# Metagenome-assembled genomes reveal many novel microbial lineages in the geothermal springs of the subantarctic Kerguelen Islands

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## Abstract

The Kerguelen Islands, located in the southern part of the Indian Ocean, are very isolated geographically. They have been the subject of very few microbiological investigations. In particular, their microbial diversity has never been analyzed with high-throughput sequencing methods and no sequencing studies of the genomes of the microbial communities have been performed. In this article we performed the first metagenomics analysis of microorganisms present in Kerguelen hot springs. From four different hot springs, we assembled metagenomes and recovered 42 metagenome-assembled genomes, mostly associated with new taxa. Bacterial and archaeal MAGs were studied in details and showed affiliations to new species, genera, families and orders. Metabolic predictions from MAGs suggest the presence of heterotrophs and primary producers involved in the sulfur cycle. This paper, which focuses on only four of the dozens of hot springs in the Kerguelen Islands, is a preliminary study of the microorganisms, particularly thermophiles, inhabiting the hot springs of these insulated islands. These results show that more efforts should be made to better understand these ecosystems as they represent a reservoir of unknown microbial lineages and potential new metabolic pathways.

## Introduction

Terrestrial hot springs are found all over the world, on all continents, and are abundant in areas of volcanic activity such as Iceland, Japan, Russia, Chile, Algeria or New Zealand [1–2]. With possible exception in the polyextreme Dallol area [3], all studied geothermal environments harbor microbial cohorts. Microbial cohorts in terrestrial hot springs are often composed of *Bacteria* belonging to *Aquificae, Chloroflexi, Deinococcus-Thermus* and *Thermotogae*, and *Archaea* belonging to *Desulfurococcaceae, Thermoproteaceae* and *Thermococcaceae* [4–6]. Most of terrestrial hot springs, like those of Yellowstone (USA) or Kamchatka (Russia) areas, have been the subject of extensive microbiological investigations, including the study of the microbial community composition, the isolation and physiological characterization of microorganisms, the investigation of adaptive mechanisms of indigenous taxa, and the mining of extremophilic species for potential enzymes, activities or molecules of biotechnological interest (*e.g.* [7–12]). Studies of bacteria and archaea living in geothermal systems are essential for our knowledge of the history of life, as these environments are early Earth analogs and one of the possible cradles of life [2, 6, 13, 14]. The microbial communities of hot springs in the polar regions are partly different in their composition. For example, the fumaroles of Deception Island (Antarctica) contain prokaryotic taxa belonging to *Verrucomicrobia*, *Proteobacteria*, *Planctomycetes*, *Patescibacteria*, *Firmicutes*, *Chloroflexi*, *Calditrichaeota*, *Bacteroidetes*, *Thaumarchaeota*, *Nanoarchaeota*, *Euryarchaeota* and *Crenarchaeota* [15–17].

The volcanic Kerguelen Archipelago, which is part of the French Southern and Antarctic Lands, is situated in the southern part of the Indian Ocean (49°S, 69°E). Located at 3300 km from the first inhabited areas, it is amongst the most isolated islands from any continental landmass and contain a large part of the limited terrestrial habitats present at these latitudes. The Kerguelen islands have the status of protected areas and is an important UNESCO's world heritage site and belongs to the national nature reserve (NNR) of the French Southern Lands.

It forms the only emerged part, together with the active volcanic Heard and MacDonald Islands, of the vast Kerguelen oceanic Plateau. Kerguelen Archipelago is the third largest volcanic island complex in the world, after Iceland and Hawaii [18]. The last volcanic activity, dated 26 ± 3 Ka, took place on the Rallier du Baty (RB) Peninsula in the south-western part of the Kerguelen Islands [19]. Current volcanic activity, due to the Kerguelen hotspot, is evidenced by fumaroles, mud pots, hydrothermal discharges and small hot springs that rise from sea level to at least 300 m altitude. Those biotopes are charged with minerals, and their pH range from acidic to alkaline, under a wide range of temperature conditions from 35 to over 100°C. The geochemical properties of the most accessible parts of this system have been monitored more or less regularly over the last decades [20, 21]. These geothermal habitats represent unique biodiversity sanctuaries in very insulated polar environments. Preliminary investigations based on 16S rRNA gene amplicon cloning and sequencing revealed a diverse collection of microbial community lineages composed of *Proteobacteria*, *Deinococcus-Thermus*, *Chloroflexi*, *Firmicutes*, *Actinobacteria* or *Aquificae*, as well as *Euryarchaeota*, *Crenarchaeota* (*Thermoproteales, Desulfurococcales, Acidilobales, Sulfolobales*) and *Thaumarchaeota* [22, 23]. Those lineages are more or less distantly related to known taxa, which were partly different from those described in Antarctic geothermal sites but also from those usually observed in similar habitats. A small number of new species have also been isolated, enriched or highlighted through molecular approaches from these regions [22, 24]. Apart from these few studies, this area has not been subjected to any comprehensive microbiological investigation to date. The microbial diversity hosted in these hot springs remains largely unknown, as well as its functional (metabolism, physiology, adaptations) potential. Microbial communities might be shaped by the biogeographic position and the physicochemical parameters of the hot springs (temperature, pH, *in situ* chemistry), that probably exert a strong selective pressure on indigenous communities [17]. Yet, these geothermal springs represent undoubtedly unique diversity, and reservoirs of new functions and innovation.

In this study, we focused on four small geothermal hot springs from the Kerguelen Islands whose microbial communities have never been studied before. We analyzed the metagenomes of the hot springs RB10, RB13 and RB32, located on the "plateau des Fumerolles" at about 300 m altitude on the west coast of the Rallier du Baty Peninsula, and of the ephemeral spring RB108 which flows slightly above sea level into the riverbed of the Infernet glacier (located at the base of the plateau des Fumerolles), in order to study the taxonomic diversity and to predict its genetic potential (Fig. 1).

