STUDIES RELATING TO THE THEORY OF PANSPERMIA

By

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Thesis submitted to the Department of Molecular Biology and Biotechnology the University of Sheffield, England, in fulfilment of the requirements for the Degree of Doctor of Philosophy.

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2017

IN THE NAME OF ALLAH MOST GRACIOUS MOST MERCIFUL

Dedication

I dedicate this work to my husband Saad, the memory of my father, affectionate Mother. This project is dedicated to my beloved sons Mohamed, Ahmed, Almoatsm and Mustafa, my sweet daughters Fatma, Zenab, Amna and Aisha, also to my brothers and sisters.

Acknowledgements

First, I thank Allah for helping me to complete this Thesis, and the messenger of Allah, Mohammad (peace be on him) who taught that the learning is from cradle to tomb.

I expand my thanks and respect to my supervisor Professor Dr. Milton Wainwright for his support, guidance, constant encouragement all time, helpful advice, assisted me during study and complete my research. I am pleased to give my special thanks to Dr. D. J. Gilmour for his co-supervision and his advice during my study. I am grateful to Professor Jeffrey Green and Dr Mesnage for their constructive discussions, helpful advice and comments throughout my study. My thanks are due to all my family for their encouragement, support and patience throughout the period of my study. The go also to my colleagues in Prof. Wainwright's laboratory (G10) for their friendship, support and continuous encouragement. I would like to thank the Cultural Attaché at Libyan Embassy in London for their support. Finally, I am grateful to Ministry of Higher Education and Scientific Research and Omar Al Mukhtar University for supplying me with a postgraduate scholarship.

Abstract

Panspermia is the theory that life on Earth originated came from space, for example from comets, or other planets. The Theory focuses on microorganisms because large organisms could not achieve the required space travel and impact events. Microorganisms could be protected from lethal factors (such as ultraviolet light, high pressure, low and high temperatures) by being entrapped in cosmic dust and bolides such as meteorites. In order to extend this theory, the following studies were undertaken:

1- Samples of an established meteorite were analysed using Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDAX). Results under scanning electron microscopy showed a presumptive bacterial biofilm consisting of a number of apparent microbial forms. Fulgurite was also analysed using SEM, EDAX and molecular biology techniques; such samples are, of course, formed on Earth are not directly relevant to investigate to panspermia, but remain of interest, nevertheless. SEM studies of fulgurite showed an unusual distribution of numerous spherules, which could be viewed as being bacterial fossils, although it is

concluded that this is unlikely. Bacteria isolated from fulgurite include, *Bacillus cereus*.

2- An attempt was made to verify the theory of neopanspermia that life is continually arriving to Earth from space. If this is the case then it should be possible to find life forms, which are incoming to the high stratosphere, a region which should not be contaminated with biology in excess of 6microns in size. A novel sampling device, carried by a hydrogen–filled weather balloon was used and a variety of so-called "biological entities" (BEs) were obtained from heights of around 30km. The BEs contain only carbon and oxygen, have biological form and were clearly not inorganic, cosmic dust. Confirmation that these BEs were incoming to Earth and not part of the planet's biota was provided by the fact that a) they exceed the theoretical size-limit for a particle to be carried from Earth to the stratosphere and b) the presence of BEs on the sampling disks was not associated with Earth materials such as grass, pollen and fungal spores. Demonstration of the existence of bacteria in the stratosphere was also made using a sampling Drone. It is concluded that BEs are continually arriving to Earth from space.

3- Bacteria isolated from geological samples (K/Boundary clay, Boulder clay, amber, and an ancient salt crystal and a stromatolite sample from Sharks Bay, Australia). The relevance of finding bacteria within these samples relates to negative panspermia (i.e. the possibility that material leaving the Earth, following and impact event, could have transmitted bacteria and other microbes from Earth into space).

4- Locally sampled iron-rich micrometeorites were analysed using Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy. Bacteria isolated from micrometeorites were found to be predominantly species of *Bacillus.* It is concluded that the spore forming genera such as *Bacillus,* would be ideal organisms in relation to panspermia because they are able to survive environmental extremes similar to those found in space.

5-An attempt was made to verify the work of Barber relating to the presence of proteolytic, silver resistant bacteria in rainwater, which Barber concluded related to the panspermia delivery of bacteria from Venus. No evidence of this was obtained in this study, although it is concluded that Barber's work remains intriguing.

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Chapter 1: General Introduction

1. Introduction

The purpose of astrobiology is to search for the origin, distribution, evolution and future of life in the universe (Des Marais *et al*., 2008). Cosmic life, beyond Earth (if it exists) will interplay between microorganisms and their environment, which will be highly extreme compared to the conditions found on Earth. On Earth microbes live at very low temperatures (~ minus 20° C), very high temperatures (up to 115° C), and over a vast range of pH values and, in terms of survival over time, bacteria have been found inside amber, where they have apparently survived for over 25 to 40 million years (Cano and Borucki,1995), and in ancient salt crystals for 250 million years (Vreeland *et al*, 2000).

1.1. Panspermia Hypothesis

The theory of panspermia provides an alternative to the generally held "chemical theory" of life's origin. It was proposed by the ancient Greeks and in its modern form by William Thomson (Lord Kelvin) in 1871 and Arrhenius (Arrhenius, 1908) and more recently by Hoyle and Wickramasinge (2010) (Weber and Greenberg, 1985; Melosh, 1988). Arrhenius suggested that microbes moved through the cosmos by solar winds and by the pressure of starlight into interstellar space (so-called

"radio panspermia"). In contrast, Lord Kelvin suggested that life forms could exist in meteorites, which would protect them from the rigours of interstellar space. Carl Sagan in the mid-1960s, broke down in detail both the physical and organic parts of the Arrhenius situation. In 1973, Crick and Orgel proposed the hypothesis of “Directed Panspermia” which suggests that an intelligent agency sent life to Earth in some kind of vehicle and presumably as part of an experiment.

The most recent and most highly developed theories of panpsermia have been proposed by Wickramasinghe *et al*. (2010) and Hoyle and Wickramasinghe (1979) have been further developed by, amongst others, Joseph and Schild 2010 and Wainwright *et al*. (2010). Hoyle and Wickramasinghe have placed emphasis on the possible commetary origin of panspermic microbes (commetary panspermia) and the possibility that new microbial diseases may have arrived to Earth from space (pathospermia) (Hoyle and Wickramasinghe 1979, Hoyle and Wickramasinghe 1981, Wainwright and Wickramasinghe (2003). Although panspermia is generally discussed in relation to the origin of life on Earth, a more modern variant of the Theory (so-called “neopanspermia”) suggests that life is continually arriving to Earth from space (Wainwright, 2003).

1.2. Lithopanspermia

This version of panpsermia suggests that life is transmitted throughout the cosmos in rocks, i.e. meteorites which are ejected from life-bearing planets by impact events (McSween, 1985, Gladman, 1997; Dones *et al*., 1999; Mileikowsky *et al*., 2000, D'Argenio *et al*., 2001 Melosh, 2003, Porras *et al*., 2003, Lada and lada, 2003). A variety of presumptive microfossils have been found in meteorites suggesting that life may at one time have existed on other planets; the Murchison, Orgeuil, Efremovka and Allen Hills meteorites provide good examples of such potential panspermic vehicles (Hoover 1997, 2006; Pflug 1984; Zhmur Also Gerasimenko 1999; Zhmur *et al*.,1997). As yet however, there is no conclusive proof that such microfossils provide evidence that life has existed elsewhere in the solar system or that living organisms can be currently transmitted via this process. Lithopanspermia has the obvious advantage in that it provides a vehicle in which life forms might be transmitted through space while being protected from the rigours of the space environment notably, exposure to high levels of UVC.

1.3. Microbes able to survive in extraterrestrial environments

Prokaryotes can live during water and nutrient deprivation over long-term periods and some bacteria produce endospores or achieve dormancy by shutting down almost all of their metabolic machinery (Rothschild and Mancinelli, 2001), while Cyanobacteria such as *Synechococcus* can survive in evaporate. Dormant bacterial spores from insects embedded in amber for 40 million- years can be resuscitated (Seckbach and Oren, 2000) and a halotolerant *Bacillus* has been apparently isolated from a 250 million years antique salt crystal (Vreeland *et al*., 2000). Microbes found in ice cores obtained from the bottom of Lake Vostok in Antarctica may serve as a model simulating conditions inside the permafrost subsurface place of Mars or Jupiter's moon Europa (Seckbach and Oren, 2000). In addition, hyperthermophilic microbes may resemble existing forms present on warm planets such as Venus or the volcanically active moon, Io (Des Marais and Walter, 1999). Such studies point to the possibility of cometary transfer with microbes surviving inside the melted centre of icy comets (Wickramasinghe, 2003). Microbes can even survive within the excessive vacuum of space and exposed to severe galactic radiation. An indication of the survivability of bacteria in the space environment is indicated by the fact that *Streptococcus mitis* was isolated from a camera on board Surveyor 3 after 31 months on the

Moon (Jones, 1995). *Deinococcus radiotrans* can survive five million years (Battista, 1997) and microorganisms also possess remarkable DNA-repair mechanisms (Battista *et al*., 1999). *Deinococcus* is capable of surviving sterilising solar photons in space and in an Earth orbit experiment, samples of *Bacillus subtilis* were shown to survive severe sun-derived ultraviolet radiation when shielded by a monolayer of dead cells (Horneck, 1998). Even if only one spore out of 10,000 can avoid lethal cosmic rays for hundreds of thousands of years this survival rate would be significant in relation to panspermia (Nicholson *et al*., 2000).

1.4. Transfer of life-bearing meteorites from Earth to other planets

A number of scientists now believe that microorganisms can be transferred to and from various planets and moons, including from Earth to different universe (Wainwright *et al*., 2010, Joseph and Schild 2010a, b; Napier and Wickramasinghe 2010;). Such "negative panspermia" could have, for example, seeded Mars, or *vice versa* (Joseph and Schild, 2010a, b ;). While panspermia has been hypothesized for quite a while, there has been a late upsurge of enthusiasm for the theory following claims of potential extraterrestrial fossils and organic markers in meteorites (Gillet *et al*., 2000; McKay *et al*., 1996), and possibly microbes in comets (Pizzarello,

2004) and other heavenly bodies (Kwok, 2009); notable amongst these is the debatable fossil evidence for microbial life in the ALH84001 meteorites (McKay *et al*., 1996).

Over 24 thousand meteorites have reached the Earth, around 130 having originated from the Moon and 35 from Mars. A number of these meteorites never achieved temperatures above 100°C, and it has been argued that three Martian meteorites - Allen Hills, Nakhla and Yamato - hold fossilised bacterial mats (Gibson *et al.*, 2001). These rocks constitute hard proof that material can be exchanged among the internal planets, and in the process do not achieve a temperature, which is sufficiently high to sterilize their interiors.

An incoming body sufficiently large to punch its way through the atmosphere (where it exists) of one of the internal planets may strike the ground at a few several kilometres a minute, and produce a hole and ejecting stones and soil at high speeds upwards. This may yield discharges into space without much warming occurring within the stones being tossed upwards at about the speed of the impactor (Melosh, 1984). A 20 km bolide striking Mars at 15 km/s, would bring about the launch of something like 30 million tonnes of debris with mean size of 6 m (i.e. more

than adequate to ensure microorganisms would be internally protected from cosmic extremes).

Calculations suggest that the portion of earthbound material ending up on Mars following a 10-million-year period of impacts would be around 0.16%. Even a gram of basalt may contain 10^{7}-10^{8} microorganisms within fissures (Haldeman *et al*., 1994), making possible the two-way transfer of microbes between Earth and nearby planets. Such possibilities rely on whether life forms are able to survive the stun-effect of being tossed into space. Burchell *et al*. (2001) utilized a gas weapon to discharge pellets containing microorganisms at high speeds onto strong surfaces. At stun weights of 30 GPa, survival divisions of 10^{-4}–10^{-6} were recorded, declining as stun weights increased, making inter-cosmic transfer of viable microbes a distinct possibility.

Horneck *et al*. (2008) have confirmed the above outcomes for an assortment of microorganisms and have shown that the associated stun weights are in the region deduced for Martian meteorites and confirm that a large number of microbes are likely to survive discharge by a substantial effect, thus opening the way to the transmission of life between planets.

Mileikowsky *et al.* (2000) have also suggested that ejected material may ride on the vapour plumes, making it simpler to accomplish escape speed.

Al-Mufti *et al.* (1986) confirm that dried microbes and spores can survive heating to no less than 350°C for 30 seconds. Wallis and Wickramasinghe (2004) have argued that over the lifetime of the close planetary system a normal of ~3 kg of un-sterilised planetary ejecta may get inserted into the surface layers of Edgeworth-Kuiper Belt comets (an arrangement of some 109 comets circling just past the edges of the planetary framework); in all probability microorganisms would need to be protected in extensive rocks for a great many years before critical quantities of them could move to the Edgeworth-Kuiper (EK) area.

Early claims by Claus and Nagy, (1961) that fossilised bacteria reside in carbonaceous meteorites were discounted because the samples were contaminated by ragweed pollen. Further work was done on the Murchison meteorite in which thin sections of the meteorite were placed on membrane filters and leached with HF gas to leave indigenous carbonaceous structures intact (Napier and Wickramasinghe, 2010). Laser ion probes established that the structures had a biological provenance, and contamination was thus ruled out. Carbonaceous chondrites constitute a

few percent of known chondrites, the most primitive of which may contain up to 20% water and various minerals as well as clay-like hydrous phyllosilicates, amino acids and aromatic hydrocarbons (Aleon *et al.*, 2009). Carbonaceous meteorites also contain many other notable strong chemical biomarkers including purines and pyrimidines (nitrogen heterocycles of nucleic acids); and phytane (components of the chlorophyll pigment); similarly, morphological biomarkers (microfossils of filamentous Cyanobacteria) have been described by Hoover (2010).

1.5. Survival of space microorganisms during panspermia

Geomicrobiolgy and astrobiology impact directly on the possible existence of life elsewhere in the cosmos. Joseph (2009) for example, has hypothesized that the beginning of life on Earth started from microbes and spores that survived in the nebula which gave birth to our plant. The modern version of the view that life did not originate on Earth but was derived from space, comets, or other planet in rock after impact events comes from the theory of panspermia (Hoyle and Wickramasinghe 1979). The theory of panspermia focuses on microorganisms because large organisms could obviously not undertake the required space travel following impact events (Wainwright, 2003). Microorganisms, in contrast,

could be protected from lethal factors such as ultraviolet by entrapment in cosmic rocks (Wainwright *et al*., 2009). Hoyle and Wickramasinghe, (2000) also suggested that bacteria such as species of *Sarcina* and *Staphylococcus* could be protected by forming clumps of individual cells, the outer ones of which become carbonized and thereby protect the inner cells from UV; a covering of cosmic dust would achieve the same effect. Studies by Al-Mufti *et al*. (1984) have shown that *Escherichia coli* can also survive high pressure. Burchell *et al.* (2004) extended the lithopanspermia theory (i.e. that life is exchanged between planets inside impact event-ejected rocks). Microbes have also been isolated from the stratosphere, some of which it has been suggested originate from space; *Bacillus simplex* and *Staphylococcus pasteuri* and the fungus *Engyodontium album* for example have been isolated from a height of 41km (Wainwright *et al*., 2003). Griffin (2004) also isolated *Bacillus luciferensis* and *Bacillus sphaericus* and a species of *Penicillium* from a height of 20Km. The stratosphere is an extreme environment and it could be argued that bacteria are unlikely to survive the low temperatures, or exposure to high ultraviolet radiation. However, a number of mechanisms have been proposed to explain how bacteria found in the stratosphere, as cells survive the extremes found in deep space (Wickramasinghe, 2004).

Vreeland *et al.* (2000) for example provided evidence that microbes can remain dormant and survive the hazards of space, perhaps for millions of years. It is also suggested that the debris produced during supernova formation can carry living spores and as some of this debris falls to Earth; as a result, life on Earth may have originated, or be supplemented by organism from other planets (Wickramasinghe, 2004).

1.6. Aims of the study reported in this Thesis

The aim of the study reported in this Thesis was to study cosmos-derived and geological materials from Earth, which have a bearing on astrobiology, and to isolate and identify bacteria from these samples. The studies were conducted in relation to adding further weight to the theory of panspermia.

Chapter 2: Studies on Presumptive Microbes in a Commercially Obtained Meteorite

2.1. Introduction

A meteorite is a hard fragment of rock originating from such sources as asteroids or comets that populate outer space and survives its impact with the Earth's surface; it is referred to as a meteoroid before its impact. The study of meteorites can reveal how the solar system first began, what life (if any) is like on other planets and how these planets were formed. In short, when rocks originating elsewhere in the solar system land on Earth, they carry with them information about the planets from which they originated.

Meteorites could also potentially bring evidence for the past existence of other life forms. Considerable debate followed claims by Glaus and Nagy (1961) of finding evidence for fossilized extraterrestrial life in a meteorite. One highly controversial meteorite, ALH84001 was discovered in Antarctica in 1984 and the electron microscopy showed that ALH84001 contains very tiny grains of a magnetic–mineral made of oxygen and iron surrounding tubes of width 20 nm and a length of 100nm, which looked like nanobacteria. A group of scientists at NASA led by David McKay, (McKay *et al.*, 1996) claimed to have found evidence of bacteria having lived on the meteorite when it existed as a rock on Mars. A huge number of

studies have been conducted to rebut or confirm this hypothesis, but no conclusive proof has emerged and the idea that these worm–like forms are fossilized microbes remains putative. Other putative fossilized bacteria have also been seen in an ancient Earth samples (Schopf and Packker, 1987).

Wainwright *et al.*, (2013) discovered fossilized "wormlike forms" in a claimed new sort of carbonaceous meteorite, which later fell down on

Polonnaruwa, Sri Lanka. Schopf has detected such worm-like forms in other meteorites notably the Martian Allen Hills sample and a lunar meteorite. In a fragment of the inner surfaces of the Ala, Ivunga, Orguell C11 carbonaceous meteorites, evidence of microfossils, similar to Cyanobacteria, has been discovered by Hoover (2011), who claimed that these fossilized bacteria were once living organisms in the original forms of meteors, moons, comets, and are not Earth contaminants. Wickramasinghe (2012) claimed the presence of fossilized diatoms in samples of a meteorite, which fell in Polonnaruwa. Putative Cyanobacteria, fungi, algae, nanobacteria, spores, diatoms, and protozoa have also been discovered by (Hoover, 2006) in deep ancient ice cores over 4,000 years old, drilled from Lake Vostok. A number of microbes recovered from Lake

Vostok also increased with increasing numbers of cosmic dust particles, which fell to Earth from space (Abyzo *et al*., 1998). A number of papers have also reported the existence of bacteria-like features in Martian and other meteorites (Claus and Nagy, 1961; Hoover, 2010; Sears and Kral, 1998, Steele *et al.*, 2000); the most famous of course starting the claimed nanobacteria-like, fossilized structures existed in the Allen Hills (AH 84001) meteorite (McKay *et al*., 1996). As was mentioned above, to date none of these claims have been fully substantiated, largely because meteorites contain mineral features which closely mimic bacteria and other microorganism making it difficult to distinguish between potential fossilized bacteria and non-biological artefacts (McSween and Harvey, 2010). The aim of the work reported here was to use scanning electron microscopy (SEM) to examine a sample of a commercial sourced Martian meteorite of known provenance to look for bacteria-like structures and if found, to give proof to show that such bacteriomorphs are biological in origin and are not mineral artefacts.

2.2. Materials and methods

2.2.1. Sampling of the Northwest Africa 4925 meteorite

The single sample supplied from a dealer was cut from an originally larger meteorite sample, which was catalogued as Northwest Africa 4925 NWA 4925; the fellow members of the International Meteorite Collectors Association confirmed the authenticity of the sample. The meteorite was recovered in 2007 from Erfoud, a town in the Sahara Desert, in the Meknès-Tafilalet of the Maghreb region in eastern Morocco. The fragment which was used was covered partially with a fusion crust, and showed a porphyritic texture with large chemically zoned olivine megacrysts set into a fine-grained groundmass composed of pyroxene and maskelynite; minor phases include chromite, sulphides, phosphates, and small Fe-rich olivines. Olivine megacrysts often contain melt inclusions and small chromites. Its mineral composition (EMPA) is: Olivine, $Fa_{27.6–46.8}$; pyroxene, $Fs_{20.0–37.7}Wo_{3–14.8}$; maskelynite, $An_{67–69}$. It is classified as an achondrite (Martian, olivine-phyric shergottite); severely shocked with some melt pockets; moderately weathered.

2.2.2. Sterilization of the meteorite sample, and lyses of potential contaminants modern biofilms

The sample was immersed in 70 percent ethanol for an hour, and then washed twice with sterile, deionized water, before being transferred to a sterile Petri dish prior to being examined under the SEM. The water used in the sterilization process was sterilized in the autoclave at 120^0C for twenty minutes, and then filtered through 0.1 µm micropore filter (Nalgene).

2.2.3. Scanning electron microscopy examination

After sterilization, the meteorite sample was positioned inside a staging chamber, with the side to be analysed flush to the base. Konductomet phenolic mounting compound (20-3375-016) was used to stage the sample. Similar procedures usually involve grinding then polishing of the surface of the sample during this stage, however, during the work described here, only an instantaneous process of grinding was performed, in the aim of removing any build- up that might be present on the upper surface to be examined, so that only freshly material would be viewed. The coarseness of pile used was 120 microns using a Bueler Automet 250 for 5 seconds with a touch force of 20N, a head speed of 50 RPM and a Platen speed of

140 RPM. A second sample was prepared presenting the outside surface of the meteorite. This was staged on top of a conductive carbon tab. Due to its relatively low conductive nature the sample was coated using an Emscope gold sputter coater, so as to minimize charging effects and optimise image acquisition. The sample was coated for a deposition-duration of 1 minute at 15 milliamps. Before being introduced into the SEM, the sample was placed in a vacuum chamber overnight to remove any remaining moisture from the porous sample. No chemicals or concentrated alcohols were introduced at any stage as a cleaning step. The sample was finally delicately irrigated using de-ionised water.

2.3. Results and Discussion

A presumptive bacterial biofilm contains a number of forms which look notably similar to bacteria and which had they been detected on the surface of a terrestrial soil or rock sample would be readily considered by most microbiologists as comprising a distinct bacterial biofilm

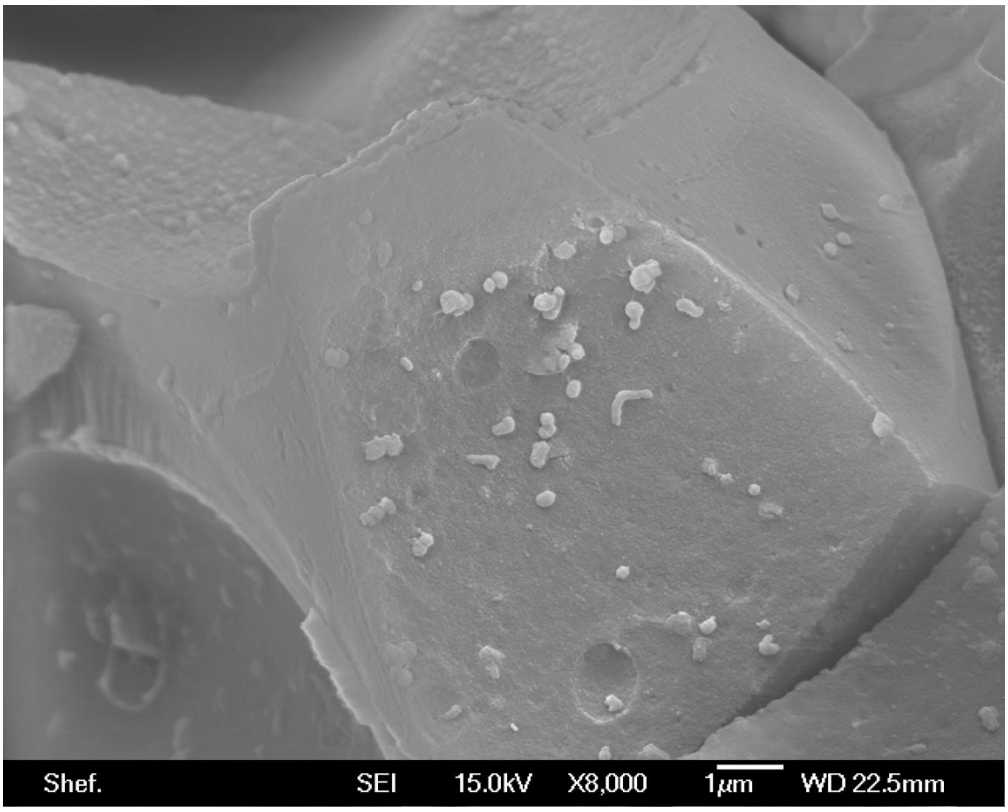
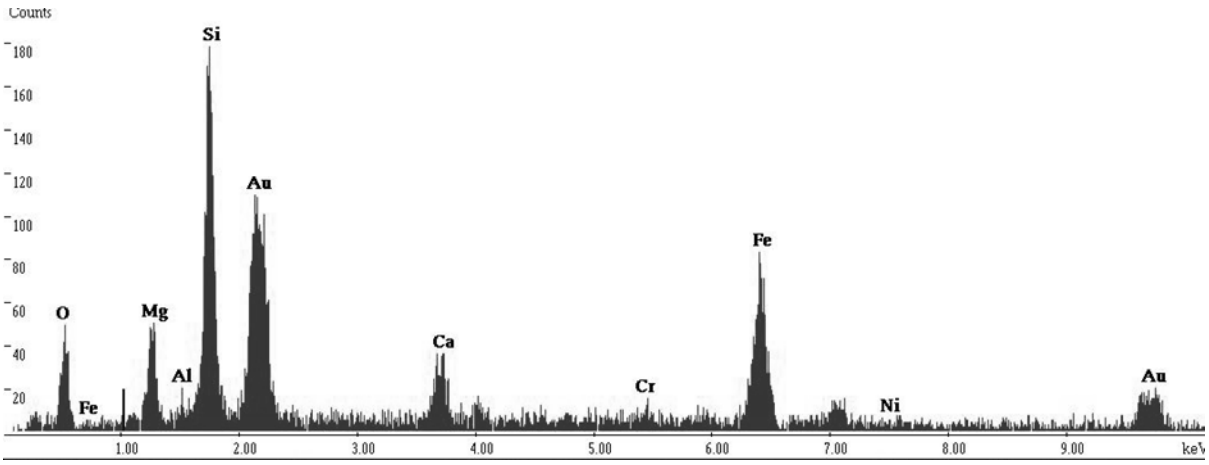


Fig.2.1. Bacteria-like objects on the cut surface of the Northwest Africa 4925 meteorite sample.

The image shows what appears to be individual bacterial types with obvious cocci, rods, individual rods and spiral shapes bacteria-like chains. The chemical composition of both an adjacent non-biofilm region and the presumptive biofilm area, as identified by EDAX, is shown in Fig 2.2 to

A\



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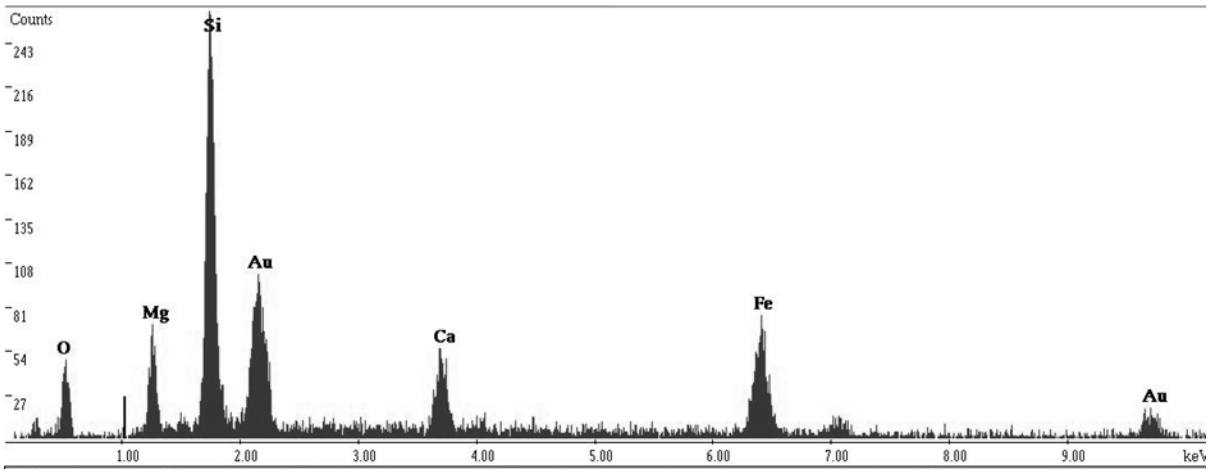

Figure2.2. The chemical composition identified by EDAX of a), the presumptive "biofilm" region, and b) an area away from the "biofilm" (non-biofilm region) be essentially similar and made up essentially of iron, magnesium, calcium, silicon and oxygen.

be basically identical and being made up essentially of iron, magnesium, calcium, silicon and oxygen (. e. a composition model of a meteorite). The sample has clearly been cut from a larger piece, so that the outside surfaces would originally have been inside the meteorite. The surface of the sample was carefully scanned and imaged.

It is suggested that Fig 2.1 shows a fossilized bacterial biofilm made up of typical bacterial forms, which would have originally been located within the original meteorite from which the sample was cut. It could be stated that the image is of a recent bacterial biofilm, which presumably formed after the meteorite landed on Earth. The fact that the bacteria show no signs of lysis, after having been exposed to 80% ethanol, however, suggests otherwise; a modern terrestrial biofilm might have undergone mineralization during the period when the meteorite resided on Earth, but this seems highly unlikely. Similarly, it is highly unlikely that a modern terrestrial biofilm could have formed from an air-derived bacterial inoculum, while, the meteorite was kept in storage.

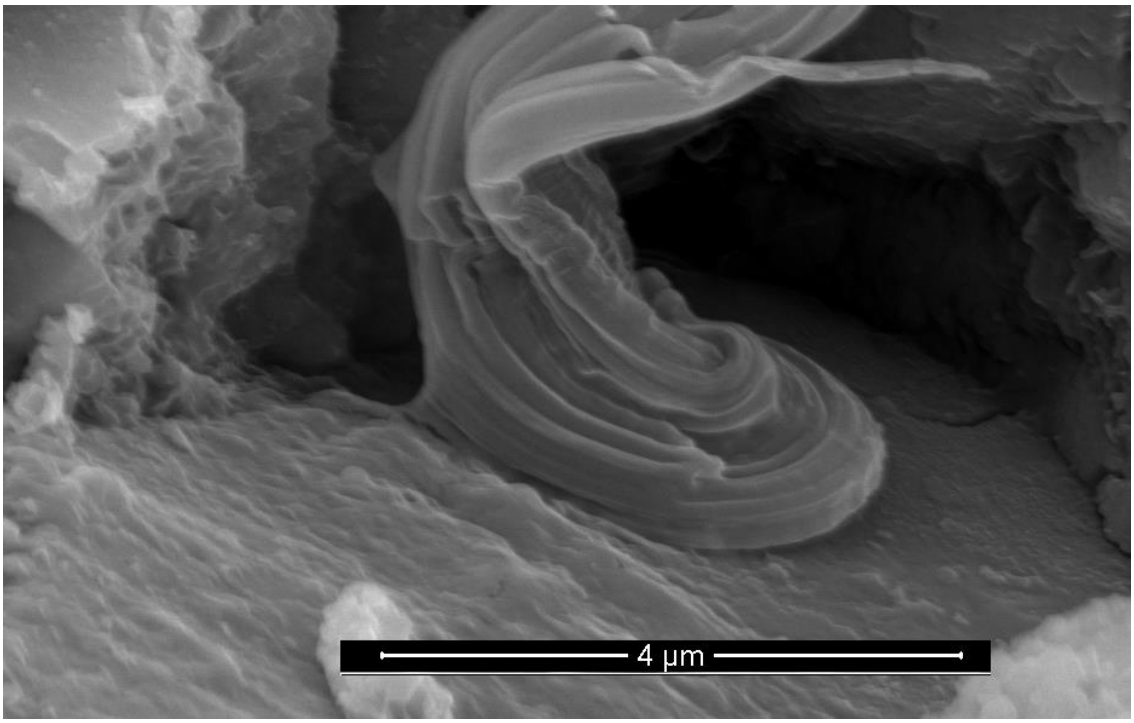

Fig.2.3. Filamentous material found inside the Northwest Africa 4925 meteorite sample.

