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Microbial ecology in Azores deep-seafloor hydrothermal environments

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"*Messieurs, les microbes auront le dernier mot*"
"*Gentlemen, the microbes will have the last word*"

Louis Pasteur, 1822-1895, French chemist and one of the fathers of Microbiology

to my family

to my parents Fernanda and João, my sister João, my brother Bernardo
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Microbial ecology in Azores deep-seafloor hydrothermal environments

Abstract

The world's oceans cover two-thirds of the planet's surface being by far the largest habitat on Earth. The marine habitats range from sunlight surface waters to ocean trenches 11,000 m deep with pressures exceeding 1000 bar. Water temperatures range from sea ice, in the polar regions, to over 300 ºC, at deep-sea hydrothermal vents. Microorganisms are able to survive and grow throughout these environments, including the subsurface and the deep-sea. Deep-sea hydrothermal systems represent an important interface between the lithosphere and the oceans, and are considered to be "windows" into the subsurface biosphere. At these locations, thermally charged hydrothermal fluids, enriched with metal compounds and dissolved gases, are ejected into the ocean, providing conditions for supporting chemosynthesis and microbial growth. Extremophile organisms have adapted to the high pressures and temperature shifts, developed novel physiological strategies to thrive in such conditions resorting to unique enzymes and proteins with interesting activities and potential biotechnological applications. Chemoautotrophic *Bacteria* and *Archaea* are the primary source of nutrition for the overall organisms dwelling around the vents. They are primary producers of organic carbon, able to establish intricate chemosynthetic symbioses with micro- and macroorganisms, and to transfer the energy up to the food web, sustaining life in the deep-sea. Because of their unique features, they are considered plausible analogues to the early microorganisms of Earth. Therefore, the study of such putative early microbes may help us understanding the origin and evolution of life, and the adaptation mechanisms to these extreme environmental conditions. Moreover, these microorganisms are potentially playing important roles in global geochemical cycling between crusts and oceans, what makes their distribution and activities in deep-sea floors interesting subjects for contemporary microbial ecologists.

So far, the diversity and distribution patterns of invertebrate vent communities have been reasonably investigated, however few studies reported on the microbial ecology of free-living *Archaea* and *Bacteria* in deep-sea hydrothermal sediments, even though their remarkable activity at these ecosystems. Prior to this work, sediment-associated microbial diversity in the Menez Gwen, Lucky Strike and Rainbow hydrothermal vent fields, southwest of the Azores, was still largely unexplored. Only three microbiological surveys based on 16S clone libraries were conducted in sediments from Rainbow field.

The overall goal of this thesis was therefore to characterize the microbiota associated with hydrothermal sediments from the three most visited deep-sea vent fields of the Azores region, and the elucidation of the microorganisms' metabolic potential. The outcome of this work gives useful insights into yet undiscovered organisms with unique living conditions, molecular mechanisms, and promising enzymes for biotechnological applications.

As most of the vent-associated microorganisms remain uncultured, diversity studies are usually based in culture-independent sequencing technologies. Here, the bacterial, archaeal and micro-eukaryotic taxonomic profiles were addressed by barcoded pyrosequencing of a

segment of the ribosomal RNA gene. The taxonomic profiles obtained were used to compare communities associated with different hydrothermal sediments retrieved from different sites. Metagenomic approaches were then used to characterize the metabolic potential of the communities.

The microbial community associated with sediments from the Menez Gwen vent system was surveyed for the first time. The microbiota of hydrothermally influenced sediments was compared with the one in non-hydrothermally influenced sediments. Microbial communities were shown to be significantly different in the two geographical areas. Specific mesophilic, thermophilic and hyperthermophilic archaeal (e.g., *Archaeoglobus*, *ANME-1*) and bacterial (e.g., *Caldithrix*, *Thermodesulfobacteria*) taxa were detected in sediments near an active vent chimney, and contrasted with members affiliating with ubiquitous deep-ocean phylogroups (e.g., *Thaumarchaeota MGI*, *Gamma- and Alphaproteobacteria*) found in the sediments from a permanently cold bathyal plain.

Similarly, the sediment-associated microbiota from three neighbouring vent fields, the Menez Gwen, Lucky Strike and Rainbow systems, was also compared. The distinct geological and ecological features allow these fields to support a diversity of vent microbial communities, which were shown to be distinct between the three separated sites. Taxonomic analyses identified anaerobic methanogens and microaerobic *Epsilonproteobacteria*, dominating the Menez Gwen community, which contrasted with the *Gammaproteobacteria Nitrosococcus*, *Acidiferrobacter* and marine benthic group *JTB255* members found to dominate both Lucky Strike and Rainbow microbial communities. Besides, the Rainbow field presented the highest archaeal diversity among all the studied sediments. Trace metal contents and distance from an active vent were suggested to influence the sediment-associated microbiota from the three vent sites. Results from this study provided a better understanding of the distribution of microorganisms in deep-sea hydrothermal sediments and allowed for specifically choosing the most interesting samples to be explored in a *a posteriori* metagenomic approach.

Accordingly, the microbial communities from the Menez Gwen and Rainbow hydrothermal sediments were investigated for the presence and diversity of genes responsible for carbon fixation and sulfur, nitrogen and methane metabolisms. A metagenomic study of the microbiotas elucidated the gene sequences responsible for carbon and energy flux in both hydrothermal ecosystems. The metagenomic analyses confirmed the microbial and geochemical variability and revealed the largely autotrophic communities thriving in both sites. Chemolithotrophy appeared to be primarily driven by sulfur oxidation, whether through the SOX-dependent pathway at Menez Gwen site or through reverse sulfate reduction at Rainbow site. Other energy-yielding processes, such as methane, nitrite or ammonia oxidation, were also detected but presumably contributing less to chemolithoautotrophy.

