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Cultured Microbial Diversity Of Parys Mountain Acidic Site

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Declaration

I hereby declare that this thesis is the results of my own investigations, except where otherwise stated. All other sources are acknowledged by bibliographic references. This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree unless, as agreed by the University, for approved dual awards.

Yr wyf drwy hyn yn datgan mai canlyniad fy ymchwil fy hun yw'r thesis hwn, ac eithrio lle nodir yn wahanol. Caiff ffynonellau eraill eu cydnabod gan droednodiadau yn rhoi cyfeiriadau eglur. Nid yw sylwedd y gwaith hwn wedi cael ei dderbyn o'r blaen ar gyfer unrhyw radd, ac nid yw'n cael ei gyflwyno ar yr un pryd mewn ymgeisiaeth am unrhyw radd oni bai ei fod, fel y cytunwyd gan y Brifysgol, am gymwysterau deuol cymeradwy.

Project Title: **Cultured Microbial Diversity Of Parys Mountain Acidic Site**

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Abstract

Acid Mine Drainage (AMD) sites are found throughout the world, formed through the runoff of acidic water and heavy metals from mining activity. Microorganisms that are able to inhabit these environments are known as acidophiles, growing at an optimal pH of < 3. The Parys Mountain located in Anglesey (UK) is an AMD site subject to studies over the years. These studies have detected the presence of many acidophiles belonging to Archaea such as *Cuniculiplasma, Ferroplasma, ''Candidatus* Scheffleriplasma hospitalis'' (also known as ''B_DKE'') and an uncultured archaeon known as ''E-plasma'' all from the same phylum *Euryarchaeota* (or ''*Ca.* Thermoplasmatota''). One issue in the research of acidophiles is the ability to cultivate them *in vitro*, as acidophiles (and archaea in general) are difficult to cultivate. Additionally, unique nano sized archaea known as ''*Ca.* Microcaldota'' (formally known as ''*Ca.* Micrarchaeota'') has very few successful cultivations *in vitro*.

The primary goal of this study was to cultivate the microbial diversity of the PM5 site (Parys Mountain). The second goal of this study was to investigate cultivation techniques in an attempt to successfully enrich elusive microorganisms such as ''*Ca.* Micrarchaeota''. To achieve these goals, aerobic and anaerobic enrichment cultures were set-up and monitored over the course of seven months. Variations included enrichment cultures containing two types of media, as well as acidic water collected from the sample site. Substates included sulfur and yeast extract, beef extract and tryptone added at 0.1% (w/v) or 0.02% (w/v). Finally, some enrichment cultures had the addition of *Cuniculiplasma* biomass at 10% (w/v). The microbial composition of cultures was monitored by the use of 16S rRNA V4 primers (as well as ITS primers on enrichment cultures that showed fungal growth) at two timepoints. Timepoint 3 and timepoint 7 (2 and 6 months after initial enrichment cultures set-up).

Sequencing of enrichment cultures revealed archaea affiliated (99.22% identity) with *C. divulgatum* in high abundance in both timepoints 3 and 7, as well as sequences affiliated with *Ferroplasma acidiphilum* (identity 100%) in near 100% abundance in a number of enrichment cultures. Sequences that could only be affiliated at the family level with *Thermoplasmataceae* were also detected in a number of enrichment cultures at timepoint 3, while timepoint 7 cultures were able to show an affiliation to ''*Ca.* S. hospitalis'' (100% identity). Additionally, sequences affiliated (100% identity) with ''E-plasma'' were detected

in low abundance in several enrichment cultures. In both timepoints 3 and 7, sequences affiliated (99.61% identity) with ''*Ca.* Micrarchaeota Mia-14'' were also detected in a number of enrichment cultures. Bacterial sequences were detected in low abundances in comparison to archaeal sequences. The highest abundance sequences were affiliated with the phyla *Actinobacteriota* and *Nitrospirota. Actinobacteria* contained sequences that could only be affiliated (100% identity) to the class *Acidimicrobiia.* Sequencing of fungal cultures also revealed the presence of sequences affiliated with phylum *Ascomycota*, specifically the genus *Aspergillus* (99.20% identity).

This study resulted in successful cultivation of ''*Ca.* Micrarchaeota ''and has confirmed the tendency for this ''*Ca.* Micrarchaeota'' phylotype, similar to ''*Ca.* Mancarchaeum acidiphilum, Mia-14'' to be associated with *Cuniculiplasma* sp. under certain laboratory conditions and shown evidence of a pH growth range of 1.7-2.2 and at 0.02% (w/v) polypeptide presence. As preferential characteristics, both parameters found to be slightly different than that of another *''Ca.* Micrarchaeota''/ *C. divulgatum* association, isolated from the PM4 site (Parys Mountain, UK). This study has also shown the first growth of ''E-plasma'' *in vitro* representing an important step in studying this microorganism. Finally, detection of sequences affiliated with ''*Ca.* S. hospitalis'' also indicate the potential aerobic growth of this microorganism previously only enriched and cultivated under anoxic conditions.

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Introduction

Our planet hosts a variety of environments in which life not only survives but thrives. Each of these environments contains its own set of selection pressures which life must adapt to in order to succeed. This is seen most clearly in environments where the selection pressures are so harsh that traditional life as we know it is unable to survive. A few examples of extreme selection pressures include environments where the temperatures are very high (or very low), water environments where the salinity is high, low water environments such as arid deserts, high pressure environments and finally environments in which the pH is very low (acidic) (Pikuta *et al.*, 2007). Microorganisms that have evolved to survive in these hostile environments are known as extremophiles. In addition to this, extremophiles are also grouped and named based on the extreme selection pressure that they have adapted to. Some examples include thermophiles (microorganisms that have adapted to high temperatures), halophiles (microorganism that have adapted to high salinity) and xerophilies (microorganisms that have adapted to low water available environments) (Pikuta *et al.*, 2007). One particular group that has gained a lot of interest among scientists (and a number of industrial companies) are extremophiles that have to adapted to survive in acidic environments. These are known as acidophiles (Johnson, 2007). Acidophiles can be further grouped depending on the pH range they optimally use for growth. Microorganisms whose optimal pH for growth is less than pH 3 are defined as ''extreme acidophiles'', whilst microorganisms which have an optimal pH for growth between pH 3-5 are known as ''moderate acidophiles'' (Johnson, 2012). These microorganisms can be found in many different types of acidic environments including natural sources, such as deep-sea vents, hydrothermal sources and sulfuric pools. They are also found in man-made areas usually where mining activities have taken place such as acid mine drainage (AMD) areas (Hedrich and Schippers, 2021). These sites are formed when mining activity exposes sulfidic minerals (such as pyrite) to air and water, causing oxidation and the formation of sulfuric acid which in turn dissolves heavy metals. This leads to the runoff of water containing a low pH and heavy metal compounds (Johnson, 2012). Two examples of AMD sites are the Iron Mountain Mine in California (USA) and the Parys Mountain located in Anglesey (Wales, UK). In these highly acidic environments, there is usually a large abundance of inorganic sulfur compounds which acidophiles are believed to play a key role in oxidising (Rohwerder and

Sand, 2007). Successful growth of these organisms depends on a number of factors, with pH and temperature considered to be the most important however other factors such as redox potential and the availability of oxygen are also considered to have influence on the growth of communities in these environments (Johnson and Quatrini, 2020).

Literature Review

Adaptations to acidic environments

Most microorganisms usually grow at a pH around 7.0 (these microorganisms are also known as neutrophiles). When exposed to a highly acidic environment, most of these microorganisms are usually destroyed by the influx of H+ ions (protons) disrupting and destroying vital functions, membranes, enzyme structure/activity and protein structure (Baker-Austin and Dopson, 2007). It is vital therefore that microorganisms maintain a constant pH inside the cell known as pH homeostasis. In acidophiles a balance must also be made for the flow of ions/protons inside the cell used for vital processes such as ATP synthesis and while also pumping out protons of the cell to maintain the constant pH. Acidophiles do this through a number of adaptations. The first is to by making entry of protons more difficult. In a number of archaea, it has been found that the cell membrane contains a complex of hydrogen bonds between tightly packed lipids located in the inner membrane and cyclopentane molecules in the outer layer (Chong *et al.,* 2012; Macalady *et al.,* 2004). Another potential adaptation that may decrease membrane permeability in acidophiles is the reduced size of membrane channels as a study of *Acidithiobacillus ferrooxidans* showed that when placed into a more acidic environment the pore and ion selectivity of the channel became smaller however, this adaptation has only been observed in a single study meaning it is unknown if other acidophiles utilise this adaptation (Amaro *et al.*, 1991). It has also been found that a number of acidophiles such as *Sulfolobus acidocaldarius* create a positive membrane potential by accumulating positively charged ions such as potassium ions (K+) (Buetti-Dinh *et al.*, 2016). By having a reversed electrostatic potential, in which the membrane has a positive potential, the influx of H+ ions is more difficult due these ions having a positive charge (Baker-Austin and Dopson, 2007). Inside the cytoplasm, buffer molecules are present which can sequester H+ ions. One of the more popular examples of this, is the decarboxylation of amino acids such as glutamate and

arginine (Richard and Foster, 2004). These compounds react with H+ ions, consuming them to form polyamines which are then transported out of cell, helping maintain a stable pH (Slonczewski *et al.*, 2009). The ability for microorganisms to repair damage to proteins is also an important factor in surviving acidic environments. While most microorganisms have some ability to repair protein damage, one study in 2005 revealed a biofilm community containing *Leptospirillum* highly expressed chaperones responsible for protein refolding, suggesting its importance in mitigating protein damage (Ram *et al.,* 2005).

Past mining activities in the Parys Mountain, UK

The Parys Mountain, located in Anglesey, UK, is an area in which mining for heavy metals such as copper and lead has taken place over a large period of time. Due to this mining, sulfide compounds are present in the area, in the form of different minerals (Johnson, 2012). These can be found deposited within the environment which has contributed to the area's low pH. Heavy mining has also meant areas within the Parys Mountain are potentially high in arsenic, cadmium, selenium and lead (Bullock *et al.*, 2017). While most mining took place in the 19th century, studies have shown evidence for the extraction of copper taking place as early as the Bronze Age (Vernon, 1996). In the last few decades, concerns with the dam in the area and safety concerns over a structural failure meant the reservoir was drained and the dam itself was dismantled (Younger and Potter, 2012). Thanks to this drainage and the long history of metal extraction in the area has made the Parys Mountain an ideal location in which to study different acidophilic microbial communities. A number of sites have been labelled with scientific interest as they contain varying conditions such as temperature, pH and redox potential (Younger and Potter, 2012; Jenkins *et al.,* 2000). Over the last few decades since the dam's removal a number of studies have been conducted gaining valuable insights of the microbial diversity of a number of sites.

Prokaryotes in the Parys Mountain

Studies on sediments and acidic water samples have shown members belonging to archaea are able to dominate the community at one particular site, making up the largest abundances within the community. In particular archaea associated with the order *Thermoplasmatales* made up the largest abundances of these communities (Korzhenkov *et al.*, 2019). In addition, bacterial constitutes only made up around one third of this

community. Another more recent study also showed a high abundance of archaea in sediment samples taken in depths of up to 20cm from an AMD stream at the same site. The order *Thermoplasmatales* once again making up the largest abundances of archaea (Distaso *et al.*, 2020). Within the order *Thermoplasmatales*, an uncultured group known as ''E-plasma'' and ''*Ca.* Scheffleriplasma hospitalis'' (also known as ''B_DKE'') were detected with high abundances, as well as *Ferroplasma* and *Cuniculiplasma* being detected in sediment and water samples (Korzhenkov *et al.*, 2019; Distaso *et al.*, 2020). With average monthly high temperatures in the Parys Mountain ranging from 8°C Jan-18°C Aug (and average low temperatures of 2°C Feb-11°C Aug) (Timeanddate, 2022), the high abundance of archaea in these relatively low temperature acidic environments is of interest, with abundance of archaea further increasing when the pH drops below pH 2 (Chen *et al.*, 2016; Kuang *et al.*, 2013). Multiple studies have been conducted to understand this tolerance to low temperatures. In 2020, one study investigated the proteome response in *Cuniculiplasma divulgatum* when exposed to temperatures of 0°C for a short period of time (3 hours). A number of proteins were found to be upregulated, which in turn showed a particular increase in expression of the protein's glutamate dehydrogenase and thermosome beta subunit; suggesting they play a role in low temperature tolerance (Bargiela *et al.*, 2020). How archaea dominate these particular communities still requires more research, but these highly successful archaea (in particular those belonging to the order *Thermoplasmatales*) do suggest that these microorganisms play a greater role in the biochemical cycle than what we currently understand.

Within a Parys Mountain site known as PM4 (this area contains an acidic stream originating from an open pit pond in which water flows through sulfidic deposits), the phylum *Proteobacteria* was one of the more abundant bacterial sequences having an average abundance of around 26% in sediment samples taken at various depths (0-20cm depth) from the acidic stream. Other bacterial phyla with a notable relative abundance in these sediment samples include *Firmicutes*, *Actinobacteria* and *Nitrospirae* (it should also be noted that water samples taken from acidic stream showed an increased relative abundance of *Nitrospirae* making 25-30% of the community) (Korzhenkov *et al.*, 2019; Distaso *et al.*, 2020). Older studies over the last twenty years have also highlighted the large abundance of *Proteobacteria* with one study from 2004 investigated subterranean water samples from the

Parys Mountain finding the dominate microbial species being *Acidothiobacilli*, an iron-oxidising bacteria belonging to the phylum *Proteobacteria* (Coupland and Johnson, 2004).

Eukarya in the Parys Mountain

Eukaryotic acidophiles are an area less well described when compared with archaea and prokaryotes. This can be due to a number of reasons, with one being that archaea and bacteria (as shown) tend to dominate acidic environments, meaning detecting and describing eukaryotic species may be more difficult. Another reason could be that eukaryotic microorganisms could be less well adapted to AMD environments, with one study in 2006 investigating the eukaryotic distribution in the Rio Tinto river located in Spain. This study showed that the diversity and abundance of the algae genera *Dunaliella* and *Chlamydomonas* was reduced with decrease to pH and was especially affected by the increasing presence of heavy metals (Aguilera *et al.*, 2006). This can give the idea that when compared with archaeal and bacterial species, eukaryotic microorganisms struggle to survive in typical AMD environments, however in the last decade studies have shown that eukarya do also form a substantial part of acidic microbial communities.

Within the Parys Mountain, a study in 2019 investigated the eukaryotic diversity of a number of acidic pools. Abundant invertebrates include *Corixidae* and *Sialidae* however, only *Chironomidae* was found in the most acidic pools, suggesting few invertebrates are adapted to AMD conditions (Dean *et al.,* 2019). The species *Chlamydomonas acidophila* (strain PM01) was detected with notable abundance in highly acidic conditions, showing a tolerance to heavy metals present in AMD sites, in particular high Cu content (Dean *et al.,* 2019).

Looking beyond the Parys Mountain, the green algae *Chlamydomonas* has since been found and well-studied in a number of AMD sites across the world, highlighting how it dominated the eukaryotic microbial community during the winter months (growing most optimally at 8°C, pH 3.0) in two AMD lakes (Lake Tafang and Lake Paitu) located in China (Xin *et al.*, 2021). This study also additionally detected the microalgae genera *Coccomyxa* and *Auxenochlorella,* with the latter being more dominant in the warmer summer months, growing optimally at a lower pH (2.5) when compared with *Chlamydomonas* (Xin *et al.*, 2021). Fungi have also been found to make up acidic microbial communities. In particular a

number of fungal microorganisms in the AMD site, Berkeley Pit Lake located in North America, USA was revealed (a lake formed from a former open pit copper mine) (Stierle *et al.*, 2007; 2003). Over the course of a decade, species belonging to the genera *Penicillium* and *Aspergillus* have been found at various depths. Additionally, *Aspergillus* was also found in an AMD site located in Gangneung, South Korea (Park *et al.*, 2009).

Archaeal 'Dark Matter'

Over the last few decades DNA sequencing capacities have dramatically increased. In the field of microbiology, this has allowed for easier and more direct methods of molecular analysis at a much lower cost (Makarova *et al.,* 2019). This has led to new methods of genetic analysis in microbiology such as the use of metagenomics, the study of genetic material which has been directly sampled from the environment. Methods like this helped lead to the discovery of unique archaeal groups (Makarova *et al.,* 2019). Despite these advances in community analysis much of what makes up many microbial communities remain unknown as culturing microorganisms can be extremely difficult. This is especially true for archaea having a much smaller rate of successful culturing when compared with bacteria and eukarya (Sun *et al.,* 2020). This large amount of uncultured and understudied microorganisms is what scientists refer to as microbial 'dark matter' (Marcy *et al.,* 2007). A number of methods can be taken in understanding the species that make up this dark matter. One of the main methods will be successfully growing enrichment cultures of communities of microorganisms that better represent the environment they were sampled in, as well as the successful growth of pure cultures of target organisms. This will require novel enrichment methods especially in targeting archaeal species. Another method will be the combined use of metagenomics and single-cell genomics of uncultured microorganisms, allowing analysis of DNA at both the community level and of single cells which could be isolated by methods such as optical tweezers (Saw *et al.*, 2015).

The smallest Archaea

A number of archaeal groups across a range of environments have the unique property of being extremely small in size when compared with other microorganisms (Nakai, 2020). The first of these species called *Nanoarchaeum equitans*, was discovered in co-culture with its host *Ignicoccus hospitalis* using samples taken from a hydrothermal vent north of Iceland

known as the Kolbeinsey Ridge (Huber *et al.,* 2002). Cells were measured 400nm in diameter and their growth was found to be dependent on an archaeal host. In addition to the small size, *N.equitans* was found to have one of the smallest genomes in archaeal species with a size of 0.49 Mbp (Waters *et al.*, 2003). In the years since this discovery, many other nano-sized archaea have been characterised to the point now that a number of phyla have been created. These members, *Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanoarchaeota* and *Nanohaloarchaeota,* are collectively grouped in the superphylum known as DPANN (Rinke *et al,.* 2013).

Within the acidophile group, archaea measuring with a diameter of under 0.45µm in were discovered in AMD site located in the Iron Mountain, California, USA (Baker *et al.*, 2006). These acidophiles were initially known as 'Archaeal Richmond Mine Acidophilic Nanoorganisms' (ARMAN) archaea, later reclassified into two distinct groups known as ''*Ca.* Parvarchaeota'' and ''*Ca.* Micrarchaeota'' (Baker *et al.,* 2010; Rinke *et al.,* 2013).

Across all the DPANN groups the genome size is drastically reduced, however the metabolic potential does vary between species with debate on how metabolically independent they can be. For a large number of DPANN species, they lack the genes that code for processes such as the electron transport chain (ETC), a vital process for the formation of ATP (Castelle and Banfield, 2018). The groups ''*Ca.* Parvarchaeota'' and ''*Ca.* Micrarchaeota'' suggest that some DPANN species may have the ability to utilise both aerobic and anaerobic metabolism. This is due to the detection of ''*Ca.* Micrarchaeota'' in anaerobic enrichment cultures as well as studies of the tricarboxylic acid (TCA) cycle showing members in both phyla processing a two-submit type OFOR, this converts 2-oxoglutarate to succinyl-CoA, a pathway utilised by a number of anaerobic microorganisms (Krause *et al.,* 2022; Chen *et al.*, 2018). This range in gene potential also extends to other vital processes such as a lack of genes in a number of DPANN species encoding for the conversion of pyruvate to acetyl-CoA (Castelle *et al.*, 2015). The lack of this metabolic pathway raises the question if these species gain vital compounds (such as acetyl-CoA) directly, perhaps as part of the relationship it forms with its host cell.

The question of DPANN's relationship with its host cell is also an interesting one as this can potentially vary across species as well as the fact it's still largely unknown whether the relationship between DPANN species and their hosts is a symbiotic or parasitic in nature. In

addition to this DPANN archaeal species within the phyla *Diapherotrites* and *Altiarchaeota,* have been shown to have the potential to lead an independent lifestyle (Dombrowski *et al.*, 2019). Another question is whether the host cell for DPANN archaea is always an archaeal species? A phylogenetic study of protein sequences in DPANN groups showed a potential homology to protein sequences found in eukaryotes which could suggest a potential relationship between eukaryotes and DPANN archaea (Narrowe *et al.*, 2018; Dombrowski *et al.*, 2019). Bacterial species could also potentially also act as host cells to DPANN organisms. One study of samples taken from the surface water of high altitude lakes noted the positive correlation of bacterial communities and the detection of 16s rRNA gene sequences assigned to DPANN archaea (Ortiz-Alvarez and Casamayor, 2016). Whilst promising, more studies are needed to confirm this particularly in the bacterial example, as archaeal species are infamously hard to grow and detect in enrichment cultures (Sun *et al.*, 2019). The lack of successful cultures for archaea can be due to a number of reasons, such as the lab conditions not correctly matching the environment the target organism was found in, and the target archaea community being outcompeted for limited resources by more rapidly reproducing bacteria (Sun *et al.*, 2019). For last few years only a small number of DPANN archaea had been successfully co-cultured in a lab, such as *N. equitans*, ''*Ca*. Mancarchaeum acidiphilium'' and *Nanobsidianus stetteri* (Dombrowski *et al.*, 2019). In the last year however, more success has been gained in the cultivation of members belonging to ''*Ca.* Micrarchaeota''. The ''*Ca.* Micrarchaeota'' known as ''A_DKE'' (under the proposed name ''*Ca.* Micrarchaeum harzensis'') was successfully co-cultured alongside "*Ca.* S. hospitalis" (also known as ''B_DKE'') at a pH of 2.5 under anoxic conditions (Krause *et al.,* 2022). Additionally, a stable co-culture was achieved between ''*Ca.* Micrarchaeota ARM-1'' and the genus *Metallosphaera* at 60°C, pH 3.0 under aerobic conditions (Sakai *et al.*, 2022). In addition, this study also proposed renaming the phylum ''*Ca.* Micrarchaeota'' to ''*Ca.* Microcaldota'' (Sakai *et al.*, 2022).

Aims

The primary aim of this study is to investigate the microbial diversity of the PM5 site via cultivation. This was done by collecting water and sediment samples. These were used to create enrichment cultures which after a period of time were sequenced to allow identification of microorganisms.

The second aim of this study is to investigate cultivation techniques, in an attempt to enrich difficult to grow acidophiles such as ''*Ca.* Micrarchaeota''. This aim was carried out by using a range of enrichment conditions, varying both the media and supplements added to them.

Hypotheses

Based on previous literature, two hypotheses were made:

The first, archaea belonging to the Order *Thermoplasmatales* will be detected in the PM5 enrichment cultures. This hypothesis is based on previous Parys Mountain studies (in particular the PM4 site) where *Thermoplasmatales* is found to be the most successful microorganisms in the ecosystem (Korzhenkov *et al.*, 2019; Distaso *et al.*, 2020).

The second, if ''*Ca.* Micrarchaeota'' is detected in enrichment cultures, it's growth will be found associated with the order *Thermoplasmatales*. This is based on a previous studies in which ''*Ca.* Micrarchaeota'' was found associated with members of *Thermoplasmatales.* Specially, the archaeal species *C. divulgatum* and ''*Ca*. S. hospitalis'' (Golyshina *et al.*, 2019; Krause *et al.*, 2022)

Materials and methods

Sample collection: PM5 Site

Samples were collected in November of 2020, from an area designated as the PM5 site located in the east of the Parys Mountain, North Wales, UK (Fig.1), (Coordinates: 53.38747; -4.33968). This site contains an Adit (horizontal passage now filled with water from both underground sources and rainwater) into which water flows into a shallow pool, with a depth of 10-15cm (Fig.2). No previous research had been conducted on the PM5 site, meaning the microbial community was unknown. Three locations at the pool were chosen from which three sediment samples were collected. The first sediment sample location (PM5.1) was to the leftmost side of the pool, where water entered the pool from underground. The second sample location (PM5.2) was collected from a more central area of the pool. The final area sediment samples were collected (PM5.3) was from a water outflow area, where water travelled out of the pool into the next basin. All sediment samples were collected in triplicate.

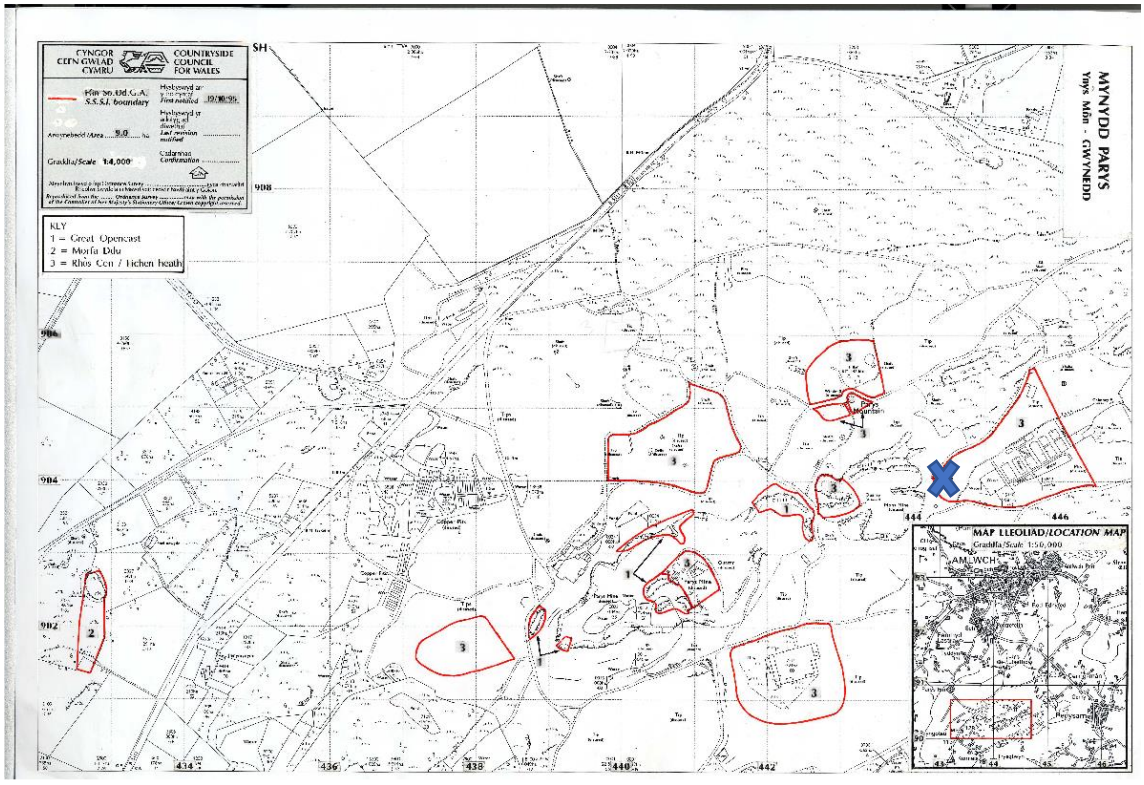

Figure 1: Map of Parys Mountain area. Located in UK, Wales, Anglesey. Blue cross indicates PM5 site area. Red lines denote areas labelled as Site of Special Scientific Interest (SSSI). Map was sourced from Countryside County for Wales.

In addition, water from the PM5 pool was also collected. These sediment and water samples were stored out of sunlight, at room temperature before cultivation experiments began. Using a SevenGo® multimeter (Mettler-Toledo, Leicester, UK), three areas had measurements taken (in triplicate) of their pH and redox potential (Eh). PM5.1 (water and sediment) had a pH of 2.25, with the water and sediment having a Eh of 620mV and 526mV, respectively. PM5.2 had a pH of 2.2 and Eh for water being 620mV and 520mV for sediment. Finally, PM5.3 had a pH of 2.19 and a Eh for water and sediment being 648mV and 620mV, respectively. Temperature of PM5 pool water and sediment at time of collection was 10°C and air temperature was 8.5°C.

Figure 2: Photo of pool located in PM5 site. This photo highlights the precipitation pool located in the PM5 site. Three areas were chosen from three sediment samples at each area was collected. Photo was taken by Dr Olga Golyshina.

Medium AB

A modified medium 88 (DSMZ) was used throughout the study (labelled as AB medium). This medium contained: $(NH_4)_2SO_4$, 1.3g; KH_2PO_4, 0.28g; $MgSO_4$ x $7H_2O$, 0.25g; $CaCl_2$ x $2H_2O$, 0.07g; $FeCl_3$ x $6H_2O$, 0.02g (DSMZ: https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium88.pdf). These compounds were added to 900ml of distilled water after which concentrated H_2SO_4 was added until the target pH was reached (this would be pH 1.0, 1,7 or 2.2), monitoring with a

pH meter calibrated before use. After target pH was reached, an additional 100ml of distilled water was added (to make a total volume of 1L), after which the pH was measured again, and adjusted to maintain the target pH. Finally, the solution was autoclaved to minimize potential contamination of culture set-up. No trace elements or vitamins were added to the medium nor were added directly to enrichment cultures at timepoint 1. From timepoint 2 onwards, two solutions were added directly to enrichment cultures during set-up. The first was Kao-Michayluk vitamin solution (Sigma-Aldrich, UK) added at a ratio of 1:100 (v/v). The second was trace element solution SL-10. Making this solution requires; HCl (25%), 10ml; $FeCl_2$ x $4H_2O$, 1.5g; $ZnCl_2$, 70mg: $MnCl_2$ x $4H_2O$, 100mg; H_3BO_3, 6mg; $CoCl_2$ x $6H_2O$, 190mg; $CuCl_2$ x $2H_2O$, 2mg; $NiCl_2$ x $6H_2O$, 24mg; Na_2MoO_4 x $2H_2O$, 36mg; distilled water, 990ml (DSMZ: https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium320.pdf). This solution was added at a ratio of 1:1000.

9K medium

In addition to AB medium, a *Ferroplasma acidiphilum* medium (labelled as 9K) was also utilised for a number of enrichment cultures. This medium contained 1L distilled water in which was added; $(NH_4)_2SO_4$, 0.2g; K_2HPO_4, 0.1g; $MgSO_4$ x $7H_2O$, 0.4g; KCl, 0.1g;, $FeSO_4$ x $7H_2O$, 25g (DSMZ: https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium874.pdf). The target pH of this solution was pH 1.6.

Enrichment cultures containing PM5 site water

Another variation included adding PM5 site water collected from the Parys Mountain. As mentioned in the sample collection, the pH of this water was around pH 2.2. Two variants were made up using this water. The first variation involved adding unfiltered PM5 site water. The second variation used PM5 site water, which was filtered using a 500ml Nalgene filter bottle under pressure (pore size of filter unit was 0.45µm for first filtration, second filtration used a 0.1µm filter).

Aerobic enrichment culture set-up

Setting up the first enrichment cultures (timepoint 1) involved weighing out 0.5g of the selected PM5 sediment sample, (sediment samples 1, 2 or 3) in a 50ml Greiner tube (all enrichment cultures were set-up in a safety cabinet). After this, 10ml of the media was added, which as mentioned could be an AB, 9K or PM5 site water depending on the variation. Depending on the type, the polypeptides; yeast extract, beef extract and tryptone (Bacto BD Biosciences, Wokingham, UK) were added at 0.1% (w/v) or 0.02% (w/v) each. This amount varied, as it may potentially influence the growth of the microbial community over time. In addition, a number of enrichment cultures were set-up with sulfur (Sigma-Aldrich) added to make up 1% (w/v). This sulfur was first sterilised by adding the powder to distilled water, after which it would be autoclaved. Using a mortar and pestle (that had been sterilised via autoclaving before use), the sulfur was crushed and added to the enrichment culture, using an electric scale to measure the correct amount. In other sets of enrichment cultures, dead cells/biomass of *C. divulgatum*, strain S5 were added at 10% (w/v). This biomass was used to provide the compounds/metabolites from *Cuniculiplasma,* and was prepared by freezing (-70°C), and melting (multiple times) cultures of *Cuniculiplasma* that were in a stationary phase. The lack of viability of biomass was checked previously (not shown). These were set up, as biomass of *Cuniculiplasma* may influence growth of microorganisms (especially ''*Ca.* Micrarchaeota''). Table 1 (as well as Table 2, Appendices) shows the full set of aerobic enrichment cultures at timepoint 1. Each variation was set-up in duplicates.

Table 1: Aerobic enrichment cultures set-up in timepoint 1. Each ID contained enrichment cultures set-up in duplicates from the three sediment samples collected from the PM5 site. Enrichment cultures using AB media were set in two sets, with one having a pH of 1.0 and the second having a pH of 1.7. Additionally, enrichment cultures containing PM5 site water were set-up with two sets, one with the PM5 site water filtered and the other set unfiltered. YE represents yeast extract, BE represents beef extract, and Trp represents tryptone. See Table 2 (Appendices) for full enrichment set up at timepoint 1.

ID	Sample	Medium	pH	Volume	Temp	Substrate 1	Substrate 2
A	PM5 1, 2, 3	AB	1/1.7	10	37	YE,BE,Trp 0.1%	
B	PM5 1, 2, 3	AB	1/1.7	10	37	Sulfur 1%	YE,BE,Trp 0.1%
C	PM5 1, 2, 3	AB	1/1.7	10	37	Sulfur 1%	YE,BE,Trp 0.02%
D	PM5 1, 2, 3	AB	1/1.7	10	37	*Cuniculiplasma* biomass	
E	PM5 1, 2, 3	9K	1.6	10	37	YE,BE,Trp 0.02%	
F	PM5 1, 2, 3	9K	1.6	10	37	*Cuniculiplasma* biomass	
G	PM5 1, 2, 3	PM water filtered/Unfiltered	2.2	10	37	YE,BE,Trp 0.1%	
H	PM5 1, 2, 3	PM water filtered/Unfiltered	2.2	10	37	Sulfur 1%	YE,BE,Trp 0.1%
I	PM5 1, 2, 3	PM water filtered/Unfiltered	2.2	10	37	Sulfur 1%	YE,BE,Trp 0.02%
J	PM5 1, 2, 3	PM water filtered/Unfiltered	2.2	10	37	*Cuniculiplasma* biomass	

In total, 108 enrichment cultures were created at timepoint 1. These were left to grow for 30 days at 37°C, being kept static (no shaking) during this time. All enrichment cultures were reinoculated at timepoint 2, with the same conditions as the previous set. 1ml (10% of the inoculate) of the previous enrichment culture, was transferred to the new set. As mentioned, Kao-Michayluk vitamin solution, and SL-10 trace element solution were added to all new enrichment cultures after timepoint 1 to support growth. During the 30 day growth period, the enrichment cultures were monitored via simple visual observation/estimation of density of cultures (Fig.3, Appendices), or microscoping if growth did not seem apparent.

At timepoint 3, samples were reinoculated into two enrichment cultures of the exact same conditions, (these were labelled T3A and T3B shorthand for Timepoint 3 Tube A or Tube B). In addition to this, samples were also reinoculated into new enrichment cultures with similar conditions, except an AB medium with a pH of 2.2 was used (these were labelled as T3C and T3D, shorthand for Timepoint 3, Tube C or Tube D)

Anaerobic enrichment culture set-up

These enrichment cultures followed a similar methodology, as laid out in the aerobic set up. To ensure anaerobic conditions, these enrichment cultures were set up in a sterile Pyrex culture tubes. After the components of the enrichment culture were added, a rubber stopper and screw cap were secured onto the tubes (having also been sterilised). These enrichment cultures were then flushed using an Anaerobic Gassing Unit, (Qcal, Messtechnik GmbH) CO_2 (10%), H_2 (10%), and N_2 (80%) gas, for 10 min to remove oxygen and substitute the atmosphere from the enrichment culture. Incubation was done at 37°C and left to grow for over a month (between 45-60 days), before reinoculation occurred. These were left to grow for a longer period, as anaerobic communities tend to grow more slowly when compared with aerobic microorganisms. Monitoring this growth also involved basic visual observations (Fig.4, Appendices), as well as use of a light microscope. Reinoculation methods followed a similar methodology to the aerobic enrichment cultures. Kao-Michayluk vitamin solution, and trace elements being added to the new enrichment cultures to support microbial growth. A sterile syringe and needle was used to transfer 1ml of the previous culture to the new one. This would penetrate the rubber stopper, avoiding introducing oxygen which could be lethal to obligate anaerobic microorganisms. Table of

enrichment culture conditions can be seen in table 3, (as well as Table 4, Appendices). In total, 132 anaerobic enrichment cultures were set-up in timepoint 1.