Figure 2.3 shows a mass of filamentous material inside the Northwest Africa 4925 meteorite sample. The filaments appear biological and are similar to those found by Hoover (2011) in a number of different meteorites, which he suggested are fossilized Cyanobacteria of extra-terrestrial origin. The EDAX of this material (Figure 2.2) was found to be the same as the meteorite background mass, so this is not a modern contaminant. Unfortunately, there is no obvious way of demonstrating the

biological nature of this filamentous mass, and the critic would argue that it is a mineral mass simulating biological material.

In both of cases of presumed fossilized microbe shown above, EDAX analysis shows that the presumptive biofilm region is mineralized and is made up essentially of the same inorganic material found in adjacent meteorite regions lacking a presumptive biofilm, results which we suggest, confirms, that the presumptive biofilm is a component part of the Mars meteorite, rather than being a modern biofilm, comprised of contaminating, terrestrial bacteria.

Critics could argue that the observed bacteria-like structures are simply mineral artefacts simulating the morphology of bacteria, which would prove to be a remarkable coincidence. The bacterial forms seen here, unlike those seen in the Allen Hills meteorite (which are nano-sized), are around 0.2 microns (i.e. similar in size to terrestrial bacteria found in natural, nutrient-limited environments found on Earth (McKay *et al*.1996) and again, in comparison to the Allen Hills form, the putative bacteria described in the current work are sufficiently large to contain a complete bacterial genome.

It could be argued that the bacteria-like forms, seen here are too similar to terrestrial bacteria, a criticism which is only valid if it is assumed that bacteria from a non-terrestrial source would, necessarily, differ in their morphology from Earth-based organisms.

2.4. Studies on the Micrometeorites

2.4.1. Introduction

The largest source of extraterrestrial material travelling to Earth is in the form of microscopic dust, known as micrometeorites, which range in size from 25 microns to 2 mm in diameter. Such micrometeorites are unlike larger meteorites, the annual flux of the former being estimated at 30,000 tonnes, whereas the meteorite flux is estimated at only 50 tonnes per year (Love and Brownlee, 1993). Micrometeorites are sourced from a much greater variety of extraterrestrial matter than are the more familiar meteorites (Brownlee, 1985). When micrometeorites enter the atmosphere they are heated by friction and most are destroyed although those that do not are generally covered with a thin, black iron oxide rim. Micrometeorites are divided by their degree of thermal change into, un-melted MMs, partially melted MMs, and extensively melted MMs (Genge

et al., 1997; Kurta *et al*., 1994). Not surprisingly, un-melted MMs provide us with the most information on the nature of their parent bodies.

Micrometeorites have been collected from a wide area of environments in space and on the Earth's surface and from deep sea sediments (Blanchard *et al*., 1980; Brownlee 1985), glacial lakes in Greenland (Maurette *et al*; 1986). A large number have also been collected from filtered Antarctic ice melt and these provide important evidence about small bodies in the solar system (Maurette *et al*; 1991; Taylor *et al*., 1998). Other large-sized cosmic spherules have been collected from Polar Regions by (Rochette *et al*., 2008), where they arrived some million years ago. Extraterrestrial dust samples, also known as interplanetary dust particles have been collected in the stratosphere by NASA ER-2 aircraft (Brownlee, 1985). Micrometeorites have a mineral composition close to that of both comets and asteroids, and most are ferromagnetic in nature due to presence of magnetite which forms during atmospheric entry. As a result, simple magnets can be used to collect them. Micrometeorites tend to be rich in olivines and pyroxenes (Imae *et al*., 2013), and most are derived from carbonaceous chondrites.

2.4.2. Material and Methods

2.4.2.1. Collection of micrometeorite from rainwater and gutters

Clean containers were left outside to collect rainwater. Any collected water was filtered by Whatman filter paper No 1, which dried and any residue was examined. A strong, praseodymium magnet (fig: 2.4) was then placed in a plastic bag and drawn gently across the collected material; any material that did not adhere to the magnet was discarded. These particles were then transferred to a microscope slide and examined under the light mioscope, finally being preserved in a glass bottle containing a small amount of distilled water.

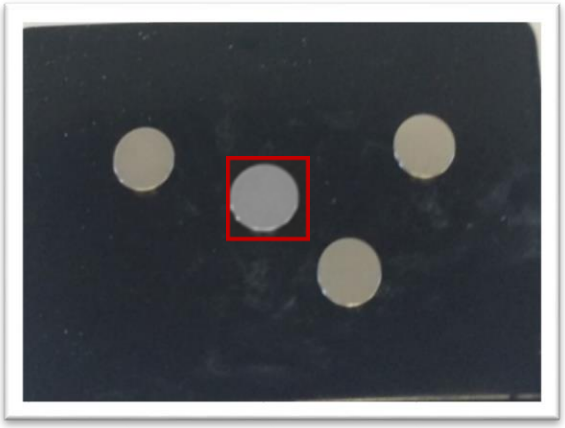

Fig.2.4. A strong, praseodymium magnets, magnet size bordered by red box.

2.4.2.2. Collection of micrometeorites from soil might be earth iron

A variety of soils was collected from the Sheffield region and then suspended in distilled water and filtered over Whatman filter paper No1.The strong magnet, used above, was then passed through the material and examined as previously described. The results show that micrometeorites were collected from all locations using magnetic separation of iron-rich material; examples are provided in Fig, 2.5.

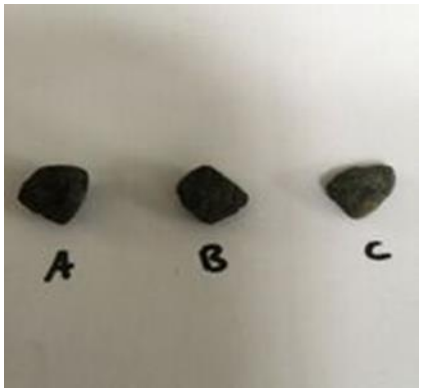

Fig: 2.5. Large micrometeorites obtained from Firth Court Building guttering.

2.4.2.3. Scanning E/M of micrometeorite

The meteorite samples were immersed in 70 per cent liquid bleach for 20 minutes, washed with sterile distilled water and moved to a sterile, plastic Petri dish before being scanned under the SEM.

2.4.3 Results

The micrometeorite samples were scanned under the scanning electron microscope (SEM). Both the outer and inner surfaces were scanned in detail; no evidence was found of any structures which could be regarded as being presumptive biological structures. The EDAX of both samples showed that the micrometeorites are rich in silicon and metals; the outer surface also contains iron (i.e. they have a composition which is typical of meteorites fig 2.5).

2.3.4 Discussion

Bacteria were isolated from micrometeorites collected from the rainwater and from Firth Court roof guttering. EDAX analysis confirmed the micrometeorite nature of the particles. Since the micrometeorite samples were sterilised before breaking, it is assumed that the bacteria originated from within the samples. However, the obvious criticism of this approach relates to contamination (i.e. the bacteria could have contaminated the

particles during transfer to the Nutrient Agar plate). Unfortunately, there are no adequate controls to demonstrate that the bacteria must have originated from inside the micrometeorite particles. It is noteworthy that the bacteria isolated from micrometeorites were predominantly species of *Bacillus.* Since these are spore formers, this would fit in with the possibility that they can survive extremes, such as those found in space, and in terms of time over which the amber samples have existed. The reality is that in relation to this kind of research, critics can (and do) always invoke contamination as an explanation for any research findings. Such criticism needs of course to be answered, but the reaction of many critics to this research is one of unremitting disbelief. Perhaps the only way in which such (sometimes valid) criticism can be answered is by building up a wide range of experimental evidence in favour of panspermia, which we hope to continue.

In conclusion, results shown are that the micrometeorites contain bacteria although there is no direct evidence that they have a space origin. The results are show, relevant to negative panspermia since micrometeorite would act as ideal vehicles for the transport of microbes from Earth to the cosmos following an impact event.

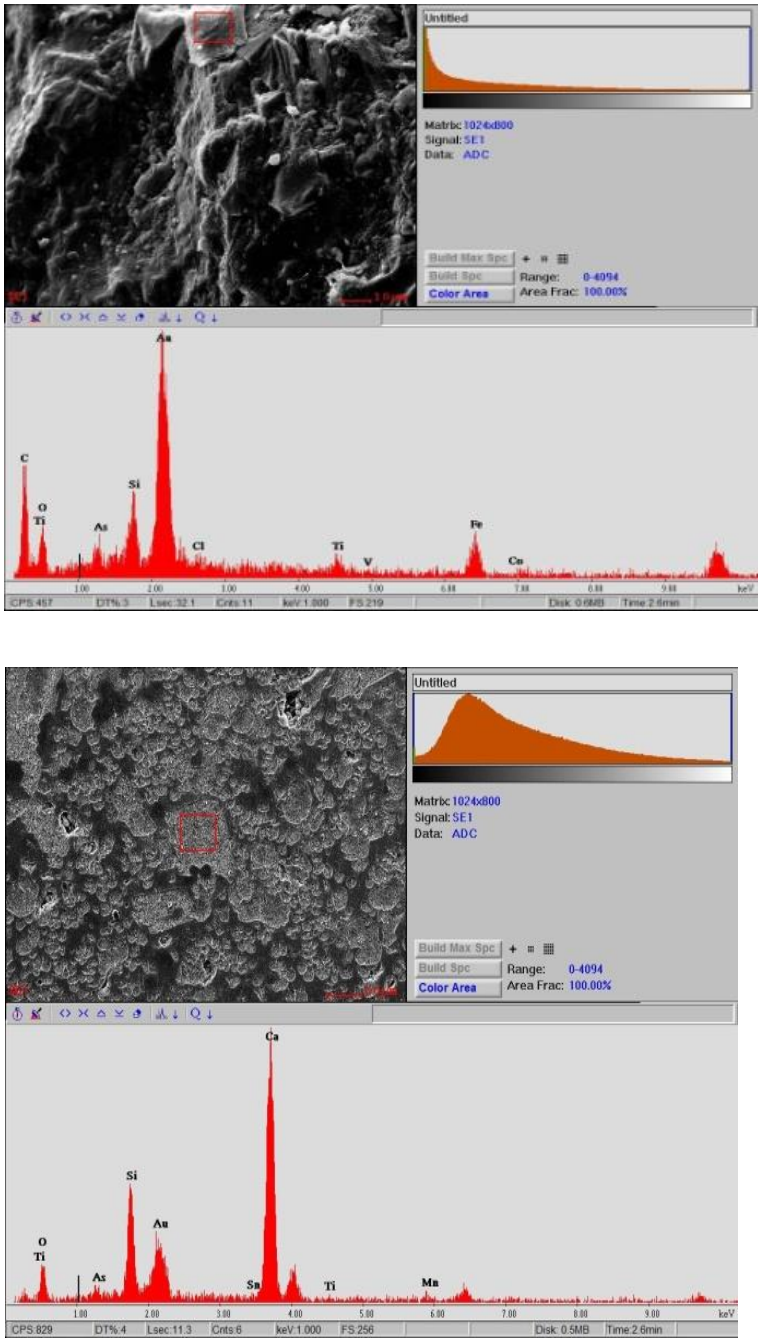

Fig.2.5. The chemical composition for micrometeorite samples determined by EDAX (confirmed that micrometeorites are rich in silicon and metals and the outer surface also contains iron).

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Chapter: 3 Isolation of Biological Entities from the Stratosphere

3.1. Introduction

The current theory of panspermia proposed by Hoyle and Wickramasinghe states that microbes are carried to Earth within meteorites and comets (Hoyle and Wickramasinghe, 1981) or are delivered in a "free state" protected by cosmic dust and carbonized cosmic organics. Neopanspermia presents an extension of the panspermia theory by suggesting that if microbes delivered life to Earth from space, then this process must be continuing, so that and life should be still being impacting our planet in the cosmic dust which is brought to Earth in a continuous stream (Wainwright, 2003). In order to demonstrate the veracity of such a possibility it would be desirable to capture, and identify, any arriving organism arriving from deep space. Such an approach is however, limited by the fact that, since space lacks an atmosphere, any arriving material (unless an aerogel-type material is employed) would be atomized on any sampler located at say the height of the International Space Station (400km) (Tabata *et al*., 2011). The stratosphere on the other hand, having an atmosphere, which can slow down incoming cosmic material, provides an ideal place from which from which to obtain biological material which is incoming to Earth from the cosmos (Wainwright, 2008; Smith *et al*., 2010). Such an approach has recently been realized using balloons sent into the stratosphere which carry

various types of samplers capable of capturing incoming microbes for subsequent analysis (Wainwright *et al*., 2004; Yang *et al*., 2008b; Smith *et al*., 2014; Wainwright *et al*., 2015).

The atmosphere of Earth comprises (starting from the bottom), the troposphere, stratosphere, mesosphere, and finally the thermosphere (Brasseur and Solomon, 2006). The troposphere, being the lowest region starts from the Earth's surface and continues to an average height of 12 km, while the maximum height of the troposphere at the equator is 17 km (extending at the poles to only 9 km). This region is warmest at the bottom and decreases with height to about −60 °C at the upper extremity; since it holds about 80% water. Since it is the densest atmospheric layer, Earth's weather changes take place within this region (Wayne, 1991).

The stratosphere extends to an altitude of 55 km above the surface and is separated from the troposphere by the tropopause. Unlike the troposphere, the temperature in the stratosphere eventually increases with height to an average of 0°C at its highest point because this layer contains ozone (O3), which absorbs any ultraviolet radiation energy coming from the sun (Brasseur and Solomon, 2006). At the upper edge of the stratosphere is

the stratopause, which separates it from the mesosphere.

Unfortunately, sampling balloons can be elevated to a height of around 42 km into the stratosphere, a fact which limits the sampling strategies used to determine if life is incoming to Earth from space (Hartmann *et al*., 2001; Wainwright *et al*., 2006). The mesosphere begins at around a height of 50 km above sea level and continues upwards to 80-85 km in the mesopause. This is the coldest layer of the atmospheric layers at about −85 °C; temperatures at the base of the mesosphere are around 0°C and continue to fall until they reach below -100°C into the mesopause region (Brasseur and Solomon, 2006; Sullivan, 2013). Finally, the thermosphere, a region which stretches from the mesopause up into thermopause (500-1000 km) has a temperature which increases with elevation to as high as 1500 °C (Figure 2 1); the lowest part, the thermosphere contains the ionosphere, a region devoid of any meteorological phenomena (Smith, 2003).

3.2. Microbes of the high atmosphere

The troposphere is the region where the majority of the weather changes take place, concentrated within the boundary layer extending to 2 km above the Earth and where most of the heavy air-mixing occurs with any particles which are small enough to be elevated, including aerosols, dust, smoke, and masses of terrestrial microbes (Kellogg and Griffin, 2006).

Industry contributes to this biology from factories, waste-water treatment, and agricultural all of which contribute microbe-rich aerosols (Smith, 2013). The flora of the lower atmosphere is by no means passive since, as Bauer *et al.* (2002) have shown bacteria act as nuclei in the formation of clouds and ice.

High altitude particles can be sampled using high flying aircraft (DeLeon-Rodriguez *et al*., 2013, Yang *et al.*, 2009) or sounding rockets (Imshenetsky *et al*., 1978), although their findings have been questioned (Smith, 2013). Balloons are cheaper than rockets and are usually made using polyethylene and are elevated with helium or the much cheaper (and more flammable) hydrogen. A sampler can then be attached to the base of the balloon and opened at the desired height, its capacity depending on the lift provided by the balloon.

The most important balloon launch related to the work described in this Thesis was elevated over India on 21 January 2001 (launched from the National Scientific Balloon Facility of the Tata Institute of Fundamental Research, Hyderabad, India (Harris *et al*., 2002). The balloon lifted a cryosampler which sampled air from 20-41 km. SEM analysis of the samples obtained provided evidence of viable microbial cells at heights of

41km. Initial analysis reported only viable, but non-culturable microbes, further isolation work by Wainwright *et al*. (2003) however, yielded *Bacillus simplex* and *Staphylococcus pasteuri*) and the fungus, *Engyodontium album*; viable staining techniques also demonstrated the presence in the stratosphere of living bacterial cells.

3.2.1. Materials and Methods

The sampling box, made primarily from Styrofoam (fig.3.1,3.2), had a build-in drawer mechanism (i.e. a standard CD tray) that could be automatically opened and closed at the desired altitude by pre-programing in the data before the launch. This drawer, shown in fig., 3.4 carried sterile SEM stubs, which collected the incoming particulate material substances, which fell onto it. The sampling apparatus was shielded from the possible downfall of particulate matter from the balloon (fig.3.3) itself by means of a cover. Before the launch, the sampling drawer was scrupulously cleaned, air-blasted, and swabbed with 70% alcohol to ensure no remaining contaminants were present.

Figure 3.1. The Styrofoam stratosphere sampling box.

Figure 3.2. Inside the sampling box and associated GPS system.

Figure 3.3. The balloon (blue), being filled with hydrogen from cylinders prior to launch. The parachute is at the top of the balloon (orange).

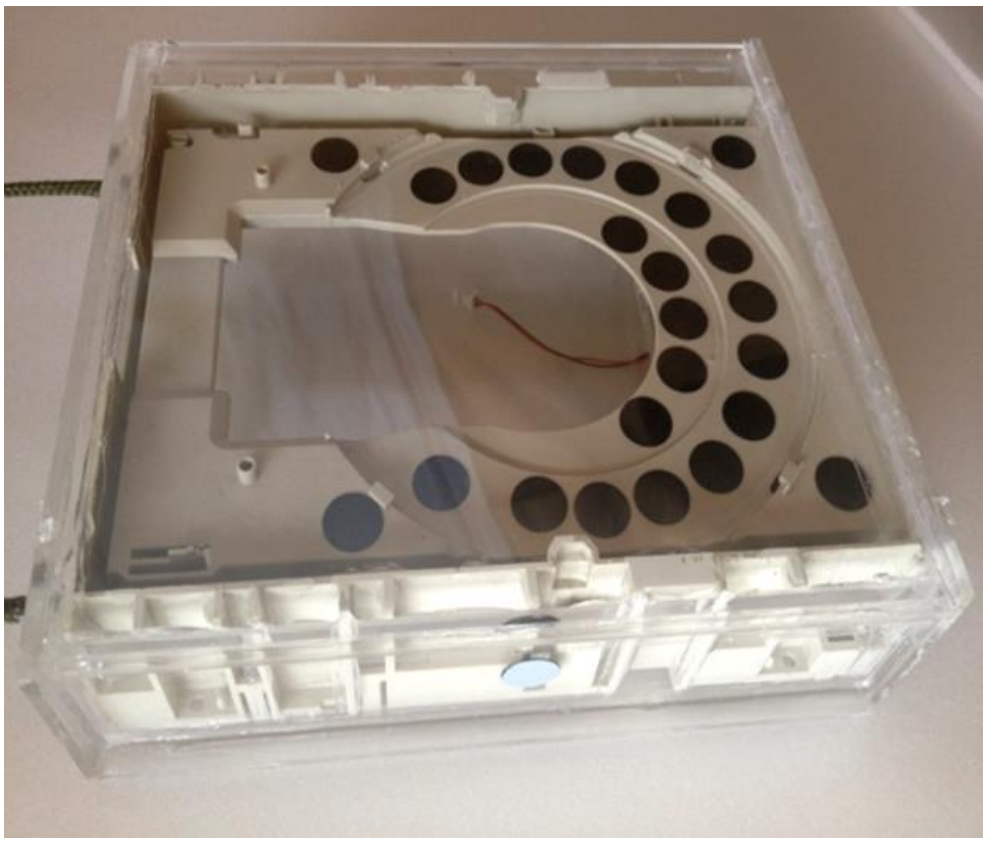


Figure 3.4. The samples drawer, which was automatically opened and closed in the stratosphere. Particles which fell on the black E/M stubs could then be analyzed.

Scanning electron stubs (adhesive carbon tabs, sometimes referred to as Leit tabs - Agar Scientific) were placed in rows inside the drawer, as shown in (Figure 3.4), with the top surface, initially covered by a protective grey tape layer, facing upwards. When the drawer is opened

during flight, the stubs capture any in-falling matter which impacts them. The protective grey layer was removed moments before launch under sterile conditions using a sterilized forceps to minimize contamination probability.

Separate control flights were also performed before each of the sampling flights. In this case, the balloons were sent up into the stratosphere but the sampling drawer remained closed so that the stubs were never exposed to the stratosphere; however, all other analysis techniques were followed in an identical manner to those conducted on the sampling launches. When SEM analysis was conducted on these control stubs, no particulate matter was found, showing the sealing of the drawer was airtight and that none of the stubs were exposed to any particles from Earth of any height during ascent or descent of the balloon. The negative findings from the controls also prove that no contamination occurred when the samples were processed and analyzed.

3.2.2. Results and Discussion

Apparent biological entities (BEs), collected from the stratosphere at heights of around 30km above the Bonneville Salt Flats, USA are shown in Figs 3.5-3.6.

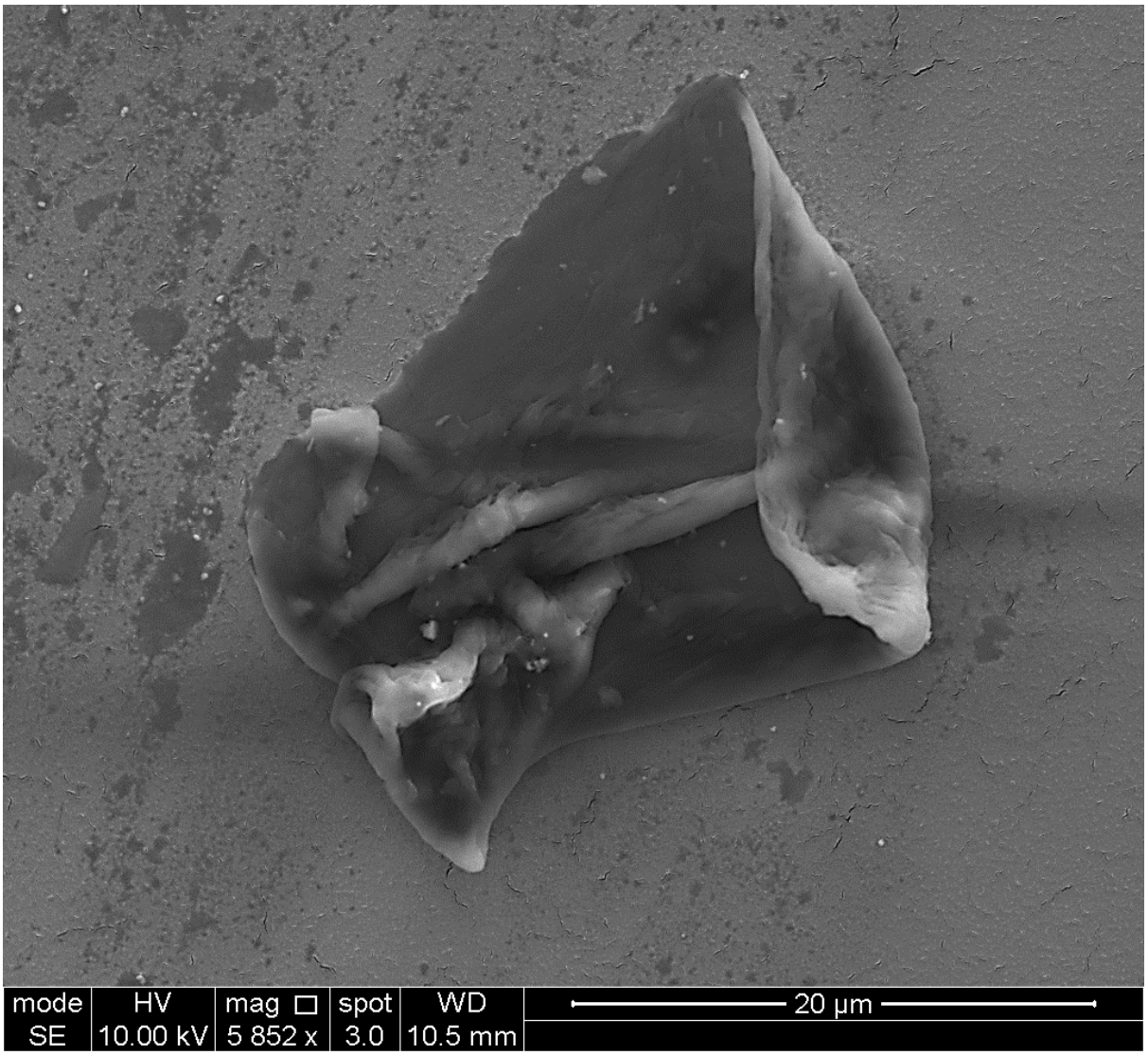


Figure 3.5.A sheet-like stratosphere mass.

Figure 3.5 details a crumpled sheet-like mass. While the top edge appears to be torn, the remaining edges have a thickened appearance, suggesting that this particle is bag-like and could possibly be inflated. There appears to be an orifice in the bottom right. EDAX shows that the particle is made

up entirely of C and O with a trace of N, showing that it is not an inorganic cosmic dust particle and is therefore a presumed BE. Note that the size of the particle, around 20 microns excludes the possibility that it was elevated to the stratosphere from Earth; note that the particles pristine and is not asssociated with common Earth materials such as grass shards, pollen or fungal spores.

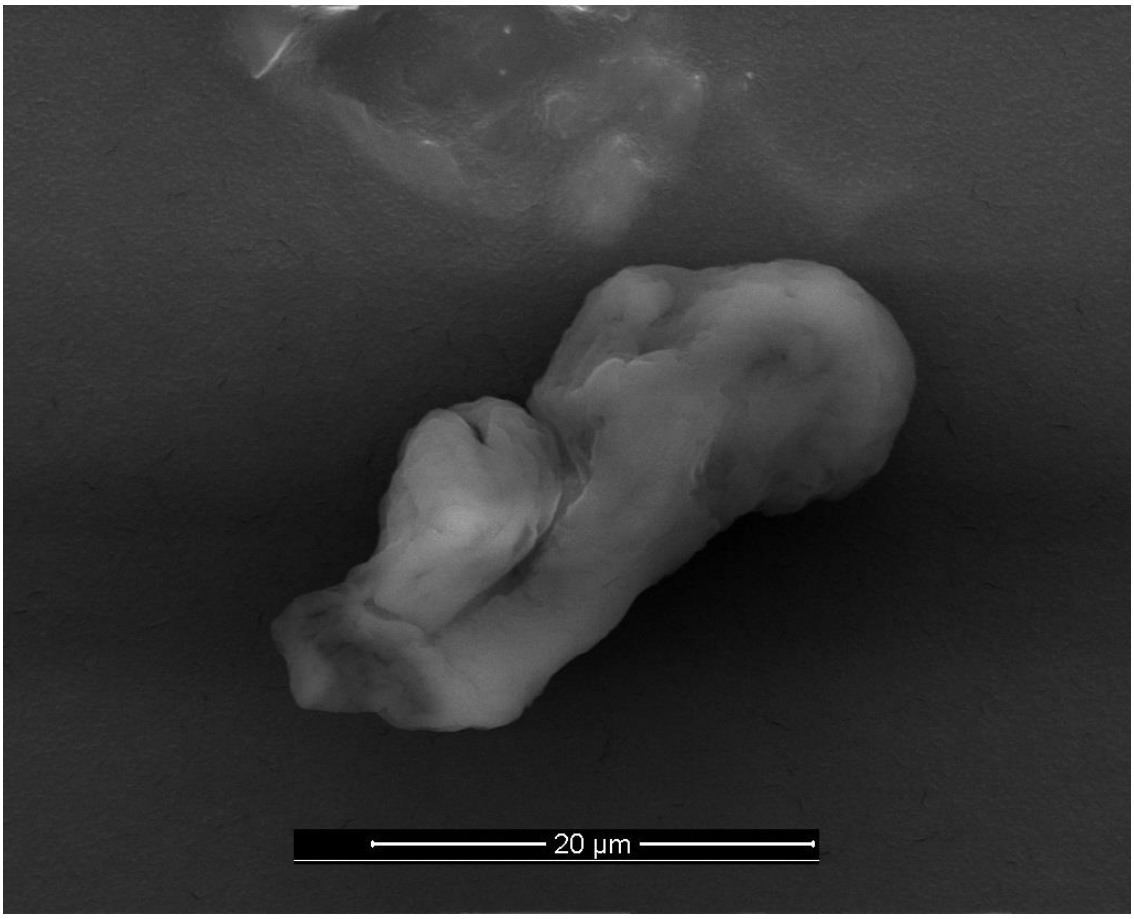


Figure 3.6. A presumed BE which was isolated from the stratosphere.

A second stratosphere-derived particle having a structure, which is biological in appearance, is shown in Fig 3.6. This appears to possess a rounded structure with a projection, which is folded back on itself. This projection or proboscis terminates in what appears to be an opening or orifice. The particle has an EDAX signature of C, N, and O and exceeds 20 microns in length. It is therefore organic in nature and is not cosmic dust; its size prevents its transport from Earth. It is extremely relevant to note that the BE shown in Fig. 3.6 is not surrounded by Earth–associated material and as a result, is presumed to be space-derived BE.