This work furthers our knowledge of the microbial ecology of deep-sea hydrothermal sediments, and represents an important repository of novel genes with potential biotechnological interest.

Resumo

Os oceanos são o maior habitat da Terra e cobrem aproximadamente dois terços da superfície do planeta. Desde as águas superficiais até às fossas abissais, os ambientes marinhos podem atingir 11 km de profundidade e pressões superiores a 1000 bar, variar entre temperaturas de 0 ºC nas regiões polares e mais de 300 ºC nas fontes hidrotermais. Em todos estes ecossistemas marinhos proliferam microorganismos que, independentemente das condições, conseguem colonizar até mesmo as regiões mais inóspitas do mar profundo e do subsolo oceânico.

As fontes hidrotermais de profundidade são ambientes marinhos reconhecidos como pontos de ligação entre a litosfera e os oceanos, sendo mesmo consideradas "janelas" para a biosfera do subsolo. Nestes locais, por entre fissuras da crosta oceânica, há emissões de fluidos hidrotermais, uma combinação de gases e metais vinda do interior da Terra que é projectada para o oceano a elevadas temperaturas. Estes fluidos transportam espécies químicas reduzidas, geradas pelo contacto entre gases e minerais existentes na crosta oceânica, que servem de alimento a microorganismos quimioautotróficos dos domínios *Bacteria* e *Archaea.* Estes microorganismos desenvolveram estratégias fisiológicas únicas para conseguirem prosperar em tais condições, como a produção de enzimas e compostos activos alternativos, despertando o interesse dos cientistas para o potencial biotecnológico dos seus recursos genéticos. Bactérias e arqueas quimioautotróficas são os únicos organismos capazes de produzir matéria orgânica a partir de energia química. Estes microorganismos asseguram a produção primária nestes ambientes e a transferência de energia para a cadeia trófica, sustentando assim a vida no mar profundo. Dado o património genético, metabolismos próprios e capacidade de estabelecer relações de simbiose com outros organismos, estas bactérias e arqueas são potencialmente representativas dos organismos primordiais da Terra, e o seu estudo pertinente na explicação da origem e evolução da vida. Por desempenharem também um papel importante no ciclo biogeoquímico entre a crosta e o oceano, existe um interesse acrescido da comunidade científica na actividade, diversidade e distribuição destes microorganismos no subsolo e fundos oceânicos.

Até à data desta dissertação, inúmeros estudos foram realizados no sentido de caracterizar a fauna hidrotermal e sua distribuição, incidindo sobretudo nas comunidades de invertebrados marinhos, mas apesar disso ainda muito se desconhece sobre a ecologia microbiana local, nomeadamente a associada a sedimentos hidrotermais de profundidade. Em particular, a informação sobre a diversidade microbiana associada a sedimentos do Menez Gwen, Lucky Strike e Rainbow, três campos hidrotermais de profundidade dos mares dos Açores, é ainda escassa. Apenas três estudos foram efectuados em sedimentos do Rainbow com recurso a métodos tradicionais de construção de bibliotecas genómicas de RNA ribossomal.

O principal objectivo deste trabalho foi, por isso, caracterizar o microbiota associado a sedimentos dos três campos hidrotermais de profundidade mais conhecidos dos Açores, e

elucidar o potencial metabólico dos microorganismos. Desta forma, obtemos informação relevante sobre formas de vida pouco ou nada conhecidas, sustentadas por mecanismos moleculares únicos e enzimas promissoras para potenciais aplicações biotecnológicas.

Visto que a maioria dos microorganismos associados ao mar profundo não são cultiváveis em laboratório, os estudos de diversidade desenvolveram-se com recurso a ferramentas de sequenciação de DNA. Assim, os perfis taxonómicos de bactérias, arqueas e micro-eucariotas foram obtidos por pirosequenciação de um segmento do gene de RNA ribossomal. As comunidades microbianas associadas a sedimentos hidrotermais distintos foram comparadas e as suas funções metabólicas identificadas, por sequenciação metagenómica do DNA obtido directamente das amostras ambientais.

Pela primeira vez, os microbiotas associados a sedimentos do Menez Gwen foram caracterizados. Verificou-se que as comunidades encontradas em sedimentos influenciados pelo hidrotermalismo diferiam significativamente daquelas encontradas em sedimentos não influenciados pelo hidrotermalismo. Em sedimentos amostrados perto de uma chaminé hidrotermal foram detectados microorganismos mesofílicos, termofílicos e hipertermofílicos pertencentes aos domínios *Archaea* (e.g., *Archaeoglobus*, *ANME-1)* e *Bacteria* (e.g., *Caldithrix*, *Thermodesulfobacteria)*. Por outro lado, associados a sedimentos amostrados numa planície batial, abundavam representantes de filogrupos ubíquos no mar profundo (e.g., *Thaumarchaeota MGI*, *Gamma-* e *Alphaproteobacteria*).

Da mesma forma, foram comparados os microbiomas associados a sedimentos recolhidos em três campos hidrotermais de profundidade dos Açores, Menez Gwen, Lucky Strike e Rainbow. Dadas as diferenças geológicas e geoquímicas registadas nos três campos, os seus sedimentos albergam comunidades microbianas distintas. Associados ao campo hidrotermal Menez Gwen foram identificados maioritariamente membros da classe *Epsilonproteobacteria* assim como alguns microorganismos metanogénicos anaeróbios pertencentes ao domínio *Archaea*. Em contrapartida, as comunidades do Lucky Strike e Rainbow revelaram ser dominadas por representantes da classe *Gammaproteobacteria* (e.g. *Nitrosococcus*, *Acidiferrobacter* e *Marine Benthic Group JTB255).* Para além disso, entre todos os sedimentos estudados, as comunidades associadas à fonte hidrotermal Rainbow foram as que apresentaram maior diversidade de representantes do domínio *Archaea*. A composição química dos sedimentos e a influência hidrotermal foram vistos como possíveis factores que explicam as diferenças encontradas nos microbiomas associados aos sedimentos de cada campo: Menez Gwen, Lucky Strike e Rainbow. Os resultados deste trabalho permitiram caracterizar a diversidade e distribuição dos microorganismos hidrotermais e permitiram seleccionar as amostras mais interessantes a serem investigadas num estudo metagenómico posterior.