## Results And Discussion

### MAG binning and general features

From the four hot springs, we assembled four associated metagenomes and then binned a total of 42 MAGs. We recovered 12 MAGs from RB10 hot spring, 13 from RB13, 14 from RB32 and 3 from RB108. Out of these 42 MAGs, 7 were of high-quality, 25 of nearly-high quality, 9 of medium quality and 1 of low quality (Table 1) from metagenomics standards [25]. The GC% was quite variable, ranging from 25.76% to 70.35% among all MAGs and between 32.15% and 69.21% only among the high- and near high-quality MAGs. With the exception of RB108 from which we only recovered bacterial MAGs, we retrieved both bacterial and

archaeal MAGs in the other hot springs. Two thirds of the MAGs (26/42) were assigned to the domain *Bacteria* and the rest to the domain *Archaea* (Table 2). Accession numbers of the 4 metagenomes and 42 MAGs are given in Table 3.

### Taxonomic and phylogenomic analyses of MAGs

The taxonomic affiliation of the MAGs was investigated in details through GTDB-Tk (release 95) (Table 2) and through phylogenomic analyses (Figure S1 A-I). We also tried to classify MAGs on the basis of overall genome relatedness indices (OGRI), which is detailed in supplementary material (Text S1, Table S2, Figure S2).

For *Bacteria*, GTDB-Tk analyses allowed us to place the MAGs in the following clades: six in the phylum *Aquificota* from the four different springs, comprising four MAGs belonging to the genus *Hydrogenivirga* (family *Aquificaceae*) (RB10-MAG07, RB13-MAG10, RB32-MAG07, RB108-MAG02), and two belonging to the family '*Hydrogenobaculaceae'* (RB10-MAG12, RB32-MAG11) (Table 2, Figure S1A). Their closest cultured relatives originated either from hot springs or from deep-sea hydrothermal vents [26]. Three MAGs from three geothermal springs belonged to the phylum *Armatimonadota* (RB10-MAG03, RB13-MAG04, RB32-MAG03) and had no close cultured relatives. Seven MAGs have been classified into the phylum *Chloroflexota:* three MAGs belonging to the genus *Thermoflexus* from three different springs (RB10-MAG04, RB13-MAG05, RB32-MAG02), one affiliating with the genus *Thermomicrobium* (RB32-MAG08), one falling into the family *Ktedonobacteraceae* (RB108-MAG03) and one belonging to the class *Dehalococcoidia* (RB32-MAG04). Six MAGs from four various hot springs belonged to the phylum *Deinococcota,* and to the genera *Thermus* (RB10-MAG08, RB10-MAG11, RB13-MAG09, RB32-MAG10, RB108-MAG01) and *Meiothermus* (RB13-MAG13). One MAG belonged to the family '*Sulfurifustaceae'* (RB13-MAG01), in the phylum *Proteobacteria* (*Gamma*- class). The MAG referenced as RB32-MAG13 was classified into the phylum '*Patescibacteria'*, in the class '*Paceibacteria'*, and was distantly related to MAGs originating from groundwater and from hot springs. Finally, two MAGs from two different springs belonged to the phylum WOR-3, in the *Candidatus* genus 'Caldipriscus*'* (RB32-MAG12, RB10-MAG09).

For *Archaea*, almost all the MAGs reconstructed in this study, *e.g.* 15 of the 16 archaeal MAGs, belonged to the phylum *Thermoproteota*. Among them, four belonged to the genus *Ignisphaera* (RB10-MAG05, RB13-MAG08, RB13-MAG11, RB32-MAG05), three to the genus *Thermofilum* (RB10-MAG06, RB13-MAG03, RB32-MAG09), two to the genus *Zestosphaera* (RB10-MAG02, RB13-MAG06), three to the family *Acidilobaceae* (RB10-MAG01, RB13-MAG02, RB32-MAG01) and two to the class *Thermoprotea* (RB10-MAG10, RB32-MAG06). Additionally, one belonged to the order *Thermoproteales* (RB13-MAG07). Lastly, the MAG belonging to another phylum (RB13-MAG12) was affiliated with the '*Aenigmatarchaeota'*, class '*Aenigmatarchaeia'*, and was distantly related to MAGs from hot springs and from deep-sea hydrothermal vent sediments [27,28].

Out of these 42 MAGs, 23 corresponded to different taxa at the taxonomic rank of species or higher. Eighteen of them belonged to lineages with several cultivated representatives and were distributed respectively, into 1 known species called *Thermus thermophilus*, 10 new genomic species within the genera *Zestosphaera*, *Thermoflexus*, *Ignisphaera* (× 2), *Thermofilum*, *Hydrogenivirga*, *Thermus*, *Meiothermus*, *Caldipriscus* and *Thermomicrobium*, 5 putative new genera belonging to the families *Acidilobaceae*, '*Thermocladiaceae*', '*Sulfurifustaceae*', '*Hydrogenobaculaceae'* and *Ktedonobacteraceae,* and 2 putative new orders within the classes *Dehalococcoidia* and *Thermoprotea*. In addition, five MAGs belonged to lineages that are predominantly or exclusively known through environmental DNA sequences. They were classified as 1 new genomic species in the phylum *Armatimonadota*, 2 putative new genera in the classes '*Aenigmatarcheia'* and '*Paceibacteria',* and 1 putative new family in the phylum *Chloroflexota*. Thus, these 42 MAGs comprised a broad phylogenetic range of *Bacteria* and *Archaea* at different levels of taxonomic organization, of which a large majority were new.

The approaches implemented here were not intended to describe the microbial diversity present in these sources in an exhaustive way and to compare them in a fine way, and do not allow it because of storage. However, they do provide an overview of the microbial diversity effectively present. If we compare the phylogenetic diversity of the MAGs found in the 4 hot springs, we can observe that 3 shared phyla (*Deinococcota*, *Aquificota* and *Chloroflexota*: phyla names according to GTDB), 2 shared families (*Thermaceae* and *Aquificaceae*), and 2 shared genera (*Hydrogenivirga* and *Thermus*) were found among the four sources (Figure 2). In addition, hot springs RB10, RB13 and RB32, that are geographically close (< 60 m), also share 2 other phyla (*Thermoproteota* and *Armatimonadota*) and 5 other families in common (*Acidilobaceae*, *Ignisphaeraceae*, *Thermofilaceae, Thermoflexaceae*, and HRBIN17) (Figure 2). These phyla and families that are shared between sources are widespread lineages in terrestrial geothermal habitats (*e.g.* [4-6,12]). Phyla and families detected in the hot environments of Antarctica are also found here, such as *Patescibacteria* for example [15].