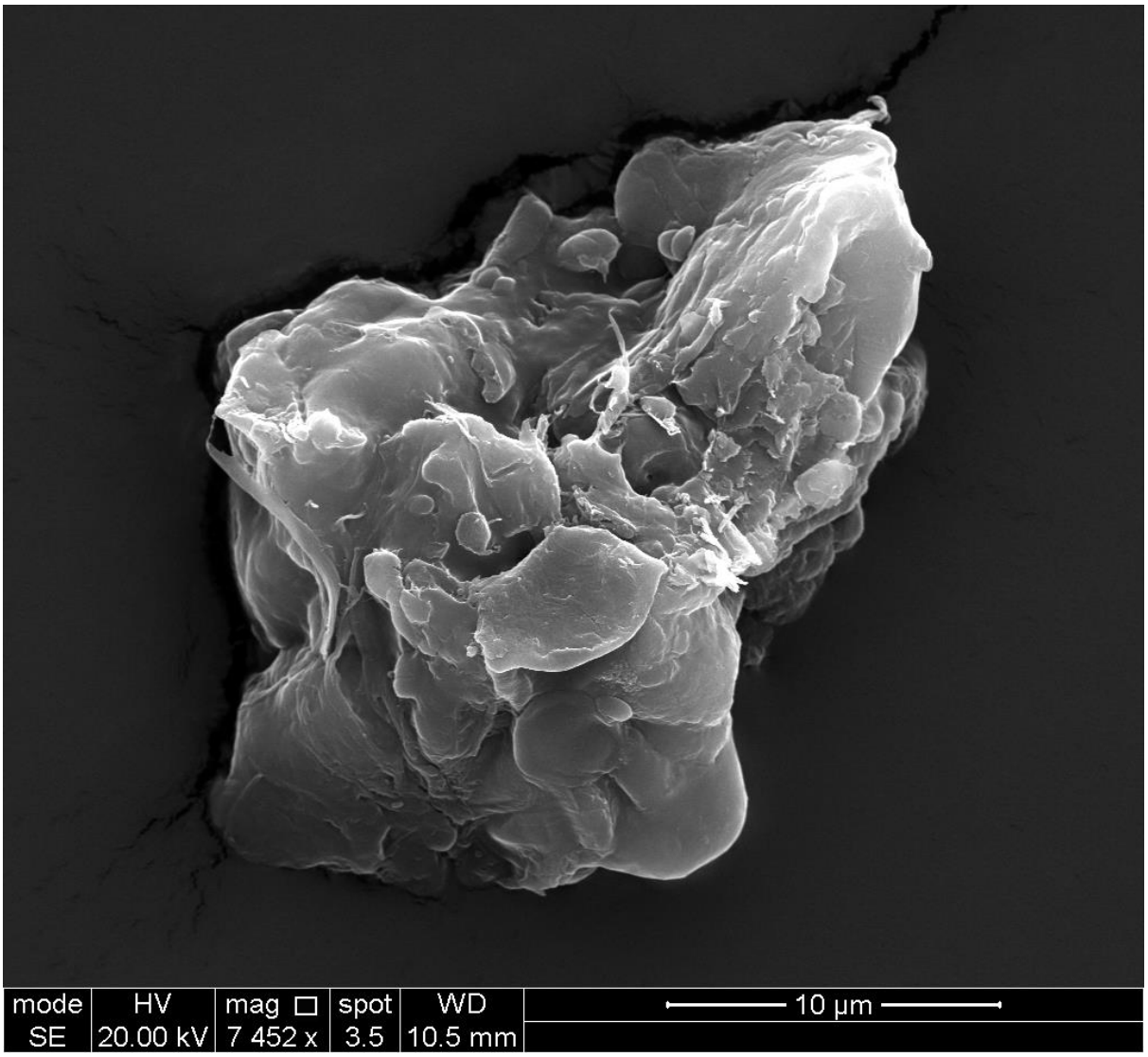


Figure 3.7. A stratosphere particle mass.

The final stratosphere derived material is shown in Fig.3.7. Here, we see a complex particle mass having an EDAX signature of C and O. This particle is therefore organic in nature and since it has structures which have

form, and are not inorganic in appearance, it can be assumed to be a BE. Its size (exceeding 20 microns) precludes its being lifted from the Earth; also note the absence of Earth-derived debris. This complex organic mass is shown in more detail in Fig.3.8. which shows a number of biological–like structures, notably filaments and ovoid bodies of size approximately 1 micron in length.

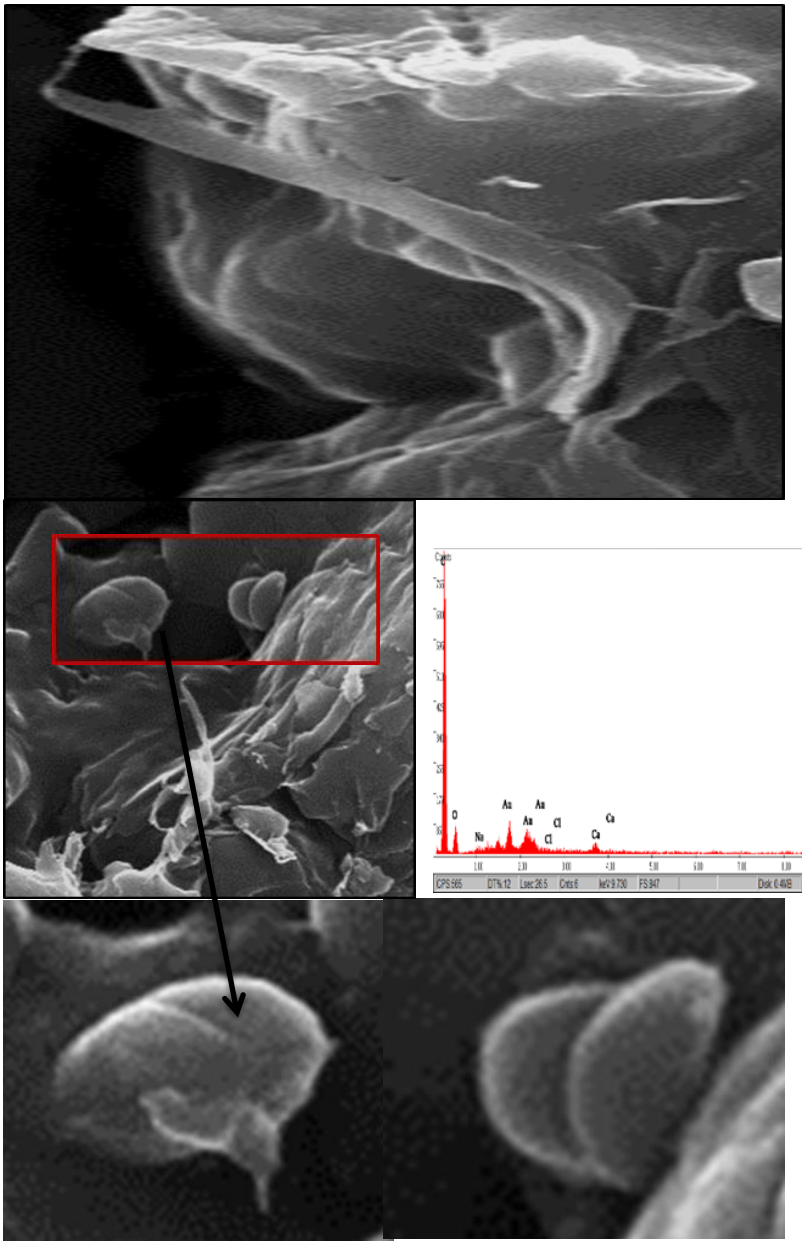


Figure3.8. An organic mass isolated from the stratosphere (The middle image is approximately 10 microns, so the oval objects images seen in the bottom image are around 1micron across. EDAX shows the material be composed of C and O.

The images shown in (Figs.3-8) perfectly illustrate the recent studies done of potential BEs of presumed space origin conducted in this laboratory over the last few years. The implications of this work are given in the Discussion.

3.3. Use of a Drone to isolate bacteria from the lower atmosphere

3.3.1. Introduction

Studies of outdoor atmospheric microbiology have largely focused on microbial air pollution and the distribution of plant and human pathogenic bacteria. The survival and distribution of such organisms are influenced by biological factors and meteorological conditions, such as solar radiation, temperature and relative humidity. Bacteria are usually determined by exposing a culture media to the atmosphere in samplers which at high altitude are carried by balloons or aircraft. However, culturable bacteria make up only a fraction of the atmospheric population and most airborne bacteria are either dead (i.e. cannot reproduce) or are viable but not culturable (VBNC) (Wainwright *et.al.* 2006). The concentration of airborne bacteria varies greatly in the short term as well as diurnally, and annually. The great majority of the genera in the outdoor atmosphere are Gram-positive and some twenty percent is unidentifiable; Micrococi and

Staphylococcus aureus tend to predominate. The Gram-negative genera made up a minor fraction of the population, with pseudomonads and xanthomonads making up around 5–10 and 0–8% of the populations, respectively. Some common culturable taxa include *Bacillus*, *Arthrobacter*, *Clavibacter*, *Curtobacterium*, *Rhodococcus*, *Micrococcus*, *Pseudomonas*, *Xanthomonas*, *Staphylococcus Micrococcus*, *Corynebacterium*, *Brevobacterium*, *Listeria* and *Bacillus.*

3.3.2. Methods used to detect airborne microbes

A number of standard methods have been used to collect microorganisms from the lower atmosphere (Davies, 1971) and these include passive monitoring which involves the use of 'settle plates'– Petri dishes containing culture media, which are opened and exposed for a given time and then incubated. Active monitoring on the other hand, involves impingers, which use a liquid medium for particle collection- sampled air being drawn by a suction pump through a narrow inlet tube into a small flask containing the collection medium. A modification is to employ a solid or adhesive medium, such as agar gel, rather than a liquid for particle collection. One of the best known impact samplers is the Andersen sampler, a multi-stage 'cascade' sieve sampler that uses perforated plates with progressively smaller holes at each stage, allowing particles to be

separated according to size (Davies, 1971). Another such sampler is the Casella slit sampler, in which the slit is positioned above a turntable on which is placed an agar plate. Air is drawn through the slit and an agar plate rotates, so that particles are deposited evenly over its surface (Davies, 1971).

The aim of the work described here was to use a drone sampler to sample microbes from the lower atmosphere. The drone sampler included a Petri dish containing a growth medium, which could be exposed at altitude. Any microbes alighting on the surface of the medium could then be cultured and identified. This work was aimed to emphasise (the well-known fact) that a diverse microbial flora is present in this region and to emphasise that if microbes could be carried up from the lower regions of the atmosphere to the stratosphere then members of this diverse flora would be expected to be found on the stratosphere sampling stubs, a fact which has been confirmed by Wainwright *et al.* (2004) and more recently by Wainwright *et al.* **(**2015) and Wainwright and Omairi (2016).

3.3.3. Material and Methods

3.3.3.1. Drone Sampler

The drone (Fig.3.9) was launched from a field near Bakewell**,** Derbyshire and the drone aperture was opened at a height of 500m above the town.

Figure 3. Image of drone in flight.

3.3.3.2. Microbe-Isolation

Bacteria were isolated using Nutrient Agar, while fungi were isolated using Czapek Dox medium. The atmosphere-exposed plates were incubated at 37^0C overnight and at 25^0C for 7 days for bacteria and fungi, respectively. The bacteria and fungi were identified using classical methods, in order to

gain experience of these approaches. Bacteria were identified using Bergey's Manual.

3.3.4 Results and Discussion

The results of the drone study confirm that the low atmosphere (at 500m) contains a diverse population of microorganisms (i.e. both of bacteria and fungi). This fact is of course well known and it may seem so prosaic that it need not be further established here. The aim of these drone experiments is twofold, however: Firstly, to demonstrate that a drone can be used effectively to sample the lower atmosphere and secondly to confirm the presence of a diverse population in the lower atmosphere. This latter aim is particularly relevant to the studies of the presence of BEs in the stratosphere, since it emphasis the fact that a large population of microorganisms is available to cross from Earth into the stratosphere. A number of studies have confirmed that this is possible. In the case of bacteria this transport across the tropopause is easy to explain, since most bacteria are around 1 micron in size and some, the nanobacteria are much smaller. All of these bacterial cells are therefore smaller than the 5-6-micron size limit which modelling studies show should prohibit the transfer of bacteria from Earth to the stratosphere (Omairi, 2017). Fungi however, are much larger that this prohibitory size limit and as result, we

have an apparent paradox here because, since most fungal spores are too large to cross the tropopause, why have they been isolated from this region? If fungal spores can reach heights of say 41km then the above made argument that BEs cannot be elevated to space based on their dimensions exceeding 6 micron falls down. Again, it should be emphasized that no fungal spores were found on the stratosphere-sampling stubs although not unexpectedly, they were found in large numbers on the sampling box; this having been simply contaminated as it passed up and down through the atmosphere. This paradox can be explained by assuming that fungal spores less than 6 microns in size can be transported to the stratosphere, but that spores larger than this cannot. The presence of fungi having larger than 6 micron spores can then be explained by assuming that, colonies (obtained from stratosphere samples) which grow on isolation media originate from viable hyphal particles of size less than 6 microns.

The second aim of these studies was to demonstrate that drones have potential in the study of aerobiology. The drone system used here worked well although its use is currently limited by UK Civil Aviation Authority's height limits for drones; these can however, be overcome with the required permission. Of particular note is that drones are sufficiently efficient to transport reasonable sampling loads. A novel sampling plate-exposure

system was used here which is currently submitted to a patent application and as a result, technical details of it cannot be given. However, it should be relatively straightforward for aerobiologists to develop a range of different drone-lofted samplers; thereby making the use of drones in aerobiogy commonplace.The following bacteria was isolated using a drone from a height of 500 meters:

Bacillus brevis, Bacillus licheniformis, Bacillus mycoides, Bacillus megaterium, Bacillus mycoides, Enterococcus faecium, Psedomona aeruginosa, Staphylococcuspasteuri, Acinetobacter baumannii, Flavobacterium columnare, Citrobacter brakkii.

The following fungi were isolated:

Aureobasidium pullulans, Aspergillus niger, Penicillium brevicopmatcum, Penicllium citrinum, Penicllium chrysogenum, Aternaria tenuis, Fusarium oxysporum, Fusarium solanum.

The organisms listed above are common inhabitants of the biosphere and all could potentially reach the stratosphere, carried by violent volcanic eruptions and possibly electrostatic phenomena (Wainwright *et.al*, 2006).

Chapter 4: Studies on the Isolation of Bacteria from Astrobiological and Earth-related Geological Samples

4.1 Visualisation of the structure and isolation of bacteria from fulgurite

4.1.1 Introduction

Fulgurites are formed by many terrestrial phenomena including lightning strikes the ground, when it heats, melts, and fuses the sand in soils to form glass tubes (Fig 4.1), or during the impact of a meteorite into a layer of Sandstone, (Elizabeth *et al*. 2010). The fulgurite has low abundances of Na, K, P, Ca and Mg, and relatively high abundances of Si, Fe, Al and Ti. Elizabeth *et al*. (2010), (Hoover 2007) explored the use of nitrogen level and biogenic element ration for characteristic between modern and fossil microorganisms as a mechanism for recognizing recent biological contaminants in meteorite and terrestrial rocks. Some critics of recent Sri Lankan meteorites have claimed that they are fulgurites. Fulgurites are of particular interest because they can be confused with meteorites, so it important to be able to differentiate the two; fulgurite is obviously also of interest in its own right.

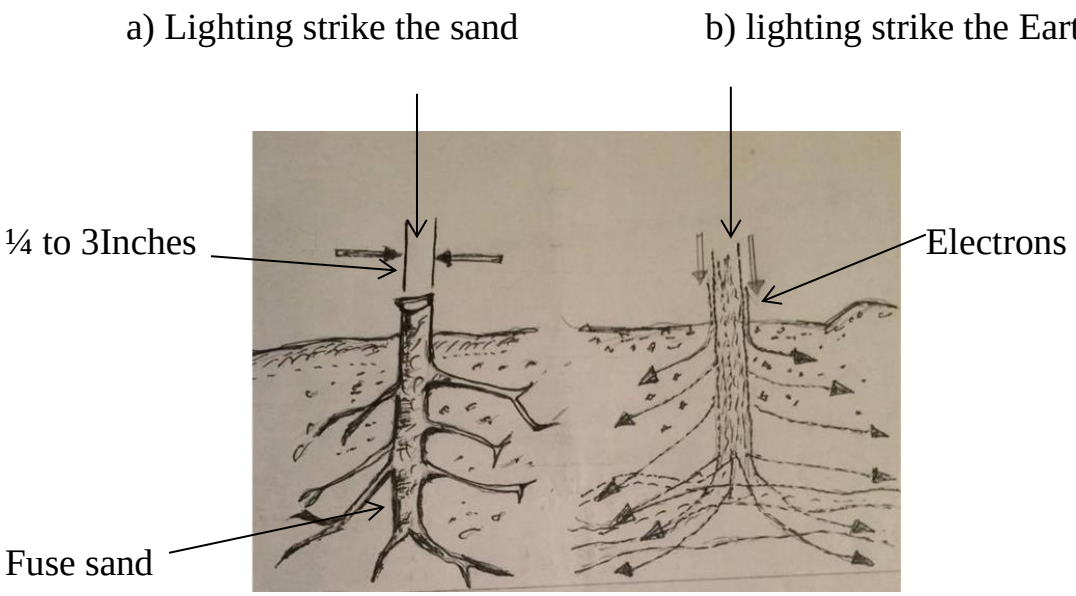
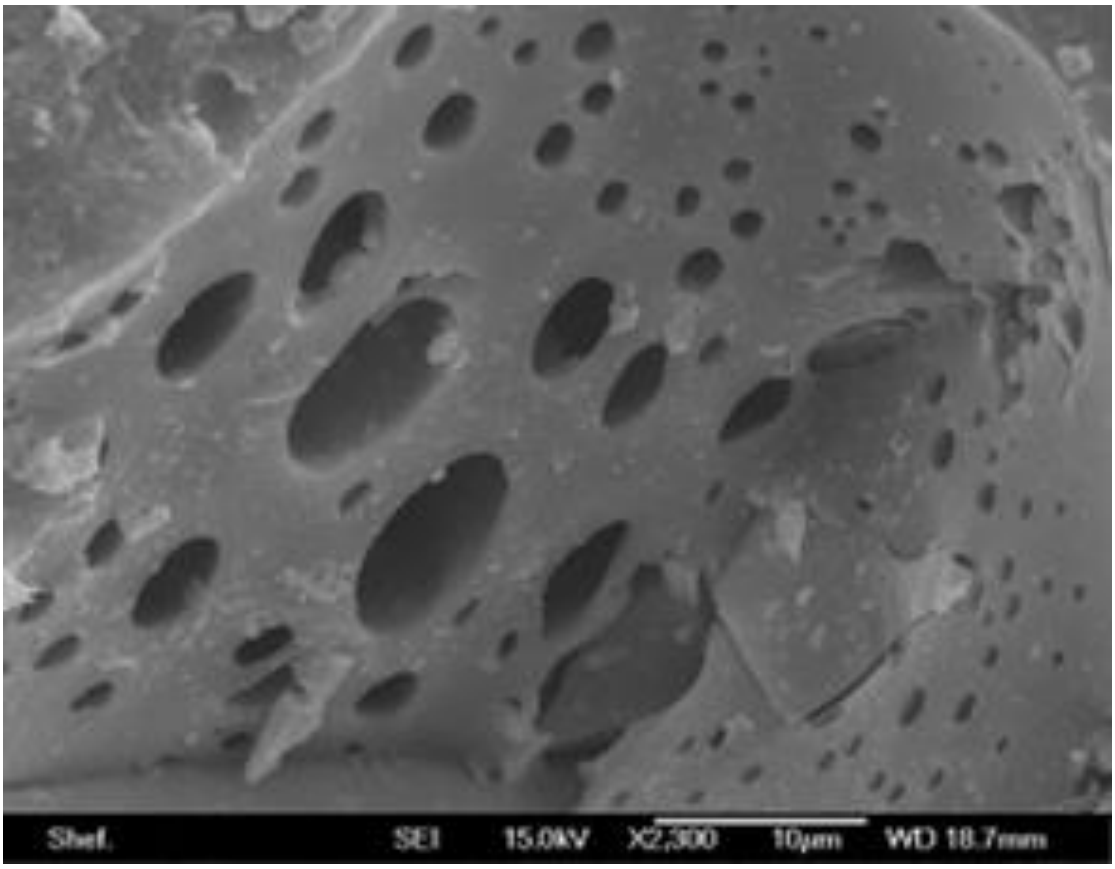


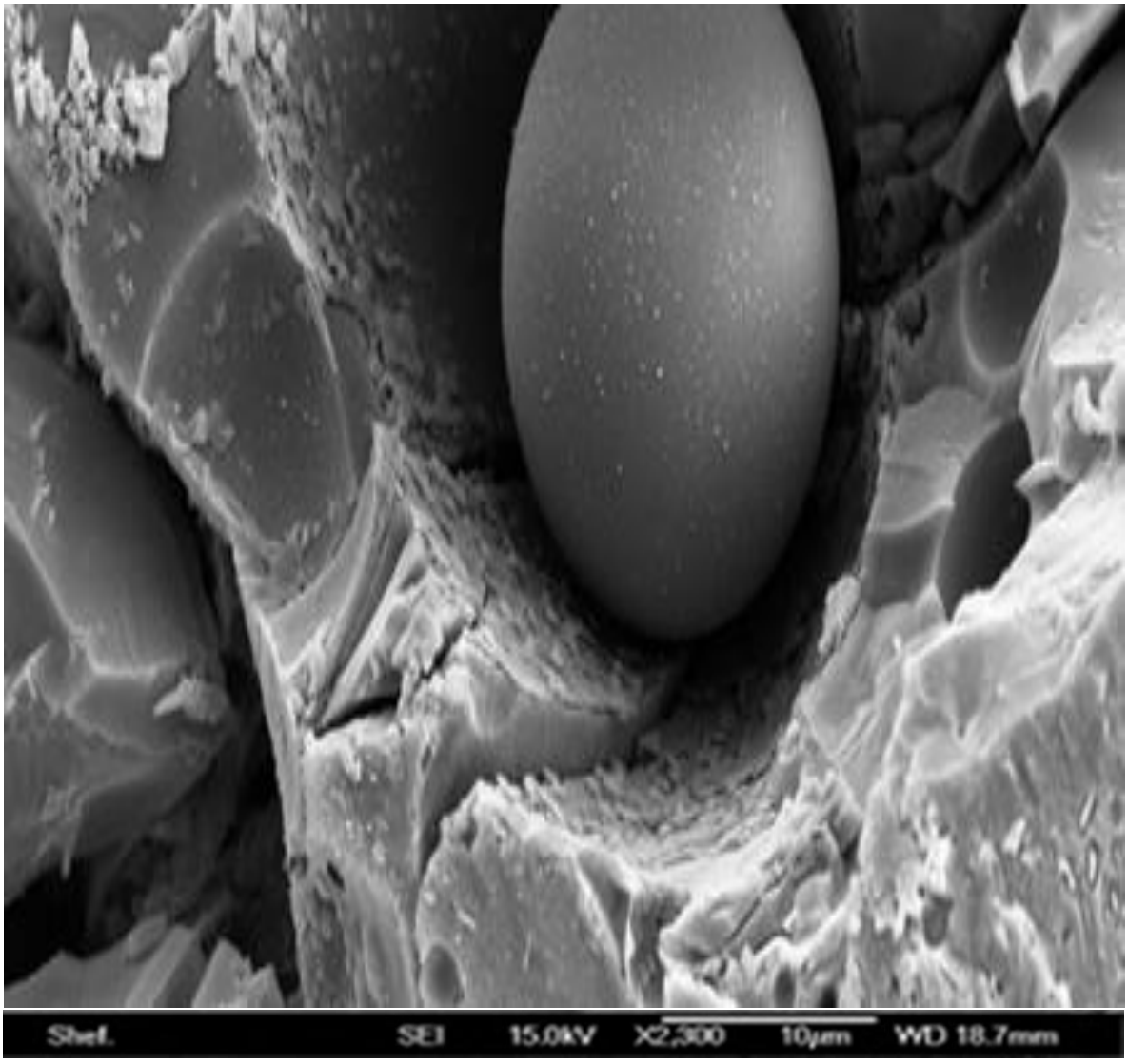
Figure 4.1. Fulgurite formed A) When lightning strikes the sand fulgurite is sometimes made and fuses certain type of sand. B) When lightning hits the Earth, the electrons flow outward in all directions.

4.1.2 Scanning E/M of fulgurites

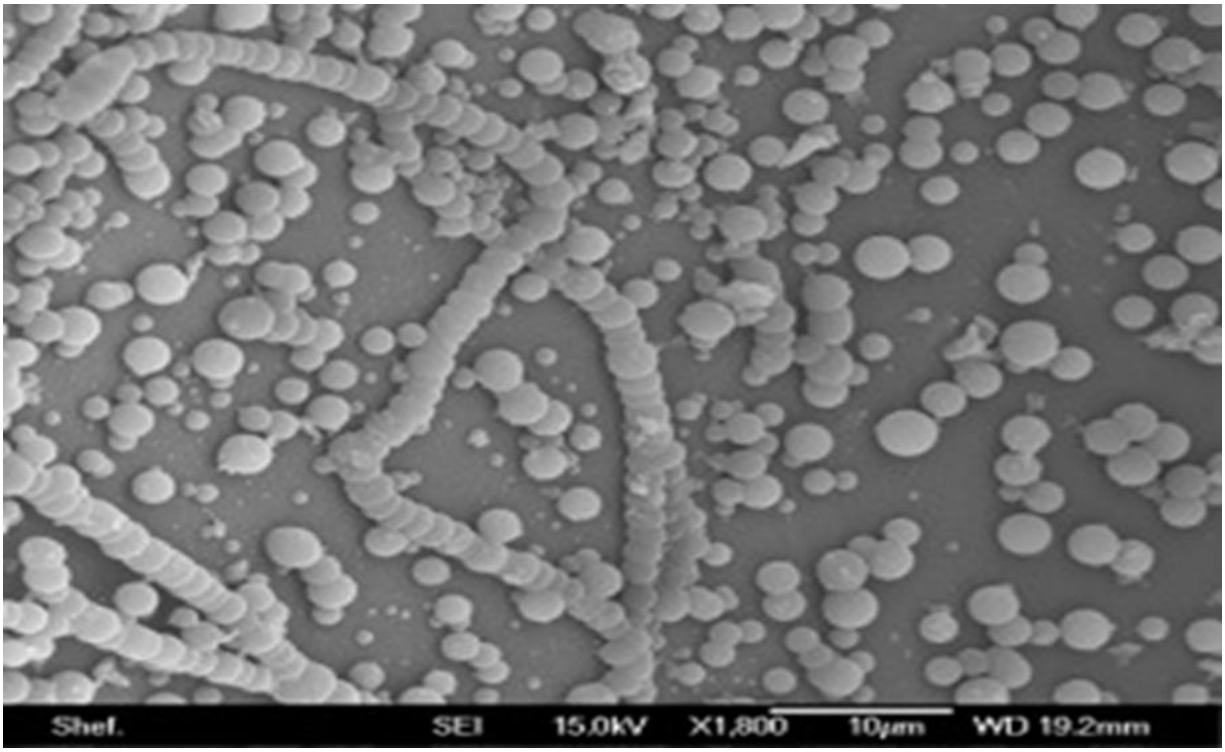
Scanning E/M images of a fulgurite sample are shown in Fig4.2 A. They show a remarkably uniform, spongiform internal structure, while Fig4.2. B shows an internal sphere, again showing unusual uniformity. Finally, Fig. 4.2 C shows an unusual distribution of numerous spherules, which could be regarded as bacterial fossils. However, their uniform size and sphericalnature suggests that they are inorganic pyrolysis (heat) derived structures.



4.2/A



4.2/B



4.2/C

Figure 4.2. Scanning E/M images of a fulgurite sample, A) remarkably uniform, spongiform internal structure. B) An internal sphere, again showing unusual uniformity. C) An unusual distribution of numerous spherules, which could be regarded as bacterial fossils.

4.1.3 Isolation of bacteria from fulgurites

Fulgurite samples (Fig. 4.3) were sterilized by liquid bleach, then shaken by hand for ten minutes and then washed with distilled water three times. Nutrient Broth was prepared by suspending 28g containing (Lab-Lemco powder 1g, yeast extract 2g, peptone 5g and NaCl 5g) in dH2O 1L and boiled to dissolve completely. The pH was then adjusted to pH 7.2 and autoclaved. The fulgurite was suspended in nutrient broth medium incubated overnight at 37º C. After this, 1-3 ml of media were streaked onto four Nutrient agar plates and incubated at 37º C under aerobic conditions for 24h to confirm that bacteria grow in Nutrient Agar medium. After incubation the colonies were identified.

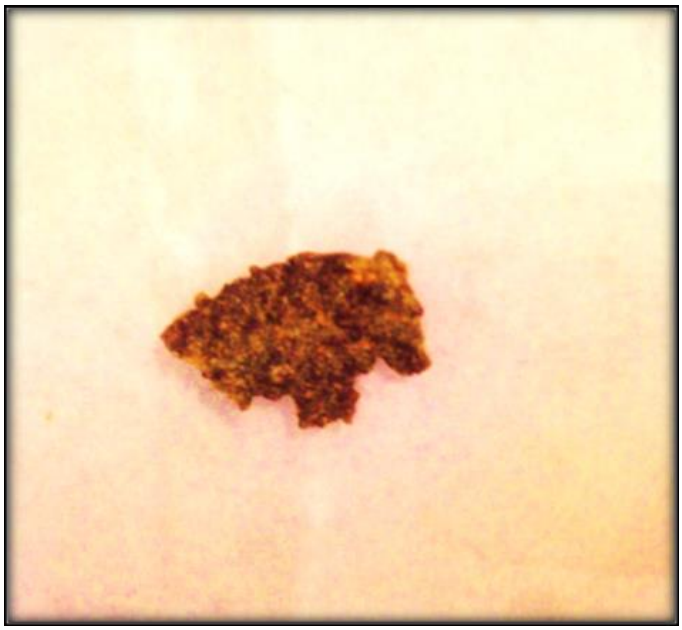


Figure 4. 3. A fulgurite sample.

4.2. Studies on a sample of K/Boundary clay

4.2.1. Introduction

The K/T boundary clay layer was formed from the impact of large extra-terrestrial material over the earth. Many studies have been conducted to prove that KT boundary results from a large meteorite impacting Earth and causes environmental catastrophes world-wide (Bohor *et al*., 1984, Pollastro and Bohor, (1993) Bohor *et al*. (1987) and Al Varez *et al.* (1980) provide evidence which confirms that the Cretaceous-Tertiary boundary

layer is the result of ejecta products. The aim of this study was to determine if K/T boundary samples contain microbes, largely in relation to the possibility of negative panspermia transfer of an ancient Earth sample. The Stevns klint sample used here was obtained from a white chalk cliff situated 6 km (3.7 miles) southeast of Store Hedding on the island of Zealand in Denmark. It stretches 17 km (11 mi) along the coast, it is geologically important because it is the best exposed Cretaceous –Tertiary (K/T) boundary in the world. The cliff contains layers from the uppermost part of the Maastrichian (72 to 66 million years ago) and from the lowest section of the Danian stage (66 to 62 million years ago). It is made up of a few centimetres of a black layer of fish clay which is rich in iridium and which clearly indicates the Cretaceous-Permian boundary.

4.2.2. Isolation of bacteria -from KT-boundary clay methods

In order to release the inclusions into media, the clay obtained from Bidart -France (Fig.4.4) was cracked open and broken into small pieces using the vessel shown in Fig.4.5.

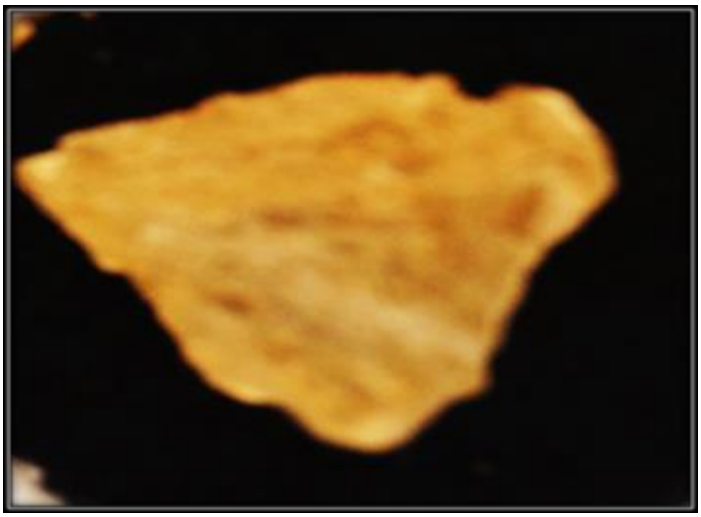


Figure.4.4. K/T Boundary clay sample.