Table 3: Anaerobic enrichment cultures set-up in timepoint 1. Each ID contained enrichment cultures set-up in duplicates from the three sediment samples collected from the PM5 site. Enrichment cultures using AB media were set in two sets with one having a pH of 1.0 and the second having a pH of 1.7. Additionally, enrichment cultures containing PM5 site water were set-up with two sets, one with the PM5 site water media filtered and the other set unfiltered. After set-up enrichment cultures were flushed with an anaerobic mix. YE represents yeast extract, BE represents beef extract, and Trp represents tryptone. See Table 4 (Appendices) for full enrichment culture set up at timepoint 1

ID	Sample	Medium	pH	Volume	Temp	Substrate 1	Substrate 2
K	PM5 1, 2, 3	AB	1/1.7	10	37	YE,BE,Trp 0.1%	
L	PM5 1, 2, 3	AB	1/1.7	10	37	Sulfur 1%	YE,BE,Trp 0.1%
M	PM5 1, 2, 3	AB	1/1.7	10	37	Sulfur 1%	YE,BE,Trp 0.02%
N	PM5 1, 2, 3	AB	1/1.7	10	37	*Cuniculiplasma* biomass	
O	PM5 1, 2, 3	AB	1/1.7	10	37	20mM Fe2(SO4)3	YE,BE,Trp 0.1%
P	PM5 1, 2, 3	AB	1/1.7	10	37	20mM Fe2(SO4)3	YE,BE,Trp 0.02%
Q	PM5 1, 2, 3	AB	2.2	10	37	20mM Fe2(SO4)3	*Cuniculiplasma* biomass
R	PM5 1, 2, 3	PM water filtered/Unfiltered	2.2	10	37	YE,BE,Trp 0.1%	
S	PM5 1, 2, 3	PM water filtered/Unfiltered	2.2	10	37	Sulfur 1%	YE,BE,Trp 0.1%
T	PM5 1, 2, 3	PM water filtered/Unfiltered	2.2	10	37	Sulfur 1%	YE,BE,Trp 0.02%
U	PM5 1, 2, 3	PM water filtered/Unfiltered	2.2	10	37	*Cuniculiplasma* biomass	

Preparing enrichment culture samples for PCR

At timepoint 3 for both aerobic enrichment cultures, (3 months after initial set-up/timepoint 1) and anaerobic enrichment cultures, (6 months after initial set-up/timepoint 1) sequencing was conducted. To do this, direct PCR was performed on all the enrichment cultures. A pellet was first extracted. This required the enrichment cultures to first be vortexed for around 30s, after which 1.5ml of the culture was transferred to a 1.5ml Eppendorf tube. This was centrifuged, (Eppendorf Centrifuge 5810R) at 17,000 x g for 12 min, after which the supernatant was discarded, taking care not to also discard the pellet (if no pellet had formed from centrifuging, most of the supernatant was discarded and the first steps were repeated again). Once a pellet was obtained it had to be prepared for direct PCR. The pellet was first washed with 500ul of (sterile) 1x Phosphate-Buffered Saline (PBS) solution. This solution contains NaCl, 137mM; KCl, 2.7mM; Na_2HPO_4, 10mM; KH_2PO_4, 1.8mM (Sigma-Aldrich: https://www.sigmaaldrich.com/GB/en/support/calculators-and-apps/1x-phosphate-buffered-saline). This was done to neutralise the acidic nature of the pellet. After resuspending the pellet in PBS, the solution was centrifuged, (Eppendorf

Centrifuge 5810R) at 10,000 x g for 2 min to reform the pellet. After centrifuging, the liquid was discarded, taking care not to discard the pellet. 20ul of 1x MyTaq Red buffer (Bioline Meridian Bioscience, UK) was added, pipetting up and down to ensure mixing. This was placed in a heating block at 95°C and left for 10 min. Finally, the pellet was centrifuged (Eppendorf Centrifuge 5810R) for 1.5 min at 10,000 x g. This solution could now be used for PCR.

16S rRNA gene V4 amplicon sequencing

The vast majority of the PCR done amplified the 16S rRNA V4 region, however with enrichment cultures containing fungal growth, ITS primers were used for the eukaryotic taxa present. For the ITS primers, the forward primer ITS1F was used, (5'-CTTGGTCATTTAGAGGAAGTAA-3') while the reverse primer used was ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') (Wang *et al.,* 2015). To prepare such large quantities of enrichment cultures for PCR, a master mix was created. From this master mix, each 0.2ml PCR tube would contain; 5ul of 5x MyTaq Red buffer (Bioline Meridian Bioscience, UK), 17.55ul of (ultra-pure) water, 0.25ul of Taq Polymerase (Bioline Meridian Bioscience, UK) and 0.6ul of the forward primer (forward primer F515 5'-GTGBCAGCMGCCGCGGTAA-3') described in Distaso *et al.* (2020) (the forward primer was only added to the master mix if the complete set of PCR being conducted used the same forward primer). Separately adding to each tube, was 0.6ul of the reverse primer described in Distaso *et al.* (2020) (reverse R806 prokaryotic primer 5'-GGACTACHVGGGTWTCTAAT-3'), and 1.0ul of the extracted pellet solution described in the previous section (if the PCR failed one method of repeat was varying the amount of the extracted pellet solution added, potentially adding 0.5ul instead). After preparation, PCR was carried with the cycles outlined in Table 5.

Table 5: PCR cycles. Temperatures and timings of the PCR cycle which amplified the V4 region of the 16 rRNA gene. Initial denaturation was carried out at 95°C for 2 min. This was followed by 30 cycles of 95°C for 45s (denaturation), 50°C for 60s (annealing), and 72°C for 30s (elongation) with final elongation at 72°C for 5 min.

Temperature (°C)	Length of time (min/s)
95	2 min
95	45s
50	60s
72	30s
72	5 min
4	10 min

After the PCR cycle was completed, the PCR products were run on a 1.2% agarose gel to confirm successful PCR reaction. Making the gel involved adding 0.6g agarose powder (Bioline Meridian Bioscience, UK) to 50ml 1xTris acetate EDTA (TAE) buffer solution. (this solution contains 0.4M tris acetate and 0.01M EDTA) after which it was heated to dissolve the powder. Once the solution was cooled enough, 0.7ul of SafeView (Bioline Meridian Bioscience, UK) was added to the 50ml solution and mixed, after which the solution was poured and left to solidify (around 30 min). Once solidified, the gel was placed in a gel chamber with 1xTAE buffer, 1.5ul of 1kb ladder (Bioline Meridian Bioscience, UK) was added to the wells on each end of the gel, and 2.0ul of the PCR products was added to each well. This was run at 90V for 30 min. This gel run would be then viewed using a GelDoc system (BioRad). Those producing no amplicons would be repeated.

Pooling and gel purification of obtained PCR products

Once gel imaging and repeats were completed for all the enrichment cultures, the positive PCR products were purified. This involved grouping samples based on their intensity shown of the gel image. Groups could vary from 8-15 PCR samples. These samples were once again ran for 30 min at 90V, on a 1.2% agarose gel, however the wells containing PCR products were much larger (enough to contain 100ul of PCR product). After running the gel, the

image was viewed using a Safe Imager 2.0 Blue-Light Transilluminator (Invitrogen), and the target region was excised and placed in a 2.0ml Eppendorf tube, (the weight of this empty tube should also be measured). To purify the DNA, the QIAEX II'' Gel Extraction kit (QIAGEN, Sigma-Aldrich) was used, following the protocol outlined by the supplier.

Measuring DNA concentration

After collection of the purified PCR samples, the concentration of the DNA solution was calculated. Following the protocol provided by the supplier. 199ul of Qubit Thermo Fisher Scientific™ 1x dsDNA working solution was added to a Qubit tube. To this solution, 1ul of the purified DNA sample was added. This solution was vortexed for 30s and left at room temperature for 5 min. Once the time had elapsed, the solution was placed in a Qubit 4 Fluorometer (Invitrogen, ThermoFisher Scientific, Altrincham, UK) with the following settings: 1x dsDNA high sensitivity and DNA amount 1ul. This would give a DNA concentration read measured in ng/uL.

Bioinformatics

Processing the raw sequencing reads was conducted by Dr Rafael Bargiela, and followed the protocol as laid out by Korzhenkov *et al.* (2019) and Distaso *et al.* (2020; 2022). This involved having barcodes removed from the sequences via pre-processing the data. Additionally, primer sequences were removed using TagCleaner. After pre-processing, an in-house Python script was used to re-match the barcodes and the sequences. After this step, the resulting reads were analysed using QIIME IME2, version 2021.2 (Caporaso *et al.,* 2010). The libraries were demultiplexed using the different barcodes from the sequences, after which sequences were subject to correction and quality control via use of the DADA2 pipeline (Callahan *et al.,* 2016), implemented for QIIME2, classifying the reads based on amplicon sequencing variants (ASVs). The SILVA database (Quast *et al.,* 2012), version 138 was used to perform taxonomic assignation of ASVs.

Statistical analysis

Construction of graphs and tables was done using Microsoft Excel, and the statistical application SHAMAN (Volant *et al.,* 2020).

Phylogenetic analysis

All phylogenetic trees were constructed using the phylogenetic analysis software MEGA X (Kumar *et al.,* 2018). Taxa that did not attain a relative abundance above 1% in any enrichment culture, were not included in phylogenetic analysis. ASV sequences were uploaded in FASTA format. These sequences were aligned using the MUSCLE alignment, and reference sequences were added using the National Center for Biotechnology Information, (NCBI) database (Table 8, Appendices). After alignment, the file was exported in MEGA format, (Non-protein coding) after which a neighbour-joining phylogenetic tree was constructed (Saitou and Nei, 1987) using a bootstrap method of 1000 replications (Felsenstein. 1985). Evolutionary distances were calculated using the p-distance method (Nei and Kumar, 2000), and the rate variation was modelled with a gamma distribution (shape parameter equals 1). Gaps and missing data were treated using complete deletion.

Results

Over the course of this study, the sampling at 9 timepoints took place for the aerobic enrichment cultures. The first timepoint (Timepoint 1/month 1), represented the initial cultures set-up, and every subsequent timepoint, were set-up after one month of growth. During this time, two timepoints were selected for 16S rRNA v4 sequencing. The two timepoints chosen represented enrichments cultures after 3 and 7 months of reinoculations (timepoints 3 and 7).

Month 3 Enrichment Major taxonomic groups

After three months, DNA sequencing was conducted on timepoint 3 enrichment cultures (Fig.5; Table 7, Appendices). At this timepoint, sequences (such as T3_ASV 7, 2, 14, and 16. Table 7, Appendices) with an 100% identity to *C. divulgatum* PM4 strain, took up the largest abundance in the majority of the enrichment cultures, with near 100% abundances in B2b_T3A and B2b_T3C. In addition, sequences with an 100% identity to the species *F. acidiphilum* strain Y, had a majority abundance in three enrichment cultures (B2a_T3A, B2a_T3B and B2a_T3C). Other archaeal sequences of note, were affiliated with the family *Thermoplasmataceae* (99.63% identity). These were detected in a number enrichment cultures, making up to 6-8% relative abundance. ''*Ca.* Micrarchaeota Mia14'' related sequences, (99.61% identity) were also detected in low abundances (between 1-2%) in a few enrichment cultures. ''E-plasma'' sequences, (100% identity) were also detected with relative abundances above 1% in two enrichment cultures (I3b_T3C and I3b_T3D).

When examining the bacterial sequences at timepoint 3, sequences affiliated with *Sulfobacillus* (97.21% identity) were detected with large relative abundances, in several enrichment cultures. These sequences made up almost 100% abundance in G5a_T3B (Fig.5). The bacterial genus *Leptospirillum* (100% identity) was also detected in a number of enrichment cultures, with I4a_T3A containing the largest abundance (54%). Additionally, sequences affiliated with *Acidithiobacillus* (99.62% identity) were detected, with I6a_T3D having an abundance of 72%, around 4 times higher than the next most abundant microorganisms (*Cuniculiplasma* with an abundance of 17.6%). Other bacterial sequences detected of note, include *Acidiphilium*, the class *Acidmicrobiia* (100% identity), *Acidibacillus* (97.66% identity), *Acetobacteraceae* (99.62% identity), and *Ferrimicrobium* (98.88%

identity). Small abundances affiliated with ‘’CPla_3 termite group’’ (98.05% identity) sequences, from the phylum *Planctomycetota* were detected in three enrichment cultures.

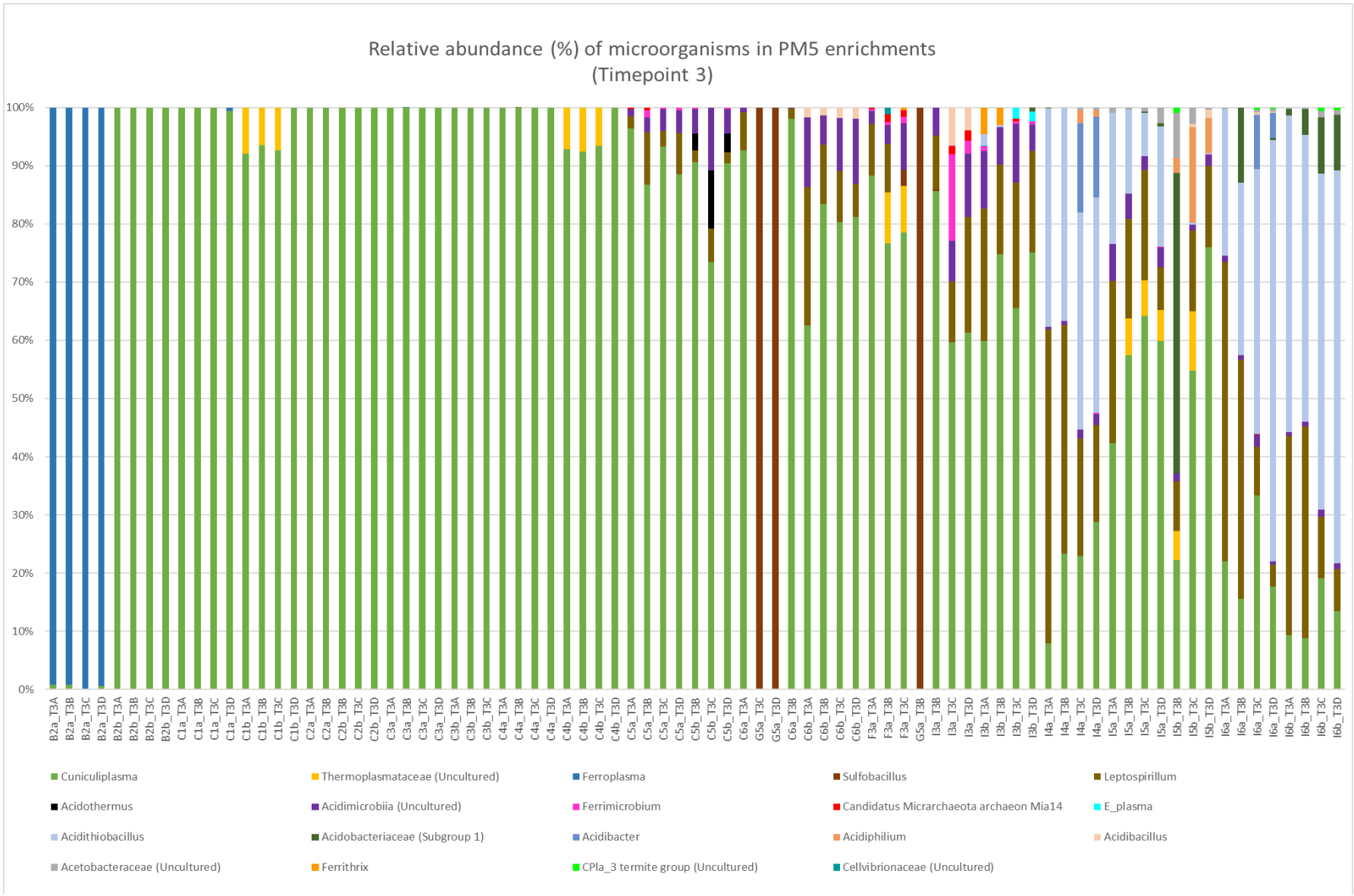

Figure 5: Major taxonomic groups found in each enrichment culture at timepoint 3. Major taxonomic groups calculated from detected reads in each enrichment culture. Microorganisms named at Genus level, exception being *Acidimicrobiia*, *Thermoplasmataceae* and *Acetobacteraceae*. Sequences with no reads above 1% in any enrichment culture were removed. Samples were reinoculated into two enrichment cultures of the exact same conditions, (these were labelled T3A and T3B shorthand for Timepoint 3 Tube A or Tube B). In addition to this, samples were also reinoculated into new enrichment cultures with similar conditions, except an AB medium with a pH of 2.2 was used (these were labelled as T3C and T3D, shorthand for Timepoint 3, Tube C or Tube D). See Table 2 (Appendices) for details on enrichment culture conditions.

Phylogeny of archaea in enrichment cultures

The use of enrichment cultures and subsequent DNA barcoding analysis allowed for the detection of a number of archaeal sequences (Table 8, Appendices) . These sequences are microorganisms affiliated with the order *Thermoplasmatales* and ''*Ca.* Micrarchaeota*.* Microorganisms within *Thermoplasmatales* belonged to the genus *Cuniculiplasma* (99.22% identity), and *Ferroplasma* (100% identity)*.* An uncultured archaeon was also detected, belonging to the family *Thermoplasmataceae* likely the microorganism ''*Ca*. S. hospitalis'' (100% identity)*.* Within ''*Ca.* Micrarchaeota*,* the microorganism affiliated with ''*Ca.* Micrarchaeota Mia 14'' (99.61% identity) was detected (Fig.6).

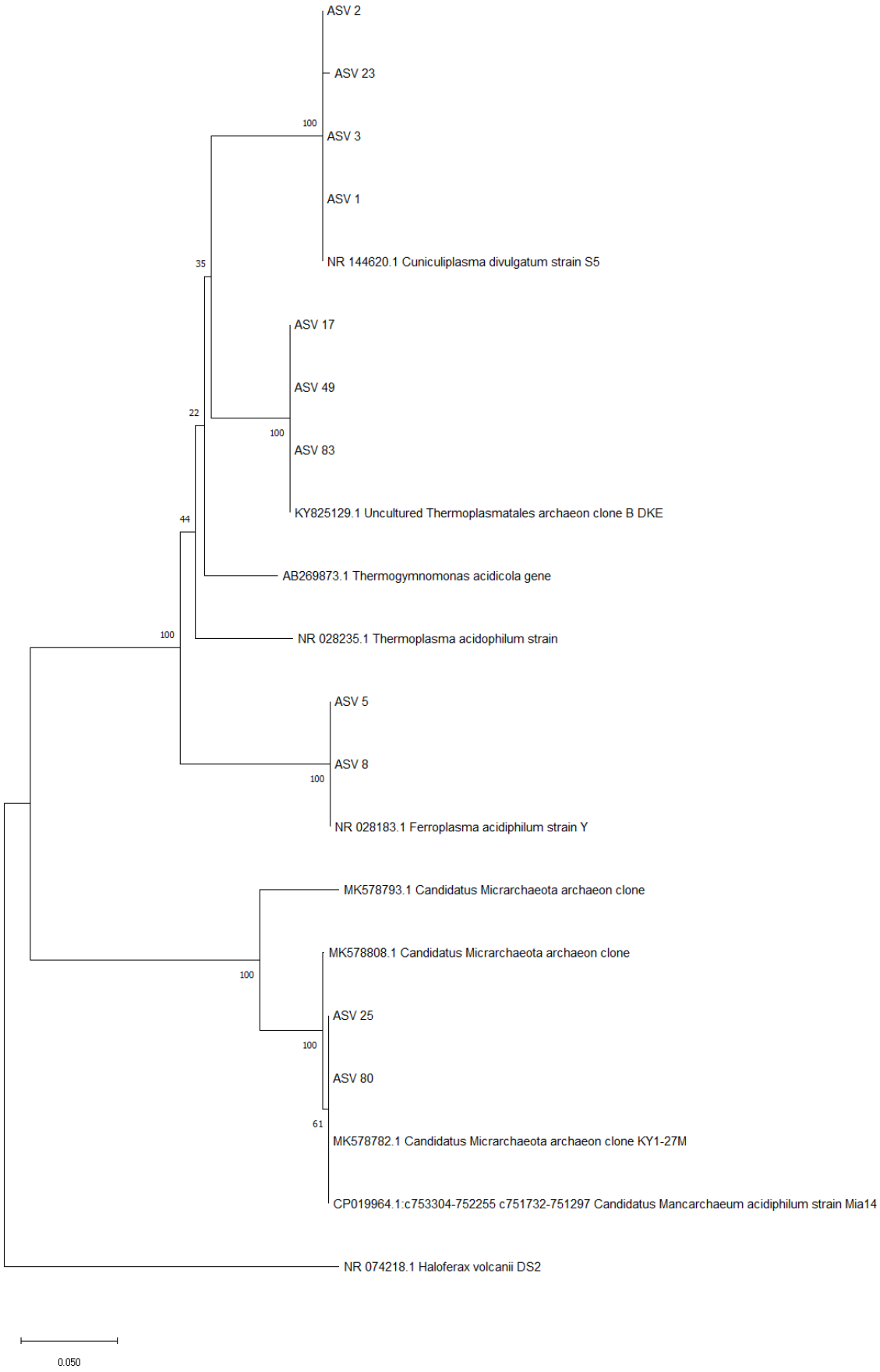

Figure 6: Neighbour-joining tree made from archaeal reads detected in PM5 enrichment cultures. Made using ASV sequences detected in Parys Mountain PM5 enrichment cultures (Timepoint 7). Bootstrap test of 1000 replicates. Evolutionary distances calculated using p-distance method. All positions containing gaps and missing data were eliminated (complete deletion). Reference strain *Haloferax volcanii* was used to root the tree. Other reference strains include *Cuniculiplasma divulgatum*, *Thermoplasmatales* archaeon B_DKE, *Thermogymnomonas acidicola*, *Thermoplasma acidophilum*, and ''*Ca.* Micrarchaeota''. See Table 8 (Appendices) for details on ASV sequences and reference strains. Tree made using MEGA X software.

Phylogeny of bacteria in enrichment cultures

In addition to the detection of archaeal sequences, a number of bacterial sequences were detected from the DNA barcoding of Timepoint 7 enrichment cultures (Table 8, Appendices). Bacterial sequences were detected that affiliated with the classes *Acidmicrobiia* (100% identity)*, Sulfobacillus* (97.27% identity)*, Bacilli* (97.66% identity)*, Leptospirillia* (100% identity) *Alphaproteobacteria* (100% identity)*,* and *Gammaproteobacteria* (100% identity) (Fig.7).

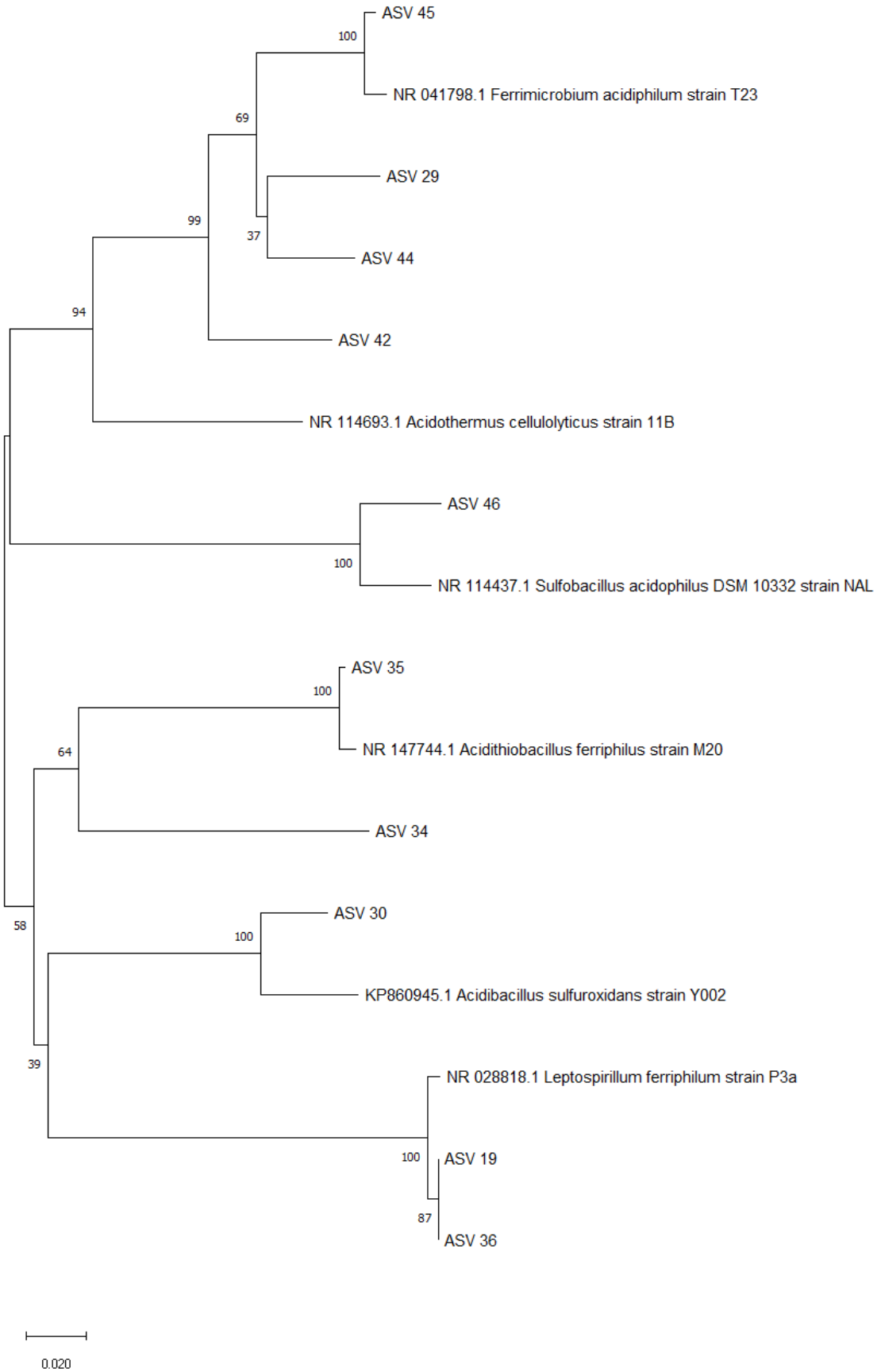

Figure 7: Neighbour-joining tree made from bacterial reads detected in PM5 enrichment cultures. Made using ASV sequences detected in Parys Mountain PM5 enrichment cultures (Timepoint 7). Sequences with no reads above 1% abundance in any enrichment culture was removed. Bootstrap test of 1000 replicates. Evolutionary distances calculated using p-distance method. All positions containing gaps and missing data were eliminated (complete deletion). Reference strains include *Leptospirillum ferriphilum*, *Sulfobacillus acidophilus*, *Acidibacillus sulfuroxidans, Ferrimicrobium acidophilum*, *Acidothermus celluloyticus*, and *Acidithiobacillus ferriphilus*. See Table 8 (Appendices) for details on ASV sequences and reference strains. Tree made using MEGA X software.

Relative abundance of PM5 sites month 7 enrichment cultures

Seven months after enrichment cultures were set up, DNA extraction and barcoding was conducted on timepoint 7 cultures. Looking at each of the sampling sites within PM5 (Fig.8), sequences affiliated with *Cuniculiplasma* (however, it should be noted some enrichment cultures contained biomass of *Cuniculiplasma*) and *Ferroplasma* had the largest abundances, with the former having a relative abundance of 93.9% in PM5.1, 59.1% in PM5.2 and 84.2% in PM5.3.

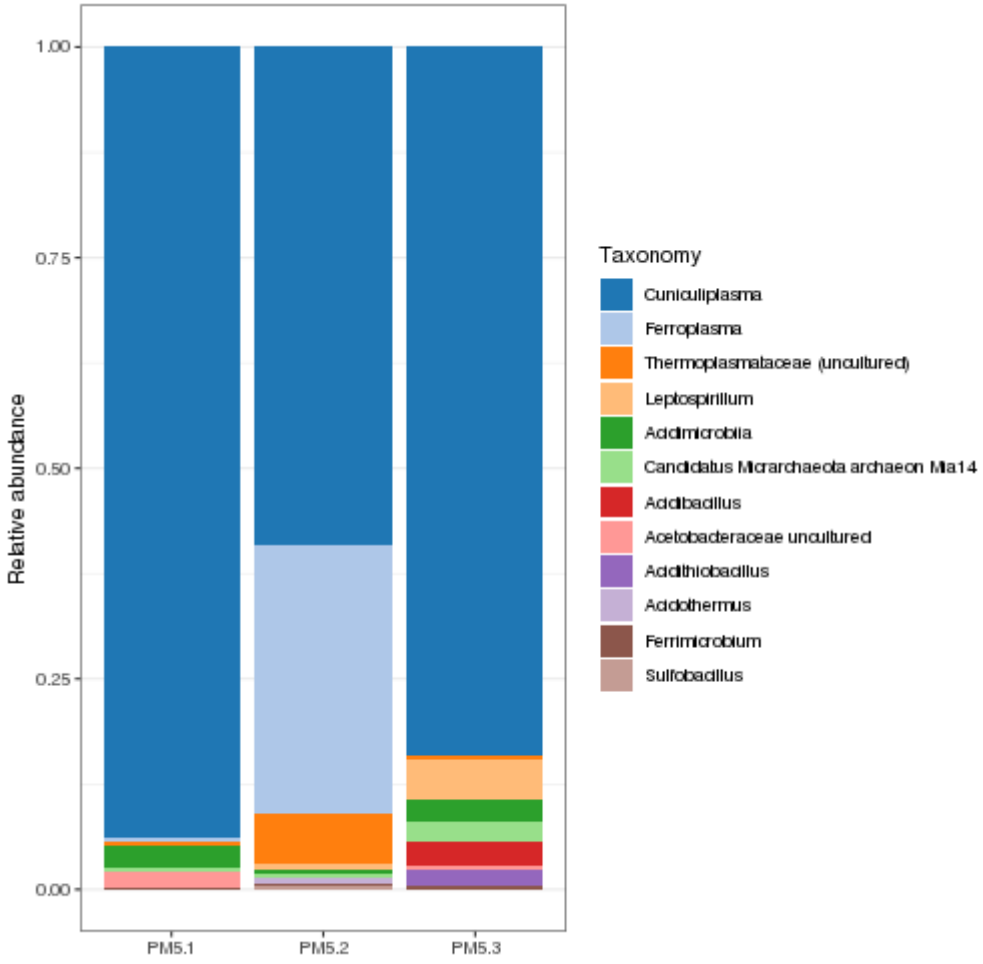

Figure 8: Relative abundance of PM5 sample site enrichment cultures: Abundance calculated from reads taken from enrichment cultures that belonged to each site. Microorganisms named at Genus level, exception being *Acidimicrobiia*, *Thermoplasmataceae* and *Acetobacteraceae*. Sequences with no reads above 1% in any enrichment culture were removed. PM5.1, PM.2 and PM5.3 represent each sample site within PM5. n=number of enrichment cultures. n for PM5.1= 4, n for PM5.2=9, n for PM5.3=9.

Enrichment cultures containing PM5.3 samples had the largest abundance of sequences outside of *Cuniculiplasma* and *Ferroplasma*. Of note are the bacterial sequences; *Leptospirillum,* having the largest abundance of bacterial sequences (4.9%), which is nearly twice the abundance of the next largest bacterial sequences detected, *Acidibacillus* and *Acidimicrobiia* with a relative abundance of 2.8% and 2.6% respectively. Additionally, in PM5.2 nearly 6% of the relative abundance is made up of an uncultured archaeal sequences affiliated to the family *Thermoplasmataceae* (''*Ca*. S. hospitalis''). Finally, sequences affiliated with ''*Ca.* Micrarchaeota Mia 14'' has the largest abundance from PM5.3 enrichment cultures with a relative abundance of 2.4%, while PM5.1 and PM5.2 had a relative abundance of under 1%.

Month 7 Enrichment Major taxonomic groups

Most enrichment cultures contained sequences affiliated with *Cuniculiplasma* as the largest abundance (Fig.9). A number of these, such as B2a_T7C and C1b_T7A contained sequences affiliated with *Cuniculiplasma* in a near pure culture.

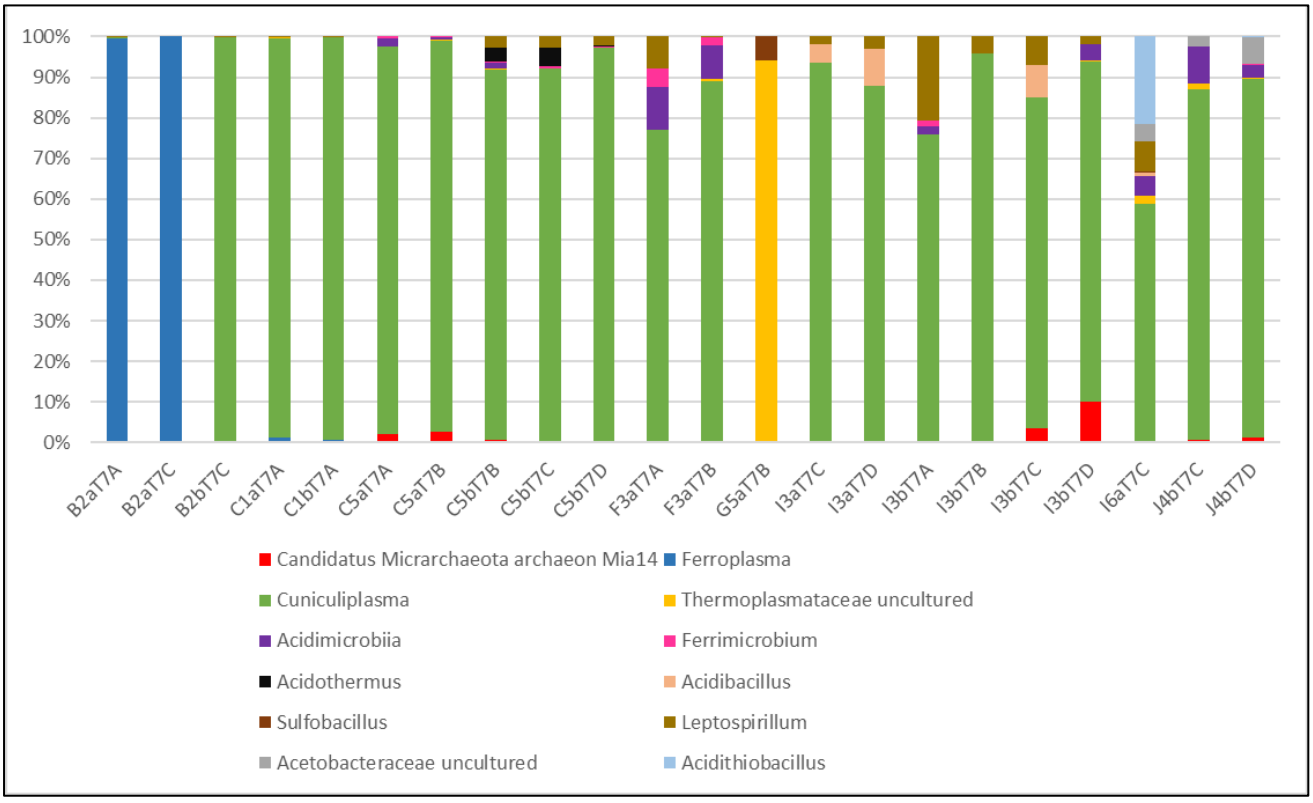

Figure 9: Major taxonomic groups found in each enrichment culture at timepoint 7. Major taxonomic groups calculated from detected reads in each enrichment culture. Microorganisms named at Genus level, exception being *Acidimicrobiia*, *Thermoplasmataceae* and *Acetobacteraceae*. Sequences with no reads above 1% in any enrichment culture were removed. See Table 2 (Appendices) for details on enrichment culture conditions

B2a_T7C is also of note with the sequences detected suggesting a pure culture of the archaeal genus *Ferroplasma*, with a near pure culture detected in B2a_T7A. An archaeal sequence affiliated to *Thermoplasmataceae* (''*Ca*. S. hospitalis'') was detected as the largest abundance microorganism in enrichment G5a_T7B, with the only other sequences detected affiliated to the bacterial genus *Sulfobacillus.*

Month 7 Enrichment culture variations

An important aspect of this study was to evaluate methods in successfully promoting the growth of acidophilic microorganisms. To do this, enrichment cultures were set up with a range of different conditions. This included variations in the media added and the supplements added to them.