Neste contexto, as comunidades microbianas dos sedimentos hidrotermais dos campos Menez Gwen e Rainbow foram investigadas quanto à presença de genes intervenientes na fixação de carbon e metabolismos do enxofre, azoto e metano. A análise metagenómica destes dois microbiotas revelou quais os genes-chave envolvidos nos fluxos de carbono e energia em ambos ecossistemas hidrotermais. Para além de confirmar a variabilidade microbiana e

geoquímica observada na composição dos diferentes sedimentos hidrotermais, os resultados revelaram o carácter amplamente autotrófico das comunidades microbianas aí residentes. A análise metagenómica aponta para a oxidação do enxofre como via metabólica dominante dos processos quimioautotróficos, que ocorre principalmente pela via metabólica das SOX nos sedimentos do Menez Gwen ou pela via reversa da redução do sulfato nos sedimentos do Rainbow. Foram igualmente detectados outros processos de obtenção de energia – como a oxidação do metano, do nitrito e do amoníaco – no entanto, parecem contribuir em menor escala para a quimioautotrofia nestes locais.

Este trabalho de investigação contribui para o conhecimento global da ecologia microbiana de sedimentos hidrotermais de profundidade, e constitui um importante repositório de sequências de novos genes com potencial interesse biotecnológico.

1

General Introduction

1. Deep-sea Hydrothermal Vents

1.1 Historical background of deep-sea biology

By the present time, the humanity is beginning to recognise that the planets and moons that surround the Earth are much more active and dynamic than what was previously guessed. The idea about the existence of life forms in the solar system somewhere other than Earth is too tempting to dismiss. At the same time, the deep-sea, the largest ecosystem on our planet, and the origin of life on Earth itself, are still rather ignored.

In the late 60's, when the space travel was finally achieved, it was suggested that more was known about the Moon than about the deepest parts of the ocean (Flannery, 2007). Currently, Europa, Jupiter's fourth-largest moon, is highlighted as the most likely extraterrestrial world to harbour life. It is said to contain a hidden liquid ocean under its icy exterior crust (Hussmann *et al.*, 2002), and one might say, where there is water there might be life (Kimura and Kitadai, 2015). However, this is a relatively modern thought.

Until the 19th century, the knowledge about the vast oceans was pretty modest. Edward Forbes (1815-1854), the first "oceanographer" established the "azoic hypothesis" (Anderson and Rice, 2006), suggesting that below 550 meters of the sea level light and photosynthetic productions did not occur, and at such depths the ocean was static, lacked oxygen and was lifeless. Forbes's theory persisted until the HMS Challenger expedition (1873-1876) when living specimens were found at great depths, and bottom dredges revealed a hidden "deep-sea biology" (Thomson *et al.*, 1889).

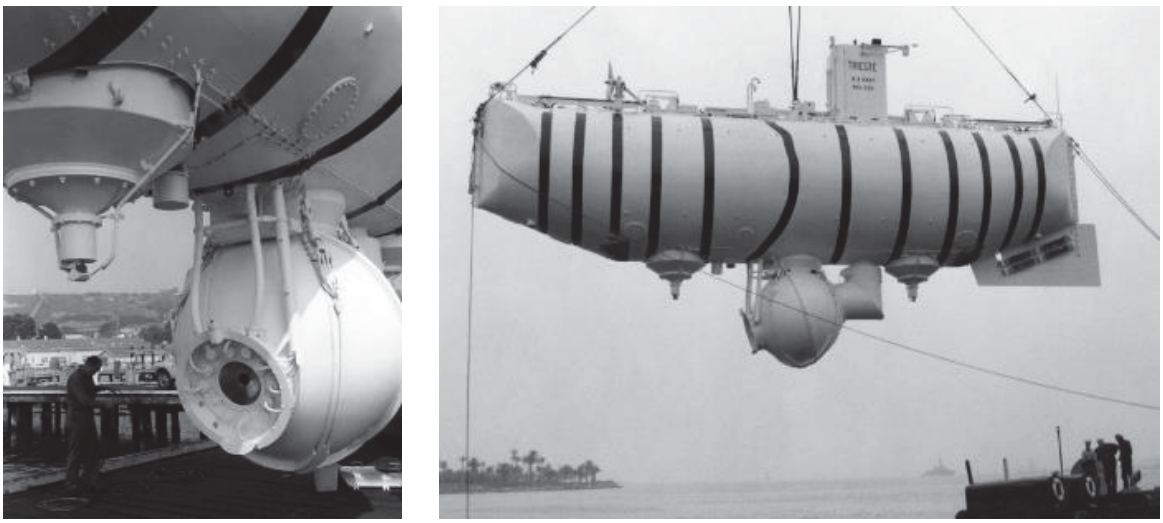

Figure I-1 - The Bathyscaph Trieste, a work of engineering art and science, built by Auguste Piccard to mankind exploration of the deep seas. The Bathyscaph Trieste was essentially a balloon which Piccard referred to as a float. The float consisted of a thin metal shell that was filled with gasoline, which would allow the float to ascend or climb in the water, once ballast was released. The two-member crew stayed in the observation gondola, a separate pressure sphere located at the bottom of the float. Source: U.S. Naval Historical Center Photograph, San Diego, California, 1958-59.