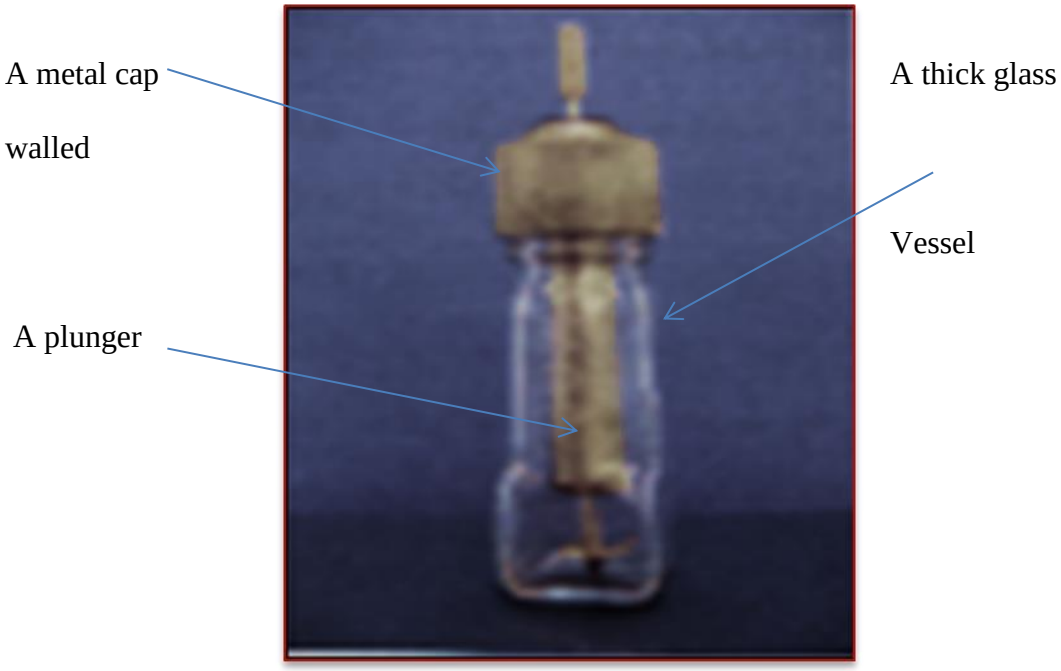


Fig.4. 5 Cracking vessel.

The cracking vessel (Fig.4.5) consisted of a thick glass-walled tissue homogenizing vessel, sealed with a metal cap. A plunger passed through the cap and touched the bottom of the vessel. The top of the cap was covered with part of an autoclave bag (using a corner cut from a large autoclave bag), attached and sealed closed using autoclave tape, attached to the cap and the top of the plunger. The sterilized clay was cracked *in situ* in the cracking vessel by placing the bottom of the plunger into a surface indentation and applying a sharp tap to the top of the plunger with a light-weight hammer, the force cracked the clay to expose any fossilized insects inside. In order to facilitate cracking, a shallow central indentation was made in the surface of the clay and four shallow grooves (1 mm) were scored (with a serrated knife) from this indentation around the clay; further crushing of the clay was then achieved by manually applying force to the top of the plunger. Any leaks in the system were checked for by placing filter paper strips inside the autoclave bag cover prior to autoclaving. When a bacterium was isolated, after sample cracking, the whole cracking vessel was immersed into a solution of coloured (red) food dye. The absence, on the filter papers, of coloured food dye demonstrated that the system was leak free; claims that bacteria were isolated from the cracked samples were only made if no leaks were observed. For the isolation of bacteria, nutrient

broth (Oxoid, 10 ml) was added to the cracking vessel which was then autoclaved for 20 minutes at 120°C. The clay was immersed in domestic bleach (10% v/v) for 20 minutes and then transferred to a closed bottle containing sterile distilled water (500 ml) and washed vigorously. The clay was then removed and immersed in membrane-filtered (0.22 mm) alcohol and transferred to a flame, using flame-sterilized forceps; the residual alcohol was then ignited. The sterilized clay was finally transferred to the growth medium (10 ml) in the cracking vessel. After inserting the sterile clay, the cracking vessel was left for four days at 37°C. All vessels in which bacteria grew in the medium were considered to be contaminated and therefore discarded. Where no bacterial growth appeared in the medium, the vessels were opened in a laminar air-flow cabinet and a small amount of a medium was poured onto a Nutrient Agar (Oxoid) plate; this was then incubated at 25°C for a further four days. If no bacteria appeared on the Nutrient Agar, or in the nutrient broth, over this time period the clay was cracked *in situ* in the vessel. If, following this period of incubation, bacterial growth appeared in the nutrient broth the vessel was opened, the neck of the vessel was thoroughly flame sterilized and a small amount of broth, was aseptically transferred to Nutrient Agar; this was then incubated at 25°C until growth appeared. Any bacterial isolates were then purified by

streaking and were independently identified using 16SrRNA analysis. All transfers were performed in a laminar air-flow cabinet, the sterility of which was checked periodically.

4.3. Studies on a sample of Earth clays

4.3.1. Introduction

Recent studies by (Wainwright *et al*., 2009) were conducted to determine if Earth materials such as amber, boulder clay and coal contain bacteria which could be ejected from Earth during impact events. They found two species of *Bacillus* in the amber samples, and a species of the same genus were found in coal; bacilli were also commonly found in clay. It is concluded that species of the spore- forming genus *Bacillus* could therefore be ejected from Earth in these geological substrates and possibly be transferred elsewhere in the cosmos.

4.4. Isolation of bacteria from Filey-Boulder clay

Boulder clay samples were used to represent Earth-derived clays. The samples were obtained from the cliffs of Filey Brig, North Yorks. In order to release the organisms from the geosamples into Nutrient Broth (Oxoid), the clay and a clay with roots samples were cracked and broken into small pieces using the cracking vessel as described for the KT boundary clay

sample. Any bacterial isolates were then purified by streaking and were independently identified using 16SrRNA analysis.

4.5. Studies on an ancient salt crystal

Vreeland *et al*. (2000) have claimed that many types of ancient contain bacteria, such as halophilic microorganisms in ancient rock salt samples. The argument centres on whether these halophiles were trapped in salt when the evaporites formed millions of years ago, or else are modern contaminants. The discovery of life in evaporates has, in turn, resulted in extrapolations to similar environments on Mars and Europa as being potential sites for the search for extraterrestrial life. Evidence of a spore forming bacteria, *Bacillus* strain 2-9-3, from ancient brine was reported by Vreeland *et al.* (2000). Stan-Lotter *et al*. (2003) have similarly explored halophilic archaebacteria in rock salt of Permo-Triassic age in Austrian salt where ancient microorganisms and have survived in the salt sediments. Again, this work was conducted in relation to the possible transfer of ancient samples from Earth to space following an impact event.

4.5.1 Methods isolation of bacteria from an ancient salt crystal

The salt crystal was obtained from the Hutchinson Salt Mines, Kansas, USA (Figure: 4.6). The salt rock sample was immersed in 70% alcohol before being passed through a blue Bunsen flame on a number of occasions. The salt sample was then broken into four pieces and each piece was placed in a flask containing Nutrient Broth medium and incubated over night at 37^0C for 24h. All transfers were carried out under a sterile hood. Any isolated bacteria were purified on Nutrient Agar and identified by 16SrRNA technique.

Figure 4.6. The ancient salt sample.

4.6. Studies on the Microbiology of a stromatolite sample from Sharks Bay, Western Australia

Studies on modern stromatolites may help us understand the formation of similar structures from the earliest geological record and important bio-signatures for the early Earth and in the search for extraterrestrial life Papineau *et al.* (2005). Stromatolites are sedimentary structures predominantly accreted by sediment trapping, binding, and *in situ* precipitation as a result of the growth and metabolic activity of microorganisms. The oldest examples of preserved fossil stromatolites are about 3.5 billion years old and are found in Western Australia and South Africa (Papineau *et al.* 2005). Stromatolites, are therefore sedimentary structures formed by microbial activity. Papineau *et al*. (2005) provide a framework for understanding the kinds of organisms that build contemporary stromatolites, their ecology, and their relevance to stromatolites preserved in the geological record. A study of stromatolites is also relevant to the potential transfer of ancient rocks in negative panspermia.

4.7. Methods -isolation of bacteria from a Shark Bay stromatolite rock

Stromatolites obtained from Hamelin Pool, Shark Bay in Western Australia were placed in tube with water. Stromatolite fragments were streaked onto four Nutrient Agar plates and incubated at 37°C under aerobic condition for 24h; after incubation the colonies were identified by using 16SrRNA technique.

4.8. Molecular biology techniques

Use of 16SrRNA to identify bacteria has totally changed our understanding of bacterial relatedness and has given us a rapid means of identifying environmentally-isolated organisms (Amann *et al*., 1995; Hill *et al*., 2000; Kirk *et al*., 2004; Lahiri, 1992 and Rogers, 2008; Wintzingerode *et al*., 1997).

4.9. Polymerase chain reaction (PCR) technique

The PCR technique was detected in the mid-1980s by Kerry Mullis who gained a Nobel Prize in 1993 for its development (Anderson, 2011; Baker *et al.*,2006; Hadidi and Candresse, 2003; Kubista *et al*., 2006; Maier *et al.*, 2009; Madigan *et al*., 2012; Holmes, 2003; Van Holde, 1989).

4.10. Identification of bacterial isolates from geological samples using the 16SrRNA technique

A bacterial suspension in Luria-Bertani (LB) medium consisted of 10g tryptone, 10g NaCl and 5g yeast extract in 1L of dH_2O, dissolved and sterilised by autoclaving at 121°C for 15 minutes and incubated overnight at 37°C. After the Incubation period, 1-3 ml of media was transferred to a sterile Eppendorf tube and centrifuged at 6000xg for 2 min at room temperature and the supernatant was decanted completely. A KeyPrep bacterial DNA extraction kit supplied by ANACHEM® was used and all steps were done described in the instructions provided by the company. Buffer (100µl) R1 was added to the pellet and the cells were re-suspended completely by pipetting up and down. After full cell homogenising, 20 µl of lysosyme was added, mixed and incubated at 37°C for 20 min. The mixture was digested by centrifugation at 10,000xg for 3 min and the supernatant was completely decanted. The pellet was then re-suspended in 180 µl of buffer R2 and 20 µl of proteinase K was added and incubated at 65°C for 20 min in a water bath with occasional mixing every 5 min. 400 µl of buffer BG was added and mixed thoroughly by inverting the tube several times until a homogeneous solution was obtained and then incubated for 10 min at 65^0C. After the incubation period, 200 µl of

absolute ethanol was added and mixed thoroughly. The sample was next transferred into a column that was assembled in a clean collection tube and centrifuged at 10,000xg for 1 min while the flow was discarded. The column was washed by addition of 750 µl of wash buffer and centrifuged at 10,000xg for 1 min while the flow was discarded. Finally, the column was placed in a clean micro centrifuge tube and 70µl of elution buffer was added and centrifuged at 10,000xg for 2 min to elute DNA. DNA was stored at 2°C until the next step.

4.10.1. Molecular Identification of Isolates

4.10.1.1. Gel electrophoresis

Gel electrophoresis was done to make sure that the bacterial DNA was well extracted and purified. The following steps were taken:

4.10.1.2. Agarose preparation

0.5 g of molecular biology grade agarose was dissolved in 50 ml of 1x TAE (Tris Acetate EDTA) buffer and 40ml distilled water by heating in a microwave oven. The solution was mixed gently and allowed to cool to 55°C, and then 2.5 µl of ethidium bromide was added. After mixing, 25-30 mL of the solution was poured into the casting tray. A comb was inserted at the one side of the gel. The gel was allowed to solidify for about 10

minutes then the comb was removed. The gel was placed into an electrophoresis tank and submerged in 1x TAE buffer.

4.10.1.3. Sample loading

DNA sample (10 µl) was mixed with 2 µl of Blue/Orange 6x loading dye with glycerine and loaded into the wells. 6 µl of hyper ladder was added into an adjacent well as a reference. Set the voltage to the desired level at 80V for 40 minutes to begin electrophoresis. The leads were attached; the DNA will migrate into the gel toward the anode or positive lead. The progress of the separation can be monitored by the migration of the dyes in the loading buffer. After the supposed period the DNA fragments were visualized under a UV transilluminator and the images were captured using a connected digital camera.

4.10.1.4. Sample amplification

Samples were amplified using the Polymerase Chain Reaction (PCR) technique. A mixture in a sterile Eppendorf tube was prepared as follows: 12 µl of Master Mix, 1 µl of forward primer, 1 µl of reverse primer, 1µl of the DNA sample, and 35 µl of sterile distilled water. The mixture was inserted in to a PCR machine and the programme was adjusted as follows (Table: 4.1)

Steps	Temperature	Time (Min)	Number of cycle
Initialization (Initial denature	94 °C	3	1
Denaturation	94°C	1	35
Annealing	60°C	1	35
Extension/Elongation	72°C	1	35
Final elongation	72°C	5	1
Hold	4°C		1

Table 4.1. The PCR Process.

4.10.1.5. 16SrRNA sequencing and phylogenetic analysis

After PCR, aliquots of 10 µl of each sample were allowed with 10µl of forward primer and 10µl of Reverse primer in a sterile small size tube and sent to the Medical School Core Genetics Unit (University of Sheffield) to be sequenced. 16SrRNA gene sequences were adapted using the Finch TV

software then exported into the Basic Local Alignment Search (BLAST), available from the website of the National Centre for Biotechnology Information (NCBI), to identify matches with existing characterized reference sequences. Partial sequences, generated in this experiment, were assembled and the errors of consensus sequences were corrected manually by using Finch TV software (version 1.4). In Finch TV software the unknown nucleotide represents as N, and it could be either A, or T, or G, or C, according to the different colours appear (Mishra *et al*., 2010).

4.11. Results and Discussion

4.11.1. 16S rRNA sequencing

trains of unknown bacteria were isolated from fulgurite, Salt crystal Samples, namely: KT-boundary clay, clay and a clay rhizosphere from Filey, and a stromatolite sample, all by using a Qlagen Spin Miniprep - Bacterial DNA Extraction Kit. A whole genomic DNA was successfully extracted from the isolates (Figure 4.7).

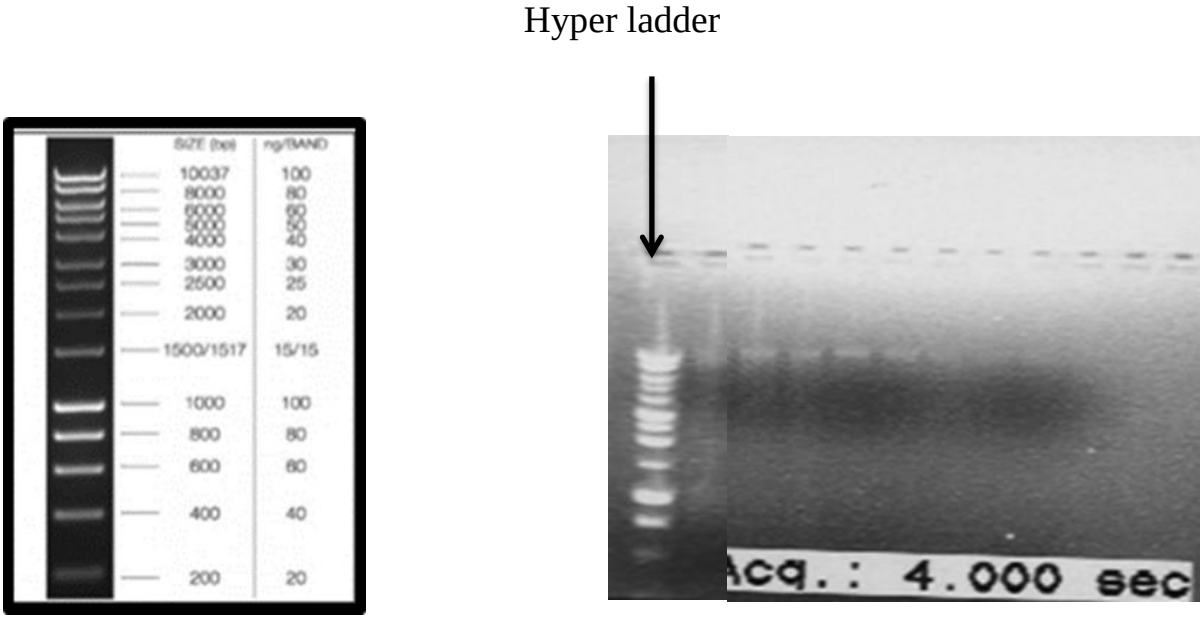


Figure.4.7: (Left) Standard Hyper ladder I produced of 14 regularly spaced bands and each lane (5 µl) provides 720 ng of DNA.

(Right) Successful genomic DNA extraction from the isolates with band size over 1000 base pairs in agarose gel.

4.11.2 PCR amplification of extracted DNA

16S rRNA gene sequences have hyper-variable regions where sequences have diverged over time and these are often flanked by strongly conserved regions. Primers targets are conserved regions and amplify variable regions. The DNA sequence gene of 16S rRNA has been determined for a

large number of species, forming a readily accessible and extensive library. Amplified 16SrRNA genes are shown in (Fig.3.8.) and the size was banded as expected around 1 Kb.

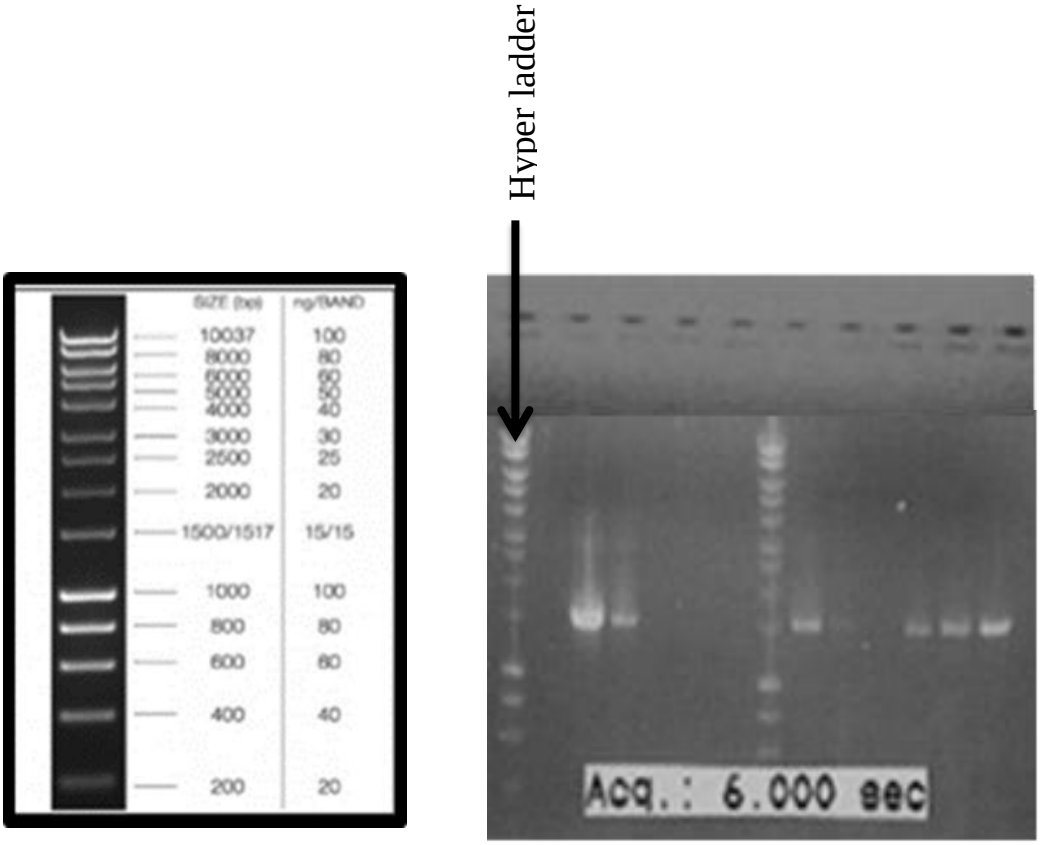


Figure 4.8. PCR amplification (PCR) on agarose gel (1%) electrophoresis with ethidium bromide from product of 16S rRNA gene (1 Kb).

4.11.3. Phylogenetic identification of unknown organisms 16S rRNA

Sequences of bacteria isolated from fulgurite, the salt crystal, KT-boundary clay, clay and clay roots and the stromatolite sample were determined and the sequence data were used to produce a phylogenetic tree providing the basis for efficient phylogenetic investigation, of each genus.

4.12. **Results and Discussion**

Table 4.2 shows that *Bacillus cereus, Staphylococcus aureus, Pseudomonas geniculate, Lysinibacillus boronitolerans, Lysinibacillus sphaericus, Brevibacillus brevis* were cultured from fulgurite, salt crystal, K/T clay, Filey Earth clay and the stromatolite rock sample. The phylogenetic analysis of the organisms is represented in Appendix A. In addition, Table 4.2 shows the 16S rRNA sequence analyses representing the closest matches of the above organisms.

Representative sequence	Closest matches identification	Sequence identity	NBCI (Accession number)
Fulgurite	*Bacillus cereus*	99%	FR865171.1
Salt Crystal	*Staphylococcus aureus*	99%	KP137514.1
KT Boundary Clay	*Pseudomonas geniculate*	99%	KC934806
Filey- Earth Clay	*Lysinibacillus boronitolerans*	98%	KJ155817
Filey Earth Clay Sample 2	*Lysinibacillus sphaericus*	99%	KM873374
Stromatolite	*Brevibacillus brevis*	100%	KP137563

Table 4.2.16srRNA sequence analyses of cultures from fulgurite, salt crystal, K/T clay, Filey- Earth clay and clay roots and from the stromatolite rock.

It is difficult to generalize on the implications of the above bacterial isolations. Further studies will be conducted to determine the complete microflora of these samples. The obvious next step in relation to the ancient salt crystal, for example, is to use salt-rich media in isolation studies, although it is notable that a non-extreme halophile (i.e. *S. aureus*)

was isolated in this instance. As was mentioned above, most of the samples studied in this section of the Thesis are not directly relevant to panspermia, with the possible exception of the K/T boundary samples which is made up of material deposited by a cosmic impact event (Cocioni and Galotti, 1994). In retrospect, it would have been desirable to have checked this sample for the presence of organism similar to the BEs obtained from the stratosphere and which, we suggest above, originate from space. However, such a check could be conducted in future studies, using freshly collected K/T boundary material.

Chapter 5. Studies on the Isolation of Bacteria from Amber

5.1. Introduction

Microorganisms are the most ancient cells and they are key to the history of the origin and development of life on Earth. For instance, amber is excellent geological substrate for studying the morphological organization of microfossils and microbial communities from ancient times. Wainwright *et al*. (2009) found two species of *Bacillus* in the amber and a species of the same genus was found in coal also bacilli found in clay, while Martin-Gonzalez *et al*. (2009) presented a critical review of fossilized prokaryotic and eukaryotic microorganisms found entrapped in Cretaceous ambers.

The aim of the work reported here was to determine if amber contains bacteria entrapped since its formation, some 40 million years ago. Amber may also act as a vehicle for the transfer of microbes via negative panspermia (i.e. the transfer of microbes from Earth to space).

Figure5.1. Amber sample.

5.2. Materials and Methods

A sample of Baltic amber (Fig.5.1), containing insect inclusions, was obtained from a variety of suppliers. In order to liberate the inclusions into media, the amber was broken open and cracked into small pieces using the vessel shown in (Fig.4.5) which was discussed in more detail in chapter (4). This consisted of a thick glass walled, tissue homogenizing vessel,

sealed with a metal cap. A plunger passed through the cap and touched the bottom of the vessel. The top of the cap was covered with part of an autoclave bag (using a corner cut from a large autoclave bag), attached and sealed using autoclave tape attached to the cap and the top of the plunger. The ability of the autoclave bag covers to act as an airtight seal, and thereby prevented ingress of contaminants, was checked by inverting it underwater and pumping air into it via the cracking vessel cap; absence of air bubbles confirmed the air-tight nature of the seal. For the isolation of bacteria, Nutrient Broth (Oxoid, 10 ml) was added to the cracking Vessel which was then autoclaved for 20 minutes at 120°C.

5.2.1. Sterilisation of the Amber

A superficial central indentation was made in the surface of the amber and shallow groves were scored (with a serrated knife) from this indentation around the amber. The amber was immersed in domestic bleach (10% v/v) for 20 minutes and then transferred to a closed bottle containing sterile distilled water (500 ml) and washed vigorously. The amber was then removed and immersed in membrane-filtered (0.22 /Am) alcohol and transferred to a flame, using flame-sterilized forceps; the residual alcohol

was then ignited. The sterilized amber was finally transferred to the growth medium (10 ml) in the cracking vessel.

5.2.2. Incubation of the Vessel and isolation of bacteria

The sterile amber was next transferred to the cracking vessel was left for 4 days at 37°C. Any vessel, in which bacteria grew in the medium, was considered to be contaminated and therefore discarded. Where no bacterial growth appeared in the medium, the vessel was opened in a laminar airflow cabinet and a small amount of medium was poured onto a Nutrient Agar plate; this was then incubated at 25°C for a further 4 days. If bacteria appeared on the Nutrient Agar, or in the Nutrient Broth, over this time period the amber was cracked *in situ* in the vessel. If following this period of incubation, bacterial growth appeared in the Nutrient Broth the vessel was opened, the neck of the vessel was thoroughly flame sterilized and a small amount of broth was aseptically transferred to Nutrient Agar; this was then incubated at 37°C until growth appeared. Any bacterial isolates were then purified by streaking and were independently identified (NCIMB, Aberdeen) using 16SrDNA analysis. All transfers were done in a laminar airflow cabinet, the sterility of which was checked periodically.

Figure 5.2. A bacterium isolated from amber.

5.3. Molecular identification techniques for bacteria

Molecular techniques used here were described in Chapter 4.

5.4. Results Strains of unknown bacteria isolated from amber were identified by using (Qlagen Spin Miniprep -Bacterial DNA Extraction Kit), (Figure: 5.3) and (Figure: 5.4).

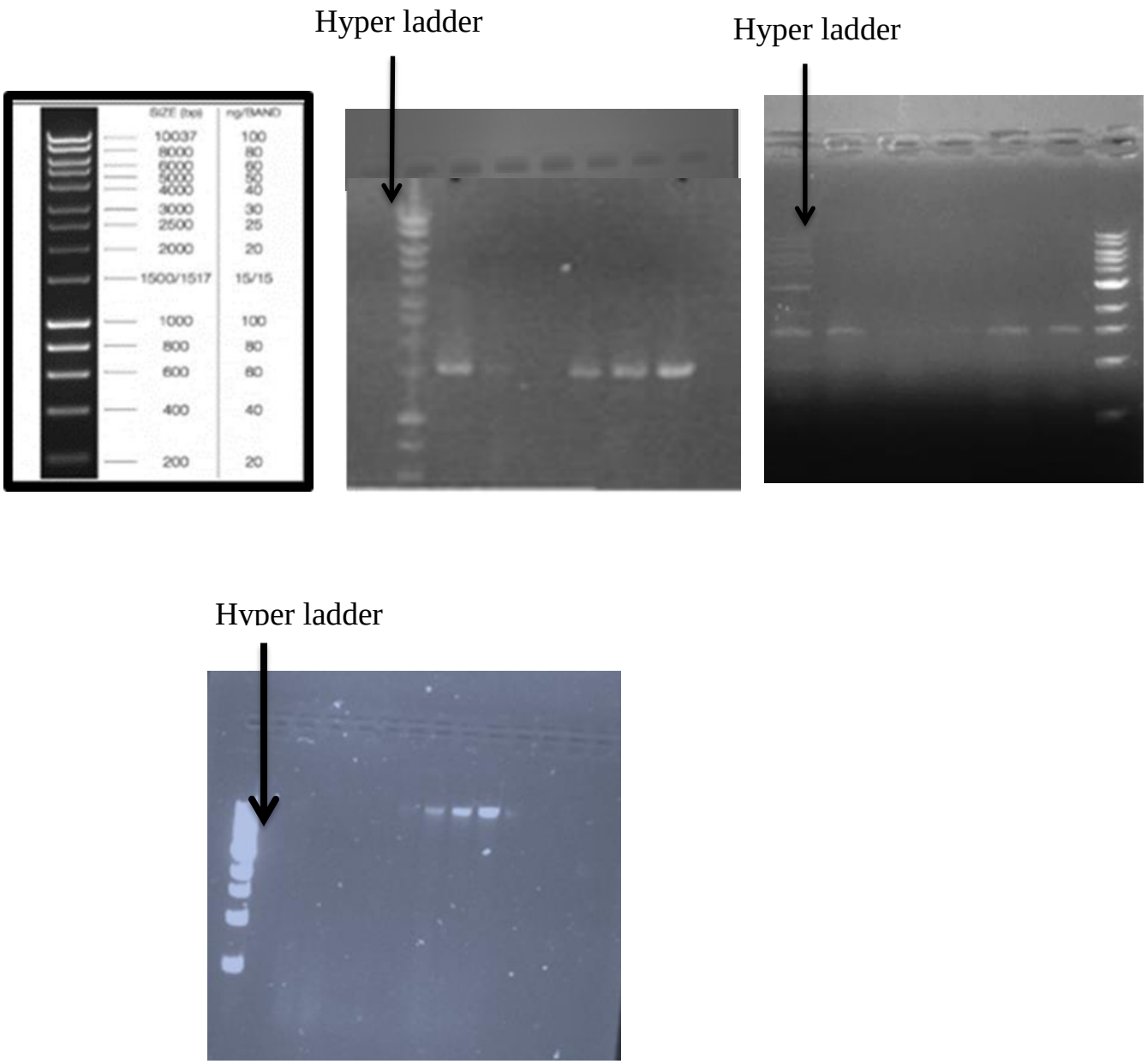


Figure 5.3: Successful genomic DNA extraction from isolated strains with band size over 1000 base pairs in agarose gel (Right). Standard

Hyper ladder I produces of 14 regularly spaced bands and each lane (5 µl) provides 720 ng of DNA (Left).