In summary, this metagenomic analysis highlighted the presence of bacterial and archaeal lineages commonly found in hot springs, and lineages found in hot habitats from polar areas (*e.g.* [4-6,15,29]). The microbial communities in these Kerguelen Islands hot springs were diverse, particularly in RB10, RB13, and RB32 hot springs. However, within these lineages that have been previously reported to occur in geothermal environments, a majority of the genomic taxa detected here were new, sometimes at a high taxonomic rank.

### Metabolic potential of MAGs

An extensive genomic characterization of the 42 MAGs has been performed to explore the metabolic pathways and the possible adaptations of the microbial populations from which these MAGs originate.

KEGG Decoder visualization highlighted various pathways associated with carbohydrate degradation, oxidative phosphorylation and sulfur, nitrogen, and amino-acid metabolisms, among others (Figure 3).

To confirm these initial metabolic predictions, further annotation was performed by combining data generated by Prokka with the MetaCyc database. Efforts have been directed at studying catabolic pathways, particularly those involving inorganic electron donors and acceptors. These results are not representative of the metabolic diversity of all the hot spring ecosystems studied, but they do reflect some of the microbial catabolisms likely to be used *in situ* to produce energy and, by assumption, the most abundant ones. Metabolic predictions are presented hereafter, at different taxonomic ranks.

MAGs belonging to the genus *Thermoflexus* (RB10-MAG04, RB13-MAG05, RB32-MAG02) encode pathways for carbon monoxide oxidation (*via* aerobic carbon monoxide dehydrogenase), hydrogen oxidation and nitrate respiration; However, the only cultivated known representative of this genus is a heterotrophic bacterium [30]. The *Dehalococcoidia*'s MAG (RB32-MAG04) encodes only a carbon monoxide oxidation pathway, whereas the genus consists of strict anaerobic hydrogenotrophic, organohalide-respiring bacteria [31]. In the MAG associated with the genus *Thermomicrobium* (RB32-MAG08), we predicted pathways for dimethylsulfide degradation, thiosulfate disproportionation and carbon monoxide oxidation, whereas only carboxydotrophic growth has been reported in this genus and demonstrated by culture [32]. In the *Chloroflexota*'s RB32-MAG14, carbon monoxide oxidation and thiosulfate disproportionation pathways are present but no CDS associated with phototrophy, which may suggest a chemoorganotrophy mode of energy production [33]. The *Ktedonobacteraceae*'s MAG (RB108-MAG03) encodes enzymes of four pathways of hydrogen oxidation (aerobic), carbon monoxide oxidation, dimethylsulfide degradation, selenate reduction, thiosulfate oxidation and disproportionation and finally tetrathionate oxidation; yet, the few taxa of this family isolated so far are mesophilic heterotrophic bacteria [34]. Within *Hydrogenobaculaceae* MAGs (RB10-MAG12, RB32-MAG11), we predicted a thiosulfate disproportionation pathway; most of the species within this family are capable of chemolithotrophic microaerophilic or anaerobic growth [35]. MAGs belonging to the genus *Hydrogenivirga* (RB10-MAG07, RB13-MAG10, RB32-MAG07, RB108-MAG02) possess genes encoding enzymes of aerobic respiration, thiosulfate oxidation, thiosulfate disproportionation, tetrathionate reduction, and hydrogen oxidation (aerobic and anaerobic), which is consistent with what is known about the genus (nitrate and molecular oxygen respiration combined to hydrogen, sulfur, or thiosulfate oxidation) [35]. In MAGs associated with the genus *Thermus* (RB10-MAG08, RB10-MAG11, RB13-MAG09, RB32-MAG10, RB108-MAG01), we predicted pathways for aerobic respiration, assimilatory sulfate reduction, hydrogen oxidation, selenate reduction, thiosulfate oxidation and thiosulfate disproportionation; cultivated species of this genus grow mainly chemoorganoheterotrophically by aerobic respiration, but some have their genome coding for chemolithotrophic and anaerobic respiration enzymes [36]. The MAG belonging to the genus *Meiothermus* (RB13-MAG13) encodes pathways for carbon monoxide oxidation, hydrogen oxidation, thiosulfate oxidation and thiosulfate disproportionation; in the current state of knowledge, the growth of *Meiothermus* strains is based on chemoorganotrophy and oxygen or nitrate respiration [37]. For the RB13-MAG01 belonging to the *Sulfurifustaceae*, we predicted the genetic potential for aerobic respiration, ammonia oxidation, dissimilatory sulfate reduction, sulfite oxidation, sulfide oxidation (to sulfur globules), tetrathionate reduction, thiosulfate oxidation and thiosulfate disproportionation; *Sulfurifustaceae* (referenced as *Acidiferrobacteraceae* in the LPSN taxonomy) are known to be able to oxidize sulfur and iron, and the microorganism corresponding to this MAG may possess a larger panel of chemolithotrophic abilities [38]. For *Armatimonadota*'s members (RB10-MAG03, RB13-MAG04, RB32-MAG03), we predicted pathways for assimilatory sulfate reduction, carbon monoxide oxidation, selenate reduction and thiosulfate disproportionation; the members of the phylum are known as aerobic heterotrophs [39]. In *Zestosphaera*'s and *Ignisphaera*'s MAGs (RB10-MAG02, RB13-MAG06) (RB10-MAG05, RB13-MAG08, RB13-MAG11, RB32-MAG05), we predicted sulfur reduction (sulfur and polysulfides) pathways; those MAGs classified in the *Desulfurococcaceae* in the LPSN taxonomy, are known as heterotrophs respiring sulfur species [40,41]. MAGs belonging to the class *Thermoproteia* (RB10-MAG10, RB13-MAG07, RB32-MAG06), encode pathway for dissimilatory sulfate reduction; various catabolic pathways are described in this class [42]. In MAGs related to the genus *Caldipriscus* (RB10-MAG09, RB32-MAG12), phylum *Patescibacteria* (RB32-MAG13), family *Acidilobaceae* (RB10-MAG01, RB13-MAG02, RB32-MAG01), family *Thermofilaceae* (RB10-MAG06, RB13-MAG03, RB32-MAG09) and class *Aenigmatarchaeia* (RB13-MAG12), we did not predict any catabolic pathway of inorganic nutrients among those reported in the MetaCyc database. This could be explained by the low completion of the MAGs and/or the fact that only well-known pathways are documented in this database. However, all these MAGs have pathways associated with carbohydrate and protein degradation. This may indicate that these taxa are chemoheterotrophs, which has already been reported in geothermal environments and already described for relatives of some of these taxa [43,44].

