculated according to formula [1].

$$\text{LOI}_{550} = [(\text{DW}_{105} - \text{DW}_{550}) / \text{DW}_{105}] \times 100 \quad [1]$$

Where LOI is the loss in ignition and DW, the dry weight.

Carbonate content of the samples was also determined by loss on ignition using the same protocol, but the combustion temperature was raised 950°C. The  $\text{LOI}_{950}$  was calculated as follows:

$$\text{LOI}_{950} = [(\text{DW}_{550} - \text{DW}_{950}) / \text{DW}_{105}] \times 100 \quad [2]$$

In order to determine the carbonate content, the assumption must be made that only calcium carbonate is present in the sample. The  $\text{LOI}_{950}$  value is multiplied by 2.27 to obtain the weight of calcium carbonate in the sample.

### 3.3 *Sample preparation for organic $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in rocks with high carbonate content*

Two to three grams of sample were sectioned using a hammer and a chisel. In order to concentrate the organic matter and to prevent interferences and clogging of the instruments, carbonates were eliminated from the samples. In order to do so, samples were soaked in 10% HCl until the dissolution reaction completely stopped after adding concentrated HCl to the sample. The samples were then centrifuged in 50mL falcon tubes at maximum speed (2500 rpm) to precipitate the organic matter. The supernatant was discarded and the organic matter pellet was washed with deionized water and centrifuged again at maximum speed. This step was repeated once more and the pellet was transferred to a Petri plate to dry at 50°C overnight. Samples were collected and weighed in tin capsules on a microbalance and submitted to the G.G. Hatch Stable isotope laboratory at the University of Ottawa to determine the carbon and nitrogen content of the

organic residue. Using the elemental analysis results, an exact amount of organic residue corresponding to 100ug of nitrogen was weighed into tin capsules and the samples were submitted for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  analysis at the same laboratory. The isotopic composition was determined by the analysis of  $\text{CO}_2$  and  $\text{N}_2$  produced by combustion on a CE 1110 Elemental Analyzer followed by gas chromatograph separation and on-line analysis by continuous-flow with a DeltaPlus Advantage isotope ratio mass spectrometer coupled with a ConFlo III carrying a routine precision of 0.20‰.

### 3.4 $^{13}\text{C}$ Nuclear Magnetic Resonance

In order to gather enough organic matter for  $^{13}\text{C}$  NMR, approximately 100g of endostromatolite was sectioned from the bedrock using a chisel and a hammer. The carbonate was eliminated by soaking in 10% HCl until complete dissolution of all carbonates (usually overnight). To ensure that no carbonate remained in the sample, concentrated HCl was also added and if no reaction occurred, the sample was deemed carbonate-free. The organic matter was collected from the liquid phase by centrifugation in 50mL falcon tubes at 2500 rpm for 15 minutes. The supernatant was discarded and the organic matter pellet was washed with 50mL deionized water and the centrifugation step was repeated twice more to remove all traces of calcium from the organic residue. The organic matter was collected in Petri dishes and dried at 50°C overnight. Samples were submitted to Glen Facey at the University of Ottawa NMR facility. Approximately 2g of sample was compacted into a capsule and analyzed on an Avance 500 with 5mm X/H/P triple resonance broadband probe.

### 3.5 Scanning Electron Microscopy (SEM)

To examine the internal growth structures and micro-morphologies of the endostromatolites, some of the deposits were thin sectioned. Others were left unprepared to observe structures that could be destroyed by the preparation step. The samples were then examined with a scanning electron microscope (SEM JEOL 6400) equipped with an energy dispersion spectrometer (EDS) at the Université du Québec à Montréal (UQAM).

Prior to the examination under SEM, some of the samples were pre-cleaned with a weak acid (2% HCl) to remove undesirable residues and mounted onto an aluminum stub using doubled-sided carbon tape and then sputter-coated with gold for 60 seconds.

### 3.6 *DNA Fingerprinting methods*

Given the fact that molecular phylogenetic analysis (DNA) of bacterial communities in endostromatolites had never been undertaken before, specific extraction techniques were used to cope with the constraints imposed by the high carbonate content of the endostromatolites. The DNA from the endostromatolites was extracted using an adapted version of the protocol developed by Barton et al. (2006) for low-biomass carbonate cave environments. Only the methods that generated positive results are explained in this section. All the methods that were attempted and failed to produce results are provided in appendix 1.

#### 3.6.1 DNA extraction from carbonate rocks

Under sterile conditions (i.e., sterile tools and counter), approximately 0.5 g of rock was subsampled and crushed in a baked mortar and pestle. The rock powder was immediately transferred to a 2.0ml Eppendorf safe lock microcentrifuge tube and all subsequent extraction steps were performed under a laminar flow hood with UV decontamination lights (Microzone #BM4-2A-49).

The following chemicals were added to the subsample rock powder: 500  $\mu$ l of 2x buffer AE (200mM Tris, (pH 8.0), 50 mM EDTA, (pH 8.0), 300 mM EGTA, (pH 8.0), 200 mM NaCl), along with 30  $\mu$ l of 10mg/ml lysozyme and 5  $\mu$ g of poly-dIdC . The tube was capped and incubated in a water bath at 37°C for 30 minutes and shaken every 10 minutes. After incubation, 6  $\mu$ l of 20 mg/ml of Proteinase K and 15  $\mu$ l of 20% SDS (0.3% wt/vol) were injected in the tube. The tube was then incubated in a water bath at 50°C for 30 minutes. Subsequently, 236  $\mu$ l of 20% SDS (5% wt/vol) and zirconium beads were added to the tube. The sample was then disrupted on a Retsch MM301 bead beater at low

speed for 2 min and high speed for 30 s in the presence of 50% (vol/vol) phenol-chloroform-isoamyl alcohol (25:24:1). The tube was then centrifuged to separate the two phases for a period of 2 minutes using a benchtop centrifuge at medium speed. After the centrifugation step, the supernatant was collected (~700  $\mu$ l) and extracted with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) into a new tube. The new tube was then centrifuged to separate the two phases, for 2 minutes at medium speed. The supernatant was then removed and 20  $\mu$ l of poly-dIdC stock solution was added. The nucleic acids were precipitated by bringing the concentration of Na-acetate to 0.3 M with 3 M Na-acetate and the addition of 2 volumes of 100% EtOH to the mixture. The mixture was then centrifuged at 13,000 x g for 20-25 minutes at 4°C. The ethanol was then poured out without disturbing the DNA pellet, which was then washed with 30  $\mu$ l of 70% ethanol, air dried in a laminar flow hood and re-suspended in 50  $\mu$ l of Hypure molecular biology grade water (Hyclone #ARH26826). The DNA was then placed in a Slide-A-Lyzer Mini Dialysis unit (Pierce Biosystems #69550) and dialyzed against 100 ml of sterile 20 mM EGTA for more than 4 hrs (usually overnight) at 4°C. The volume of sample generally increased by 3-5 fold during the dialysis step, therefore the DNA was concentrated again by precipitation with 0.3 M sodium acetate and 2 volumes of ethanol followed by centrifugation at 13 000 x g. The final DNA product was washed in 70% ethanol, air dried in a laminar flow hood and re-suspended in 50  $\mu$ l of molecular biology grade water. The DNA extract was then stored at -20°C until the next extraction steps.

### 3.6.2 Polymerase Chain Reaction from extraction product

PCR reactions were carried out in a total volume of 25  $\mu$ l using Qiagen Core PCR Kit with Taq DNA polymerase (# 201223). All PCR reactions were prepared under laminar flow hood using aerosol resistant pipette tips. In order to amplify bacteria, we used the general bacterial primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 805R (5'-GAC TAC CAG GTT ATC TAA T-3') (Invitrogen Life Technologies), known to have successfully amplified bacterial 16S rDNA from cave environments (Barton et al. 2006). To amplify archaea, we used the general archaea primers ARCH 344F (5'-ACG GGG TGC AGG CGC GA-3') and ARCH 915R (5'-GTG CTC CCC CGC AAT TCC T-



3') (Invitrogen Life Technologies), known to have successfully amplified Archaea from lakes in Antarctica (Karr et al., 2006). PCR reactions were carried out on an Eppendorf epgradient Mastercycler thermal cycler. In order to amplify for bacteria, the program cycle was set at 94°C for 4 min of initial denaturation followed by thirty cycles of 94°C 45s, 55°C 1 min, 72°C 2 min and a final extension at 72°C for 10 min. For Archaea amplification, the cycling program was 94°C for 15 sec of initial denaturation followed by thirty cycles of 94°C 15 s, 54°C, 20s, 72°C 20s and a final extension period of 1 min at 72°C. PCR products were then checked by Agarose gel electrophoresis with Eithidium Bromide staining and visualized in a Gel DOC-IT Ultraviolet Transilluminator (BioImaging Systems). The sample were then kept at 4°C or frozen at -20°C for subsequent analysis.

### 3.6.3 Cloning PCR product into *E. coli* vector

The PCR product was subsequently cloned using Invitrogen Life Technologies TOPO TA Cloning® kit with pCR® 2.1-TOPO® vector (#1393286) with chemically competent cells. Specific details of the material and methods used are available in the InVitrogen TOPO TA cloning® kit manual. Very briefly, fresh PCR product, (less than 24 hrs old), was ligated in a prepared plasmid vector with specifically designed T overhangs at either side and then inserted into chemically competent *E. coli* cells. The bacteria were incubated overnight at 37°C in LB agar with 1X Kanamycin and X-gal which produced hundreds of *E. coli* colonies. White colonies (indicating ligation product present in the cells) were picked for subsequent analysis.

### 3.6.4 Extraction of DNA insert and analysis by restriction enzyme digestion of DNA.

#### i) Extraction of colonies and DNA extraction

Approximately 140 colonies were picked from the LB medium with sterile plastic toothpicks and transferred to microplate wells each containing approximately 100 µl of

sterile water. The microplates were sealed with a sterile polypropylene sheet and put in a microwave for approx. 45 s at 800W in order to lyse the *E. coli* cells (release DNA) and inactivate the nucleases. The microplates were immediately put on ice for 10 minutes and subsequently centrifuged at a maximum speed for 20 minutes at 4°C. 50 µl from each microplate well were subsampled and transferred to a new sterile microplate and centrifuged at maximum speed for 20 minutes at 4°C. 40 µl were subsampled and transferred to a fresh microplate and frozen at -20°C for subsequent analysis.

#### ii) M13 PCR

PCR reactions were carried out in a total volume of 25 µl using Invitrogen Taq DNA polymerase, PCR buffer, MgCl<sub>2</sub> (#18038-018) and ABgene dNTP's (#AB-0315). The commonly known M13 primers M13F (5'-GTA AAA CGA CGG C-3') and M13R (5'-CAG GAA ACA GCT A-3') (Invitrogen Life Technologies) were used. PCR reactions were carried out on an Eppendorf thermal cycler at 94°C for 5 min of initial denaturation followed by thirty cycles of 94°C 45 s, 54°C 45 s, 72°C 2 min and a final extension at 72°C for 10 min. The samples were then kept at 4°C or frozen at -20°C for subsequent analysis.

#### iii) Restriction digestion

For some of the samples, 3 µl of the M13 PCR reaction were subsampled and added to a 7 µl mixture of MSP1 restriction enzyme (Fermentas Life sciences #ER0541 (1ul)), enzyme buffer (1 µl) and water (5 µl)). The reaction volumes were then enclosed in PCR tubes and incubated at 37°C for 90 minutes in order to allow the restriction enzyme to completely cut the DNA in the target spots. The reactions were then transferred to a 68°C oven for 20 minutes to inactivate the restriction enzyme. Samples were put on ice or frozen for subsequent analysis.

#### iv) Restriction digestion gel electrophoresis

For the samples where restriction digestion was performed, a 300 ml volume of 1X TBE buffer was mixed with 6g of MetaPhor® high resolution Agarose gel to make a 2% Agarose solution. The Agarose was dissolved in TBE buffer by heating it in a microwave until complete dissolution of the Agarose powder. The Agarose solution was cooled to approximately 45°C on the benchtop at which point 6 µl of 10 µg/ml of ethidium bromide solution was added to the gel. The gel was then poured into a 300 ml Fisher Biotech Electrophoresis system (FB-SB-2025) with 2 combs of 24 wells (1.5 mm x 5 mm) and allowed to solidify for 60-90 minutes on the counter. The gel was submerged in a 1X TBE buffer solution. Wells were loaded with the restriction digestion product, which were mixed with 2 µl of loading dye prior to insertion into the wells. The last wells of every comb were kept for a 50 bp DNA ladder (InVitrogen #10416-014). The gel was run for 2 hours at 170 volts or 16 hours at 30 volts. The temperature of the gel increased significantly during the electrophoresis process, notably when run at 170 V. Before visualization, the gel was put at 4°C for 10 minutes to allow cooling and full re-solidification before handling. The gel was visualized in a BioImaging Systems gel doc under UV light and digital pictures were taken for analysis of the results. The different restriction enzyme patterns were grouped visually according to the sizes of the different DNA fragments produced by the enzymatic reaction for subsequent analysis.

### 3.6.5 Sequencing

One or two representatives of each restriction pattern group were chosen and re-amplified using the M13 protocol. For the samples where restriction digestion and grouping were not performed, all the clones were selected for sequencing. The fresh PCR reactions were purified using a Qiagen MinElute® PCR Purification Kit (#28006) according to the manufacturer's protocol. The product was then checked on Agarose gel to certify that the DNA concentration was at least 20ng/µl. The samples were subsequently frozen at -20°C and sequenced using the M13 primer on a CEQ™ 8000 Genetic Analysis System.

### 3.6.6 Analysis of Results

#### i) Construction of phylogenetic trees

Sequences were converted to Fasta format and the unnecessary parts of the sequences were removed (TOPO insert fragments, M13 primers) using the freeware program Bioedit. Sequences were checked for chimeric sequences using Chimera Check on the Ribosomal Database Project II release #9.50 (<http://rdp.cme.smu.edu/>) (RDP) website. The sequences suspected to be chimeric were removed from the database. If the chimera breakpoint was situated at the end of the sequence and did not significantly affect the sequence, it was deleted and the rest of the sequence was conserved for further analysis. The sequences were then matched to relevant known matches using the Basic Local Alignment Search Tool (BLAST) on the NCBI database (Altschul et al., 1990) and the Seqmatch program on the Ribosomal Database Project for more commonly known sequences. The selected sequences were imported into a fasta file containing their designated clone library. The sequences were aligned using Clustal X version 1.8 (Thompson et al. 1997) using pairwise alignment parameters of 10.00 for gap openings and 0.1 for gap extensions and multiple parameters of 10 for gap openings and 0.2 for gap extensions. Sequences that were too divergent to be aligned by Clustal X to the others in the clone library were removed from the analysis. From the resulting alignments, a Neighbor-Joining tree topology was inferred and tested with 1000 bootstrap re-samplings and a DNA distance matrix was created for the statistical analysis. The resulting unrooted tree was rooted using *Aquifex pyrophilus* as the root with the freeware program Treeview (Win32) version 1.6.6. The tree was then copied as an enhanced metafile (emf) into Microsoft PowerPoint, ungrouped and edited with representative names because Clustal X only allows a string of 10 characters as name.

#### ii) Statistical analysis

Two different methods were used to statistically compare the different clone libraries. First, the RDP classifier was used to assign the different clones to respective

phyla using a confidence threshold of 80% on classification and leaving out of the analysis any clones which did not fit into the 80% confidence threshold established. Secondly, in order to include in the statistical analysis all of the clones from each clone library, operational taxonomic units (OTUs) were defined for each clone library using a DNA distance matrix created simultaneously with the Clustal X alignments which was run with DOTUR, a computer program for defining OTUs and estimating Species Richness (Shloss et al., 2005). Additional information on the DOTUR computer program can be found in the DOTUR manual available on the following web site: <http://www.plantpath.wisc.edu/fac/joh/DOTUR/DOTURManual.pdf>. DOTUR is a computer program using distance matrix which assigns an OTU for every distance level that can be used to form OTUs using (in our case) furthest neighbor clustering algorithms.

From the output files created by DOTUR, rarefaction curves were constructed to verify if a sufficient number of clones were sequenced and to compare richness of the clone libraries. The output files were simplified because the number of calculations was too large and values were subsequently analyzed exclusively at OTU distance selection levels of 0, 0.05, 0.10, ~0.20 and ~0.30, corresponding respectively to approximate number of differences in the DNA that could be expected at different classification stages using conventional methods, which will be discussed in section 5. However, even with the simplification of the DOTUR data, there is an overwhelming amount of information and in order to build a comprehensive study, some of the data can only be analyzed for the 0.10 difference in DNA, a value we have found to be optimal for our clone libraries to show contrasts between communities. Tables were built from the DOTUR data to compare the various clone libraries with the frequency distribution.