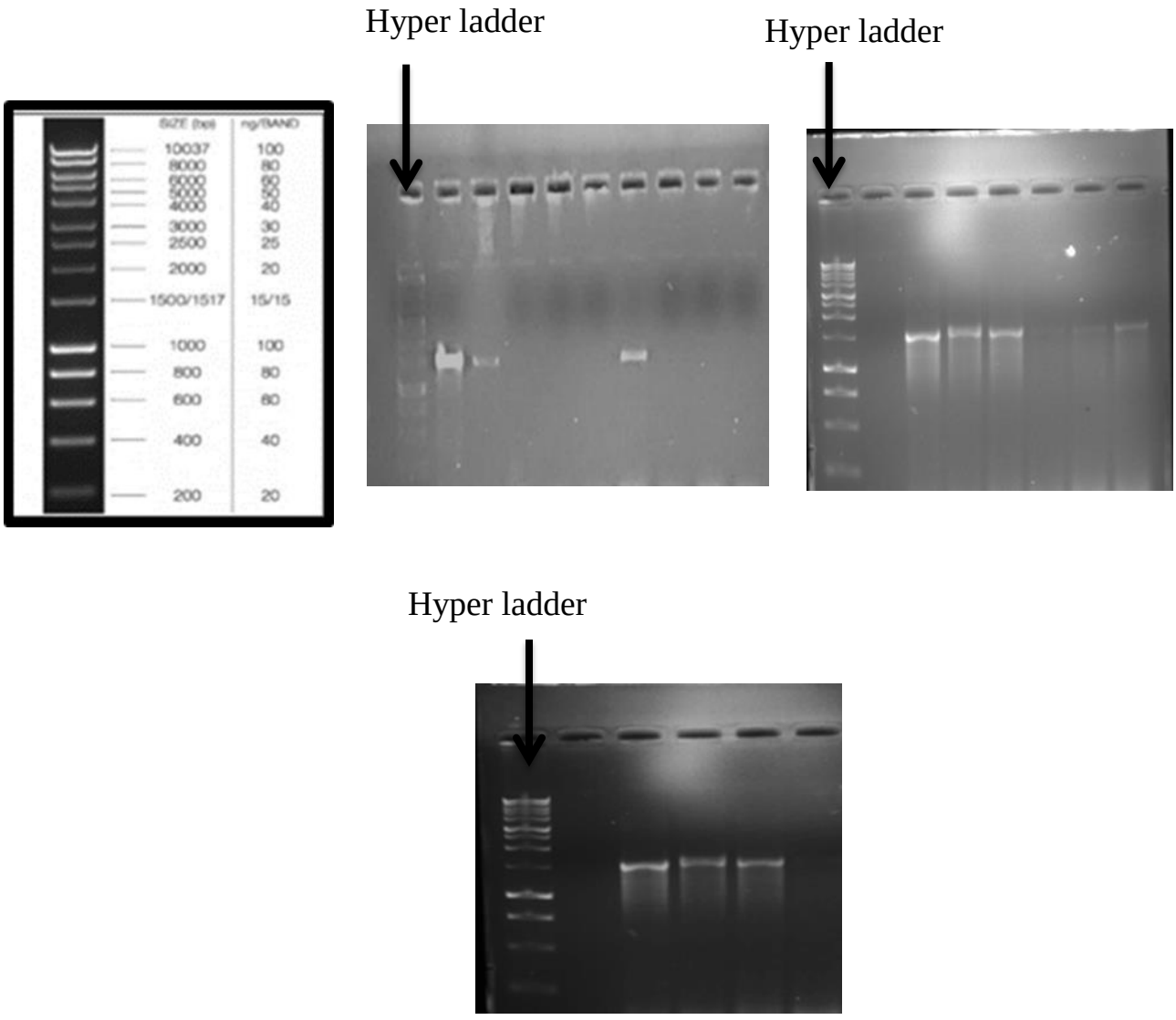


Figure5.4: The polymerase chain reaction (PCR) of extracted DNA from isolated strains on agarose gel (1%) electrophoresis with ethidium bromide.

5.4.1Phylogenetic identification of unknown organisms 16SrRNA

Sequences from isolates obtained from amber were determined and the sequence data was used to produce a phylogenetic tree providing the basis for efficient phylogenetic investigation, of each genus.Table:5.1 shows that *Bacillus amyloiquefaciens, Bacillus methylotrophicus, Bacillus subtilis*, *Cupriavidus respiraculi* were isolated from micrometeorites and *Bacillus thuringiensis*, *Bacillus pumilus, Bacillus cereus, Bacillus toyonensis*, *Anerinibacillus migulanus*, *Anerinibacillus sp.*, *Bacillus altitudinis*, Endophytic bacterium from amber. Tables: 5 (1and2) show 16SrRNA sequence analyses representing the closest matches of the above organisms.

5.4.2. Bacteria Isolation Results

Representative sequence	Closest matches identification	Sequence identity	NBCI(Accession number)
micrometeorite	*Bacillus amyloiquefaciens*	99%	gblcp007165.1
	Bacillus methylotrophicus	99%	gblkF054862.1
	Bacillus subtilis	97%	GbkT026098.1
	Cupriavidu srespiraculi	99%	gblAY860237.1

Table 5.1. 16SrRNA sequence analyses of samples of bacteria cultured from a micrometeorite.

Representative sequence	Closest matches identification	Sequence identity	NBCI (Accession number
Amber	*Bacillus thuringiensis*	100%	gblcpo15150.1
	Bacillus pumilus	93%	gblkF68897.1
	Bacillus cereus	100%	gblkF228908.1
	Bacillustoyonensis	100%	GblkP407115.1
	Anerinibacillus migulanus	95%	gblGu397386.1
	Anerinibacillus sp.	98%	gblkp980744.1
	Bacillus altitudinis	100%	Gblku898277.1
	Endophytic bacterium	100%	Gblkp757642.1

Table 5.2. 16SrRNA sequence analyses of samples of bacteria cultured from amber.

5.5. Discussion

In 1990, Louis and Frank suggested that millions of tonnes of micrometeorites impact the Earth each year Frank and Huyges (1990). At first this suggestion was met with derision, but over the years has become increasing accepted. The relevance of this to panspermia relates to the size of micrometeorites and the fact that they are known to contain organic materials and water. When it was first suggested that lager meteorites might have brought these components to the prebiotic Earth it was pointed out that that such large bolides would have burnt up or atomized on impact, making this suggestion impossible, especially since at this time there would have been no atmosphere top slow them down. The situation changes dramatically however, when considering micrometeorites because these should be able to arrive to the Earth undamaged (except for their outer surfaces and bring with them water and organics (Frank and Huyghe, 1990). The partial aim of the work reported in this chapter was to isolate micrometeorites locally, and determine if they contain bacteria.

Bacteria were isolated from micrometeorites collected from the rainwater and from Firth Court roof guttering. EDAX analysis confirmed the micrometeorite nature of the particles. Since the micrometeorite samples

were sterilised before breaking, it is assumed that the bacteria originated from within the samples. However, the obvious criticism of this approach relates to contamination (i.e. the bacteria could have contaminated the particles during transfer to the Nutrient Agar plate). Unfortunately, there are no adequate controls to demonstrate that the bacteria must have originated from inside the micrometeorite particles. This problem with contamination is dramatically reduced in the case of the amber sampling approach, since the system includes a built-in control (i.e. the amber is only cracked when no bacteria are present in the medium and any bacteria, which grow after cracking, must, it is argued, have originated from inside the meteorite). It is noteworthy that the bacteria isolated from both micrometeorites and amber were predominantly species of *Bacillus*. Since these are spore formers, this would fit in with the possibility that they can survive extremes, such as those found in space, and in terms of time over which the amber samples have existed. The reality is that in relation to this kind of research, critics can (and do) always invoke contamination as an explanation for any research findings. Such criticism needs of course to be answered, but the reaction of many critics to this research is one of unremitting disbelief. Perhaps the only way in which such (sometimes

valid) criticism can be answered is by building up a wide range of experimental evidence in favour of panspermia.

Chapter 6: Short Experiments Relevant to Panspermia

6.1. Attempt to repeat Barber's work related to panspermia

6.1.1. Introduction

In the nineteen thirties, the astronomer Donald Barber, made an unusual observation while working in the Lockyer Laboratory in England. He noticed that exposed photographic plates which had been washed with well-rain water became covered in small areas where the coating was dissolved to form craters and in some case such that the photographic emulsion slid of the glass plate (Barber, 1963). *From the centre of which the silver deposit had been eroded, and transferred to the perimeter* Barber used his knowledge of astronomy to suggest that the bacteria arrived to Earth from space (i.e. they provided evidence in support of the theory of panspermia).

The photographic plates showed bacterial contamination of photographic plates which had been exposed when astronomically observing the night sky between the years 1936 and 1961 and associated with a definite pattern appearing thirty days following the inferior conjunctions of Venus. It was claimed that bacteria contaminated the film during film processing, from and local spring water supply (which was used in the film processing), and that their appearance followed rains and a northerly wind. Barber

suggested the well-known condition, known to photographers who process film as "flying saucers," which appear in the processed film-sky when a water droplet remains on the film during drying. Barber commented as follows:

"At the height of each major 'invasion' irreparable damage to freshly processed spectrographic plates was caused by numerous quasi-circular crater-like defects (0.05 to 0.25 mm in diameter) from the centre of which the silver deposit had been eroded, and transferred to the perimeter. In a badly affected 1/4 plate negative some 5100 of these craters could be seen at low magnification (x15). In several instances the photographic film was completely liquefied and was seen to slide off the glass base intact."

The bacteria which damaged the film appeared in each case some thirty days after an inferior conjunction of Venus, and after northerly winds (actually winds from the northwest) and rain. From these observations, Barber claimed that the bacteria entered the Earth's upper atmosphere (presumably at the aurora borealis) with the solar wind after it passed by Venus. The thirty-day delay is about equal to a terrestrial wind speed of fifteen miles per hour (24 km per h) from the auroral region. Such events only occurred nine times in a 25-year period. Each followed an inferior conjunction of Venus

and the simultaneous occurrence of a “geomagnetic storm," (i.e., an auroral display). Barber claimed that the bacteria were proteolytic (possibly *Pseudomonnas fluorescens*) and had arrived from Venus, (i.e. via panspermia). The bacteria were subsequently shown to be resistant to silver.

The aim of the work reported here was to simulate Barber’s experiments by collecting rainwater over an extended period and determine if any bacteria are present which can solubilize gelatine and are resistant to silver.

6.1.2. Material and Methods

Rainwater samples were collected using a rain collector, comprised of a plastic funnel containing a Whatman Number 1 filter paper and inserted in to a plastic container. Samples were collected on the roof of a building of the Sheffield University each month from May 2016-January, 2017. The samples were placed in glass-sterilized bottles for microbiological analysis and transported to the laboratory the same day. During the 1-year period, 12 seasonal samplings were performed and 300 samples were collected.

The basal medium used for isolation and growth for bacteria was Nutrient agar. Rainwater (1ml) was added to a Nutrient Agar plate; this was then incubated at 25°C under an aerobic condition for 24h to confirm that

bacteria grow in Nutrient Agar. After incubation, the colonies were identified using 16SrRNA analysis.

6.1.3. Results and Discussion

6.1.3.1. Identification of bacteria by 16SrRNA sequencing

Strains of unknown bacteria were isolated from rainwater by using a Qlagen Spin Miniprep - Bacterial DNA Extraction Kit. A whole genomic DNA has successfully been extracted from the strains (fig: 6.2).

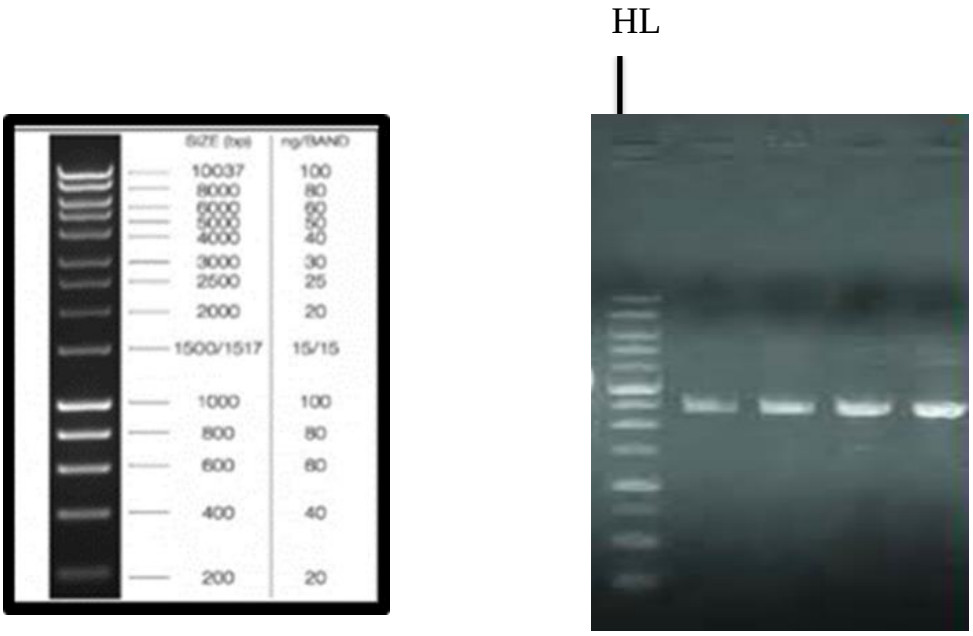


Figure 6.2. Successful genomic DNA extraction from the strains with band size over 1000 base pairs in agarose gel (Right). Standard Hyper ladder produces 14 regularly spaced bands and each lane (5 µl) provides 720 ng of DNA (Left).

6.1.3.2 PCR amplification of extracted DNA

16S rRNA gene sequences have hyper-variable regions where sequences have diverged over time and these are often flanked by strongly conserved regions. Primers targets are conserved regions and amplify variable regions. The DNA sequence gene of 16S rRNA has been determined for a large number of species, forming a readily accessible and extensive library. Amplified 16SrRNA genes are shown in (Fig: 6.3) and the size was banded as expected around 1 kb.

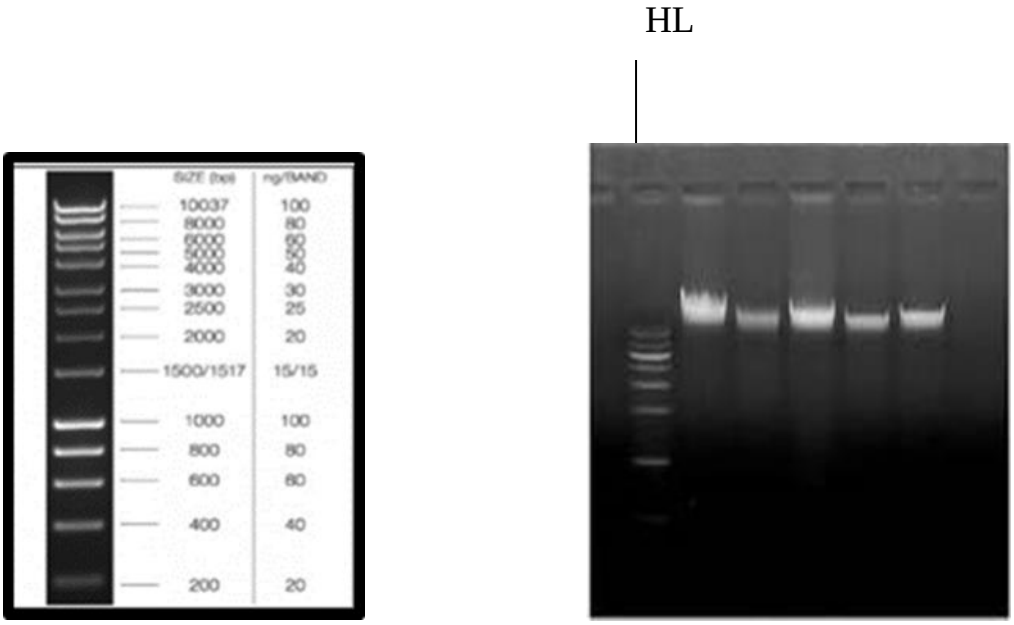


Figure 6.3. Polymerase chain reaction (PCR) of extracted DNA from the strains on agarose gel (1%) electrophoresis with ethidium ,HL stands for the l-kb DNA ladder. Standard Hyperladder I produces of 14 regularly spaced bands and each lane (5 µl) provides 720 ngof DNA (Left).

6.1.3.3. Phylogenetic identification of unknown organisms 16SrRNA

Sequences from rain water were determined and the sequence data used to produce a phylogenetic tree providing the basis for efficient phylogenetic investigation, of each genus. Table 6.1 shows the species were *Bacillus cereus, Bacillus sp., Bacillus thuringiensis,* Uncultured prokaryote, *Bacillus sp, Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus mojavensis , Bacillu velezensis*, *Micrococcus luteus, Enterobacter cloacae.*

The phylogenetic analysis of the organisms (Table: 6.1) shows 16SrRNA sequence analyses representing the closest matches of the above organisms.

6.1.4. Bacteria Isolation Results

Representative sequence	Closest matches identification	Sequence identity	NBCI (Accession number
rain water:	*Bacillus cereus*	99%	CP015589.1
	Bacillus sp	98%	KP 708598.1
	Bacillus thuringiensis	98%	EU939700.1
	Uncultured prokaryote	98%	kp409600.1
	Bacillus sp	98%	km675965.1
	Bacillus subtilis	99%	KF 460575.1
	Bacillus amyloliquefaciens	99%	KM191359.1
	Bacillus mojavensis	99%	JF414769.1
	Bacillus velezensis	99%	CP017775.1
	Micrococcus luteus	99%	KX5277242
	Enterobacter cloacae	99%	KU7470821

Table 6.1: 16SrRNA sequence analyses of samples of bacteria cultured from rainwater.

6.1.5. Results and Discussion

The study showed that no bacteria grew before three days of incubation. Growth occurred in Nutrient Agar supplemented with silver nitrate 0.30, 1.69 and 2.40 rates but not in 3, 10 rates. Fig: 5.6 (A, B, C, D).

A **B**

C **D**

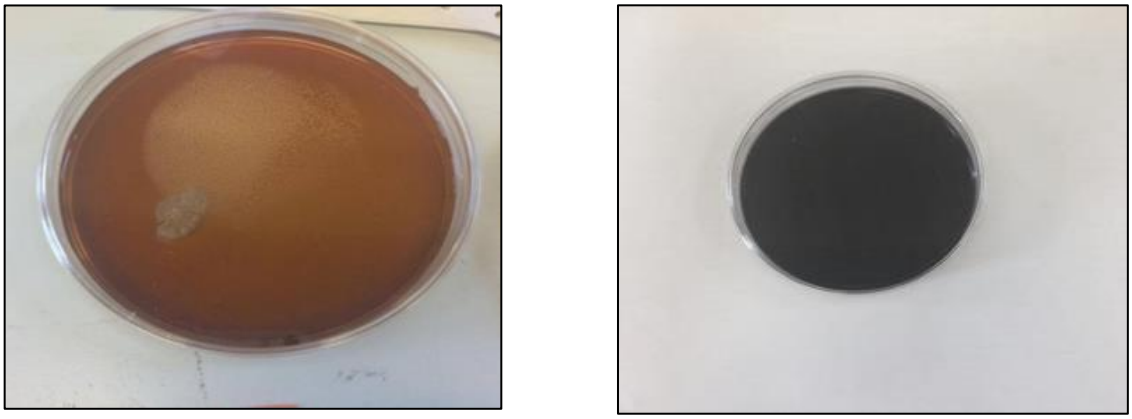


Fig: 5.6 (A, B, C and D) Bacterial Growth in Nutrient Agar supplemented with silver nitrate 0.30, 1.69 and 2.40 rates but not in 3, 10 rates.

The three important points about Barber's findings are:

1) Isolation of gelatine-decomposing bacteria

2) Isolation of bacteria resistant to high concentrations of silver

3) Isolation of bacteria having these properties, which mirrored the orbit of Venus

These studies could provide a negative result when attempting to confirm Barber's studies by showing that silver resistant bacteria could not be isolated from rainwater over a period of a year; sufficient time one would

consider any pansperic-silver resistant bacteria to have been isolated. Had such resistant bacteria been found, then their appearance could have been correlated with the orbit of Venus. On the other hand, had silver resistant bacteria been readily isolated, then it would have made Barber's emphasis on them of little interest.

1) Critics argued that, Barber's findings, although intriguing, do not prove the arrival of bacteria from Venus. They point out that it is difficult to understand why organisms from Venus, should they exist, would be adapted to consuming gelatine, and to do so in the relative cold temperatures of the observatory location and were not adapted to the hot, extreme climate of their supposed parent planet.
2) Despite this, even the critics admitted that the correlation of the findings to Venus's position and the geomagnetic storms remains puzzling.

6.2. Experiments to demonstrate the potential of Morning Glory seeds as a vehicle for panspermia

6.2.1. Introduction

During 2008, the space shuttle, "Atlantis" transported an experiment (called"Exposed") to the International Space Station. The experiment involved biological samples including organic molecules, microorganisms, as well as lichens and fungi and two thousand seeds of tobacco and *Arabidopsis thaliana*, a European weed widely used as a model organism in plant research. The samples were exposed to the outside of the Space Station and exposed for 558 days to the extreme space environment, and thereby exposed to lethal UV light, cosmic radiation, and extreme temperature fluctuations.

Figure 6 .5. A flower of Morning Glory.

When the samples were returned to Earth in 2009, roughly 20% germinated and grew into normal plants10 years later, Tepler and Leach (2008), an emeritus physicist at Paris-Meudon Observatory in France, have taken a closer look at the DNA of some of these space-traveling seeds. In a follow-on experiment in the laboratory, Tepler and Leach (2006) exposed three types of seeds—morning glory, tobacco, and *A. thaliana*—to high doses of UV light. Tepler and Leach (2017) thought morning glory seeds might do well based on their large size, tough seed coats, and ability to survive for more than 50 years in soil. They found that only the Morning Glory seeds germinated after being exposed to UV light approximately six million times the dose generally used in the sterilization of drinking water

(*Arabidopsis* seeds lost their ability to germinate after exposure to some 87 megajoules per square meter of radiation, while all of the morning glories survived even after exposure to a massive dose of 2420 megajoules). Such exposure however, killed the much smaller tobacco and *A. thaliana* seeds. It was suggested that the heavy seed coat of the morning glory protected it, and that flavonoids-compounds act as a cosmic sunscreen. Much of the genome of Morning Glory seeds is redundant, which means they have multiple copies of genetic information should some get damaged. Seeds are also designed to survive long stretches of cold with no water and carry bacteria and fungi that could hitch a ride to a new planet. And even if a seed doesn't survive a long journey through space, it still brings organic materials like proteins, nucleic acids and ribosomes to wherever it lands, which could help jump-start primitive forms of life.

The aim of the wok described here was to determine if Morning Glory seeds carry microbes on the outside of the seed coat and also within the seed, in relation to the possibility that they may act as vehicles of panspermia.

6.2.2. Materials and Methods

Seeds of Morning Glory were obtained from a commercial seed supplier. Around 10 seed were surface sterilized with sodium hypochlorite (10% v/v) and washed twice with 100 ml of sterile distilled water. They were then transferred aseptically to the surface of nutrient agar and crushed *in situ* in order to isolate microbes from the inside of the seeds. Unsterilized seeds were also transferred to nutrient agar and remained uncrushed in order to isolate surface microbes.

6.2.3. Results and Discussion

Microorganisms were isolated from the unsterilized seed coat and from within the seeds. Microorganisms were isolated from the unsterilized seed coat and from within the seeds. Three organisms were isolated, two species of *Bacillus* and colonies of the dimorphic yeast-like fungus, *Aureobasidium pullulans*. The identity of the organisms is not, however, directly relevant to this research, merely the fact that they are present is sufficient. This simple experiment shows that Morning Glory seeds could act as vectors of microbes through space. The microorganisms isolated from within the seeds would be protected from cosmic and UV radiation and be capable of obtaining nutrients and water supplies from within the

seed. It is of course likely that any microbes on the surface of the seeds would be exposed to radiation and the seed would be surface-sterilized. Microbes might however, gain protection from residing within cracks in the rough surface of the seeds or the outer seed microflora might be protected from radiation-killing by a coating of cosmic dust. The results therefore point to the possibility that seeds of angiosperms and analogues of other plants might act as vehicles for panspermic transportation.

Chapter 7: General Discussion

General discussion

The work described in this Thesis was aimed at exploring evidence for the theory of panspermia and neopanspermia. Panspermia relates to the overall theory that life comes from space and relates to the origin of life on Earth, while neopanspermia refers to the possibility that life is continuously arriving to the planet from space. The latter possibility is based on the obvious fact that nothing has fundamentally changed in relation the relationship between Earth, the cosmos since life originally appeared on Earth, and that if life came in then it must continue to do so today.

Most studies on astrobiology relate to studies on the microbiology of extreme environments on Earth, with the hope that they will relate in some way to the possible growth of microbes on other planets. The reason for this is that it is difficult and costly to work on space-derived samples. An alternative is to study microbes, should they exist at high altitude in the stratosphere the hope that the sampling points are sufficiently high to avoid them being contaminated by the fecund Earth biota. Another alternative is to examine material like meteorites which are incoming to Earth from space; both of these approaches have been used here.

The possibility that microbes might be isolated from meteorites and thereby demonstrate that life a exits elsewhere was broached by Lipman (1932) who claimed to have isolated bacilli from the inside of surface-sterilized meteorites. Not surprisingly this claim was met with derision and the usual knee-jerk reaction to studies such as these, namely contamination. More recently studies have focused on looking for fossilised microbes in bolides. The work of Hoover (2006, 2007, 2010) has loomed large in such studies and the many resulting electron microscope images of presumptive microbes (notably Cyanobacteria) in meteorites is impressive; despite this, the work has not been generally accepted by the scientific community. The most famous claim for fossilized microbial life in meteorites comes from studies on the famous Allen Hills meteorite (Mckay *et al*., 1996).

In 1996 it was claimed by NASA and publicised by President Clinton that this bolide provide evidence for life on Mars. Since then, this meteorite has become the most extensively studied rock in history and to date there is still no firm conclusion that the nanobacteria it contains represent fossil life from Mars. The current thinking however, is moving towards a negative with regard to this possibility.

The images provided here of presumptive microbes in a meteorite are amongst a number which have been seen in this laboratory and reported in the literature. While the worm-like and filamentous structures have been shown here not to be recently deposited microbes, it remains difficult to prove that they are not geological artefacts. They certainly appear microbial, but the problem of peridolia sets in that the human mind is structured to look for familiar objects in unfamiliar ones. Unfortunately, did not have the resources in terms of both the manpower and equipment spent on the Allen Hills meteorite, to study the microbe like structures seen in the meteorite studied here. It is important to note that the microbe –like structure found here, and by other workers in this laboratory, as well as by Hoover (2006,2007, 2010) are of the size of bacteria found on Earth and are not similar to the apparent nanobacteria found in the Allen Hills meteorite, the criticism that there is insufficient space in the presumptive organism to contain the necessary genetic material, while relevant to the Allen Hills nanobes, is not relevant in the case of the bacteria seen in meteorites in this laboratory.

Isolation of presumed biological entities from the stratosphere

A balloon launched sampler was developed which could be opened at any required height in the stratosphere up to a theoretical limit of around 42 km. The balloon carries with it GPS location and height devices and a parachute. The sampler is contained in a black Styrofoam box which is tightly sealed. The sampler consists of a drawer which opens at the desired height and collects any stratospheric material which falls onto electron microscope stubs. The sampler box is then returned to Earth by parachute and the samples are removed to a clean room for preparation. The stubs were then coated with gold and examined using and SEM to which is attached an EDAX machine, which can be used to determine the elemental composition of any captured particles.

In the above mentioned studies, two distinct types of particles were captured from the stratosphere, namely cosmic dust and organic particles having the appearance of biology which are termed biological entitles (Bes). EDAX analysis showed that the cosmic dust particles are composed of inorganic elements, such as silicon, iron and calcium while the BEs were found to be composed of carbon and oxygen with occasional traces of

Nitrogen; this signature is regarded as indicating biology. The presumed biological nature of the BEs is based on the following; the particles:

1) Are organic in nature.
2) Are of a size indicative of microbes.
3) Show biology-like form including bilateral symmetry.
4) It is important to note, however, that BEs do not represent any type of organisms currently known to occur on Earth.

Critics of the above findings argue (based on Occam's Razor) that the BEs isolated from the stratosphere have been lifted in some way from Earth; a knee-jerk reaction amongst most critics. The commonly held view is countered by the following:

1) BEs are generally between 10-40 microns in size, which is larger than the 5 microns which is held to be the theoretical limit for the transfer of particles from Earth to the stratosphere at the sampling heights used.
2) BEs are not associated with any Earth-derived debris, such as grass shards, pollen grains or fungal spores.

3) BEs are occasionally associated with impact craters on the stubs, which suggest that they are arriving to the sampler at speed (i.e. from space, rather than lazily drifting up from Earth).

It could of course be argued that the theoretical Earth to the stratosphere transfer-size limit is wrong. The "clincher" here is the lack of common Earth debris in the sampling stubs. One would expect, had the BEs arrived from Earth, that they would be surrounded by a mass of common Earth debris, so much so, in fact that it would be difficult to distinguish the BEs from such debris. In all cases the BEs occur pristinely on the stubs. While control flights show the expected contamination of the outside of the sampling box with grass, pollen and fungal spores, these have never been found on the inside of the box or on the stubs. This would appear to provide irrefutable evidence that the BEs are not from Earth. The apparent non-Earth-biology appearance of the BEs could be explained by the argument that they are Earth organisms but have not yet been found here, an argument which can, of course, never be refuted.

Origin of BEs

While the non-Earth origin of the BEs is convincing it is not clear from where they originated. The Hoyle-Wickramasinghe theory of panspermia

suggests that life is ejected by comets as they travel through the cosmos. Comets may be a source therefore of BEs. Such particles may be ejected in large ice particles which progressively become smaller as they travel through space. Any BEs in such ice masses would be protected from

U|V-C and other extreme conditions (note that such ice would be covered in black commentary debris). Eventually such particles would arrive at the edge of the Earth's atmosphere and depending on the angle of approach would either burn up or descend into the stratosphere. Some BEs would still be contained in ice participles which would explain why in rare cases BEs are associated on the sampling stubs with impact events. It is interesting to note that the successful entry of BEs to a planet needs an atmosphere and that Earth is the perfect planet to sample such particles. Any BEs arriving on the Moon, for example, would almost certainly be atomised because of the lack of an atmosphere. Tonnes of cosmic dust arrive to the Earth annually and BEs would likely make up only a small, but important part of this debris. It is probable that most would be dead, although the occasional viable BEs would likely get through. Their DNA and any other information they possess would however, be delivered by this process.

The above findings, if proven correct by others, would have a major impact on our view of biology. The results strongly suggest that non–Earth organisms, not only possibly led to the origin of life on Earth (panspermia), but are continually arriving here from space (neopanspermia), and bring with them DNA, and other sources of information which might be able to integrate with the Earths biome. Such information could have a major impact on the course of the evolution of life on Earth. If correct, studies like these, and the others conducted in this laboratory on neopanspermia could be paradigm-shifting and force biologists away from their current, largely Earth-centric view of biology.

The last two experiments described here relate to the unusual studies of Barber on panspermia and the possibility that morning glory, and thereby other seeds could act as vehicles for panspermia. Barber's work on panspermia is highly speculative, but at the same time intriguing. This researcher observed that the presence of bacteria in rainwater, which are able to dissolve photographic emulsion (largely based on gelatine) and are highly resistant to silver ions seemed to correlate with the movements of Venus. Barber then went on to make the highly speculative claim that these bacteria (*Pseudomonas fluroscens)* originated from Venus. It was considered worthwhile (although recognized from the outset as something

of a long-shot) to attempt to isolate silver-resistant bacteria from rainwater collected in Sheffield over a one-year period. No such bacteria were isolated over a year-long sampling period.

Such confirmatory studies are of course only of interest if they produce a positive result since it can always be argued that a negative result is based on difference in samples times and approaches. Barber's work will doubtless be shrugged –off as a fantasy by most microbiologists and astrobiologist and we have found nothing to confirm it. The suggestion that it is very unlikely that Earth-like bacteria, such as species of *Pseudomonas* could survey the rigours of Venus is of course compelling. Despite this, astronomers will point to the remarkable apparent coincidence between the isolation of bacteria from rain samples and the movements of Venus.

