


REVIEW

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Unveiling microbial diversity in deep geothermal fluids, from current knowledge and analogous environments

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Abstract

Extreme environments on Earth host a large diversity of microbial life. Bacteria, archaea, and fungi are able to survive under one or several extreme conditions including extreme ranges of temperature, pressure, pH or salinity. Despite extensive research on extremophilic microorganisms, a relatively unexplored frontier within the study of the deep biosphere is the survey of the diversity of microorganisms inhabiting deep geothermal reservoirs used for energy production. These sites offer unique access to investigate life in the deep biosphere. The conditions in these reservoirs are often within the range of the known limits of life, which makes them a suitable habitat for various extremophilic microorganisms. Moreover, microbial-driven processes such as microbially induced scaling or corrosion can decrease the efficacy of geothermal power plant systems. The present review summarizes the current knowledge and uncertainties surrounding microbial life in deep geothermal reservoirs. As the knowledge in deep geothermal fluids is still scarce, the microbial diversity in analogous environments, such as surface geothermal springs, deep-sea hydrothermal vents or deep subsurface environments, is also summarized here. The high diversity of microorganisms inhabiting these analogous environments suggests that deep geothermal fluids may host an unsuspected microbial diversity. Moreover, the challenges associated to the study of microorganisms in geothermal fluids are reviewed. These include notably challenges linked to sampling, DNA extraction from low biomass samples, DNA amplification and sequencing of unknown communities, and biases induced by comparison of the sequences obtained to reference databases. Such biases are even stronger concerning fungi and archaea, as specific databases are less extensive than those for bacteria. A broader knowledge on microorganisms in deep geothermal fluids may not only allow to reduce the negative impact of microbial activity in geothermal power plants, but could also provide new insights into the evolution of microorganisms and their survival in extreme environments.

Keywords: Deep geothermal fluids, Extreme microorganisms, Deep biosphere, 16S rRNA gene sequencing, Molecular methods

Introduction

The productivity of geothermal systems depends not only on the rock properties of the reservoir but also on the fluid-chemical composition and physical properties (Finster et al. 2015). Despite the worldwide use of geothermal fluids, the complexity of these systems makes them hard to characterize precisely. One additional factor that is becoming increasingly relevant is the diversity of life (mostly microbial) hosted in geothermal waters (Gniese et al. 2014). Microbial life in such fluids is often ignored, despite the growing evidence of the presence of microorganisms in geothermal power plant systems (Inagaki et al. 2003; Filippidou et al. 2016; Westphal et al. 2019). The impact of microbial diversity associated with geothermal energy production is a two-way road. Indeed, microorganisms (or microbial activity) are known to be involved in the formation of scales (e.g. silica scales), thus decreasing the power plant's efficacy (Inagaki et al. 2003). At the same time, the exploitation of a geothermal reservoir might change the environmental conditions, resulting in changes on the composition and metabolic processes of deep geothermal microbial communities. These changes could set off new metabolic reactions, for instance leading to silica precipitation or hydrogen sulphide production (Hao et al. 1996; Inagaki et al. 2003).

To date, the knowledge on the microbial diversity present in deep geothermal fluids is extremely scarce. Deep geothermal reservoirs can be considered more generally as a combination of the deep biosphere, which encompasses any ecosystem located below the seafloor or the terrestrial surface, from 1 m depth and beyond (Edwards et al. 2012; Shu and Huang 2021) and geothermal systems. Therefore, this review presents not only the limited number of studies performed in deep geothermal systems, but also an overview of the known microbial diversity present in other environments from the deep biosphere and in extreme environments that share some of the key environmental constraints with deep geothermal fluids used for electricity production. This comparison is intended as a source of baseline information for the identification of the potential diversity of microbial life to be found in deep geothermal fluids. The parallels made are meant to underline the probability to find diverse microorganisms in deep geothermal fluids, but cannot replace microbial diversity studies on deep geothermal fluids. Moreover, it is important to highlight that even though microorganisms may be found in an environment, this does not necessarily mean that they are metabolically active, as they can also be present in a dormant state. Hence, one needs to establish the metabolic state of the observed microorganisms at the time of investigation, which can be aided, for instance, by hydrochemical and isotopic investigations. In addition to this diversity overview, we highlight the potential impacts of microorganisms on the functioning of geothermal power stations and the challenges to study them in these specific settings.

Generalities about bacteria, archaea and fungi

By definition, microorganisms consist of all living forms with a cellular state that is invisible to the naked eye. This term includes prokaryotes (organisms lacking a nucleus), such as Archaea and Bacteria, as well as eukaryotes (organisms having their DNA encapsulated into a nucleus inside the cells) such as fungi, protists and micro-algae, and non-cellular life forms such as viruses or prions. Current knowledge on the diversity of life

in geothermal environments indicates that the microorganisms potentially present in geothermal power plants could belong to all these microbial groups (Ghozzi et al. 2013; Inskeep et al. 2013; Strazzulli et al. 2017; Oliverio et al. 2018; Prieto-Barajas et al. 2018). However, the focus of this review is on the archaeal and bacterial domains, as well as on the fungal kingdom. Archaea and bacteria are prokaryotic organisms, usually unicellular, and their size ranges from 0.2 to 50 μm . Fungi are eukaryotic organisms and may either be unicellular, such as yeasts or chytrids, or pluricellular, such as filamentous fungi, for which the vast majority of the organism is represented by a mycelium that is within microbial size range.

Many bacteria and most fungi are able to form resistant cells, such as spores, which allow them to survive under conditions unsuitable for active growth (Kües and Fischer 2006; Setlow 2010). In bacteria, known types of resistant cells include endospores (Firmicutes) (Nicholson et al. 2002), exospores (Actinobacteria) (Beskrovnaya et al. 2021), myxospores (Myxococcales) (Bui et al. 2009), cysts (*Azotobacter*) (Whittenbury, Davies and Davey 1970) or akinetes (Cyanobacteria) (Kaplan-Levy et al. 2010). Fungi can also form multiple types of spores, which can either be asexual (clones of the parental cell) or sexual (produced by sexual reproduction, which involves the fusion of two parental nuclei and the return to a haploid state through meiosis, including genetic recombination through crossing-overs). In addition to the formation of spores, some fungal extremophiles may adapt their cell wall morphology or composition to extreme conditions by increasing its thickness or by including melanin and mycosporins. This is typically the case of black-fungi, which are considered as poly-extremophilic fungi (Gostinčar, Muggia and Grube 2012). Archaea are not known to form spores, but they are highly resistant to extreme conditions thanks to, for instance, the unique lipid structure of the cell membrane (Siliakus, van der Oost and Kengen 2017). The ability to produce resistant structures and to resist to diverse environmental conditions allow archaea, bacteria and fungi to colonise all known environments, including extreme environments.

Extreme environments and the known limits of life

Extreme environments can be defined in different ways. However, the factors usually used to define extreme environments are salinity, pH, temperature, pressure, radiation, water activity, and energy and nutrient limitations (Rothschild and Mancinelli 2001; Merino et al. 2019). Other factors, such as the presence of heavy metals or electric currents may also create extreme conditions (Rothschild and Mancinelli 2001). Organisms thriving and requiring extreme conditions to complete their life cycles are called extremophiles, while extremotolerant organisms only tolerate them (Rampelotto 2013). The different categories of extremophiles of interest for this review are summarized in Table 1. So far, in surface extreme environments, the main driver of microbial biogeography is either temperature (in environments with temperature above 70 °C) or pH (in environments with a temperature below 70 °C) (Power et al. 2018). However, such biogeographic considerations are currently unknown for deep geothermal environments.