AB, 9K and Site water

Two main types of media were used during this study, namely AB and 9K. Additionally, PM5 site water (filtered and unfiltered) was used, however this is technically not a medium. Within each medium variant, sequences affiliated to *Cuniculiplasma* made up a substantial number of reads, having the highest relative abundance in the 9K enrichment cultures, with an overall relative abundance of around 85% and the lowest abundance occurring in the site water cultures with an overall relative abundance of around 61.4% (Fig.10). Within enrichment cultures containing AB medium, the archaeal genus *Ferroplasma* had an overall relative abundance of 19%, while site water variants contained high abundance of sequences affiliated with the family *Thermoplasmataceae* (''*Ca*. S. hospitalis''). Looking at bacterial reads, sequences affiliated with *Acidimicrobiia* had the highest overall relative abundance (9.2%) in 9K medium (however had an overall abundance of less than 1.5% in AB and site water). Within enrichment cultures containing site water, *Leptospirillum* had the largest relative abundance of bacterial reads (6.9%), over three times the relative abundance of next largest bacterial sequence, *Sulfobacillus* (1.8%). Overall relative abundances within AB medium showed a number of sequences with 1-2% abundances including *Leptospirillum* (1.5%)*, Acidimicrobiia* (1.2%)*,* and *Acidibacillus* (1.2%).

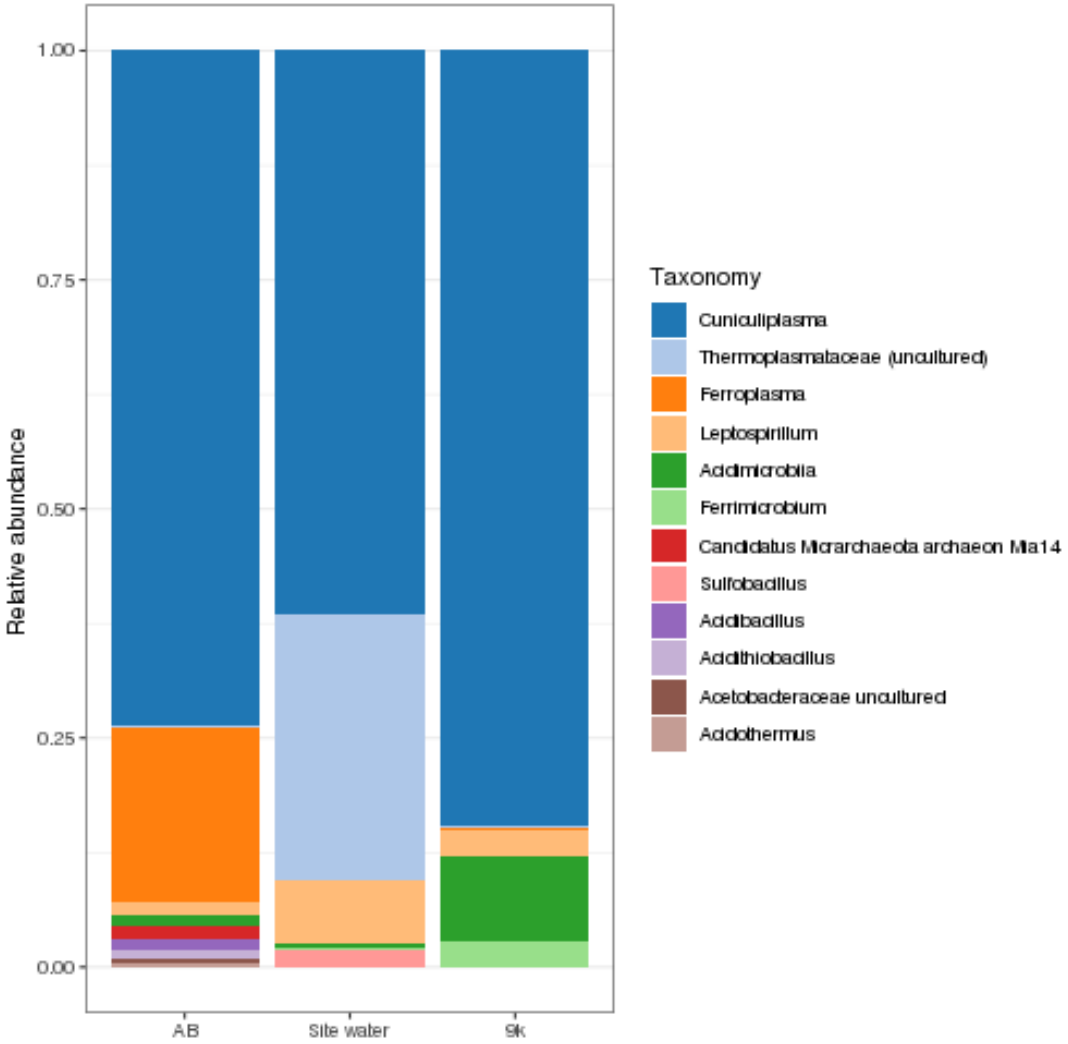

Figure 10: Relative abundance of microorganisms in different enrichment media: Abundance calculated from reads taken from enrichment cultures. Microorganisms named at Genus level, exception being *Acidimicrobiia*, *Thermoplasmataceae* and *Acetobacteraceae*. Sequences with no reads above 1% in any enrichment culture were removed. Media used were AB, 9K and Site water. n=number of enrichment cultures, n for AB= 17, n for Site water=3, n for 9K= 2

An analysis of the diversity (Fig. 11) highlights enrichment cultures made using 9K conditions contain the highest diversity values (Alpha: 5, Shannon: 0.607, and Simpson: 0.295). AB conditions had the second highest diversity value in an Alpha diversity test (4.58) however scored the lowest diversity values in the other two tests (Shannon: 0.345, and Simpson: 0.155). Site water had diversity values closer to AB than that of 9K (Alpha: 2.67, Shannon: 0.354, and Simpson: 0.188).

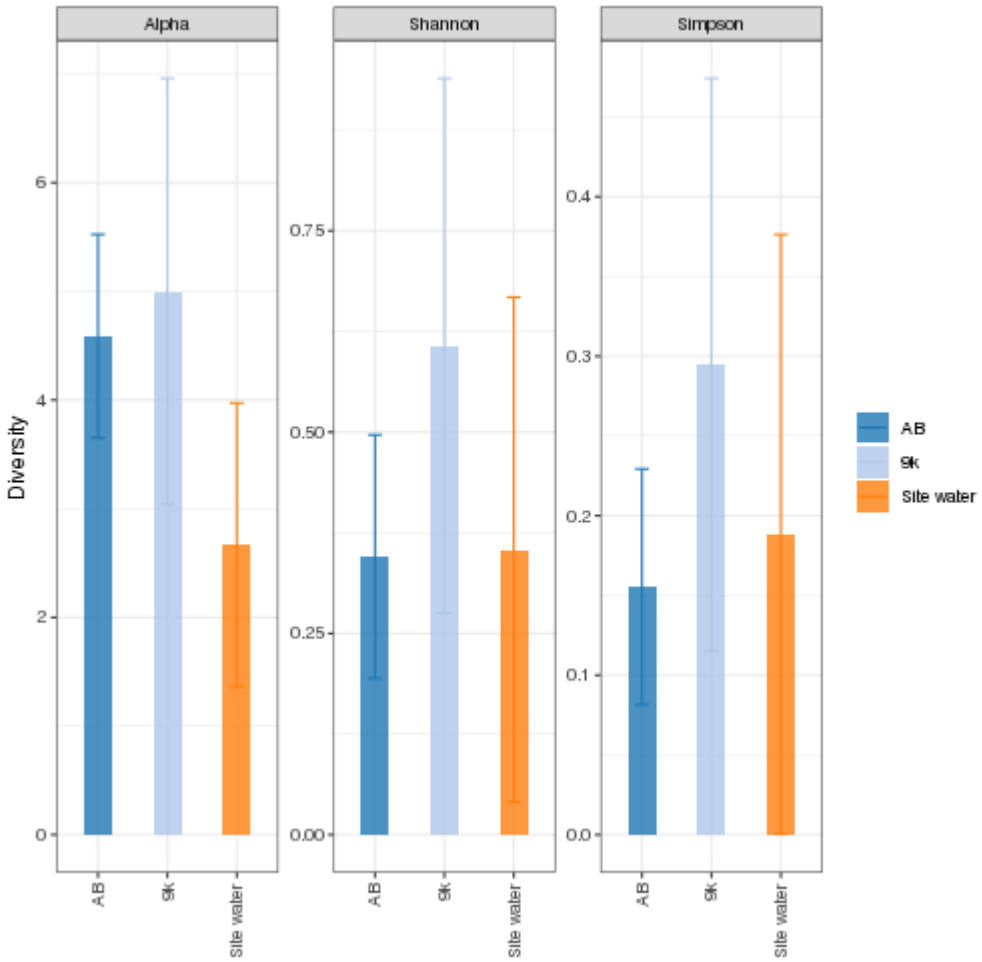

Figure 11 : Microbial diversity of AB, Site water and 9K enrichment cultures at timepoint 7. Diversity measured by varying diversity indices: Alpha diversity (Chao1), Shannon index and Simpson index. Bars represent standard error. Sequences with no reads above 1% in any enrichment culture were removed. n=number of enrichment cultures, n for AB= 17, n for Site water=3, n for 9K= 2

pH of enrichment cultures

A range of pH was utilised throughout the study, AB medium being used had a pH of 1.0, 1.7 and 2.2, PM5 site water had a pH of around 2.2 and the 9K medium had a pH of 1.6.

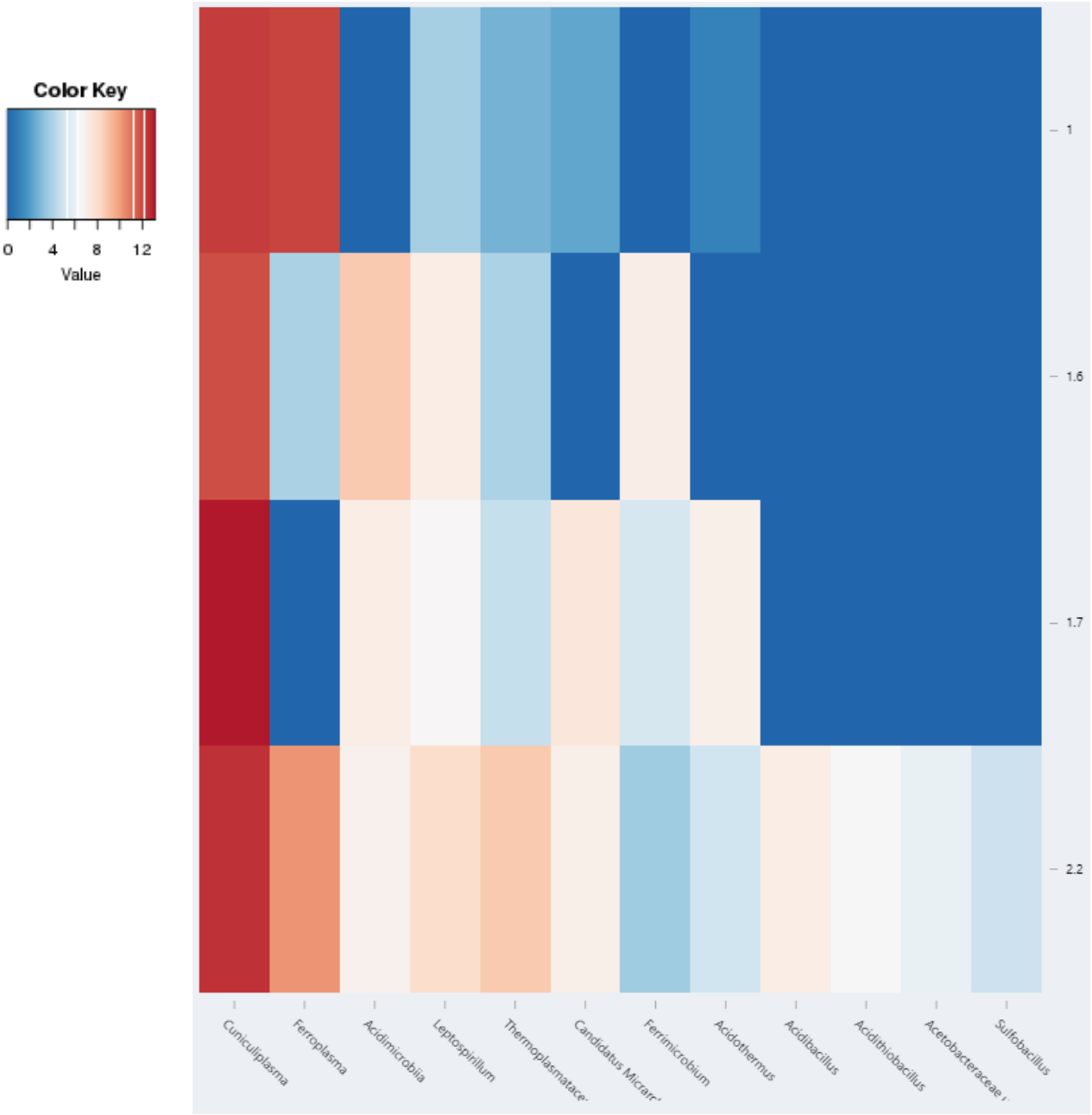

Figure 12: Heatmap of microorganisms from enrichment cultures at varying pH: Abundance calculated from reads taken from enrichment cultures. 1-12 key used with higher number/red colour indicating higher abundance while lower number/blue colour indicates lower abundance. Microorganisms named at Genus level, exception being *Acidimicrobiia*, *Thermoplasmataceae* and *Acetobacteraceae*. Sequences with no reads above 1% in any enrichment culture were removed. pH used were 1.0, 1.6, 1.7 and 2.2. n=number of enrichment cultures, n for 1.0= 3, n for 1.6=2, n for 1.7= 3, n for 2.2= 14.

A high abundance of sequences matching *Cuniculiplasma* sp. were detected regardless of pH, with the highest abundance at pH 1.7 (94.4%) (Fig.12). *Ferroplasma* sp. should also be noted of its successful growth in pH 1.0 and 2.2 enrichment cultures (with the former having the highest abundance of 47.3%) while having low to no success in pH 1.6 and 1.7, respectively. Comparison of pH 1.0 and 2.2 shows higher abundances of microorganisms as a whole at pH 2.2. This is shown with pH 1.0 having no abundances for six microorganisms (as indicated by the dark blue colour) and low success for four microorganisms, *Acidothermus* sp., ''*Ca.* Micrarchaeota'', *Thermoplasmataceae* (Uncultured) and *Leptospirillum* sp (under 1% abundance). In contrast with overall abundance of pH 2.2 enrichment cultures, no microorganism has no abundance at this pH. Microorganisms with no abundances at pH 1.0, but detection at pH 2.2 include *Sulfobacillus*, *Acetobacteraceae* (Uncultured), *Acidithiobacillus* sp., *Acidibacillus* sp., *Ferrimicrobium* sp. and *Acidimicrobiia*. Fig.12 also highlights the higher abundances of ''*Ca.* Micrarchaeota'' phylotype at pH 1.7 and 2.2 (1.7% and 1.4% respectively), when compared to its low overall abundance in pH 1.0 (<1% abundance) enrichment cultures and no abundance at pH 1.6.

Diversity analysis of pH enrichment cultures (Fig.13) indicates that of the three diversity tests conducted (Alpha, Shannon and Simpson), pH 1.0 consistently scored the lowest value indicating the lowest overall diversity (Alpha: 3.33, Shannon: 0.0578 and Simpson: 0.0184). Of note while pH 2.2 showed higher diversity values (Alpha: 4.29, Shannon: 0.420 and Simpson: 0.201) when compared to pH 1.0 and 1.7 (aside from the alpha diversity test where pH 1.7 scored highest with a value of 5.33), pH 1.6 scored the higher diversity values from the three tests (Alpha: 5.0, Shannon: 0.607 and Simpson: 0.295).

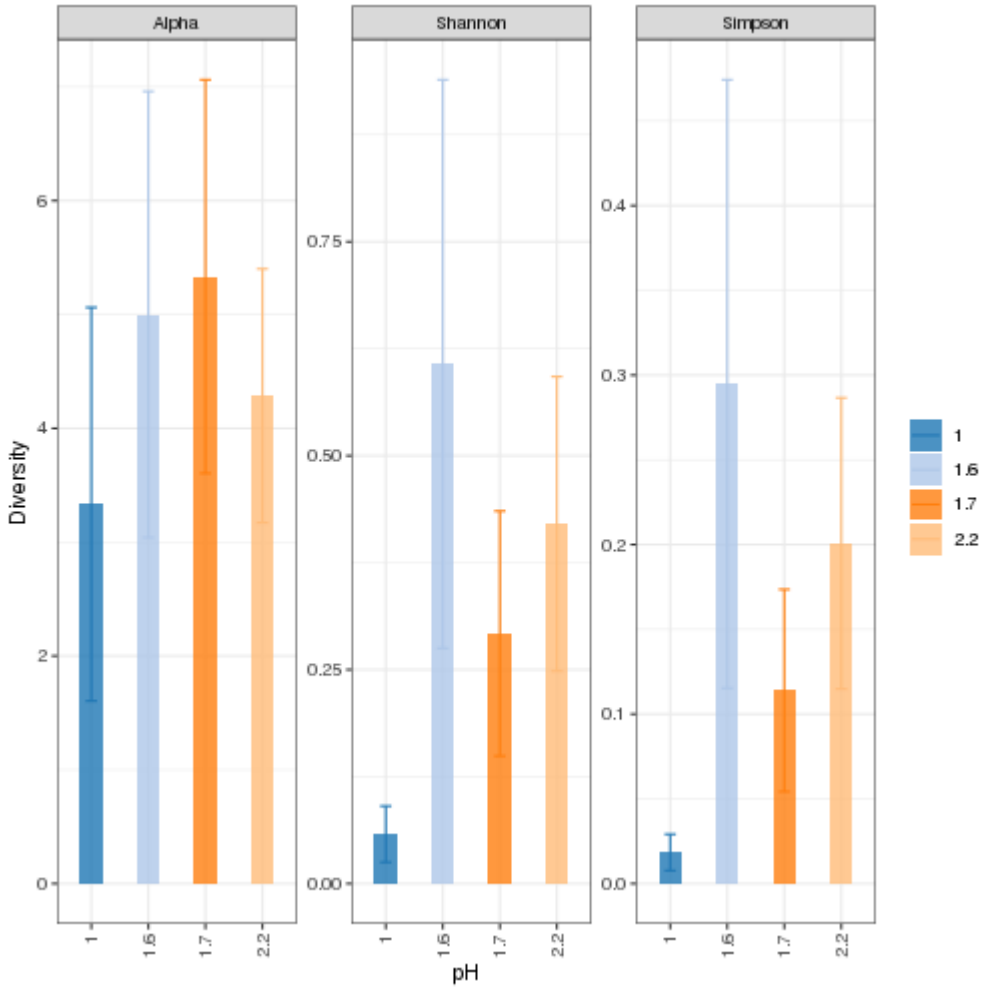

Figure 13: Microbial diversity of varying pH enrichment cultures at timepoint 7. Diversity measured by varying diversity indices: Alpha diversity (Chao1), Shannon index and Simpson index. Bars represent standard error. Sequences with no reads above 1% in any enrichment culture were removed. pH used were 1.0, 1.6, 1.7 and 2.2. n=number of enrichment cultures, n for 1.0= 3, n for 1.6=2, n for 1.7= 3, n

Sulfur presence in enrichment cultures

In a number of enrichment cultures, sulfur was added to make up 1% of the content. In those that contain sulfur, sequences affiliated with *Cuniculiplasma* had the largest overall relative abundance of 73.9%, nearly 4 times higher than the next largest archaeal sequence detected, *Ferroplasma* with a relative abundance of 18.8% (Fig.14). In enrichment cultures absent of sulfur, sequences affiliated with *Cuniculiplasma* once again had the highest overall relative abundance with a relative abundance of around 67.4%. A high abundance of uncultured sequences affiliated to the family *Thermoplasmataceae* (21.1%) was also detected from enrichment cultures absent of sulfur. For bacterial sequences, all relative abundances were below 3%, with the highest abundance coming from sequences affiliated

with *Leptospirillum* in sulfur present enrichment cultures followed by an uncultured sequences affiliated to the family *Acetobacteraceae* (Fig.14).

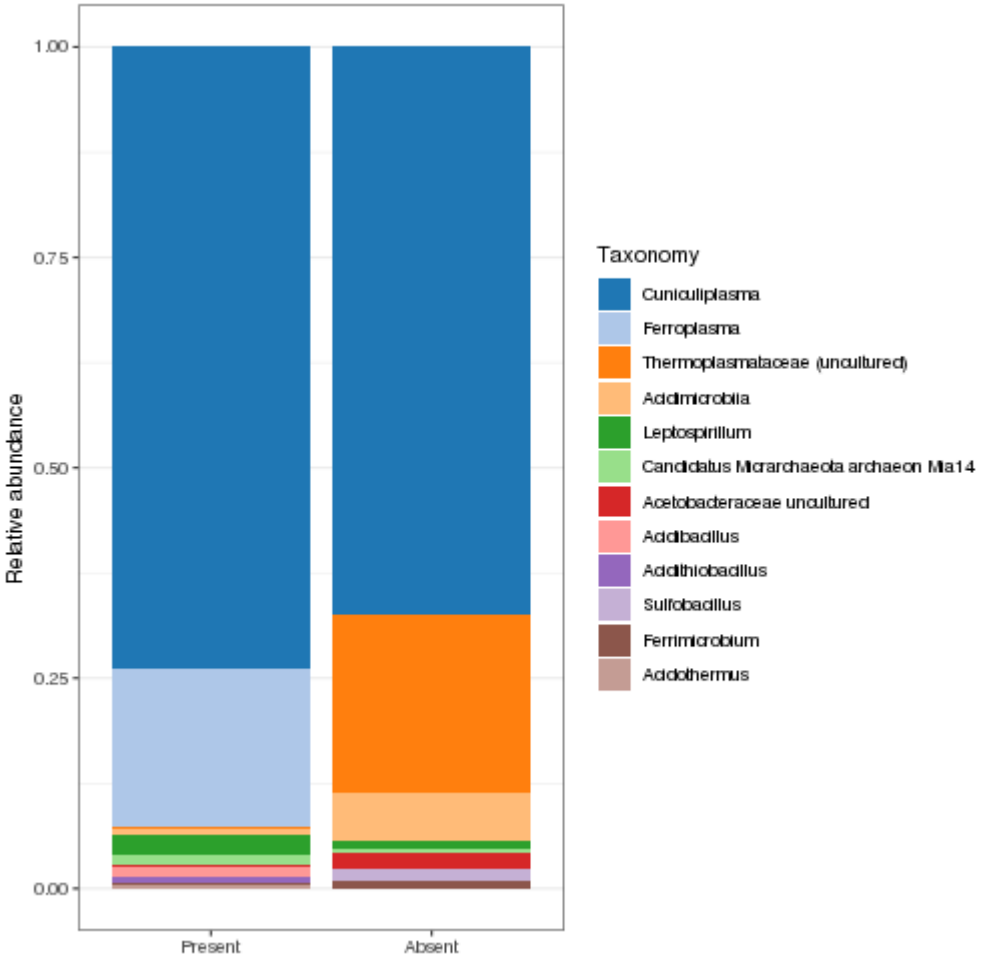

Figure 14: Relative abundance of microorganisms from enrichment cultures with Sulfur present and absent: Abundance calculated from reads taken from enrichment cultures Microorganisms named at Genus level, exception being *Acidimicrobiia*, *Thermoplasmataceae* and *Acetobacteraceae*. Sequences with no reads above 1% in any enrichment were removed. Sulfur added represented 1% of the total enrichment (0.1g). n= number of enrichment culture, n for Sulfur present=17, n for Sulfur absent= 5

Looking at the diversity analysis (Fig.15), each test in enrichment cultures containing sulfur (Alpha: 4.12, Shannon: 0.336, and Simpson: 0.156), diversity values were lower than that compared to those absent of sulfur (Alpha: 5.2, Shannon: 0.488, and Simpson: 0.230). Enrichment cultures absent of sulfur have a diversity value 1.2-1.5 times higher than that of those containing sulfur.

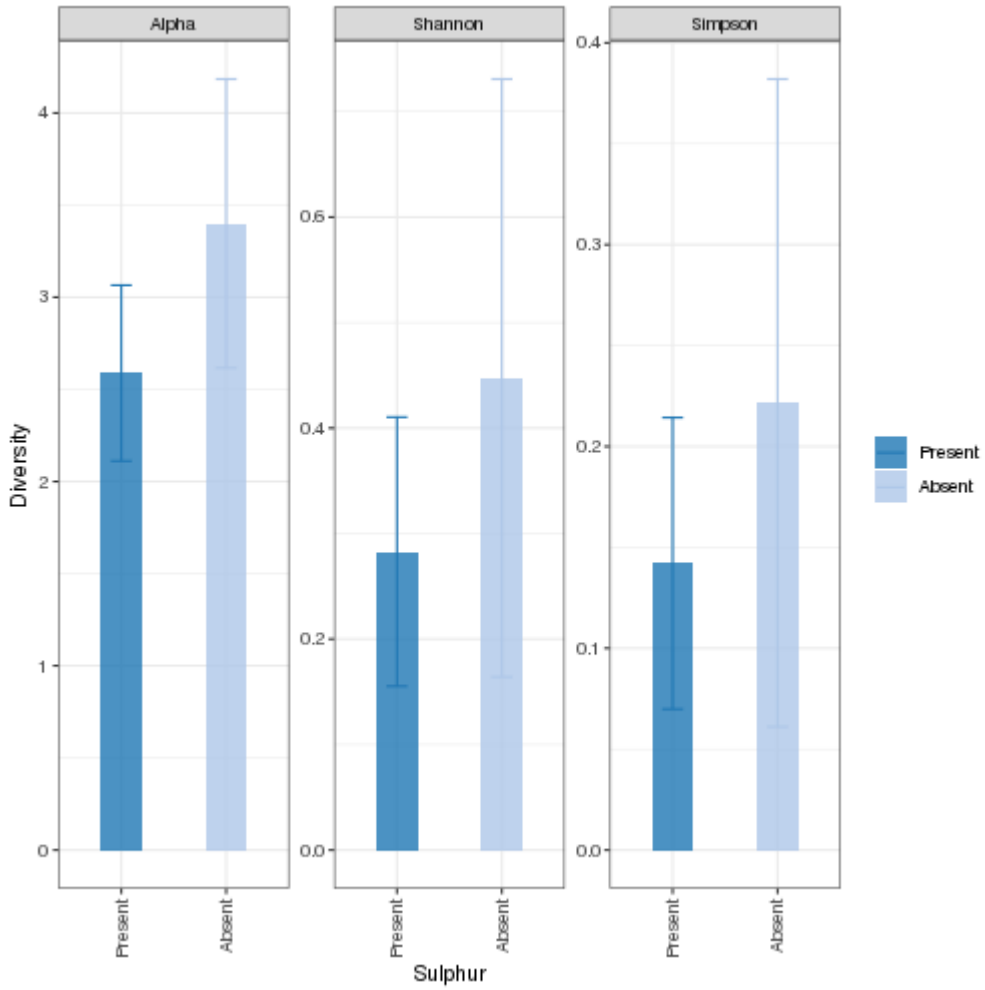

Figure 15: Microbial diversity of sulfur present/absent enrichment cultures at timepoint 7. Diversity measured by varying diversity indices: Alpha diversity (Chao1), Shannon index and Simpson index. Bars represent standard error. Sequences with no reads above 1% in any enrichment were removed. Sulfur added represented 1% of the total enrichment culture (0.1g). n= number of enrichment cultures, n for Sulfur present=17, n for Sulfur absent= 5

Effect of *Cuniculiplasma* Biomass in enrichment cultures

Another variation was the addition of *Cuniculiplasma* biomass (Fig.16). This biomass contained no living *Cuniculiplasma* (not shown), and was added to make up 10% of the enrichment culture. By timepoint 7, only four enrichment cultures containing this dead biomass were successfully used in DNA barcoding. With the biomass added, sequences matching with *Cuniculiplasma* had a larger overall abundance of 86.3%, while with no biomass added *Cuniculiplasma* sequences were detected with an overall abundance of 71.3%.

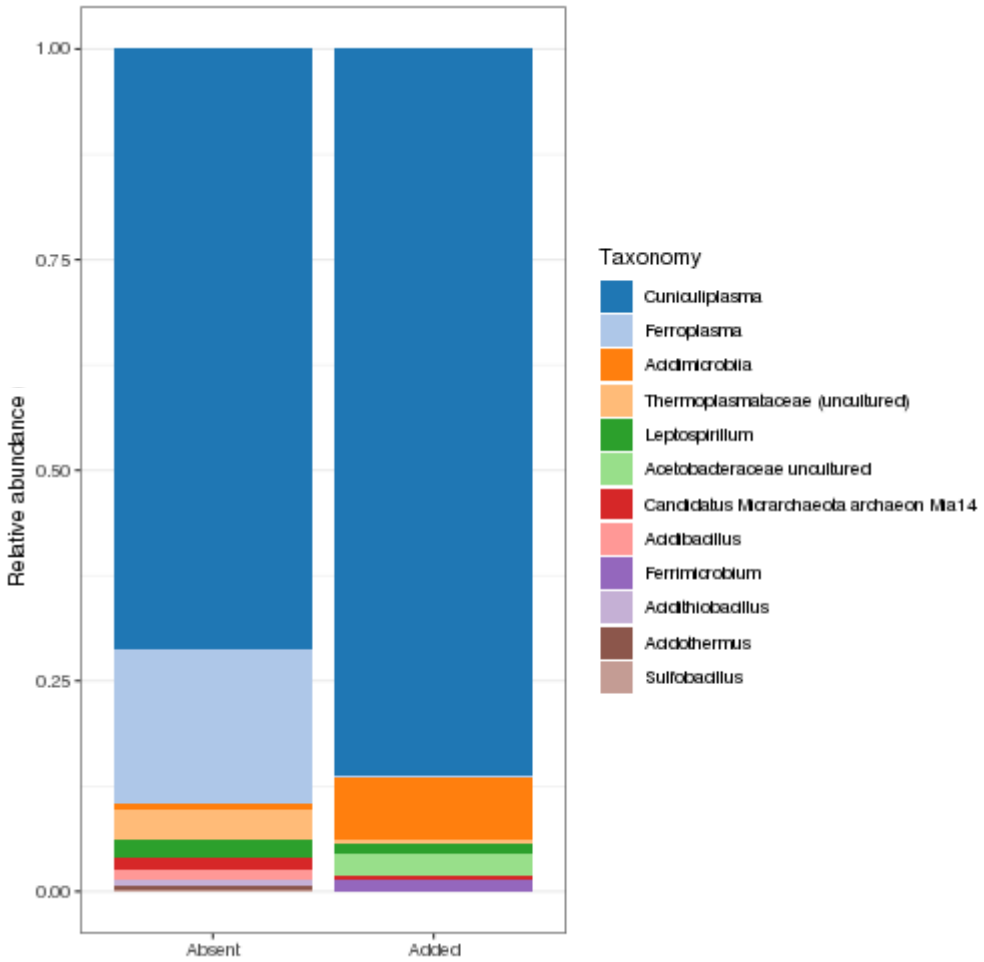

Figure 16: Relative abundance of microorganisms from enrichment cultures with *Cuniculiplasma* biomass added and absent: Abundance calculated from reads taken from enrichment cultures. Microorganisms named at Genus level, exception being *Acidimicrobiia*, *Thermoplasmataceae* and *Acetobacteraceae*. Sequences with no reads above 1% in any enrichment culture were removed. Biomass added represented 10% of the total enrichment culture (1ml). n= number of enrichment cultures, n for biomass added=4, n for biomass absent= 18.

With enrichment cultures not containing *Cuniculiplasma* biomass, higher abundances of sequences matching *Ferroplasma* (18.2%), ''*Ca.* Micrarchaeota'' (1.3%), *Leptospirillum* (2.2%), *Acidibacillus* (1.1%), and *Thermoplasmataceae* (Uncultured) (3.5%), were detected when compared with those containing *Cuniculiplasma* biomass. Additionally, with biomass added, a higher overall relative abundance of the bacterial sequences *Acidimicrobiia* (7.4%), *Ferrimicrobium* (1.3%) and *Acetobacteraceae* (uncultured) (2.6%) are noted when compared against overall abundance of biomass absent enrichment cultures.

The diversity analysis of *Cuniculiplasma* biomass being present and absent in enrichment cultures shows that those containing the biomass scored higher diversity values in the three tests (Fig.17). With biomass present, Alpha: 6, Shannon: 0.557, and Simpson: 0.261. With biomass absent, Alpha: 4, Shannon: 0.329, and Simpson: 0.153. Biomass present enrichment cultures scored a diversity value 1.5-1.7 times higher than that of biomass absent.

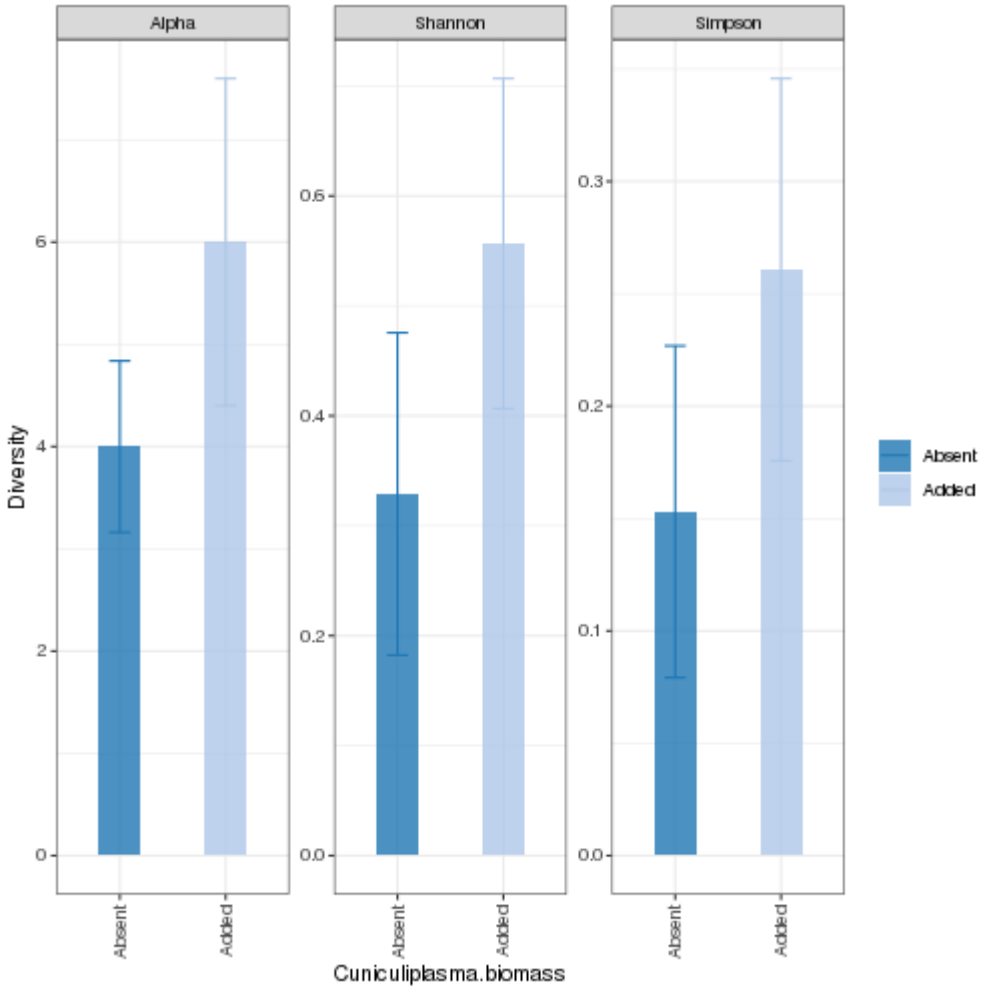

Figure 17: Diversity analysis *Cuniculiplasma* biomass present/absent at timepoint 7. Diversity measured by varying diversity indices: Alpha diversity (Chao1), Shannon index and Simpson index. Bars represent standard error. Sequences with no reads above 1% in any enrichment culture were removed. n= number of enrichment cultures, n for 0% =4, n for 0.02%= 14, n for 0.1%=4.