The rarefaction curves were constructed, using the equation developed by Simberloff, (1978):

$$E(S_n) = \sum_{i=1}^s \left( 1 - \frac{\binom{N - N_i}{n}}{\binom{N}{n}} \right) \quad [3]$$

where  $E(S_n)$  is the expected number of groups in a random sample of individuals,  $S$ , the total number of groups in the entire collection,  $N_i$ , the number of clones in group  $I$ ,  $N$ , the total number of clones in the collection  $E N_i$  and  $N$ , the value of sample size (number of clones) chosen for standardization ( $n \leq N$ )

### iii) Comparing clone libraries: Similarity indices

In order to compare the variability in between clone libraries and compare the two classification methods (RDP classifier and OTU), two indexes were calculated. The first index, known as the Renkonen index (Renkonen, 1938), was calculated as follows:

$$P = \sum \min(p_{1i}, p_{2i}) \quad [4]$$

where  $P$  is the similarity between community 1 and 2,  $P_{1i}$ , the proportion of group  $i$  in community 1 and  $P_{2i}$ , the proportion of group  $I$  in community 2.

The second index used is the simplified Morisita index of similarity (Morisita, 1959):

$$C_H = \frac{2 \sum X_{ij} X_{ik}}{(\sum X_{ij}^2) + (\sum X_{ik}^2)} \quad [5]$$

where  $C_H$  is the simplified Morisita index of similarity,  $X_{ij}$ , the proportion of group  $i$  in sample  $j$ , and  $X_{ik}$ , the proportion of group  $I$  in sample  $k$ . The similarity index calculations were done for one OTU classification level and for the RDP classification.

## 4. Results

### 4.1 *Sample location and analysis*

Samples from two distinct locations were used in this study, i.e., Haughton Crater and Yukon Territory. The type of samples and the analyses performed on them are listed in Table 4.1.

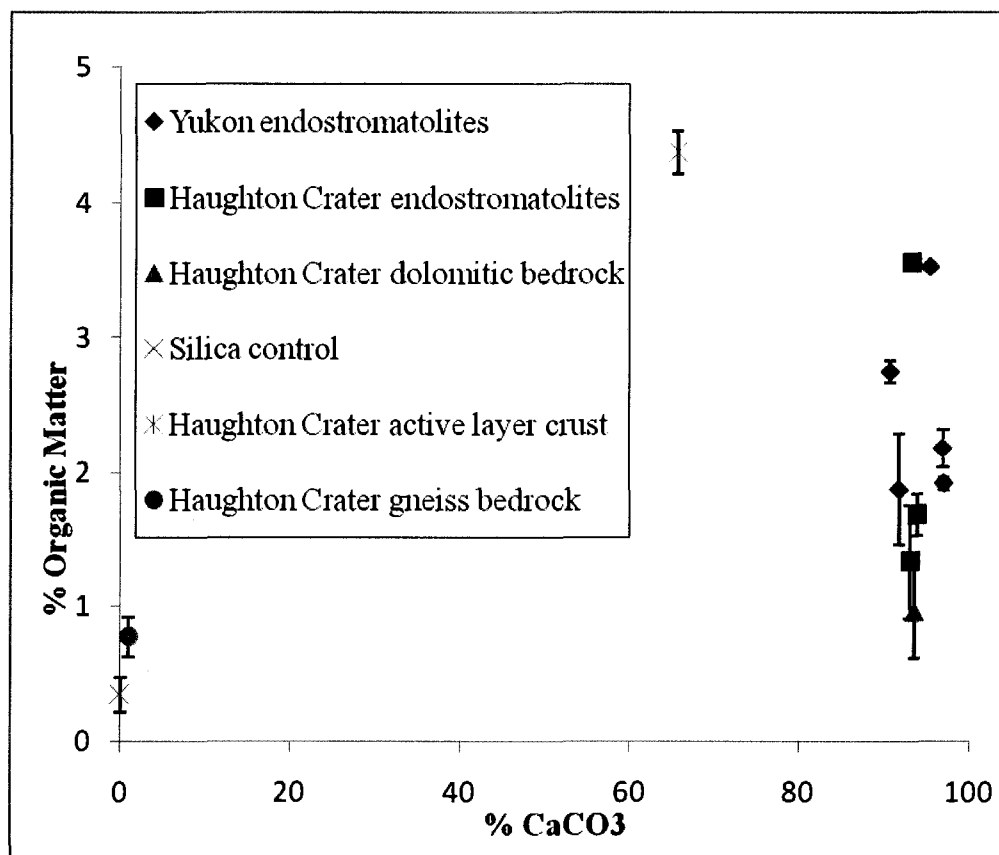
**Table 4.1:** Description, location and analysis of the various samples studied in this project

<b>Sample name and location</b>	<b>Analyses performed</b>
Haughton Crater endostromatolite	LOI, SEM, DNA, $^{13}\text{C}$ NMR, $\delta^{13}\text{C}$ , $\delta^{15}\text{N}$
Haughton Crater silica-rich crust	DNA
Haughton Crater active layer crust	LOI, $\delta^{15}\text{N}$
Haughton Crater dolomitic bedrock	LOI, SEM, $^{13}\text{C}$ NMR
Haughton Crater soil located above endostromatolite outcrop	DNA
Haughton Crater soil located below endostromatolite outcrop	DNA
Control soil outside Haughton Crater	SEM, DNA
Haughton Crater gneiss bedrock	LOI
Yukon endostromatolite	LOI, $\delta^{13}\text{C}$ , $\delta^{15}\text{N}$

### 4.2 *Organic and inorganic carbon composition*

The organic matter content (OMC), as well as the carbonate content (expressed as  $\text{CaCO}_3$ ) of the different samples are shown in Figure 4.1. The Haughton Crater active layer crust had the highest OMC with an average of 4.38% (n=3) but the lowest amount of carbonate (65.62%). The organic matter content of the endostromatolites from the Haughton impact structure varied from 1.33% (n=3) to 3.55% (n=4), which is similar to the OMC of The Yukon endostromatolites, i.e., 1.87% (n=3) and 3.53% (n=1). The carbonate content of the endostromatolites from both locations ranged between 90.69% (n=2) and 96.98% (n=3). The Haughton Crater Gneiss bedrock was used as a negative control and had 0.77% OMC and only 0.92% carbonate (n=4), whereas the Haughton

Crater dolomitic bedrock contained 0.92% of organic matter and 93.56% of carbonate (expressed as  $\text{CaMg}(\text{CO}_3)_2$ ) ( $n=3$ ). The silica standard used ( $n=6$ ) contained 0.34% of organic matter, which is within the error margin associated with the LOI method.



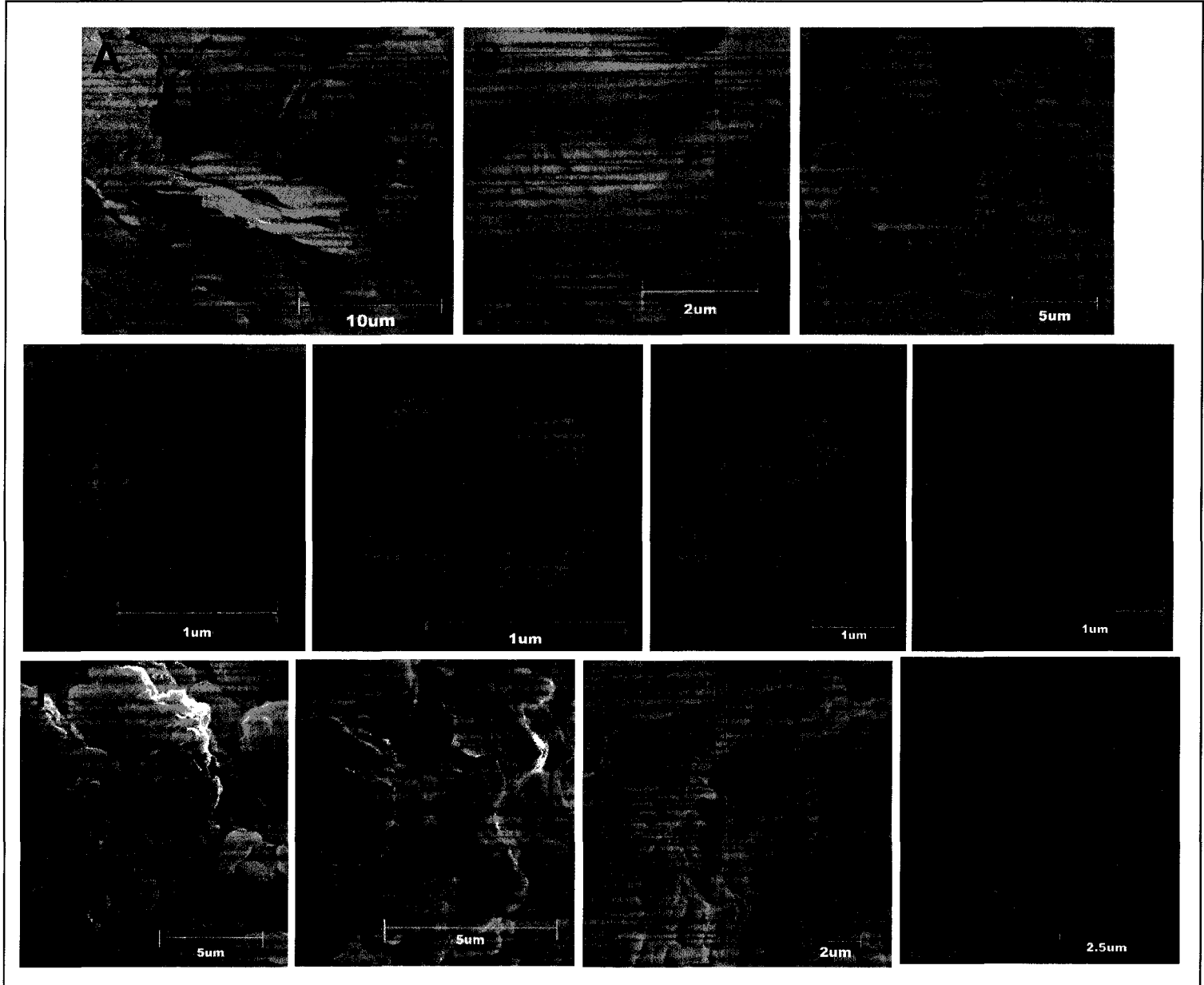
**Figure 4.1:** Organic matter and carbonate contents of the endostromatolites from two different locations and surrounding bedrocks and active layer crusts from Haughton Crater. Reference material, such as silica standard, is also shown.

### 4.3 Scanning Electron Microscopy (SEM)

SEM observations of the Haughton Crater endostromatolites revealed the presence of several structures (Figure 4.2), but energy dispersive spectroscopy (EDS) analyses indicated that they were essentially composed of calcium carbonate (See Appendix 1.1). The endostromatolites are composed of heterogeneous and porous, laminated structures and plaquettes (Figures 4A, B and C), along with nodules of various



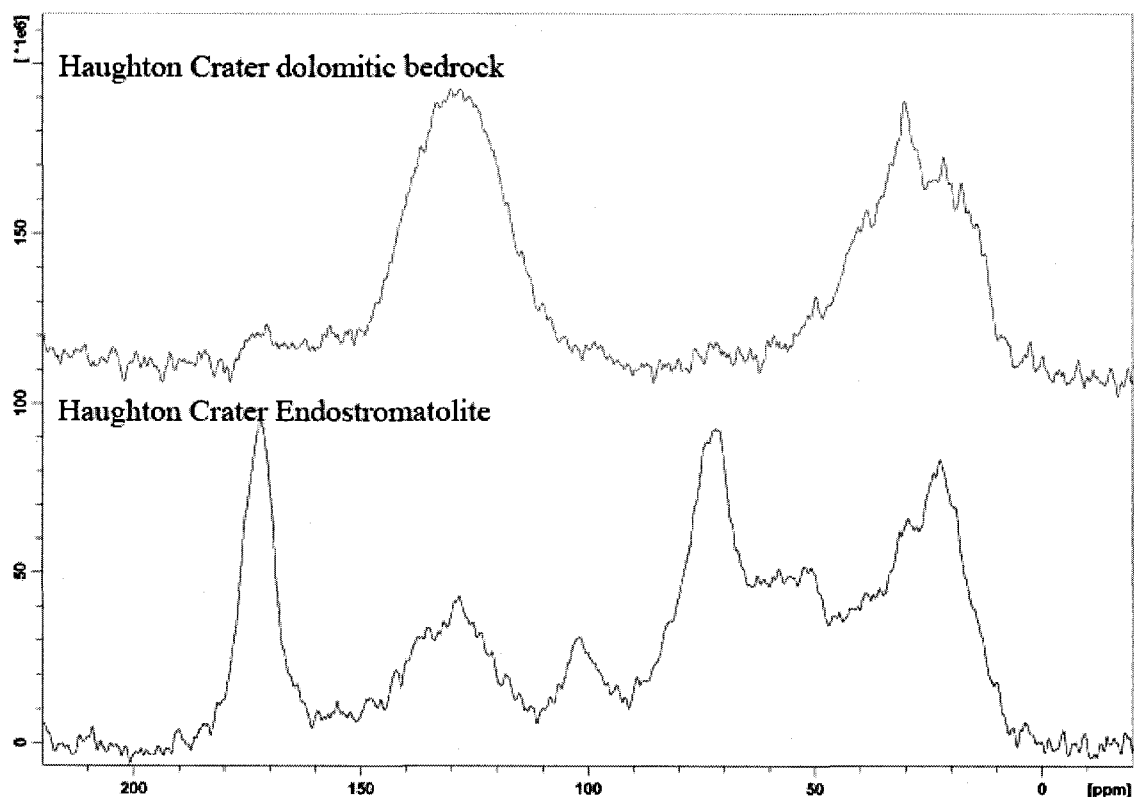
sizes and morphologies (Figures 4D, E, F and G). The nodules are either rounded and smooth or consist of aggregates of smaller particles. Smaller amorphous Ca-rich crystals were also present (Figure 4H). In addition, rod shaped particles and filamentous structures reminiscent of biological forms were observed (Figures 4I and J). Finally, detrital material was present, but not in great abundance. It was generally embedded in calcite (Figure 4K). EDS analyses performed on polished thin sections of endostromatolites from Haughton Crater and from Yukon Territory as well as from the active layer crusts sampled from Haughton Crater showed similar elemental enrichment patterns within the different visible layers of the calcite precipitate (Appendix 1.6). The most important finding is that endostromatolites sampled at the two different locations (Haughton Crater and Yukon Territory) have consistent elemental contents as is shown in Appendix 1.6. On the other hand, the active layer crust has significantly higher concentrations of Si and Fe embedded within the calcite matrix than all endostromatolites samples.



**Figure 4.2:** SEM images showing characteristic features frequently encountered on the surface of Haughton crater endostromatolites. Planar calcitic plaquettes mineralization (A), nodular surface of unknown origin showing a porous nature and likely a great habitat for small bacteria (B), Dissolution/precipitation patterns also observed throughout Haughton Crater soils (C), nodules of various sizes and shapes commonly associated with carbonate precipitation in the presence of large amounts of organic matter (D,E,F), Double spheroid shaped calcite nodule found in bacterially mediated calcite precipitates (G), amorphous calcite crystals covering widespread surfaces examined of endostromatolites (H), structures reminiscent of bacterial colonies (I and J) and commonly observed detritic grains partially embedded in calcite matrix, a common occurrence in Haughton Crater and Yukon endostromatolites (K).

#### 4.4 $^{13}\text{C}$ NMR results

Organic carbon preserved in the Haughton Crater endostromatolites and Haughton Crater dolomitic bedrock was also characterized by  $^{13}\text{C}$  NMR. The bedrock's  $^{13}\text{C}$  NMR spectrum (Figure 4.3) shows two broad peaks centered at frequencies of 30 and 130 ppm, corresponding to the  $\text{CH}_3\text{CO}$ - carbon group and to the carbonyl group (aromatic carbon region typical of mature carbon following thermocatalytic reaction during burial and diagenesis), respectively. The  $^{13}\text{C}$  NMR spectrum of the organic matter extracted from the endostromatolite displays a series of narrow peaks centered at frequencies of 30 ppm ( $\text{CH}_3\text{CO}$ - carbon group), ~ 70 ppm (alkyne group), ~ 100 ppm (alkene group), ~ 130 ppm and 170 ppm (carbonyl group).