Sulfide oxidation may be a possible energy production pathway for 28 MAGs based on KEGG Decoder (Figure 3), since they code for a sulfide:quinone oxidoreductase (K17218) and a flavoprotein chain of sulfide dehydrogenase (K17229), but this hypothesis was not confirmed by MetaCyc except for RB13-MAG01. Due to high representations of sulfur metabolisms, genes encoded in MAGs were evaluated with DiSCo, which gave similar results to those obtained when analyzed with Pathway tools. DiSCo confirmed complete dissimilatory sulfate reduction pathways for two MAGs, predicted for sulfate reduction process (RB13-MAG07) or sulfide oxidation process by reverse sulfate reduction pathway (RB13-MAG01). The assimilatory sulfate reduction pathway is more represented in the overall dataset formed by all MAGs than the dissimilatory pathway, which is consistent with the low sulfate concentration measured in the four hot springs (Table S1). The thiosulfate disproportionation pathway predicted by MetaCyc in many MAGs simply refers to the detection of an enzyme, the rhodanese-type thiosulfate sulfurtransferase. However, in the current state of knowledge on the disproportionation pathways of inorganic sulfur compounds [45,46], this enzyme alone does not allow the implementation of this catabolic pathway. If we consider all the genes present in these MAGs, nothing indicates that the microorganisms from which these MAGs originate can achieve the disproportionation of inorganic sulfur compounds.

No enzymes clearly associated with photosystems I and II were found. Nevertheless, it cannot be ruled out that these energy production pathways are absent in microorganisms indigenous to these sources. On the other hand, our results show that these sources host chemolithoautotrophic taxa involved in the carbon and sulfur cycle, and to a lesser extent in the hydrogen and nitrogen cycles. Several taxa are likely to be involved in the primary production of these sources through chemolithoautotrophy, but in addition, heterotrophs appear to be very present and diverse in the collected samples. However, additional studies will be required to better apprehend the metabolic diversity, the trophic webs of these hot springs and their microbial actors, and to better understand the functioning of the microbial communities of the Kerguelen hot springs and their interactions with their biotic and abiotic environment.

In conclusion, this first metagenomic overview of the microbial diversity of Kerguelen hot springs allowed the assembly of 42 MAGs, from 4 hot springs, many of which correspond to putative new taxa, namely 11 new genomic species, 7 new putative genera, 1 new putative family and 2 new putative orders affiliated to *Bacteria* and *Archaea*. Based on their genetic potential, these taxa appear to be chemolithoautotrophs and chemoheterotrophs and thus probably involved in the carbon, sulfur, hydrogen and nitrogen cycle. As geographically isolated sites, the Kerguelen Islands are reservoirs of diversity and taxa of novel microorganisms that should be interesting to study the evolution of microbial life and speciation processes. It has been difficult to fully assess the microbial metabolic diversity in these geothermal pools due to the inherent limitations of MAG reconstruction and the state of knowledge of microbial pathways that remains limited. However, these geothermal ecosystems could be reservoirs of novel metabolic pathways, physiological properties and adaptive mechanisms and should be examined in detail through further and broader metagenomic studies and cultural approaches.

## Methods

# Sample collection

Water samples were collected from four hot springs during the 2016–2017 austral summer TALISKER field campaign (1st of December – 11th of February) organized by the French Polar Institute Paul Emile Victor (https://institut-polaire.fr/en/). Different aliquots were collected among which water samples and water samples mixed with surficial sediments. Water samples were collected in 250ML LDPE Nalgene bottles stored at 4°C until ionic chromatography analysis. Mixed water and sediment samples were collected aseptically in sterile 50 mL Becton- Dickinson and Company-syringes, then stored (anaerobically) in sterile glass bottles at 4°C. Field measurements of fluid parameters consisted of pH, temperature (°C), alkalinity (mg/l), and electrical conductivity measurements (mS/cm) (Table S1).

# Major elements analysis in water samples

The major anions and cations were analyzed at LGL-TPE using ion-chromatography (Methrom ECO IC). A mixture of 3.2 mM $Na_2CO_3$ and 1 mM $NaHCO_3$ was used as an eluent for analysis of anions and a chemical suppression module (MSM) was used to suppress the conductivity. For cations, 1.7 mM $HNO_3$ was used as an eluent. The anions and cations were separated using analytical columns, Metrosep A Supp5 Guard/4.0 and Metrosep C4 250/4.0, respectively. Data are presented in Table S1.

# DNA extraction and sequencing

Hot spring's samples analyzed here were originally collected to grow thermophilic taxa. They were stored at 4°C for two years before DNA was extracted. This long storage has probably led to changes in microbial communities and to the selective loss or enrichment of some taxa. As a result, no analysis of abundance or absence of taxa can be conducted from these metagenomes and the results are discussed taking this bias into account.