The true limits of life are likely still unknown today but our knowledge on microorganisms thriving or surviving under extreme conditions is constantly increasing (Merino et al. 2019). Additionally, the presence of dormant resistant structures might push even further the presence of microbial life in extreme environments, especially pushing the

Table 1 Different categories of extremophiles and their respective limits of the concerned parameters

Parameters	Category	Limits
High temperature [°C]	Thermophile	45–80 °C
High temperature [°C]	Hyperthermophile	> 80 °C
Pressure [MPa]	Barophile (or piezophile)	10–50 MPa
Pressure [MPa]	Hyperbarophile	> 50 MPa
Salinity	Halophile	NaCl (w/v) > 8.8%
High pH	Alkaliphile	pH > 9
Low pH	Acidophile	pH < 5

Adapted from (Merino et al. 2019). Salinity is only represented by NaCl concentration, however other salts could be considered.

limits of environments inhabited by dormant bacteria and fungi, two microbial groups for which the limits of active growth are below those reported so far for archaea. Accordingly, nearly all Earth's environments studied so far do host life (Cottin et al. 2017). As shown in Table 2, which illustrates the currently known limits of life, extremophilic or extremotolerant microorganisms can withstand temperatures of up to 122 °C (the archaeon *Methanopyrus kandleri*) (Takai et al. 2008), pressures of up to 125 MPa (the archaeon *Thermococcus piezophilus*) (Dalmasso et al. 2016), or a pH from 0 (the archaea *Picrophilus oshimae*, *Picrophilus torridus*, *Acidiplasma aelicum*) (Schleper et al. 1995; Golyshina et al. 2009; Merino et al. 2019) to 14 (the fungus *Penicillium* spp.) (Dhakar et al. 2014). For all of the above, even though archaea hold many of the most extreme records, bacteria and fungi are not far behind. Radiation requires particular mention in the subsurface, where microorganisms may be exposed to alpha-, beta- and gamma-radiation due to the decay of different radioactive elements (uranium-238, thorium-232 and potassium-40) (Blair et al. 2007; Lollar et al. 2014; Sauvage et al. 2021). Members of the archaeal, bacterial and fungal kingdom are known to resist to radiation, going as up as 30kGy of gamma radiation for the archaea *Thermococcus gammatolerans* EJ3 and the bacteria *Deinococcus hohokamensis* and 6kGy for the fungus *Exophila dermatitis* (Jolivet et al. 2003; Rainey et al. 2005; Romsdahl et al. 2021).

Characteristics of deep geothermal fluids and potential energy sources for microbial life

Properties of deep geothermal fluids vary greatly from site to site (Finster et al. 2015). Deep geothermal fluids present various parameters that are often considered as extreme for life, such as high temperature, high pressure or high salinity (main parameters summarized in Table 3). Moreover, the presence of radioactive elements, heavy metals, dissolved gas, wide ranges of pH in geothermal reservoirs (Bertani 2005; Finster et al. 2015; Regenspurg et al. 2016; Moya et al. 2018), as well as the flow induced by the use of the fluids for energy or heat production, are all expected to constrain microbial life (Simões et al. 2010). Nevertheless, these parameters often fall within the known limits of microbial life (see Table 2). Moreover, the presence of diverse organic carbon sources in geothermal fluids suggests once more that such environments may not be as hostile to life as previously thought (Leins et al. 2022). Indeed, organic compounds in deep geothermal fluids, in particular dissolved organic carbon (DOC), may be used as a nutrient source for microorganisms, allowing their survival in such environments. Moreover, it was

Table 2 Known limits of life for Archaea, Bacteria and Fungi

Parameter	Archaea			Bacteria			Fungi		
	Limits	Organisms	References	Limits	Organisms	References	Limits	Organisms	References
High temperature [°C]	122 °C	<i>Methanopyrus kandleri</i> 116	(Takai et al. 2008)	100 °C	<i>Geothermobacterium ferriducens</i>	(Kashefi et al. 2002)	61 °C	<i>Chaetomium thermophile</i> LaTouche, <i>Thermoascus aurantiacus</i> , <i>Thermomyces ibadanensis</i> Apinis & Eggins	(Maheshwari et al. 2000)
Salinity	125 MPa	<i>Thermococcus piezophilus</i>	(Dalmaso et al. 2016)	100 MPa	<i>Shewanella benthica</i>	(Kato et al. 1998)	111 MPa**	Ascomycota, Basidiomycota	(Xu et al. 2014)
High pH	37%	<i>Haloferax sulfurifontis</i>	(Elishahed et al. 2004)	35% NaCl	<i>Halarsenatibacter silvermanii</i> SLAS-1 T	(Oremland et al. 2005; Blum et al. 2009)	32% NaCl	<i>Hortaea werneckii</i>	(Gunde-Cimerman et al. 2000)
Low pH	10,5	<i>Natronolimnobius aegyptiacus</i>	(Mesbah and Wiegel 2012; Zhao et al. 2018)	12.5	<i>Serpentinomonas</i> sp. B1	(Suzuki et al. 2014; Bird et al. 2021)	14	<i>Penicillium</i> spp.	(Dhakar et al. 2014)
Radioactivity (gamma radiations)	0	<i>Picrophilus oshimae</i> , <i>Picrophilus torridus</i> / <i>Acidiplasma aeolicum</i> strain VT	(Schleper et al. 1995; Golyshina et al. 2009; Merino et al. 2019)	1.1	<i>Sulfobacillus sibiricus</i> N1	(Melamud et al. 2003)	1.5	<i>Penicillium</i> spp.	(Dhakar et al. 2014)
Radioactivity (gamma radiations)	30 kGy	<i>Thermococcus gammatolerans</i> EJ3	(Jolivet et al. 2003)	30 kGy	<i>Deinococcus hohokamensis</i>	(Rainey et al. 2005)	6 kGy	<i>Exophiala dermatitidis</i>	(Romsdahl et al. 2021)

Limits that were not clearly found are represented by N.D. (Not Defined)

** Only DNA detected (uncultured organisms)

Table 3 General characteristics of fluids inside reservoirs of geothermal power plants used for energy production

Geothermal power plants used for electricity production		
Parameters	Typical ranges	References
Temperature [°C]	73°–450 °C	(Zarrouk and Moon 2014; Khare et al. 2019)
Depth	300 m–5000 m	(Sanjuan et al. 2016; Moya et al. 2018)
Pressure [MPa]	Up to 48 Mpa	(Árpási, Lorberer and Pap 2000)
Cl ⁻	Up to 160 g/L	(Leins et al. 2022)
TDS	95–280'000 mg/L	(Finster et al. 2015)
pH	0.9–11.8	(Finster et al. 2015)
Radioactivity	> 200 pCi/L (equal to > 7.4 Bq/L)	(Finster et al. 2015)
DOC	Up to 25.9 mg C/L	(Leins et al. 2022)
Formate	Up to 40.4 mg/L	(Feldbusch 2015)
Acetate	Up to 47.6 mg/L	(Leins et al. 2022)
Propionate	Up to 2.64 mg/L	(Leins et al. 2022)