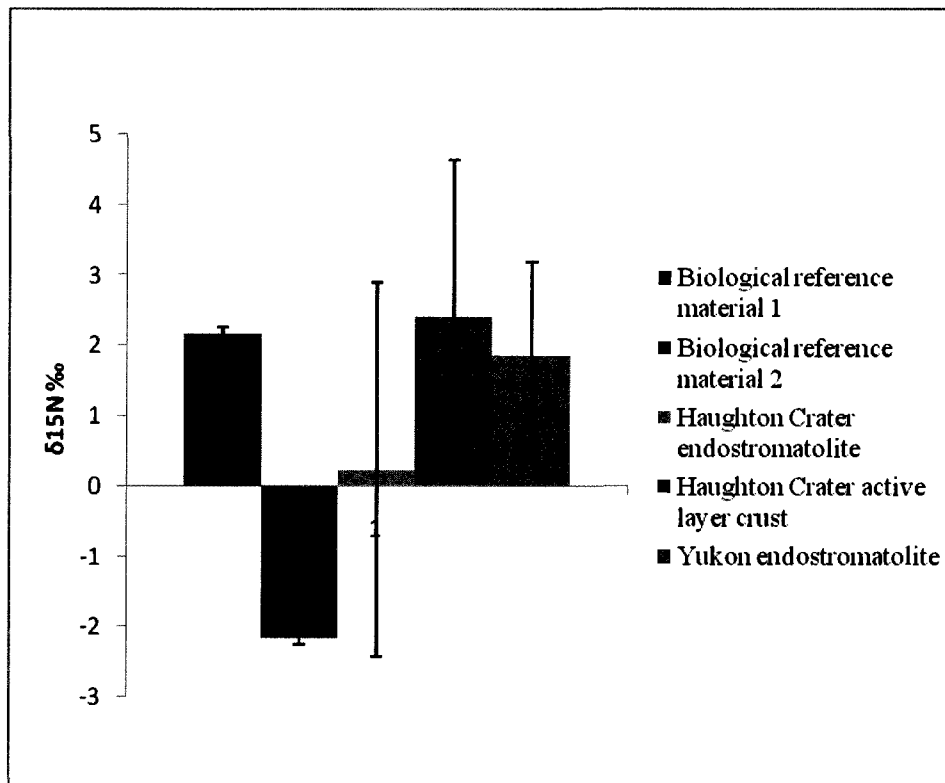


**Figure 4.3:**  $^{13}\text{C}$  NMR analysis of the Haughton Crater dolomitic bedrock (upper spectrum) and the Haughton Crater endostromatolites (lower spectrum). The X axis corresponds to the chemical shift (ppm) while the Y axis measures the intensity of the peak.

## 4.5 *Carbon and Nitrogen isotopic composition*

### 4.5.1 Nitrogen

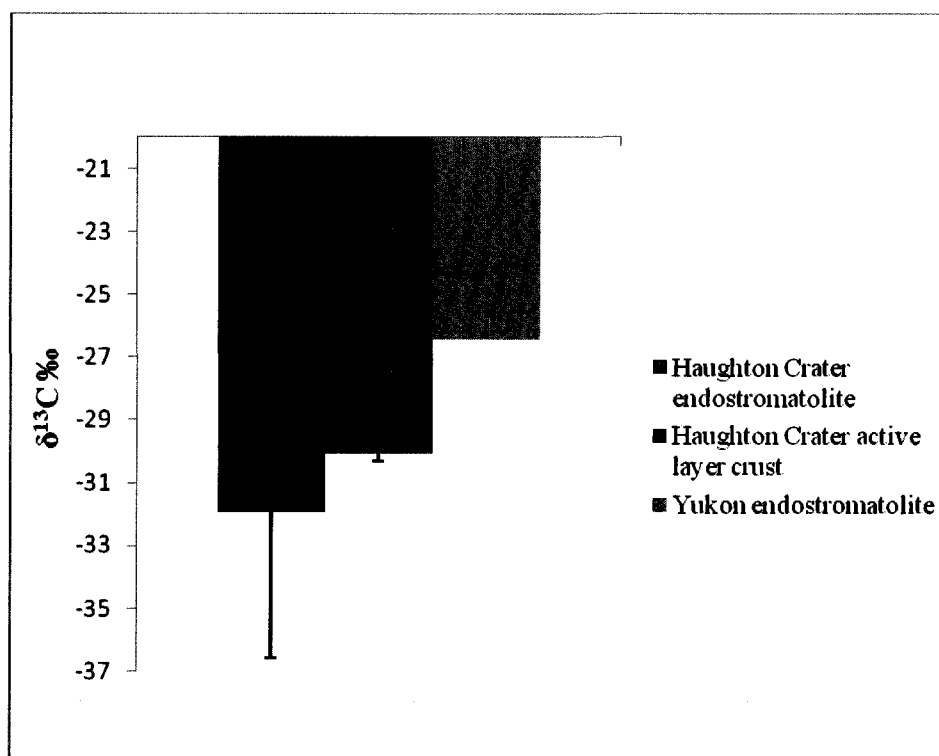
The nitrogen isotopic composition of the endostromatolites from the two different locations, along with the active layer crust from Haughton crater are shown in Figure 4.4. Reference biological materials are also included for comparison purposes. Sample #1, corresponds to a lake biofilm sampled from within the Haughton impact structure, whereas the reference sample #2 is a stream algae sampled in a tributary of the lake where the lake biofilm was taken. The reference material # 1 showed an enrichment in heavy ( $\delta^{15}\text{N}$ ) isotope of 2‰ (+/- 0.10) from atmospheric nitrogen (n=3), whereas the reference biological material #2 had an average nitrogen composition of -2.16‰ (n=3) with an SD of 0.10 on the mean. The isotopic values of the endostromatolites samples through the Haughton impact structure are very inconsistent, ranging from within -5.26‰ to 3.74‰, averaging at 0.23‰ (SD= 2.66, n=14). The same high variability is present in the Haughton Crater active layer crust sample; values ranged from 1.32‰ to 5.76‰, with an average of 2.40‰ (SD=2.24, n=4). Yukon endostromatolites also showed the same variability between samples, with values ranging from 0.8‰ to 3.8‰ with an average of 1.86‰ (SD=1.33, n=4).



**Figure 4.4:**  $\delta^{15}\text{N}$  composition of the endostromatolites and other materials measured from Haughton Crater as well as Yukon endostromatolites

#### 4.5.2 Carbon

The  $\delta^{13}\text{C}_{\text{org}}$  values of the various endostromatolite samples ranged between  $-33.4$  and  $-29.8\text{‰}$  (Figure 4.5). All samples are relatively depleted compared to typical  $\text{C}_3$ -type vegetation ( $-27$  to  $-23\text{‰}$ ; O'Leary, 1988), but are within the range of  $\delta^{13}\text{C}$  values obtained for hydrocarbons trapped in the bedrock (Eglinton et al., 2006)



**Figure 4.5:**  $\delta^{13}\text{C}$  of organic matter contained within endostromatolites from the Haughton Crater, active layers and Yukon endostromatolites for comparison.

#### 4.6 *Molecular phylogenetic analysis of Haughton Crater endostromatolites and soils*

Several types of samples for Haughton Crater were submitted to phylogenetic analysis, they are shown in Table 4.2.

**Table 4.2:** List of samples analyzed for phylogenetic analysis

<b>Sample name</b>	<b>Sample code</b>	<b>Description</b>
Endostromatolite sample number 1	N93	Endostromatolite sampled from the edge of Haughton crater (Allen bay formation)
Endostromatolite sample number 2	N74	Endostromatolite sampled outside of Haughton Crater (Allen bay formation)
Soil above endostromatolite	LH1	Soil sample from carbonate-rich breccias located above the outcrop with endostromatolite N93
Soil below endostromatolite	LH2	Soil sample from soil located below the outcrop with endostromatolite N93
Dolomitic sand	DS1	Dolomitic sand sampled far outside of Haughton Crater
Silica crust	SC1	Crust with similar structures as endostromatolites but composed of silica

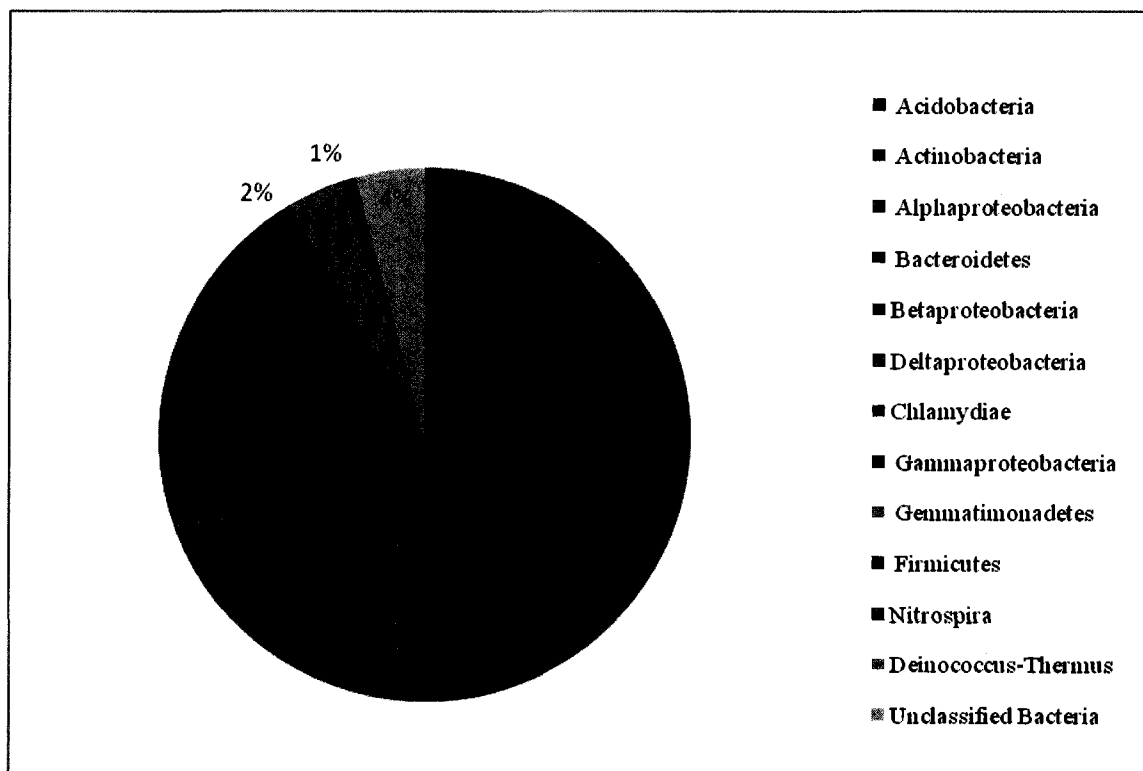
##### 4.6.1 Clone libraries

Clone libraries were built from the results of the DNA extraction, PCR amplification, cloning and sequencing. The raw results of the sequencing are available in Appendix 2 for all clone libraries. The phylogenetic tree construction files are also available as Appendix 3. PCR amplification only yielded positive results for bacteria. Amplification of archaeal DNA was also attempted but was unsuccessful because of limited time constraints to develop a reliable method. This study therefore solely focuses on the bacterial community found within endostromatolites.

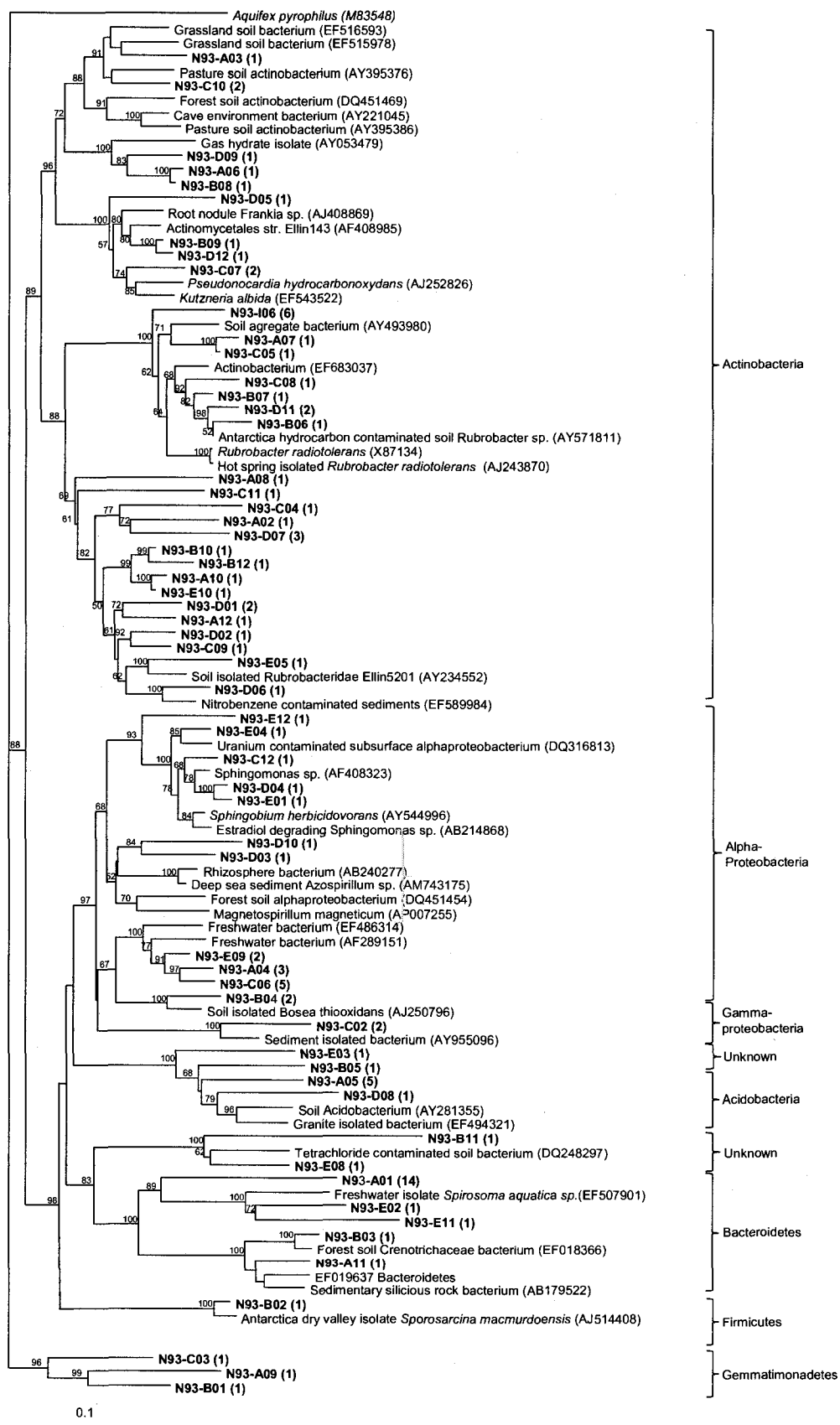
##### *i) Endostromatolite clone libraries*

The clone library of the endostromatolite from Haughton Crater N93 was composed of 95 clones. According to the RDP classifier (Figure 4.6) and phylogenetic tree branching (Figure 4.7), the clones could be divided into six different phyla: *Actinobacteria* (44% of the total clones), *Proteobacteria* (22%), *Bacteroidetes* (19%), *Acidobacteria* (7%), *Gemmatimonadetes* (3%) and *Firmicutes* (1%). Four percent of the clones could not be classified into known phylogenetic groups. The family *Rubrobacteridae* represented over half of the clones classified as *Actinobacteria*. The most closely correlated cultivable bacteria of these clones is *Rubrobacter radiotolerans*, a singular oxygenic, chemoheterophilic bacterium. The other matches still classified as *Actinobacteria* had a range of different isolates closely correlated to *Pseudonocardia hydrocarbonoxydans* and *Kutzneria albida*, as well as a number of uncultured *Actinobacterium* from different soil environments. The phyla *Alphaproteobacteria* composed almost the entire clone library of *Proteobacteria*. *Sphingobium herbicidovorans*, as well as other *Sphingomonas sp.* which were close matches to the clones. Two percent of the *proteobacteria* were however closely correlated to *Gammaproteobacteria*. Members classified as *Bacteroidetes* had as closest cultivated match *Spirosoma aquatica*, although more closely related matches did exist and are shown in Figure 4.7. *Acidobacteria* and *Gemmatimonadetes* were minor constituents of the community and were closely related within each group but lacked interesting matches in the NCBI database. The only clone classified as a *Firmicutes* had a very close relation to *Sporosarcina macmurdoensis*, a bacterium first isolated from the McMurdo Dry Valleys in Antarctica.