The first records of organisms found to live in the deep ocean were suggested to depend on sinking organic material from sunlit surface waters, due to the foreseen nutrient unavailability in the remote, extreme cold, dark and high-pressure deep-sea. The existence of any living forms in both deep-sea and Europa's oceans would have to rely on a source of energy other than photosynthesis.

The rational that life was sustained in such extreme deep conditions was reinforced in 1960 when Jacques Piccard and Lieutenant Don Walsh saw living creatures at the bottom of the Mariana Trench. They piloted the deep-submersible "Bathyscaph Trieste" (**Figure I-1**) reaching a record depth of about 11,000 meters, in the deepest known part of the oceans – the Challenger Deep (Emery, 1961). By that time, the idea that life could endure at the deepest parts of the sea was so tempting as it is now the possibility of alien life. Today it is widely believed that the birthplace of life on Earth may rely on the ocean depths (Wächtershäuser, 1990; Huber and Wächtershäuser, 1997), and besides that the deep-sea microbiology will help us understand how life began.

Explorations to the Ocean sea-floor have continued and, by the late 1970s, one of the expeditions to the Galapagos Rift ended-up with the discovery of the first hydrothermal vent system. This incident became one of the most significant events in the Earth Science of the last 40 years and set the beginning of an age of research into the deep-sea. An underwater oasis of 2m tall forests of tube worms, dense settlements of clams, mussels, crabs, sea anemones, and other animals was detected at more than 2500 m deep, during dives of the submersible "Alvin" in the Galápagos hotspot (Lonsdale, 1977). The discovery of extraordinary faunal assemblages around thermal springs in deep-sea waters (Corliss and Ballard, 1977) brought evidence supporting that life was flourishing at those extreme environments compared to the surrounding deep seafloor.

Today it is known that in hydrothermal vents food chains do not begin with photosynthetic plants or algae. Instead, life is based on primary production undergone by chemosynthetic organisms (namely Archaea and Bacteria) able to convert sulfides, hydrocarbons or hydrogen from the vent fluids into useful energy (Jannasch and Wirsen, 1979; Karl *et al.*, 1980). The chemosynthetic microbes are the primary source of nutrition for the higher organisms living around the vents. While some of the vent animals consume microbes directly, others incorporate microbes into or on their bodies as symbionts (Cavanaugh, 1983). Because of the unique features of deep-sea hydrothermal vent ecosystems, they are considered plausible analogues to the early ecosystems of Earth (Wächtershäuser, 2000). As such, the study of Earth putative early microorganisms may help us understanding the extremes and evolution of life on our planet, and also facilitate the search for life elsewhere, in singular extreme environmental conditions (Jannasch and Mottl, 1985; Foster and Mobberley, 2010; Nakamura and Takai, 2014). At this stage, the biodiversity of deep-sea hydrothermal vents is beginning to be understood, and thus the colonizing microbes, potentially playing important roles in global geochemical cycling between crusts and oceans (Orcutt et al., 2011), are interesting study models for contemporary microbial ecologists.

1.2 Distribution of Hydrothermal activity in the oceans

Since the first discovery of hydrothermal activity, a series of other hydrothermal sites have been found along all mid-ocean ridges (MORs), back-arc spreading centers and volcanic hot spots (**Figure I-2**). It is clear that hydrothermal activity is widespread across all oceans, where around 285 hydrothermal vent fields are presently confirmed and 347 inferred to be active (Beaulieu, 2013).