DOC dissolved organic carbons, TDS total dissolved solids

shown that in geothermal fluids used for heat storage, the presence of DOC and sulphate (SO₄²⁻) can provide enough electron donors and acceptors to allow microbial respiration (Vetter et al. 2012). It is thus imaginable that similar processes happen in higher temperature fluids as well. More generally speaking, in the terrestrial subsurface, the presence of sulphate, which can originate from the oxidation of pyrite for instance, can sustain the metabolism of sulphate-reducing organisms (Lau et al. 2016). On the contrary, low amounts of DOC in low temperature geothermal fluids (< 80 °C) may not necessarily be due to low concentrations in the original fluids, but to the degradation of organic carbon by microorganisms (Leins et al. 2022). The presence and origin of organic compounds in deep geothermal fluids is extensively discussed in (Leins et al. 2022). Furthermore, the presence of CO₂ in deep geothermal fluids (Alt-Epping et al. 2013; Canbaz, Ekren and Aksoy, 2022), may also serve as a carbon source for autotrophic microorganisms (Saini et al. 2011), further sustaining life in these extreme environments. The presence of hydrogen (H₂) in some geothermal fluids (Leila et al., 2021) is another energy source that can sustain microbial life. In the deep biosphere, H₂, which can come from tectonic activity, serpentinization or water radiolysis, can serve as an energy source for chemolithotrophic growth (Freund et al. 2002; Blair et al. 2007; Colman et al. 2017; Sauvage et al. 2021).

Microbial life in deep geothermal fluids and other related extreme environments

Diversity of microorganisms in environments analogous to geothermal power plants

Deep geothermal fluids display several extreme parameters, which are at the interface between environments from the deep biosphere and geothermal environments, making possible formulating parallels between these environments (Fig. 1). In fact, the microbial diversity found in systems with some of the same extreme conditions defined for deep geothermal fluids can provide hints on the microbial life that can be expected in the latter.

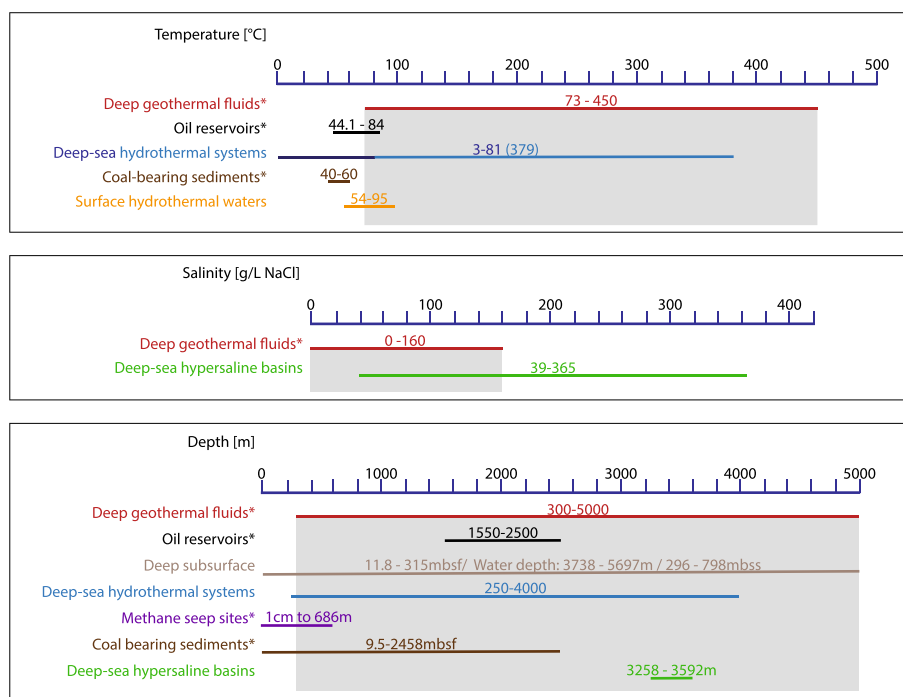


Fig. 1 Overlap in environmental conditions between deep geothermal fluids used for electricity production and other extreme environments. The parameters shown are temperature [°C], salinity [NaCl in g/L], and depth [m]. The presence of hydrocarbons is marked by an Asterisk (*). For depth, the following abbreviations are used: meters below sea floor (mbsf), meters below soil surface (mbss).

The microbial diversity currently known in the deep biosphere and in geothermal environments is summarized in Additional file 1: Table S1. From this information, the overlap of the reported microbial genera found in these different environments was represented graphically in Fig. 2. Despite the scarcity of knowledge on the microbial diversity in deep geothermal fluids used for electricity production, it is noteworthy to see that the known diversity at the genus level largely overlaps with the diversity reported for each analogous environment considered here. The overlap underlines the fact that the microbial diversity present in other extreme analogous environments can provide useful hints on the microbial diversity that can be expected in deep geothermal fluids used for electricity production (Additional file 2).

The deep biosphere

Deep geothermal fluids are part of the deep biosphere, which includes all environments from 1 m below the surface (Edwards et al. 2012; Shu and Huang 2021). This includes the terrestrial subsurface, the marine seafloor and all kinds of deep-sea environments. Generally speaking, the deep biosphere is known to host a large diversity of microorganisms. Indeed, it is estimated that most prokaryotic life on Earth is located in the deep biosphere (Bar-On et al. 2018; Magnabosco et al. 2018; Drake and Reiners 2021) and the discoveries of life in such extreme habitats challenge our understanding of the limits of life (Inagaki et al. 2003; Orsi, Biddle and Edgcomb 2013; Ivarsson et al. 2018; Zain Ul Arifeen et al. 2020). These deep environments

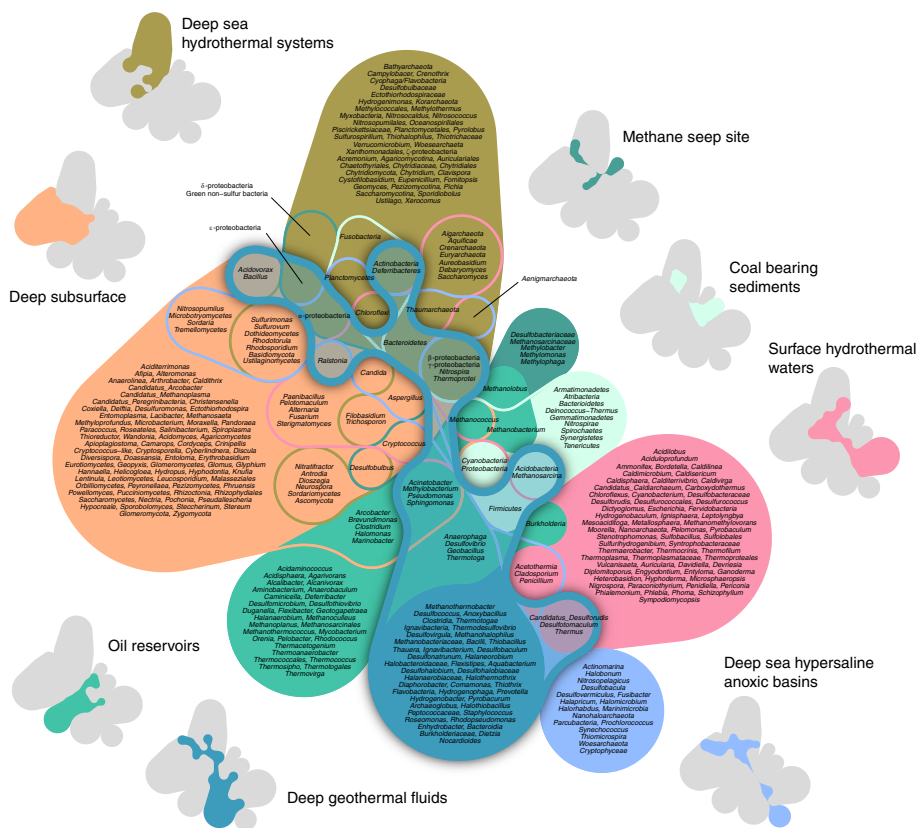


Fig. 2 Main bacterial, archaeal and fungal genera reported so far in deep geothermal fluids and the analogous environments considered in this review. This graphical representation (Venn diagram) highlights the overlaps between environments. Taxonomic classification was only kept up to the genus level