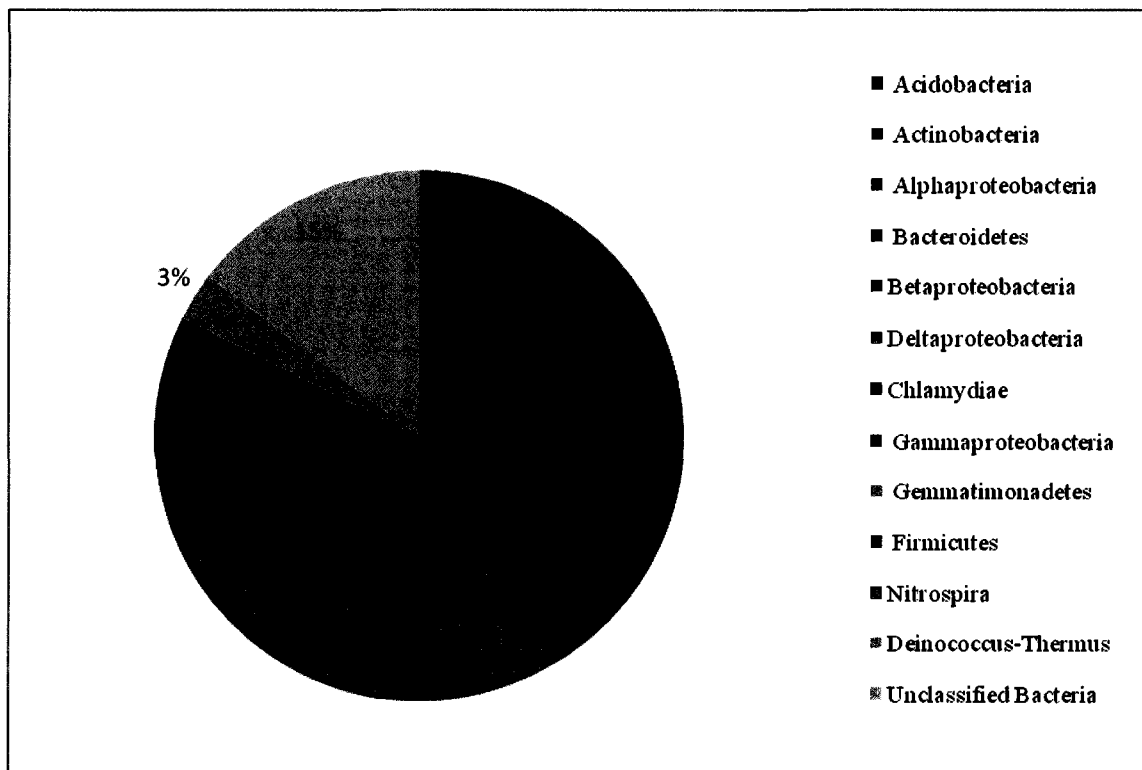


**Figure 4.6:** Abundances of different phyla in clone library N93 (endostromatolite from Haughton Crater) built from 95 clones.

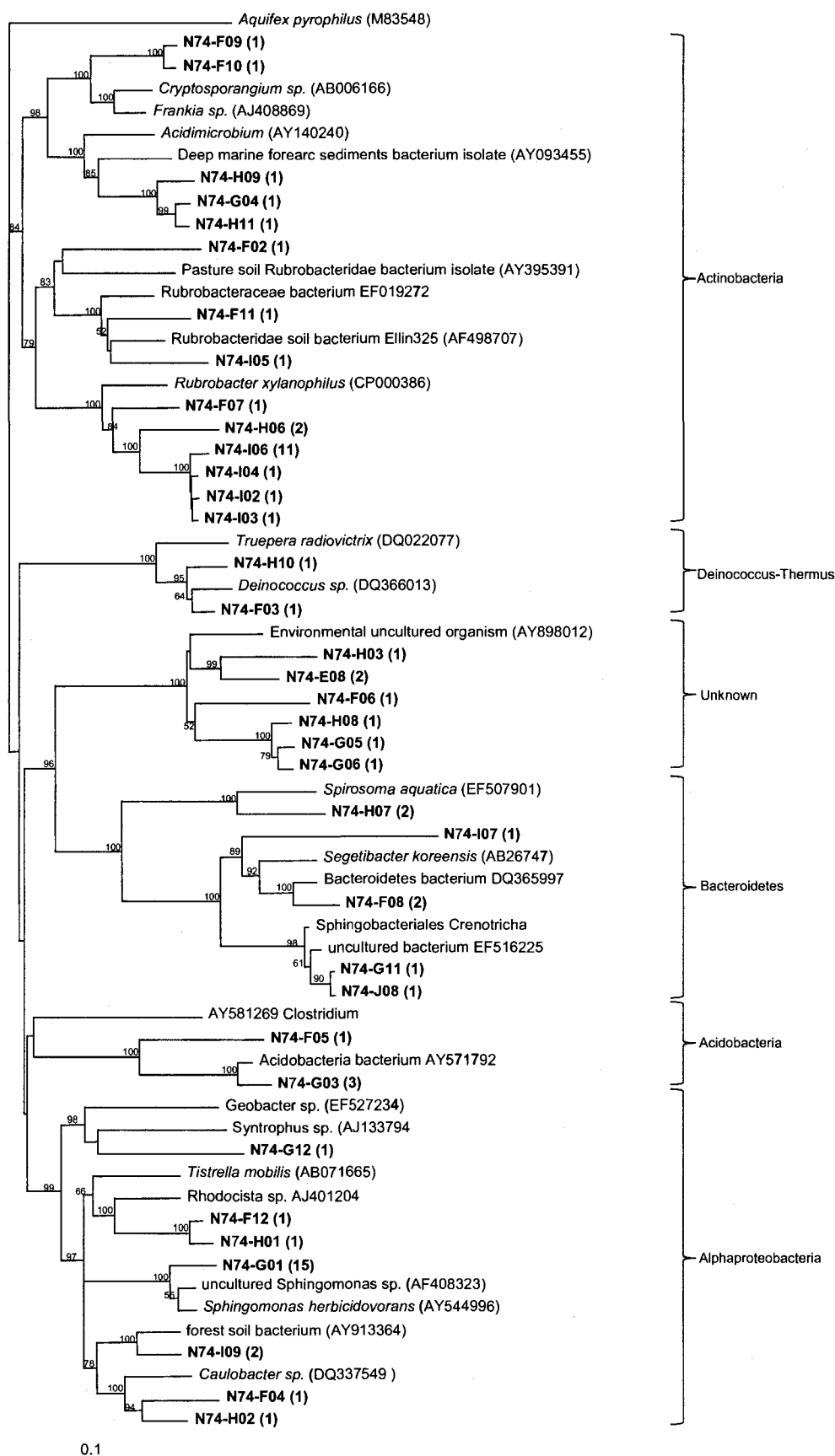


**Figure 4.7:** Phylogenetic relationships of the bacterial 16S rRNA gene sequences obtained from the N93 clone library. The tree was inferred by neighbor-joining analysis of 800 homologous positions of sequence from each clone. *Aquifex pyrophilus* was used as the outgroup. Numbers on the nodes are the bootstrap values (percentages based on 1000 replicates). The scale bar indicates the estimated number of base changes per nucleotide sequence position. Bold type indicates N93 clones with their prevalence in the library in brackets.

The clone library of the second endostromatolite sampled from Haughton Crater (N74) was composed of 68 clones. The clones could be divided into five different phyla: *Actinobacteria* (37%), *Proteobacteria* (31%), *Bacteroidetes* (10%), *Acidobacteria* (4%) and *Deinococcus-Thermus* (3%). 15% of the clones could not be ascribed to a known phylogenetic group by the RDP classifier (Figure 4.8) or by phylogenetic tree branching (Figure 4.9) with known published sequences. All of these unclassified clones were closely related to each other and correlated most closely to the *Bacteroidetes* branch as shown in Figure 4.9. As for the first sample, the subclass *Rubrobacteridae* composed a large fraction of the *Actinobacteria*, the closest cultured species to the majority being *Rubrobacter Xylanophilus*. Some clones classified as *Actinobacteria* did also have other close correlations in that phylum such as *Cryptosporangium sp.* and *Frankia sp.* The phyla *Proteobacteria* was mostly composed of clone isolates classified as *Alphaproteobacteria*, more than half of which correlated most closely to *Sphingomonas sp.*, the rest in close correlation with *Tristella mobilis* or other related matches. One clone was closely matched to *Geobacter sp.* an anaerobic *Deltaproteobacteria* strain. Among the *Bacteroidetes*, *Spirosoma aquatica* as well as some *Sphingobacteria* bacteria were closely correlated to a part of the clones.



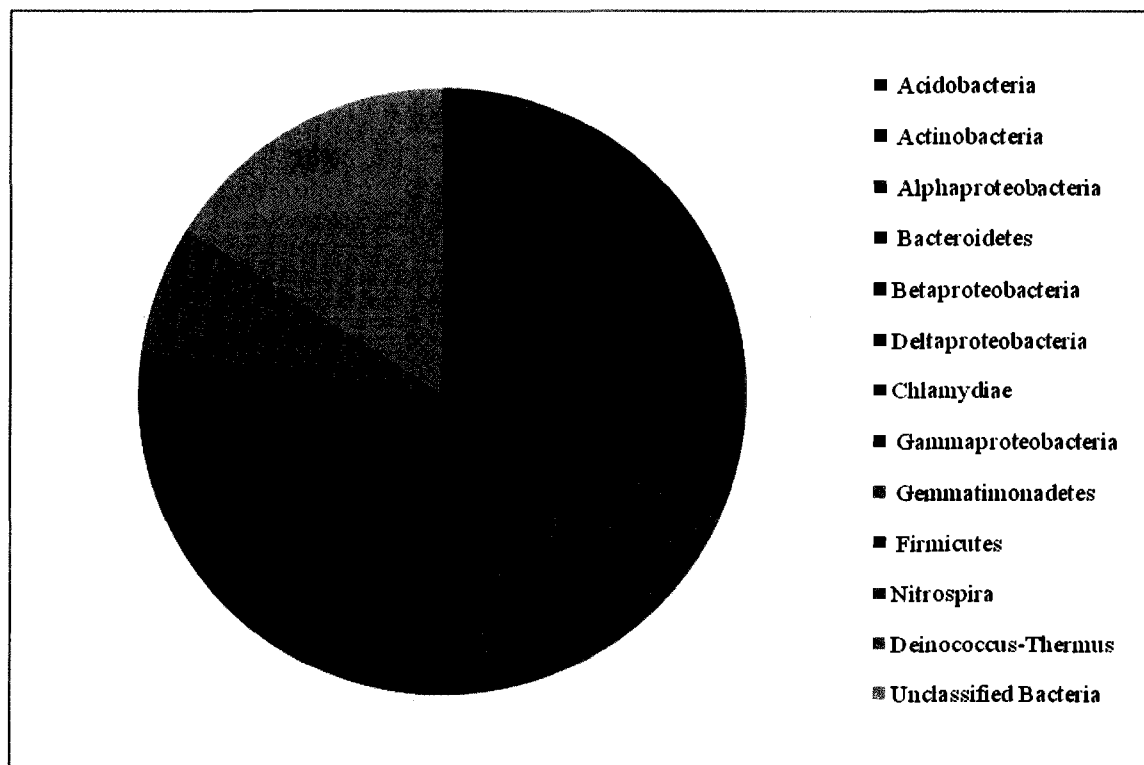
**Figure 4.8:** Abundances of different phyla in clone library N74 (endostromatolite from Haughton Crater) built from 68 clones



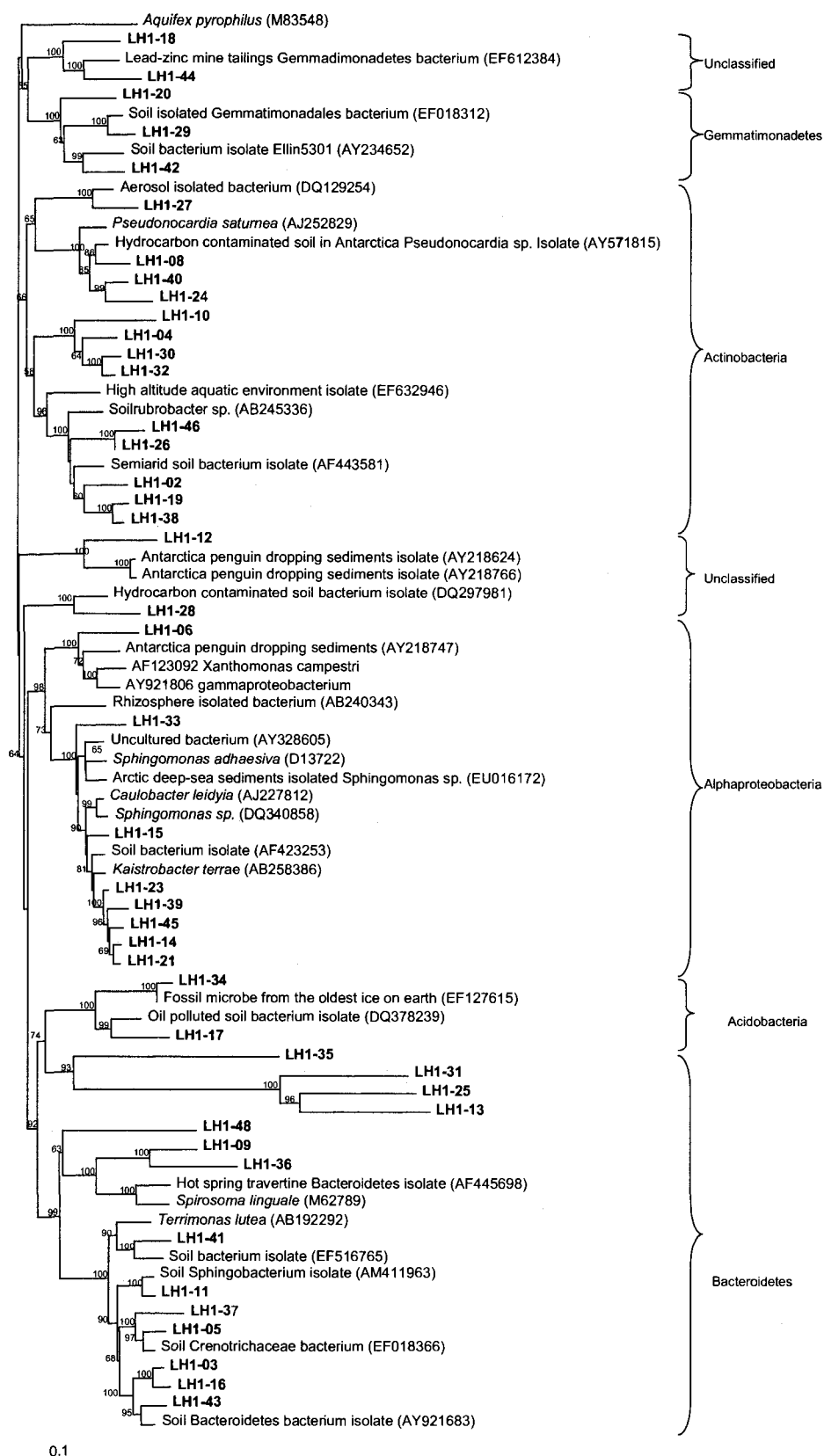
**Figure 4.9:** Phylogenetic relationships of the bacterial 16S rRNA gene sequences obtained from the N74 clone library. The tree was inferred by neighbor-joining analysis of 800 homologous positions of sequence from each clone. *Aquifex pyrophilus* was used as the outgroup. Numbers on the nodes are the bootstrap values (percentages based on 1000 replicates). The scale bar indicates the estimated number of base changes per nucleotide sequence position. Bold type indicates N74 clones with their prevalence in the library in brackets

ii) Soil clone libraries

The clone library of the soil sample located above the outcrop showing endostromatolites (LH1) was composed of 44 clones. The clones could be divided into five different phyla: *Actinobacteria* (27%), *Bacteroidetes* (25%), *Proteobacteria* (20%), *Gemmatimonadetes* (7%) and *Acidobacteria* (5%). 16% of the clones could not be classified at the phyla level by the RDP classifier (Figure 4.10) or by phylogenetic tree branching (Figure 4.11). As for the two endostromatolite samples, approximately half of the clones assigned as *Actinobacteria* could also be classified at the subclass level as *Rubrobacteridae*, but some also correlated most closely with *Pseudonocardia saturnea*. *Proteobacteria* clones classified as *Alphaproteobacteria* were mostly all closely related with the species *Kaistobacter terrae* or other *sphingomonas* sp. One Gammaproteobacteria related clone was also found in this library. Surprisingly, a larger proportion of clones matched Bacteroidetes.