For each hot spring sample, three replicates of DNA extraction were conducted individually, and combined as a composition sample, before the sequencing. DNA was extracted with a standard PCI (Phenol: Chloroform: Isoamyl Alcohol (25:24:1)) protocol, as described elsewhere, from 10 g environmental matrix [47], with the exception that 50 µM linear acrylamide were added to enhance nucleic acids precipitation (Invitrogen™). One negative control was included and contained 10 mL of DNA-free sterile water. Elution of total DNA extracts was performed in 30 to 50 µL EB buffer (10 mM Tris-Cl, pH 8.5). Nucleic acid solution quality was determined using the NanoDrop™ 8000 (Thermo Scientific, Waltham, MA, USA) spectrophotometer. Double-strand DNA concentration was measured using the kit Quantifluor™ dsDNA system. DNA samples were sequenced by NovaSeq 6000 (2 × 150 bp, paired-end reads) technology by the Duke Center for Genomic and Computational Biology (GCB) (https://genome.duke.edu/).

# Sequence processing, metagenomic assembly and binning

Metagenome sequences' quality were controlled by FastQC (v0.11.9 - https://github.com/s-andrews/FastQC) and MultiQC (v1.9 - https://github.com/ewels/MultiQC). Sequences were then processed with the snakemake of Anvi'o [48–50] (v7 - https://github.com/merenlab/anvio), filtered by integrated minoche script (v2.8 - https://github.com/merenlab/illumina-utils). Next, we used MetaSpades [51] (v3.14.1 - https://github.com/ablab/spades) as genome assembler and Concoct [52] (v1.1.0 - https://github.com/BinPro/CONCOCT) as genome binner with anvi_cluster_contigs function with "all against all" mode. Furthermore, MAGs were manually refined with anvi-refine function. Genome mapping was performed with bowtie2 [53] (v2.4.2 - https://sourceforge.net/projects/bowtie-bio/files/bowtie2/2.4.2/) and samtools [54] (v1.7 - https://samtools.github.io/). MAGs' quality were estimated by Anvi'o and furthermore by CheckM [55] (v1.1.3 - https://ecogenomics.github.io/CheckM/), both with default parameters. Total length, number of contigs, N50, and GC% content were extracted with anvi-summarize function.

# Taxonomic and phylogenetic inference of metagenomic assemblies and MAGs

According to the standards proposed elsewhere [25], bins were defined as high-quality MAGs (> 90% completion, < 5% contamination, presence of the 23S, 16S and 5S rRNA genes and at least 18 tRNAs), nearly high-quality MAGs (> 90% completion, < 5% contamination, other criteria partially covered), medium-quality MAGs (≥ 50% completion, < 10% contamination) and poor-quality MAGs (< 50% completion, < 10% contamination). The taxonomic affiliation of the MAGs was first investigated by placing the MAGs in a phylogenomic context. The phylogenetic reconstructions were based on 122 archaeal or 120 bacterial single copy conservative marker genes according to the Genome Taxonomy Data Base (R95 release) and were constructed using *de novo* workflow implemented in GTDB-Tk (v1.4.1 - https://github.com/Ecogenomics/GTDBTk) [56, 57]. Visualization and analysis of trees were done using ARB software [58].

As the taxonomy proposed by GTDB is new and does not correspond exactly to the one recognized by the International Code of Nomenclature of prokaryotes (ICNP), we also analyzed data according to the rules of the Code and its nomenclature. For this purpose, we implemented a combination of genomic indices classically used for the delineation of the different taxonomic ranks, namely: 16S rRNA gene sequence similarity, average nucleotide identity score (ANI) and average amino-acid identity value (ANI). The approach followed and the results are given in supplementary material (Text S1, Table S2, Figure S2).

# Metabolic profiling

MAGs were processed with KEGG Decoder script (https://github.com/bjtully/BioData/tree/master/KEGGDecoder) from Anvi'o gene calls tables generated with kegg_kofams and then plotted with R packages (ComplexHeatmap, circlize, RColorBrewer, and dplyrt) to get a general annotation with R version 3.6.3 [60, 61]. A more accurate annotation was performed with Prokka [62] (v1.14.6 - https://github.com/tseemann/prokka) and its outputs were analyzed by using the Pathway Tools software (v.24.5) [63] with the MetaCyc database (v.24.5) [64] to explore in details the putative metabolisms encoded in MAGs. Regarding sulfur metabolisms, for dsr genes, the perl script DiSCo (v.1.0.0, https://github.com/Genome-Evolution-and-Ecology-Group-GEEG/DiSCo) was used on the Prokka protein sequences ouputs of each MAG to highlight the specific genes [65].

## Declarations

### Data availability

The metagenome bins generated and analyzed during the current study are available in the European Nucleotide Archive (ENA) (https://www.ebi.ac.uk/ena/browser/home), under the Project PRJEB46766 (Table 3).

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### Author contributions

Conceptualization, M.A. and K.A.; Formal analysis, M.A., S.Y., A.M., V.L., D.G., J.A., and K.A.; Funding acquisition, D.G., M.L.R. and K.A.; Investigation, M.A., S.Y., A.M., M.C., D.G., J.A., and K.A; Supervision, K.A.; Writing—original draft, M.A., S.Y. and K.A.; Review & editing, all coauthors. All authors have read and agreed to the published version of the manuscript.

### Competing interests

The authors declare no conflict of interest.