are characterized by their high pressure and some, such as deep-sea hydrothermal vents, also involve geothermal fluids. The pressure in the deep biosphere, whether it is below water or below the Earth surface, increases gradually. Under water, the pressure increases by 10 MPa per km against 30 MPa per km underground (Jebbar et al. 2015). Besides pressure, organisms inhabiting the deep biosphere may have to deal with temperature extremes (high or low), high salinity, low concentrations of nutrients, anaerobic conditions or the presence of toxic compounds (Rastogi et al. 2010; Zain Ul Arifeen et al. 2020). Despite these harsh conditions, various prokaryotic and eukaryotic microorganisms have been frequently found in the deep biosphere (Schipers et al. 2005; Orsi, Biddle and Edgcomb 2013; Borgonie et al. 2015; Zain Ul Arifeen et al. 2020). Interestingly, fungi are the dominant group among eukaryotes in such environments (Orsi, Biddle and Edgcomb 2013; Rédou et al. 2015; Zain Ul Arifeen et al. 2020).

The subsurface, which is part of the deep biosphere, is one of the largest microbial environments on Earth (McMahon and Ivarsson 2019). In subsurface environments, temperature is one of the most important parameters limiting life (Hoehler 2004). The temperature gradient in the continental subsurface is 25 °C km⁻¹ and thus, the habitable zone is theoretically limited to 5 km below the surface (Takai et al. 2008;

Jebbar et al. 2015). Life has been detected in waters, sediments or crust samples at various depths below the sea floor (Schippers et al. 2005; Mason et al. 2010; Inagaki et al. 2015; Ivarsson, Bengtson and Neubeck 2016; Liu et al. 2017; Purkamo et al. 2020; Quemener et al. 2020; Zain Ul Arifeen et al. 2020; Merino et al. 2022). Bacteria detected in a 4.4 km deep crystalline bedrock correspond to the deepest evidence of microbial life in the subsurface (Purkamo et al. 2020). Beside direct detection of microorganisms in the subsurface, the microbial degradation of hydrocarbons in fossil fuel reservoirs up to 4 km below the seafloor further support the presence of active microbial life in the deep subsurface (Head et al. 2003; Jones et al. 2008). However, in fossil fuel reservoirs, microorganisms were found to be present only in reservoirs with a maximum temperature of 80 °C (Head et al. 2003). The search for fossils in the subsurface or subseafloor can also give some hints on the presence and diversity of microorganisms in such environments. In fact, fossils of bacteria, fungi, and prokaryotic microorganisms have been found in deep subseafloor basalts (Ivarsson et al. 2012; Bengtson et al. 2014), notably pointing out to the importance of this environment for fungi (Ivarsson 2012). Biogeographical patterns of microbial communities are still not completely understood for the deep subsurface, and probably differ from one type of environment to another. For instance, it has been shown that the observed changes in microbial communities can be driven by advection in fractured rock aquifers (Zhang et al. 2022) or a combination between nutrients, connectivity to the surface and geological processes in subsurface aquifers (Hubalek et al. 2016). Moreover, in some subsurface aquifers, it was shown that microbial communities are more similar to each other if environmental conditions are similar, and if they are in close geographic proximity (Magnabosco et al. 2014). Thus, changes in microbial communities could also be used to track changes in fractures systems and connectivity between wells or to the surface (Magnabosco et al. 2014; Hubalek et al. 2016; Zhang et al. 2022). This is something that can be of relevance in the planning and management of deep geothermal drilling projects.

Beside terrestrial subsurface and deep subseafloor, deep-sea environments are habitats in which microorganisms proliferate. In these deep-sea environments, some of the most diverse microbial hotspots can be found in hydrothermal areas. Indeed, microorganisms can thrive in such environments, whether in the hydrothermal fluids, around hydrothermal vents or in surrounding sediments and numerous bacteria, archaea, and fungi have been found there (Blöchl et al. 1997; Edgcomb et al. 2002; López-García et al. 2003; López-García et al. 2007; Calvez et al. 2009; Connell et al. 2009; Anantharaman et al. 2016; Ding et al. 2017; Dick 2019) (Additional file 1: Table S1). Deep-sea hypersaline anoxic basins (DHABs), such as the L'Atalante basin in the Mediterranean sea, can be considered as some of the most hostile environments on Earth (anoxic and sulfidic conditions, combined with high salinity, high hydrostatic pressure, presence of toxic compounds such as hydrogen sulphide, manganese, and ammonium, as well as the complete absence of light) (Barone et al. 2019). Nevertheless, communities of archaea, bacteria, and fungi has been discovered recently in several DHABs, including some metabolically active fungi (Alexander et al. 2009; Stock et al. 2012; Bernhard et al. 2014; Steinle et al. 2018).

Geothermal environments outside of the deep biosphere

The best characterized geothermal environments in terms of their microbiology correspond to surface geothermal systems. Therefore, the microbial diversity present in such systems can also provide important indications as to the diversity of microorganisms that can be found in deep geothermal fluids. Geothermal environments other than deep geothermal fluids or hydrothermal vent systems include all sorts of geothermal surface waters, such as hot springs and geothermal lakes, which can be subjected to extremes in temperatures and pH. In these geothermal surface waters, the microbial diversity is driven mainly by temperature but also by pH, and *in-situ* chemistry (Sharp et al. 2014; Iacono et al. 2020). The best-known example of surface geothermal hot springs are the manifestations of Yellowstone National Park (USA). Since the pioneering studies by Brock in 1978 (Brock 1978), the archaeal and bacterial communities of this area have been extensively studied through molecular analyses and isolation of organisms, notably underlying the presence of Aquificales, Thermodesulfobacteriales, Caldiserica, Crenarchaea, Desulphurococcales and Thermoproteales in hot springs (Sand 2003; Meyer-Dombard et al. 2005; Inskeep et al. 2013; Colman, Lindsay and Boyd 2019). Studies on archaea and bacteria in geothermal environments are numerous, not only in Yellowstone, but in various geothermal waters and soils (Power et al. 2018; Prieto-Barajas et al. 2018). Moreover, in the last decade, fungi have been also found in several geothermal environments including alkaline and acidic surface thermal hot springs and their surrounding soil. These studies have shown the dominance in one case of *Penicillium*, *Entyloma*, *Cladosporium*, *Engyodontium* and *Schizophyllum* and in the other case of Ascomycota, with *Thermomyces lanuginosus* (34.78%) and *Scytalidium thermophilum* (28.26%) as dominant species (Pan et al. 2010; Liu et al. 2018). Fungi have been reported also in acidic geothermal lakes (Ascomycota, including *Aspergillus*, *Paecilomyces*, *Ochroconis*, *Acidomyces*, *Penicillium*, *Cladosporium* and *Phialophora*) (Ervin 2014; Wolfe et al. 2014), hot springs of soda lakes (mainly Ascomycota (83.3%), followed by Basidiomycota (15.8%) and Glomeromycota (0.02%)) (Salano et al. 2017) or surface geothermal water sources (Chytridiomycota: *Batrachochytrium dendrobatidis*) (Forrest and Schlaepfer 2011).