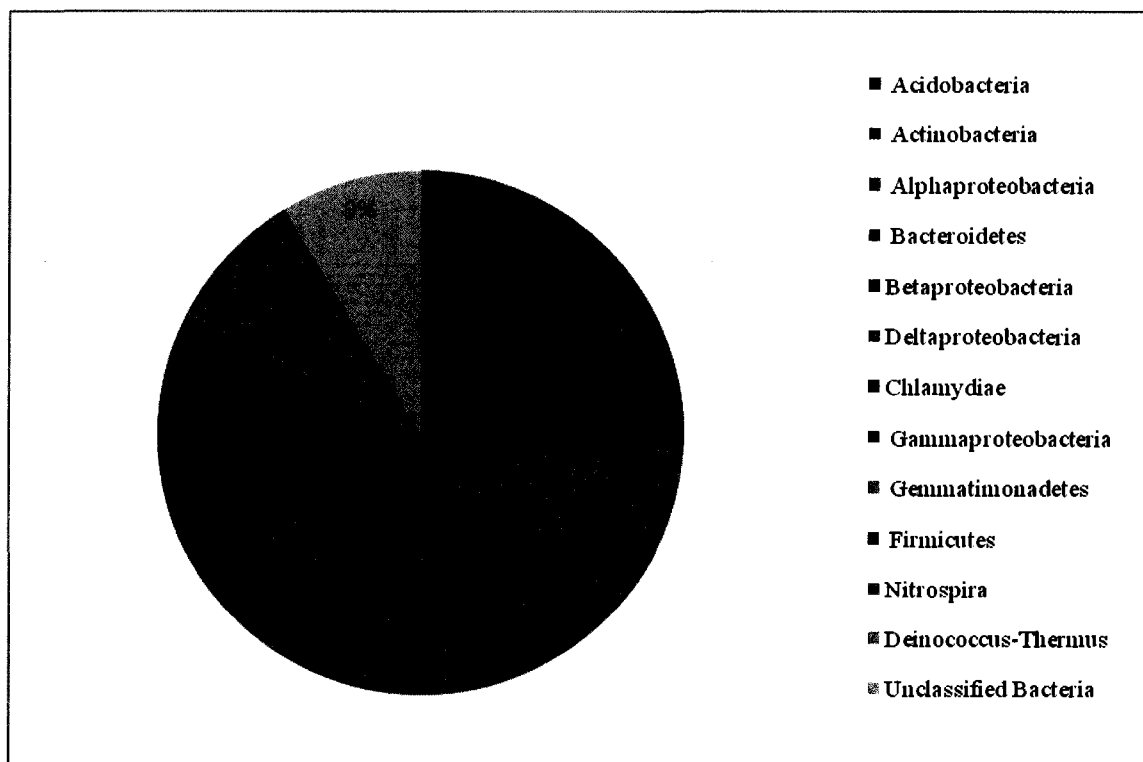


**Figure 4.10:** Abundances of different phyla in clone library LH1 (soil within Haughton Crater) built from 44 clones



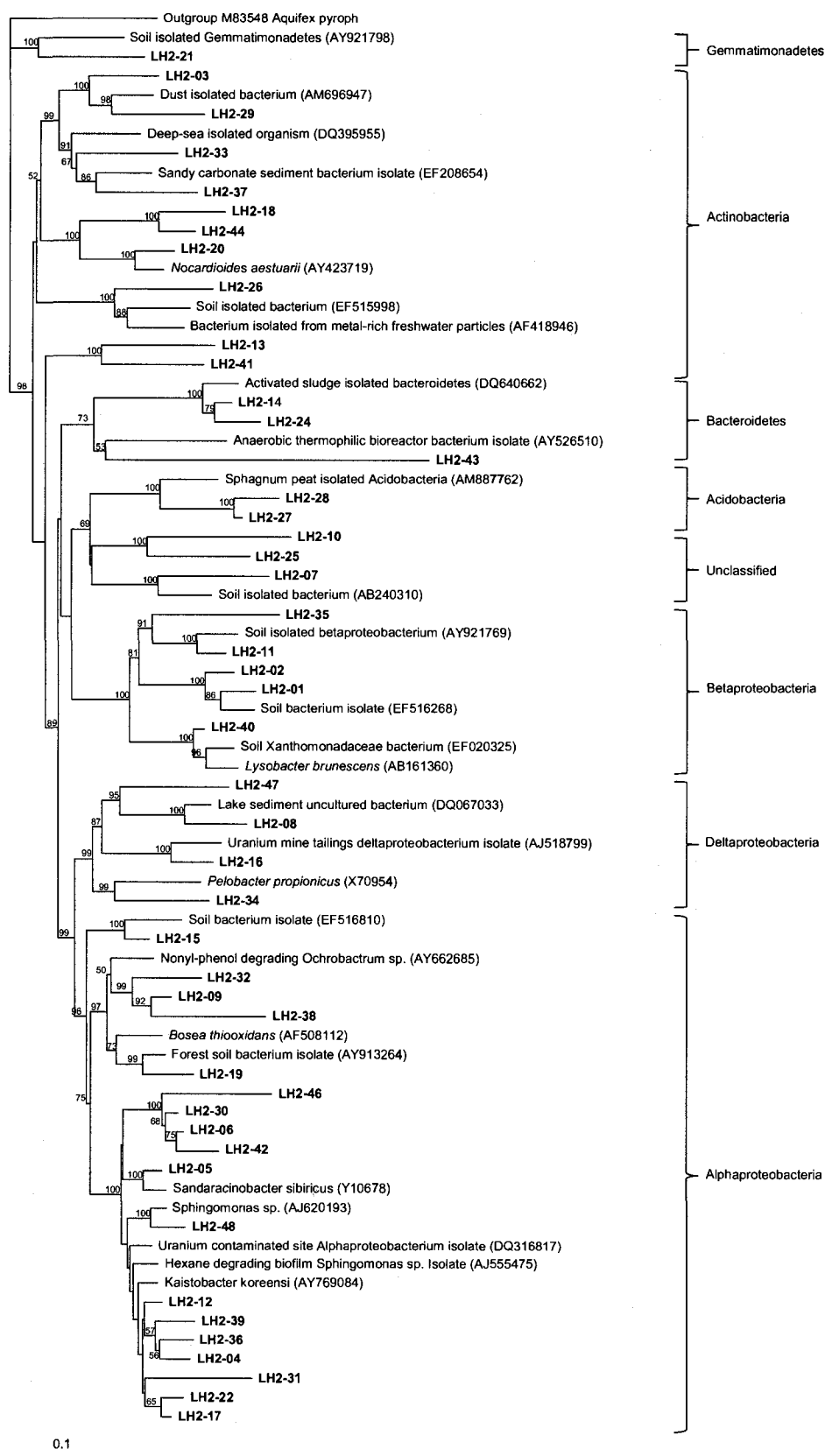
**Figure 4.11:** Phylogenetic relationships of the bacterial 16S rRNA gene sequences obtained from the LH1 clone library. The tree was inferred by neighbor-joining analysis of 800 homologous positions of sequence from each clone. *Aquifex pyrophilus* was used as the outgroup. Numbers on the nodes are the bootstrap values (percentages based on 1000 replicates). The scale bar indicates the estimated number of base changes per nucleotide sequence position. Bold type indicates LH1 clones.

The clone library of the soil sample located below the outcrop showing endostromatolites (LH2) was composed of 46 clones. The clones could be divided into four different phyla: *Proteobacteria* (60%), *Actinobacteria* (20%), *Bacteroidetes* (7%), and *Acidobacteria* (4%). Nine percent of the clones could not be classified at the phyla level by the RDP classifier (Figure 4.12) or by phylogenetic tree branching (Figure 4.13). *Betaproteobacteria* composed 10% of the total community while 8% of the clones were classified as *Deltaproteobacteria*. The remaining *Proteobacteria* (36%) were classified as *Alphaproteobacteria* and again, most of the clones classified as *Alphaproteobacteria* were classified in the *Sphingomonadales* subclass and closely correlated to *Kaistobacter* sp. This was the sample with the most abundant number of *Betaproteobacteria* classified clones from which those that could be classified at a lower level were classified as *Burkholderiales*. *Actinobacteria*, representing around 20% of the community, were not composed mostly of *Rubrobacteridae*, as the other clone libraries were. Instead, *Nocardioides aestuarii*, a strictly aerobic bacterium which was isolated from tidal flat sediments was the dominant *Actinobacteria* in this sample.



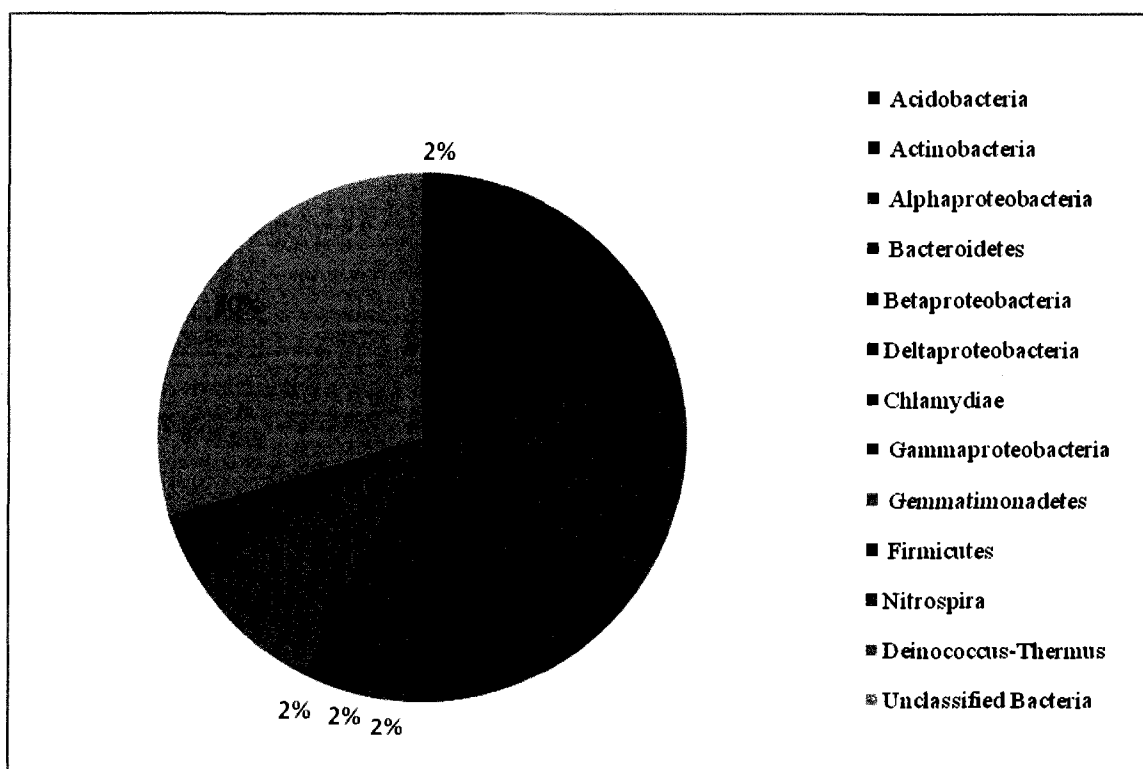
**Figure 4.12:** Abundances of different phyla in clone library LH2 (soil within Haughton Crater) built from 46 clones



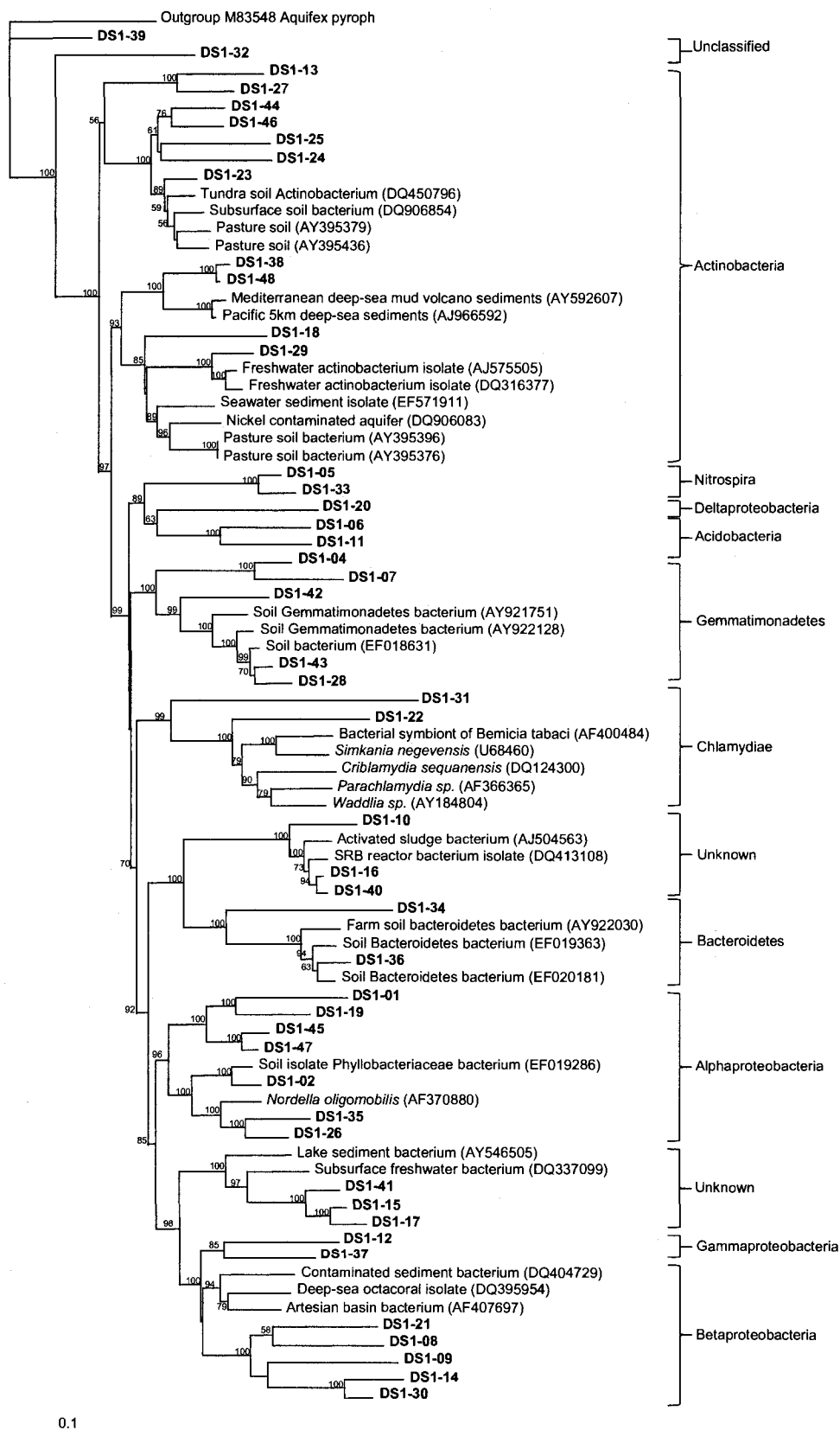


**Figure 4.13** Phylogenetic relationships of the bacterial 16S rRNA gene sequences obtained from the LH2 clone library. The tree was inferred by neighbor-joining analysis of 800 homologous positions of sequence from each clone. *Aquifex pyrophilus* was used as the outgroup. Numbers at the nodes are the bootstrap values (percentages based on 1000 replicates). The scale bar indicates the estimated number of base changes per nucleotide sequence position. Bold type indicates LH2 clones.

The clone library of the dolomitic sand sample collected outside of Haughton Crater (DS1) was composed of 47 clones. The clones could be divided into seven different phyla: *Proteobacteria* (32%), *Actinobacteria* (17%), *Gemmatimonadetes* (9%), *Nitrospira* (4%), *Bacteroidetes* (4%), *Acidobacteria* (2%), *Chlamydiae* (2%). Thirty percent, the largest fraction from all the clone libraries, could not be assigned to specific phyla by the RDP classifier (Figure 4.14) or by phylogenetic tree branching (Figure 4.15). This was the site where the highest number of phyla was observed but concomitantly, it was the site which contained the highest uncertainty because a large fraction of the clone library could not be reliably assigned to a phylum. Novel phyla found at this site were classified as *Chlamydiae* and had as nearest match, bacterial symbionts of organisms. The portions of the clone libraries that could not be classified using NCBI databases were closely correlated to each other, suggesting that novel types of bacteria may exist at this site.