Discussion have been made in this Thesis concerning the possibility of so-called reverse panspermia, implies that microorganism's on Earth could be ejected into space by an impact event, such as the one which has been suggested resulted in the death of the dinosaurs. Such impacts could be caused by meteorites or comets hitting the Earth, leading to the ejection of vast amounts of debris, some of which would be sub-5 microns and could therefore be sent to the atmosphere by such a violent release of energy.

Since microbes exit in all substrates on Earth this possibility is obvious but still needs to be backed up by evidence. Here we show that a boulder clay and stromatolite contain a wide range of bacteria which could, if ejected by an impact event be sent into space. Wainwright (2013) showed that the materials used in buildings which support our civilisation, including bricks, stone, road-tarmac and concrete all contain microbes within them and coined the term “archipansperma” to suggest the possibility that all the man-made material around the world and particularly in big cities like London and New York could be ejected into space following an impact event and carry with them living material which would be protected by being inside hard material.

The final part of this study is based on the recent claim that morning glory, and other, seeds might act as vehicles for panspermia themselves and in relation to the bacteria and fungi they might contain. Morning glory seeds are extremely resistant to radiation and could possibly survive in space and be transmitted via panspermia. The results presented here show that they contain bacteria and the fungus, *Aureobasidum pullulans* which could, by being protected inside the seeds themselves survive passage through space. It is worth reiterating that we have not seen similar seed or pollen grains to indicate that plant life is coming into Earth in the

stratosphere-studies reported here and elsewhere. While such studies are suggestive of the possibility that microbes present within seeds could be transported through space, the idea that morning glory and other plants are growing on some distant planet would seem to most scientists to be incredulous.

At the time of concluding this Thesis, Russian scientists have reported finding plankton and other microbes on the outside windows of the international space station. This remarkable finding is one, which was earlier reported in 2014, but unlike the original report, this 2017 claim is backed up by a scientific paper, which unfortunately is in Russian, making it difficult to interpret. A copy of the English Abstract is, however, included here:

STUDY OF THE ENVIRONMENT FINELY DISPERSED ON THE OUTER SURFACE OF THE INTERNATIONAL SPACE STATION AND DETECTION OF MICROBIOLOGICAL OBJECTS IN SPACE EXPERIMENT «TEST»

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Results of space experiment «Test», aimed at studying the state of the outer surface of the modules of the Russian Segment of the International Space Station (ISS), functioning in an aggressive external environment are considered in the work. The space experiment, which aims – identify prerequisites and possible mechanisms of the emergence and development of destructive processes on the surface of the ISS is described. Space experiment «Test» consists of two phases of research: the orbital and ground. Task of orbital phase is accompanying sampling of gas and dust precipitation on the surface of the station from the environment and degradation products of fine material of structural elements caused by exposure to cosmic radiation, corrosion processes, particle bombardment and other debris. For the first time in the world samples have been collected by astronaut-operator during operation «extravehicular Activities» («EVA») in a container-monoblok (sampler), which is supported by sterilized and hermetic throughout the experiment, including delivery to Earth. Task of ground phase is conducting physic-chemical, toxicological, microbiological and molecular analysis. The analysis showed the presence of viable microorganisms on the outer surface of the ISS, which allows you to put the question of the boundaries of the spread of the Earth's biosphere and continue more in-depth study of the ecological state of isolated inhabited space objects.

Key words: *International Space Station, dust and gas deposits, the fine environment degradation products of matrices, sampling viable organisms, Earth's biosphere.*

As can be inferred from this, the Russians are claiming that a number of bacterial species are present in the cosmic dust found on the outside windows of the ISS. Scientists on board the ISS are reported to have discovered such living organisms when taking samples from windows.

Vladimir Solovyev, head of the Russian ISS orbital mission said the results of the experiment "are absolutely unique". Solovyev further informed the Russian Itar-Tass news agency that the tiny marine life-forms were not native to the launch site in Kazakhstan and concluded that the microbes were being uplifted to the ISS from Earth. NASA scientists appear not to be party to the discovery, but a spokesperson said that the windows must have been contaminated on Earth, this despite the fact that rigorous cleaning is done on any object that is sent into space. The assumption that the organisms were lifted from Earth to the height of the ISS orbit (400km) is, as was mentioned earlier in this Thesis, highly unlikely since plankton are too large and heavy to have been lifted to this extreme height by any known mechanism.

Wainwright *et al*. (2013) reported the isolation of a partial diatom frustule from the stratosphere at a height approaching 30km and suggested that this, and other Biological Entities, are incoming to Earth from space. This published conclusion was questioned on the basis of Occam's razor, since

it was argued the plankton must have come from Earth because of the known vast numbers of such organisms in the oceans. Wainwright *et al*. (2013) however, have concluded that Biological Entities are incoming to Earth all of the time, thereby advocating the theory of Neopanspermia. From a seemingly outlandish idea which few scientists even considered until recently, the theory of panspermia has now become widely accepted and even fashionable, in large part due to the work of Hoyle and Wickramasinghe which has been widely discussed in this Thesis. It is hoped that some of the work described in the Thesis will add further to the view that panspermia is a respectable scientific idea and that biologists of the future will need to move away from an Earth–centric view of the world and accept that our planet is an open system which continually interacts with the cosmos, for good and for ill.

Conclusion

The results presented in this Thesis, are both fascinating and often frustratingly inconclusive. Many of the findings are open to reasonable attack by critics, although some published work by members of this laboratory in this area of work has been subjected to knee-jerk response of contamination. Every attempt has been made here to avoid this obvious criticism. Work on panspermia is limited by the fact that so few scientists are working in this field so that there is little in the way of back-up information to strengthen any research findings. Without doubt, the most

convincing part of this research relates the studies of BEs in the stratosphere. The finding of unusual biological entities in the stratosphere is in itself obviously notable. The fact that I, and others from this laboratory, provides sound evidence that this biological material is arriving to Earth from space is potentially paradigm-changing. To date, no serious arguments have been provided to discredit our view that BEs are not Earth organisms- based on their size, morphology and the fact that they are not associated with a stratosphere-obtained sample- SEM stubs with grass, pollen and fungal spores. It is imperative that these stratosphere-studies be repeated by other workers in labratoes across the world, because if the

findings are indeed correct (as they appear to be) then biologists will have to completely change their mind-sets from being Earth centred to astrobiologists.

Studies on the microbiology of fulgurites were conducted here in relation to the criticism that perhaps, I and others in this laboratory have not been working on meteorites (this, despite the fact that samples have been authenticated as such) but they are fulgurite samples which have been formed here on Earth and therefore have been readily contaminated by Earth organisms. Such fulgurites are formed when lightning hits sand on Earth. They have a characteristic structure under the SEM and can be easily distinguished from carbonaceous chondrite, meteorites.

It was clear from the studies conducted here that fulgurites contain a diverse living bacterial flora and they do not possess presumptive fossilized bacteria like those seen in meteorites. It is clear then that fulgurites are completely distinct from meteorites, both in relation to their active bacterial flora and lack presumptive fossilized bacteria-morphs.

Amber and the other samples used here, such as stromalolite samples represent an ancient material which could provide evidence for the potential longevity of life. Critics of the panspermia-theory, claim that microorganisms cannot possibly live long enough to be transported over

the vastness of the cosmos, which would involve journey times of millions of years. Since the work of Lipman (Lipman, 1932) there have been claims that bacteria can be isolated from ancient amber of ages around 40 million years. Studies in the laboratory have had variable results in relation to isolating bacteria from this source, with some studies being positive for bacteria, while others produced no living bacteria. The basis of this work is the possibility that bacteria were associated with insect and plant debris at the time when they were sealed into amber and have remained dormant ever since. Although substantiated by a number of very careful studies by Canto and Boruki (1995), such claims are of course very difficult to believe. The work described here provided positive results relating to the isolation of bacteria from ancient amber and is based on a meticulous isolation technique containing built-in controls which apparently demonstrates, beyond reasonable doubt, that bacteria are present inside amber; the critics of course respond that these are modern bacteria and have entered the amber sample, this despite the fact that there were no cracks evident on the amber surface. Canto and Boruki (1995) also claim that their isolated bacteria were associated with gas bubbles in the amber which were laid down when the amber formed.

As with many aspects of work like that described in the Thesis, it seems almost impossible to satisfy the demands of the critics.

Suggestions for Further Studies

The following suggestions are given for potential further studies:

1. Perform studies on the survivability of microbes in the stratosphere and the upper layers of the atmosphere by exposing different microbial samples with different shielding material to the stratospheric environment.
2. Collaborate (if possible) with NASA and the Russian Space Agency on studies on the possible microbial contamination of the outside of the Space Station.

3. Encourage (and collaborate with) other groups to attempt to replicate studies on the presence of BEs in the stratosphere.

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Appendices

Appendix A

16srRNA sequence analyses of cultured from fulgurite, salt crystal, K/T clay, Filey- Earth clay and clay roots and from the stromatolite rock.

Bacillus cereus partial 16S rRNA gene, strain IICTSVMH1

Sequence ID: emb|FR865171.1| Length: 5572 Number of Matches: 7

Range 1: 79 to 485 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
726 bits(804)	0.0	405/407(99%)	0/407(0%)	Plus/Plus

```
Query  38   GACTGCGATAACTCCGGGAAACGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTC  97
            ||||| ||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  79   GACTGGGATAACTCCGGGAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTC  138

Query  98   GAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTT  157
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  139  GAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTT  198

Query  158  GGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCA  217
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  199  GGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCA  258

Query  218  CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCA  277
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  259  CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCA  318

Query  278  ATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAA  337
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  319  ATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAA  378

Query  338  CTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAA  397
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  379  CTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAA  438

Query  398  CCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTA  444
            ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  439  CCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTA  485
```

Staphylococcus aureus strain MASMIN29 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|KP137514.1| Length: 1200 Number of Matches: 1