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## Tables

**Table 1**. General characteristics of the 42 MAGs obtained from RB10, RB13, RB32 and RB108.

| Sample | MAG quality | Percent complet. | Percent contam. | Mean Coverage | GC content | Size (Mbp) | Contig number | N50 | tRNA | *rrn* sequences (5S-16S-23S) |
|---|---|---|---|---|---|---|---|---|---|---|
| RB10-MAG01 | NHQ | 96.84 | 0.95 | 61.98 | 57.01 | 1.78 | 59 | 78.449 | 35 | 1-0-0 |
| RB10-MAG02 | NHQ | 98.00 | 0.63 | 40.11 | 38.88 | 1.49 | 212 | 15.625 | 33 | 1-0-0 |
| RB10-MAG03 | NHQ | 92.59 | 0.19 | 38.28 | 56.24 | 2.65 | 72 | 76.979 | 47 | 1-1-0 |
| RB10-MAG04 | HQ | 92.73 | 1.82 | 125.94 | 65.49 | 2.50 | 119 | 38.887 | 45 | 1-1-1 |
| RB10-MAG05 | NHQ | 94.34 | 0.00 | 20.12 | 33.97 | 1.60 | 309 | 8.060 | 17 | 1-0-0 |
| RB10-MAG06 | HQ | 97.79 | 0.74 | 74.51 | 53.43 | 1.72 | 322 | 11.699 | 19 | 1-1-1 |
| RB10-MAG07 | HQ | 97.56 | 0.41 | 4822.70 | 53.78 | 1.44 | 153 | 17.997 | 43 | 1-1-1 |
| RB10-MAG08 | HQ | 96.75 | 0.85 | 448.27 | 69.15 | 2.01 | 196 | 20.594 | 45 | 0-0-0 |
| RB10-MAG09 | MQ | 54.88 | 0.00 | 12.64 | 42.63 | 0.75 | 417 | 1.860 | 16 | 0-0-0 |
| RB10-MAG10 | MQ | 57.09 | 0.00 | 12.99 | 33.71 | 0.86 | 455 | 2.000 | 10 | 0-0-0 |
| RB10-MAG11 | NHQ | 91.61 | 0.14 | 118.61 | 65.66 | 1.78 | 159 | 22.734 | 33 | 0-0-0 |
| RB10-MAG12 | MQ | 54.21 | 0.81 | 13.87 | 31.69 | 0.75 | 413 | 1.839 | 12 | 1-0-0 |
| RB13-MAG01 | NHQ | 93.29 | 1.22 | 50.58 | 67.39 | 2.20 | 188 | 24.091 | 35 | 1-0-1 |
| RB13-MAG02 | NHQ | 96.52 | 0.63 | 1385.16 | 56.92 | 1.82 | 69 | 74.252 | 33 | 1-0-1 |
| RB13-MAG03 | NHQ | 97.79 | 0.74 | 189.70 | 53.64 | 1.67 | 246 | 22.516 | 47 | 1-0-0 |
| RB13-MAG04 | NHQ | 90.74 | 0.19 | 23.91 | 56.24 | 2.56 | 329 | 11.396 | 45 | 1-0-0 |
| RB13-MAG05 | NHQ | 93.03 | 1.01 | 31.18 | 65.49 | 2.35 | 243 | 14.935 | 17 | 1-0-1 |
| RB13-MAG06 | NHQ | 97.20 | 1.58 | 224.61 | 38.67 | 1.41 | 228 | 9.959 | 19 | 1-0-0 |
| RB13-MAG07 | NHQ | 92.65 | 0.74 | 36.10 | 52.52 | 1.24 | 149 | 14.053 | 43 | 1-0-0 |
| RB13-MAG08 | NHQ | 92.72 | 1.58 | 209.57 | 36.07 | 1.23 | 126 | 22.470 | 45 | 0-0-0 |
| RB13-MAG09 | NHQ | 100.00 | 0.00 | 118.59 | 65.33 | 2.01 | 179 | 35.402 | 16 | 0-0-0 |
| RB13-MAG10 | HQ | 97.43 | 0.41 | 118.59 | 53.62 | 1.41 | 147 | 14.581 | 10 | 1-1-1 |
| RB13-MAG11 | MQ | 87.62 | 0.95 | 574.42 | 34.11 | 1.50 | 306 | 7.292 | 33 | 0-0-0 |
| RB13-MAG12 | MQ | 72.90 | 3.27 | 125.85 | 25.76 | 0.74 | 154 | 8.912 | 12 | 0-1-0 |
| RB13-MAG13 | PQ | 30.02 | 0.43 | 90.33 | 66.35 | 0.83 | 520 | 1.613 | 35 | 1-0-1 |
| RB32-MAG01 | NHQ | 97.47 | 0.95 | 29.75 | 56.80 | 1.75 | 57 | 88.979 | 33 | 0-0-0 |
| RB32-MAG02 | HQ | 94.55 | 1.82 | 421.42 | 65.38 | 2.54 | 148 | 29.008 | 47 | 1-1-2 |
| RB32-MAG03 | HQ | 92.59 | 0.19 | 845.13 | 56.23 | 2.67 | 84 | 95.702 | 45 | 1-1-1 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| RB32-MAG04 | NHQ | 94.72 | 0.00 | 24.31 | 64.77 | 2.45 | 128 | 40.031 | 17 | 1-0-1 |
| RB32-MAG05 | NHQ | 98.73 | 0.00 | 28.06 | 34.36 | 1.76 | 238 | 16.008 | 19 | 1-0-1 |
| RB32-MAG06 | NHQ | 91.18 | 0.00 | 302.91 | 34.75 | 1.96 | 305 | 12.851 | 43 | 1-0-0 |
| RB32-MAG07 | NHQ | 97.83 | 0.41 | 1025.50 | 53.50 | 1.45 | 157 | 17867.000 | 45 | 1-0-1 |
| RB32-MAG08 | NHQ | 94.95 | 0.93 | 1025.50 | 65.25 | 2.55 | 629 | 5.755 | 16 | 0-0-0 |
| RB32-MAG09 | NHQ | 94.85 | 1.47 | 94.92 | 53.78 | 1.57 | 334 | 8.549 | 10 | 1-0-0 |
| RB32-MAG10 | NHQ | 100.00 | 1.27 | 184.55 | 65.39 | 1.99 | 128 | 41.211 | 33 | 1-0-1 |
| RB32-MAG11 | NHQ | 94.70 | 2.28 | 20.34 | 32.15 | 1.30 | 295 | 6.491 | 12 | 0-0-0 |
| RB32-MAG12 | MQ | 80.35 | 0.00 | 11.95 | 42.38 | 1.20 | 362 | 20.577 | 35 | 1-0-0 |
| RB32-MAG13 | MQ | 70.82 | 0.00 | 129.49 | 27.82 | 0.55 | 176 | 19.960 | 33 | 0-0-1 |
| RB32-MAG14 | MQ | 55.12 | 0.00 | 11.32 | 70.35 | 1.47 | 834 | 1.812 | 47 | 0-0-0 |
| RB108-MAG01 | NHQ | 99.15 | 1.27 | 1120.09 | 69.21 | 2.04 | 126 | 30.509 | 45 | 0-0-0 |
| RB108-MAG02 | NHQ | 96.75 | 0.00 | 579.13 | 53.28 | 1.45 | 75 | 29.169 | 17 | 1-0-0 |
| RB108-MAG03 | MQ | 53.38 | 4.73 | 86.68 | 56.73 | 3.96 | 2105 | 1.944 | 19 | 0-0-0 |