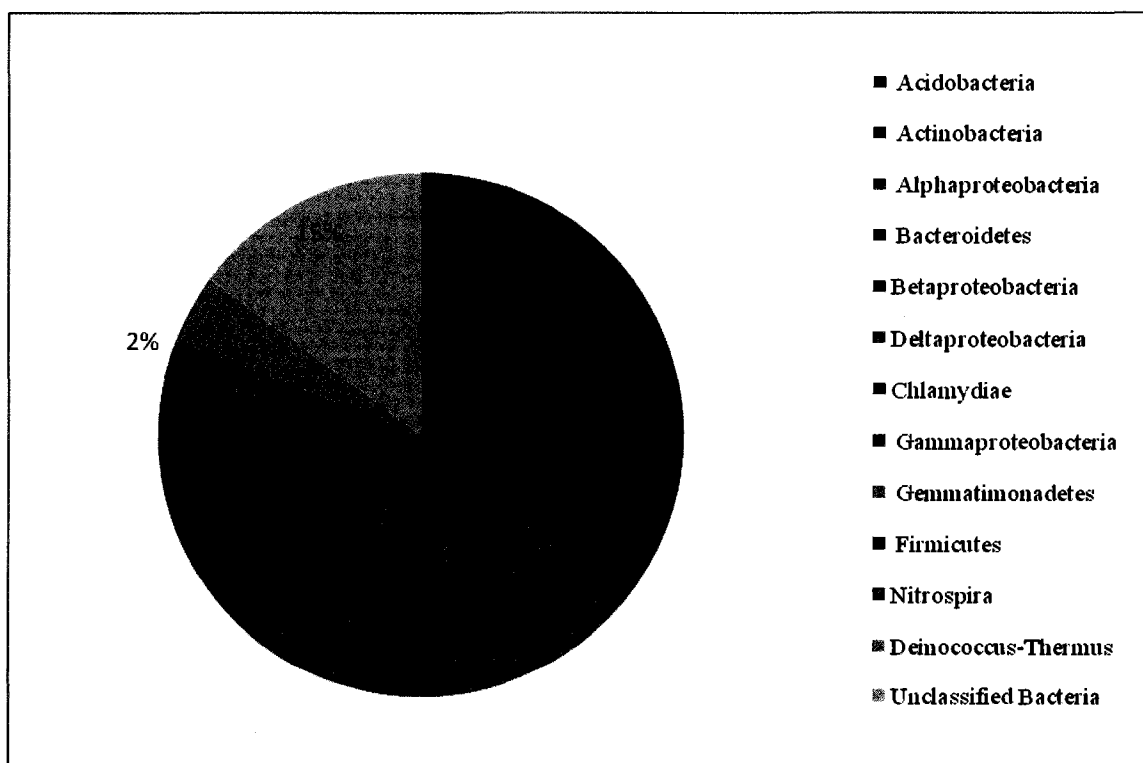
**Figure 4.14:** Abundances of different phyla in clone library DS1 (soil outside Haughton Crater) built from 47 clones



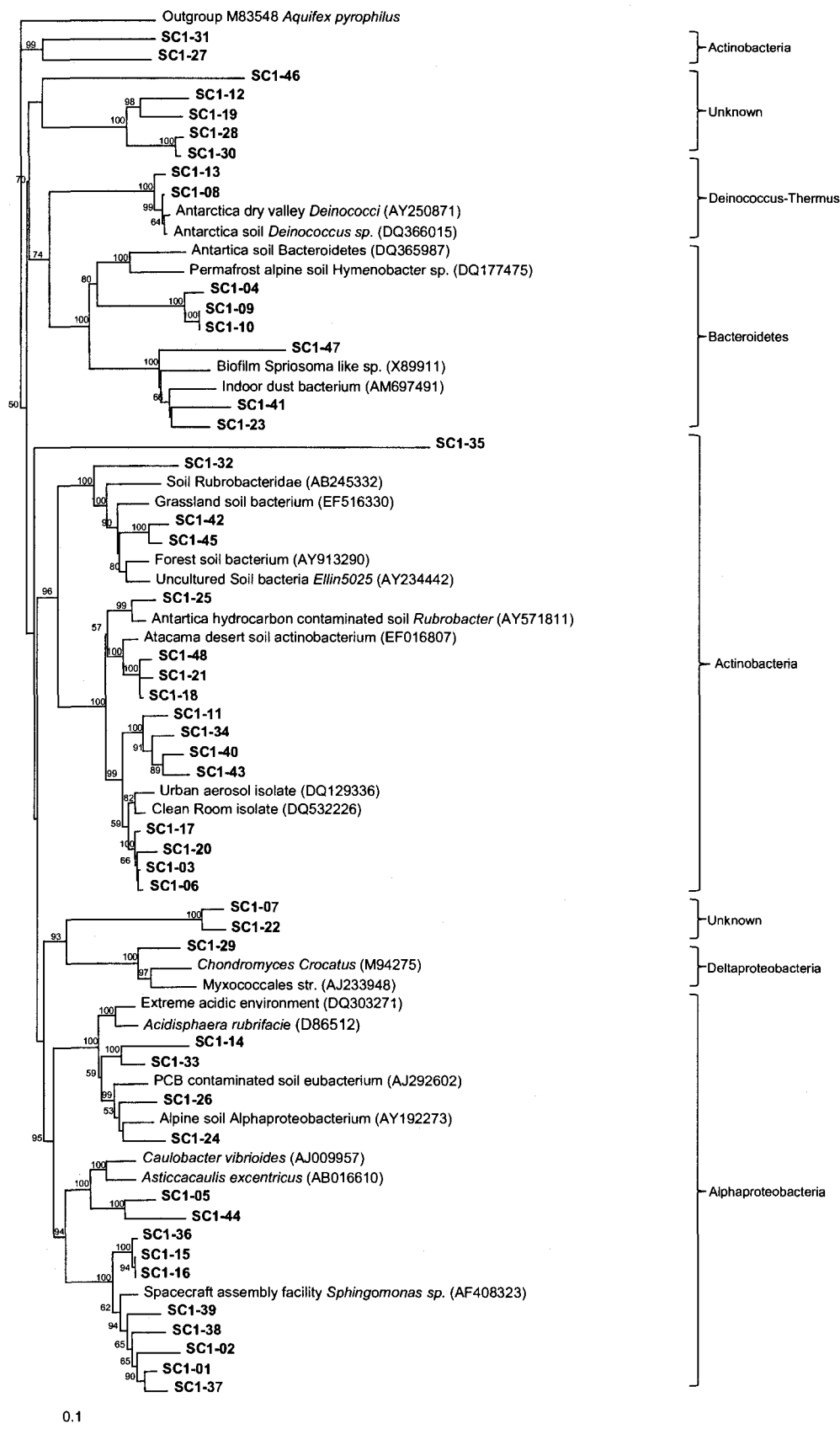
**Figure 4.15:** Phylogenetic relationships of the bacterial 16S rRNA gene sequences obtained from the DS1 clone library. The tree was inferred by neighbor-joining analysis of 800 homologous positions of sequence from each clone. *Aquifex pyrophilus* was used as the outgroup. Numbers on the nodes are the bootstrap values (percentages based on 1000 replicates). The scale bar indicates the estimated number of base changes per nucleotide sequence position. Bold type indicates DS1 clones.

iii) Silica crust clone library

The clone library of the silica crust sample (SC1) was composed of 47 clones. The clones could be divided into four different phyla: *Actinobacteria* (36%), *Proteobacteria* (32%), *Bacteroidetes* (13%) and *Deinococcus-Thermus* (4%). Fifteen percent of the clones could not be classified into specific phyla according to the RDP database (Figure 4.16) or phylogenetic branching (Figure 4.17). Although the mineralogical composition of this sample is different from the other samples studied in this thesis, the bacterial community was very similar to the soils and carbonate endostromatolites. A large fraction of the clones that were classified as *Actinobacteria* were also grouped into the *Rubrobacteriidae* subphyla. The Proteobacteria were almost entirely (all but one clone) classified as *Alphaproteobacteria*, the other being *Deltaproteobacteria*. The *Alphaproteobacteria*, were closely matched to *Sphingomonas* sp. for the large part, but others were also matched with *Caulobacter vibrioides*, a methanotrophic bacterium (Poindexter, 1981).



**Figure 4.16:** Abundance of different phyla in clone library SC1 (Silica crust within Haughton Crater) built from 47 clones.



**Figure 4.17:** Phylogenetic relationships of the bacterial 16S rRNA gene sequences obtained from the SC1 clone library. The tree was inferred by neighbor-joining analysis of 800 homologous positions of sequence from each clone. *Aquifex pyrophilus* was used as the outgroup. Numbers on the nodes are the bootstrap values (percentages based on 1000 replicates). The scale bar indicates the estimated number of base changes per nucleotide sequence position. Bold type indicates SC1 clones.

#### 4.6.2 Statistical analysis of clone libraries

##### i) Application of DOTUR to compare soil clone libraries: Diversity

The methods used to generate the data presented in section 4.5.2 are unsuccessful in assigning with confidence a group to all sequences. The methods used were phylogenetic tree branching and the use of the RDP classifier from which important information has been extracted namely by assigning a likely phylum name and a potential classification branch through matching with known sequences from the RDP and NCBI databases. This yields insights into the representative environment's general ecological purpose and suggests possible environmental adaptations of communities, but this type of classification has left out a varying degree of the population (up to 30%) from being classified. As a result, a true picture of the diversity within each clone library and between each clone library cannot be drawn.

Assigning operation taxonomic units (OTUs) to all clones in a clone library can alleviate this problem and by utilizing different classification parameters, a true view of the diversity within a sample can be resolved. Performing the DOTUR analysis with the different clone libraries obtained in this study highlighted the fact that the frequency distribution of sequences in OTUs were comparable within each other, especially for the soil libraries (Table 4.3).

**Table 4.3:** Comparison of various clone libraries to determine the frequency distribution and richness estimates when OTUs are assigned at a level of 10% difference by DOTUR

16s rRNA gene library	Soil samples			Endostromatolites			Silica crust
	LH1	LH2	DS1	N93	N74	SC1	
Total # of sequences	44	46	48	95	66	45	
# of OTUs	31	34	36	37	22	19	
No. of OTUs with $n_x$ sequences							
$n_1$	24	27	27	21	8	6	
$n_2$	4	5	6	4	9	9	
$n_3$	2	1	3	3	3	1	
$n_4$	0	0	0	3	0	0	
$n_5$	0	0	0	3	0	1	
$n_6$	1	1	0	1	0	0	
$n_7$	0	0	0	0	0	1	
$n_8$	0	0	0	0	0	1	
$n_9$	0	0	0	0	0	0	
$n_{10}$	0	0	0	1	0	0	
$n_{11}$	0	0	0	0	0	0	
$n_{12}$	0	0	0	0	0	0	
$n_{13}$	0	0	0	0	0	0	
$n_{14}$	0	0	0	1	0	0	
$n_{15}$	0	0	0	0	1	0	
$n_{16}$	0	0	0	0	1	0	

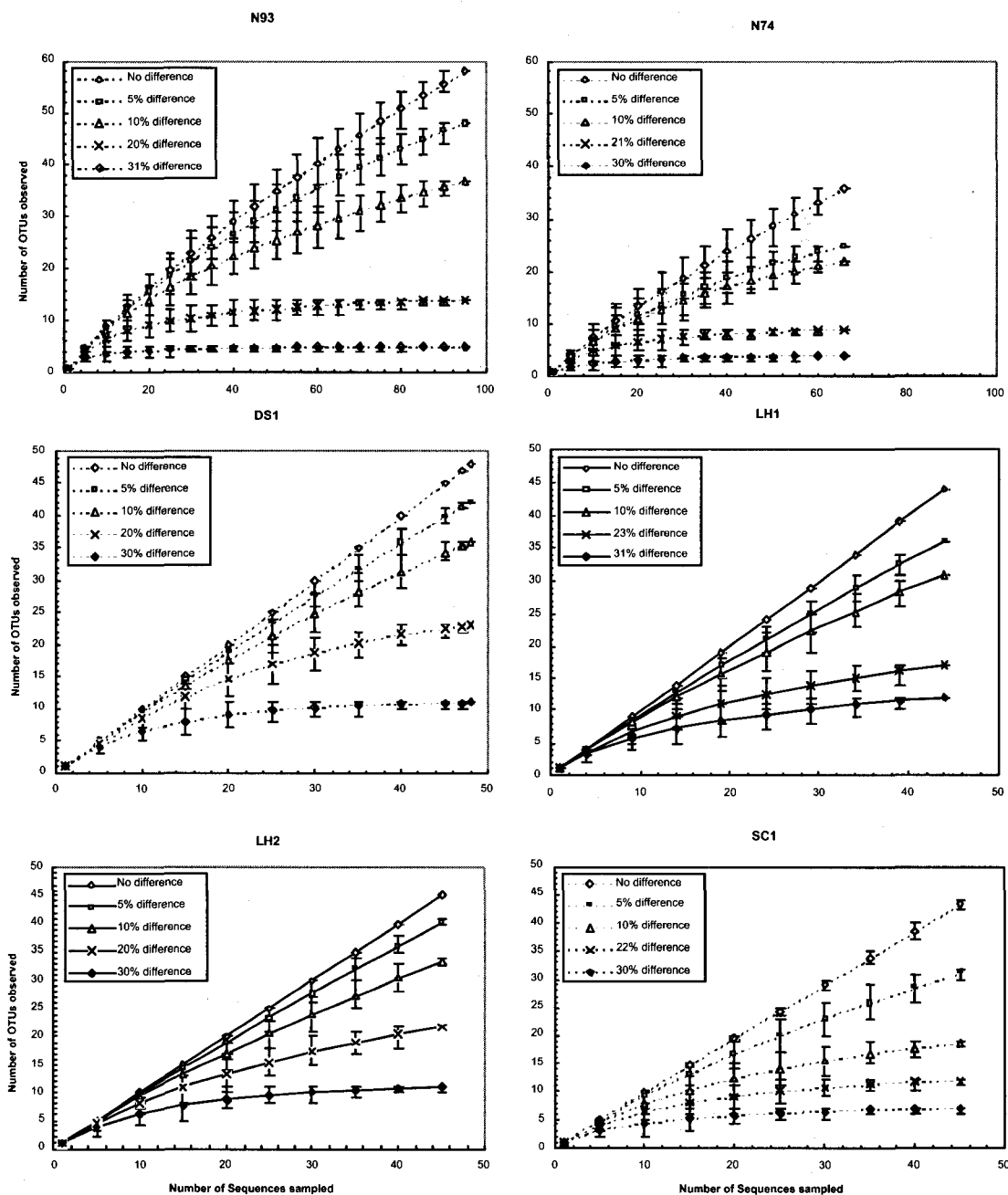
\*OTUs were defined by using a distance level of 10% difference. The frequency distribution of the endostromatolite and Haughton Crater soil 16S rRNA gene libraries were performed by using furthest neighbor assignment algorithms implemented in DOTUR.

It is however clear that the endostromatolites and the silica crust sample have OTU groups with greater abundances than the soil samples (up to 16 sequences in one OTU in clone library N74). Rarefaction curves generated from the clone libraries indicated that by using a OTU threshold of 10% difference, there was a 95% chance that if only 44 sequences had been sampled for each clone library (corresponding to the lowest amount that was sequenced for one clone library, LH1, which gave 31 OTUs), then we would have identified between 32 and 34 OTUs for library LH2, 20 and 27

OTUs for N93, 31 and 35 OTUs for DS1, 17 and 19 OTUs for SC1, 15 and 21 OTUs for N74.

The results can be visualized as rarefaction curves in Figure 4.18 for many OTU classification levels although the level of choice for this study was set at 10%. The full results of the DOTUR output are available in Appendix 4. It is clear that when using a parameter of 10% difference to classify OTUs, it becomes impossible to conclude that there is a difference in the richness between LH1 and DS1 because the number of observed OTUs from the LH1 soil falls within the 95% CI of the DS1 soil after the sampling of 44 sequences. In the same way, at the maximum value where all libraries can be compared, the libraries N93 and N74 have no difference in richness and neither do N74 and SC1 or DS1, LH1 and LH2. We can however state that there is a difference in richness between many of the samples in such a way that the crust samples (N93, N74 and SC1) are different than the soil samples. Interestingly, the soil clone libraries and the crust libraries formed two distinct groups of different richness. It is probable that with further sequencing and as the resolution of the study increases, more difference in richness between the libraries would be observed.





**Figure 4.18:** Rarefaction curves for endostromatolites, silica crust and soil clone libraries using the furthest neighbor assignment algorithm. Error bars represent the 95% CI. Note that the libraries N93 and N74 have different X and Y axis values.

*ii) Comparing clone libraries: Similarity*

The number of clones and similarity indices (Renkonen index of similarity and simplified Morisita index of similarity) calculated for the six clone libraries are summarized in Table 4.4. Again it is important to note that the statistical analyses of the clone libraries labeled under “RDP classifier” were undertaken on the fraction of the communities which could be classified under a known phyla, while the fraction of the communities which could not be ascribed with confidence to a known phyla were omitted from the statistical analysis. The number of clones of the different libraries ranged between 44 (LH1) and 95 (N93) for the different communities. These numbers were variable dependent on (1) the numbers of clones initially believed to be necessary to take a representative sample of a particular community and (2) the number of these that were successful. The number of clones used for the statistical analysis “RDP classified” however ranged between 32 (DS1) and 91 (N93).