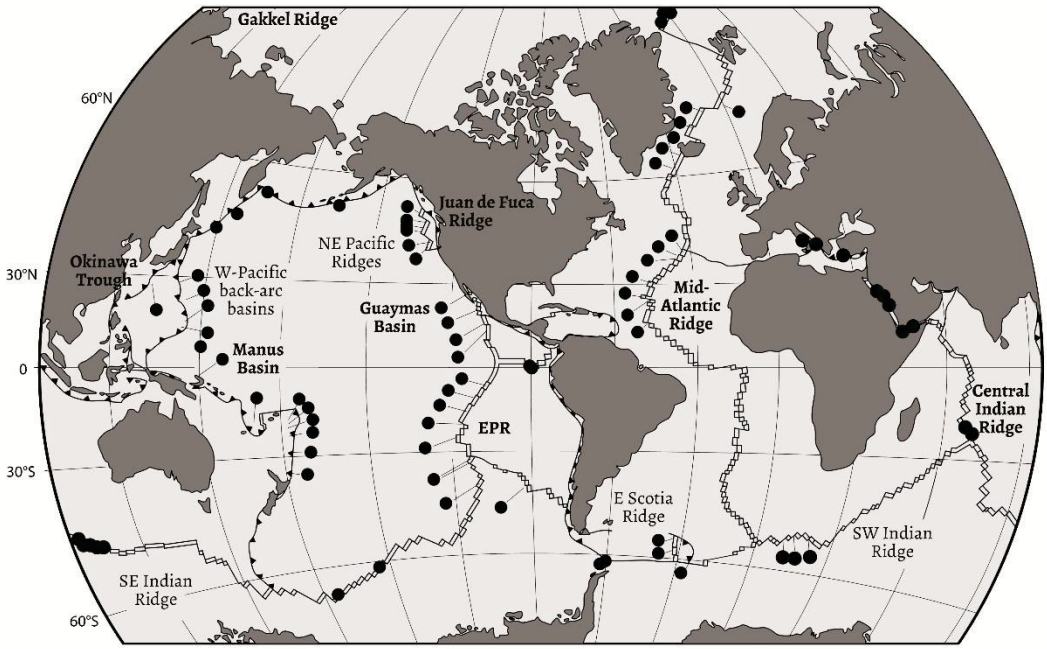

Figure I-2 - Distribution of hydrothermal vent fields along Mid-ocean ridges, island arcs, and back-arc spreading centers. Spreading centers are shown with double lines, and areas of subduction are marked with arrowheads that point in the direction of subduction. Black dots represent the most well-known vent systems in fast- to intermediate spreading ridges (e.g. East Pacific Rise – EPR, Juan de Fuca Ridge, Central Indian Ridge), slow- to ultraslow-spreading ridges (e.g. Mid-Atlantic Ridge), sediment-covered ridges (e.g. Guaymas Basin, Okinawa Trough) and back-arc basins (e.g. Manus Basin). Adapted from Census of Marine Life (ChEss project) (Baker *et al.*, 2010).

Most seafloor hydrothermal vent systems are associated with common tectonic activity and heated by magmatic heat as it is transferred into the crust (Seyfried and Mottl, 1995). The most well-studied areas of hydrothermal activity are along MORs, located at boundaries between tectonic plates. Where the ridge plates separate, molten mantle rocks rise from deep in the earth to fill the cracks created by spreading, giving rise to a new basaltic seafloor and a volcanically active ridge. According to their spreading rate, MORs are classified from superfast-spreading (i.e., those spreading more than 40 mm a^{-1} full rate) to ultralow-spreading ridges (i.e., spreading at less than 40 mm a^{-1}) (MacDonald *et al.*, 1991). Hydrothermal circulation along MORs results from the active heating of seawater that flows through the newly formed crust.

Besides, hydrothermal venting is commonly found along convergent boundaries, where an oceanic plate descends beneath the adjacent plate, forming island arc volcanoes and back-arc basins. As the descending plate bends downward at the surface releases water into the base of the overriding plate, changing the rock composition, and causing rock to melt and rise. This creates a large linear depression known as oceanic trench and leads to the eruption of chains of andesitic arc volcanoes near the edge of the overriding plate (Martinez *et al.*, 2007). Back-arc basins are formed behind subduction zones by rifting volcanic arcs and growing new volcanic seafloor. Hydrothermal vents along back-arc basins are actively heated in the same way as those along MOR, although the fluids tend to be more heterogeneous due to the variability in magma composition, and additional inputs from the subducting plate (**Table I-1**). Seafloor hydrothermal systems can also be found at intra-plate volcanic hot spots, which are not directly associated with tectonic plate boundaries. Volcanic hot spot systems are presumed to be actively heated by plumes of hot molten magma rising from the Earth's mantle and crust (Seyfried and Mottl, 1995).

1.3 Hydrothermal systems at Mid-Ocean Ridges

Rock-hosted hydrothermal systems are found in MOR spreading areas, associated with basalt or peridotite rocks. During hydrothermal circulation, dense and cold seawater passes downward through the fractured oceanic crust, becomes heated and modified, and exits again at the vent site (Ramirez-Llodra *et al.*, 2007) (**Figure I-3**).

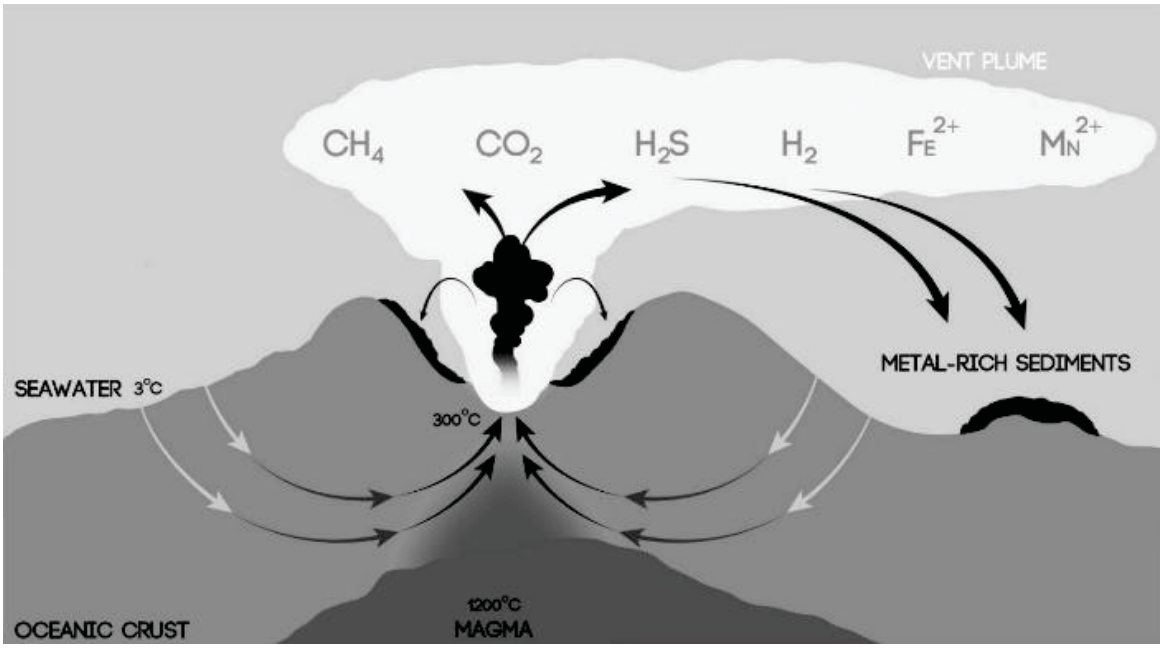

Figure I-3 - Scheme of hydrothermal circulation at MORs. Cold seawater percolates the permeable seafloor. A series of chemical reactions with high-temperature subsurface rocks occur. The chemically modified and superheated seawater is discharged back into the ocean as hot hydrothermal fluid. The rapid mixing with cold, oxygenated and alkaline seawater causes fine-grained metal sulfide minerals to precipitate out, forming buoyant plumes and sediments from hydrothermally-derived particles. © Tomás Melo.