Diversity studies in deep geothermal fluids used for energy production

In terms of sustainability, one of the major challenges for the exploitation of geothermal resources is assessing the role of geothermal systems as a biodiversity reservoir. Geothermal systems provide a unique set of environmental conditions that will result in very specialized ecosystems often harbouring a unique set of living organisms well adapted to survive under those conditions. Therefore, as part of the development of geothermal projects, understanding the biodiversity associated with each individual system is required to assess its impact. Moreover, as for any other ecosystem on Earth, the organisms within a geothermal ecosystem can have a direct impact on the efficiency of the use of the energy resource. These two elements combined make the assessment of biodiversity an important component of studies aiming at improving geothermal energy development. There is a risk of contamination of geothermal fluids during drilling (Regenspurg et al. 2018), fluid sampling, or enhanced geothermal systems (EGS) stimulation (which stimulates the rock reservoir to increase the efficacy of the power plant) (Blöcher et al.

2016) and therefore, microorganisms detected in geothermal power plants may not all come directly from the fluids or the reservoir (Dahle et al. 2008). However, the consistent detection of microorganisms in diverse geothermal environments suggest that some of them are indigenous to these environments.

The diversity of microorganisms detected or isolated in geothermal fluids used for electricity production is summarized in Table 4. It is interesting to note that all of these studies concern Archaea and Bacteria, but not Fungi. Even though the knowledge of the microbial diversity present in deep geothermal fluids is still scarce, some studies have shown the presence of microorganisms in fluids of up to 250 °C (in situ) and at 4200 m depth (Takai, Komatsu and Horikoshi 2001; Filippidou, Jaussi, et al. 2015). However, the active metabolic state of microorganisms under these extreme conditions was not demonstrated. In fact, biomarkers of bacteria and archaea have been detected in different types of geothermal fluids (Takai and Horikoshi 1999; Takai, Komatsu and Horikoshi, 2001; Fardeau et al. 2009; Alawi et al. 2011; Lerm et al. 2011; Morozova, Alawi et al. 2011; Lerm et al. 2013; Westphal et al. 2019; Kalwasińska et al. 2020; Purkamo et al. 2020). Moreover, biofilms (i.e., organised form of multispecies collective growth of organisms on surfaces) are common as deposits inside geothermal power plant pipes (Inagaki et al. 1997; Inagaki et al. 2003; Fujino et al. 2008; Filippidou, Jaussi, et al. 2015; Filippidou et al. 2016). In addition to the detection of biomarkers and biofilms, several bacterial strains have been isolated from geothermal fluids (Table 4). Those include *Anoxybacillus geothermalis* (Filippidou, Jaussi, et al., 2015; Filippidou et al. 2016), *Hydrogenobacter subterraneus* (Takai, Komatsu and Horikoshi 2001), *Thermotoga elfii* (Fardeau et al. 2009), *Bacillus paralicheniformis* (Kalwasińska et al. 2020), and other bacteria belonging to the *Thermus* and *Hydrogenobacter* genera (Inagaki et al. 2003). The archaeon *Archaeoglobus fulgidus* was also isolated from deep geothermal fluids (Fardeau et al. 2009). The absence of fungi in these studies does not necessarily derive from their true absence from the fluids, but rather from the fact that most studies did not even consider investigating fungi. However, the discovery of numerous fungal extremophiles during the last decades shows the equal importance of searching for fungi in extreme environments (see for instance Nagano and Nagahama 2012).

A broad range of metabolically distinct bacteria and archaea has been detected in geothermal fluids, such as sulphate-reducing bacteria (ex. *Desulfatamaculum* and *Desulfococcus*) (Alawi et al. 2011), hydrogen-oxidizing bacteria (*Hydrogenophaga* spp., *Acidovorax* spp., *Ralstonia* spp., *Pseudomonas* spp.), thiosulfate-oxidizing bacteria (*Dia-phorobacter* sp.) or biocorrosive strains (Morozova, Zettlitzer et al. 2011). Additionally, the archaeal and bacterial community of some saline geothermal fluids were shown to be dominated by Firmicutes, but the heat extraction or the addition of scaling inhibitors or nitrate changed this community to a community dominated by Firmicutes and Proteobacteria (Westphal et al. 2019).

Issues caused by microorganisms in geothermal power plants: mineral precipitation, corrosion and biofilm formation

Microorganisms inhabiting geothermal fluids are thought to contribute to some of the most important issues affecting the performance of geothermal power plants, namely mineral scaling and corrosion (Westphal et al. 2019). On the one hand, any type of

Table 4 Diversity studies on deep geothermal fluids or deposits in side geothermal power plants

Site or region	Temperature [°C]	Depth [m]	pH	Other measured conditions	Type(s) of sample	Archaea	Bacteria	Fungi	Reference
Molasse Basin (DE)	61–103 °C	NA	6.6–7.3	salinity 600–900 mg/l; DOC: 6.4–19.3 mg C/l	Fluids	X	X		(Alawi et al. 2011)
Bruchsal (DE)	132 °C (deepest drilling point)	2500 m	5.4–5.8	2.2 MPa (inside drilling pipes)	Biofilms		X		(Filippidou et al. 2016)
Gross Schönebeck (DE)	150 °C (reservoir)	4200 m	5.5 at 25 °C		Deposits		X		(Filippidou, Jaussi, et al. 2015; Filippidou et al. 2016)
Bad Blumau (AT)	107 °C	2800 m	8	salinity 20 g/l; sulfate 500 mg/l	Fluids	X	X		(Westphal et al. 2019)
Neu-Brandenburg (DE)	45–54 °C or 65–85 °C	1250–1335 m	6	mineralization 131 g/l; DOC 3.5 g/l	Fluids (different sources) and filter samples		X		(Lerm et al. 2013)*
Berlin (DE)	6–30 °C	30–60 m	7	low salinity (< 1 g/l)	Fluids (different sources) and filter samples		X		(Lerm et al. 2011)*
Northest Germany (DE)	> 120 °C	3500 m	5.5–5.9	salinity up to 420 g/l; 20 MPa	Fluids		X		(Morozova, Zettlitzer et al. 2011)*
Otake (JP)	75–90 °C	NA	NA	NA	Silica scale in an aging tank of geothermal hot water		X		(Inagaki et al. 1997; Inagaki et al. 2003)
Hatchobaru (JP)	In situ: 250 °C, collected at 96 °C	1500 m	NA	NA	Fluids; production well		X		(Takai, Komatsu and Horikoshi 2001)**
Hatchobaru (JP)	In situ: 250 °C, collected at 96 °C	1500 m	NA	NA	Fluids; production well	X			(Takai and Horikoshi 1999)
Otake (JP)	NA	NA	NA	NA	Silica scale		X		(Fujino et al. 2008)
Melun-L'Almont (FR)	70 °C	2000 m	6.4	DOC 2.3 mg/L	Fluids	X	X		(Fardeau et al. 2009)
Pyrzyce (PL)	61 °C	1640 m	6.2	TDS 121 g/L	Fluids	X	X		(Kalwasińska et al. 2020)
Stargard (PL)	85 °C	2578 m	5.9	TDS 140 g/L	Fluids	X	X		(Kalwasińska et al. 2020)
Otaniemi (FI)	46–76 °C	3203 m to 4375 m	NA	NA	Fluids		X		(Purkamo et al. 2020)

The detection of microorganisms is marked by an X. A more detailed table is presented in Additional file 2

* Geothermal power plant not used for electricity production

** Deep subsurface geothermal water not used in a power plant

mineral depositions inside the pipes is problematic. Indeed, these deposits can clog the wells or any part of the system, thus disturbing the normal operation of geothermal power plants (Regenspurg et al. 2015). Beside abiotic factors contributing to it (i.e. temperature variations), microorganisms in geothermal environments are often involved in silica precipitation (Inagaki et al. 2003). Thermophilic bacteria such as *Thermus thermophilus* are known to induce silica scaling (Fujino et al. 2008). Even though the role of microorganisms has not been assessed in all scaling types, such as in the case of copper-based scaling (Regenspurg et al. 2015), their impact cannot be excluded.