For the RDP classified analysis, the Renkonen similarity indices between each community ranged between 0.383 (DS1 and N74) and 0.792 (SC1 and N74) with an average similarity index of 0.572, but all low values were caused by community DS1 which was the most dissimilar community from all the others (maximum dissimilarity was between DS1 and N74 (0.383) and DS1 and SC1 (0.383)) according to the Renkonen index. The simplified Morisita index values ranged from 0.659 to 0.993 between the different clone libraries. According to this index, the most dissimilar two libraries were DS1 and N93 (0.659) while the most similar two clone libraries were SC1 and N74. Interestingly, the values of the Morisita index between libraries DS1 and N74 (0.703) and DS1 and SC1 (0.703) were not the lowest as indicated by the Renkonen index. The data for both the Renkonen index and Morisita index for the RDP classified analysis are in brackets in Table 4.4.

The results of the simplified Morisita index of similarity performed on the output from DOTUR did yield different values from the ones obtained by RDP classification. This is to be expected since a fraction of the clone libraries was not incorporated into this

equation for the RDP classified values. Values of similarity ranged between 0.384 and 0.978 between the different clone libraries and, unexpectedly, did not show any increasing or decreasing trends in between different levels of OTUs although variation was present. Utilizing a criterion of 10% difference on the selection of OTUs, library LH1 and LH2 were the most similar (0.978) and N74 and DS1 the most dissimilar (0.469). N74 was the most dissimilar community from all the others, although it had high similarity to the other endostromatolite library (0.918). The results for the simplified Morisita index of similarity are shown in Table 4.4 for the OTU selection of 10% difference. The Renkonen index of similarity's highest value was again between LH2 and LH1 (0.935) while the lowest was also between N74 and DS1 (0.600).

**Table 4.4:** Renkonen index of similarity and Simplified Morisita index of similarity in between each clone library

	DS1	LH1	LH2	N74	N93	SC1
		Morisita index of similarity				
DS1		0.91 (0.71)	0.92 (0.75)	0.47 (0.70)	0.61 (0.66)	0.75 (0.70)
LH1	0.86 (0.47)		0.98 (0.73)	0.59 (0.88)	0.69 (0.76)	0.85 (0.89)
LH2	0.90 (0.51)	0.94 (0.51)		0.59 (0.89)	0.68 (0.76)	0.82 (0.99)
N74	0.60 (0.38)	0.68 (0.60)	0.66 (0.63)		0.92 (0.96)	0.82 (0.99)
N93	0.79 (0.43)	0.80 (0.74)	0.79 (0.53)	0.74 (0.71)		0.82 (0.99)
SC1	0.65 (0.38)	0.71 (0.58)	0.72 (0.60)	0.79 (0.79)	0.79 (0.79)	
		Renkonen index of similarity				

\*Upper right triangle gives the Simplified Morisita index of similarity values calculated from OTU criteria of 10% similarity. Brackets are the values obtained from the RDP classification. Lower left triangle gives the Renkonen index of similarity. Brackets are the values obtained from the RDP classification. Colors link the two indexes.

## 5. Discussion

### 5.1 *Organic matter content and morphology of the endostromatolites*

#### 5.1.1 Origin of the organic matter in the endostromatolites

Organic matter enclosed within endostromatolites can be as high as 7% (Lauriol and Clark, 1999), but normal values are situated between 2 and 6% (Clark et al. 2004), especially from specimens found in the Yukon. The present study of the endostromatolites and active layer crusts from Haughton Crater reports organic carbon values (Figure 4.1) similar to those found by Clark et al. (2004). The origin of the organic matter in endostromatolites has however been the subject of some debate. In the Yukon Territory endostromatolites, it has been argued that the organic matter could originate from the overlying soils, be transported by water in the fissures and get immobilized by calcite precipitation (Lauriol and Clark, 1999). This hypothesis is supported by the similarity between the  $^{13}\text{C}$  NMR spectra of the organic matter from the Arctic vegetation and the Yukon endostromatolites (Clark et al. 2004). However, in Haughton Crater, the near absence of overlying vegetation does not support the above interpretation although such a trend is uncertain for the Holocene hypithermal. On the other hand, the dolomitic bedrock contains around 1% of organic matter, mainly as hydrocarbon fluid inclusions (Figure 4.1), (Parnell et al., 2007)). The  $^{13}\text{C}$  NMR spectrum of endostromatolites from Haughton Crater (Figure 4.3) indicates that the organic matter may be in part derived from the hydrocarbons contained in the host rock along with a contribution from alkynes from an additional unknown source. This is in agreement with the carbon isotopic signatures of the organic matter (Fig. 4.5), which indicates that the values reported for the endostromatolites and active layer from Haughton Crater are very similar to those generally measured for hydrocarbons (Eglinton et al., 2006). On the other hand, the nitrogen isotopic composition of the organic matter (Fig. 4.4) is highly variable and cannot be used to identify the type of organic material in the endostromatolite samples and active layer. The lack of reproducibility likely stems from the fact that the amount of nitrogen in the endostromatolite and active layer samples was too low compared to the

quantity present in the fresh biological reference material (i.e., biofilms and algae, Fig. 4.4).

In summary, the results show that the endostromatolites and active layer crusts from both locations are composed of a complex mixture of organic material originating from either the surrounding bedrock and vegetation or which may partially be generated in-situ by an unaccounted process.

### 5.1.2 Morphology

Electron microscopy is commonly used to identify biological material and textures. It was used in the study of the ALH 84001 Martian meteorite to infer evidence of life on Mars (McKay et al. 1996), but care must be taken when interpreting the results because several morphologies and textures can resemble biotic formations but can have an abiotic origin (Fortin et al., 2007). As a result, morphological observations must be supported by molecular, chemical and isotopic data.

The various morphologies shown in Figure 4.2 are similar to those observed in endostromatolites from the Yukon Territory (Chauret 1999). The same author reported morphologies resembling the shape of *Staphylococcus*, *Bacilli* and an unknown type of fungus and attributed these to microorganisms entrapped by the calcite matrix of the endostromatolites. In the Haughton crater endostromatolites, there were small, micrometer sized carbonate spheroids (Fig. 4.2), having similar characteristics (size, shape, roundness) as those reported by Braissant et al. (2004) during their study of the role of exopolysaccharides (EPS) on the roundness of calcite crystals. These authors concluded that the organic matter content of a solution will have an effect on the precipitation morphology of calcite crystals. EPS are mostly known to be secreted by bacteria in biofilms (Sutherland, 2001), but other sources of organic carbon, susceptible of affecting the morphology of calcite crystals, can also be found in the environment, i.e., hydrocarbons, humic and fulvic acids, etc. Given the fact that organic matter can originate from both abiotic and biotic processes (Anders, 1989), it is therefore impossible

to ascertain the presence of microbially-derived organic matter in the formation of spheroid particles in the Haughton Crater endostromatolites.

Despite the fact that the organic carbon and morphology results cannot be used alone to ascertain the biological origin of the endostromatolites, it is clear that there are several chemical and morphological similarities between the endostromatolites and active layer crusts from Haughton Crater and the samples from the Yukon Territory.

## 5.2 *Bacterial communities present in the Haughton Crater samples*

Understanding soil microbial diversity and the environmental processes they control require an in-depth understanding of how microbial diversity influences and is influenced by the environment (Mummey et al., 2006). Microbial communities are composed of bacteria, archaea and eukaryotes, but the results presented in this study focused solely on bacteria found within the Haughton Crater endostromatolites, soils and crusts because archaea were not detected in the samples. It is however possible that the archaeal DNA recovered (if any) within the endostromatolites was too little and was overwhelmed by the bacterial DNA signal, thus preventing its amplification. Future work on endostromatolite should focus on the development and use of specifically designed PCR techniques that would allow for the amplification of archaeal DNA.

### 5.2.1 Building clone libraries

Molecular techniques have greatly contributed to our understanding of microbial ecology but as with any method, it does have biases. It is widely known that PCR primers can influence the sequence diversity within rDNA clone libraries (Marchesi et al., 1998) and that many factors like PCR cycling parameters and amount of DNA template used in PCR reactions may affect the coverage and hence the overall diversity in uncultured 16S rDNA libraries (Shrivage et al., 2007). Since there are known biases associated with DNA extraction and PCR amplification (Perreault et al. 2007, Von Wintzingerode et al.

1997, Martin-Laurent et al. 2001), the abundance of a phyla in a clone library does not necessarily reflect its abundance in the sample, and the corresponding ecological function cannot be inferred with certainty from phylogenetic affiliation (Achenbach and Coates, 2000). According to Perreault et al., (2007) such assumptions should be made only when there is a high degree of sequence similarity between the phylotypes and known cultivated species. In the present study, the sequence similarity has been demonstrated through phylogenetic branching with published BLAST matches and cultivated species. Although most clones from the libraries did have a sequence homology with cultivated organisms, allowing some prediction of ecological function within the endostromatolites or soil samples, some rather large clusters of clones did not (e.g. Figures 4.7, 4.9, 4.11, 4.13, 4.15 and 4.17). In these cases, clones were closely related to each other suggesting the possibility of novel taxonomic units.

### 5.2.2 Operational Taxonomic Unit classification

The computer program used in this study to assign OTUs was DOTUR. It rapidly assigns sequences to OTUs at all distances and assists in assessing the reliability of the richness estimates. For this study, the determination of the quantitative richness of individual clone libraries was not our primary goal, but the richness estimates were however necessary to compare the different libraries. DOTUR permitted the evaluation of the clone libraries on multi-levels by removing a certain degree of uncertainty. In addition, DOTUR also enabled the quick construction of rarefaction curves. Rarefaction is a statistical method for estimating the number of species expected in a random sample of individuals taken from a collection (Kreb, 1999). Since the size of the clone libraries differed for all samples, it would have been impossible to compare them from a statistical point of view. For instance, directly comparing the clone library of the sample N93, which contains 95 clones, with the clone library of the sample DS1, which only has 48 clones, shows that N93 has 37 OTUs while DS1 has 36 OTUs, bringing us to the conclusion that the community richness is practically the same between both libraries. This approach is however wrong because the sample size is not the same for both samples. One way to overcome this problem is to standardize all samples from different

communities to a common sample size of the same number of individuals. Once the rarefaction analysis is performed, sample N93 only contains 24 OTUs and DS1 has 33 OTUs, which brings us to a different conclusion, i.e., DS1 has a higher richness than N93.

Generally speaking, values greater than 97% identity between ribosomal RNA sequences serve as a general but imperfect guideline for defining membership in the same microbial species (Huber et al., 2007). However, the level chosen can either over or under estimate species-level boundaries. Furthermore, the 3% level is mostly simply used because “everyone is doing it” and therefore enables comparison of the results. For the purpose of defining membership in different bacterial populations, we have adopted the 10% level instead of the 3% level because it was not necessary for us to see the species diversity but rather to draw contrast between the clone libraries using a small number of sequences. Furthermore, given the low number of sequences, our objective was not to identify all species, of bacteria, but only the major ones. This was achieved through phylogenetic tree construction. The “true” microbial diversity of the samples is undoubtedly much greater than what has been identified using the 10% level. However, at that level, the contrast necessary to achieve our objectives was attained. Furthermore, given the low number of sequences, the 10% level enables us to be confident that we are describing biologically relevant richness. In the future, more extensive sequencing may increase the resolution of such an analysis and indicate the richness of libraries with a greater certainty.

The DOTUR computer program can also assist in assessing the completeness of a sequencing effort (Schloss and Handelsman, 2005) but this analysis was not performed because the order in which the sequences were sampled was not available. This should however be performed during the analysis of future clone libraries.

Because the DOTUR analysis calculates the richness at every possible level of OTU, a full overview of the richness in the clone libraries is available, significantly reducing a bias and a degree of controversy (Bond et al., 1995, Borneman et al., 1997,



Hugenholtz, 1998). However, displaying the full amount of information generated by DOTUR would be overwhelming to the reader. In order to represent the DOTUR data, careful studying of the case and the representation of “typical” trends need to be presented. This led to our choice of 10% difference as OTU selection criterion. In the cases where DOTUR found that two clone libraries were not different, phylogenetic branching with known matches from the NCBI database can show that the phylogenetic composition of these databases are different even if DOTUR finds the richness to be undifferentiable. Furthermore, because a connection between species richness and ecological mechanisms cannot be resolved, it is possible that two communities have considerably different membership (richness) and yet conduct similar biological processes (Schloss and Handelsman, 2005).

### 5.2.3 Description of bacterial diversity and inference of ecological function

In cryptoendolithic environments, (such as the environments where endostromatolites are growing), we can expect specific types of micro-organisms (Cockell et al., 2005). The present study provides, to our knowledge, the first characterization of the bacterial diversity present in endostromatolites of Northern Canada. Although the physico-chemical parameters that existed within the fissures were not studied, they could be inferred by the metabolism pathways of the microorganisms found within the fissures and might explain how those structures formed.

#### 5.2.3.1 Actinobacteria

The phylum *Actinobacteria* is known to include some of the most common soil microorganisms playing an important role in the decomposition of organic matter (Pankratov et al., 2006)). *Actinobacteria* represented the largest portion of the bacterial community in the endostromatolites from Haughton Crater. This is similar to other high carbonate endolithic environments where molecular phylogenetic analysis of the bacterial community was studied (McNamara et al., 2006). These authors found that 46% of the endolithic community from the limestone of a Maya Archeological site was *Actinobacteria* while the epilithic community was much lower, at 22%. Both of the

endostromatolite sites sampled in our study had a high percentage of *Actinobacteria* closely related to *Rubrobacter sp.*, an aerobe genus known to survive in high UV environments (Yoshinaka et al. 1973). This particular characteristic is however not consistent with the environment in which endostromatolites are found, because they occur in fissures where there is very little light. On the other hand, some of the *Rubrobacter*-related species (*Rubrobacteridae*) present in the Haughton impact structure had as a nearest known match in the NCBI database, with isolates from soil environments from Antarctica soils (Yergeau et al., 2007), which have similar properties or similar ecological constraints as the soils of Haughton Crater. In addition, some of the nearest matches also corresponded to samples originating from hydrocarbon contaminated soils (Saul et al., 2005) and heavy metal contaminated soils (Moffett et al., 2003), which shares similarities with the chemical characteristics of the dolomitic bedrock in Haughton Crater. These similarities do not however indicate that *Rubrobacteridae* are exclusively found in environments similar to our study site because they have also been reported as an important component of desert varnish microbial communities (i.e., microbial communities living on the surface of sun exposed rocks in very hot and dry environments), which include *Rubrobacter radiotolerans* (Kuhlman et al. 2006) In addition, *Rubrobacter*-related species have been identified as the dominant microbial fraction (60-80% of the community) associated with rosy discolorations in endolithic lime wall paintings communities (Imperi et al., 2007, Schabereiter-Gurtner et al., 2001) and shown to be diverse and widespread in arid soils of Australia (Holmes et al. 2000). It is clear from the above studies that *Actinobacteria*, and more specifically *Rubrobacteridae* isolates are widespread and abundant in selected soil-type terrestrial environments, and climate does not seem to affect their presence. Their metabolic activity at low or high temperatures has however never been investigated.

It was initially thought that because so many clones were related to *Rubrobacter*, that the bacteria present in the endostromatolites utilized hydrocarbons enclosed within the bedrock of Haughton Crater as an electron donor. Their metabolic activity could have led to calcite precipitation (as a result of alkalinity generation following the oxidation of organic carbon) which would account for the presence of endostromatolites.

However, analyses of the bacterial communities in the soil environments of Haughton Crater showed that *Rubrobacter* was also found throughout the landscape, although as a lesser fraction of the community, and was not restricted to secondary carbonate precipitates, such as endostromatolites where hypothetically they could have consumed the hydrocarbons. Since the dolomitic bedrock of Haughton Crater contains hydrocarbons, this is somewhat in agreement with the study of Saul et al., (2005) or Yergeau et al., (2007), on hydrocarbon contaminated sites in Antarctica which revealed that *Rubrobacter sp* was present in hydrocarbon contaminated sites. Our results however differ from a previous study of the Haughton Crater environments which did not show any microorganisms closely related to *Rubrobacter sp*. (Fike et al., 2003), but the specific environments studied by these authors were not analyzed in detail and the methods used to analyse the living microorganisms were significantly different. Nonetheless, our results show that *Actinobacteria*, especially *Rubrobacter*-related species, are found as a higher fraction of the total community in endostromatolites (in samples N93 and N74) than in surrounding soils environments (samples LH1, LH2, DS1). Hypothetically, this could be because the environment provided by endostromatolites coincided with specific adaptations/requirements of this group of bacteria (or that it disfavored other types of bacteria). Interestingly, the silica crust had a similar abundance of *Actinobacteria* (sample SC1; Fig. 4.16) even though it was not composed of calcite. This suggests that environments conducive to endostromatolite formation might host various physico-chemical conditions favoring *Actinobacteria* although these conditions cannot be determined from this study.