Communities in PM5 water medium with and without filtration

Two types of PM5 site water were used throughout the study. PM5 water unfiltered, and PM5 water that was filtered. It should be noted that PM5 unfiltered water was the source of fresh portion of organisms populating lotic fraction of the site during re-inoculation time points. Only three enrichment cultures remained that made use of the PM5 site water, with only one of these containing PM5 site water that was unfiltered (Fig.18). In filtered PM5 water, *Cuniculiplasma* makes up the largest relative abundance with 88.8% of reads (Fig.18). The next largest sequences detected were affiliated with the bacterial genus *Leptospirillum* with a 10% relative abundance.

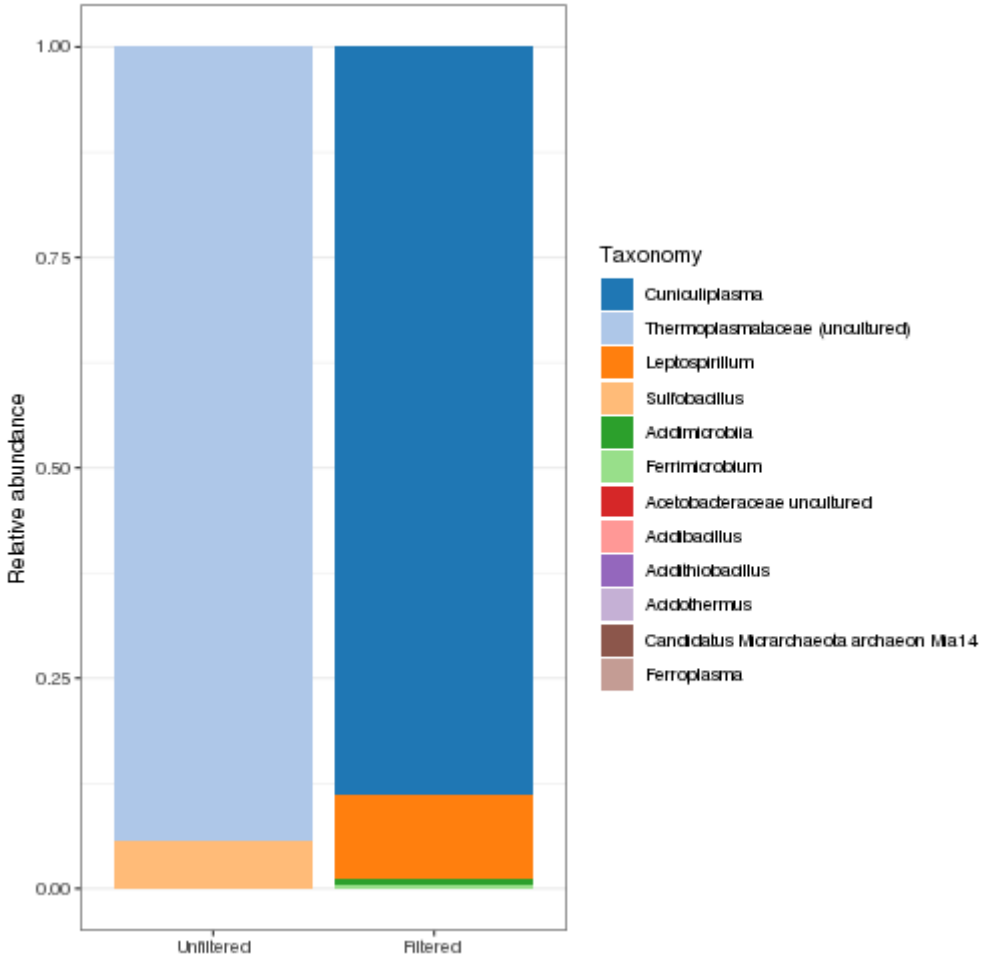

Figure 18: Relative abundance of microorganisms from enrichment cultures with PM5 site water medium present: Abundance calculated from reads taken from enrichment cultures. Microorganisms named at Genus level, exception being *Acidimicrobiia*, *Thermoplasmataceae* and *Acetobacteraceae*. Sequences with no reads above 1% in any enrichment culture were removed. PM5 site water was used in two types of medium, unfiltered and filtered. n= number of enrichment cultures, n for unfiltered=1, n for filtered=2.

In enrichment cultures containing PM5 unfiltered site water, the overall abundance was made up of uncultured sequences matching to the archaeal family *Thermoplasmataceae*, with an overall relative abundance of 94.3%. The rest of the reads in unfiltered PM5 site water, was made up by sequences matching with the bacterial genus *Sulfobacillus,* with an overall relative abundance of 5.7%.

A diversity analysis (Fig.19) shows enrichment cultures containing filtered PM5 site water (Alpha: 3, Shannon: 0.426, and Simpson: 0.226) had a higher diversity than that of unfiltered PM5 site water (Alpha: 2, Shannon: 0.215, and Simpson: 0.105).

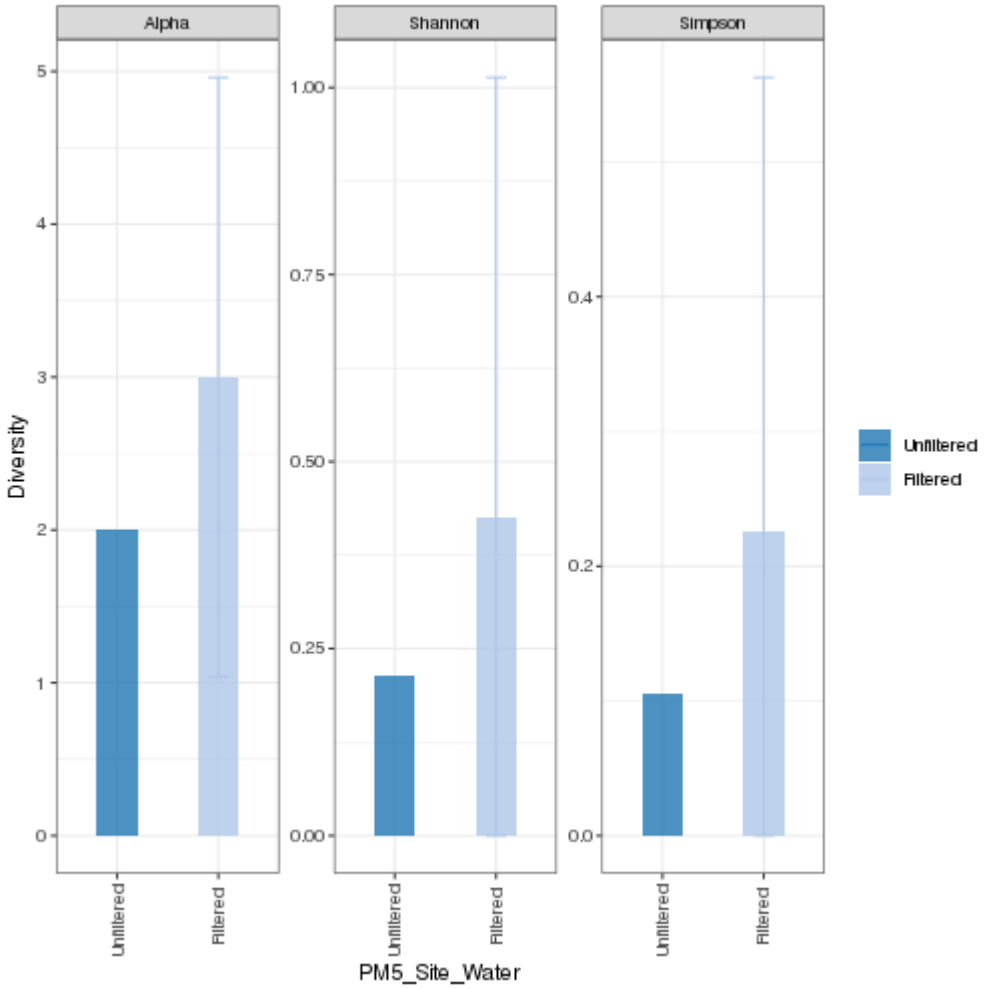

Figure 19: Diversity tests of enrichment cultures containing filtered/unfiltered PM5 site water at timepoint 7. Diversity measured by varying diversity indices: Alpha diversity (Chao1), Shannon index and Simpson index. Bars represent standard error. Sequences with no reads above 1% in any enrichment culture were removed. n= number of enrichment cultures, n for unfiltered=1, n for filtered=2.

Influence of beef extract, yeast extract and tryptone amendments

The final variation added to the enrichment cultures, was the addition of yeast and beef extracts, as well as tryptone. This was done, as previous was shown that these polypeptides are substrates for acidophiles, such as for *Cuniculiplasma* and *Ferroplasma* (Golyshina *et al.*, 2017; 2000). These were added in varying amounts, either making up 0.1%, or 0.02% of the enrichment culture, while in some was not present at all (at timepoint 7 variants that did not contain extracts were F3a, G5a and J4b).

Sequences affiliated with *Cuniculiplasma* consistently score a high abundance at each percentage variation, with the highest value occurring at 0.02% (abundance of 90%) (Fig.20). In the 0.1% variation, sequences matching the microorganisms *Thermoplasmataceae* (uncultured), and *Ferroplasma* , had a moderate to high abundance (11.7% and 63.6% abundance respectively), with lower values in other polypeptide variations. Of note, the bacterial sequences matching *Acidibacillus*, *Acidithiobacillus*, and *Acidothermus* appear to have low to no success in the 0.1% and 0% variations(under 1% abundance), while have moderate success in the 0.02% (above 1% abundance). Finally, sequences highly affiliated with ''*Ca.* Micrarchaeota'' appears to have the largest overall abundance from enrichment cultures containing 0.02% (2% abundance).

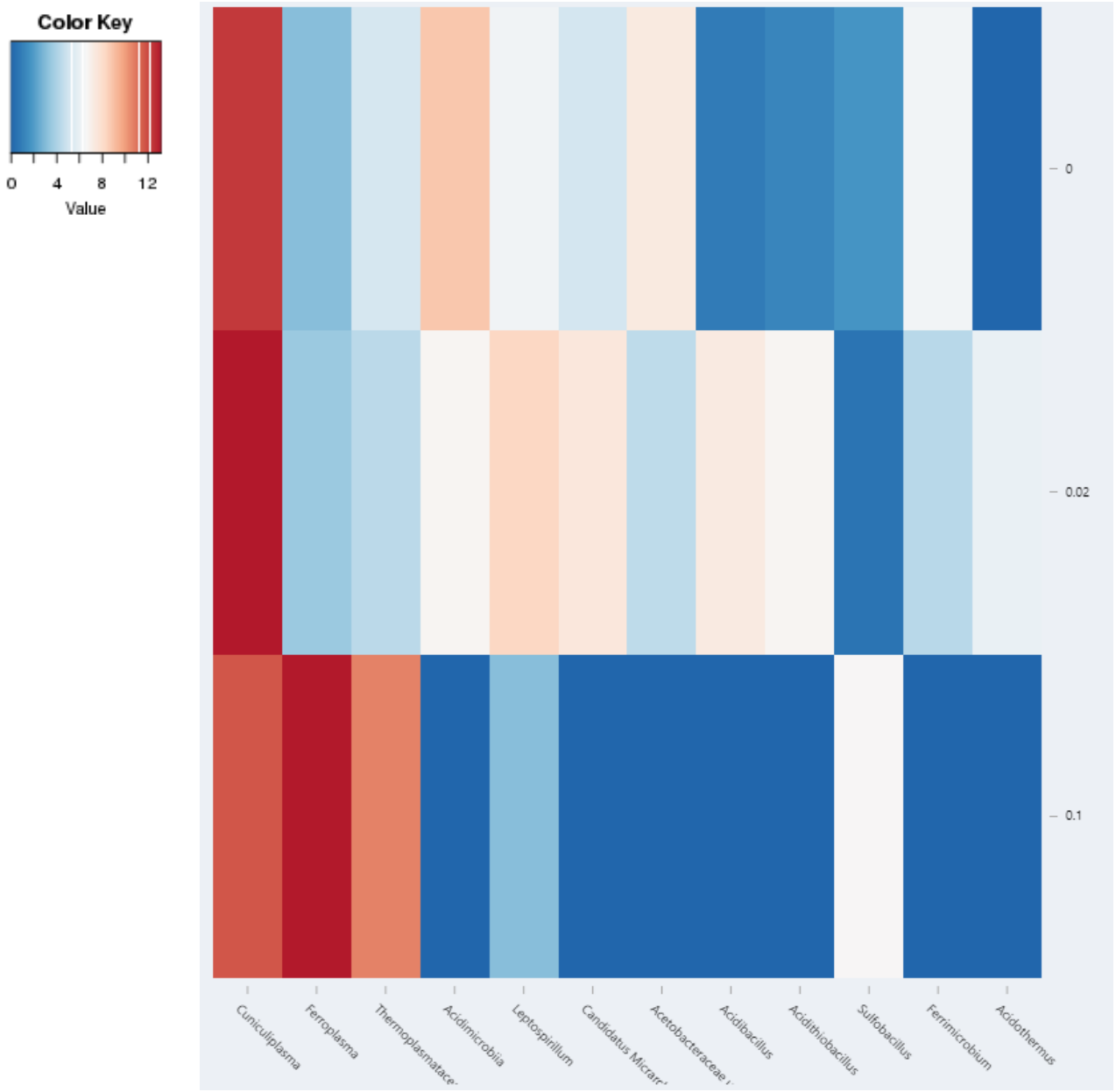

Figure 20: Heatmap of microorganisms from enrichment cultures with yeast extract, beef extract and tryptone added: Abundance calculated from reads taken from enrichment cultures. 0% represents no extracts added, 0.02% represents extracts added to make up 0.02% of the enrichment, 0.1% represents extracts added to make up 0.1% of the enrichment. Microorganisms named at Genus level, exception being *Acidimicrobiia*, *Thermoplasmataceae* and *Acetobacteraceae*. Sequences with no reads above 1% in any enrichment culture were removed. n= number of enrichment cultures, n for 0% =4, n for 0.02%= 14, n for 0.1%=4.

A diversity analysis of these extract variations (Fig.21) reveals that enrichment cultures with no extracts present had the highest diversity values across the three tests (Alpha: 6.0, Shannon: 0.557 and Simpson: 0.267). Looking at just alpha diversity, 0% extract presence has a value over 3 times higher than 0.1% extract presence which consistently scores the lowest diversity values across the three tests (Alpha: 1.75, Shannon: 0.0640 and Simpson: 0.0294).

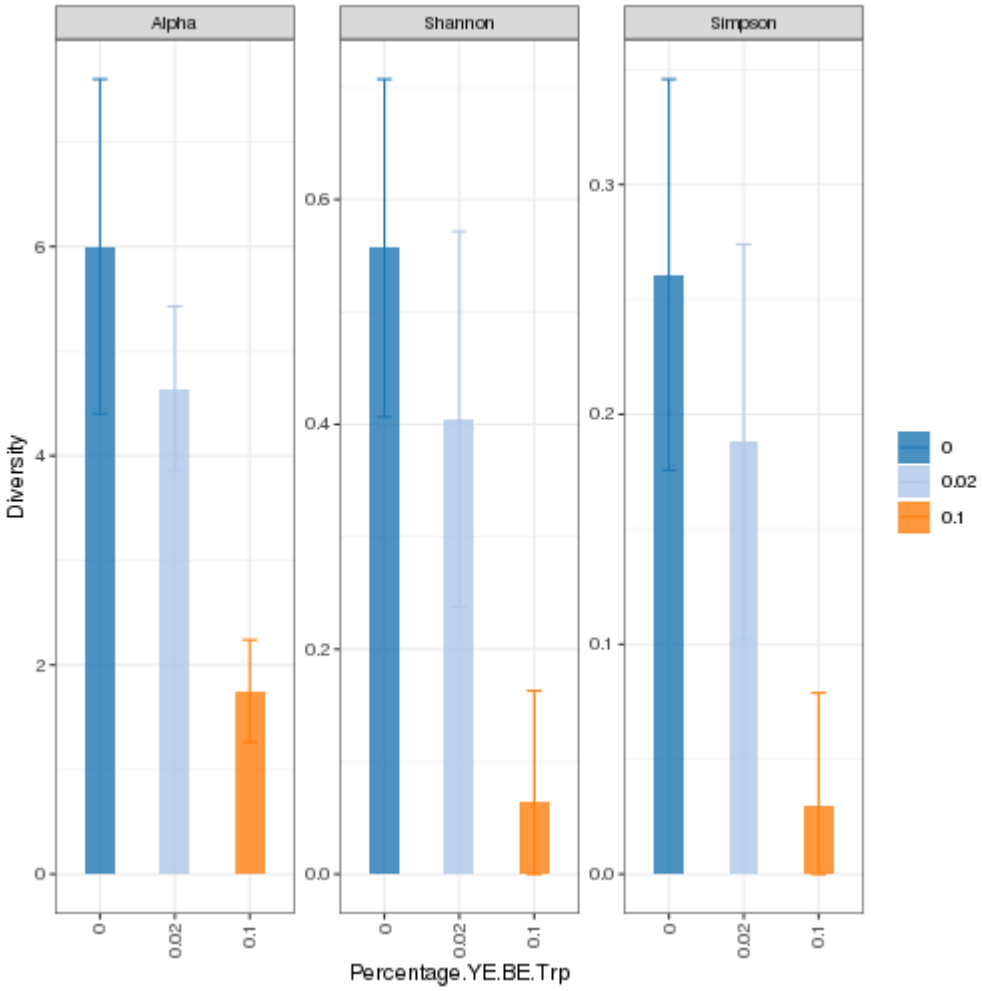

Figure 21: Diversity tests of Beef extract, yeast extract and Tryptone variations at timepoint 7. Diversity measured by varying diversity indices: Alpha diversity (Chao1), Shannon index and Simpson index. Bars represent standard error. Sequences with no reads above 1% in any enrichment culture were removed. n= number of enrichment cultures, n for 0% =4, n for 0.02%= 14, n for 0.1%=4.

Detection of ''*Ca.* Micrarchaeota'' in enrichment cultures

As stated, one of the goals of this research was to evaluate various enrichment techniques to improve growth of difficult to cultivate microorganisms such as ''*Ca.* Micrarchaeota''. Fig.22 shows a scatterplot of ''*Ca.* Micrarchaeota'' against *Cuniculiplasma*. In this scatterplot, it is shown that enrichment cultures that contain reads of ''*Ca.* Micrarchaeota'' also contain high reads of *Cuniculiplasma* with a Pearson correlation of 0.367.

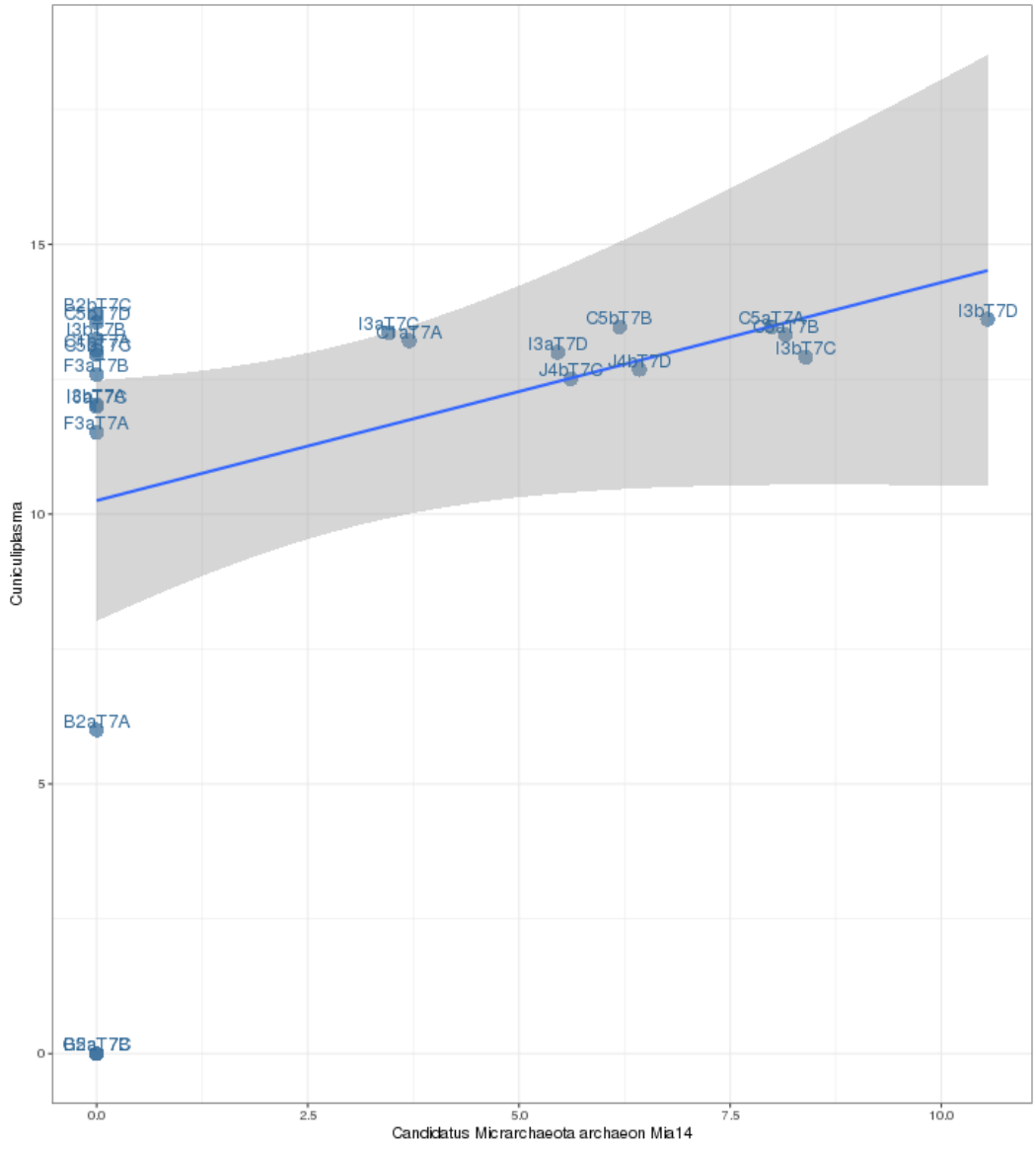

Figure 22: Scatterplot of ''*Ca.* Micrarchaeota Mia 14'' with *Cuniculiplasma*: Made using enrichment cultures that contain reads of both *Cuniculiplasma* and ''*Ca.* Micrarchaeota''. Data transformation: Log2+1. Correlation method: Pearson. Correlation= 0.367.

Fungal enrichment cultures

During the course of the study, it was visually observed that four enrichment cultures grew fungal microorganisms at each timepoint. These cultures contained sulfur making up 1% of the enrichment culture, and beef extract, yeast extract and tryptone making up 0.02% (w/v). Two enrichment cultures, (H2a T_A, H2A T_B) contained the medium PM5 filtered water (pH 2.2), while the other contained the AB medium (pH 2.2). Due to the presence of fungal growth, it was decided that in addition to 16S rRNA V4 primers being used, ITS primers would be used to conduct DNA barcoding analysis, at timepoints 3 and 6. Both these primers identified sequences affiliated to the fungus *Aspergillus* (99.20% identity) (Table 8, Appendices). Basic Local Alignment Search Tool (BLAST) (Altschul *et al.,* 1990) searches of these sequences show an affiliation to *Aspergillus hiratsukae* strain E9 and *Aspergillus tsunodae*. One other fungal sequence was detected (ITS_ASV13). BLAST searches of this sequence show affiliation to many uncultured fungal microorganisms, with the highest being with phylum *Mucoromycota* (93.33% identity), from an uncultured Glomeromycota clone 18S rRNA gene (accession number EF619906.1).

Anaerobic enrichment cultures

In addition to aerobic enrichment cultures, anaerobic cultures were set up and monitored over the course of six months. After two months of reinoculations, many of the anaerobic enrichment cultures were discarded due to lack of growth through observation. Due to the slow growth of anaerobic microorganisms only one DNA barcoding analysis took place (using V4 primers). Out of the anaerobic enrichment cultures that remained, only three produced a positive result from PCR/gel electrophoresis so only these underwent DNA barcoding analysis (Table 10, Appendices).

Examining archaea reads firstly (Table 11), two sequences were detected, affiliated with the order *Thermoplasmatales*, specifically with an identity 100% to *C. divulgatum* (PM4 strain) . Within the bacterial reads, sequences affiliated with the orders *Gammaproteobacteria incertae sedis* (99.22% identity), *Leptospirillales* (100% sequence identity) and *Acetobacterales* (96.88% identity) were detected. The largest abundance of bacterial sequences were affiliated to an uncultured *Proteobacteria* from the genus *Acidibacter*.

Table 11: Community composition from anaerobic enrichment cultures at phylum-level. DNA barcoding took place after 6 months of reinoculations. N= number of enrichment cultures, N=3. Sequences with no abundance above 1% in any enrichment culture were removed from table.

Phylum	Average relative abundance (%)
Thermoplasmatota	**75.14%**
Proteobacteria	**20.58%**
Nitrospirota	**2.98%**
Proteobacteria	**1.12%**

Discussion

The primary aim of this research project was to study the cultivated microbial diversity of the PM5 site, located in the Parys Mountain. In addition to this, the secondary aim of this project involved investigating cultivation techniques to improve growth of difficult to cultivate acidophiles. With these aims in mind, two hypotheses were made. The first, archaea belonging to the order *Thermoplasmatales* would be found present in this site. The second hypothesis was that the nano-sized archaea ''*Ca.* Micrarchaeota'' would be detected associated to the Order *Thermoplasmatales*.

Amplification of the 16S rRNA V4 region revealed the vast majority of enrichment cultures at timepoint 3 contained sequences identical with *C. divulgatum*, with it having the largest relative abundance in 60 out the 81 enrichment cultures at this timepoint (Fig. 5). Previous studies have stated this microorganism to be a heterotroph. As well as having the ability to grow at a pH range between pH 1-4 (Golyshina *et al.*, 2016). This would explain its success across the different variations, with the highest relative abundances occurring in enrichment cultures that contain; AB medium, sulfur and the addition of organic compounds (0.02% each).

In enrichment cultures, B2a_T3A, B2a_T3B, B2a_T3C, and B2a_T3D, sequences affiliated with the archaeal genus *Ferroplasma* were detected as the largest abundance microorganism, with other sequences detected registering at under 1% abundance (Fig.5). These sequences had 100% of identity to an isolated strain known as *F. acidiphilum* Strain Y^T (Golyshina *et al.*, 2000). As Table 1 shows, these enrichment cultures contain an AB medium with B2a_T3A and B2a_T3B having a pH of 1.0, while B2a_T3C, and B2a_T3D have a pH of 2.2. These enrichment cultures also contain the yeast extract, beef extract and tryptone (0.1% w/v) as well as sulfur (1%). Some of conditions match that of the characteristic growth conditions described in Golyshina *et al.* (2000), such as growth occurring close to the optimum growth temperature (35°C) and for two enrichment cultures (B2a_T3C, and B2a_T3D) pH being in the growth range (pH 1.3-2.2). Key differences between enrichment cultures of sequence detected, and *F. acidiphilum* Strain Y^T include growth at a lower pH (1.0) than the range suggested (pH 1.3-2.2), as well growth occurring in 0.1% presence yeast

extract whereas *F. acidiphilum* Strain Y^T optimal growth occurs at 0.02% yeast extract presence (Golyshina *et al.,* 2000).

Within enrichment cultures I3a_T3C and I3a_T3D sequences were detected with relative abundances of nearly 2% affiliated with microorganisms from the Order *Thermoplasmatales* known as ''E-plasma'' (Fig.5). This of note due to the fact in previous studies this microorganism has been detected in previous Parys Mountain sediment and water samples, ranging from 6% to around 45% abundance (Korzhenkov *et al.*, 2019; Distaso *et al.*, 2020). Despite the large potentially large abundances in Parys Mountain samples, successful *in vitro* cultivation had not occurred yet. The conditions of I3a_T3C and I3a_T3D in which affiliated sequences of ''E-plasma'' were detected include AB medium with a pH 2.2, the presence of sulfur, and yeast extract, beef extract and tryptone (0.02%). Unfortunately, sequencing at the later timepoint 7 of these enrichment cultures did not detect any sequences affiliated with ''E-plasma''.

Examining the bacterial sequences detected at timepoint 3, sequences matching *Sulfobacillus* were detected making up nearly 100% abundance in three enrichment cultures, G5a_T3B, G5a_T3C, and G5a_T3D (Fig.5). These contained AB medium (pH 2.2) (except from G5a_T3B which contained PM5 unfiltered site water) into which yeast extract, beef extract and tryptone were added to make up 0.1% of the enrichment culture. BLAST searches of these sequences determine the microorganism to be similar to *Sulfobacillus acidophilus* (16S rRNA gene sequence identity of 97.21%). It was shown, growth occurs at a similar pH of 2.0 as well the ability for autotrophic growth in the presence of ferrous iron or sulfur (Norris *et al.*, 1996). In addition, it was shown these microorganisms can utilise heterotrophic metabolism in the presence of yeast exact as well mixotrophic growth when ferrous iron is present. (Norris *et al.*, 1996).

Large abundance of *Cuniculiplasma*

A recurring theme within each enrichment variation, is the high abundance of sequences affiliated with *C. divulgatum* (Fig.9). Similar to timepoint 3, these sequences contain the largest abundances in the majority of enrichment cultures (19 out of the 27). This abundance of *Cuniculiplasma* is higher than the levels they are detected in Parys Mountain

sites. Previous studies detected *Cuniculiplasma* with a relative abundance of less than 5% in both sediment and water samples in the PM4 site and even less at the PM5 site (Korzhenkov *et al.*, 2019; Distaso *et al.*, 2020; 2022). The success of this microorganism in enrichment cultures is most likely due to the known physiology and conditions of cultivation for these archaea (Golyshina *et al.,* 2016).

AB and 9K media enrich *Ferroplasma* spp. and *Acidimicrobiia*

The overall relative abundance of microorganisms within AB medium variations, showed a substantial percentage belonging to sequences affiliated with *Ferroplasma* (Fig.10). This abundance is due to the detection of near pure *Ferroplasma* enrichment cultures in B2a_T7A (16025 out of 16113 reads detected belonged to *Ferroplasma*) and B2a_T7C (19798 out of 19810 reads detected belonged to *Ferroplasma*) (Fig.9), these sequences had a 100% identity match to *F. acidiphilum* strain Y^T (Golyshina *et al.,* 2000). Of particular note, *F. acidiphilum* strain Y^T was cultivated in a 9K medium which contained 25g $FeSO_4$, while the strain detected in this study had the largest abundance in a medium that did not contain $FeSO_4$ (Golyshina *et al.,* 2000).

In addition to *Ferroplasma*, a higher species richness is shown in AB medium when compared with 9K medium (11 microorganisms detected in AB medium and 6 detected in 9K) (Fig.9). AB medium having a larger quantity of microorganisms detected, could be due to the larger number of enrichment cultures containing this type of medium throughout the study, with a large pH range (with pH 2.2 being closest to the PM5 site water) while 9K media only used a pH of 1.6.

Looking at 9K media, overall abundance of bacterial sequences is higher, mainly due to 9% of the abundance made up of sequences matching with the class *Acidimicrobiia* (Fig.10). A substantial percentage (9.2%) of these sequences could not be identified beyond the class level, however, sequences making up a small relative abundance (2.8%) could be matched with the genus *Ferrimicrobium*. The class *Acidimicrobiia* has been detected in previous Parys Mountain studies, in both sediment and water samples (Korzhenkov *et al.*, 2019; Distaso *et al.*, 2020). It was also detected in the direct sequencing of PM5 samples with a similar abundance (Distaso *et al.*, 2022). The success of microorganisms belonging to the class

Acidimicrobiia may be due to the iron-oxidising ability of these organisms. A larger presence of iron sulfate ($FeSO_4$) was present in 9K compared with AB. Previous research has shown *Ferrimicrobium* has the ability to use iron oxidation to perform heterotrophic growth (Johnson *et al.*, 2009).

Effect of pH on enrichment culture composition

As shown in previous sections, four pH variations were used in this study (Table 1). These were pH 1.0, 1.6, 1.7 and 2.2 (all pH 1.6 enrichment cultures contained 9K media and *Cuniculiplasma* biomass, so variations at this pH may be due to these factors). The results show a low diversity of microorganisms at pH 1.0, scoring the lowest diversity values (Fig.13). The only two sequences with significant abundance were matched with *Cuniculiplasma* and *Ferroplasma* (Fig.12). Sequences matching ''*Ca.* Micrarchaeota Mia-14'', *Leptospirillum*, *Acidothermus* and the uncultured *Thermoplasmataceae* were also detected however these had a very low overall relative abundance at this pH. As the pH increases, the results indicate abundance and diversity of microorganisms also increases, with pH 2.2 containing the highest number of microorganisms detected (Fig.12). This pH most closely resembles the pH of the PM5 site, meaning it is most likely the optimum pH for most microorganisms in this community. This is especially true for microorganisms belonging to bacteria, as previous studies have shown lower pH values (less than pH 2) favour the growth of archaea, and in particular *Thermoplasmatales* (Chen *et al.*, 2016; Kuang *et al.*, 2013).

The results of this study appear to indicate the preferential pH range for growth of ''*Ca.* Micrarchaeota'' organism as pH 1.7-2.2 (Fig.12). This range would be slightly different to the range reported of the closely related ''*Ca.* Micrarchaeum harzensis'' (referred to as ''A_DKE'' in previous studies) which was shown to grow at a range of pH 2.0-2.5 (Krause *et al.,* 2022). ''*Ca.* Micrarchaeota'' organism was revealed to be associated under particular laboratory conditions with *Cuniculiplasma* sp., populating the PM5 site. In Parys Mountain environments such as the PM5 site where the pH is around 2.2 and *C. divulgatum* makes up a small abundance of the community, other microorganisms might be the preferred host for ''*Ca.* Micrarchaeota''. Enrichment cultures from this study do continue to support the association between these two microorganisms, with them having a positive correlation of 0.367 (Fig.22). This may mean ''*Ca.* Micrarchaeota'' has more than one potential host in

nature. ''*Ca.* Micrarchaeota'' having multiple hosts has been put forward in previous studies, as this would be advantageous over depending on one host species for vital organic compounds (Chen *et al.*, 2018; Jarett *et al.*, 2018). No suitable second host could be identified in this study, under checked laboratory conditions.

Elemental sulfur promotes ''*Ca.* Micrarchaeota''

Previous studies have shown the presence of elemental sulfur can have an effect on the growth on certain acidophiles (Kawano *et al.*, 2018). In addition, previous unpublished data has indicated the presence of elemental sulfur may have a positive effect on the growth of ''*Ca.* Micrarchaeota''. The results of this this study continue to support the proposition sulfur has a positive effect on the growth of ''*Ca.* Micrarchaeota'', with nearly all detection of sequences matching ''*Ca.* Micrarchaeota'' organisms occurring in enrichment cultures containing sulfur. One example is I3b_T7D (10% abundance), an enrichment culture containing sulfur (pH 2.2, AB medium and 0.02% yeast extract, beef extract and tryptone) (Fig.9). It is unknown what role sulfur is playing in the growth of Mia-14, recent research on ''*Ca.* Micrarchaeota ARM-1'' highlighted a lack of genes used in the assimilation of sulfur suggesting it relies on a host cell to gain metabolites (Sakai *et al.*, 2022). One proposition is that micrarchaeotal cells use the sulfur crystals for attachment, however, this requires further research.