The modified and heated seawater injected back into the ocean is called hydrothermal fluid, and its chemical composition is defined by the system's host rock. Most of the MORs are rich in the extrusive igneous rock called mid-ocean ridge basalt (MORB). Basalts contain olivine, pyroxene, and feldspar. The sulfur concentrations in submarine mafic rocks are substantially high, which result in elevated sulfide concentrations in fluids from basalt-hosted hydrothermal systems (Alt and Shanks, 1998). Moreover, these fluids are characterized by being slightly acidic, anoxic, Mg-poor, and enriched in transition metals (e.g. Fe, Zn, Cu, Mn) (Tivey, 2007) (**Table I-1**).

Table I-1 - Representative chemical compositions of hydrothermal fluids from different geologic systems. Adapted from (Mccollom, 2007; Tivey, 2007; Nakamura and Takai, 2014).

	Bottom seawater	Ultramafic-hosted	Basal-hosted MORB	Sediment-hosted	Back-arc Basin
T (°C)	2	347 – 365	≤ 405	100 – 315	278 – 334
pH (25°C)	7.8	2.8 – 3.9	2.5 – 6.6	5.1– 5.9	< 1 – 5.0
CO_2, mmol/kg	2.36	10 – 16	3.56 – 39.9	n.d.	4.4 – 274
H_2S, mmol/kg	0	1 – 2.5	0–19.5	1.10 – 5.98	1.3–13.1
CH_4, mmol/kg	3.00 E-07	0.13 – 4	0.007 – 2.58	2 – 52	0.007 – 2.58
H_2, mmol/kg	4.00 E-07	13 – 16	0.0005 – 38	< 1 – 13	0.035 – 0.5
Fe, mmol/kg	0	2.5 – 24.0	0.007–18.7	< 0.180	0.013 – 2.5
Mn, mmol/kg	0	0.3 – 2.3	0.06 – 3.3	0.01 – 0.24	0.01 – 7.1
Na, mmol/kg	464	438 – 600	10.6 – 983	315 – 560	210 – 590
Cl, mmol/kg	550	515 – 750	30.5 – 1245	412 – 668	255 – 790
Ca, mmol/kg	10.2	20 – 67	4.02 – 109	160 – 257	6.5 – 89
K, mmol/kg	10.1	10 – 22	1.17 – 58.7	13.5 – 49.2	10.5 – 79
Cu, µmol/kg	0.007	15 – 162	0 – 150	< 0.02 – 1.1	0.003 – 34
Zn, µmol/kg	0.012	25 – 185	0 – 780	0.1 – 40	7.6 – 3000
Ba, µmol/kg	0.14	> 67	1.64 – 18.6	> 12	5.9 – 100

n.d., not determined

Slow-spreading ridges may also support hydrothermal systems hosted in peridotite rocks, which are referred to as peridotite-hosted or ultramafic-hosted systems. These rocks are mainly composed of olivine and pyroxene (Mg and Fe^{2+} silicates), which react with seawater at near surface environments. The oxidation of Fe^{2+} and the reduction of water through the net exothermic reaction $3FeO + H_2O \rightarrow Fe_3O_4 + H_2$ occurs during serpentinization processes and leads to the release of molecular hydrogen (Frost, 1985; Frost and Beard, 2007). Fluids of hydrothermal systems hosted by ultramafic rocks reveal significantly high hydrogen contents. Such fluids are also typically enriched in CH_4, with high Co, Ni and Fe concentrations, and low Mg and Si levels (Wetzel and Shock, 2000; Charlou *et al.*, 2002; Douville *et al.*, 2002) (**Table I-1**).

In contrast, sediment-covered ridges occur close to continental margins, where the MOR is influenced by the close landmasses. The Guaymas Basin, located in the Gulf of California, is one of the best studied sediment-hosted systems. In general, the emitted fluids have a higher pH and lower metal contents than those from unsedimentary systems (Tivey, 2007) (**Table I-1**). It is worth mentioning that understanding these interactions and how the composition of the host rock affect the composition of hydrothermal fluids is important, as the fluids set the physiological parameters and provide the metabolic menu for colonizing microbes.

1.4 Deep-sea hydrothermal sediments

Besides chimney and seafloor structures, rock-hosted hydrothermal systems are sometimes overlain by a thick sediment layer, which is one of the products of hydrothermal activity. During hydrothermal circulation, hydrothermal fluids can either mix with cold seawater and emanate at low speed and mild temperatures into the overlying seawater (diffuse fluids), or be ejected directly into the cold seawater. In the latter case, Fe and Mn salts precipitate as black particle clouds reminiscent of smoke, which is known as vent plume. Initially, the plume has positive buoyancy relatively to seawater and thus ascends from the seafloor. Once the plume reaches density equilibrium it attains neutral buoyancy and begins to spread out laterally, away from the vent sites, driven by deep-sea currents (Lupton, 1995). The precipitation of dissolved materials and hydrothermally-derived particles can either form solid surfaces such as chimney structures or hydrothermal sediments (Rona, 1984) (**Figure I-3**). Sedimentation process may also be derived from oxidative degradation of hydrothermal structures and deposits, which cause the underlying rocks, predominantly a mixture of Cu, Zn and Fe sulfides and Ca and Ba sulfates, to leach (German *et al.*, 1993; Mills *et al.*, 1993).
The distinction of hydrothermal sediments from non-hydrothermally influenced sediments is not straightforward, and in reality, a continuous gradient exists between highly concentrated sediment deposits, consisting of nearly 100% hydrothermally-derived components, and sediments where the hydrothermal component is negligible.