Finally, some of the clones in the endostromatolites are related to known cultured species of *Rubrobacter* (*Rubrobacter radiotolerans*, *Rubrobacter taiwanensis*, *Rubrobacter xylanophilus*) which have been mostly isolated from hot springs and cultured as thermophilic bacteria with optimal growth temperatures from 37°C to 60 °C. Such growth conditions are obviously very different from those prevailing in the Arctic setting, however, it is possible that their ability to grow under extreme conditions might help them adapt to harsh conditions and resist to long periods of desiccation, such as those found in an Arctic desert environment. This will be discussed further in section 5.2.5 of the thesis.

### 5.2.3.2 Bacteroidetes

It is not surprising to find *Bacteroidetes* throughout all the clone libraries built from Haughton crater environments because they are widely distributed over a diverse range of ecological niches (Dong-Shan et al., 2007) *Bacteroidetes* are found in soil, freshwater, plants and in the air (Buczolits et al., 2002). Cultured members of the *Bacteroidetes* are known to proliferate on degrading biopolymers, such as cellulose and chitin, along with the high molecular weight fraction of dissolved organic material (Kirchman, 2002) and are aerobic heterotrophic bacteria (Dong-Shan et al., 2007). The metabolic properties of this phyla isolated from environments of Haughton Crater is not dissimilar from the metabolic properties of the other major phyla isolated from endostromatolites and soils in our study area.

### 5.2.3.3 *Deinococcus-Thermus*

Although *Deinococcus-Thermus* was only present in one sample of endostromatolite (N74) and in the silica crust (SC1), it not unexpected to find that phyla in the samples since it is an opportunistic strain. Its thick membrane allows it to survive high doses of radiation (Anderson et al., 1956) and dessication (Dyer, 2003)). Genetically, it is closely related to *Actinobacteria* (Figure 4.9) and should display similar properties to *Actinobacteria* (Kausar, 1997). *Deinococcus-Thermus* is thought to live in every environment on planet Earth because it can withstand extreme conditions. As a result, this bacterium is an extraordinarily resistant opportunist capable of surviving dry conditions for years (Stolp, 1988). It has been isolated from soils (Saul et al., 2005), spacecraft assembly facilities (Moissl et al., 2007), desert varnishes (Perry et al., 2003), which represent extreme environments. It is therefore not surprising to find it in the endostromatolites from Haughton Crater.

### 5.2.3.4 *Proteobacteria*

While *Actinobacteria* were clearly the dominant microbial fraction in the endostromatolites, it was not necessarily the case for the soils samples, which clearly showed a more diverse array of bacteria. *Proteobacteria* were the most abundant group

in the soil samples LH2 and DS1, but they were also found in significant numbers in all other clone libraries. They are gram negative bacteria grouped into five classes; Alpha, Beta, Proteo, Gamma and Epsilon. The abundance of *Proteobacteria* varied throughout the soil samples of Haughton Crater, but as a general rule, *Alphaproteobacteria* were the most abundant group. Their presence is not surprising because *Alphaproteobacteria* are “everywhere” (Dyer, 2003). In all but one of the samples, the predominant number of clones which were classified as *Alphaproteobacteria* were closely related to *Sphingomonas sp.* Members of the genus *Sphingomonas* have an aerobic heterotrophic soil-based lifestyle somehow similar to *Pseudomonas* (White et al., 1997). They are also capable of degrading extraordinarily recalcitrant carbon sources (Harms et al., 1995) and producing gellan and related exopolysaccharides (EPS) (White et al., 1997). Interestingly, EPS has been shown to affect calcite formation, as discussed earlier (section 5.1.2) by forming rounded agglomerates. Furthermore, the genus *Sphingomonas* also contains many representatives that are able to degrade compounds such as polynuclear aromatic or halogenated molecules that might be present at low concentrations in the atmosphere (Kuhlman et al. 2006). *Sphingomonas sp.* is therefore a perfect candidate to inhabit arctic soils and other environments of Haughton Crater because hydrocarbons are part of the host bedrock.

*Betaproteobacteria* were only encountered in soil samples in this study. *Betaproteobacteria* consist of several groups of aerobic or facultative aerobic bacteria which are highly versatile in their degradation pathways but also contain chemolithotrophic genera (Dyer, 2003). Many *Betaproteobacteria* are found in environmental samples, such as wastewater or soil (Yeates et al., 2003) and some strains also participate in calcite precipitation. For instance, *Macromonas bipunctata*, a strictly aerobic *Betaproteobacteria* classified as *Burkholderiales*, has been isolated from moonmilk along with *Actinobacteria* and algae (Broughton, 1972). Moonmilk is a soft, white, cream cheese like substance found coating walls and precipitated dripstone deposits in certain caves (Broughton, 1972). These bacteria, assist in the breaking down of wall-rock minerals and their conversion to moonmilk (which is  $\text{CaCO}_3$ ).

*Betaproteobacteria* identified in the soils of Houghton Crater are however not from the genus found in Moonmilk, but some clones were closely related to *Burkholderiales*.

*Deltaproteobacteria* are a minor constituent of the bacterial community in the samples studied. Consequently an ecological function cannot be inferred without more extensive sampling of the bacterial community.

#### 5.2.3.5 *Acidobacteria*

Physiological data about *Acidobacteria* are very limited (only 3 described species). Most of them are uncultured and only known by their 16S rDNA sequences (Quaiser, 2003). They are acidophilic bacteria but they are not necessary limited to acidic environments (Barns et al., 1999). *Acidobacteria* have been identified in various types of soils (Barns et al., 1999), but also in lakes, snow and sludges (Ludwig et al., 1997). Knowledge about their ecological functions remains limited. The fact that *Acidobacteria* were found in low quantities in all the clone libraries (with the exception of the silica crust SC1) shows how they are widespread in soil-type environments.

#### 5.2.3.6 *Gemmatimonadetes*

*Gemmatimonadetes* is a phylum very recently described that contains only four isolates (Joseph et al., 2003). Based on Mummey et al. (2006), *Gemmatimonadetes* seems to be common in soil environments. This phylum was observed in four of the six clone libraries studied here but no apparent trends were observed. These results suggest that *Gemmatimonadetes* may be widespread in Houghton Crater in low numbers and that a higher resolution of the bacterial communities may find a more even distribution of its presence among the libraries.

### 5.2.4 Ecological fingerprint of the microbial community in endostromatolites from Houghton Crater.

The ecological niches described in the previous sections for the bacteria found in the endostromatolites of Houghton Crater allow us to hypothesize about the

environmental conditions prevailing within the fissures of the carbonate precipitates. Given the fact that the vast majority bacteria found within the endostromatolites closely match several chemoheterotrophic aerobic bacteria, it is most likely that within the fissures, the redox conditions are oxidizing. In addition, we can rule out that seasonal reducing conditions occurred in the fissures, because no anaerobic bacteria were identified. This has important implications because it contradicts previous studies which have suggested that endostromatolites formed in anoxic water-saturated fissures as a result of the metabolic activity of methanogenic or acetogenic microorganisms (Clark et al. 2004, Marschner, 2007). The different findings could be due in part to the fact that endostromatolites from Haughton Crater are not found as deep within the bedrock as those from the Yukon, as previously reported by Lauriol and Clark (1999) and Clark et al. (2004). The endostromatolites observed at Haughton Crater are also smaller than those from the Yukon. However, based on our field observations and laboratory tests, the Haughton Crater samples fit the description of “endostromatolite”, as proposed by Clark et al., (2004).

Finally, it is important to mention that endostromatolites have likely grown over a period of >10 000 years (Clark et al., 2004) and as a result, it remains difficult to assess the age of the DNA extracted from the samples. DNA extraction procedures were designed to extract any DNA that could be found within the endostromatolites. Under ideal conditions, DNA has been shown to remain PCR amplifiable for 400 Kyr (Willerslev et al., 2004). Therefore, the community composition and structure of the endostromatolites could represent a mixture of all the microorganisms that have been mineralized within the structure for the last thousands of years but certainly are not contaminant bacteria carried over from the bedrock when it was deposited & lithified. Unfortunately, the age of the extracted DNA cannot be assessed, but if “ancient” DNA was indeed extracted, it reinforces the proposed hypothesis that the conditions prevailing during the formation of the endostromatolites in Haughton Crater were suitable for aerobic bacteria to grow and potentially participate in the formation of those unique carbonate structures.

### 5.2.5 Radiotolerance and Thermotolerance in endolithic Arctic soils?

A fair portion of the bacterial communities isolated in this study are thermophilic bacteria and/or radiotolerant bacteria. On one hand, one might think that there was a contamination problem during the extraction/amplification/sequencing processes but on the other hand, thermophilic bacteria have been isolated from Arctic soils (McBee and McBee, 1956). These authors indicated that there is no doubt that thermophilic bacteria are present in Arctic soils and water and that they may constitute an appreciable portion of the bacterial population. In addition, their presence is not due to fecal contamination by birds and other animals, but the same authors pointed out that thermophilic bacteria are present in higher number close to human settlements. According to Boyd and Boyd (1971), thermophilic bacteria are however abundant in certain soils of northern Canada, where there is no known history of sewage, contamination or cultivation, suggesting that some strains occur naturally in water and soils of northern regions. In addition, Antarctica soils also harbour thermophilic strains in the northern Victoria Land (Forsyth and Logan, 2000) and in the McMurdo Dry Valleys (Shravage et al., 2007). The discovery of bacteria normally qualified by their resistance to heat should therefore not be surprising but expected in cold dry environments.

The presence of bacteria resistant to radiation in endolithic environments protected from atmospheric radiation might seem odd at first, but when one knows that bacteria can adapt to various environments, it is not that surprising. *Deinococcus radiodurans* is the most radiation-resistant organisms currently known and is capable of withstanding 1500 kilorads of radiation. The strongest natural radiation sources on Earth only provide 0.05 to 20 rads/year, which is far less than what *D. radiodurans* can withstand (Daly, 2000; Makarova et al., 2001). Other studies have also demonstrated that *D. radiodurans* is resistant to damage from UV, oxidizing agents, electrophilic mutagens, nitric acid and other chemical and most importantly, desiccation (Daly and Minton, 1995; Daly and Minton, 1996; Lange et al., 1998; Makarova et al., 2001). It is also thought that desiccation might have caused *D. radiodurans* to evolve its radiation resistance because dry conditions caused very similar DNA damage (Daly, 2000, Mrazek, 2002). Those



findings therefore indicate that after all, it is not that surprising to find a radiation tolerant microorganism in the Haughton Crater endostromatolites.

#### 5.2.6 Microbial diversity in soils and endostromatolites

Our results indicate that there are several similarities between the Haughton Crater soil and endostromatolite microbial communities, but the soils appear to be more diversified. This is to be expected because soils are widely known to be heterogeneous in nature (Mummey et al., 2003) and they possess microenvironments which can increase the biological diversity of the microorganisms. It is however not surprising to find somewhat similar microbial communities in both endostromatolite and soil samples because soils are composed of weathering products of the surrounding dolomitic bedrock, which provide similar physico-chemical conditions and nutrient levels to both environments. However, endostromatolites offer a much more homogeneous environment in terms of mineralogical composition. This might account for the lower diversity.

#### 5.2.7 Endostromatolites: biomineralization or mineralization?

The microbial analyses suggest that aerobic conditions are currently present in endostromatolites in Haughton Crater. There is however still no direct evidence that bacteria participated in the precipitation of carbonates which composed the laminated columnar structures or that they are currently forming. As mentioned earlier, abiotic mineralization processes can produce the same isotopic signatures as bacterial mineralization under aerobic conditions. If endostromatolites are currently forming, the role of bacteria in the mineralization process cannot be ascertained but at the same time, it cannot be ruled out, because some of the bacteria present in the endostromatolites have previously been linked with carbonate biomineralization (Schabereiter-Gurtner et al., 2001, Imperi et al., 2007, Broughton, 1972). In addition, the presence of bacteria capable of secreting copious amounts of EPS, which can affect the morphology of carbonate crystals, have also been identified in the Haughton Crater endostromatolites. These

bacteria are abundant in the endostromatolites and could potentially be involved in the mineralization process of carbonates.

In summary, the molecular phylogenetic assessment of endostromatolites, silica crust and soils sampled in Haughton Crater has enabled us to conclude that all the samples contain mostly aerobic, chemoheterotrophic bacteria. Based on the community compositions in the soils and crust samples, the predominant environmental conditions were aerobic. This is in accordance with our hypothesis that suggested that if the conditions within the fissures were aerobic, similar microbial communities would be present within the fissures and in adjacent soils. The role of bacteria in carbonate formation however remains unresolved because the isotopic composition of the carbonates within the endostromatolites does not differentiate between biotic and abiotic pathways.

### 5.3 *Extraterrestrial endostromatolites?*

Due to the conditions under which endostromatolites form (i.e., dry and aerobic conditions), they could potentially be considered as a biosignature of past life on Earth and other planetary bodies, such as Mars. The red planet, as we know it today, cannot support life, but past conditions might have been conducive to life. For instance, carbonates have been spectroscopically identified in Martian dust (Quinn et al., 2006). Recent imaging of the Martian surface by thermal emission spectrometry aboard the Martian Global Surveyor has also identified hydrated sulfates on light-toned layered terrains which constitute outcrops in Valles Marineris, Margaritifer Sinus and Terra Meridiani (Smith et al., 2001). These show evidence for kieserite, gypsum and polyhydrated sulfates presence (Gendrin et al., 2005), which suggest that there was once water on Mars. If water was present, it therefore indicates that Mars might have harbored life. Recently, Bibring et al. (2007) suggested that the low level of surface alteration following the Naochim era (where conditions were favorable for life on Mars) could have preserved most of the record of biological molecules, structures or other diagnostic features in clay-rich surface or subsurface rocks. These authors concluded that areas of

high habitability potentially offer exciting targets for future in situ exploration. The work presented in this study offers valuable lessons for the detection of life on Mars, because when this study began, speculative hypotheses were put forward as what type of microorganisms would be present in the endostromatolites. Such was done with morphological studies of structures found in endostromatolites which were inferred to be of biological origin. As well, despite the fact that previous isotopic data indicated that we should look for anaerobic microorganisms, the present results indicate that the microbial community found within endostromatolites from Haughton Crater is essentially composed of aerobic bacteria. This should be taken in account when Mars returned samples are analyzed because unless PCR analyses are performed, one cannot be sure about the presence of microorganisms and the type of environment they lived in. With billions of sequences readily available from planet Earth, the sequences should be compared within a few seconds to all life forms on planet Earth. This technique does however remain a challenge due to its high sensitivity and necessitates optimal conditions in order to obtain confident results. Finally, DNA extraction from endostromatolites and the problems encountered because of calcium binding to DNA, should be taken in account when analyzing samples from Mars.

## 6. Conclusion

Through the molecular phylogenetic assessment of endostromatolites, silica crust and soils sampled in Haughton Crater, this study has found that all environments sampled are harboring mostly aerobic, chemoheterotrophic bacteria. This provides some evidence of an aerobic environment currently existing within endostromatolites. Furthermore, although the biological diversity of the communities in the soil, endostromatolite and silica crust samples is somewhat different in species richness, most of the bacteria that are found in endostromatolites are also found in the other local environments sampled, leading to the belief that the environmental conditions may be similar in all the materials studied.

Whether the endostromatolites are actively forming or are fossil entities cannot however be determined by DNA analysis. Further investigation of the microbial community with more sensitive methods may detect the presence of Archaea or other microorganisms unaccounted by this study. According to the results of this study, biomineralization of carbonate in anoxic water-saturated fissures seems an unlikely scenario for Haughton Crater endostromatolites given the current results unless significantly different environmental conditions were present when these structures formed, which may be the case. As stated before, the findings of this study do provide some evidence for a currently aerobic setting within the endostromatolites but it was unsuccessful in answering the fundamental question regarding the abiotic or biotic origin of endostromatolites. This may in the future be resolved by microcosm experiments but experimental design is hindered by the long period needed to form endostromatolites. As a final thought, this study contributes to understanding the variability of microbial life between specific environments in an arctic desert setting and provides useful data for contrasting abiotic and biotic systems on earth and other planetary bodies, such as Mars.

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