Two sequences detected in the presence of sulfur were affiliated with *Ferroplasma*. Like the sequences detected in Timepoint 3, these sequences were highly affiliated with the *F. acidiphilum* Strain Y^T. This could suggest the sulfur in these enrichment cultures were not utilised by *Ferroplasma* as previous research has shown *F. acidiphilum* Strain Y^T does not use sulfur either in its elemental or reduced forms (Golyshina *et al.*, 2000). Other studies have shown however strains of *Ferroplasma* such as ''*Ferroplasma acidarmanus*'' 'fer 1' (which also had a 16S rRNA gene sequence identity of 100%) and '*Ferroplasma* like strains' detected in microbial fuel cells, do have the ability to metabolize sulfur compounds (Baumler *et al.*, 2005; Ni *et al.,* 2018). The ability for this strain of *Ferroplasma* detected to utilise sulfur should therefore not be ruled out.

Presence of Cuniculiplasma biomass in enrichment cultures

By timepoint 7, only 4 enrichment cultures remained that had *Cuniculiplasma* biomass added. Overall relative abundance of *Cuniculiplasma* is higher in the enrichment cultures containing the biomass than those absent of it (Fig.16). This higher abundance is most likely due to the biomass added. The addition of *Cuniculiplasma* biomass also did not appear to have any positive effect on the growth of ''*Ca.* Micrarchaeota'' organisms, and a greater overall abundance was detected in enrichment cultures absent of the biomass (Fig.16). This variation alone does not gain any insight of ''*Ca.* Micrarchaeota'', as overall more enrichment cultures were set-up that were absent of *Cuniculiplasma* biomass.

The addition of *Cuniculiplasma* biomass did have a positive effect on the abundance of sequences matching with the class *Acidimicrobiia* (including sequences matching with the genus *Ferrimicrobium*) and the family *Acetobacteraceae* (Fig.16). Within the biomass present, abundance of *Acidimicrobiia* was higher in enrichment cultures that contained 9K medium (Fig.9). As mentioned in the 'AB and 9K media' section, the higher abundance in 9K media is most likely is due to the larger presence of iron sulphate, as *Ferrimicrobium* has been described with the ability to oxidise/reduce ferrous iron compounds (Johnson *et al.,* 2009). While it was described as only able to do this in the presence of yeast extract, it may have gained the necessary organic compounds from the *Cuniculiplasma* biomass.

Detection of ''*Ca*. S. hospitalis'' in enrichment cultures containing unfiltered PM5 site water

Looking at the overall relative abundance of enrichment cultures containing filtered PM5 site water, a large majority is taken up by sequences matching with *Cuniculiplasma* (Fig.18). The next largest overall abundance present in this variation were sequences which matched with the bacterial species *Leptospirillum ferriphilum*. This microorganism has been consistently detected in acidic environments including the Parys Mountain PM4 site (Korzhenkov *et al.,* 2019; Distaso *et al.,* 2020).

In timepoint 7, sequences likely belonging to uncultured microorganisms were detected in large abundances (Fig.9). This is especially notable in timepoint 7 enrichment culture G5a_T7B, in which over 90% of the relative abundance is made up by these sequences. The

remaining abundance was taken up by sequences affiliated with the bacterial genus *Sulfobacillus* (Fig.9). This enrichment culture contains unfiltered PM5 site water into which yeast extract, beef extract and tryptone were added to make up 0.1% of the enrichment. BLAST searches of these sequences reveals matches to a number of different microorganisms, including uncultured *Acidimicrobiia,* an uncultured DPANN archaeon, and *Thermoplasmata ''Ca.* S. hospitalis''. It is most likely these sequences belong to the *Thermoplasmata* group ''*Ca*. S. hospitalis'' based on the BLAST search as well as these sequences position on the neighbour joining phylogenetic tree, in which its placed on the same branch as a ''*Ca*. S. hospitalis'' (''B_DKE'') reference strain (Fig.6). Sequences described as ''*Ca.* S. hospitalis'' have been previous detected in large abundances Parys Mountain sediment samples in the PM4 site, as well as being detected in surface water samples in a separate study (Korzhenkov *et al.,* 2019; Distaso *et al.,* 2020). In addition, sequences identified as being ''*Ca*. S. hospitalis'' were detected in enrichment cultures set-up using biofilms obtained from the Harz Mountains, Germany (a pyrite mine) (Krause *et al.,* 2017). These enrichment cultures were set-up under anoxic conditions, with one of the concluding points being ''*Ca*. S. hospitalis'' microorganisms likely lead an anaerobic lifestyle (Krause *et al.,* 2017). If the sequences detected in G5a_T7B are indeed ''*Ca*. S. hospitalis'' this would indicate the ability of aerobic growth for these microorganisms, with the possibility to enrich them under aerobic conditions. Additionally, it was proposed that ''*Ca*. S. hospitalis'' is a host for the closely related archaeal species *''Ca.* Micrarchaeum harzensis'' (Krause *et al.,* 2022). Enrichment cultures from this study found no evidence of ''Ca. S. hospitalis'' being associated with sequences affiliated with ''*Ca.* Micrarchaeota Mia-14''. This indicates it is unlikely ''*Ca.* S. hospitalis'' is a host organism for ''*Ca.* Micrarchaeota'' (''*Ca*. Mancarchaeum acidiphilum'').

Presence of beef extract, yeast extract and tryptone can decrease microbial diversity indices

The results indicate placing substrates at a presence of 0.1% (w/v) of the enrichment is detrimental to the growth of most acidophiles. This is shown through a low species richness, as well as 0.1% substate present enrichment cultures consistently having the lowest diversity values (Fig.21). The three archaeal sequences that have a high abundance at this variation are affiliated with *Cuniculiplasma*, *Ferroplasma* and ''*Ca*. S. hospitalis'' (Fig.20).

Success of *Ferroplasma* has been previously discussed in the timepoint 3 section. Most sequence matches were with *F. acidiphilum* Strain Y^T where ideal growth occurs at 0.02% yeast extract presence (Golyshina *et al.,* 2000), however no sequences matching with *Ferroplasma* were detected at this variation. Previous studies on *Cuniculiplasma* have found growth occurs at 0.05-0.5% (w/v) beef/yeast extract (with the strain PM4 also requiring the addition of 0.1% w/v tryptone) so its success in these enrichment cultures is further confirmation (Golyshina *et al.,* 2016). The success of sequences matching with ''*Ca*. S. hospitalis'' has similarities to previous studies, in which cultures that detected ''*Ca*. S. hospitalis'' also contained yeast extract at a presence of 0.1% (w/v) (Krause *et al.,* 2017). It is unknown whether beef extract and tryptone had any influence over the success of this organism, as the previously mentioned study added casein (0.1%) and ferric sulfate (20mM) instead (Krause *et al.,* 2017). The same study proposed ''*Ca*. S. hospitalis'' uses ferric iron as an electron acceptor in respiratory metabolism (Krause *et al.,* 2017). It is possible ''*Ca.* S. hospitalis'' was still able to obtain ferric iron in this study as the enrichment culture which contained the largest abundance of sequences matching ''*Ca.* S. hospitalis'' (G5a_T7B) contained unfiltered PM5 site water. AMD sites contain heavy metals such as iron compounds (Johnson, 2012). A chemical analysis of water samples taken from the PM5 site showed presence of sulfur and iron being on average 648.39 mg/l and 393.76 mg/l respectively (Distaso *et al.,* 2022).

Species richness and overall diversity levels were higher in enrichment culture variations that contained 0.02% polypeptides, as well as enrichment cultures that did not contain any added polypeptides (Fig.21). Looking at the overall abundance of sequences that matched with ''*Ca.* Micrarchaeota Mia-14'', it appears growth is best supported in enrichment cultures that contain 0.02% polypeptide presence (Fig.20). A previous study which was able to cultivate ''*Ca.* Micrarchaeota Mia 14'' alongside *C. divulgatum* contained these polypeptides at a higher concentration of 0.1% w/vol (Golyshina *et al*., 2017). While it was detected in enrichment cultures with no polypeptides added, these cultures contained added *Cuniculiplasma* biomass (J4b_T7C and J4b_T7D) .The detection of ''*Ca.* Micrarchaeota'' in these two variations may indicate an oligotrophic lifestyle via scavenging and growing on small amounts of organic matter.

Fungal component is represented by *Aspergillus* spp.

As shown in the results, four enrichment cultures H2aT7A, H2aT7B, H2aT7C and H2aT7B, were unique due the presence of eukaryotic cells. Sequencing using ITS primers revealed these enrichment cultures to be dominated by sequences affiliated with the fungus, *Aspergillus* (Table 9, Appendices). While strains of *Aspergillus* have not been documented from Parys Mountain samples before, this study confirmed its presence. A number of *Aspergillus* species have been documented to be able to survive in acidic conditions with one study in 2009, isolating a strain of *Aspergillus fumigatus* from an AMD site in Gangneung, South Korea (Park *et al.,* 2009). Additionally, the presence of mechanisms in *Aspergillus niger* to be able to adapt and process copper metals in cultures was revealed (Šimonovičová *et al.,* 2013). This may help explain why *Aspergillus* has been documented in AMD sites as its ability to process metals such as copper would be advantageous as copper ions can form a major part of these sites.

Detection of *Cuniculiplasma* and *Acidibacter* spp. in anaerobic enrichment cultures

Despite numerous enrichment cultures being set-up, only three successfully enriched microorganisms under anaerobic conditions. These were U1a_A, U5b_A, and U5b_B, the results of which are shown in Table 11. The microorganism *C. divulgatum* was detected with the largest abundance (and the only archaeal sequences detected in these enrichment cultures). This may have been detected due to the fact *Cuniculiplasma* biomass was added to these enrichment cultures however, this microorganism has been documented as being facultatively anaerobic so its presence in these cultures is further confirmation of its ability to grow under anaerobic conditions (Golyshina *et al.,* 2016). Despite previous studies finding *C. divulgatum* being associated with growth of ''*Ca.* Micrarchaeota Mia-14'' as well as this study showing a positive correlation between growth of these microorganisms (Fig.22), no sequences matching with ''*Ca.* Micrarchaeota Mia-14'' were detected any anaerobic enrichment culture. This would suggest that this microorganism prefers aerobic conditions, however the small number of successful anaerobic enrichment cultures in this study as well as the ability of the closely related ''*Ca.* Micrarchaeum harzensis'' to grow in anoxic conditions means the potential of anaerobic grow of ''*Ca*. Mancarchaeum acidiphilum'' shouldn't be completely ruled out (Golyshina *et al.,* 2019; Krause *et al*., 2022).

The next largest abundance microorganisms detected closely matched the bacterial genus *Acidibacter*. While detected in a number of AMD sites, it has not being detected in anaerobic cultures. The study which first described *Acidibacter* noted that growth did not occur under anaerobic conditions but may under micro-aerobic (Falagán and Johnson, 2014). This may indicate conditions in these enrichment cultures were not ideal for anaerobic growth, however since few studies have been published on *Acidibacter* further research should be done to examine growth conditions of this microorganism.

Limitations

One limitation of this study was the lack of positive PCR results from the anaerobic enrichment cultures. While a number of papers have described the ability for a number of acidophilic microorganisms to survive under anoxic conditions, having only 3 out of an initial 138 enrichment cultures produce successful PCR results gives limited insight in the potential effect anaerobic microorganisms may be having on the PM5 site and acidic environments more generally.

Another limitation of this study was the relatively short time period for culture growth. Successful cultivation of microorganisms such as ''*Ca.* Micrarchaeota'' may take years due to slow growth. This means that potential microorganisms present in these enrichment cultures could be undetectable at timepoint 7 but given more time could become more prevalent.

Future research

One possible area to research further would be the anaerobic potential of the PM5 site. Further research in *Acidibacter* is needed to confirm its anaerobic potential. Anaerobic enrichment cultures set up and monitored over longer time periods may offer further insights into the PM5 site. Additionally, this project has shown the first success in cultivating ''E-plasma'', further success in cultivating them is needed in understanding their potential effect on AMD sites as they have been detected in large abundances in other Parys Mountain sites (Korzhenkov *et al*., 2019; Distaso *et al.,* 2020).

Conclusion

Through the set-up and monitoring of various aerobic and anaerobic enrichment cultures using sediment (and water samples) collected from the PM5 site, this study has been able to investigate the cultivable fraction of microbial diversity of this acidic site as well as techniques in enriching various unique microorganisms.

Cultures of *Ferroplasma* and *Cuniculiplasma* were successfully cultivated. Additionally, ''*Ca*. S. hospitalis'' cultures were cultivated using unfiltered PM5 site water, demonstrating the aerobic potential of these microorganisms previously thought to only grow in anoxic conditions. Another insight of this study has been the first documented detection of the archaeal sequence ''E-plasma'' in enrichment cultures (despite having a notable abundance in Parys Mountain environments), representing an important step in cultivation of this microorganism.

Additionally, this study has gained further insight in the potential behaviour of ''*Ca.* Micrarchaeota''/ ''*Ca*. Mancarchaeum acidiphilum'', Mia-14 related organism. Results show further correlation between its growth and *C. divulgatum*. Additionally, no detection of ''*Ca.* Micrarchaeota Mia-14'' related organism in anaerobic enrichment cultures despite the presence of sequences belonging to *C. divulgatum* suggests an aerobic lifestyle for this microorganism (although few anaerobic cultures were successfully enriched, warranting further study). The presence of sulfur also seems to have a positive effect on the growth of this archaeon*,* however what role it plays is currently unknown.

Finally, other sequences affiliated to various microorganisms were also detected in this study. In particular large relative abundances matching with previously uncultured organisms of the class *Acidimicrobiia* and family *Acetobacteraceae* were detected in enrichment cultures containing 9K medium and *Cuniculiplasma* biomass. Use of ITS primers also detected sequences matching with the fungal genus *Aspergillus,* this is the first time this microorganism has been detected in Parys Mountain samples.

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Appendices

Table 2: Full list of aerobic enrichment cultures set-up at timepoint 1. First enrichment cultures to be inoculated. YE, BE, and Trp represent Yeast extract, Beef extract and Tryptone. Sample 1, 2 and 3 represent area in PM5 site from which sample was collected, these samples were collected in triplicate represented here as .1 .2 and .3.

ID	Sample	Medium	pH	Volume (ml)	Temp (°C)	Substrate 1	Substrate 2
A1a	PM5 1.1	AB	1	10	37	YE,BE,Trp 0.1%	
A1b	PM5 1.1	AB	1	10	37	YE,BE,Trp 0.1%	
A2a	PM5 2.1	AB	1	10	37	YE,BE,Trp 0.1%	
A2b	PM5 2.1	AB	1	10	37	YE,BE,Trp 0.1%	
A3a	PM5 3.1	AB	1	10	37	YE,BE,Trp 0.1%	
A3b	PM5 3.1	AB	1	10	37	YE,BE,Trp 0.1%	
A4a	PM5 1.1	AB	1.7	10	37	YE,BE,Trp 0.1%	
A4b	PM5 1.1	AB	1.7	10	37	YE,BE,Trp 0.1%	
A5a	PM5 2.1	AB	1.7	10	37	YE,BE,Trp 0.1%	
A5b	PM5 2.1	AB	1.7	10	37	YE,BE,Trp 0.1%	
A6a	PM5 3.1	AB	1.7	10	37	YE,BE,Trp 0.1%	
A6b	PM5 3.1	AB	1.7	10	37	YE,BE,Trp 0.1%	
B1a	PM5 1.1	AB	1	10	37	YE,BE,Trp 0.1%	Sulfur 1%
B1b	PM5 1.1	AB	1	10	37	YE,BE,Trp 0.1%	Sulfur 1%
B2a	PM5 2.1	AB	1	10	37	YE,BE,Trp 0.1%	Sulfur 1%
B2b	PM5 2.1	AB	1	10	37	YE,BE,Trp 0.1%	Sulfur 1%
B3a	PM5 3.1	AB	1	10	37	YE,BE,Trp 0.1%	Sulfur 1%
B3b	PM5 3.1	AB	1	10	37	YE,BE,Trp 0.1%	Sulfur 1%
B4a	PM5 1.1	AB	1.7	10	37	YE,BE,Trp 0.1%	Sulfur 1%
B4b	PM5 1.1	AB	1.7	10	37	YE,BE,Trp 0.1%	Sulfur 1%
B5a	PM5 2.1	AB	1.7	10	37	YE,BE,Trp 0.1%	Sulfur 1%
B5b	PM5 2.1	AB	1.7	10	37	YE,BE,Trp 0.1%	Sulfur 1%
B6a	PM5 3.1	AB	1.7	10	37	YE,BE,Trp 0.1%	Sulfur 1%
B6b	PM5 3.1	AB	1.7	10	37	YE,BE,Trp 0.1%	Sulfur 1%
C1a	PM5 1.1	AB	1	10	37	YE,BE,Trp 0.02%	Sulfur 1%
C1b	PM5 1.1	AB	1	10	37	YE,BE,Trp 0.02%	Sulfur 1%
C2a	PM5 2.1	AB	1	10	37	YE,BE,Trp 0.02%	Sulfur 1%
C2b	PM5 2.1	AB	1	10	37	YE,BE,Trp 0.02%	Sulfur 1%
C3a	PM5 3.1	AB	1	10	37	YE,BE,Trp 0.02%	Sulfur 1%
C3b	PM5 3.1	AB	1	10	37	YE,BE,Trp 0.02%	Sulfur 1%
C4a	PM5 1.1	AB	1.7	10	37	YE,BE,Trp 0.02%	Sulfur 1%
C4b	PM5 1.1	AB	1.7	10	37	YE,BE,Trp 0.02%	Sulfur 1%
C5a	PM5 2.1	AB	1.7	10	37	YE,BE,Trp 0.02%	Sulfur 1%
C5b	PM5 2.1	AB	1.7	10	37	YE,BE,Trp 0.02%	Sulfur 1%
C6a	PM5 3.1	AB	1.7	10	37	YE,BE,Trp 0.02%	Sulfur 1%
C6b	PM5 3.1	AB	1.7	10	37	YE,BE,Trp 0.02%	Sulfur 1%

D1a	PM5 1.1	AB	1	10	37	*Cuniculiplasma* biomass	
D1b	PM5 1.1	AB	1	10	37	*Cuniculiplasma* biomass	
D2a	PM5 2.1	AB	1	10	37	*Cuniculiplasma* biomass	
D2b	PM5 2.1	AB	1	10	37	*Cuniculiplasma* biomass	
D3a	PM5 3.1	AB	1	10	37	*Cuniculiplasma* biomass	
D3b	PM5 3.1	AB	1	10	37	*Cuniculiplasma* biomass	
D4a	PM5 1.1	AB	1.7	10	37	*Cuniculiplasma* biomass	
D4b	PM5 1.1	AB	1.7	10	37	*Cuniculiplasma* biomass	
D5a	PM5 2.2	AB	1.7	10	37	*Cuniculiplasma* biomass	
D5b	PM5 2.2	AB	1.7	10	37	*Cuniculiplasma* biomass	
D6a	PM5 3.1	AB	1.7	10	37	*Cuniculiplasma* biomass	
D6b	PM5 3.1	AB	1.7	10	37	*Cuniculiplasma* biomass	
E1a	PM5 1.1	9K	1.6	10	37	YE,BE,Trp 0.02%	
E1b	PM5 1.1	9K	1.6	10	37	YE,BE,Trp 0.02%	
E2a	PM5 2.2	9K	1.6	10	37	YE,BE,Trp 0.02%	
E2b	PM5 2.2	9K	1.6	10	37	YE,BE,Trp 0.02%	
E3a	PM5 3.1	9K	1.6	10	37	YE,BE,Trp 0.02%	
E3b	PM5 3.1	9K	1.6	10	37	YE,BE,Trp 0.02%	
F1a	PM5 1.1	9K	1.6	10	37	*Cuniculiplasma* biomass	
F1b	PM5 1.1	9K	1.6	10	37	*Cuniculiplasma* biomass	
F2a	PM5 2.2	9K	1.6	10	37	*Cuniculiplasma* biomass	
F2b	PM5 2.2	9K	1.6	10	37	*Cuniculiplasma* biomass	
F3a	PM5 3.1	9K	1.6	10	37	*Cuniculiplasma* biomass	
F3b	PM5 3.1	9K	1.6	10	37	*Cuniculiplasma* biomass	
G1a	PM5 1.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	
G1b	PM5 1.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	
G2a	PM5 2.2	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	
G2b	PM5 2.2	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	

G3a	PM5 3.2	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	
G3b	PM5 3.2	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	
G4a	PM5 1.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	
G4b	PM5 1.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	
G5a	PM5 2.2	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	
G5b	PM5 2.2	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	
G6a	PM5 3.2	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	
G6b	PM5 3.2	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	
H1a	PM5 1.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
H1b	PM5 1.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
H2a	PM5 2.2	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
H2b	PM5 2.2	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
H3a	PM5 3.2	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
H3b	PM5 3.2	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
H4a	PM5 1.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
H4b	PM5 1.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
H5a	PM5 2.2	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
H5b	PM5 2.2	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
H6a	PM5 3.2	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
H6b	PM5 3.2	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
I1a	PM5 1.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
I1b	PM5 1.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
I2a	PM5 2.2	PM water filtered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
I2b	PM5 2.2	PM water filtered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
I3a	PM5 3.2	PM water filtered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%

I3b	PM5 3.2	PM water filtered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
I4a	PM5 1.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
I4b	PM5 1.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
I5a	PM5 2.2	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
I5b	PM5 2.2	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
I6a	PM5 3.2	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
I6b	PM5 3.2	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
J1a	PM5 1.1	PM water filtered	2.2	10	37	*Cuniculiplasma* biomass	
J1b	PM5 1.1	PM water filtered	2.2	10	37	*Cuniculiplasma* biomass	
J2a	PM5 2.2	PM water filtered	2.2	10	37	*Cuniculiplasma* biomass	
J2b	PM5 2.2	PM water filtered	2.2	10	37	*Cuniculiplasma* biomass	
J3a	PM5 3.2	PM water filtered	2.2	10	37	*Cuniculiplasma* biomass	
J3b	PM5 3.2	PM water filtered	2.2	10	37	*Cuniculiplasma* biomass	
J4a	PM5 1.1	PM water unfiltered	2.2	10	37	*Cuniculiplasma* biomass	
J4b	PM5 1.1	PM water unfiltered	2.2	10	37	*Cuniculiplasma* biomass	
J5a	PM5 2.2	PM water unfiltered	2.2	10	37	*Cuniculiplasma* biomass	
J5b	PM5 2.2	PM water unfiltered	2.2	10	37	*Cuniculiplasma* biomass	
J6a	PM5 3.2	PM water unfiltered	2.2	10	37	*Cuniculiplasma* biomass	
J6b	PM5 3.2	PM water unfiltered	2.2	10	37	*Cuniculiplasma* biomass	

Table 4: Full list of anaerobic enrichment cultures set-up at timepoint 1. First enrichment cultures to be inoculated. YE, BE, and Trp represent Yeast extract, Beef extract and Tryptone.Fe2(SO4)3 represents Iron (III) sulphate (also known as ferric sulphate). Sample 1, 2 and 3 represent area in PM5 site from which sample was collected, these samples were collected in triplicate represented here as .1 .2 and .3.

ID	Sample	Medium	pH	Volume (ml)	Temp (°C)	Substrate 1	Substrate 2
K1a	PM5 1.1	AB	1	10	37	YE,BE,Trp 0.1%	
K1b	PM5 1.1	AB	1	10	37	YE,BE,Trp 0.1%	
K2a	PM5 2.1	AB	1	10	37	YE,BE,Trp 0.1%	
K2b	PM5 2.1	AB	1	10	37	YE,BE,Trp 0.1%	
K3a	PM5 3.1	AB	1	10	37	YE,BE,Trp 0.1%	
K3b	PM5 3.1	AB	1	10	37	YE,BE,Trp 0.1%	
K4a	PM5 1.1	AB	1.7	10	37	YE,BE,Trp 0.1%	
K4b	PM5 1.1	AB	1.7	10	37	YE,BE,Trp 0.1%	
K5a	PM5 2.1	AB	1.7	10	37	YE,BE,Trp 0.1%	
K5b	PM5 2.1	AB	1.7	10	37	YE,BE,Trp 0.1%	
K6a	PM5 3.1	AB	1.7	10	37	YE,BE,Trp 0.1%	
K6b	PM5 3.1	AB	1.7	10	37	YE,BE,Trp 0.1%	
L1a	PM5 1.1	AB	1	10	37	YE,BE,Trp 0.1%	Sulfur 1%
L1b	PM5 1.1	AB	1	10	37	YE,BE,Trp 0.1%	Sulfur 1%
L2a	PM5 2.1	AB	1	10	37	YE,BE,Trp 0.1%	Sulfur 1%
L2b	PM5 2.1	AB	1	10	37	YE,BE,Trp 0.1%	Sulfur 1%
L3a	PM5 3.1	AB	1	10	37	YE,BE,Trp 0.1%	Sulfur 1%
L3b	PM5 3.1	AB	1	10	37	YE,BE,Trp 0.1%	Sulfur 1%
L4a	PM5 1.1	AB	1.7	10	37	YE,BE,Trp 0.1%	Sulfur 1%
L4b	PM5 1.1	AB	1.7	10	37	YE,BE,Trp 0.1%	Sulfur 1%
L5a	PM5 2.1	AB	1.7	10	37	YE,BE,Trp 0.1%	Sulfur 1%
L5b	PM5 2.1	AB	1.7	10	37	YE,BE,Trp 0.1%	Sulfur 1%

L6a	PM5 3.1	AB	1.7	10	37	YE,BE,Trp 0.1%	Sulfur 1%
L6b	PM5 3.1	AB	1.7	10	37	YE,BE,Trp 0.1%	Sulfur 1%
M1a	PM5 1.1	AB	1	10	37	YE,BE,Trp 0.02%	Sulfur 1%
M1b	PM5 1.1	AB	1	10	37	YE,BE,Trp 0.02%	Sulfur 1%
M2a	PM5 2.1	AB	1	10	37	YE,BE,Trp 0.02%	Sulfur 1%
M2b	PM5 2.1	AB	1	10	37	YE,BE,Trp 0.02%	Sulfur 1%
M3a	PM5 3.1	AB	1	10	37	YE,BE,Trp 0.02%	Sulfur 1%
M3b	PM5 3.1	AB	1	10	37	YE,BE,Trp 0.02%	Sulfur 1%
M4a	PM5 1.1	AB	1.7	10	37	YE,BE,Trp 0.02%	Sulfur 1%
M4b	PM5 1.1	AB	1.7	10	37	YE,BE,Trp 0.02%	Sulfur 1%
M5a	PM5 2.1	AB	1.7	10	37	YE,BE,Trp 0.02%	Sulfur 1%
M5b	PM5 2.1	AB	1.7	10	37	YE,BE,Trp 0.02%	Sulfur 1%
M6a	PM5 3.1	AB	1.7	10	37	YE,BE,Trp 0.02%	Sulfur 1%
M6b	PM5 3.1	AB	1.7	10	37	YE,BE,Trp 0.02%	Sulfur 1%
N1a	PM5 1.1	AB	1	10	37	*Cuniculiplasma* biomass	
N1b	PM5 1.1	AB	1	10	37	*Cuniculiplasma* biomass	
N2a	PM5 2.1	AB	1	10	37	*Cuniculiplasma* biomass	
N2b	PM5 2.1	AB	1	10	37	*Cuniculiplasma* biomass	
N3a	PM5 3.1	AB	1	10	37	*Cuniculiplasma* biomass	
N3b	PM5 3.1	AB	1	10	37	*Cuniculiplasma* biomass	
N4a	PM5 1.1	AB	1.7	10	37	*Cuniculiplasma* biomass	
N4b	PM5 1.1	AB	1.7	10	37	*Cuniculiplasma* biomass	
N5a	PM5 2.1	AB	1.7	10	37	*Cuniculiplasma* biomass	
N5b	PM5 2.1	AB	1.7	10	37	*Cuniculiplasma* biomass	
N6a	PM5 3.1	AB	1.7	10	37	*Cuniculiplasma* biomass	

N6b	PM5 3.1	AB	1.7	10	37	*Cuniculiplasma* biomass	
O1a	PM5 1.1	AB	1	10	37	YE,BE,Trp 0.1%	20mM Fe2(SO4)3
O1b	PM5 1.1	AB	1	10	37	YE,BE,Trp 0.1%	20mM Fe2(SO4)3
O2a	PM5 2.1	AB	1	10	37	YE,BE,Trp 0.1%	20mM Fe2(SO4)3
O2b	PM5 2.1	AB	1	10	37	YE,BE,Trp 0.1%	20mM Fe2(SO4)3
O3a	PM5 3.1	AB	1	10	37	YE,BE,Trp 0.1%	20mM Fe2(SO4)3
O3b	PM5 3.1	AB	1	10	37	YE,BE,Trp 0.1%	20mM Fe2(SO4)3
O4a	PM5 1.1	AB	1.7	10	37	YE,BE,Trp 0.1%	20mM Fe2(SO4)3
O4b	PM5 1.1	AB	1.7	10	37	YE,BE,Trp 0.1%	20mM Fe2(SO4)3
O5a	PM5 2.1	AB	1.7	10	37	YE,BE,Trp 0.1%	20mM Fe2(SO4)3
O5b	PM5 2.1	AB	1.7	10	37	YE,BE,Trp 0.1%	20mM Fe2(SO4)3
O6a	PM5 3.1	AB	1.7	10	37	YE,BE,Trp 0.1%	20mM Fe2(SO4)3
O6b	PM5 3.1	AB	1.7	10	37	YE,BE,Trp 0.1%	20mM Fe2(SO4)3
P1a	PM5 1.1	AB	1	10	37	YE,BE,Trp 0.02%	20mM Fe2(SO4)3
P1b	PM5 1.1	AB	1	10	37	YE,BE,Trp 0.02%	20mM Fe2(SO4)3
P2a	PM5 2.1	AB	1	10	37	YE,BE,Trp 0.02%	20mM Fe2(SO4)3
P2b	PM5 2.1	AB	1	10	37	YE,BE,Trp 0.02%	20mM Fe2(SO4)3
P3a	PM5 3.1	AB	1	10	37	YE,BE,Trp 0.02%	20mM Fe2(SO4)3
P3b	PM5 3.1	AB	1	10	37	YE,BE,Trp 0.02%	20mM Fe2(SO4)3
P4a	PM5 1.1	AB	1.7	10	37	YE,BE,Trp 0.02%	20mM Fe2(SO4)3
P4b	PM5 1.1	AB	1.7	10	37	YE,BE,Trp 0.02%	20mM Fe2(SO4)3
P5a	PM5 2.1	AB	1.7	10	37	YE,BE,Trp 0.02%	20mM Fe2(SO4)3
P5b	PM5 2.1	AB	1.7	10	37	YE,BE,Trp 0.02%	20mM Fe2(SO4)3
P6a	PM5 3.1	AB	1.7	10	37	YE,BE,Trp 0.02%	20mM Fe2(SO4)3
P6b	PM5 3.1	AB	1.7	10	37	YE,BE,Trp 0.02%	20mM Fe2(SO4)3

Q1a	PM5 1.1	AB	1	10	37	*Cuniculiplasma* biomass	20mM Fe2(SO4)3
Q1b	PM5 1.1	AB	1	10	37	*Cuniculiplasma* biomass	20mM Fe2(SO4)3
Q2a	PM5 2.1	AB	1	10	37	*Cuniculiplasma* biomass	20mM Fe2(SO4)3
Q2b	PM5 2.1	AB	1	10	37	*Cuniculiplasma* biomass	20mM Fe2(SO4)3
Q3a	PM5 3.1	AB	1	10	37	*Cuniculiplasma* biomass	20mM Fe2(SO4)3
Q3b	PM5 3.1	AB	1	10	37	*Cuniculiplasma* biomass	20mM Fe2(SO4)3
Q4a	PM5 1.1	AB	1.7	10	37	*Cuniculiplasma* biomass	20mM Fe2(SO4)3
Q4b	PM5 1.1	AB	1.7	10	37	*Cuniculiplasma* biomass	20mM Fe2(SO4)3
Q5a	PM5 2.1	AB	1.7	10	37	*Cuniculiplasma* biomass	20mM Fe2(SO4)3
Q5b	PM5 2.1	AB	1.7	10	37	*Cuniculiplasma* biomass	20mM Fe2(SO4)3
Q6a	PM5 3.1	AB	1.7	10	37	*Cuniculiplasma* biomass	20mM Fe2(SO4)3
Q6b	PM5 3.1	AB	1.7	10	37	*Cuniculiplasma* biomass	20mM Fe2(SO4)3
R1a	PM5 1.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	
R1b	PM5 1.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	
R2a	PM5 2.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	
R2b	PM5 2.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	
R3a	PM5 3.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	
R3b	PM5 3.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	
R4a	PM5 1.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	
R4b	PM5 1.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	
R5a	PM5 2.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	
R5b	PM5 2.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	
R6a	PM5 3.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	
R6b	PM5 3.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	
S1a	PM5 1.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%

S1b	PM5 1.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
S2a	PM5 2.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
S2b	PM5 2.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
S3a	PM5 3.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
S3b	PM5 3.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
S4a	PM5 1.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
S4b	PM5 1.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
S5a	PM5 2.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
S5b	PM5 2.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
S6a	PM5 3.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
S6b	PM5 3.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.1%	Sulfur 1%
T1a	PM5 1.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
T1b	PM5 1.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
T2a	PM5 2.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
T2b	PM5 2.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
T3a	PM5 3.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
T3b	PM5 3.1	PM water filtered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
T4a	PM5 1.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
T4b	PM5 1.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
T5a	PM5 2.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
T5b	PM5 2.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
T6a	PM5 3.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
T6b	PM5 3.1	PM water unfiltered	2.2	10	37	YE,BE,Trp 0.02%	Sulfur 1%
U1a	PM5 1.1	PM water filtered	2.2	10	37	*Cuniculiplasma* biomass	
U1b	PM5 1.1	PM water filtered	2.2	10	37	*Cuniculiplasma* biomass	

U2a	PM5 2.1	PM water filtered	2.2	10	37	*Cuniculiplasma* biomass	
U2b	PM5 2.1	PM water filtered	2.2	10	37	*Cuniculiplasma* biomass	
U3a	PM5 3.1	PM water filtered	2.2	10	37	*Cuniculiplasma* biomass	
U3b	PM5 3.1	PM water filtered	2.2	10	37	*Cuniculiplasma* biomass	
U4a	PM5 1.1	PM water unfiltered	2.2	10	37	*Cuniculiplasma* biomass	
U4b	PM5 1.1	PM water unfiltered	2.2	10	37	*Cuniculiplasma* biomass	
U5a	PM5 2.1	PM water unfiltered	2.2	10	37	*Cuniculiplasma* biomass	
U5b	PM5 2.1	PM water unfiltered	2.2	10	37	*Cuniculiplasma* biomass	
U6a	PM5 3.1	PM water unfiltered	2.2	10	37	*Cuniculiplasma* biomass	
U6b	PM5 3.1	PM water unfiltered	2.2	10	37	*Cuniculiplasma* biomass	

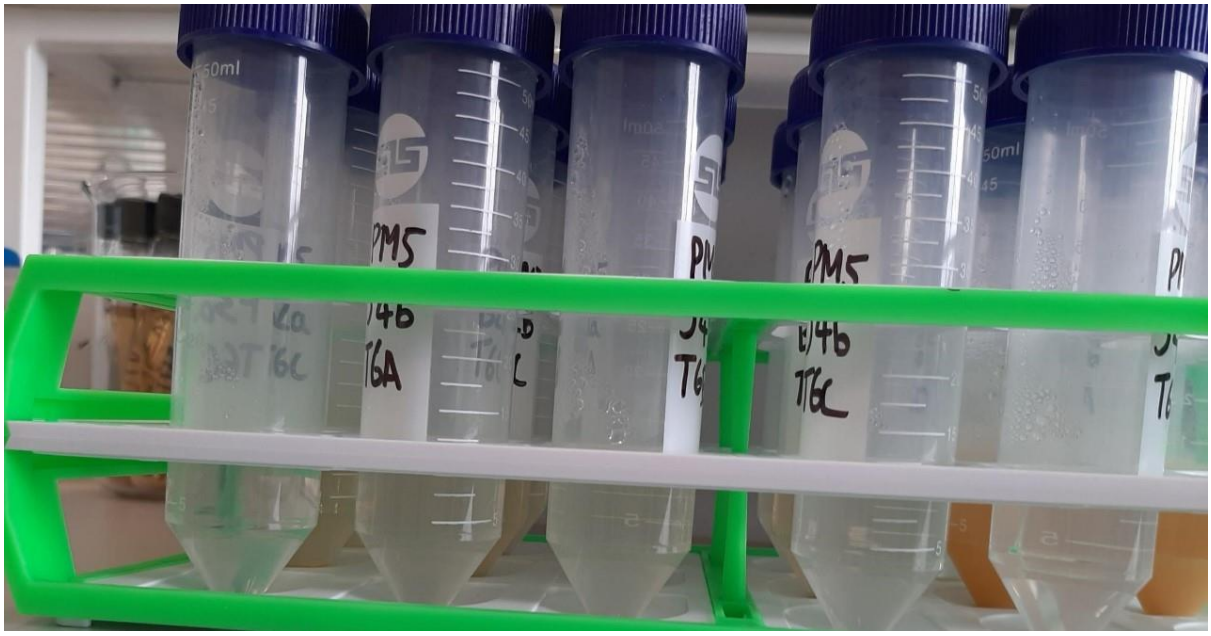

Figure 3: Photo of aerobic enrichment cultures. Pictured at timepoint 6. Made up in 50ml Greiner tube. Cultivated at 37°C for around 30 days (Kept static/no shaking). Photo taken by Dr Olga Golyshina.