On the other hand, corrosion of metals induced by microorganisms (microbially induced corrosion, MIC) is also common in infrastructures, such as pipelines or power production infrastructures (Beech et al. 2005; Little and Lee 2007). Despite several examples of the highly problematic consequences of MIC in these systems (e.g., costly maintenance and efficacy reduction), the relation between microorganisms and corrosion issues is still not fully understood (Ibrahim et al. 2018). Mechanisms leading to MIC are still under investigation and might be multiple (Tripathi et al. 2021). One specific case of MIC that has been more thoroughly investigated is the case of sulphate reducing bacteria (SRB), whose metabolism notably results in H_2S production, a highly corrosive compound (Ibrahim et al. 2018). SRB are involved in MIC in wastewater treatment, oil or gas facilities or other pipeline systems (Hao et al. 1996; Sherar et al. 2011; Ibrahim et al. 2018), or in geothermal power plants with fluids containing sulphate or another suitable electron acceptor, seriously affecting the power plant operation (Valdez et al. 2009; Lerm et al. 2013). In addition, sulfur-oxidizing bacteria (SOB) can oxidize H_2S into H_2SO_4 , which is a highly corrosive compound (Muthukumar et al. 2003; Ibrahim et al. 2018). Bacteria producing acids, reducing or oxidizing iron or oxidizing manganese all have the potential to induce corrosion in infrastructures (Muthukumar et al. 2003).

The formation of biofilms inside pipes happens frequently. Indeed, biofilms are an important growth form (Flemming and Wuertz 2019) providing survival benefits in various environments and are thought to be a well-conserved trait throughout microbial evolution (Hall-Stoodley et al. 2004; Harding et al. 2009). Inside biofilms, microbial cells benefit of a higher protection thanks to the surrounding cells and all cells may benefit from compounds produced by others. Therefore, communities living in a biofilm are often more resistant than free-living cells to biotic (i.e., predation) or abiotic challenges. Moreover, an extracellular matrix, composed of extracellular polymeric substances (EPSs), is formed to keep the cells together (Hall-Stoodley et al. 2004). Therefore, in geothermal power plants, it is not only the cells themselves that can cause issues, but the sticky matrix compounds may also be problematic and cause, for instance, the blocking of filters.

The different issues mentioned above rarely happen alone and combinations of problems are common, with microbially induced mineral precipitation, H_2S production or corrosion happening simultaneously (Ibrahim et al. 2018). The issues caused by microbially induced mineral precipitation and corrosion, as well as biofilm formation, are not specific to geothermal power plants, but are also common in the oil and gas industries (Ibrahim et al. 2018; Madirisha et al. 2022). Thus, a better understanding of the issues caused by microorganisms in one of these industries could also help to better understand what happens in the others.

Challenges and risks of studying microbial diversity in deep geothermal fluids

As for other extreme and hardly accessible environments, literature on microorganisms present in deep geothermal fluids has to be evaluated carefully, notably because of uncertainties in sampling and data processing. In the following section, an overview of potential problems associated with sampling and potential biases in data analysis are presented.

Microbial activity produces, in some cases, a macroscopic manifestation such as a colour change or the appearance of an odour. However, most of the microbes remain undetectable without any prior amplification step or by their indirect detection using biomolecules or metabolic products. The presence of microorganisms in environments such as deep geothermal fluids, can be shown by the detection of biomarkers, such as phospholipids or phospholipid fatty acids (Vetter et al. 2012). Such methods do not allow the identification of specific microorganisms present in the environment, but can be used to monitor changes in the microbial communities present in the fluids (e.g., SRB present branched fatty acids and monoenoic fatty acids, thus the presence of these fatty acids in the fluids may indicate the presence of such bacteria). Therefore, phospholipid profiles can be useful indicators of disturbances or changes in the system (Vetter et al. 2012). As only a very small fraction of microorganisms is amenable to cultivation in the laboratory, a very significant experimental bias is introduced in microbial diversity studies if only culture-dependent techniques are applied (Amann, Ludwig and Schleifer 1995; Amann 2000). This bias is exacerbated when extreme environments are considered, as the microorganisms living under extreme conditions tend to be highly unculturable (Lewin et al. 2013). For this reason, the advancement of technologies relying on DNA or RNA detection (also called culture-independent or molecular methods) has unravelled an unprecedented diversity on and within the Earth. Indeed, the use of genomic tools (i.e., the analysis of the genetic material present in an environment either through targeted sequencing or whole-genome sequencing) allows to bypass cultivation in order to have a better understanding of the microbial diversity in different ecosystems (Ye et al. 2019). These methods are therefore of particular interest for the investigation of microorganisms in extreme environments (Cowan et al. 2015; Garrido-Cardenas and Manzano-Agugliaro 2017). However, as in other extreme environments, genomic studies of microorganisms in deep geothermal fluids possess challenges at each step of the process: from the sampling of the fluids to the final analysis of the genomic data generated (Fig. 3).

Sampling and biomass concentration

There are multiple challenges in the sampling of geothermal fluids (Arnórsson et al. 2006), but one that is particularly relevant when studying microorganisms is the high temperature of the fluids. Since the brines will be cooled down to ambient conditions after extraction from the reservoir, the possibility for contamination during sampling is large and has to be minimized. For this, avoiding the use of open-air systems, and the use of pre-sterilized containers are required. In addition, maintaining conditions as aseptic as possible should be considered, whenever this is feasible. This might be highly challenging considering the set-up of the sampling site and therefore, including controls