1.5 Mid-Atlantic hydrothermal vents

Until the discovery of the first hydrothermal vent field detected along the slow-spreading Mid-Atlantic Ridge (MAR) – the TAG hydrothermal system (Rona *et al.*, 1986) – it was thought that hydrothermal activity would be restricted to fast-spreading ridges, due to insufficient heat flow and much lower magma supply along slow-spreading ridges. Contrarily to these predictions, hydrothermal activity in slow-spreading ridges is more localized, intense and produces larger deposits than in fast-spreading ridges (Hannington *et al.*, 1995). Since TAG

discovery, the MAR has been extensively surveyed between 14ºN and the Azores Triple Junction (ATJ) (Charlou *et al.*, 2000).

Research expeditions conducted on the south-western branch of the ATJ resulted in the discovery of many other hydrothermal fields. To date, sixteen active vent fields have been visually confirmed in the axial valley of the Northern MAR, between the equator and the ATJ at ~39°N, and only one has been detected between the ATJ and the Charlie Gibbs Fracture Zone at 52.5°N, which divides the MAR from the Reykjanes Ridge (Beaulieu, 2013) (**Figure I-4**).

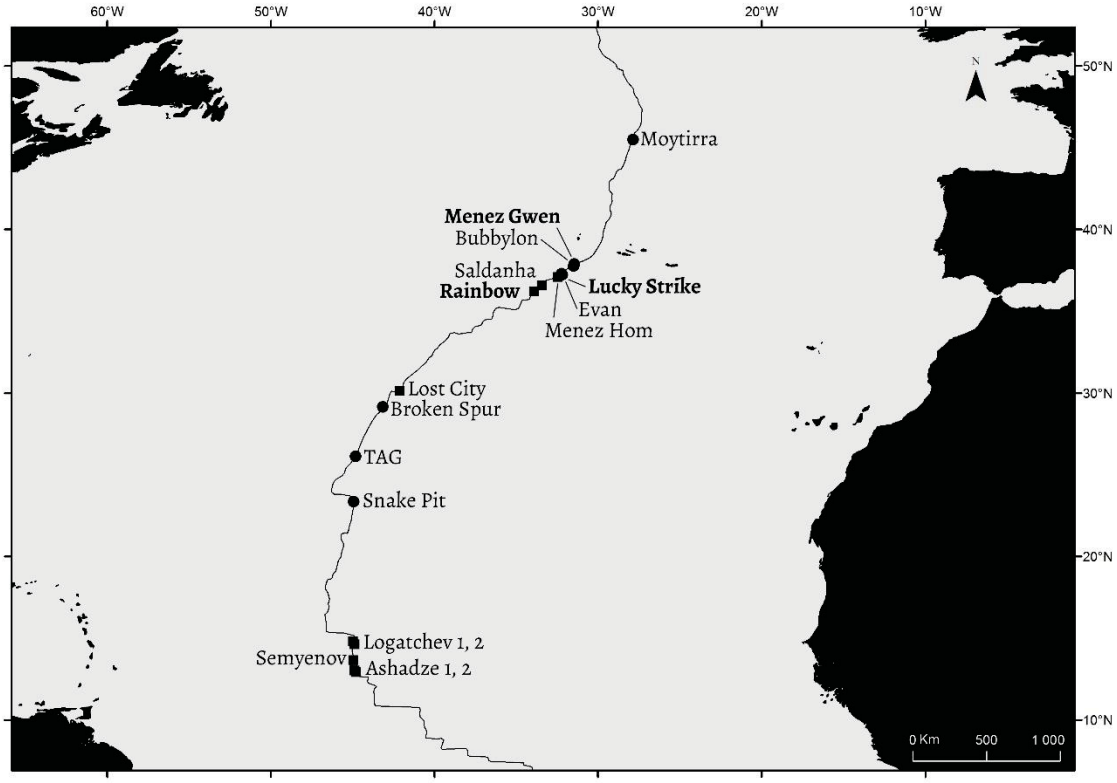

Figure I-4 - Currently known active hydrothermal vent fields along the Northern Mid-Atlantic Ridge, between the equator and the Charlie Gibbs Fracture Zone at 52.5°N. Circles denote basalt-hosted whereas squares ultramafic-hosted systems. © Tomás Melo and Ricardo Medeiros, ImagDOP.

The three major vent systems in the ATJ area, are located on three distinct MAR segments, at different depths, and within 2 degrees of latitude. These are the Menez Gwen, Lucky Strike and Rainbow vent sites, the three most well-studied high-temperature deep-sea hydrothermal vent fields of the Azores (**Figure I-4**). The region is marked by the intersection of three tectonic plates, being the seafloor shaped by a discontinuous bathymetry, changing source rocks, and geochemically distinct hydrothermal vent fields.

The three neighbouring systems were the chosen sites for this work. The Menez Gwen and Lucky Strike vent systems are located on axial seamounts and on a relatively shallow portion of the MAR, at 850 and 1700 m depth, respectively. Both are basalt-hosted fields influenced by an area of enhanced melt production in the mantle (Parson *et al.*, 2000). By contrast, the

Rainbow field, located at 2300 m depth, is associated with upper-mantle ultramafic rocks. The three vent fields have been relatively well characterized with respect to fluid chemistry (Langmuir *et al.*, 1997; Charlou *et al.*, 2000; Charlou *et al.*, 2002; Douville *et al.*, 2002; Seyfried *et al.*, 2011), geologic setting (Humphris *et al.*, 2002; Ondreas *et al.*, 2009; Amend *et al.*, 2011), deposit mineralogy and macrobiological communities (Colaço *et al.*, 1998; Desbruyères *et al.*, 2001; Riou *et al.*, 2008; Cuvelier *et al.*, 2011; Bettencourt *et al.*, 2014). However little is known about the microbial ecology associated with the hydrothermal environments in these fields.