Figure 4: Photo of anaerobic enrichment cultures. Pictured at timepoint 2. Set-up in Pyrex culture tubes. Cultivated at 37°C for around 45-60 days (Kept static/no shaking). Photo taken by Dr Olga Golyshina.

Table 6. Reference strains added to phylogenetic trees. All strains sourced from the NCBI database. 16S rRNA region used in phylogenetic tree.

Reference strain	**Paper**
MK578782.1 ''*Ca.* Micrarchaeota archaeon clone KY1-27M''	Golyshina *et al* 2019
NR_144620.1 *Cuniculiplasma divulgatum* strain S5	Golyshina *et al* 2016
NR_028183.1 *Ferroplasma acidiphilum* strain Y	Golyshina *et al* 2000
NR_074218.1 *Haloferax volcanii* DS2	Hartman *et al* 2010
NC_002578.1:c1475770-1474299 *Thermoplasma acidophilum*	Ree *et al* 1987
MK578808.1 ''*Ca.* Micrarchaeota archaeon clone''	Golyshina *et al* 2019
MK578793.1 ''*Ca.* Micrarchaeota archaeon clone''	Golyshina *et al* 2019
KY825129.1 Uncultured Thermoplasmatales archaeon clone B_DKE	Krause *et al* 2017
CP019964.1:c753304-752255,c751732-751297 ''*Ca.* Mancarchaeum acidiphilum strain Mia14''	Golyshina *et al* 2017
AB269873.1 *Thermogymnomonas* acidicola gene	Itoh, Yoshikawa, and Takashina 2007
NR_114693.1 *Acidothermus cellulolyticus* strain 11B	Rainey and Stackebrandt 1993
NR_114437.1 *Sulfobacillus acidophilus* DSM 10332 strain NAL	Norris *et al* 1996
NR_041798.1 *Ferrimicrobium acidiphilum* strain T23	Johnson *et al* 2009
NR_028818.1 *Leptospirillum ferriphilum* strain P3a	Coram and Rawlings 2002
KP860945.1 *Acidibacillus sulfuroxidans* strain Y002	Holanda *et al* 2016
NR_147744.1 *Acidithiobacillus ferriphilus* strain M20	Falagan and Johnson 2016

Table 7: ASV sequences detected in aerobic enrichment cultures at Timepoint 3. Detected through the use of 16S rRNA V4 primers. Sequences were analysed using QIIME. The libraries were demultiplexed using the different barcodes from the sequences after which SILVA version 138 database was used to classify the reads based on Operational Taxonomic Units (OTUs).

ASV sequence	ASV lineage	ASV ID
TTCTAATCCGATTCGTTCTCCTAGCCTTCGCTCCTCACCGTCGGATGCGTTCTGGTCAAGCGCCTTCGCCACCGTTAGTCCTTATAGGATTACAGGATTTTACCCCTCCCCTATAAGTACTCTTGACCTCACCCGCTCCCTAGTCAATGGGTATCTCATGCACGCATTGATGTTGAGCACCAATATTTCACATAAGACGGCATTGACCGGCTACGAGCGCTTTAAGCCCAATAATCGTGGACACCACTTGTGCTGCGA	*Archaea;*Micrarchaeota;Micrarchaeia;Micrarchaeales*;Candidatus* Micrarchaeum*;Candidatus* Micrarchaeota archaeon Mia14	**T3_ASV 64**
TCTAATCCGATTCGTTCTCCTAGCCTTCGCTCCTCACCGTCGGATGCGTTCTGGTCAAGCGCCTTCGCCACCGTTAGTCCTTATAGGATTACAGGATTTTACCCCTCCCCTATAAGTACTCTTGACCTCACCCGCTCCCTAGTCAATGGGTATCTCATGCACGCATTGATGTTGAGCACCAATATTTCACATAAGACGGCATTGACCGGCTACGAGCGCTTTAAGCCCAATAATCGTGGACACCACTTGTGCTGCGA	*Archaea;*Micrarchaeota;Micrarchaeia;Micrarchaeales*;Candidatus* Micrarchaeum*;Candidatus* Micrarchaeota archaeon Mia14	**T3_ASV 79**
TTGCTACCCTAGCCTTCGTTCCTTACCGTCAGATTCGTTCTAGTTAAACGCTTTCGCCACTGGTCGTCCTTCCGGGATTACAGGATTTTACCCCTACCCTGAAAGTACGTTTAACCTCACCCGATCTCAAGTCTTGCAGTCTCTTCAGACTTTCTGGAGTTAAGCTTCAGGCTTTATCTGAAGATTTACAAAACCGGCTACGAACGCTTTAGGCTCAATAAAAGTGACCACTACTCGTGCTGCGGGT	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Ferroplasmaceae;Ferroplasma;Ferroplasma acidiphilum*	**T3_ASV 5**
TCTAATCCGGTTTGCTACCCTAGCCTTCGTTCCTTACCGTCAGATTCGTTCTAGTTAAACGCTTTCGCCACTGGTCGTCCTTCCGGGATTACAGGATTTTACCCCTACCCTGAAAGTACGTTTAACCTCACCCGATCTCAAGTCTTGCAGTCTCTTCAGACTTTCTGGAGTTAAGCTTCAGGCTTTATCTGAAGATTTACAAAACCGGCTACGAACGCTTTAGGCTCAATAAAAGTGACCACTACTCGTGCTGCGGGT	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Ferroplasmaceae;Ferroplasma;Ferroplasma acidiphilum*	**T3_ASV 8**
TTGCTCCCCTCTCCTTCGTTCCTCCCCGTCGGATCCGTTCTAGTTGAACGCCTTCGCC	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae*	**T3_ASV 34**
TTGCTCCCCTCTCCTTCGTTCCTCCCCGTCGGATCCGTTCTAGTTGAACGCCTTC	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae*	**T3_ASV 69**
TCTAATCCGGTTTTCTCCCCTCTCCTTCGTTCCTCCCCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGGATTACAGGATTTT	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae*	**T3_ASV 109**
TTCTAATCCGGTTTGCTCCCCTCTCCTTCGTTCCTCCCCGTCGGATCCGTTCTAGTTGAACGCCTTCGCC	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae*	**T3_ASV 143**
TTCTAATCCGGTTTTCTCCCCTCTCCTTCGTTCCTCCCCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGGATTACAGGATTTT	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae*	**T3_ASV 75**
TTGCTCCCCTAGCCTTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGGATTACAGGATTTTACCCCTACCCCAGAAGTACATTCAACCTCACCCGGTCCCTAGATTTGCAGTCTCTCCAAAATTTCTTCAGTTAAGCAGAAGTATTTTCCTAGAGATTTACAAATCCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACTACCACTCGAGCCGCGGGTGTTACCGC	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae;Cuniculiplasma;Cuniculiplasma divulgatum*	**T3_ASV 7**

TTCTAATCCGGTTTGCTCCCCTAGCCTTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGGATTACAGGATTTTACCCCTACCCCAGAAGTACATTCAACCTCACCCGGTCCCTAGATTTGCAGTCTCTCCAAAATTTCTTCAGTTAAGCAGAAGTATTTTCCTAGAGATTTACAAATCCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACTACCACTCGAGCCGCGGGTGTTACCGC	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae;Cuniculiplasma;Cuniculiplasma divulgatum*	**T3_ASV9**
TTGCTCCCCTAGCCTTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGGATTACAGGATTTTACCCCTACCCCAGAAGTACATTCAACCTCACCCGGTCCCTAGATTTGCAGTCTCTCCAAAATTTCTTCAGTTAAGCAGAAGTATTTTCCTAGAGATTTACAAATCCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACTACCACTCGAGCCGCGGGT	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae;Cuniculiplasma;Cuniculiplasma divulgatum*	**T3_ASV2**
TTCTAATCCGGTTTGCTCCCCTAGCCTTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGGATTACAGGATTTTACCCCTACCCCAGAAGTACATTCAACCTCACCCGGTCCCTAGATTTGCAGTCTCTCCAAAATTTCTTCAGTTAAGCAGAAGTATTTTCCTAGAGATTTACAAATCCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACTACCACTCGAGCCGCGGGT	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae;Cuniculiplasma;Cuniculiplasma divulgatum*	**T3_ASV1**
TCTAATCCGGTTTGCTCCCCTAGCCTTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGGATTACAGGATTTTACCCCTACCCCAGAAGTACATTCAACCTCACCCGGTCCCTAGATTTGCAGTCTCTCCAAAATTTCTTCAGTTAAGCAGAAGTATTTTCCTAGAGATTTACAAATCCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACTACCACTCGAGCCGCGGGTGTTACCGC	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae;Cuniculiplasma;Cuniculiplasma divulgatum*	**T3_ASV14**
TCTAATCCGGTTTGCTCCCCTAGCCTTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGGATTACAGGATTTTACCCCTACCCCAGAAGTACATTCAACCTCACCCGGTCCCTAGATTTGCAGTCTCTCCAAAATTTCTTCAGTTAAGCAGAAGTATTTTCCTAGAGATTTACAAATCCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACTACCACTCGAGCCGCGGGT	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae;Cuniculiplasma;Cuniculiplasma divulgatum*	**T3_ASV16**
TTGCTCCCCTAGCCTTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGGATTACAGGATTTTACCCCTACCCCAGAAGTACATTCAACCTCACCCGGTCCCTAGATTTGCAGTCTCTCCAAAATTTCTTCAGTTAAGCAGAAGTATTTTCCTAGAGATTTACAAATCCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACTACCACTCGAGCCGCGGGTGTTACCGCGGC	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae;Cuniculiplasma;Cuniculiplasma divulgatum*	**T3_ASV19**
TTCTAATCCGGTTTGCTCCCCTAGCCTTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGGATTACAGGATTTTACCCCTACCCCAGAAGTACATTCAACCTCACCCGGTCCCTAGATTTGCAGTCTCTCCAAAATTTCTTCAGTTAAGCAGAAGTATTTTCCTAGAGATTTACAAATCCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACTACCACTCGAGCCGCGGGTGTTACCGCGGC	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae;Cuniculiplasma;Cuniculiplasma divulgatum*	**T3_ASV40**
TTCTAATCCGGTTTGCTCCCCTAGCCCTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGGATTACAGGATTTTACCCCTACCCCAGAAGTACATTCAACCTCACCCGGTCCCTAGATCCGCAGTCTCTCCAGAAGGTCCTTCGTTGAGCGAAGGAATTTTCCTGGAGATTTACGGATCCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACCACCACTCGAGCTGCGGGTGTTACCGC	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae;*E-plasma;uncultured archaeon	**T3_ASV95**
TTGCTCCCCTAGCCCTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGGATTACAGGATTTTACCCCTACCCCAGAAGTACATTCAACCTCACCCGGTCCCTAGATCCGCAGTCTCTCCAGAAGGTCCTTCGTTGAGCGAAGGAATTTTCCTGGAGATTTACGGATCCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACCACCACTCGAGCTGCGGGTGTTACCGC	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae;*E-plasma;uncultured archaeon	**T3_ASV97**

TTGCTCCCCTAGCCCTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGGATTACAGGATTTTACCCCTACCCCAGAAGTACATTCAACCTCACCCGGTCCCTAGATCCGCAGTCTCTCCAGAAGGTCCTTCGTTGAGCGAAGGAATTTTCCTGGAGATTTACGGATCCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACCACCACTCGAGCTGCGGGTGTTACCGCGGC	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae;*E-plasma;uncultured archaeon	**T3_ASV 149**
TTCTAATCCGGTTTGCTCCCCTAGCCCTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTTGTCCTTCCAGGATTACAGGATTTTACCCCTACCCCGGAAGTACATTCAACCTCACCCGGTCCCAAGTTCTACAGTCTCTTCAGAAGATCATCCGTTGAGCGAATGAATTTATCTGAAGATTTACAGAACCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACCACCACTAGAGCTGCGGGTGTTACCGCGGC	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae;*uncultured;uncultured archaeon	**T3_ASV 209**
TTCTAATCCTGTTTGCTCCCCTAGCTTTCGTGCATCAGCGTCAGTTATGGTCCAGTGAGCCGCTTTCGCCACAGGTGTTCCTTCCGATATCTACGCATTTCACCGCTACACCGGAAATTCCACTCACCTCTCCCATACTCAAGCCCGACAGTTTCCGATGCAGACTCTGGGTTGAGCCCAGAGATTTCACATCAGACTTGTCAAACCGCCTACGCACCCTTTACGCCCAATAATTCCGAACAACGCTTGCCCCCCTCGTATTACCGC	*Bacteria;Acidobacteriota;Acidobacteriae;Acidobacteriales;Acidobacteriaceae* (*Subgroup 1)*	**T3_ASV 22**
TTCTAATCCTGTTTGCTCCCCTAGCTTTCGTGCATCAGCGTCAGTTATGGTCCAGTGAGCCGCTTTCGCCACAGGTGTTCCTTCCGATATCTACGCATTTCACCGCTACACCGGAAATTCCACTCACCTCTCCCATACTCAAGCCCGACAGTTTCCGATGCAGACTCTGGGTTGAGCCCAGAGATTTCACATCAGACTTGTCAAACCGCCTACGCACCCTTTACGCCCAATAATTCCGAACAACGCTTGCCCCCCTCGTGTTACCGC	*Bacteria;Acidobacteriota;Acidobacteriae;Acidobacteriales;Acidobacteriaceae* (Subgroup 1)	**T3_ASV 36**
TTCTAATCCTGTTTGCTCCCCTCTCTTTCGTGCATCCGCGTCAGTTATGGTCCAGTGAGCCGCTTTCGCCACAGGTGTTCCTTCCGATATCTACGCATTTC	*Bacteria;Acidobacteriota;Acidobacteriae;Acidobacteriales;Acidobacteriaceae* (Subgroup 1)	**T3_ASV 56**
TTCTAATCCTGTTTTCTCCCCTCTCTTTCGTGCATCCGCGTCAGTTATGGTCCAGTGAGCCGCTTTCGCCACAGGTGTTCCTTCCGATATCTACGCATTTC	*Bacteria;Acidobacteriota;Acidobacteriae;Acidobacteriales;Acidobacteriaceae* (Subgroup 1)	**T3_ASV**
TCTAATCCTGTTTGCTCCCCTAGCTTTCGTGCATCAGCGTCAGTTATGGTCCAGTGAGCCGCTTTCGCCACAGGTGTTCCTTCCGATATCTACGCATTTCACCGCTACACCGGAAATTCCACTCACCTCTCCCATACTCAAGCCCGACAGTTTCCGATGCAGACTCTGGGTTGAGCCCAGAGATTTCACATCAGACTTGTCAAACCGCCTACGCACCCTTTACGCCCAATAATTCCGAACAACGCTTGCCCCCCTC	*Bacteria;Acidobacteriota;Acidobacteriae;Acidobacteriales;Acidobacteriaceae* (Subgroup 1)	**T3_ASV 65**
TCTAATCCTGTTTGCTCCCCTAGCTTTCGTGCATCAGCGTCAGTTATGGTCCAGTGAGCCGCTTTCGCCACAGGTGTTCCTTCCGATATCTACGCATTTCACCGCTACACCGGAAATTCCACTCACCTCTCCCATACTCAAGCCCGACAGTTTCCGATGCAGACTCTGGGTTGAGCCCAGAGATTTCACATCAGACTTGTCAAACCGCCTACGCACCCTTTACGCCCAATAATTCCGAACAACGCTTGCCCCCCTCGTATTACCGC	*Bacteria;Acidobacteriota;Acidobacteriae;Acidobacteriales;Acidobacteriaceae* (Subgroup 1)	**T3_ASV 82**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACCGGCCCAGGCCACCGCCTTCGCCACCGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCGTGACCCCCTACCGGACTCTAGCCACGACAGTATCGGGTGCAATCTCCAGGTTGAGCCTGGAGTTTTCACACCCGACTTATCGAGCCGCCTACGAGCTCTTTACGCCCAATAAATCCGGACAACGCTCGCCCCCTAC	*Bacteria;Actinobacteriota; Acidimicrobiia*	**T3_ASV 60**
TCTAATCCTGTTTGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACCGGCCCAGGCCACCGCCTTCGCCACCGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCGTGACCCCCTACCGGACTCTAGCCACGACAGTATCGGGTGCAATCTCCAGG	*Bacteria;Actinobacteriota; Acidimicrobiia*	**T3_ASV 83**

TTGAGCCTGGAGTTTTCACACCCGACTTATCGAGCCGCCTACGAGCTCTTTACGCCCAATAAATCCGGACAACGCTCGCCCCCTAC		
TTCTAATCCTGTTTGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACCGGCCCAGGCCACCGCCTTCGCCACCGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCGTGACCCCCTACCGGACTCTAGCCACGACAGTATCGGGTGCAATCTCCAGGTTGAGCCTGGAGTTTTCACACCCGACTTATCGAGCCGCCTACGAGCTCTTTACGCCCAATAAATCCGGACAACGCTCGCCCCCTACGTGTTACCGC	*Bacteria;Actinobacteriota; Acidimicrobiia*	**T3_ASV 86**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACCGGCCCAGGCCACCGCCTTCGCCACCGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCGTGACCCCCTACCGGACTCTAGCCACGACAGTATCGGGTGCAATCTCCAGGTTGAGCCTGGAGTTTTCACACCCGACTTATCGAGCCGCCTACGAGCTCTTTACGCCCAATAAATCCGGACAACGCTCGCCCCCTACGTATTACCGC	*Bacteria;Actinobacteriota; Acidimicrobiia*	**T3_ASV 87**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACCGGCCCAGGCCACCGCCTTCGCCACCGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCGTGACCCCCTACCGGACTCTAGCCACGACAGTATCGGGTGCAATCTCCAGGTTGAGCCTGGAGTTTTCACACCCGACTTATCGAGCCGCCTACGAGCTCTTTACGCCCAATAAATCCGGACAACGCTCGCCCCCTACGTGTTACCGCGGC	*Bacteria;Actinobacteriota; Acidimicrobiia*	**T3_ASV 117**
TTCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTTCCGGCCCAGACCACCGCCTTCGCCACTGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCATGGTCCCCTACCGGACTCTAGCCATAGCAGTATCGAGTGGCGACTCCAGGTTGAGCCTGGAGATTTCACACCCGACTTGCTAAGCCGCCTACGAGCTCTTTACGCCCAATGAATCCGGACAACGCTCGCCCCCTACGTGTTACCGCGGC	*Bacteria;Actinobacteriota; Acidimicrobiia*	**T3_ASV 124**
TTCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTTCCGGCCCAGACCACCGCCTTCGCCACTGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCATGGTCCCCTACCGGACTCTAGCCATAGCAGTATCGAGTGGCGACTCCAGGTTGAGCCTGGAGATTTCACACCCGACTTGCTAAGCCGCCTACGAGCTCTTTACGCCCAATGAATCCGGACAACGCTCGCCCCCTACGTATTACCGC	*Bacteria;Actinobacteriota; Acidimicrobiia*	**T3_ASV 139**
TCTAATCCTGTTTGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACCGGCCCAGGCCACCGCCTTCGCCACCGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCGTGACCCCCTACCGGACTCTAGCCACGACAGTATCGGGTGCAATCTCCAGGTTGAGCCTGGAGTTTTCACACCCGACTTATCGAGCCGCCTACGAGCTCTTTACGCCCAATAAATCCGGACAACGCTCGCCCCCTACGTATTACCGC	*Bacteria;Actinobacteriota; Acidimicrobiia*	**T3_ASV 140**
TCTAATCCTGTTTGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACCGGCCCAGGCCACCGCCTTCGCCACCGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCGTGACCCCCTACCGGACTCTAGCCACGACAGTATCGGGTGCAATCTCCAGGTTGAGCCTGGAGTTTTCACACCCGACTTATCGAGCCGCCTACGAGCTCTTTACGCCCAATAAATCCGGACAACGCTCGCCCCCTACGTGTTACCGCGGC	*Bacteria;Actinobacteriota; Acidimicrobiia*	**T3_ASV 158**
TCTAATCCTGTTTGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACCGGCCCAGGCCACCGCCTTCGCCACCGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCGTGACCCCCTACCGGACTCTAGCCACGACAGTATCGGGTGCAATCTCCAGGTTGAGCCTGGAGTTTTCACACCCGACTTATCGAGCCGCCTACGAGCTCTTTACGCCCAATAAATCCGGACAACGCTCGCCCCCTACGTATTACCGCGGC	*Bacteria;Actinobacteriota; Acidimicrobiia*	**T3_ASV 177**

TTCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACCGGCCCAGATCGCTGCCTTCGCCGTTGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCACGATCCTCTACCGGACTCTAGCCATAGCAGTATCGGATGGCGACTCCAGGTTAAGCCTGGAGATTTCACATCCGACTTGCTAAGCCGCCTACGAGCTCTTTACGCCCAATAAATCCGGACAACGCTTGCCCCCTAC	*Bacteria;Actinobacteriota;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ferrimicrobium*	**T3_ASV 44**
TTCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACCGGCCCAGATCGCTGCCTTCGCCGTTGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCACGATCCTCTACCGGACTCTAGCCATAGCAGTATCGGATGGCGACTCCAGGTTAAGCCTGGAGATTTCACATCCGACTTGCTAAGCCGCCTACGAGCTCTTTACGCCCAATAAATCCGGACAACGCTTGCCCCCTACGTCTTACCGC	*Bacteria;Actinobacteriota;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ferrimicrobium*	**T3_ASV 104**
TTCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACCGGCCCAGATCGCTGCCTTCGCCGTTGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCACGATCCTCTACCGGACTCTAGCCATAGCAGTATCGGATGGCGACTCCAGGTTAAGCCTGGAGATTTCACATCCGACTTGCTAAGCCGCCTACGAGCTCTTTACGCCCAATAAATCCGGACAACGCTTGCCCCCTACGTCTTACCGCGGC	*Bacteria;Actinobacteriota;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ferrimicrobium*	**T3_ASV 113**
TCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACCGGCCCAGATCGCTGCCTTCGCCGTTGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCACGATCCTCTACCGGACTCTAGCCATAGCAGTATCGGATGGCGACTCCAGGTTAAGCCTGGAGATTTCACATCCGACTTGCTAAGCCGCCTACGAGCTCTTTACGCCCAATAAATCCGGACAACGCTTGCCCCCTACGTCTTACCGC	*Bacteria;Actinobacteriota;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ferrimicrobium*	**T3_ASV 133**
TCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACCGGCCCAGATCGCTGCCTTCGCCGTTGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCACGATCCTCTACCGGACTCTAGCCATAGCAGTATCAGATGGCGACTCCAGGTTAAGCCTGGAGATTTCACATCTGACTTGCCAAGCCGCCTACGAGCTCTTTACGCCCAATAAATCCGGACAACGCTTGCCCCCTAC	*Bacteria;Actinobacteriota;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ferrimicrobium;uncultured bacterium*	**T3_ASV 173**
TTCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACCGGCCCAGATCGCTGCCTTCGCCGTTGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCACGATCCTCTACCGGACTCTAGCCATAGCAGTATCAGATGGCGACTCCAGGTTAAGCCTGGAGATTTCACATCTGACTTGCCAAGCCGCCTACGAGCTCTTTACGCCCAATAAATCCGGACAACGCTTGCCCCCTACGTCTTACCGC	*Bacteria;Actinobacteriota;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ferrimicrobium;*uncultured bacterium	**T3_ASV 179**
TTCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTAATGGCCCAGACTACCGCCTTCGCCACTGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCGTAGTCCCCTACCATACTCTAGCCATAGCAGTATCAGATGGCATCCCCAGGTTGAGCCTGAGGGTTTCACATCTGACTTGCCAAGCCGCCTACGAGCTCTTTACGCCCAATGAATCCGGACAACGCTTGCACCCTACGTATTACCGC	*Bacteria;Actinobacteriota;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ferrithrix;*uncultured actinobacterium	**T3_ASV 71**
TTCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTAATGGCCCAGACTACCGCCTTCGCCACTGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCGTAGTCCCCTACCATACTCTAGCCATAGCAGTATCAGATGGCATCCCCAGGTTGAGCCTGAGGGTTTCACATCTGACTTGCCAAGCCGCCTACGAGCTCTTTACGCCCAATGAATCCGGACAACGCTTGCACCCTAC	*Bacteria;Actinobacteriota;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ferrithrix;*uncultured actinobacterium	**T3_ASV 70**
TCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTAATGGCCCAGACTACCGCCTTCGCCACTGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCGTAGTCCCCTACCATACTCTAGCCATAGCAGTATCAGATGGCATCCCCAGGTTGAGCCTGAGGGTTTCACATCTGACTTGCCAAGCCGCCTACGAGCTCTTTACGCCCAATGAATCCGGACAACGCTTGCACCCTACGTGTTACCGC	*Bacteria;Actinobacteriota;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ferrithrix;*uncultured actinobacterium	**T3_ASV 150**

TTCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTAATGGCCCAGACTACCGCCTTCGCCACTGGTGTTCCTCCTG ATATCTGCGCATTTCACCGCTACACCAGGAATTCCGTAGTCCCCTACCATACTCTAGCCATAGCAGTATCAGATGGCATCCCCAG GTTGAGCCTGAGGGTTTCACATCTGACTTGCCAAGCCGCCTACGAGCTCTTTACGCCCAATGAATCCGGACAACGCTTGCACCCT ACGTGTTACCGC	*Bacteria;Actinobacteriota;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ferrithrix;*uncultured actinobacterium	**T3_ASV 171**
TTCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTGTTGGCCCAGACCACCGCCTTCGCCGCTGGTGTTCCTCCTG ATATCTGCGCATTTCACCGCTACACCAGGAATTCCGTGGTCCCCTACCAAACTCTAGCCATGGCAGTATCGGATGGCGGCTCCAG GTTAAGCCTGGAGGTTTCACATCCGACTTGCCAAGCCGCCTACGAGCTCTTTACGCCCAATGAATCCGGACAACGCTCGCCCCCT AC	*Bacteria;Actinobacteriota;Acidimicrobiia;uncultured*	**T3_ASV 24**
TTCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTGTTGGCCCAGACCACCGCCTTCGCCGCTGGTGTTCCTCCTG ATATCTGCGCATTTCACCGCTACACCAGGAATTCCGTGGTCCCCTACCAAACTCTAGCCATGGCAGTATCGGATGGCGGCTCCAG GTTAAGCCTGGAGGTTTCACATCCGACTTGCCAAGCCGCCTACGAGCTCTTTACGCCCAATGAATCCGGACAACGCTCGCCCCCT ACGTATTACCGC	*Bacteria;Actinobacteriota;Acidimicrobiia;uncultured*	**T3_ASV 47**
TCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTGTTGGCCCAGACCACCGCCTTCGCCGCTGGTGTTCCTCCTGA TATCTGCGCATTTCACCGCTACACCAGGAATTCCGTGGTCCCCTACCAAACTCTAGCCATGGCAGTATCGGATGGCGGCTCCAG GTTAAGCCTGGAGGTTTCACATCCGACTTGCCAAGCCGCCTACGAGCTCTTTACGCCCAATGAATCCGGACAACGCTCGCCCCCT AC	*Bacteria;Actinobacteriota;Acidimicrobiia;*uncultured	**T3_ASV 66**
TCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTGTTGGCCCAGACCACCGCCTTCGCCGCTGGTGTTCCTCCTGA TATCTGCGCATTTCACCGCTACACCAGGAATTCCGTGGTCCCCTACCAAACTCTAGCCATGGCAGTATCGGATGGCGGCTCCAG GTTAAGCCTGGAGGTTTCACATCCGACTTGCCAAGCCGCCTACGAGCTCTTTACGCCCAATGAATCCGGACAACGCTCGCCCCCT ACGTATTACCGC	*Bacteria;Actinobacteriota;Acidimicrobiia;*uncultured	**T3_ASV 72**
TCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTGTTGGCCCAGACCACCGCCTTCGCCGCTGGTGTTCCTCCTGA TATCTGCGCATTTCACCGCTACACCAGGAATTCCGTGGTCCCCTACCAAACTCTAGCCATGGCAGTATCGGATGGCGGCTCCAG GTTAAGCCTGGAGGTTTCACATCCGACTTGCCAAGCCGCCTACGAGCTCTTTACGCCCAATGAATCCGGACAACGCTCGCCCCCT ACGTGTTACCGC	*Bacteria;Actinobacteriota;Acidimicrobiia;*uncultured	**T3_ASV 96**
TCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTGTTGGCCCAGACCACCGCCTTCGCCGCTGGTGTTCCTCCTGA TATCTGCGCATTTCACCGCTACACCAGGAATTCCGTGGTCCCCTACCAAACTCTAGCCATGGCAGTATCGGATGGCGGCTCCAG GTTAAGCCTGGAGGTTTCACATCCGACTTGCCAAGCCGCCTACGAGCTCTTTACGCCCAATGAATCCGGACAACGCTCGCCCCCT ACGTATTACCGCGGC	*Bacteria;Actinobacteriota;Acidimicrobiia;*uncultured	**T3_ASV 100**
TTCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTGTTGGCCCAGACCACCGCCTTCGCCGCTGGTGTTCCTCCTG ATATCTGCGCATTTCACCGCTACACCAGGAATTCCGTGGTCCCCTACCAAACTCTAGCCATGGCAGTATCGGATGGCGGCTCCAG GTTAAGCCTGGAGGTTTCACATCCGACTTGCCAAGCCGCCTACGAGCTCTTTACGCCCAATGAATCCGGACAACGCTCGCCCCCT ACGTGTTACCGC	*Bacteria;Actinobacteriota;Acidimicrobiia;*uncultured	**T3_ASV 106**
TTCTAATCCTGTTTGCTCCCCACGCCTTCGCGCCTCAGCGTCAGGTTTCGTCCAGTCAGGCGCCTTCGCCACTGGTATTCCTCCAC ATCTCTACGCATTTCACCGCTACACGTGGAATTCCCCTGACCTCTCCGACCCTCAAGTCCACCCGTTTCCAAGGCCATCTCAGGGT TGAGCCCTGAACTTTCACCCCAGACGTGATAGACCGCCTGCGCGCGCTTTACGCCCAGTCATTCCGGACAACGCTTGCCCCCTAC	*Bacteria;Firmicutes;Bacilli;Alicyclobacillales;Alicyclobacillaceae;Acidibacillus*;uncultured low G+C Gram-positive bacterium	**T3_ASV 41**

TCTAATCCTGTTTGCTCCCCACGCCTTCGCGCCTCAGCGTCAGGTTTCGTCCAGTCAGGCGCCTTCGCCACTGGTATTCCTCCACA TCTCTACGCATTTCACCGCTACACGTGGAATTCCCCTGACCTCTCCGACCCTCAAGTCCACCCGTTTCCAAGGCCATCTCAGGGTT GAGCCCTGAACTTTCACCCCAGACGTGATAGACCGCCTGCGCGCGCTTTACGCCCAGTCATTCCGGACAACGCTTGCCCCCTACG TATTACCGC	*Bacteria;Firmicutes;Bacilli;Alicyclobacillales;Alicyclobacillaceae;Acidibacillus;*uncultured low G+C Gram-positive bacterium	**T3_ASV 115**
TCTAATCCTGTTTGCTCCCCACGCCTTCGCGCCTCAGCGTCAGGTTTCGTCCAGTCAGGCGCCTTCGCCACTGGTATTCCTCCACA TCTCTACGCATTTCACCGCTACACGTGGAATTCCCCTGACCTCTCCGACCCTCAAGTCCACCCGTTTCCAAGGCCATCTCAGGGTT GAGCCCTGAACTTTCACCCCAGACGTGATAGACCGCCTGCGCGCGCTTTACGCCCAGTCATTCCGGACAACGCTTGCCCCCTAC	*Bacteria;Firmicutes;Bacilli;Alicyclobacillales;Alicyclobacillaceae;Acidibacillus;*uncultured low G+C Gram-positive bacterium	**T3_ASV 120**
TCTAATCCTGTTTGCTCCCCACGCCTTCGCGCCTCAGCGTCAGGTTTCGTCCAGTCAGGCGCCTTCGCCACTGGTATTCCTCCACA TCTCTACGCATTTCACCGCTACACGTGGAATTCCCCTGACCTCTCCGACCCTCAAGTCCACCCGTTTCCAAGGCCATCTCAGGGTT GAGCCCTGAACTTTCACCCCAGACGTGATAGACCGCCTGCGCGCGCTTTACGCCCAGTCATTCCGGACAACGCTTGCCCCCTACG TGTTACCGC	*Bacteria;Firmicutes;Bacilli;Alicyclobacillales;Alicyclobacillaceae;Acidibacillus;*uncultured low G+C Gram-positive bacterium	**T3_ASV 132**
TTCTAATCCTATTTGCTCCCCACACTTTCGAGCCTAAGCGTCAGTTATAGCCTAAGTTTTCGCCTTCGCCTCTGGTGTTCTTCCATA TATCTACGCATTCCACCGCTCCACATGGAGTTCCAAAACTCTCTACTATACTCTAGACTTACAGTTTCCAATGCAAACAACCGTTA AGCAGTTGTCTTTAACATCAGACTTGTAAATCCGCCTGCGCTCGCTTTACGCCCAGTAAATCCGGATAACGCTTGCATCCTAT	*Bacteria;Firmicutes;Bacilli;Mycoplasmatales;Mycoplasmataceae;Mycoplasma*	**T3_ASV 98**
TCTAATCCTATTTGCTCCCCACACTTTCGAGCCTAAGCGTCAGTTATAGCCTAAGTTTTCGCCTTCGCCTCTGGTGTTCTTCCATAT ATCTACGCATTCCACCGCTCCACATGGAGTTCCAAAACTCTCTACTATACTCTAGACTTACAGTTTCCAATGCAAACAACCGTTAA GCAGTTGTCTTTAACATCAGACTTGTAAATCCGCCTGCGCTCGCTTTACGCCCAGTAAATCCGGATAACGCTTGCATCCTAT	*Bacteria;Firmicutes;Bacilli;Mycoplasmatales;Mycoplasmataceae;Mycoplasma*	**T3_ASV 108**
TTCTAATCCTGTTTGCTACCCACGCTTTCGAGCCTCAGCGTCAGTTAGAGCCCAGTAAACCGCCTTCGCCACTGGTGTTCCTCCTA ATATCTACGCATTTCACCGCTACACTAGGAATTCCGCTTACCTCTACTCCACTCAAGAGAAATAGTTTTGAACGCTGCTATCGGTT GAGCCGATAGTTTTAACATTCAACTTATTCCCCCGCCTACGCTCCCTTTACACCCAGTAATTCCGGACAACGCTTGCCACCTAC	*Bacteria;Firmicutes;Clostridia;Oscillospirales;Ruminococcaceae;Harryflintia*	**T3_ASV 73**
TCTAATCCTGTTTGCTACCCACGCTTTCGAGCCTCAGCGTCAGTTAGAGCCCAGTAAACCGCCTTCGCCACTGGTGTTCCTCCTAA TATCTACGCATTTCACCGCTACACTAGGAATTCCGCTTACCTCTACTCCACTCAAGAGAAATAGTTTTGAACGCTGCTATCGGTTG AGCCGATAGTTTTAACATTCAACTTATTCCCCCGCCTACGCTCCCTTTACACCCAGTAATTCCGGACAACGCTTGCCACCTAC	*Bacteria;Firmicutes;Clostridia;Oscillospirales;Ruminococcaceae;Harryflintia*	**T3_ASV 78**
TCTAATCCTGTTTGCTACCCACGCTTTCGAGCCTCAGCGTCAGTTAGAGCCCAGTAAACCGCCTTCGCCACTGGTGTTCCTCCTAA TATCTACGCATTTCACCGCTACACTAGGAATTCCGCTTACCTCTACTCCACTCGAGAGAAATAGTTTTGAACGCTGCTATCGGTTG AGCCGATAGTTTTAACATTCAACTTATTCCCCCGCCTACGCTCCCTTTACACCCAGCAATTCCGGACAACGCTTGCCACCTAC	*Bacteria;Firmicutes;Clostridia;Oscillospirales;Ruminococcaceae;Harryflintia*	**T3_ASV 184**
TTCTAATCCTGTTTGCTCCCCATGCTTTCGTACCTCAGCGTCAGTTACAGTCCAGAGAGTCGCCTTCGCCACTGGTATTCCTCCTA ATATCTACGCATTTCACCGCTACACTAGGAATTCCACTCTCCCATCCTGTACTCAAGCTCTGTAGTTTCAAATGCTTACCACAGTTA AGCCATAGTCTTTCACATCTGACTTACAAAGCCGCCTGCGTACCCTTACGCCCAGTAATTCCGGACAACGCTTGCCCCCTAC	*Bacteria;Firmicutes;Clostridia;Peptostreptococcales-Tissierellales;*Family XI*;Sporanaerobacter;*uncultured bacterium	**T3_ASV 215**
TCTAATCCCGTTCGCTCCCCACGCTGTCGCGCCTCAGCGTCAGGGTCAGGCCAGTGCACCGCCTTCGCCACCGGTGTTCTTCCTG ATCTCTACGCATTTCACCGCTCCACCAGGAATTCCATGCACCTCTCCTGCCCTCCAGCCGATCCGTTTAGCCGCCCTCCGTCGGGT GAGCCGACGGCTGAAAACCGCTACGTGACCGGCCGCCTACACGCCCTTTACGCCCAGTCATTCCGGACAACGCTCGCCCCCTAC	*Bacteria;Firmicutes;Sulfobacillia;Sulfobacillales;Sulfobacillaceae;Sulfobacillus*	**T3_ASV 38**
TTCTAATCCCGTTCGCTCCCCACGCTGTCGCGCCTCAGCGTCAGGGTCAGGCCAGTGCACCGCCTTCGCCACCGGTGTTCTTCCT GATCTCTACGCATTTCACCGCTCCACCAGGAATTCCATGCACCTCTCCTGCCCTCCAGCCGATCCGTTTAGCCGCCCTCCGTCGGG	*Bacteria;Firmicutes;Sulfobacillia;Sulfobacillales;Sulfobacillaceae;Sulfobacillus*	**T3_ASV 131**