**Table 2.** Classification of the MAGs based on the taxonomic classification of GTDB-Tk.

| MAG ID | Domain | Phylum | Class | Order | Family | Genus | Species |
|---|---|---|---|---|---|---|---|
| RB10-MAG07 | *Bacteria* | *Aquificota* | *Aquificae* | *Aquificales* | *Aquificaceae* | *Hydrogenivirga* | ***New*** |
| RB10-MAG12 | *Bacteria* | *Aquificota* | *Aquificae* | *Aquificales* | *Hydrogenobaculaceae* | ***New*** | |
| RB13-MAG10 | *Bacteria* | *Aquificota* | *Aquificae* | *Aquificales* | *Aquificaceae* | *Hydrogenivirga* | ***New*** |
| RB32-MAG07 | *Bacteria* | *Aquificota* | *Aquificae* | *Aquificales* | *Aquificaceae* | *Hydrogenivirga* | ***New*** |
| RB32-MAG11 | *Bacteria* | *Aquificota* | *Aquificae* | *Aquificales* | *Hydrogenobaculaceae* | ***New*** | |
| RB108-MAG02 | *Bacteria* | *Aquificota* | *Aquificae* | *Aquificales* | *Aquificaceae* | *Hydrogenivirga* | ***New*** |
| RB10-MAG03 | *Bacteria* | *Armatimonadota* | HRBIN17 | HRBIN17 | HRBIN17 | HRBIN17 | ***New*** |
| RB13-MAG04 | *Bacteria* | *Armatimonadota* | HRBIN17 | HRBIN17 | HRBIN17 | HRBIN17 | ***New*** |
| RB32-MAG03 | *Bacteria* | *Armatimonadota* | HRBIN17 | HRBIN17 | HRBIN17 | HRBIN17 | ***New*** |
| RB10-MAG04 | *Bacteria* | *Chloroflexota* | *Anaerolineae* | *Thermoflexales* | *Thermoflexaceae* | *Thermoflexus* | ***New*** |
| RB13-MAG05 | *Bacteria* | *Chloroflexota* | *Anaerolineae* | *Thermoflexales* | *Thermoflexaceae* | *Thermoflexus* | ***New*** |
| RB32-MAG02 | *Bacteria* | *Chloroflexota* | *Anaerolineae* | *Thermoflexales* | *Thermoflexaceae* | *Thermoflexus* | ***New*** |
| RB32-MAG04 | *Bacteria* | *Chloroflexota* | *Dehalococcoidia* | ***New*** | | | |
| RB32-MAG08 | *Bacteria* | *Chloroflexota* | *Chloroflexia* | *Thermomicrobiales* | *Thermomicrobiaceae* | *Thermomicrobium* | ***New*** |
| RB32-MAG14 | *Bacteria* | *Chloroflexota* | FW602-bin22 | FW602-bin22 | ***New*** | | |
| RB108-MAG03 | *Bacteria* | *Chloroflexota* | *Ktedonobacteria* | *Ktedonobacterales* | *Ktedonobacteraceae* | ***New*** | |
| RB10-MAG01 | *Archaea* | *Thermoproteota* | *Thermoproteia* | *Sulfolobales* | *Acidilobaceae* | ***New*** | |
| RB10-MAG02 | *Archaea* | *Thermoproteota* | *Thermoproteia* | *Sulfolobales* | NBVN01 | *Zestosphaera* | ***New*** |
| RB10-MAG05 | *Archaea* | *Thermoproteota* | *Thermoproteia* | *Sulfolobales* | *Ignisphaeraceae* | *Ignisphaera* | ***New*** |
| RB10-MAG06 | *Archaea* | *Thermoproteota* | *Thermoproteia* | *Thermofilales* | *Thermofilaceae* | *Thermofilum_A* | ***New*** |
| RB10-MAG10 | *Archaea* | *Thermoproteota* | *Thermoproteia* | ***New*** | | | |
| RB13-MAG02 | *Archaea* | *Thermoproteota* | *Thermoproteia* | *Sulfolobales* | *Acidilobaceae* | ***New*** | |
| RB13-MAG03 | *Archaea* | *Thermoproteota* | *Thermoproteia* | *Thermofilales* | *Thermofilaceae* | *Thermofilum_A* | ***New*** |
| RB13-MAG06 | *Archaea* | *Thermoproteota* | *Thermoproteia* | *Sulfolobales* | NBVN01 | *Zestosphaera* | ***New*** |
| RB13-MAG07 | *Archaea* | *Thermoproteota* | *Thermoproteia* | *Thermoproteales* | *Thermocladiaceae* | ***New*** | |
| RB13-MAG08 | *Archaea* | *Thermoproteota* | *Thermoproteia* | *Sulfolobales* | *Ignisphaeraceae* | *Ignisphaera* | ***New*** |
| RB13-MAG11 | *Archaea* | *Thermoproteota* | *Thermoproteia* | *Sulfolobales* | *Ignisphaeraceae* | *Ignisphaera* | ***New*** |
| RB32-MAG01 | *Archaea* | *Thermoproteota* | *Thermoproteia* | *Sulfolobales* | *Acidilobaceae* | ***New*** | |
| RB32- | *Archaea* | *Thermoproteota* | *Thermoproteia* | *Sulfolobales* | *Ignisphaeraceae* | *Ignisphaera* | ***New*** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| MAG05 | | | | | | | |
| RB32-MAG06 | *Archaea* | *Thermoproteota* | *Thermoproteia* | ***New*** | | | |
| RB32-MAG09 | *Archaea* | *Thermoproteota* | *Thermoproteia* | *Thermofilales* | *Thermofilaceae* | *Thermofilum_A* | ***New*** |
| RB10-MAG08 | *Bacteria* | *Deinococcota* | *Deinococci* | *Deinococcales* | *Thermaceae* | *Thermus* | *thermophilus* |
| RB10-MAG11 | *Bacteria* | *Deinococcota* | *Deinococci* | *Deinococcales* | *Thermaceae* | *Thermus* | ***New*** |
| RB13-MAG09 | *Bacteria* | *Deinococcota* | *Deinococci* | *Deinococcales* | *Thermaceae* | *Thermus* | ***New*** |
| RB13-MAG13 | *Bacteria* | *Deinococcota* | *Deinococci* | *Deinococcales* | *Thermaceae* | Meiothermus_B | ***New*** |
| RB32-MAG10 | *Bacteria* | *Deinococcota* | *Deinococci* | *Deinococcales* | *Thermaceae* | *Thermus* | ***New*** |
| RB108-MAG01 | *Bacteria* | *Deinococcota* | *Deinococci* | *Deinococcales* | *Thermaceae* | *Thermus* | *thermophilus* |
| RB13-MAG12 | *Archaea* | *Aenigmatarchaeota* | *Aenigmatarchaeia* | CG10238-14 | EX4484-224 | ***New*** | |
| RB13-MAG01 | *Bacteria* | *Proteobacteria* | *Gammaproteobacteria* | *Acidiferrobacterales* | *Sulfurifustaceae* | ***New*** | |
| RB32-MAG13 | *Bacteria* | *Patescibacteria* | *Paceibacteria* | *UBA6257* | *HR35* | ***New*** | |
| RB32-MAG12 | *Bacteria* | *WOR-3* | *Hydrothermia* | *LBFQ01* | *LBFQ01* | *Caldipriscus* | ***New*** |
| RB10-MAG09 | *Bacteria* | *WOR-3* | *Hydrothermia* | *LBFQ01* | *LBFQ01* | *Caldipriscus* | ***New*** |