Range 1: 346 to 1096 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1337 bits(1482)	0.0	748/752(99%)	1/752(0%)	Plus/Minus

```
Query  1     GTGTGACGGGCGGTGTGTACAAGACCCGGGAACGTATTCACCGTAGCATGCTGATCTACG  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1096  GTGTGACGGGCGGTGTGTACAAGACCCGGGAACGTATTCACCGTAGCATGCTGATCTACG  1037

Query  61    ATTACTAGCGATTCCAGCTTCATGTAGTCGAGTTGCAGACTACAATCCGAACTGAGAACA  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1036  ATTACTAGCGATTCCAGCTTCATGTAGTCGAGTTGCAGACTACAATCCGAACTGAGAACA  977

Query  121   ACTTTATGGGATTTGCTTGACCTCGCGGTTTCGCTGCCCTTTGTATTGTCCATTGTANCA  180
             |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  976   ACTTTATGGGATTTGCTTGACCTCGCGGTTTCGCTGCCCTTTGTATTGTCCATTGTAGCA  917

Query  181   CGTGTGTAGCCCAAATCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGG  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  916   CGTGTGTAGCCCAAATCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGG  857

Query  241   TTTGTCACCGGCAGTCAACTTANAGTGCCCAACTTAATGATGGCAACTAAGCTTAAGGGT  300
             |||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  856   TTTGTCACCGGCAGTCAACTTAGAGTGCCCAACTTAATGATGGCAACTAAGCTTAAGGGT  797

Query  301   TGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCAC  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  796   TGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCAC  737

Query  361   CACCTGTCACTTTGTCCCCCGAAGGGGAAGGCTCTATCTCTAGAGTTGTCAAAGGATGTC  420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  736   CACCTGTCACTTTGTCCCCCGAAGGGGAAGGCTCTATCTCTAGAGTTGTCAAAGGATGTC  677

Query  421   AAGATTTGGTAAGGTTCTTCGCGTTGCTTCGGATTAAACCACATGCTCCACCGCTTGTGC  480
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Sbjct  676   AAGATTTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGC  617

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Query  541   TAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTT  600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  556   TAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTT  497

Query  601   ACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGATCCCCACGCTTTCGCACATCAGCG  660
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Query  721   CACCGCTACACATGGAATTTCCACTTTCCTCT  752
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Sbjct  376   CACCGCTACACATGGAA-TTCCACTTTCCTCT  346
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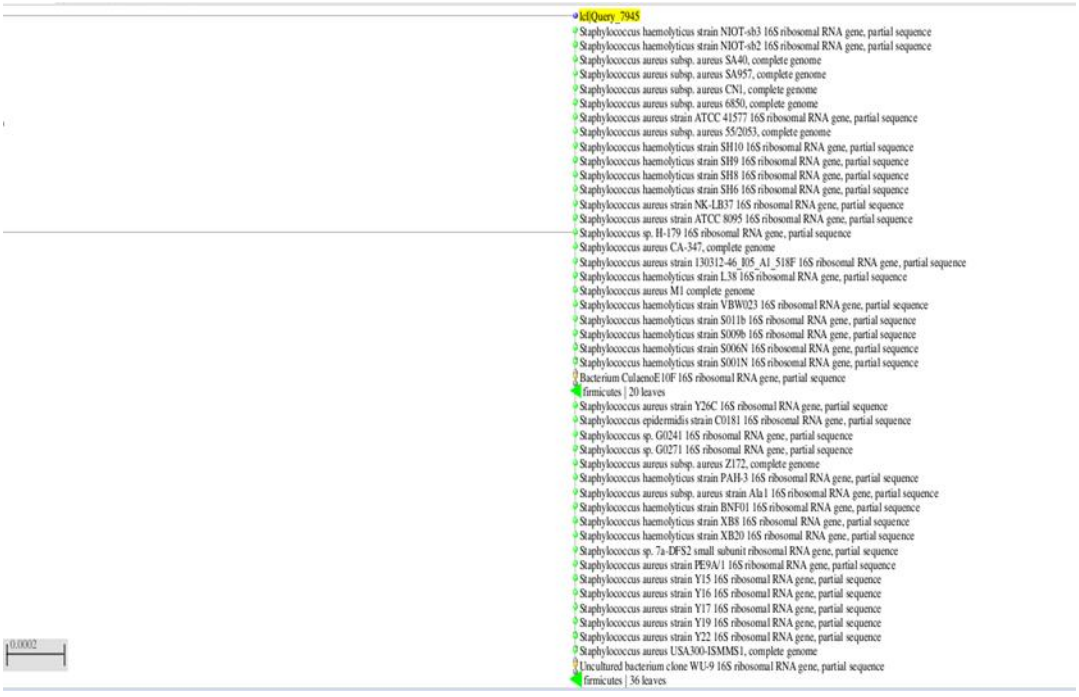

[Pseudomonas] geniculata strain H10 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|KC934806.1| Length: 1504 Number of Matches: 1

Range 1: 981 to 1395 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
731 bits(810)	0.0	411/415(99%)	0/415(0%)	Plus/Minus

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Query  61    TAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTGAGATAGGGTTT  120
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Sbjct  1335  TAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTGAGATAGGGTTT  1276

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Query  241   CACCGGCGGTCTCCTTAGAGTTCCCACCATTACGTGCTGGCAACTAAGGACAAGGGTTGC  300
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Sbjct  1155  CACCGGCGGTCTCCTTAGAGTTCCCACCATTACGTGCTGGCAACTAAGGACAAGGGTTGC  1096

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Sbjct  1035  CTGTGTTCGAGTTCCCGAAGGCACCAATCCATCTCTGGAAAGTTCTCGACATGTC  981
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lcl|Query_23621
Uncultured Stenotrophomonas sp. clone 101.2-10 small subunit ribosomal RNA gene, partial sequence
Brugia timori genome assembly B_timori_Indonesia_v1_0_4, scaffold BTMF_scaffold0005881
Schistosoma rodhaini genome assembly S_rodhaini_Burundi, scaffold SROB_scaffold0016780
Stenotrophomonas sp. enrichment culture clone MTS-2 16S ribosomal RNA gene, partial sequence
Uncultured bacterium partial 16S rRNA gene, clone SIHA.1.1224_N9D0_16S_A
Uncultured bacterium partial 16S rRNA gene, clone SIHA.1.1177_N9D0_16S_A
Uncultured bacterium partial 16S rRNA gene, clone SIHA.1.947_N9D0_16S_A
Uncultured bacterium partial 16S rRNA gene, clone SIHA947_N9D0_16S_B
Uncultured bacterium partial 16S rRNA gene, clone SIHA.1.451_N9D0_16S_A
Uncultured bacterium partial 16S rRNA gene, clone SIHA.1.740_N9D0_16S_A
Uncultured bacterium partial 16S rRNA gene, clone SIHA.1.1414_N9D0_16S_A
Uncultured bacterium partial 16S rRNA gene, clone SIHA832_N9D0_16S_B
Uncultured bacterium partial 16S rRNA gene, clone SIFF597_N9D4_16S_B
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Uncultured bacterium partial 16S rRNA gene, clone SIFF1131_N9D4_16S_B
Uncultured bacterium partial 16S rRNA gene, clone SIGX674_N9D4_16S_B
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Uncultured bacterium partial 16S rRNA gene, clone SIBS776_N12D0_16S_B
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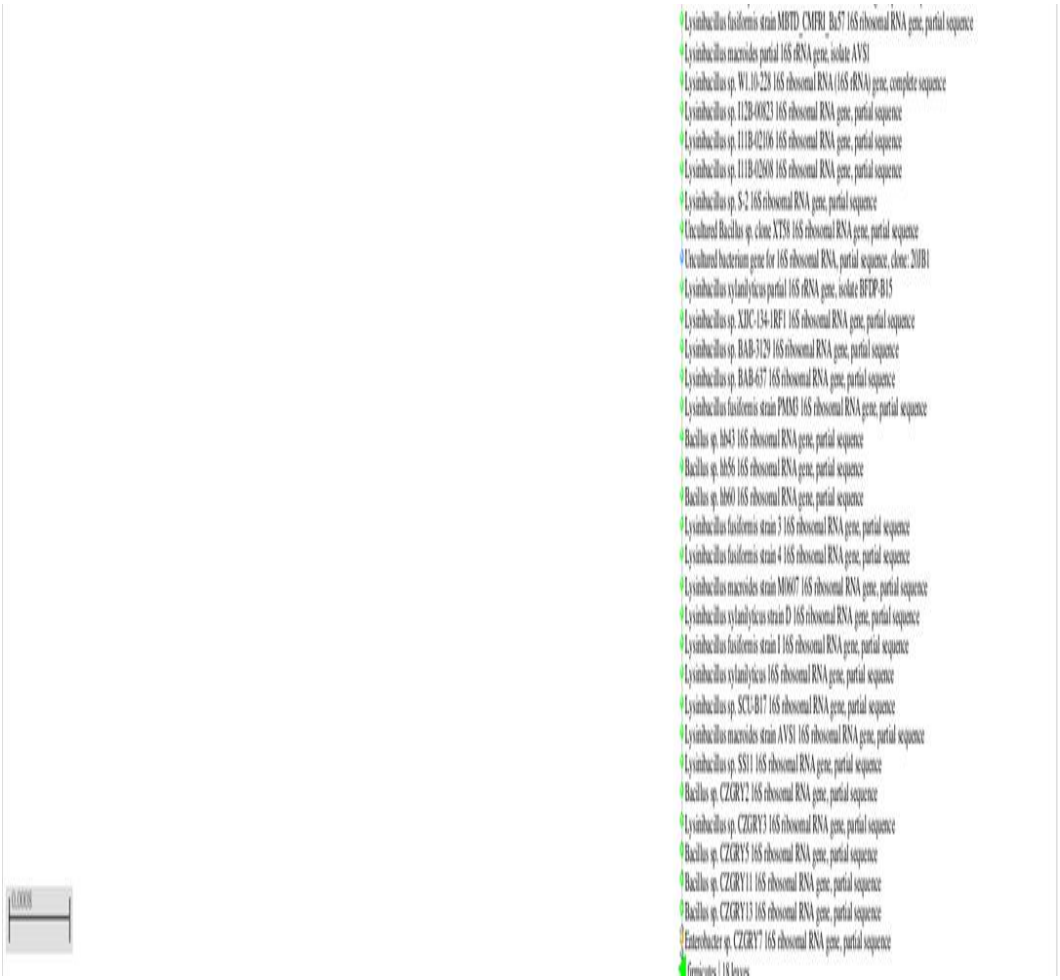
Uncultured bacterium partial 16S rRNA gene, clone SIBN1182_N12D1_16S_B
Uncultured bacterium partial 16S rRNA gene, clone SIBN1333_N12D1_16S_B
Uncultured bacterium partial 16S rRNA gene, clone SIBN1052_N12D1_16S_B
Stenotrophomonas maltophilia partial 16S rRNA gene, isolate AAIII-2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Lysinibacillus fusiformis strain MBTD_CMFRI_Bs57 16S ribosomal RNA gene, partial sequence
Lysinibacillus macroides partial 16S rRNA gene, isolate AVS1
Lysinibacillus sp. W1.10-228 16S ribosomal RNA (16S rRNA) gene, complete sequence
Lysinibacillus sp. 112B-08823 16S ribosomal RNA gene, partial sequence
Lysinibacillus sp. 111B-02106 16S ribosomal RNA gene, partial sequence
Lysinibacillus sp. 111B-02608 16S ribosomal RNA gene, partial sequence
Lysinibacillus sp. S-2 16S ribosomal RNA gene, partial sequence
Uncultured Bacillus sp. clone XT58 16S ribosomal RNA gene, partial sequence
Uncultured bacterium gene for 16S ribosomal RNA, partial sequence, clone: 20IB1
Lysinibacillus xylanilyticus partial 16S rRNA gene, isolate BFDP-B15
Lysinibacillus sp. XJIC-134-1RF1 16S ribosomal RNA gene, partial sequence
Lysinibacillus sp. BAB-3129 16S ribosomal RNA gene, partial sequence
Lysinibacillus sp. BAB-637 16S ribosomal RNA gene, partial sequence
Lysinibacillus fusiformis strain PMMD 16S ribosomal RNA gene, partial sequence
Bacillus sp. bb43 16S ribosomal RNA gene, partial sequence
Bacillus sp. bb56 16S ribosomal RNA gene, partial sequence
Bacillus sp. bb60 16S ribosomal RNA gene, partial sequence
Lysinibacillus fusiformis strain 3 16S ribosomal RNA gene, partial sequence
Lysinibacillus fusiformis strain 4 16S ribosomal RNA gene, partial sequence
Lysinibacillus macroides strain M9607 16S ribosomal RNA gene, partial sequence
Lysinibacillus xylanilyticus strain D 16S ribosomal RNA gene, partial sequence
Lysinibacillus fusiformis strain I 16S ribosomal RNA gene, partial sequence
Lysinibacillus xylanilyticus 16S ribosomal RNA gene, partial sequence
Lysinibacillus sp. SCU-B17 16S ribosomal RNA gene, partial sequence
Lysinibacillus macroides strain AVS1 16S ribosomal RNA gene, partial sequence
Lysinibacillus sp. SS11 16S ribosomal RNA gene, partial sequence
Bacillus sp. CZGRY2 16S ribosomal RNA gene, partial sequence
Lysinibacillus sp. CZGRY3 16S ribosomal RNA gene, partial sequence
Bacillus sp. CZGRY5 16S ribosomal RNA gene, partial sequence
Bacillus sp. CZGRY11 16S ribosomal RNA gene, partial sequence
Bacillus sp. CZGRY13 16S ribosomal RNA gene, partial sequence
Enterobacter sp. CZGRY7 16S ribosomal RNA gene, partial sequence

Lysinibacillus boronitolerans strain OSR5 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|KJ155817.1| Length: 826 Number of Matches: 1

Related Information

Range 1: 87 to 654 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
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Sbjct  87   TTGGGATAACTCCGGGAAACCGGGGCTAATACCGAATAATCTCTTTTGCTTCATGGTGAA  146

Query  92   AGACTGAAAGACGGTTTCGGCTGTCGCTATAGGAAGGGCCCGCGGGCGCATTAGCTAGTTG  151
            ||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||
Sbjct  147  AGACTGAAAGACGGTTTCGGCTGTCGCTATAGGATGGGCCCGCGGGCGCATTAGCTAGTTG  206

Query  152  GTGAGGTAACGGCTCNCCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCAC  211
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Sbjct  207  GTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCAC  266

Query  212  ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAA  271
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  267  ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAA  326

Query  272  TGGGCGAAAGCCTGATGGAGCAACGCCGCGTGAGTGAACAAGGTTTTCGGATCGTAAAAC  331
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Sbjct  327  TGGGCGAAAGCCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAAC  386

Query  332  TCTGTTGTAAGGGAAGAACAAGTACAGTAGTAACTGGCTGTACCTTGAAGGTACCTTATT  391
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Sbjct  387  TCTGTTGTAAGGGAAGAACAAGTACAGTAGTAACTGGCTGTACCTTGACGGTACCTTATT  446

Query  392  AGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGT  451
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Sbjct  447  AGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGT  506

Query  452  CCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTCCTTTAAGTCTGATGTGAAAGCCCA  511
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Sbjct  507  CCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTCCTTTAAGTCTGATGTGAAAGCCCA  566

Query  512  CGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGGACTTGAGTGCAGAAGAGGAAAGTGG  571
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Sbjct  567  CGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGGACTTGAGTGCAGAAGAGGAAAGTGG  626

Query  572  AATTCCAAGTGTACCGGTGAAAATGCGTA  600
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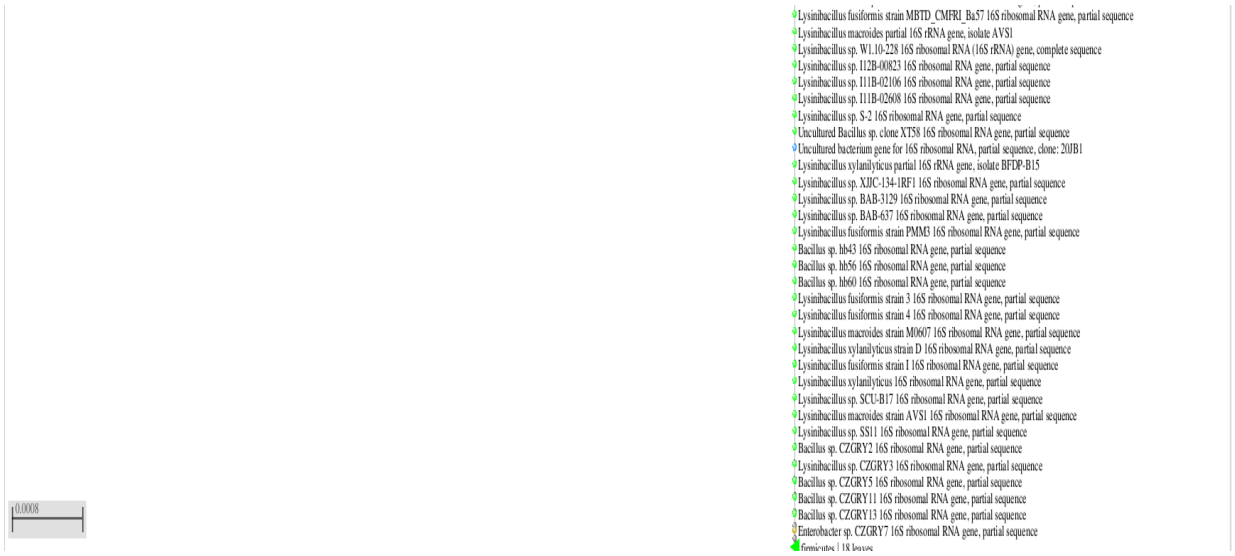
Lysinibacillus fusiformis strain MBTD_CMFRI_Ba57 16S ribosomal RNA gene, partial sequence
Lysinibacillus macroides partial 16S rRNA gene, isolate AVS1
Lysinibacillus sp. W1.10-228 16S ribosomal RNA (16S rRNA) gene, complete sequence
Lysinibacillus sp. I12B-00823 16S ribosomal RNA gene, partial sequence
Lysinibacillus sp. I11B-02106 16S ribosomal RNA gene, partial sequence
Lysinibacillus sp. I11B-02608 16S ribosomal RNA gene, partial sequence
Lysinibacillus sp. S-2 16S ribosomal RNA gene, partial sequence
Uncultured Bacillus sp. clone XTS8 16S ribosomal RNA gene, partial sequence
Uncultured bacterium gene for 16S ribosomal RNA, partial sequence, clone: 20JB1
Lysinibacillus xylanilyticus partial 16S rRNA gene, isolate BFDP-B15
Lysinibacillus sp. XJJC-134-1RF1 16S ribosomal RNA gene, partial sequence
Lysinibacillus sp. BAB-3129 16S ribosomal RNA gene, partial sequence
Lysinibacillus sp. BAB-637 16S ribosomal RNA gene, partial sequence
Lysinibacillus fusiformis strain PMM3 16S ribosomal RNA gene, partial sequence
Bacillus sp. hb43 16S ribosomal RNA gene, partial sequence
Bacillus sp. hb56 16S ribosomal RNA gene, partial sequence
Bacillus sp. hb60 16S ribosomal RNA gene, partial sequence
Lysinibacillus fusiformis strain 3 16S ribosomal RNA gene, partial sequence
Lysinibacillus fusiformis strain 4 16S ribosomal RNA gene, partial sequence
Lysinibacillus macroides strain M0607 16S ribosomal RNA gene, partial sequence
Lysinibacillus xylanilyticus strain D 16S ribosomal RNA gene, partial sequence
Lysinibacillus fusiformis strain I 16S ribosomal RNA gene, partial sequence
Lysinibacillus xylanilyticus 16S ribosomal RNA gene, partial sequence
Lysinibacillus sp. SCU-B17 16S ribosomal RNA gene, partial sequence
Lysinibacillus macroides strain AVS1 16S ribosomal RNA gene, partial sequence
Lysinibacillus sp. SS11 16S ribosomal RNA gene, partial sequence
Bacillus sp. CZGRY2 16S ribosomal RNA gene, partial sequence
Lysinibacillus sp. CZGRY3 16S ribosomal RNA gene, partial sequence
Bacillus sp. CZGRY5 16S ribosomal RNA gene, partial sequence
Bacillus sp. CZGRY11 16S ribosomal RNA gene, partial sequence
Bacillus sp. CZGRY13 16S ribosomal RNA gene, partial sequence
Enterobacter sp. CZGRY7 16S ribosomal RNA gene, partial sequence
0.0008

Lysinibacillus sphaericus strain YH4 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|KM873374.1| Length: 1451 Number of Matches: 1

Range 1: 780 to 1388 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1085 bits(1202)	0.0	605/609(99%)	0/609(0%)	Plus/Minus

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Sbjct  1388  TCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGAT  1329

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Query  481   TGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGA  540
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Query  601   CGTTTACGG  609
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015_fs_Sh-1-R_16srRNA
Brevibacillus sp. strain RHH26 16S ribosomal RNA gene, partial sequence
Brevibacillus brevis strain YQH9 16S ribosomal RNA gene, partial sequence
Brevibacillus brevis strain YQH40 16S ribosomal RNA gene, partial sequence
Brevibacillus brevis strain ZAQ6 16S ribosomal RNA gene, partial sequence
Brevibacillus brevis strain YQH18 16S ribosomal RNA gene, partial sequence
Brevibacillus brevis strain FM4B 16S ribosomal RNA, partial sequence
Brevibacillus agri partial 16S rRNA gene, strain 7-2AIA
Brevibacillus reuszeri strain Ns2-14 16S ribosomal RNA gene, partial sequence
Brevibacillus parabrevis strain DYJK4-8 16S ribosomal RNA gene, partial sequence
Bacillus amyloliquefaciens strain GSLS11 16S ribosomal RNA gene, partial sequence
Brevibacillus parabrevis strain Ks8-12 16S ribosomal RNA gene, partial sequence
Brevibacillus brevis strain Hs8-13 16S ribosomal RNA gene, partial sequence
Brevibacillus formosus strain NBRC 15716 16S ribosomal RNA gene, partial sequence
Brevibacillus parabrevis strain KT6-18 16S ribosomal RNA gene, partial sequence
Brevibacillus brevis strain JSZYS8 16S ribosomal RNA gene, partial sequence
Brevibacillus brevis strain HNYM3 16S ribosomal RNA gene, partial sequence
Brevibacillus reuszeri strain HNYM37 16S ribosomal RNA gene, partial sequence
Brevibacillus reuszeri strain HNYM43 16S ribosomal RNA gene, partial sequence
Brevibacillus sp. SE12 16S ribosomal RNA gene, partial sequence
Brevibacillus sp. F51 16S ribosomal RNA gene, partial sequence
Brevibacillus parabrevis strain SH37 16S ribosomal RNA gene, partial sequence
Brevibacillus sp. SAUBC3-2 16S ribosomal RNA gene, partial sequence
Brevibacillus formosus strain TJKB-24 16S ribosomal RNA gene, partial sequence
Brevibacillus agri strain SSA834 16S ribosomal RNA gene, partial sequence
Brevibacillus sp. AT05 16S ribosomal RNA gene, partial sequence
Brevibacillus formosus strain N26-1 16S ribosomal RNA gene, partial sequence
Brevibacillus agri 16S ribosomal RNA gene, partial sequence
Brevibacillus brevis strain RZ-17 16S ribosomal RNA gene, partial sequence
Brevibacillus sp. ZK3 16S ribosomal RNA gene, partial sequence
Brevibacillus sp. BAB-4179 16S ribosomal RNA gene, partial sequence
Brevibacillus brevis strain N-134 16S ribosomal RNA gene, partial sequence
Brevibacillus brevis strain X09 16S ribosomal RNA gene, partial sequence
Brevibacillus sp. AMBR2 16S ribosomal RNA gene, partial sequence
Brevibacillus brevis strain GZDF2 16S ribosomal RNA gene, partial sequence
Brevibacillus brevis strain GZDF3 16S ribosomal RNA gene, partial sequence
Brevibacillus brevis strain EB1 16S ribosomal RNA gene, partial sequence
Brevibacillus agri 5-2 16S ribosomal RNA gene, partial sequence
Brevibacillus sp. KT84 16S ribosomal RNA gene, partial sequence
Brevibacillus agri strain DH-1 16S ribosomal RNA gene, partial sequence
Brevibacillus brevis gene for 16S ribosomal RNA, partial sequence
Brevibacillus agri strain RH01_05 16S ribosomal RNA gene, partial sequence
Brevibacillus sp. BAB-99 16S ribosomal RNA gene, partial sequence
Brevibacillus agri strain Y12 16S ribosomal RNA gene, partial sequence
Brevibacillus brevis strain St-2 16S ribosomal RNA gene, partial sequence
Brevibacillus agri strain SSA837 16S ribosomal RNA gene, partial sequence
Brevibacillus agri strain VSD814 16S ribosomal RNA gene, partial sequence
Brevibacillus sp. SAUBC3-5 16S ribosomal RNA gene, partial sequence
[Streptomyces] sp. TSA-KSA gene for 16S rRNA, partial sequence
firmicutes | 19 leaves
Uncultured bacterium gene for 16S rRNA, partial sequence, clone: smkt_Fir_004_003
0.0004

Brevibacillus brevis strain GZDF4 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|KP137563.1| Length: 1430 Number of Matches: 1

Range 1: 741 to 1348 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
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Sbjct  1348  GTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCG  1289

Query  61    ATTACTAGCGATTCCGACTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAGATTG  120
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Sbjct  1288  ATTACTAGCGATTCCGACTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAGATTG  1229

Query  121   GTTTTAAGAGATTGGCGTCCTCTCGCGAGGTAGCATCCCGTTGTACCAACCATTGTAGCA  180
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Sbjct  1228  GTTTTAAGAGATTGGCGTCCTCTCGCGAGGTAGCATCCCGTTGTACCAACCATTGTAGCA  1169

Query  181   CGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCCGCCTTCCTCCGT  240
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Sbjct  1168  CGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCCGCCTTCCTCCGT  1109

Query  241   CTTGTCGACGGCAGTCTCTCTAGAGTGCCCAACTGAATGCTGGCAACTAAAGATAAGGGT  300
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Sbjct  1108  CTTGTCGACGGCAGTCTCTCTAGAGTGCCCAACTGAATGCTGGCAACTAAAGATAAGGGT  1049

Query  301   TGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCAC  360
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Sbjct  1048  TGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCAC  989

Query  361   CACCTGTCACCGCTGCCCCGAAGGGAAGCTCTGTCTCCAGAGCGGTCAGCGGGATGTCCC  420
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Sbjct  988   CACCTGTCACCGCTGCCCCGAAGGGAAGCTCTGTCTCCAGAGCGGTCAGCGGGATGTCAA  929

Query  421   GACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGG  480
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Sbjct  928   GACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGG  869

Query  481   GCCCCCGTCAATTCCTTTGAGTTTCACTCTTGCGAGCGTACTCCCCAGGCGGAGTGCTTA  540
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Sbjct  868   GCCCCCGTCAATTCCTTTGAGTTTCACTCTTGCGAGCGTACTCCCCAGGCGGAGTGCTTA  809

Query  541   TTGCGTTAGCTGCGGCACTGCGGGTATTGCAACCCCCAACACCTAGCACTCATCGTTTAC  600
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Sbjct  808   TTGCGTTAGCTGCGGCACTGAGGGTATTGAAACCCCCAACACCTAGCACTCATCGTTTAC  749

Query  601   GGCGTGGA  608
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Sbjct  748   GGCGTGGA  741
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16SrRNA sequence analyses of samples of bacteria cultured from micrometeorite

Bacillus amyloliquefaciens subsp. plantarum NJN-6, complete genome

Sequence ID: gb|CP007165.1| Length: 4052546 Number of Matches: 8

Related Information

Range 1: 97951 to 98764 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1434 bits(1590)	0.0	806/814(99%)	0/814(0%)	Plus/Minus

Features: rRNA-16S ribosomal RNA

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Query  8       GGGGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGTTGATCCGCGATTACTAGCGA  67
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Sbjct  98764   GGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGA  98705

Query  68      TTCCAGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAACAGATTTGTGGGA  127
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Sbjct  98704   TTCCAGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAACAGATTTGTGGGA  98645

Query  128     TTGGCTTAACCTCGCGGTTTCGCTGCCCTTTGTTCTGTCCATTGTAGCACGTGTGTAGCC  187
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Sbjct  98644   TTGGCTTAACCTCGCGGTTTCGCTGCCCTTTGTTCTGTCCATTGTAGCACGTGTGTAGCC  98585

Query  188     CAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGG  247
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Sbjct  98584   CAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGG  98525

Query  248     CAGTCACCTTANAGTGCCCAACTGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTG  307
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Sbjct  98524   CAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTG  98465

Query  308     CGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACT  367
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Sbjct  98464   CGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACT  98405

Query  368     CTGNCCCCGAAGGGGACGTCCTATCTCTAGGATTGTCAGAGGATGTCAAGACCTGGTAAG  427
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Sbjct  98404   CTGCCCCCGAAGGGGACGTCCTATCTCTAGGATTGTCAGAGGATGTCAAGACCTGGTAAG  98345

Query  428     GTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAA  487
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Sbjct  98344   GTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAA  98285

Query  488     TTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCT  547
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Sbjct  98284   TTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCT  98225

Query  548     GCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTTACGGCGTGGACTA  607
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Query  608     CCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTTACAGACC  667
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Sbjct  98164   CCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTTACAGACC  98105

Query  668     AGAGAGTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGT  727
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Sbjct  98104   AGAGAGTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGT  98045

Query  728     GGAATTCCCCTCTCCTCTTCTGCACTCAAGTTTCCCCAGTTTCCAATGACCCTCCCCGGT  787
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Sbjct  98044   GGAATTCCACTCTCCTCTTCTGCACTCAAGTTTCCCCAGTTTCCAATGACCCTCCCCGGT  97985

  Query  788    TGACCGGGGGGCTTTNACATCAGACTTAAAAAAC  821
                ||| |||||||||| |||||||||||| ||||
  Sbjct  97984  TGAGCCGGGGGCTTTCACATCAGACTTAAGAAAC  97951
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Bacillus methylotrophicus strain IARI-BHD-4 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|KF054862.1| Length: 1375 Number of Matches: 1

▶ See 2 more title(s)

Range 1: 528 to 1341 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1431 bits(1586)	0.0	805/814(99%)	0/814(0%)	Plus/Minus

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Query  8     GGGGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGTTGATCCGCGATTACTAGCGA  67
             || |||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct  1341  GGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGA  1282

Query  68    TTCCAGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAACAGATTTGTGGGA  127
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1281  TTCCAGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAACAGATTTGTGGGA  1222

Query  128   TTGGCTTAACCTCGCGGTTTCGCTGCCCTTTGTTCTGTCCATTGTAGCACGTGTGTAGCC  187
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1221  TTGGCTTAACCTCGCGGTTTCGCTGCCCTTTGTTCTGTCCATTGTAGCACGTGTGTAGCC  1162

Query  188   CAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGG  247
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Sbjct  1161  CAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGG  1102

Query  248   CAGTCACCTTANAGTGCCCAACTGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTG  307
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Sbjct  1101  CAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTG  1042

Query  308   CGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACT  367
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Sbjct  1041  CGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACT  982

Query  368   CTGNCCCCGAAGGGGACGTCCTATCTCTAGGATTGTCAGAGGATGTCAAGACCTGGTAAG  427
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Sbjct  981   CTGCCCCCGAAGGGGACGTCCTATCTCTAGGATTGTCAGAGGATGTCAAGACCTGGTAAG  922

Query  428   GTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAA  487
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Sbjct  921   GTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAA  862

Query  488   TTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCT  547
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Sbjct  861   TTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCT  802

Query  548   GCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTTACGGCGTGGACTA  607
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Sbjct  801   GCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTTACGGCGTGGACTA  742

Query  608   CCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTTACAGACC  667
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Sbjct  741   CCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTTACAGACC  682

Query  668   AGAGAGTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGT  727
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Sbjct  681   AGAGAGTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGT  622

Query  728   GGAATTCCCCTCTCCTCTTCTGCACTCAAGTTTCCCCAGTTTCCAATGACCCTCCCCGGT  787
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Sbjct  621   GGAATTCCACTCTCCTCTTCTGCACTCAAGTTCCCCCAGTTTCCAATGACCCTCCCCGGT  562
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Query  728   GGAATTCCCCTCTCCTCTTCTGCACTCAAGTTTCCCCAGTTTCCAATGACCCTCCCCGGT  787
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Sbjct  561   TGAGCCGGGGGCTTTCACATCAGACTTAAGAAAC  528
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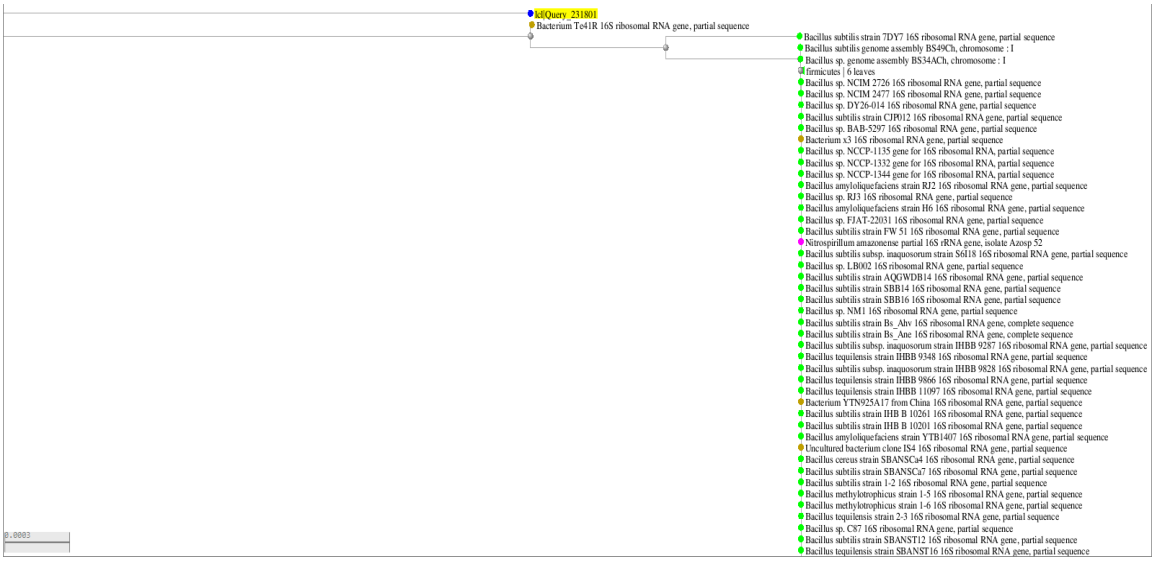
Related Information


kfQuery_231801
Bacterium Te41R 16S ribosomal RNA gene, partial sequence
Bacillus subtilis strain 7DY7 16S ribosomal RNA gene, partial sequence
Bacillus subtilis genome assembly BS49Ch, chromosome : I
Bacillus sp. genome assembly BS34ACh, chromosome : I
firmicutes | 6 leaves
Bacillus sp. NCIM 2726 16S ribosomal RNA gene, partial sequence
Bacillus sp. NCIM 2477 16S ribosomal RNA gene, partial sequence
Bacillus sp. DY26-014 16S ribosomal RNA gene, partial sequence
Bacillus subtilis strain CJP012 16S ribosomal RNA gene, partial sequence
Bacillus sp. BAB-5297 16S ribosomal RNA gene, partial sequence
Bacterium x3 16S ribosomal RNA gene, partial sequence
Bacillus sp. NCCP-1135 gene for 16S ribosomal RNA, partial sequence
Bacillus sp. NCCP-1332 gene for 16S ribosomal RNA, partial sequence
Bacillus sp. NCCP-1344 gene for 16S ribosomal RNA, partial sequence
Bacillus amyloliquefaciens strain RJ2 16S ribosomal RNA gene, partial sequence
Bacillus sp. RJ3 16S ribosomal RNA gene, partial sequence
Bacillus amyloliquefaciens strain H6 16S ribosomal RNA gene, partial sequence
Bacillus sp. FJAT-22031 16S ribosomal RNA gene, partial sequence
Bacillus subtilis strain FW 51 16S ribosomal RNA gene, partial sequence
Nitrospirillum amazonense partial 16S rRNA gene, isolate Azosp 52
Bacillus subtilis subsp. inaquosorum strain S6I18 16S ribosomal RNA gene, partial sequence
Bacillus sp. LB002 16S ribosomal RNA gene, partial sequence
Bacillus subtilis strain AQGWDB14 16S ribosomal RNA gene, partial sequence
Bacillus subtilis strain SBB14 16S ribosomal RNA gene, partial sequence
Bacillus subtilis strain SBB16 16S ribosomal RNA gene, partial sequence
Bacillus sp. NM1 16S ribosomal RNA gene, partial sequence
Bacillus subtilis strain Bs_Abv 16S ribosomal RNA gene, complete sequence
Bacillus subtilis strain Bs_Ane 16S ribosomal RNA gene, complete sequence
Bacillus subtilis subsp. inaquosorum strain IHBB 9287 16S ribosomal RNA gene, partial sequence
Bacillus tequilensis strain IHBB 9348 16S ribosomal RNA gene, partial sequence
Bacillus subtilis subsp. inaquosorum strain IHBB 9828 16S ribosomal RNA gene, partial sequence
Bacillus tequilensis strain IHBB 9866 16S ribosomal RNA gene, partial sequence
Bacillus tequilensis strain IHBB 11097 16S ribosomal RNA gene, partial sequence
Bacterium YTN925A17 from China 16S ribosomal RNA gene, partial sequence
Bacillus subtilis strain IHB B 10261 16S ribosomal RNA gene, partial sequence
Bacillus subtilis strain IHB B 10201 16S ribosomal RNA gene, partial sequence
Bacillus amyloliquefaciens strain YTB1407 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone IS4 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain SBANSCa4 16S ribosomal RNA gene, partial sequence
Bacillus subtilis strain SBANSCa7 16S ribosomal RNA gene, partial sequence
Bacillus subtilis strain 1-2 16S ribosomal RNA gene, partial sequence
Bacillus methylotrophicus strain 1-5 16S ribosomal RNA gene, partial sequence
Bacillus methylotrophicus strain 1-6 16S ribosomal RNA gene, partial sequence
Bacillus tequilensis strain 2-3 16S ribosomal RNA gene, partial sequence
Bacillus sp. C87 16S ribosomal RNA gene, partial sequence
Bacillus subtilis strain SBANST12 16S ribosomal RNA gene, partial sequence
Bacillus tequilensis strain SBANST16 16S ribosomal RNA gene, partial sequence
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Bacillus subtilis 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|KT026098.1| Length: 1223 Number of Matches: 1

Related Information

Range 1: 370 to 1160 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1308 bits(1450)	0.0	770/794(97%)	5/794(0%)	Plus/Minus

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Sbjct  1040  TTGGCTTAACCTCGCGGTTTCGCTGCCCTTTGTTCTGTCCATTGTAGCACGTGTGTAGCC  981

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Sbjct  980   CAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGG  921

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Sbjct  920   CAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTG  861

Query  308   CGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACT  367
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Sbjct  800   CTGCCCCCGAAGGGGACGTCCTATCTCTAGGATTGTCAGAGGATGTCAAGACCTGGTAAG  741

Query  428   GTTCTTCGCGTTGCTTCNAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAA  487
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Query  488   TTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAATGCGTTACCT  547
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Sbjct  680   TTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCT  621

Query  548   GCA-CACTAAGGGGCGGAAACCCCCTAACCCTTAGCACTCATCGTTTACGGCGTGGACTA  606
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Sbjct  620   GCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTTACGGCGTGGACTA  561

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Sbjct  560   CCAGGGTATCTAATCCTGTTCGCT-CCCCACGCTTTTCGCTCCTCAGCGTCAGTTACAGA  502

Query  667   CCAGAGAGCCCCCTTCCCCACTGGGGGTTCCTCCCCATCTCTACGCATTTTCACCGCTAC  726
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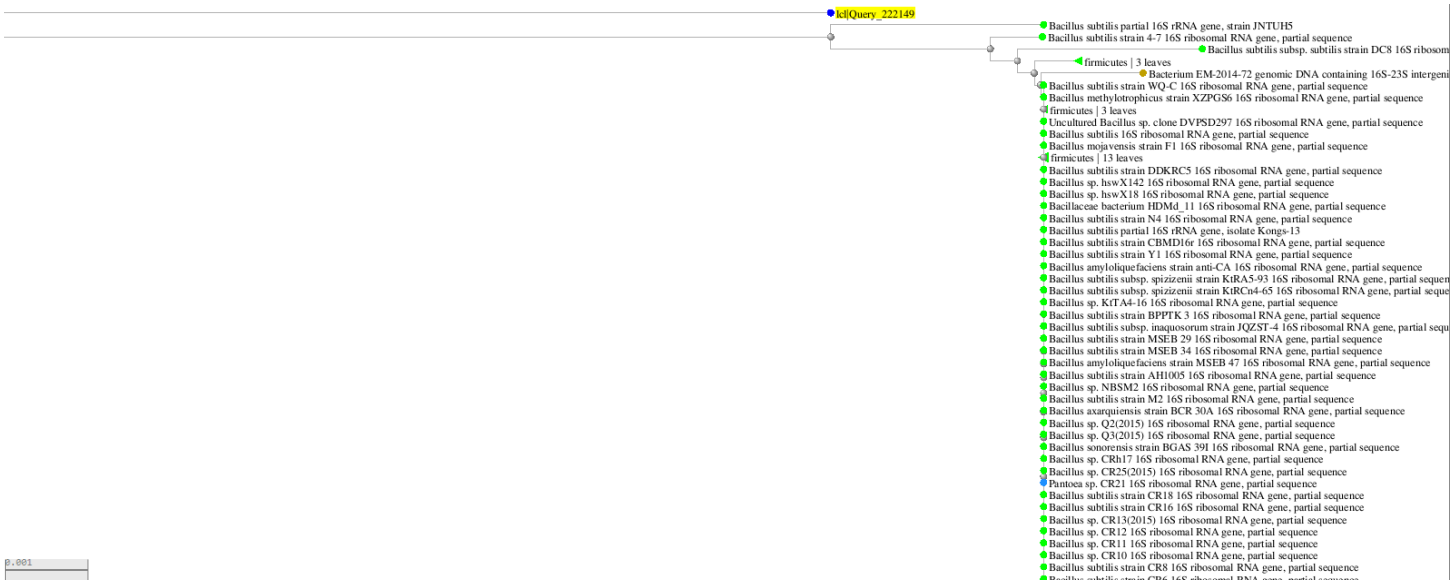

Cupriavidus respiraculi strain AU3775 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|AY860237.1| Length: 1429 Number of Matches: 1

Range 1: 532 to 1354 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1476 bits(1636)	0.0	821/823(99%)	0/823(0%)	Plus/Minus

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Sbjct  1234  ATTAGCTCCCCCTCGCGGGGTTGGCAACCCTCTGTACGCACCATTGTATGACGTGTGAAGC  1175

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Sbjct  1174  CCTACCCATAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCG  1115

Query  241   GCAGTCTCTCTAGAGTGCCCTTGCGTAGCAACTAGAGACAAGGGTTGCGCTCGTTGCGGG  300
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Sbjct  1054  ACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTCCACTT  995

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Sbjct  994   TCCCTTTCGGGCACCTGATGCATCTCTGCTTCGTTAGTGGCATGTCAAGGGTAGGTAAGG  935

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Query  481   TCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGTCAACTTCACGCGTTAGCTA  540
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Sbjct  874   TCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGTCAACTTCACGCGTTAGCTA  815

Query  541   CGTTACTGAGGAAATGAATCCCCAACAACTAGTTGACATCGTTTAGGGCGTGGACTACCA  600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  814   CGTTACTGAGGAAATGAATCCCCAACAACTAGTTGACATCGTTTAGGGCGTGGACTACCA  755

Query  601   GGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGTGCATGAGCGTCAGTGACGTCCCAGG  660
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  754   GGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGTGCATGAGCGTCAGTGACGTCCCAGG  695

Query  661   NGGCTGCCTTCGGCATCGGTATTCCTCCACATCTCTACGCATTTCACTGCTACACGTGAA  720
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  694   GGGCTGCCTTCGGCATCGGTATTCCTCCACATCTCTACGCATTTCACTGCTACACGTGGA  635

Query  721   ATTCTACCCCCCCTCTGACATACTCTAGCCTTGCAGTCACAAGCGCCATTCCCAAGTTGAG  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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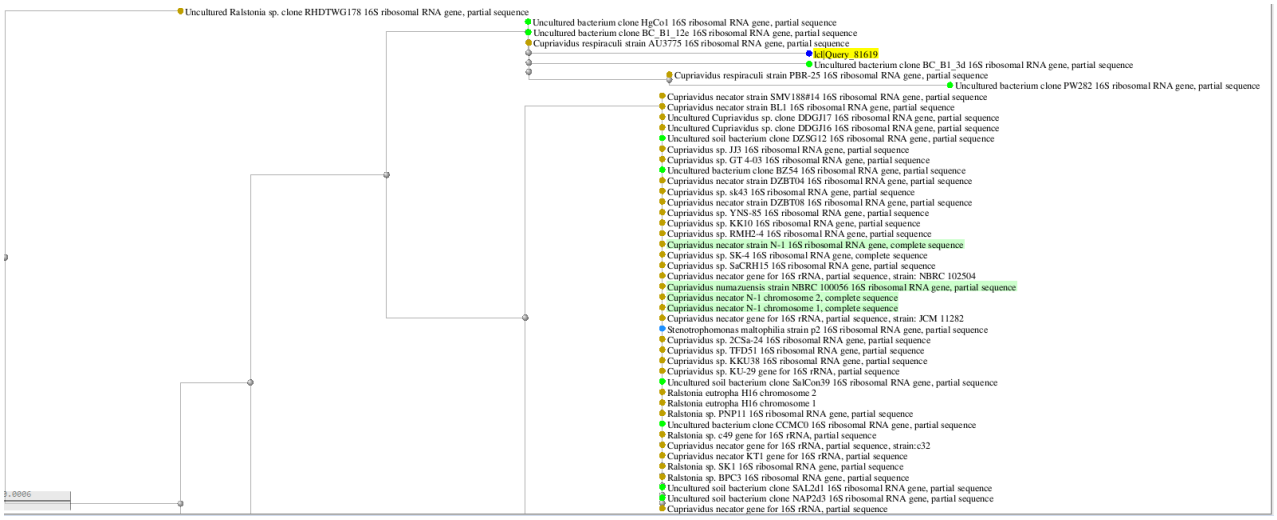
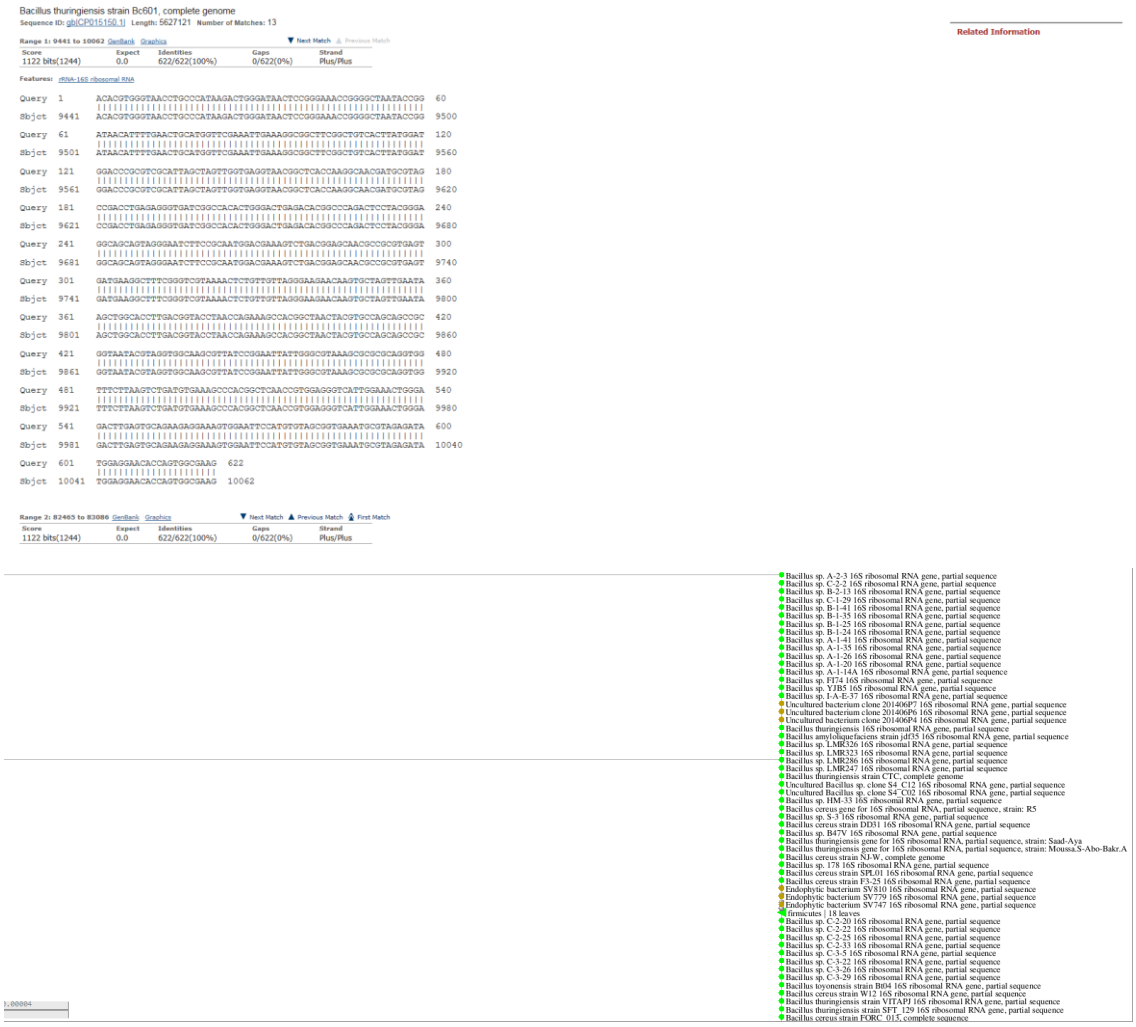


16SrRNA sequence analyses of samples of bacteria cultured from amber



Bacillus pumilus strain IND15 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|KF688987.1| Length: 493 Number of Matches: 1

Range 1: 31 to 493 GenBank Graphics

Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
682 bits(756)	0.0	429/463(93%)	0/463(0%)	Plus/Plus

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Query  1    GTGAGTAACACGTGGGCAACCTGCCTGTACGACTGGGATAACTCCGGGAAACCGGAGCTA  60
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Sbjct  31   GTGAGTAACACGTAGGCAACCTGCCTGTACGACTGGGATAACTCCGGGAAACCGGAGCTA  90

Query  61   ATACCGGATACTTCTTTCAGACCGCAGGGTCTGAAGGGGAAAGACCTTTGGTCAGGTACT  120
            |||||||||| | ||||| |||||| ||||||||   ||||||||||||||| ||||
Sbjct  91   ATACCGGATACGTTTTTCAAACCGCATGGTCTGAATTAGAAAGACCTTTGGTCAGGTACA  150

Query  121  TATGGGCCTGCCGCGCATTAACTAGTTGGTGGGGGAACGGCCTACCCAGGCGACCATGCA  180
             | ||||||||| |||||||| |||||||||||||| |||||||||| ||||||| ||||
Sbjct  151  GATGGGCCTGCGGCGCATTAGCTAGTTGGTGGGGTAACGGCCTACCAAGGCGACGATGCG  210

Query  181  TGCCCGACCTGAGAGGGTGATCGGTCACACTGGGAGTGACACACAGCCCAGACTCCTACG  240
             | ||||||||| |||||||||| |||||||||||||||||||||||||||||||||||
Sbjct  211  TACCCGACCTGACAGGGTGATCGGCCACACTGGGAGTGACACAGGCCCAGACTCCTACG  270

Query  241  GGAGGCAGCAATAGGGAATCTTCCGCAATGGATGAAAGTCTGACTGAGCAACGCCGCGTG  300
             |||||||||| ||||||||||||||||||||| |||||||||| |||||||||||| |
Sbjct  271  GGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTG  330

Query  301  AACGATGAAGGTTTTCTGATCGTACAGTTCTGTTGTTAGGGAAGAACCACCGGGATGACC  360
             |||||||||||||||| |||||| ||||||||||||||||||||||||||||||||||
Sbjct  331  AACGATGAAGGTTTTCGGATCGTAAAGTTCTGTTGTTAGGGAAGAACCGCCGGGATGACC  390

Query  361  TCCCGGTCTGAGGGTACCTAACGAGAACGCCCCGGATAACTACGTGCCACCACCGCGGT  420
             |||||||||| |||||||||||||| ||||||| |||||||||||| ||||||||||
Sbjct  391  TCCCGGTCTGAGGGTACCTAACGAGAAACGCCCCGGCTAACTACGTGCCAGCACCCGCGGT  450

Query  421  CATACGTAGGGGGCAAGCGTTGTCCGGAATTATTGGGAGTAAA  463
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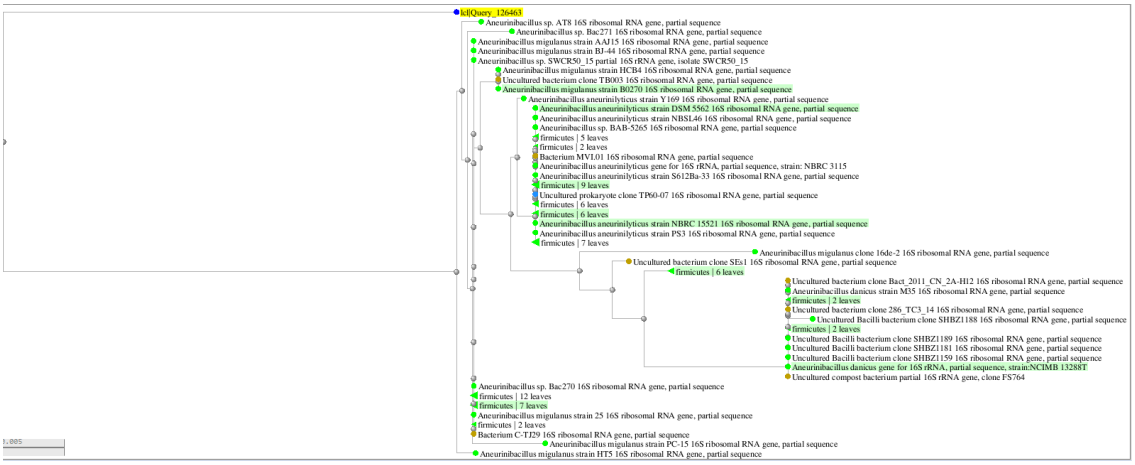

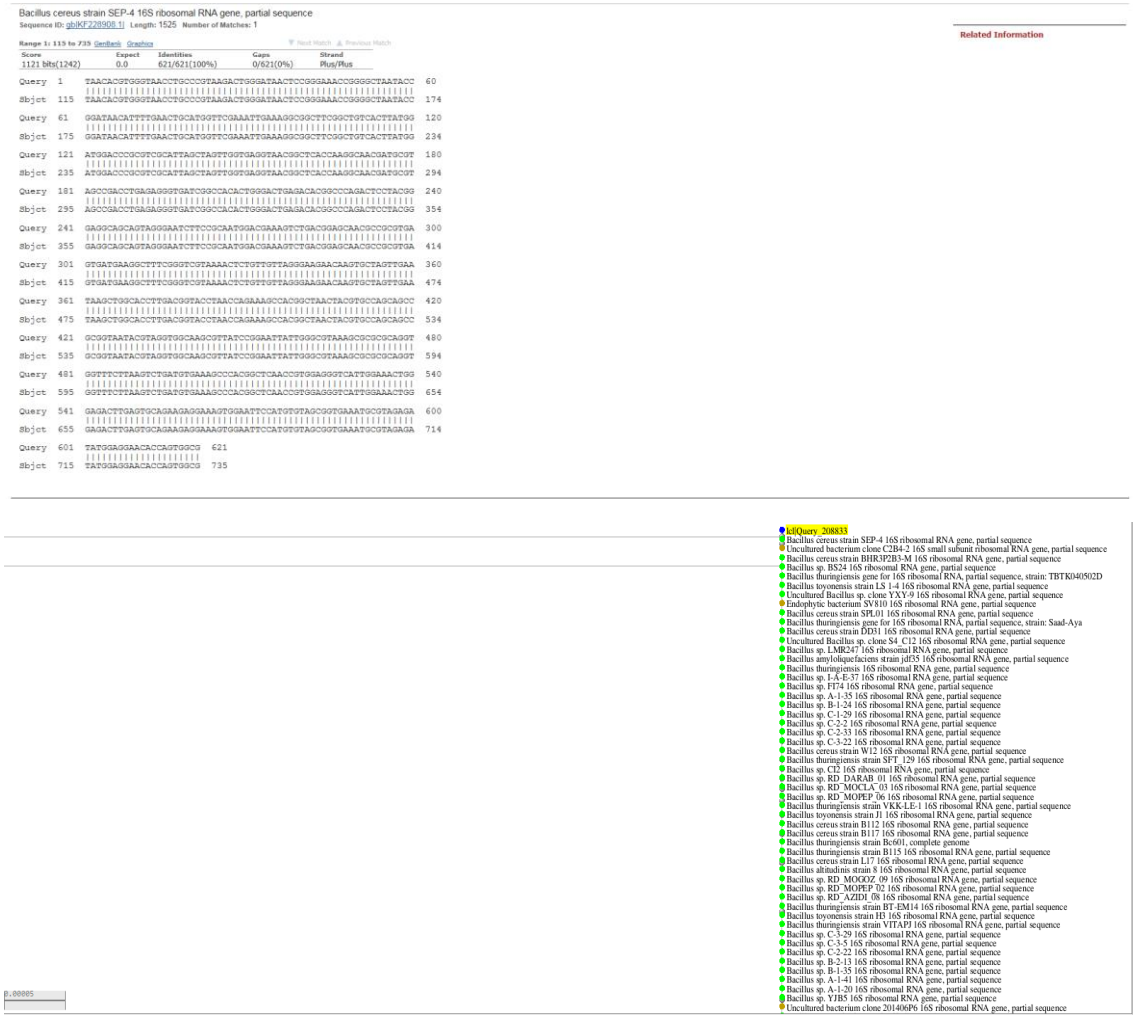
Bacillus cereus strain SEP-4 16S ribosomal RNA gene, partial sequence
Sequence ID: gb|KF228908.1| Length: 1525 Number of Matches: 1
Range 1: 115 to 735 GenBank Graphics
Next Match Previous Match
Score Expect Identities Gaps Strand
1121 bits(1242) 0.0 621/621(100%) 0/621(0%) Plus/Plus
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Sbjct 115 TAACACGTGGGTAACCTGCCCGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACC 174
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Query 121 ATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGT 180
Sbjct 235 ATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGT 294
Query 181 AGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGG 240
Sbjct 295 AGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGG 354
Query 241 GAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGA 300
Sbjct 355 GAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGA 414
Query 301 GTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAA 360
Sbjct 415 GTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAA 474
Query 361 TAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCC 420
Sbjct 475 TAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCC 534
Query 421 GCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGT 480
Sbjct 535 GCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGT 594
Query 481 GGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGG 540
Sbjct 595 GGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGG 654
Query 541 GAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGA 600
Sbjct 655 GAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGA 714
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Sbjct 715 TATGGAGGAACACCAGTGGCG 735
Related Information
lcl|Query_208833
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Uncultured bacterium clone C2B4-2 16S small subunit ribosomal RNA gene, partial sequence
Bacillus cereus strain BHR3P2B3-M 16S ribosomal RNA gene, partial sequence
Bacillus sp. BS24 16S ribosomal RNA gene, partial sequence
Bacillus thuringiensis gene for 16S ribosomal RNA, partial sequence, strain: TBTKI040502D
Bacillus toyonensis strain LS 1-4 16S ribosomal RNA gene, partial sequence
Uncultured Bacillus sp. clone YXY-9 16S ribosomal RNA gene, partial sequence
Endophytic bacterium SV810 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain SPL01 16S ribosomal RNA gene, partial sequence
Bacillus thuringiensis gene for 16S ribosomal RNA, partial sequence, strain: Saad-Aya
Bacillus cereus strain DD31 16S ribosomal RNA gene, partial sequence
Uncultured Bacillus sp. clone S4_C12 16S ribosomal RNA gene, partial sequence
Bacillus sp. LMR247 16S ribosomal RNA gene, partial sequence
Bacillus amyloliquefaciens strain jdf35 16S ribosomal RNA gene, partial sequence
Bacillus thuringiensis 16S ribosomal RNA gene, partial sequence
Bacillus sp. I-A-E-37 16S ribosomal RNA gene, partial sequence
Bacillus sp. FT4 16S ribosomal RNA gene, partial sequence
Bacillus sp. A-1-35 16S ribosomal RNA gene, partial sequence
Bacillus sp. B-1-24 16S ribosomal RNA gene, partial sequence
Bacillus sp. C-1-29 16S ribosomal RNA gene, partial sequence
Bacillus sp. C-2-2 16S ribosomal RNA gene, partial sequence
Bacillus sp. C-2-33 16S ribosomal RNA gene, partial sequence
Bacillus sp. C-3-22 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain W12 16S ribosomal RNA gene, partial sequence
Bacillus thuringiensis strain SFT_129 16S ribosomal RNA gene, partial sequence
Bacillus sp. CI2 16S ribosomal RNA gene, partial sequence
Bacillus sp. RD_DARAB_01 16S ribosomal RNA gene, partial sequence
Bacillus sp. RD_MOCLA_03 16S ribosomal RNA gene, partial sequence
Bacillus sp. RD_MOPEP_06 16S ribosomal RNA gene, partial sequence
Bacillus thuringiensis strain VKK-LE-1 16S ribosomal RNA gene, partial sequence
Bacillus toyonensis strain J1 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain B112 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain B117 16S ribosomal RNA gene, partial sequence
Bacillus thuringiensis strain Bc601, complete genome
Bacillus thuringiensis strain B115 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain L17 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain 8 16S ribosomal RNA gene, partial sequence
Bacillus sp. RD_MOKOZ_09 16S ribosomal RNA gene, partial sequence
Bacillus sp. RD_MOPEP_02 16S ribosomal RNA gene, partial sequence
Bacillus sp. RD_AZIDI_08 16S ribosomal RNA gene, partial sequence
Bacillus thuringiensis strain BT-EM14 16S ribosomal RNA gene, partial sequence
Bacillus toyonensis strain H3 16S ribosomal RNA gene, partial sequence
Bacillus thuringiensis strain VITAP3 16S ribosomal RNA gene, partial sequence
Bacillus sp. C-3-29 16S ribosomal RNA gene, partial sequence
Bacillus sp. C-3-5 16S ribosomal RNA gene, partial sequence
Bacillus sp. C-2-22 16S ribosomal RNA gene, partial sequence
Bacillus sp. B-2-13 16S ribosomal RNA gene, partial sequence
Bacillus sp. B-1-35 16S ribosomal RNA gene, partial sequence
Bacillus sp. A-1-41 16S ribosomal RNA gene, partial sequence
Bacillus sp. A-1-20 16S ribosomal RNA gene, partial sequence
Bacillus sp. YJB5 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone 201406P6 16S ribosomal RNA gene, partial sequence
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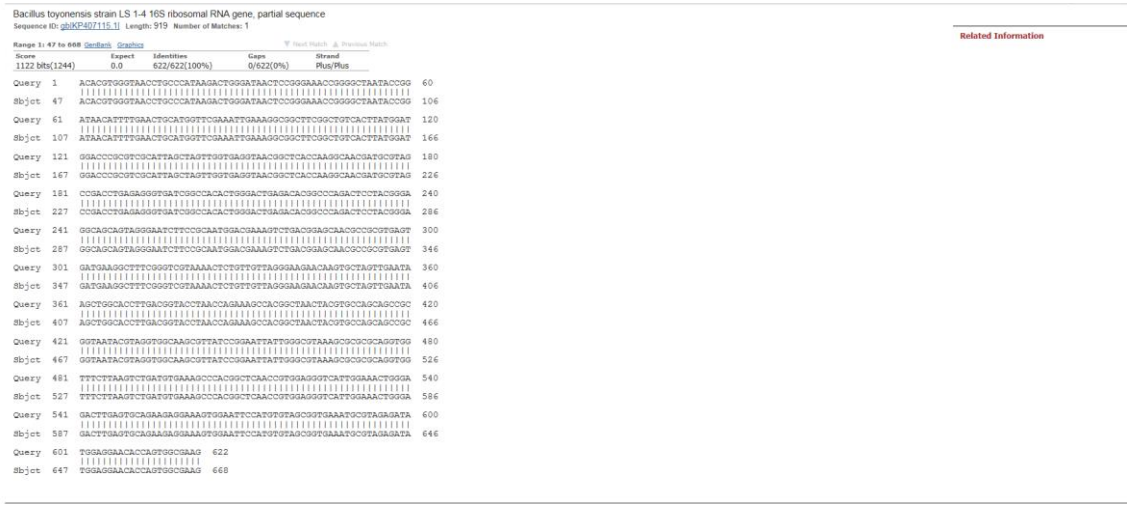

Bacillus toyonensis strain LS 1-4 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|KP407115.1| Length: 919 Number of Matches: 1

Range 1: 47 to 668 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
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Query  61   ATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  107  ATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGAT  166

Query  121  GGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAG  180
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Sbjct  167  GGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAG  226

Query  181  CCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGA  240
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Sbjct  227  CCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGA  286

Query  241  GGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGT  300
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Sbjct  287  GGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGT  346

Query  301  GATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  347  GATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATA  406

Query  361  AGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGC  420
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Sbjct  407  AGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGC  466

Query  421  GGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGG  480
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Query  481  TTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGA  540
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Sbjct  647  TGGAGGAACACCAGTGGCGAAG  668
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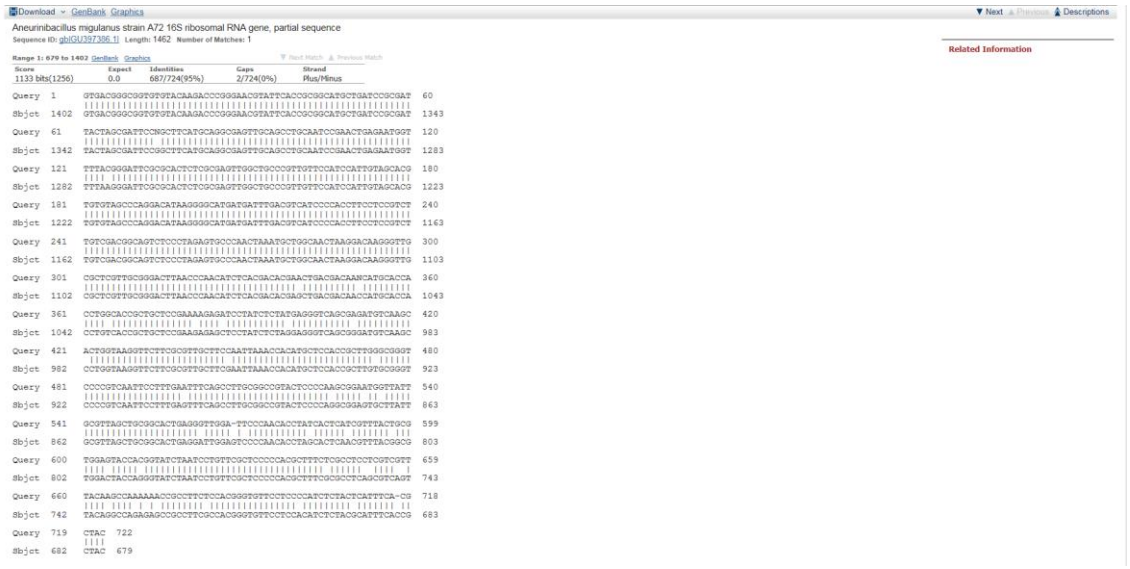
Related Information

- Bacillus sp. A-2-3 16S ribosomal RNA gene, partial sequence
- Bacillus sp. C-2-2 16S ribosomal RNA gene, partial sequence
- Bacillus sp. B-2-13 16S ribosomal RNA gene, partial sequence
- Bacillus sp. C-1-29 16S ribosomal RNA gene, partial sequence
- Bacillus sp. B-1-41 16S ribosomal RNA gene, partial sequence
- Bacillus sp. B-1-35 16S ribosomal RNA gene, partial sequence
- Bacillus sp. B-1-25 16S ribosomal RNA gene, partial sequence
- Bacillus sp. B-1-24 16S ribosomal RNA gene, partial sequence
- Bacillus sp. A-1-41 16S ribosomal RNA gene, partial sequence
- Bacillus sp. A-1-35 16S ribosomal RNA gene, partial sequence
- Bacillus sp. A-1-26 16S ribosomal RNA gene, partial sequence
- Bacillus sp. A-1-20 16S ribosomal RNA gene, partial sequence
- Bacillus sp. A-1-14A 16S ribosomal RNA gene, partial sequence
- Bacillus sp. FI74 16S ribosomal RNA gene, partial sequence
- Bacillus sp. YJB5 16S ribosomal RNA gene, partial sequence
- Bacillus sp. 1-A-E-37 16S ribosomal RNA gene, partial sequence
- Uncultured bacterium clone 201406P7 16S ribosomal RNA gene, partial sequence
- Uncultured bacterium clone 201406P6 16S ribosomal RNA gene, partial sequence
- Uncultured bacterium clone 201406P4 16S ribosomal RNA gene, partial sequence
- Bacillus thuringiensis 16S ribosomal RNA gene, partial sequence
- Bacillus amyloliquefaciens strain jdf35 16S ribosomal RNA gene, partial sequence
- Bacillus sp. LMR326 16S ribosomal RNA gene, partial sequence
- Bacillus sp. LMR323 16S ribosomal RNA gene, partial sequence
- Bacillus sp. LMR286 16S ribosomal RNA gene, partial sequence
- Bacillus sp. LMR247 16S ribosomal RNA gene, partial sequence
- Bacillus thuringiensis strain CTC, complete genome
- Uncultured Bacillus sp. clone S4_C12 16S ribosomal RNA gene, partial sequence
- Uncultured Bacillus sp. clone S4_C02 16S ribosomal RNA gene, partial sequence
- Bacillus sp. HM-33 16S ribosomal RNA gene, partial sequence
- Bacillus cereus gene for 16S ribosomal RNA, partial sequence, strain: R5
- Bacillus sp. S-3 16S ribosomal RNA gene, partial sequence
- Bacillus cereus strain DI311 16S ribosomal RNA gene, partial sequence
- Bacillus sp. B47V 16S ribosomal RNA gene, partial sequence
- Bacillus thuringiensis gene for 16S ribosomal RNA, partial sequence, strain: Saad-Aya
- Bacillus thuringiensis gene for 16S ribosomal RNA, partial sequence, strain: Moussa;S-Abo-Bakr.A
- Bacillus cereus strain NJ-W , complete genome
- Bacillus sp. 178 16S ribosomal RNA gene, partial sequence
- Bacillus cereus strain SPL01 16S ribosomal RNA gene, partial sequence
- Bacillus cereus strain F3-25 16S ribosomal RNA gene, partial sequence
- Endophytic bacterium SV810 16S ribosomal RNA gene, partial sequence
- Endophytic bacterium SV779 16S ribosomal RNA gene, partial sequence
- Endophytic bacterium SV747 16S ribosomal RNA gene, partial sequence
- firmicutes | 18 leaves
- Bacillus sp. C-2-20 16S ribosomal RNA gene, partial sequence
- Bacillus sp. C-2-22 16S ribosomal RNA gene, partial sequence
- Bacillus sp. C-2-25 16S ribosomal RNA gene, partial sequence
- Bacillus sp. C-2-33 16S ribosomal RNA gene, partial sequence
- Bacillus sp. C-3-5 16S ribosomal RNA gene, partial sequence
- Bacillus sp. C-3-22 16S ribosomal RNA gene, partial sequence
- Bacillus sp. C-3-26 16S ribosomal RNA gene, partial sequence
- Bacillus sp. C-3-29 16S ribosomal RNA gene, partial sequence
- Bacillus toyonensis strain Bt04 16S ribosomal RNA gene, partial sequence
- Bacillus cereus strain W12 16S ribosomal RNA gene, partial sequence
- Bacillus thuringiensis strain VITAPJ 16S ribosomal RNA gene, partial sequence
- Bacillus thuringiensis strain SFT_129 16S ribosomal RNA gene, partial sequence
- Bacillus cereus strain F08C_013, complete sequence
- Bacillus toyonensis strain H5 16S ribosomal RNA gene, partial sequence
- Bacillus cereus strain AHK3 16S ribosomal RNA gene, partial sequence
- Bacillus sp. C12 16S ribosomal RNA gene, partial sequence
- Bacillus thuringiensis strain BT-EM14 16S ribosomal RNA gene, partial sequence


Download GenBank Graphics
Next Previous Descriptions
Aneurinibacillus migulanus strain A72 16S ribosomal RNA gene, partial sequence
Sequence ID: gb|GU397386.1| Length: 1462 Number of Matches: 1
Related Information
Range 1: 670 to 1402 GenBank Graphics
Next Match Previous Match
Score Expect Identities Gaps Strand
1133 bits(1256) 0.0 687/724(95%) 2/724(0%) Plus/Minus
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Sbjct 1402 GTGACGGGCGGTGTGTACAAGACCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGAT 1343
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Query 541 GCGTTAGCTGCGGCACTGAGGGTTGGA-TTCCCAACACCTATCACTCATCGTTTACTGCG 599
Sbjct 862 GCGTTAGCTGCGGCACTGAGGATTGGAGTCCCCAACACCTAGCACTCAACGTTTACGGCG 803
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Sbjct 802 TGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCCACGCTTTCGCGCCTCAGCGTCAGT 743
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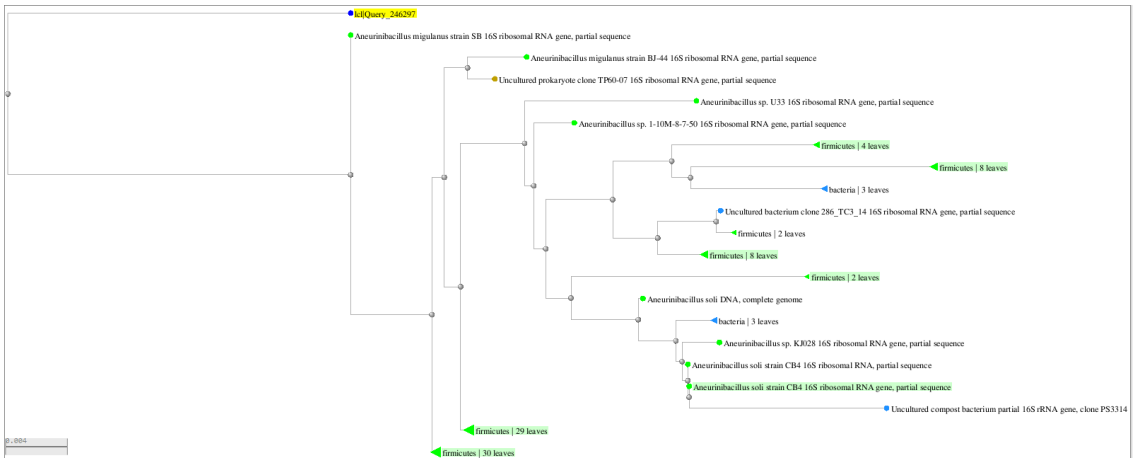
Query 719 CTAC 722
Sbjct 682 CTAC 679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Aneurinibacillus migulanus strain BJ-44 16S ribosomal RNA gene, partial sequence
Uncultured prokaryote clone TP60-07 16S ribosomal RNA gene, partial sequence
Aneurinibacillus sp. U33 16S ribosomal RNA gene, partial sequence
Aneurinibacillus sp. 1-10M-8-7-50 16S ribosomal RNA gene, partial sequence
firmicutes | 4 leaves
firmicutes | 8 leaves
bacteria | 3 leaves
Uncultured bacterium clone 286_TC3_14 16S ribosomal RNA gene, partial sequence
firmicutes | 2 leaves
firmicutes | 8 leaves
firmicutes | 2 leaves
Aneurinibacillus soli DNA, complete genome
bacteria | 3 leaves
Aneurinibacillus sp. KJ028 16S ribosomal RNA gene, partial sequence
Aneurinibacillus soli strain CB4 16S ribosomal RNA, partial sequence
Aneurinibacillus soli strain CB4 16S ribosomal RNA gene, partial sequence
Uncultured compost bacterium partial 16S rRNA gene, clone PS3314
firmicutes | 29 leaves
firmicutes | 30 leaves
0.004

Aneurinibacillus sp. Bac270 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|KP980744.1| Length: 1410 Number of Matches: 1

Related Information

Range 1: 832 to 1352 GenBank Graphics Next Match Previous Match

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Sbjct  1172  GTGTAGCCCAGGACATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGTCTT  1113

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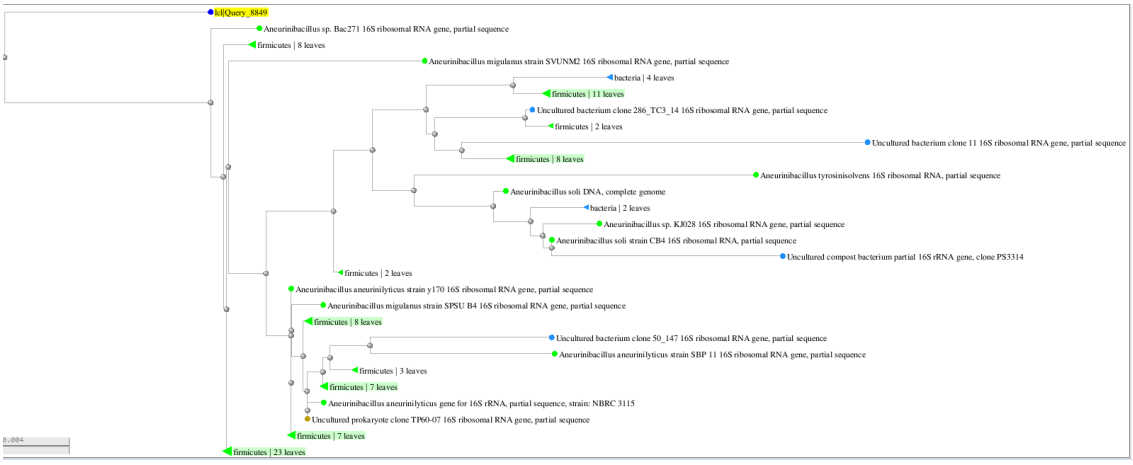

Bacillus altitudinis strain 8 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|KU898277.1| Length: 1458 Number of Matches: 1

Range 1: 86 to 707 GenBank Graphics

Next Match Previous Match

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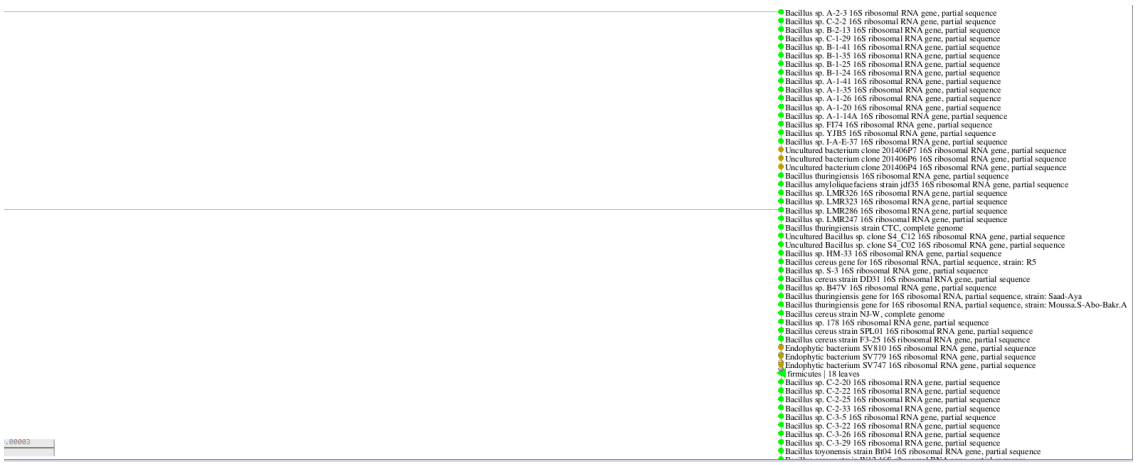

Endophytic bacterium SV779 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|KP757642.1| Length: 1399 Number of Matches: 1

Range 1: 57 to 678 GenBank Graphics

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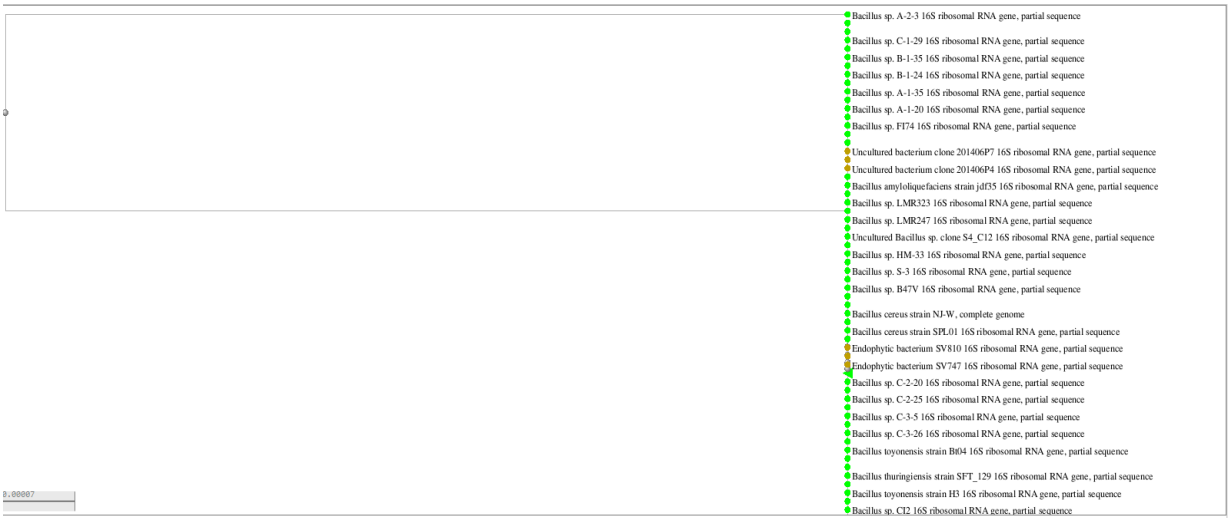
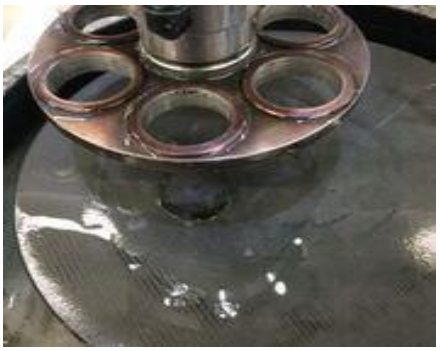
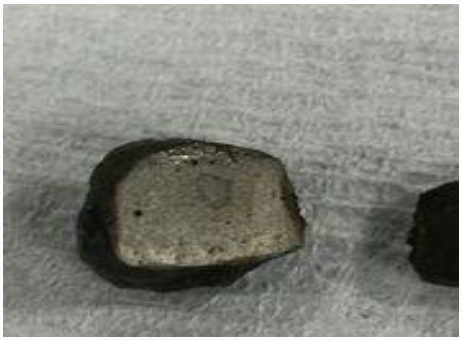


Appendix B

Micrometeorite Samples Prepare





Experiments to demonstrate the potential of Morning Glory seeds as a vehicle for panspermia