TGAGCCGACGGCTGAAAACCGCTACGTGACCGGCCGCCTACACGCCCTTTACGCCCAGTCATTCCGGACAACGCTCGCCCCCTAC		
TCTAATCCCGTTTGCTCCCCACGCTATCGCGCCTCAGCGTCAGGGACAGGCCAGTGCGTCGCCTTCGCCACTGGTGTTCTTCCTGATCTCTACGCATTTCACCGCTCCACCAGGAATTCCACGCACCTCTCCTGCCCTCCAGTCGCGCCGTTTAGCGACCATCCGTCGGGTGAGCCGACGGCTTATAACCGCTACGTACGCCACCGCCTACACGCCCTTTACGCCCAGTAATTCCGGACAACGCTTGCCCCCTAC	*Bacteria;Firmicutes;Sulfobacillia;Sulfobacillales;Sulfobacillaceae;Sulfobacillus;* uncultured bacterium	**T3_ASV 91**
TTCTAATCCCGTTTGCTCCCCACGCTATCGCGCCTCAGCGTCAGGGACAGGCCAGTGCGTCGCCTTCGCCACTGGTGTTCTTCCTGATCTCTACGCATTTCACCGCTCCACCAGGAATTCCACGCACCTCTCCTGCCCTCCAGTCGCGCCGTTTAGCGACCATCCGTCGGGTGAGCCGACGGCTTATAACCGCTACGTACGCCACCGCCTACACGCCCTTTACGCCCAGTAATTCCGGACAACGCTTGCCCCCTAC	*Bacteria;Firmicutes;Sulfobacillia;Sulfobacillales;Sulfobacillaceae;Sulfobacillus;* uncultured bacterium	**T3_ASV 175**
TCTAATCCTGTTCGCTCCCCACGCTTTCGTCCCTCAGCGTCAGTAATAGGCCAGAAAGCTGCCTTCGCCATTGATGTTCCTCCTGATATCTACGCATGTCACCGCTACACCAGGAATTCCACTTTCCTCTCCTATACTCTATTTCAACAGTTTCAAATGCATGTCACCGGTTAAGCCGGTGTCTTTAACATATGACTTATTGAAACGCCTACGGACCCTTTACGCCCAGTGATTCCGGACAACGCTTGCACCCCTC	*Bacteria;Marinimicrobia* (SAR406 clade);uncultured bacterium MB13C05	**T3_ASV 191**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGTCTCTCAGTGTCAGGCCTGTTCCAGGAAGCCGCCTTCGCCACCGGCCTTCCTTCTGATATCTACGCATTTCACCGCTACACCAGAAATTCCGCTTCCCTCTCCCAGCCTCCAGTCTGCCAGTCTCTTTGGCATTCCCATGGTTAAGCCACGGCCTTTCACCAAAGACTTGACAAACCACCTACAGACTCTTTACGCCCAGTAACTCCGAACAACGCTTGCCACCTCTGTCTTACCGCGGC	*Bacteria;Nitrospirota;Leptospirillia;Leptospirillales;Leptospirillaceae;Leptospirillum;Leptospirillum ferriphilum*	**T3_ASV 4**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGTCTCTCAGTGTCAGGCCTGTTCCAGGAAGCCGCCTTCGCCACCGGCCTTCCTTCTGATATCTACGCATTTCACCGCTACACCAGAAATTCCGCTTCCCTCTCCCAGCCTCCAGTCTGCCAGTCTCTTTGGCATTCCCATGGTTAAGCCACGGCCTTTCACCAAAGACTTGACAAACCACCTACAGACTCTTTACGCCCAGTAACTCCGAACAACGCTTGCCACCTCTGTCTTACCGC	*Bacteria;Nitrospirota;Leptospirillia;Leptospirillales;Leptospirillaceae;Leptospirillum;Leptospirillum ferriphilum*	**T3_ASV 20**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGTCTCTCAGTGTCAGGCCTGTTCCAGGAAGCCGCCTTCGCCACCGGCCTTCCTTCTGATATCTACGCATTTCACCGCTACACCAGAAATTCCGCTTCCCTCTCCCAGCCTCCAGTCTGCCAGTCTCTTTGGCATTCCCATGGTTAAGCCACGGCCTTTCACCAAAGACTTGACAAACCACCTACAGACTCTTTACGCCCAGTAACTCCGAACAACGCTTGCCACCTCT	*Bacteria;Nitrospirota;Leptospirillia;Leptospirillales;Leptospirillaceae;Leptospirillum;Leptospirillum ferriphilum*	**T3_ASV 18**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGTCTCTCAGTGTCAGGACTGTTCCAGGAAGCCGCCTTCGCCACCGGCCTTCCTTCTGATATCTACGCATTTCACCGCTACACCAGAAATTCCGCTTCCCTCTCCCAGCCTCCAGTCTGCCAGTCTCTTTGGCATTCCCATGGTTAAGCCACGGCCTTTCACCAAAGACTTGACAAACCACCTACAGACTCTTTACGCCCAGTAATTCCGAACAACGCTTGCCACCTCT	*Bacteria;Nitrospirota;Leptospirillia;Leptospirillales;Leptospirillaceae;Leptospirillum;Leptospirillum ferrooxidans*	**T3_ASV 28**
TTCTAATCCCGTTCGCTACCCACGCTTTCGTGCCTCAGTGTCAGAACCGGCCCAGTTACCCGTCTTC	*Bacteria;Planctomycetota;Phycisphaerae;Tepidisphaerales;*CPla-3 termite group	**T3_ASV 130**
TTCTAATCCCGTTCGCTACCCACGCTTTCGTGCCTCAGTGTCAGAACCGGCCCAGTTACCCGTCTTCACCACTGGCGTTCCAGACGATATCTACGCATTTCACCGCTCCACCGTCCGTTCCAGTAACCTATACCGGCCTCAAGCACGCCAGTATCAGAAGCAATTCCCGGGTTGAGCCCGGGGATTTCACAACTGACTTAACGCGCCACCTACGCACCCTTTAAACCCAGTGAATCTCAGTAACGCTTGGGACCTCT	*Bacteria;Planctomycetota;Phycisphaerae;Tepidisphaerales*;CPla-3 termite group;uncultured bacterium	**T3_ASV 94**
TCTAATCCCGTTCGCTACCCACGCTTTCGTGCCTCAGTGTCAGAACCGGCCCAGTTACCCGTCTTCACCACTGGCGTTCCAGACGATATCTACGCATTTCACCGCTCCACCGTCCGTTCCAGTAACCTATACCGGCCTCAAGCACGCCAGTATCAGAAGCAATTCCCGGG	*Bacteria;Planctomycetota;Phycisphaerae;Tepidisphaerales;*CPla-3 termite group;uncultured bacterium	**T3_ASV 101**

TTGAGCCCGGGGATTTCACAACTGACTTAACGCGCCACCTACGCACCCTTTAAACCCAGTGAATCTCAGTAACGCTTGGGACCTCT		
TCTAATCCCGTTCGCTACCCACGCTTTCGTGCCTCAGTGTCAGAACCGGCCCAGTTACCCGTCTTCACCACTGGCGTTCCAGACGATATCTACGCATTTCACCGCTCCACCGTCCGTTCCAGTAACCTATACCGGCCTCAAGCACGCCAGTATCAGAAGCAATTCCCGGGTTGAGCCCGGGGATTTCACAACTGACTTAACGCGCCACCTACGCACCCTTTAAACCCAGTGAATCTCAGTAACGCTTGGGACCTCTGTATTACCGC	*Bacteria;Planctomycetota;Phycisphaerae;Tepidisphaerales;*CPla-3 termite group*;*uncultured bacterium	**T3_ASV 116**
TTCTAATCCCGTTCGCTACCCACGCTTTCGTGCCTCAGTGTCAGAACCGGCCCAGTTACCCGTCTTCACCACTGGCGTTCCAGACGATATCTACGCATTTCACCGCTCCACCGTCCGTTCCAGTAACCTATACCGGCCTCAAGCACGCCAGTATCAGAAGCAATTCCCGGGTTGAGCCCGGGGATTTCACAACTGACTTAACGCGCCACCTACGCACCCTTTAAACCCAGTGAATCTCAGTAACGCTTGGGACCTCTGTGTTACCGC	*Bacteria;Planctomycetota;Phycisphaerae;Tepidisphaerales;*CPla-3 termite group*;*uncultured bacterium	**T3_ASV 126**
TTCTAATCCCGTTCGCTACCCACGCTTTCGTGCCTCAGTGTCAGAACCGGCCCAGTTACCCGTCTTCACCACTGGCGTTCCAGACGATATCTACGCATTTCACCGCTCCACCGTCCGTTCCAGTAACCTATACCGGCCTCAAGCACGCCAGTATCAGAAGCAATTCCCGGGTTGAGCCCGGGGATTTCACAACTGACTTAACGCGCCACCTACGCACCCTTTAAACCCAGTGAATCTCAGTAACGCTTGGGACCTCTGTATTACCGCGGC	*Bacteria;Planctomycetota;Phycisphaerae;Tepidisphaerales;*CPla-3 termite group*;*uncultured bacterium	**T3_ASV 199**
TTCTAATCCTGTTTTCTCCCCCCTCTTTCGCGCCTCCGCGTCAGTCAAGGACCAGGTCGCCGCCTTCGCCACCGGTGTTCTTTCCAATATCTACGAATTTC	*Bacteria;Proteobacteria;Alphaproteobacteria;Acetobacterales;Acetobacteraceae*	**T3_ASV 85**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTCAAGGACCAGGTCGCCGCCTTCGCCACCGGTGTTCTTTCCAATATCTACGAATTTCACCTCTACACTGGAAATTCCACGACCCTCTTCCTCACTCTAGATCACACGTCTCAGACGCAGTCCCCAGGTTAAGCCCAGGAATTTCACGCCTGACTGTGCAGTCCGCCTACGCGCCCTTTACGCCCAGTCATTCCGAGCAACGCTAGCCCCCTTC	*Bacteria;Proteobacteria;Alphaproteobacteria;Acetobacterales;Acetobacteraceae;Acidiphilium*	**T3_ASV 33**
TCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTAATGGACCAGGTTGCCGCCTTCGCCACCGGTGTTCTTTCCAATATCTACGAATTTCACCTCTACACTGGAAATTCCACAACCCTCTTCCATACTCAAGACAACACGTCTCAGACGCAGTCCCCAGGTTGAGCCCAGGAATTTCACGCCTGACTGTGCCGTCCGCCTACGCGCCCTTTACGCCCAGTCATTCCGAGCAACGCTAGCCCCCTTCGTATTACCGC	*Bacteria;Proteobacteria;Alphaproteobacteria;Acetobacterales;Acetobacteraceae;Acidiphilium*	**T3_ASV 182**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTCAAGGACCAGGTCGCCGCCTTCGCCACCGGTGTTCTTTCCAATATCTACGAATTTCACCTCTACACTGGAAATTCCACGACCCTCTTCCTCACTCTAGATCACACGTCTCAGACGCAGTCCCCAGGTTAAGCCCAGGAATTTCACGCCTGACTGTGCAGTCCGCCTACGCGCCCTTTACGCCCAGTCATTCCGAGCAACGCTAGCCCCCTTCGTGTTACCGC	*Bacteria;Proteobacteria;Alphaproteobacteria;Acetobacterales;Acetobacteraceae;Acidiphilium;*uncultured *Acidiphilium* sp.	**T3_ASV 68**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTCAAGGACCAGGTCGCCGCCTTCGCCACCGGTGTTCTTTCCAATATCTACGAATTTCACCTCTACACTGGAAATTCCACGACCCTCTTCCTCACTCTAGATCACACGTCTCAGACGCAGTCCCCAGGTTAAGCCCAGGAATTTCACGCCTGACTGTGCAGTCCGCCTACGCGCCCTTTACGCCCAGTCATTCCGAGCAACGCTAGCCCCCTTCGTATTACCGCGGC	*Bacteria;Proteobacteria;Alphaproteobacteria;Acetobacterales;Acetobacteraceae;Acidiphilium;*uncultured *Acidiphilium* sp.	**T3_ASV 74**
TCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTCAAGGACCAGGTCGCCGCCTTCGCCACCGGTGTTCTTTCCAATATCTACGAATTTCACCTCTACACTGGAAATTCCACGACCCTCTTCCTCACTCTAGATCACACGTCTCAGACGCAGTCCCCAGGTTAAGCCCAGGAATTTCACGCCTGACTGTGCAGTCCGCCTACGCGCCCTTTACGCCCAGTCATTCCGAGCAACGCTAGCCCCCTTCGTATTACCGCGGC	*Bacteria;Proteobacteria;Alphaproteobacteria;Acetobacterales;Acetobacteraceae;Acidiphilium;*uncultured *Acidiphilium* sp.	**T3_ASV 114**

TTCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTAAAGGACCAGGTCGCCGCCTTCGCCACCGGTGTTCTTCCCAATATCTACGAATTTCACCTCTACACTGGGAATTCCACGACCCTCTTCCTCACTCAAGTCTACACGTATCAAGCGCAGTCCCCAGGTTGAGCCCAGGAATTTCACGCCTGACTATATAAACCGCCTACGCGCCCTTTACGCCCAGTCATTCCGAGCAACGCTAGCCCCCTTCGTATTACCGC	*Bacteria;Proteobacteria;Alphaproteobacteria;Acetobacterales;Acetobacteraceae;*uncultured	**T3_ASV 43**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTAAAGGACCAGGTCGCCGCCTTCGCCACCGGTGTTCTTCCCAATATCTACGAATTTCACCTCTACACTGGGAATTCCACGACCCTCTTCCTCACTCAAGTCTACACGTATCAAGCGCAGTCCCCAGGTTGAGCCCAGGAATTTCACGCCTGACTATATAAACCGCCTACGCGCCCTTTACGCCCAGTCATTCCGAGCAACGCTAGCCCCCTTC	*Bacteria;Proteobacteria;Alphaproteobacteria;Acetobacterales;Acetobacteraceae;*uncultured	**T3_ASV 45**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTAAAGGACCAGGTCGCCGCCTTCGCCACCGGTGTTCTTCCCAATATCTACGAATTTCACCTCTACACTGGGAATTCCACGACCCTCTTCCTCACTCAAGTCTACACGTATCAAGCGCAGTCCCCAGGTTGAGCCCAGGAATTTCACGCCTGACTATATAAACCGCCTACGCGCCCTTTACGCCCAGTCATTCCGAGCAACGCTAGCCCCCTTCGTGTTACCGC	*Bacteria;Proteobacteria;Alphaproteobacteria;Acetobacterales;Acetobacteraceae;*uncultured	**T3_ASV 103**
TCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTAAAGGACCAGGTCGCCGCCTTCGCCACCGGTGTTCTTCCCAATATCTACGAATTTCACCTCTACACTGGGAATTCCACGACCCTCTTCCTCACTCAAGTCTACACGTATCAAGCGCAGTCCCCAGGTTGAGCCCAGGAATTTCACGCCTGACTATATAAACCGCCTACGCGCCCTTTACGCCCAGTCATTCCGAGCAACGCTAGCCCCCTTCGTATTACCGC	*Bacteria;Proteobacteria;Alphaproteobacteria;Acetobacterales;Acetobacteraceae;*uncultured	**T3_ASV 105**
GGGTTTCTAATCCTGTTTGCTCCCCCCGCTTTCGCGCCTCAGCGTCAGTAAAGGACCAGGTCGCCGCCTTCGCCACCGGTGTTCTTCCCAATATCTACGAATTTCACCTCTACACTGGGAATTCCACGACCCTCTTCCTCACTCAAGTCTACACGTATCAAGCGCAGTCCCCAGGTTGAGCCCAGGAATTTCACGCCTGACTATATAAACCGCCTACGCGCCCTTTACGCCCAGTCATTCCGAGCAACGCTAGCCCCCTTC	*Bacteria;Proteobacteria;Alphaproteobacteria;Acetobacterales;Acetobacteraceae;*uncultured	**T3_ASV 118**
TCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTAAAGGACCAGGTCGCCGCCTTCGCCACCGGTGTTCTTCCCAATATCTACGAATTTCACCTCTACACTGGGAATTCCACGACCCTCTTCCTCACTCAAGTCTACACGTATCAAGCGCAGTCCCCAGGTTGAGCCCAGGAATTTCACGCCTGACTATATAAACCGCCTACGCGCCCTTTACGCCCAGTCATTCCGAGCAACGCTAGCCCCCTTC	*Bacteria;Proteobacteria;Alphaproteobacteria;Acetobacterales;Acetobacteraceae;*uncultured	**T3_ASV 88**
TTCTAATCCTGTTTGCTCCCCCCGCTTTCGCGCCTCAGCGTCAGTAAAGGACCAGGTCGCCGCCTTCGCCACCGGTGTTCTTCCCAATATCTACGAATTTCACCTCTACACTGGGAATTCCACGACCCTCTTCCTCACTCAAGTCTACACGTATCAAGCGCAGTCCCCAGGTTGAGCCCAGGAATTTCACGCCTGACTATATAAACCGCCTACGCGCCCTTTACGCCCAGTCATTCCGAGCAACGCTAGCCCCCTTCGTATTACCGC	*Bacteria;Proteobacteria;Alphaproteobacteria;Acetobacterales;Acetobacteraceae;*uncultured	**T3_ASV 123**
TCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTAAAGGACCAGGTCGCCGCCTTCGCCACCGGTGTTCTTCCCAATATCTACGAATTTCACCTCTACACTGGGAATTCCACGACCCTCTTCCTCACTCAAGTCTACACGTATCAAGCGCAGTCCCCAGGTTGAGCCCAGGAATTTCACGCCTGACTATATAAACCGCCTACGCGCCCTTTACGCCCAGTCATTCCGAGCAACGCTAGCCCCCTTCGTATTACCGCGGC	*Bacteria;Proteobacteria;Alphaproteobacteria;Acetobacterales;Acetobacteraceae;*uncultured	**T3_ASV 127**
TCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTAAAGGACCAGGTCGCCGCCTTCGCCACCGGTGTTCTTCCCAATATCTACGAATTTCACCTCTACACTGGGAATTCCACGACCCTCTTCCTCACTCAAGTCTACACGTATCAAGCGCAGTCCCCAGGTTGAGCCCAGGAATTTCACGCCTGACTATATAAACCGCCTACGCGCCCTTTACGCCCAGTCATTCCGAGCAACGCTAGCCCCCTTCGTATTACCGCGGC	*Bacteria;Proteobacteria;Alphaproteobacteria;Acetobacterales;Acetobacteraceae;*uncultured	**T3_ASV 152**

TCTAATCCTCTTCGCTACCCCTGCTTTCGCTCCTCAGTGTCAGTAGTGACCCAGTAAGTTGCCTTCGCATTTGGTGTTCTTTCTAATATCTACGAATTTCACCTCTACACTAGAAATTCCACTTACCTCTATCACACTCTAGCTAAAAAGTTTTGATGGCAGTTCCAAGGTTGAGCCTTGGGATTTCACCATCAACTTTTTTAACCACCTACGAGCTCTTTAAGCCCAGTAATTCCGAACTACGCTAGGTCCCTTC	*Bacteria;Proteobacteria;Alphaproteobacteria;*SAR11 clade;Clade II	**T3_ASV 195**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTCAGTGTCAGTTTTAGTCCAGGAAGCCGCCTTCGCCACTGGTGTTCCTCCACATATCTACGCATTTCACCGCTACACGTGGAATTCCACTTCCCTCTACCAAACTCTAGTCCGACAGTATCAAACGCAATTCCCAGGTTAAGCCCAGGGCTTTCACATCTGACTTACCGAACCACCTACGCACGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCTGTATTACCGC	*Bacteria;Proteobacteria;Gammaproteobacteria;Acidiferrobacterales;Acidiferrobacteraceae;Acidiferrobacter;Acidiferrobacter thiooxydans*	**T3_ASV 154**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTCAGCGTCAGTATTGGGCCAGGTGGCCGCCTTCGCCACTGATGTTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCCACCACCCTCTCCCATACTCTAGTGCGCCGGTTTCCACCGCCATTCCCAGGTTGAGCCCGGGGATTTCACGACAGACCTAACGTACCGCCTACGCACCCTTTACGCCCAGTGATTCCGATTAACGCTTGCACCCCCC	*Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobacillales;Acidithiobacillaceae;Acidithiobacillus*	**T3_ASV 13**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTCAGCGTCAGTATTGGGCCAGGTGGCCGCCTTCGCCACTGATGTTCCTCCAGATATCTACGCATTTCACCGCTACACCTGGAATTCCACCACCCTCTCCCATACTCTAGTGCGCCGGTTTCTACCGCCATTCCCAGGTTGAGCCCGGGGATTTCACGACAGACCTAACGTACCGCCTACGCACCCTTTACGCCCAGTGATTCCGATTAACGCTTGCACCCCCCGTATTACCGC	*Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobacillales;Acidithiobacillaceae;Acidithiobacillus*	**T3_ASV 15**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTCAGCGTCAGTATTGGGCCAGGTGGCCGCCTTCGCCACTGATGTTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCCACCACCCTCTCCCATACTCTAGTGCGCCGGTTTCCACCGCCATTCCCAGGTTGAGCCCGGGGATTTCACGACAGACCTAACGTACCGCCTACGCACCCTTTACGCCCAGTGATTCCGATTAACGCTTGCACCCCCCGTATTACCGC	*Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobacillales;Acidithiobacillaceae;Acidithiobacillus*	**T3_ASV 17**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTCAGCGTCAGTATTGGGCCAGGTGGCCGCCTTCGCCACTGATGTTCCTCCAGATATCTACGCATTTCACCGCTACACCTGGAATTCCACCACCCTCTCCCATACTCTAGTGCGCCGGTTTCTACCGCCATTCCCAGGTTGAGCCCGGGGATTTCACGACAGACCTAACGTACCGCCTACGCACCCTTTACGCCCAGTGATTCCGATTAACGCTTGCACCCCCCGTGTTACCGC	*Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobacillales;Acidithiobacillaceae;Acidithiobacillus*	**T3_ASV 26**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTCAGCGTCAGTATTGGGCCAGGTGGCCGCCTTCGCCACTGATGTTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCCACCACCCTCTCCCATACTCTAGTGCGCCGGTTTCCACCGCCATTCCCAGGTTGAGCCCGGGGATTTCACGACAGACCTAACGTACCGCCTACGCACCCTTTACGCCCAGTGATTCCGATTAACGCTTGCACCCCCCGTGTTACCGC	*Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobacillales;Acidithiobacillaceae;Acidithiobacillus*	**T3_ASV 31**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTCAGCGTCAGTATTGGGCCAGGTGGCCGCCTTCGCCACTGATGTTCCTCCAGATATCTACGCATTTCACCGCTACACCTGGAATTCCACCACCCTCTCCCATACTCTAGTGCGCCGGTTTCTACCGCCATTCCCAGGTTGAGCCCGGGGATTTCACGACAGACCTAACGTACCGCCTACGCACCCTTTACGCCCAGTGATTCCGATTAACGCTTGCACCCCCC	*Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobacillales;Acidithiobacillaceae;Acidithiobacillus*	**T3_ASV 35**
TCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTCAGCGTCAGTATTGGGCCAGGTGGCCGCCTTCGCCACTGATGTTCCTCCAGATATCTACGCATTTCACCGCTACACCTGGAATTCCACCACCCTCTCCCATACTCTAGTGCGCCGGTTTCTACCGCCATTCCCAGGTTGAGCCCGGGGATTTCACGACAGACCTAACGTACCGCCTACGCACCCTTTACGCCCAGTGATTCCGATTAACGCTTGCACCCCCCGTATTACCGC	*Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobacillales;Acidithiobacillaceae;Acidithiobacillus*	**T3_ASV 84**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTATTGGGCCAGGTGACCGCCTTCGCCACTGGTGTTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCCATCACCCTCTCCCATACTCCAGTCAGCCCGTTTCCACCGCCATTCCCAGGTTGAGCCCGGGGATTTCACGGCAGACGTAACCCACCGCCTACGCGCCCTTTACGCCCAGTAATTCCGATTAACGCTCGCACCCTCC	*Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobacillales;Acidithiobacillaceae;Acidithiobacillus*	**T3_ASV 11**

TTCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTCAGCGTCAGTATTGGGCCAGGTGGCCGCCTTCGCCACTGATGTTCCTCCAG ATCTCTACGCATTTCACCGCTACACCTGGAATTCCACCACCCTCTCCCATACTCTAGTCCGCCGGTTTCCACCGCCATTCCCAGGTT GAGCCCGGGGATTTCACGACAGACCTAACGTACCGCCTACGCACCCTTTACGCCCAGTGATTCCGATTAACGCTTGCACCCCCC	*Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobacillales;Acidithiobacillaceae;Acidithiobacillus*	**T3_ASV 134**
TCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTCAGCGTCAGTATTGGGCCAGGTGGCCGCCTTCGCCACTGATGTTCCTCCAGA TCTCTACGCATTTCACCGCTACACCTGGAATTCCACCACCCTCTCCCATACTCTAGTGCGCCGGTTTCCACCGCCATTCCCAGGTT GAGCCCGGGGATTTCACGACAGACCTAACGTACCGCCTACGCACCCTTTACGCCCAGTGATTCCGATTAACGCTTGCACCCCCC	*Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobacillales;Acidithiobacillaceae;Acidithiobacillus*	**T3_ASV 62**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTCAGCGTCAGTATTGGGCCAGGTGGCCGCCTTCGCCACTGATGTTCCTCCAG ATCTCTACGCATTTCACCGCTACACCTGGAATTCCACCACCCTCTCCCATACTCTAGTCCGCCGGTTTCCACCGCCATTCCCAGGTT GAGCCCGGGGATTTCACGACAGACCTAACGTACCGCCTACGCACCCTTTACGCCCAGTGATTCCGATTAACGCTTGCACCCCCCG TATTACCGC	*Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobacillales;Acidithiobacillaceae;Acidithiobacillus;Acidithiobacillus ferridurans*	**T3_ASV 12**
TTCTAATCCTGTTTGCTCCCCCCGCTTTCGTGCCTCAGCGTCAGTATTGGGCCAGGTGGCCGCCTTCGCCACTGATGTTCCTCCAG ATCTCTACGCATTTCACCGCTACACCTGGAATTCCACCACCCTCTCCCATACTCTAGTCCGCCGGTTTCCACCGCCATTCCCAGGTT GAGCCCGGGGATTTCACGACAGACCTAACGTACCGCCTACGCACCCTTTACGCCCAGTGATTCCGATTAACGCTTGCACCCCCCG TATTACCGC	*Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobacillales;Acidithiobacillaceae;Acidithiobacillus;Acidithiobacillus ferridurans*	**T3_ASV 151**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTATTGGGCCAGGTGACCGCCTTCGCCACTGATGTTCCTCCAG ATCTCTACGCATTTCACCGCTACACCTGGAATTCCATCACCCTCTCCCATACTCTAGTCCGCCCGTTTCCACCGCCATTCCCAGGTT GAGCCCGGGGATTTCACGGCAGACGTAACAAACCGCCTACGCGCCCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCC GTATTACCGC	*Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobacillales;Acidithiobacillaceae;Acidithiobacillus;*uncultured *Acidithiobacillus* sp.	**T3_ASV 32**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTATTGGGCCAGGTGACCGCCTTCGCCACTGATGTTCCTCCAG ATCTCTACGCATTTCACCGCTACACCTGGAATTCCATCACCCTCTCCCATACTCTAGTCCGCCCGTTTCCACCGCCATTCCCAGGTT GAGCCCGGGGATTTCACGGCAGACGTAACAAACCGCCTACGCGCCCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCC	*Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobacillales;Acidithiobacillaceae;Acidithiobacillus;*uncultured *Acidithiobacillus* sp.	**T3_ASV 54**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTATTGGGCCAGGTGACCGCCTTCGCCACTGATGTTCCTCCAG ATCTCTACGCATTTCACCGCTACACCTGGAATTCCATCACCCTCTCCCATACTCTAGTCCACCCGTTTCCACCGCCATTCCCAGGTT GAGCCCGGGGATTTCACGGCAGACGTAACAAACCGCCTACGCGCCCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCC	*Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobacillales;Acidithiobacillaceae;Acidithiobacillus;*uncultured *Acidithiobacillus* sp.	**T3_ASV 23**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTATTGATCCAGGGAGCCGCCTTCGCCACTGGTGTTCCTCCCG ATATCTACGCATTTCACCGCTACACCGGGAATTCCACTCCCCTCTATCATACTCAAGCCGGACAGTATCGACTGCACTTCCCAGGT TAAGCCCGGGGCTTTCACAACCGACTTATTCAACCGCCTACGTGCGCTTTACGCCCAGTAATTCCGATTAACGCTCGCACCCCCC	*Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobacillales;Acidithiobacillaceae;*RCP1-48;uncultured *Acidithiobacillus* sp.	**T3_ASV 214**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGTGCATGAGCGTCAGTGTTATCCCAGGGGGCTGCCTTCGCCATTGGTATTCCTCCAA ATCTCTACGCATTTCACTGCTACACTTGGAATTCTACCCCCCTCTGACACACTCTAGTCTTACAGTTTCAAACGCAGTTCCCAAGTT GAGCTCGGGGATTTCACATCTGACTTATAAAACTGCCTGCGCACGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTAC	*Bacteria;Proteobacteria;Gammaproteobacteria;Burkholderiales;Methylophilaceae;*OM43 clade	**T3_ASV 174**
TTCTAATCCTGTTCGCTCCCCACGCTTTCGTTCCTCAGCGTCAGTATCTGTCCAGGTGGCCGCCTTCGCCACTGATGTTCCTTCCAA TCTCTACGCATTTCACCGCTACACTGGAAATTCCACCACCCTCTACAGTACTCTAGCCTAACAGTTCAAAATGCAGTTCCAAGGTT GAGCCCTGGGCTTTCACATCTTGCTTATTAAACCGCCTACGAACGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCCTC	*Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Colwelliacea*	**T3_ASV 197**

	*e;Colwellia;*uncultured marine bacterium	
TTCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTGAACGTCGGTGTCGGACCAGGAAGCCGCTTTCGCCACTGGTGTTCCTTCCG ATATCTACGCATTTCACCGCTACACCGGAAATTCCGCTTCCCTCTTCCGCACCCTAGCCGTGCAGTCTCGAATGCAATTCCCAGGT TGAGCCCGGGGCTTTCACACCCGACTTACACGACCGTCTACGCACGCTTTACGCCCAGTAATTCCGATTAACGCTCGCACCCTCT GTATTACCGC	*Bacteria;Proteobacteria;Gammaproteobacteria;Gammaproteobacteria Incertae Sedis;*Unknown Family*;Acidibacter*	**T3_ASV 27**
TCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTGAACGTCGGTGTCGGACCAGGAAGCCGCTTTCGCCACTGGTGTTCCTTCCG ATATCTACGCATTTCACCGCTACACCGGAAATTCCGCTTCCCTCTTCCGCACCCTAGCCGTGCAGTCTCGAATGCAATTCCCAGGT TGAGCCCGGGGCTTTCACACCCGACTTACACGACCGTCTACGCACGCTTTACGCCCAGTAATTCCGATTAACGCTCGCACCCTCT GTATTACCGC	*Bacteria;Proteobacteria;Gammaproteobacteria;Gammaproteobacteria Incertae Sedis;*Unknown Family*;Acidibacter;*uncultured gamma proteobacterium	**T3_ASV 153**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTGAACGTCGGTGTCGGACCAGGAAGCCGCTTTCGCCACTGGTGTTCCTTCCG ATATCTACGCATTTCACCGCTACACCGGAAATTCCGCTTCCCTCTTCCGCACCCTAGCCGTGCAGTCTCGAATGCAATTCCCAGGT TGAGCCCGGGGCTTTCACACCCGACTTACACGACCGTCTACGCACGCTTTACGCCCAGTAATTCCGATTAACGCTCGCACCCTCT	*Bacteria;Proteobacteria;Gammaproteobacteria;Gammaproteobacteria Incertae Sedis;*Unknown Family*;Acidibacter;*uncultured gamma proteobacterium	**T3_ASV 208**
TCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTGAACGTCGGTGTCGGACCAGGAAGCCGCTTTCGCCACTGGTGTTCCTTCCG ATATCTACGCATTTCACCGCTACACCGGAAATTCCGCTTCCCTCTTCCGCACCCTAGCCGAGCAGTCTCGAATGCAATTCCCAGGT TGAGCCTGGGGCTTTCACACCCGACTTGCACGACCGTCTACGCACGCTTTACGCCCAGTAATTCCGATTAACGCTCGCACCCTCT	*Bacteria;Proteobacteria;Gammaproteobacteria;Gammaproteobacteria Incertae Sedis;*Unknown Family*;Acidibacter;*uncultured *Sinobacteraceae* bacterium	**T3_ASV 163**
TTCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTATCAGTCCAGGTGGTCGCCTTCGCCACTGATGTTCCTTCCA ATCTCTACGCATTTCACCGCTACACTGGAAATTCCACCACCCTCTTCTGTACTCTAGCTTGCCAGTTCGAAATGCAATTCCAAGGT TGAGCCCTGGGCTTTCACATCTCGCTTAACAAACCGCCTACGCGCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCC	*Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Cellvibrionaceae;*uncultured gamma proteobacterium	**T3_ASV 107**
TCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTATCAGTCCAGGTGGTCGCCTTCGCCACTGATGTTCCTTCCAA TCTCTACGCATTTCACCGCTACACTGGAAATTCCACCACCCTCTTCTGTACTCTAGCTTGCCAGTTCGAAATGCAATTCCAAGGTT GAGCCCTGGGCTTTCACATCTCGCTTAACAAACCGCCTACGCGCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCC	*Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Cellvibrionaceae;*uncultured gamma proteobacterium	**T3_ASV 189**
TCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTATCAGTCCAGTGAGTCGCCTTCGCCACTGATGTTCCTTCCTAT ATCTACGCATTTCACCGCTACACAGGAAATTCCACTCACCTCTACTGTACTCTAGTCAGACAGTTCTGGATGCAATTCCCAGGTTG AGCCCAGGGCTTTCACATCCAGCTTATCAAACCGCCTACGCGCGCTTTACGCCCAGTAATTCCGATTAACGCTCGCACCCTCC	*Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Porticoccaceae;Porticoccus*	**T3_ASV 203**
TTCTAATCCTATTTGATCCCCACACTTTCGCGCCTCAGCGTCAATCTCGGCATAGACAGCTGCCTTCGCCTTTGGTGTTCCTTCCCA TATCTATGCATTCCACCGCTACACAGGAAATTCCGCTGTCTTCCACCAGATTCTAGCCAACCGGTTCTGAATGCCTTTCTGGAGTT GAGCCCCAGTCTTTAACACTCAGCCTAATCGGCCGCCTACACGCCCTTTACGCCCAGTAATTCCGAATAACGCTCGTCCCGTAC	*Bacteria;Spirochaetota;Brevinematia;Brevinematales;Brevinemataceae;Brevinema;*uncultured *spirochete*	**T3_ASV 190**

Table 8: ASV sequences detected in aerobic enrichment cultures at Timepoint 7. Detected through the use of 16S rRNA V4 primers. Sequences were analysed using QIIME. The libraries were demultiplexed using the different barcodes from the sequences after which SILVA version 138 database was used to classify the reads based on Operational Taxonomic Units (OTUs).