**Table 3.** Metagenomes and MAGs accessions numbers on ENA (Study ID: PRJEB46766).

| Assembly name | Assembly accession number | Sample accession number |
|---|---|---|
| **RB10** | ERZ4120739 | ERS7179886 |
| RB10-MAG01 | GCA_916099525 | ERS7299774 |
| RB10-MAG02 | GCA_916101595 | ERS7299775 |
| RB10-MAG03 | GCA_916102575 | ERS7299776 |
| RB10-MAG04 | GCA_916102815 | ERS7299777 |
| RB10-MAG05 | GCA_916103375 | ERS7299778 |
| RB10-MAG06 | GCA_916103935 | ERS7299779 |
| RB10-MAG07 | GCA_916103025 | ERS7299780 |
| RB10-MAG08 | GCA_916101605 | ERS7299781 |
| RB10-MAG09 | GCA_916098365 | ERS7299782 |
| RB10-MAG10 | GCA_916100445 | ERS7299783 |
| RB10-MAG11 | GCA_916100255 | ERS7299784 |
| RB10-MAG12 | GCA_916103405 | ERS7299785 |
| **RB13** | ERZ4120744 | ERS7179887 |
| RB13-MAG01 | GCA_916101585 | ERS7299786 |
| RB13-MAG02 | GCA_916101625 | ERS7299787 |
| RB13-MAG03 | GCA_916101685 | ERS7299788 |
| RB13-MAG04 | GCA_916101675 | ERS7299789 |
| RB13-MAG05 | GCA_916104055 | ERS7299790 |
| RB13-MAG06 | GCA_916104685 | ERS7299791 |
| RB13-MAG07 | GCA_916099935 | ERS7299792 |
| RB13-MAG08 | GCA_916104555 | ERS7299793 |
| RB13-MAG09 | GCA_916098735 | ERS7299794 |
| RB13-MAG10 | GCA_916103555 | ERS7299795 |
| RB13-MAG11 | GCA_916103205 | ERS7299796 |
| RB13-MAG12 | GCA_916101905 | ERS7299797 |
| RB13-MAG13 | GCA_916101985 | ERS7299798 |
| **RB32** | ERZ4120748 | ERS7179888 |
| RB32-MAG01 | GCA_916099235 | ERS7299799 |
| RB32-MAG02 | GCA_916099675 | ERS7299800 |
| RB32-MAG03 | GCA_916102075 | ERS7299801 |
| RB32-MAG04 | GCA_916098805 | ERS7299802 |
| RB32-MAG05 | GCA_916098685 | ERS7299803 |
| RB32-MAG06 | GCA_916102535 | ERS7299804 |
| RB32-MAG07 | GCA_916109635 | ERS7299805 |
| RB32-MAG08 | GCA_916109905 | ERS7299806 |
| RB32-MAG09 | GCA_916110065 | ERS7299807 |
| RB32-MAG10 | GCA_916105685 | ERS7299808 |
| RB32-MAG11 | GCA_916112325 | ERS7299809 |
| RB32-MAG12 | GCA_916112475 | ERS7299810 |
| RB32-MAG13 | GCA_916111365 | ERS7299811 |
| RB32-MAG14 | GCA_916108235 | ERS7299812 |
| **RB108** | ERZ4120752 | ERS7179889 |

| | | |
|---|---|---|
| RB108-MAG01 | GCA_916116595 | ERS7299813 |
| RB108-MAG02 | GCA_916109075 | ERS7299814 |
| RB108-MAG03 | GCA_916109065 | ERS7299815 |

## Figures

**Figure 1**

Sampling locations at the “plateau des Fumerolles” in the Rallier du Baty Peninsula, Kerguelen Islands, French Southern and Antarctic Lands, and photographs of the 4 hot springs studied here, and their temperature and pH conditions.

**Figure 2**

Venn diagrams showing the shared phyla, families and genera according to GTDB classification in the reconstructed MAGs from the hot springs RB10, RB13, RB32 and RB108.

**Figure 3**

Metabolic pathway diagram of the 42 MAGs based on KEGG Decoder annotations, showing MAG classification according to GTDB-Tk and estimated genome completion.

## Supplementary Files

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