1.5.1 The basalt-hosted Menez Gwen

The Menez Gwen hydrothermal field (37°50′N), discovered in 1994 (Fouquet *et al.*, 1994), is located on a circular volcano of 17 km in diameter and 700 m height, in the central part of the ridge segment. At the top of this volcano lies an axial graben of 6 km long, 2 km wide and 300 m deep. At the graben's northern end is set a new structure with 600 m in diameter and 120 m in height, composed entirely of fresh pillow lavas and scarce sedimentary cover, indicating that the basaltic rocks are young, only some decades old (Fouquet *et al.*, 1995). Hydrothermal activity is found at a depth between 840 to 865 m, on the northern end of this graben, covering around 200 m^2 (Fouquet *et al.*, 1994). Small and white anhydrite chimneys, typically less than 5 m high, discharge clear hydrothermal fluids with temperatures ranging between 265°C and 281° C, and pH between 4.2 and 4.8 (Charlou *et al.*, 2000; Desbruyères *et al.*, 2001). Low-temperature diffusing flows may also be found throughout the area. Hydrothermal fluids are usually enriched in CH_4 (1.4 – 2.6 mM) and H_2S (1.5–2 mM), while depleted in H_2 when compared to other MAR sites (Charlou *et al.*, 2000). The faunal composition is dominated by large colonies of *Bathymodiolus azoricus* mussels which cohabit with limpets (among others *Lepetodrilus atlanticus* and *Protolira valvatoides*), shrimps (*Chorocacis chacei* and *Mirocaris fortunata*), and crabs (*Chaecon affinis*) (Colaço *et al.*, 1998).

1.5.2 The basalt-hosted Lucky Strike

Ninety-one kilometres south of Menez Gwen vent field lies the Lucky Strike vent field (37°17′N), discovered in 1992 (Langmuir *et al.*, 1993; Langmuir *et al.*, 1997). It is one of the largest vent fields along the MAR, covering an area of about 150 Km^2, with a great extent of sulfide deposits. The system is set at 1700 m depth, on a prominent volcano without an axial graben, with three circular volcanic cones at the summit. Within the depression between them, and surrounding a central "lava lake", on-going hydrothermal activity is detected, extending over nearly 1 Km^2 (Fouquet *et al.*, 1994; Langmuir *et al.*, 1997; Charlou *et al.*, 2000). The three

volcanic cones and most of the summit area consist of pillow basalts and basaltic breccia with scarce sedimentary cover, evidencing a relatively young age of the volcanic rocks (Fouquet *et al.*, 1995). The Lucky Strike field supports several active hydrothermal sulfide structures ranging from black smoker chimneys with temperatures up to 333°C to mounds and cracks discharging shimmering fluids and cooler diffusing outflows (Fouquet *et al.*, 1995; Humphris *et al.*, 2002; Ondreas *et al.*, 2009). The predominant gas detected in the hydrothermal fluids is CO_2 (8.9–133 mM), being H_2 and CH_4 at low (< 1 mM) and H_2S at moderate (1.4–3.4 mM) levels (Charlou *et al.*, 2002; Flores *et al.*, 2011). Hydrothermal sediments in Lucky Strike are reported to be enriched in elements derived from high-temperature hydrothermalism (Fe, Cu, Zn and Ba) (Humphris *et al.*, 2002; Ondreas *et al.*, 2009).

Faunal assemblages are dominated by shrimp aggregations of *Mirocaris fortunata* and *Chorocaris chacei* living close to the vent outflows, and dense beds of *Bathymodiolus azoricus* mussels widely distributed within the field, generally in warm diffuse venting zones (Desbruyères *et al.*, 2001). The crab *Segonzacia mesatlantica* and other less conspicuous species, like polychaetes and limpets have been widely detected.

1.5.3 The ultramafic-hosted Rainbow

The Rainbow vent field (36°14'N) was discovered in 1997 (Fouquet *et al.*, 1997), 187 Km southwest of the Lucky Strike system. It is set at 2300 m deep, on the western flank of a nonvolcanic ridge, lying at the intersection between the nontransform system faults and the ridge faults, which implies that this vent field is tectonically controlled (Douville *et al.*, 2002). Rainbow field is associated with upper-mantle ultramafic rocks, and high-temperature (up to 370 °C) serpentinization processes. This confers a highly acidic character to vent fluids (pH 2.8), which are enriched in H_2 and present the highest dissolved Fe concentrations of any mid-ocean ridge vent fluid yet studied (Seyfried *et al.*, 2011). Rainbow fluids also present high concentrations of CH_4 (2.0–2.5 mM) and quite low concentrations of H_2S (1–3.3 mM) relative to their basalt-hosted counterparts (Charlou *et al.*, 2002; Mccollom, 2007; Flores *et al.*, 2011). Hydrothermal activity takes place through several groups of extremely active and large black smokers and hundreds of small chimneys. There are numerous inactive structures amongst a large number of rather short-lived active venting sites, dispersed over the entire field in an area of nearly 15 km^2 (Fouquet *et al.*, 1997).

The main vent associated fauna consists of *Rimicaris exoculata* and *Mirocaris fortunata* shrimps, whereas *Bathymodiolus azoricus* and *Bathymodiolus seepensis* mussels dominate the faunal communities on surrounding blocks within the active area.