ASV sequence	**Lineage**	**ASV ID**
TTCTAATCCGATTCGTTCTCCTAGCCTTCGCTCCTCACCGTCGGATGCGTTCTGGTCAAGCGCCTTCGCCACCGTTAGTCCTTATAGGA TTACAGGATTTTACCCCTCCCCTATAAGTACTCTTGACCTCACCCGCTCCCTAGTCAATGGGTATCTCATGCACGCATTGATGTTGAGC ACCAATATTTCACATAAGACGGCATTGACCGGCTACGAGCGCTTTAAGCCCAATAATCGTGGACACCACTTGTGCTGCGA	*Archaea ;*Micrarchaeota; Micrarchaeia; Micrarchaeales*; Candidatus* Micrarchaeum*; Candidatus* Micrarchaeota archaeon Mia14	ASV 25
TCTAATCCGATTCGTTCTCCTAGCCTTCGCTCCTCACCGTCGGATGCGTTCTGGTCAAGCGCCTTCGCCACCGTTAGTCCTTATAGGAT TACAGGATTTTACCCCTCCCCTATAAGTACTCTTGACCTCACCCGCTCCCTAGTCAATGGGTATCTCATGCACGCATTGATGTTGAGC ACCAATATTTCACATAAGACGGCATTGACCGGCTACGAGCGCTTTAAGCCCAATAATCGTGGACACCACTTGTGCTGCGA	*Archaea;* Micrarchaeota; Micrarchaeia; Micrarchaeales*; Candidatus* Micrarchaeum*; Candidatus* Micrarchaeota archaeon Mia14	ASV 80
TTGCTACCCTAGCCTTCGTTCCTTACCGTCAGATTCGTTCTAGTTAAACGCTTTCGCCACTGGTCGTCCTTCCGGGATTACAGGATTTT ACCCCTACCCTGAAAGTACGTTTAACCTCACCCGATCTCAAGTCTTGCAGTCTCTTCAGACTTTCTGGAGTTAAGCTTCAGGCTTTATC TGAAGATTTACAAAACCGGCTACGAACGCTTTAGGCTCAATAAAAGTGACCACTACTCGTGCTGCGGGT	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Ferroplasmaceae;Ferroplasma;Ferroplasma acidiphilum*	ASV 5
TCTAATCCGGTTTGCTACCCTAGCCTTCGTTCCTTACCGTCAGATTCGTTCTAGTTAAACGCTTTCGCCACTGGTCGTCCTTCCGGGAT TACAGGATTTTACCCCTACCCTGAAAGTACGTTTAACCTCACCCGATCTCAAGTCTTGCAGTCTCTTCAGACTTTCTGGAGTTAAGCTT CAGGCTTTATCTGAAGATTTACAAAACCGGCTACGAACGCTTTAGGCTCAATAAAAGTGACCACTACTCGTGCTGCGGGT	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Ferroplasmaceae;Ferroplasma;Ferroplasma acidiphilum*	ASV 8
TTGCTCCCCTAGCCTTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGGATTACAGGATTTT ACCCCTACCCCAGAAGTACATTCAACCTCACCCGGTCCCTAGATTTGCAGTCTCTCCAAAATTTCTTCAGTTAAGCAGAAGTATTTTCC TAGAGATTTACAAATCCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACTACCACTCGAGCCGCGGGT	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae;Cuniculiplasma;Cuniculiplasma divulgatum*	ASV 1
TCTAATCCGGTTTGCTCCCCTAGCCTTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGGAT TACAGGATTTTACCCCTACCCCAGAAGTACATTCAACCTCACCCGGTCCCTAGATTTGCAGTCTCTCCAAAATTTCTTCAGTTAAGCAG AAGTATTTTCCTAGAGATTTACAAATCCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACTACCACTCGAGCCGCGGGT	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae;Cuniculiplasma;Cuniculiplasma divulgatum*	ASV 3
TTCTAATCCGGTTTGCTCCCCTAGCCTTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGGA TTACAGGATTTTACCCCTACCCCAGAAGTACATTCAACCTCACCCGGTCCCTAGATTTGCAGTCTCTCCAAAATTTCTTCAGTTAAGCA GAAGTATTTTCCTAGAGATTTACAAATCCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACTACCACTCGAGCCGCGGGT	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae;Cuniculiplasma;Cuniculiplasma divulgatum*	ASV 2
TTTCTACTCCGGTTTTCTCCCCTAGCCTTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGG ATTACAGGATTTTACCCCTACCCCAGAAGTACATTCAACCTCACCCGGTCCCTAGATTTGCAGTCTCTCCAAAATTTCTTCAGTTAAGC AGAAGTATTTTCCTAGAGATTTACAAATCCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACTACCACTCGAGCCGCGGGT	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae;Cuniculiplasma;Cuniculiplasma divulgatum*	ASV 23

TTGCTACCCTAGCCCTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGGATTACAGGATTTTACCCCTACCCCGGAAGTACCTTCAACCTCACCCGGTCCCAAGTCTCGCAGTCTCTTCAGAAGATCATCTGTTAAGCAGAGGAATTTATCTGAAGATTTGCAAAACCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACCACCACTCGAGCTGCGGGT	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae*	ASV 17
TTCTAATCCGGTTTGCTACCCTAGCCCTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGGATTACAGGATTTTACCCCTACCCCGGAAGTACCTTCAACCTCACCCGGTCCCAAGTCTCGCAGTCTCTTCAGAAGATCATCTGTTAAGCAGAGGAATTTATCTGAAGATTTGCAAAACCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACCACCACTCGAGCTGCGGGT	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae*	ASV 49
TCTAATCCGGTTTGCTACCCTAGCCCTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGGATTACAGGATTTTACCCCTACCCCGGAAGTACCTTCAACCTCACCCGGTCCCAAGTCTCGCAGTCTCTTCAGAAGATCATCTGTTAAGCAGAGGAATTTATCTGAAGATTTGCAAAACCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACCACCACTCGAGCTGCGGGT	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae*	ASV 83
TTCTAATCCTGTTTGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACCGGCCCAGGCCACCGCCTTCGCCACCGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCGTGACCCCCTACCGGACTCTAGCCACGACAGTATCGGGTGCAATCTCCAGGTTGAGCCTGGAGTTTTCACACCCGACTTATCGAGCCGCCTACGAGCTCTTTACGCCCAATAAATCCGGACAACGCTCGCCCCCTAC	*Bacteria; Actinobacteriota; Acidimicrobiia*	ASV 42
TTCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACCGGCCCAGATCGCTGCCTTCGCCGTTGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCACGATCCTCTACCGGACTCTAGCCATAGCAGTATCGGATGGCGACTCCAGGTTAAGCCTGGAGATTTCACATCCGACTTGCTAAGCCGCCTACGAGCTCTTTACGCCCAATAAATCCGGACAACGCTTGCCCCCTAC	*Bacteria;Actinobacteriota;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae; Ferrimicrobium*	ASV 45
TTCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACCGGCCCAGATCGCTGCCTTCGCCGTTGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCACGATCCTCTACCGGACTCTAGCCATAGCAGTATCAGATGGCGACTCCAGGTTAAGCCTGGAGATTTCACATCTGACTTGCCAAGCCGCCTACGAGCTCTTTACGCCCAATAAATCCGGACAACGCTTGCCCCCTAC	*Bacteria;Actinobacteriota;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae; Ferrimicrobium*;uncultured bacterium	ASV 55
TTCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACCGGCCCAGATCGCTGCCTTCGCCGTTGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCACGATCCTCTACCGGACTCTAGCCATAGCAGTATCAGATGGCGACTCCAGGTTAAGCCTGGAGATTTCACATCTGACTTGCCAAGCCGCCTACGAGCTCTTTACGCCCAATAAATCCGGACAACGCTTGCCCCCGACGTCTTACCGCGGC	*Bacteria;Actinobacteriota;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae; Ferrimicrobium;*uncultured bacterium	ASV 93
TCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACCGGCCCAGCCCACCGCCTTCGCCACTGGTGTTCTTCCTGATATCTGCGCATTTCACCGCTACACCAGGAGTTCCGTGGGCCCCTACCGGACTCTAGCTATGACAGTATCGGATGGCGACTCCAGGTTGAGCCTGGAGGTTTCACATCCGACTTATCAAGCCGCCTACGAGCTCTTTACGCCCAATGAATCCGGACAACGCTCGCTCCCGAC	*Bacteria;Actinobacteriota;Acidimicrobiia;*IMCC26256	ASV 112
TTCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTGTTGGCCCAGACCACCGCCTTCGCCGCTGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCGTGGTCCCCTACCAAACTCTAGCCATGGCAGTATCGGATGGCGGCTCCAGGTTAAGCCTGGAGGTTTCACATCCGACTTGCCAAGCCGCCTACGAGCTCTTTACGCCCAATGAATCCGGACAACGCTCGCCCCCTAC	*Bacteria; Actinobacteriota; Acidimicrobiia*	ASV 29
TTCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACCGGCCCAGACCACCGCCTTCGCCACTGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCATGGTCCCCTACCGGACTCTAGCCATGACAGTATCGAGTGGCGACTCCAGGTTGAGCCTGGAGATTTCACACTTGACTTGTCAAGCCGCCTACGAGCTCTTTACGCCCAATGAATCCGGACAACGCTCGCCCCCTAC	*Bacteria; Actinobacteriota; Acidimicrobiia;*	ASV 44
TTCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTTCCGGCCCAGACCACCGCCTTCGCCACTGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCATGGTCCCCTACCGGACTCTAGCCATAGCAGTATCGAGTGGCGACTCCAGGTTGAGCCTGGAGATTTCACACCCGACTTGCTAAGCCGCCTACGAGCTCTTTACGCCCAATGAATCCGGACAACGCTCGCCCCCTAC	*Bacteria; Actinobacteriota; Acidimicrobiia*	ASV 47

TTCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGAACAGGCCCAGAGAACCGCCTTCGCCACCGGTGTTCCTCCCGATATCTGCGCATTTCACCGCTACACCGGGAATTCCGTTCTCCCCTGCCTGCCTCCAGCCTGCCCGTATCCACTGCACGCCCGGGGTTAAGCCCCGGGATTTCACAGCAGACGCGACAAGCCGCCTACGAGCTCTTTACGCCCAATAATTCCGGACAACGCTCGCGCCCTAC	*Bacteria;Actinobacteriota;Actinobacteria;Frankiales;Acidothermaceae;Acidothermus;*uncultured actinobacterium	ASV 39
TTCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGAACAGGCCCAGAGAACCGCCTTCGCCACCGGTGTTCCTCCCGATATCTGCGCATTTCACCGCTACACCGGGAATTCCGTTCTCCCCTGCCTGCCTCCAGCCTGCCCGTATCCACTGCACGCCCGGGGTTAAGCCCCGGGATTTCACAGCAGACGCGACAAGCCGCCTACGAGCTCTTTACGCCCAATAATTCCGGACAACGCTCGCGCCCGAC	*Bacteria;Actinobacteriota;Actinobacteria;Frankiales;Acidothermaceae;Acidothermus;*uncultured actinobacterium	ASV 134
TTCTAATCCTGTTTGCTCCCCACGCCTTCGCGCCTCAGCGTCAGGTTTCGTCCAGTCAGGCGCCTTCGCCACTGGTATTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCCCTGACCTCTCCGACCCTCAAGTCCACCCGTTTCCAAGGCCATCTCAGGGTTGAGCCCTGAACTTTCACCCCAGACGTGATAGACCGCCTGCGCGCGCTTTACGCCCAGTCATTCCGGACAACGCTTGCCCCCTAC	*Bacteria;Firmicutes;Bacilli;Alicyclobacillales;Alicyclobacillaceae;Acidibacillus;*uncultured low G+C Gram-positive bacterium	ASV 30
TCTAATCCTATTTGCTCCCCACACTTTCGAGCCTAAGCGTCAGTTATAGCCTAAGTTTTCGCCTTCGCCTCTGGTGTTCTTCCATATATCTACGCATTCCACCGCTCCACATGGAGTTCCAAAACTCTCTACTATACTCTAGACTTACAGTTTCCAATGCAAACAACCGTTAAGCAGTTGTCTTTAACATCAGACTTGTAAATCCGCCTGCGCTCGCTTTACGCCCAGTAAATCCGGATAACGCTTGCATCCTAT	*Bacteria;Firmicutes;Bacilli;Mycoplasmatales;Mycoplasmataceae;Mycoplasma*	ASV 108
TTCTAATCCTATTTGCTCCCCACACTTTCGAGCCTAAGCGTCAGTTATAGCCTAAGTTTTCGCCTTCGCCTCTGGTGTTCTTCCATATATCTACGCATTCCACCGCTCCACATGGAGTTCCAAAACTCTCTACTATACTCTAGACTTACAGTTTCCAATGCAAACAACCGTTAAGCAGTTGTCTTTAACATCAGACTTGTAAATCCGCCTGCGCTCGCTTTACGCCCAGTAAATCCGGATAACGCTTGCATCCTAT	*Bacteria;Firmicutes;Bacilli;Mycoplasmatales;Mycoplasmataceae;Mycoplasma*	ASV 139
TTCTAATCCTGTTTGATCCCCACGCTTTCGCACATCAGCGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTGCGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGACTTTCACATCAGACTTAAGAAACCGCCTACGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTAC	*Bacteria;Firmicutes;Bacilli;Staphylococcales;Staphylococcaceae;Staphylococcus*	ASV 132
TCTAATCCTGTTTGCTACCCACGCTTTCGAGCCTCAGCGTCAGTTAGAGCCCAGTAAACCGCCTTCGCCACTGGTGTTCCTCCTAATATCTACGCATTTCACCGCTACACTAGGAATTCCGCTTACCTCTACTCCACTCAAGAGAAATAGTTTTGAACGCTGCTATCGGTTGAGCCGATAGTTTTAACATTCAACTTATTCCCCCGCCTACGCTCCCTTTACACCCAGTAATTCCGGACAACGCTTGCCACCTAC	*Bacteria;Firmicutes;Clostridia;Oscillospirales;Ruminococcaceae;Harryflintia*	ASV 76
TCTAATCCCGTTCGCTCCCCACGCTGTCGCGCCTCAGCGTCAGGGTCAGGCCAGTGCACCGCCTTCGCCACCGGTGTTCTTCCTGATCTCTACGCATTTCACCGCTCCACCAGGAATTCCATGCACCTCTCCTGCCCTCCAGCCGATCCGTTTAGCCGCCCTCCGTCGGGTGAGCCGACGGCTGAAAACCGCTACGTGACCGGCCGCCTACACGCCCTTTACGCCCAGTCATTCCGGACAACGCTCGCCCCCTAC	*Bacteria;Firmicutes;Sulfobacillia;Sulfobacillales;Sulfobacillaceae;Sulfobacillus*	ASV 46
TTCTAATCCCGTTCGCTCCCCACGCTGTCGCGCCTCAGCGTCAGGGTCAGGCCAGTGCACCGCCTTCGCCACCGGTGTTCTTCCTGATCTCTACGCATTTCACCGCTCCACCAGGAATTCCATGCACCTCTCCTGCCCTCCAGCCGATCCGTTTAGCCGCCCTCCGTCGGGTGAGCCGACGGCTGAAAACCGCTACGTGACCGGCCGCCTACACGCCCTTTACGCCCAGTCATTCCGGACAACGCTCGCCCCCTAC	*Bacteria;Firmicutes;Sulfobacillia;Sulfobacillales;Sulfobacillaceae;Sulfobacillus*	ASV 99
TTCTAATCCTGTTTGCTCCCCACGCTTTCGTCTCTCAGTGTCAGGCCTGTTCCAGGAAGCCGCCTTCGCCACCGGCCTTCCTTCTGATATCTACGCATTTCACCGCTACACCAGAAATTCCGCTTCCCTCTCCCAGCCTCCAGTCTGCCAGTCTCTTTGGCATTCCCATGGTTAAGCCACGGCCTTTCACCAAAGACTTGACAAACCACCTACAGACTCTTTACGCCCAGTAACTCCGAACAACGCTTGCCACCTCT	*Bacteria;Nitrospirota;Leptospirillia;Leptospirillales;Leptospirillaceae;Leptospirillum;Leptospirillum ferriphilum*	ASV 19
TCTAATCCTGTTTGCTCCCCACGCTTTCGTCTCTCAGTGTCAGGCCTGTTCCAGGAAGCCGCCTTCGCCACCGGCCTTCCTTCTGATATCTACGCATTTCACCGCTACACCAGAAATTCCGCTTCCCTCTCCCAGCCTCCAGTCTGCCAGTCTCTTTGGCATTCCCATGGTTAAGCCACGGCCTTTCACCAAAGACTTGACAAACCACCTACAGACTCTTTACGCCCAGTAACTCCGAACAACGCTTGCCACCTCT	*Bacteria;Nitrospirota;Leptospirillia;Leptospirillales;Leptospirillaceae;Leptospirillum;Leptospirillum ferriphilum*	ASV 36

TTCTAATCCTGTTTGCTCCCCACGCTTTCGTCTCTCAGTGTCAGGCCTGTTCCAGGAAGCCGCCTTCGCCACCGGCCTTCCTTCTGATA TCTACGCATTTCACCGCTACACCAGAAATTCCGCTTCCCTCTCCCAGCCTCCAGTCTGCCAGTCTCTTTGGCATTCCCATGGTTAAGCC ACGGCCTTTCACCAAAGACTTGACAAACCACCTACAGACTCTTTACGCCCAGTAACTCCGAACAACGCTTGCCACCGC	*Bacteria;Nitrospirota;Leptospirillia;Leptospirillales;Leptospirillaceae;Leptospirillum;Leptospirillum ferriphilum*	ASV 98
TTCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTAAAGGACCAGGTCGCCGCCTTCGCCACCGGTGTTCTTCCCAAT ATCTACGAATTTCACCTCTACACTGGGAATTCCACGACCCTCTTCCTCACTCAAGTCTACACGTATCAAGCGCAGTCCCCAGGTTGAG CCCAGGAATTTCACGCCTGACTATATAAACCGCCTACGCGCCCTTTACGCCCAGTCATTCCGAGCAACGCTAGCCCCCTTC	*Bacteria;Proteobacteria;Alphaproteobacteria;Acetobacterales;Acetobacteraceae;*uncultured	ASV 34
TTCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTACCGAGCCAGTGAGCCGCCTTCGCCACTGGTGTTCTTTCCAATA TCTATGAATTTCACCTCTACACTGGAAATTCCACTCACCTCTCTCGGTCTCTAGACTGACAGTATCAAAGGCAGTTCCAGGGTTGAGC CCTGGGATTTCACCTCTGACTAATCAATCCGCCTACGCGCGCTTTACGCCCAGTAATTCCGAACAACGCTAGCTCCCTCC	*Bacteria;Proteobacteria;Alphaproteobacteria;Parvibaculales;Parvibaculaceae;*Mf105b01;uncultured *bacterium*	ASV 137
TTCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTCAGCGTCAGTATTGGGCCAGGTGGCCGCCTTCGCCACTGATGTTCCTCCAGAT CTCTACGCATTTCACCGCTACACCTGGAATTCCACCACCCTCTCCCATACTCTAGTCCGCCGGTTTCCACCGCCATTCCCAGGTTGAGC CCGGGGATTTCACGACAGACCTAACGTACCGCCTACGCACCCTTTACGCCCAGTGATTCCGATTAACGCTTGCACCCCCC	*Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobacillales;Acidithiobacillaceae;Acidithiobacillus*	ASV 35
TCTACTCCTGTTTTCTCCCCACGCTTTCGTGCCTCAGCGTCAGTATTGGGCCAGGTGGCCGCCTTCGCCACTGATGTTCCTCCAGATCT CTACGCATTTCACCGCTACACCTGGAATTCCACCACCCTCTCCCATACTCTAGTGCGCCGGTTTCCACCGCCATTCCCAGGTTGAGCC CGGGGATTTCACGACAGACCTAACGTACCGCCTACGCACCCTTTACGCCCAGTGATTCCGATTAACGCTTGCACCCCCC	*Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobacillales;Acidithiobacillaceae;Acidithiobacillus*	ASV 130
TCTAATCCTATTTGATCCCCACACTTTCGCGCCTCAGCGTCAATCTCGGCATAGACAGCTGCCTTCGCCTTTGGTGTTCCTTCCCATAT CTATGCATTCCACCGCTACACAGGAAATTCCGCTGTCTTCCACCAGATTCTAGCCAACCGGTTCTGAATGCCTTTCTGGAGTTGAGCC CCAGTCTTTAACACTCAGCCTAATCGGCCGCCTACACGCCCTTTACGCCCAGTAATTCCGAATAACGCTCGTCCCTTAC	*Bacteria;Spirochaetota;Brevinematia;Brevinematales;Brevinemataceae;Brevinema;*uncultured *spirochete*	ASV 122

Table 9: ASV sequences detected in fungal enrichment cultures. Detected through the use of ITS primers. Sequences were analysed using QIIME. The libraries were demultiplexed using the different barcodes from the sequences after which SILVA version 138 database was used to classify the reads based on Operational Taxonomic Units (OTUs).

ASV sequence	Lineage	ASV ID
CCAAGAGATCCGTTGTTGAAAGTTTTAACTGATTATGATAATCAAACTCAGACTGCATACTTTCAGAACAGCGTTCATGTTGGGGTCTTCG GCGGGCGCGGGCCCGGGGGCGCGAGGGCCTCCCCGGCGGCCGTCGAAACGGCGGGCCCGCCGAAGCAACAAGGTACAATAGACACG GGTGGGAGGTTGGACCCAGAGGGCCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTAC	k__*Fungi*;p__*Ascomycota*;c__*Eurotiomycetes*;o__*Eurotiales*;f__*Aspergillaceae*;g__*Aspergillus*	ITS_ASV 1
CCAAGAGATCCGTTGTTGAAAGTTTTAACTGATTATGATAATCAAACTCAGACTGCATACTTTCAGAACAGCGTTCATGTTGGGGTCTTCG GCGGGCGCGGGCCCGGGGGCGCGAGGGCCTCCCCGGCGGCCGTCGAAACGGCGGGCCCGCCGAAGCAACAAGGTACAATAGACACG GGTGGGAGGTTGGACCCAGAGGGCCCGCACTCGGTAATGATCCTTCCGCAGGGTCACCTACGGAAACCTTGTTAC	k__*Fungi*;p__*Ascomycota*;c__*Eurotiomycetes*;o__*Eurotiales*;f__*Aspergillaceae*;g__*Aspergillus*	ITS_ASV 2
CCCAGAGATCCGTTGTTGAAAGTTTTAACTGATTATGATCATCAAACTCAGACTGCATACTTTC	k__*Fungi*;p__*Ascomycota*;c__*Eurotiomycetes*;o__*Eurotiales*;f__*Aspergillaceae*;g__*Aspergillus*	ITS_ASV 3
CCCAGAGATCCGTTGTTGAAAGTTTTAACTGATTATGATAATCAAACTCAGACTGCATACTTTCAGAACAGCGTTCATGTTGGGGTCTTCG GCGGGCGCGGGCCCGGGGGCGCGAGGGCCTCCCCGGCGGCCGTCGAAACGGCGGGCCCGCCGAAGCAACAAGGTACAATAGACACG GGTGGGAGGTTGGACCCAGAGGGCCCGCACTCGGTAATGATCCTTCCGCAGGGTCACCTACGGAAACCTTGTTAC	k__*Fungi*;p__*Ascomycota*;c__*Eurotiomycetes*;o__*Eurotiales*;f__*Aspergillaceae*;g__*Aspergillus*	ITS_ASV 4
CCAAGAGATCCGTTGTTGAAAGTTTTAACTGATTATGATAATCAAACTCAGACTGCATACTTTCAGAACAGCGTTCATGTTGGGGTCTTCG GCGGGCGCGGGCCCGGGGGCGCGAGGGCCTCCCCGGCGGCCGTCGAAACGGCGGGCCCGCCGAAGCAACAAGGTACAATAGACACG GGTGGGAGGTTGGACCCAGAGGGCCCGCACTCGGTAATGATCCTTCCGCAGGGTCACCTACGGAAACCGTGTTAC	k__*Fungi*;p__*Ascomycota*;c__*Eurotiomycetes*;o__*Eurotiales*;f__*Aspergillaceae*;g__*Aspergillus*	ITS_ASV 5
CCAAGAGATCCGTTGTTGACAGTTTTAACTGATTATGATCATCAAACTCAGACTGCATACTTTCAGAACAGCGTTCATGTTGGGGTCTTCG GCGGGCGCGGGCCCGGGGGCGCGAGGGCCTCCCCGGCGGCCGTCGAAACGGCGGGCCCGCCGAAGCAACAAGGTACAATAGACACG GGTGGGAGGTTGGACCCAGAGGGCCCGCACTCGGTAATGATCCTTCCGCAGGGTCACCTACGGAAACCTTGTTACGACTTTTACTTCC	k__*Fungi*;p__*Ascomycota*;c__*Eurotiomycetes*;o__*Eurotiales*;f__*Aspergillaceae*;g__*Aspergillus*	ITS_ASV 6
CCACGAGATCCGTTGTTGACAGTTTTAACTGATTATGATAATCAAACTCAGACTGCATACTTTCAGAACAGCGTTCATGTTGGGGTCTTCG GCGGGCGCGGGCCCGGGGGCGCGAGGGCCTCCCCGGCGGCCGTCGAAACGGCGGGCCCGCCGAAGCAACAAGGTACAATAGACACG GGTGGGAGGTTGGACCCAGAGGGCCCGCACTCGGTAATGATCCTTCCGCAGGGTCACCTACGGAAACCTTGTTACGACTTTTACTTCC	k__*Fungi*;p__*Ascomycota*;c__*Eurotiomycetes*;o__*Eurotiales*;f__*Aspergillaceae*;g__*Aspergillus*	ITS_ASV 7
CCAAGAGATCCGTTGTTGAACGTTTTAACTGATTATGATACTCAAACTCAGACTGCATACTTTCAGAACAGCGTTCATGTTGGGGTCTTCG GCGGGCGCGGGCCCGGGGGCGCGAGGGCCTCCCCGGCGGCCGTCGAAACGGCGGGCCCGCCGAAGCAACAAGGTACAATAGACACG GGTGGGAGGTTGGACCCAGAGGGCCCGCACTCGGTAATGATCCTTCCGCAGGGTCACCTACGGAAACCTTGTTACGACTTTTACTTCC	k__*Fungi*;p__*Ascomycota*;c__*Eurotiomycetes*;o__*Eurotiales*;f__*Aspergillaceae*;g__*Aspergillus*	ITS_ASV 8
CCACGAGATCCGTTGTTGAAAGTTTTAACTGATTATGATACTCAAACTCAGACTGCATACTTTCAGAACAGCGTTCATGTTGGGGTCTTCG GCGGGCGCGGGCCCGGGGGCGCGAGGGCCTCCCCGGCGGCCGTCGAAACGGCGGGCCCGCCGAAGCAACAAGGTACAATAGACACG GGTGGGAGGTTGGACCCAGAGGGCCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTACTTCC	k__*Fungi*;p__*Ascomycota*;c__*Eurotiomycetes*;o__*Eurotiales*;f__*Aspergillaceae*;g__*Aspergillus*	ITS_ASV 9
CCAAGAGATCCGTTGTTGAAATTTTTAACTGATTATGATAATCAAACTCAGACTGCATACTTTCAGAACAGCGTTCATGTTGGGGTCTTCG GCGGGCGCGGGCCCGGGGGCGCGAGGGCCTCCCCGGCGGCCGTCGAAACGGCGGGCCCGCCGAAGCAACAAGGTACAATAGACACG GGTGGGAGGTTGGACCCAGAGGGCCCGCACTCGGTAATGATCCTTCCGCAGGGTCACCTACGGAAACCTTGTTAC	k__*Fungi*;p__*Ascomycota*;c__*Eurotiomycetes*;o__*Eurotiales*;f__*Aspergillaceae*;g__*Aspergillus*	ITS_ASV 10
CCCAGAGATCCGTTGTTGAAAGTTTTAACTGATTATGATAATCAAACTCAGACTGCATACTTTCAGAACAGCGTTCATGTTGGGGTCTTCG GCGGGCGCGGGCCCGGGGGCGCGAGGGCCTCCCCGGCGGCCGTCGAAACGGCGGGCCCGCCGAAGCAACAAGGTACAATAGACACG GGTGGGAGGTTGGACCCAGAGGGCCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCGTGTTAC	k__*Fungi*;p__*Ascomycota*;c__*Eurotiomycetes*;o__*Eurotiales*;f__*Aspergillaceae*;g__*Aspergillus*	ITS_ASV 11

CCACGAGATCCGTTGTTGAAAGTTTTAACTGATTATGATAATCAAACTCAGACTGCATACTTTCAGAACAGCGTTCATGTTGGGGTCTTCG GCGGGCGCGGGCCCGGGGGCGCGAGGGCCTCCCCGGCGGCCGTCGAAACGGCGGGCCCGCCGAAGCAACAAGGTACAATAGACACG GGTGGGAGGTTGGACCCAGAGGGCCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCGTGTTAC	k__*Fungi*;p__*Ascomycota*;c__*Eurotiomycetes*;o__*Eurotiales*;f__*Aspergillaceae*;g__*Aspergillus*	ITS_ASV 12
GAGCCAAGAGATCCATTGTTAAAAGTTGTCTTTGTTTATGGCACAAATAACTCTGTCAAACATACAAGCGTGAACATCGGAACCTTGCGG CCCCGTGCTAATGTGCCCTGTCAAAGTGCGAGCAGCAAGTGGAATACACTCACTCACCCACCACCCTCCCCCCAACAGAGCTCAATAATG ATTTGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTAC	k__*Fungi*	ITS_ASV 13

Table 10: ASV sequences detected in anaerobic enrichment cultures. Detected through the use of 16S V4 primers. Sequences were analysed using QIIME. The libraries were demultiplexed using the different barcodes from the sequences after which SILVA version 138 database was used to classify the reads based on Operational Taxonomic Units (OTUs).

ASV sequence	Taxon	ASV ID
TCTAATCCGGTTTGCTCCCCTAGCCTTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAG GATTACAGGATTTTACCCCTACCCCAGAAGTACATTCAACCTCACCCGGTCCCTAGATTTGCAGTCTCTCCAAAATTTCTTCAGTT AAGCAGAAGTATTTTCCTAGAGATTTACAAATCCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACTACCACTCGAGCCGCGG GTGTTACCGC	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae;Cuniculiplasma;Cuniculiplasma divulgatum*	AN_ASV 23
TTGCTCCCCTAGCCTTCGTTCCTCACCGTCGGATCCGTTCTAGTTGAACGCCTTCGCCACCGGTCGTCCTTCCAGGATTACAGGAT TTTACCCCTACCCCAGAAGTACATTCAACCTCACCCGGTCCCTAGATTTGCAGTCTCTCCAAAATTTCTTCAGTTAAGCAGAAGTA TTTTCCTAGAGATTTACAAATCCGGCTACAAACGCTTTAGGCTCAATAAAAGTGACTACCACTCGAGCCGCGGGT	*Archaea;Thermoplasmatota;Thermoplasmata;Thermoplasmatales;Thermoplasmataceae;Cuniculiplasma;Cuniculiplasma divulgatum*	AN_ASV 4
TTCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCTGAACGTCGGTGTCGGACCAGGAAGCCGCTTTCGCCACTGGTGTTCCTTCCG ATATCTACGCATTTCACCGCTACACCGGAAATTCCGCTTCCCTCTTCCGCACCCTAGCCGTGCAGTCTCGAATGCAATTCCCAGGT TGAGCCCGGGGCTTTCACACCCGACTTACACGACCGTCTACGCACGCTTTACGCCCAGTAATTCCGATTAACGCTCGCACCCTCT	*Bacteria; Proteobacteria; Gammaproteobacteria; Gammaproteobacteria Incertae Sedis*; Unknown Family; *Acidibacter*; uncultured gamma proteobacterium	AN_ASV 22
TTCTAATCCTGTTTGCTCCCCACGCTTTCGTCTCTCAGTGTCAGGACTGTTCCAGGAAGCCGCCTTCGCCACCGGCCTTCCTTCTG ATATCTACGCATTTCACCGCTACACCAGAAATTCCGCTTCCCTCTCCCAGCCTCCAGTCTGCCAGTCTCTTTGGCATTCCCATGGTT AAGCCACGGCCTTTCACCAAAGACTTGACAAACCACCTACAGACTCTTTACGCCCAGTAATTCCGAACAACGCTTGCCACCTCTG TCTTACCGC	*Bacteria;Nitrospirota;Leptospirillia;Leptospirillales;Leptospirillaceae;Leptospirillum;Leptospirillum ferrooxidans*	AN_ASV 86
TTCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTAAAGGACCAGGTCGCCGCCTTCGCCACCGGTGTTCTTCCCA ATATCTACGAATTTCACCTCTACACTGGGAATTCCACGACCCTCTTCCTCACTCAAGTCTACACGTATCAAGCGCAGTCCCCAGGT TGAGCCCAGGAATTTCACGCCTGACTATATAAACCGCCTACGCGCCCTTTACGCCCAGTCATTCCGAGCAACGCTAGCCCCCTTC	*Bacteria; Proteobacteria; Alphaproteobacteria; Acetobacterales; Acetobacteraceae*; uncultured	AN_ASV 30
TCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTAAAGGACCAGGTCGCCGCCTTCGCCACCGGTGTTCTTCCCAA TATCTACGAATTTCACCTCTACACTGGGAATTCCACGACCCTCTTCCTCACTCAAGTCTACACGTATCAAGCGCAGTCCCCAGGTT GAGCCCAGGAATTTCACGCCTGACTATATAAACCGCCTACGCGCCCTTTACGCCCAGTCATTCCGAGCAACGCTAGCCCCCTTC	*Bacteria; Proteobacteria; Alphaproteobacteria; Acetobacterales; Acetobacteraceae*; uncultured	AN_ASV 105
TTCTAATCCTATTTGCTCCCCACACTTTCGAGCCTAAGCGTCAGTTATAGCCTAAGTTTTCGCCTTCGCCTCTGGTGTTCTTCCATA TATCTACGCATTCCACCGCTCCACATGGAGTTCCAAAACTCTCTACTATACTCTAGACTTACAGTTTCCAATGCAAACAACCGTTA AGCAGTTGTCTTTAACATCAGACTTGTAAATCCGCCTGCGCTCGCTTTACGCCCAGTAAATCCGGATAACGCTTGCATCCTAT	*Bacteria; Firmicutes; Bacilli; Mycoplasmatales; Mycoplasmataceae; Mycoplasma*	AN_ASV 138