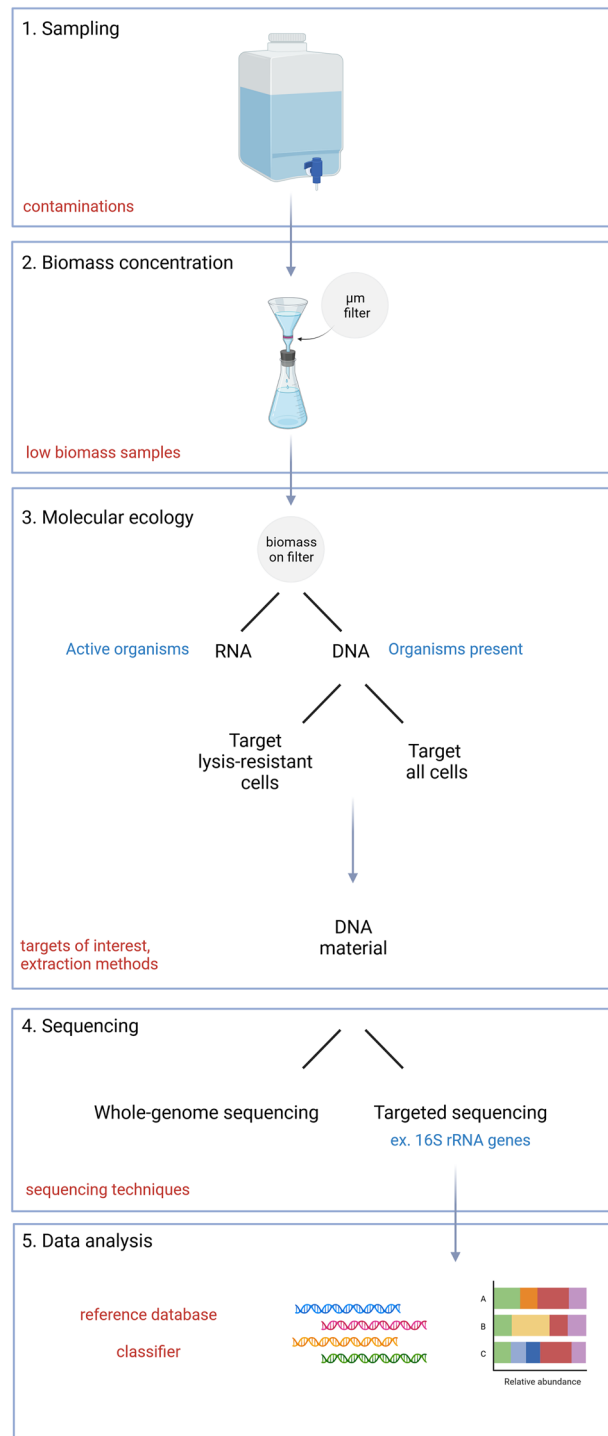


Fig. 3 Summary of the steps required to analyse the microbial diversity in deep geothermal fluids using a culture-independent approach based on the detection of genetic material. Challenges linked to each step of the analysis are mentioned in red. Figure created with BioRender.com

for contamination is required. Contaminations that can occur at this step include all types of DNA contaminants, which can come from the sampling environment (air for instance), material or researchers themselves (Eisenhofer et al. 2019). Moreover, during sampling, the fluids, which are originally in an anoxic and light-deprived state, will be exposed to oxygen and light. Such exposure may influence the microbial community composition, as both oxygen and light can be toxic compound for organisms (Downes and Blunt 1877; Fenchel and Finlay 2008).

Collecting the fluids under sterile conditions is not the only challenge during sampling, as the recovery of enough DNA for further analysis is also needed. As microbial biomass in geothermal fluids may be extremely low, the recovery of enough DNA (or other macromolecule) from low biomass samples is difficult (Barton et al. 2004; Barton et al. 2006; Lewin et al. 2013). One way to improve the recovery of microbial DNA from low biomass samples is to concentrate this biomass by filtration before DNA extraction. Using 0.22 μm or 0.1 μm pore size nitrocellulose filters will select microbial biomass (bacteria, archaea and fungi) while using 0.02 μm pore size filters would allow to capture viruses (Miyoshi et al. 2005; Russell, Zydney and Gomez 2023). In the case of geothermal fluids, filtering up to 5 L of fluids for DNA extraction only produced low amounts of DNA (unpublished) and thus the filtration of larger volumes (for instance 40 L) is often required.

DNA extraction

Once the biomass is concentrated on filters, DNA needs to be extracted. Geothermal fluid samples may not only contain vegetative cells but also resistant cells like spores, which are commonly found in extreme environments (Filippidou et al. 2019). Thus, extracting DNA from both cell types is important. Extracting DNA from spores is obviously more difficult than from vegetative cells and usual DNA extraction techniques do not allow the efficient recovery of DNA from resistant structures (structures resistant to lysis) (Belgrader et al. 2000; Filippidou, Junier, et al. 2015). Therefore, an enrichment treatment, which will concentrate the resistant cells and maximize the extraction of their DNA, can be applied on the samples, for instance (Wunderlin et al. 2016; Corona Ramirez et al. 2023). As this additional step destroys vegetative cells, each sample needs to be split into two to recover DNA from the vegetative cells (direct DNA extraction) and from resistant cells (enrichment treatment). It is also important to keep in mind that the choice of different DNA extraction techniques will undeniably introduce biases (Brooks et al. 2015).

Another issue of DNA extractions is the unavoidable presence of contaminants in DNA extraction kits. Indeed, microbial contaminants are common in these kits and are even more problematic when working with low biomass samples (Salter et al. 2014; Glassing et al. 2016; de Goffau et al. 2018; Karstens et al. 2019). In fact, the DNA of contaminants will overpower data in samples with low DNA content, therefore hiding true results (Stinson et al. 2019). Consequently, performing contamination controls during DNA extraction is mandatory (de Goffau et al. 2018). Moreover, the UV sterilization of the different components of DNA extraction kits might also help to mitigate these types of contamination sources (Gefrides et al. 2010).

In addition to using the DNA present in the samples, one could also use RNA (ribonucleic acid involved in the synthesis of proteins) (Madigan and Martinko 2007). The use of DNA allows to target all the organisms present, whether they are in an active or dormant (resistant) form, while targeting RNA allows to target only the active organisms, as RNA is only expressed by active life forms. Different RNA sequencing technologies are available and allow to precisely monitor the state of the targeted cells (Finotello and Di Camillo 2015).

Sequencing methods and data analysis pipelines

After DNA extraction, the choice of the sequencing technology (i.e., how the DNA sequences of interest will be obtained and how long they will be) will induce differences in the resulting datasets. Nowadays, available technologies are numerous, allowing either for whole genome or marker genes analyses (i.e., analysis using selected genes as molecular markers, also called targeted sequencing or amplicon sequencing; Fig. 3). To analyse samples containing low amounts of biomass, analysis of marker genes is usually more feasible (Knight et al. 2018). Thus, in this review, only the method using molecular marker genes will be discussed in further details.

Molecular markers correspond to genes shared by all the organisms and exerting the same function, allowing to appropriately compare species diversity. Ideally, a molecular marker should contain both, regions that are conserved (i.e., very few variations among the organisms in the group considered) and some that are highly variable (i.e., large differences among the organisms in the group considered to allow to identify the organisms). One of the most widely used molecular markers to study microbial diversity are the genes encoding ribosomal RNAs. Bacteria, archaea and fungi (and other eukaryotes) all have ribosomes, which are responsible for the synthesis of proteins. These ribosomes are composed of proteins, but also by RNA fragments (ribosomal RNA or rRNA) that are structural and play a key role in the synthesis of proteins. These rRNA and the genes coding (rDNA) for them are well conserved throughout evolution. Given their conservation, common ancestry and evolution, rDNA are suitable markers to compare the phylogenetic distance of organisms (i.e., how organisms are related to each other) and are often used to define “microbial species”. Using marker genes, several amplification methods are available, including Polymerase Chain Reaction (PCR) and quantitative Polymerase Chain Reaction (qPCR). A PCR will amplify all the marker genes present in the sample, allowing to identify the species present. However, a PCR does not inform on the real abundance of species, on the contrary to qPCR, which is a real-time amplification method. This means that a qPCR allows to follow in real time the amplification of the marker genes and to enumerate them, either in a relative or absolute manner. These methods of amplification can be also used to target RNA, but using a modified version (reverse transcription PCR (RT-PCR) and reverse transcription quantitative PCR (RT-qPCR)) (Jalali, Zaborowska and Jalali 2017). To target for organisms by PCR or qPCR, corresponding amplification primers have to be chosen depending on the type of microorganism studied and the desired amplification target. The choice of primers will also impact the obtained results, as different regions will be amplified. For archaea and bacteria, different variable regions of the 16S rRNA gene are widely used in diversity studies and the entire gene is used as standard for identification. For fungi, the ITS (Internal

Transcribed Spacer) regions, which also belong to ribosomal RNA operon, are commonly used (Tedersoo and Lindahl 2016). However, for the analysis of specific regions of the 16S rRNA gene and the ITS, only partial regions are often used and selected thanks to different primer pairs.

Finally, following an amplification of a marker gene through a PCR, amplicons need to be sequenced to determine the nucleotide sequences of the initial DNA fragment. This can be achieved by different technologies, such as short read sequencing techniques (e.g. Illumina sequencing (Illumina, CA, USA)) or long read sequencing techniques (e.g. Pacific Biosciences (CA, USA)) (Garrido-Cardenas and Manzano-Agugliaro 2017). Afterwards, the sequences obtained (the amplicon sequencing data) are compared between them. At this step, various combination of classification algorithms and reference databases can be set up to identify the organisms present in the sample.

While processing these amplicon sequencing data, several decisions have to be made and those will drastically influence the results (Xue, Kable and Marco 2018; Pauvert et al. 2019). First, raw sequencing reads have to be processed using different software and pipelines, such as QIIME2 or mothur (Schloss et al. 2009; Bolyen et al. 2019). Processing of these raw sequencing reads will allow the removal of sequencing errors and to group the reads according to their sequence similarity (Callahan et al. 2017). Sequences above a given threshold of similarity can be grouped into Operational Taxonomic Units (OTUs) or kept as Amplicon Sequence Variants (ASVs; exact sequences). OTUs and ASVs are two different approaches to identify the number of distinct biological entities in the sample and to classify the sequences of a dataset. The use of OTUs or ASVs has its respective drawbacks and benefits and the choice of one or the other will mostly depend on the study objectives. Recently, the benefits conferred by ASVs, such as an improvement in reproducibility and a better accuracy to assess diversity in communities not well covered in reference databases, seem to tip the scale in their favour (Callahan et al. 2016; Callahan et al. 2017; Knight et al. 2018). At this stage of the analysis, the read count of biological entities (OTUs or ASVs) in the samples is translated into an OTUs or ASVs table. The next critical step is the choice of a reference database and of a classifier to add a taxonomic information to the sequences. Indeed, OTUs or ASVs present in the dataset first need to be compared to known reference sequences of organisms in a database in order to know to which organisms these sequences initially belonged to. To do that, different classifiers can be used, such as RDP, UCLUST, consensus vsearch, consensus BLAST (basic local alignment and search tool) and scikit-learn naïve Bayes, which were all compared using mock (artificially produced) communities (Bokulich et al. 2018).

Various reference databases for microorganisms exist, such as Greengenes (DeSantis et al. 2006) and Silva (Quast et al. 2012) for the 16S rRNA gene of bacteria and archaea, or UNITE (Nilsson et al. 2019) and ITSoneDB (Santamaria et al. 2012) for the ITS region of fungi. As several databases exist and are built and curated differently, results will not be the same if one or the other database is used. The biases induced by different databases and different extraction methods are often addressed separately, but only few comparative studies on combined biases exist (Abellan-Schneyder et al. 2021; Ramakodi 2021). Actually, the database problem does not only occur when working with largely unknown environments, but also while working with extremely well-known microbiomes, such as the human gut microbiome. While mock communities (i.e., artificially

created communities in which all members are known) can be used to test if the study design is suitable to a certain type of sample (Abellan-Schneyder et al. 2021), it is not possible to create mock communities for environments in which the microbial diversity is unknown such as in the case of deep geothermal fluids.

Once a taxonomy has been assigned by a classifier in order to link OTUs or ASVs with organisms, the final step is the choice of correct and judicious normalisation and analysis methods to ensure results of good quality (McMurdie and Holmes 2014; Weiss et al. 2017; Knight et al. 2018; Calle 2019). In summary, various techniques can be applied to study microorganisms in deep geothermal fluids, including amplicon sequencing techniques, and several steps imply crucial decisions. Informed decisions from the start to the end of the process will ensure a proper analysis of this poorly known, but nonetheless rich of life, extreme environment.

Conclusions

By summarizing the current knowledge on the microbial diversity present in deep geothermal fluids and in analogous environments (the deep biosphere and geothermal environments), this review highlighted the probable habitability of deep geothermal reservoirs for electricity production. Indeed, the past few decades of research demonstrate that microorganisms persist in geothermal ecosystems and the deep biosphere, underlying an astonishing diversity of life even in the most extreme conditions (Shu and Huang 2021). This review further underlines the scarcity of knowledge on the microorganisms, and in particular a large knowledge gap for fungi inhabiting deep geothermal fluids. This supports the need for further studies to establish the diversity of microorganisms present in those environments and their potential impacts on geothermal power plants, to help mitigating MIC or microbial induced scaling inside the power plants. This knowledge will impact not only the geothermal industry but will also be essential to improve our understanding of the evolution of life, as these ecosystems can be used as analogues to Early Earth environments (Rampelotto 2013; Brown and Fritz 2019; Filippidou et al. 2019). Moreover, understanding the deep subsurface biosphere will give valuable clues in the search for life outside Earth (Hoehler 2004; von Hegner 2020). In fact, most, if not all, known exoplanets display extreme environmental conditions, hence the interest to study extreme microorganisms present on Earth in order to have a better knowledge on how to search for life elsewhere in the universe (Lal 2008; Cottin et al. 2017; Martins et al. 2017).

Finally, this review also illustrated the numerous challenges and biases induced while studying the microbial diversity in a mostly unexplored extreme environment. Careful attention should be given to the analytical methods used to study microorganisms in deep geothermal fluids and common ground between different studies should be considered before direct comparison. For instance, even though it would not be possible to use the same database for all diversity studies, raw sequencing data should always be made available with publications to allow re-analysis of data before comparison if necessary. Moreover, for the same samples, different treatments, such as different DNA extraction methods, should be applied in order to gain a better understanding of the general diversity of microorganisms present in these fluids (Additional file 3).

Definitions

Diploid cell: cell containing two exemplars of each chromosome.

Extremophile: organism that requires one or several extreme environmental parameters to grow.

Extremotolerant: organism that tolerates one or several extreme conditions to grow.

Genome: All genes of an organism.

Haploid cell: cell containing only one exemplar of each chromosome.

ITS (internal transcribed spacer) rRNA amplicon sequencing: amplified region of the ribosomal genes used to detect and identify fungi.

Marker genes: DNA regions shared by all the organisms compared; also called molecular markers.

Nucleotides: the five organic molecules that compose DNA or RNA sequences. These nucleotides are adenine (A), thymine (T), guanine (G), cytosine (C) and uracil (U).

Sequencing: determination of one/several DNA sequences (i.e. search what is the order of the nucleotides (A, T, G or C) composing the DNA).

16S rRNA amplicon sequencing: amplified region of the ribosomal genes used both to detect and identify bacteria and archaea.

Abbreviations

Bs	Below soil surface
Bsf	Below sea floor
Bw	Below water surface
DOC	Dissolved organic carbons
NGS	Next generation sequencing
TDS	Total dissolved solids

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40517-023-00269-z>.

Additional file 1: Table S1. Diversity of microorganisms in analogous environments of geothermal power plants.

Additional file 2: Table S2. Diversity of microorganisms in geothermal power plants.

Additional file 3. Additional references for table.

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Author contributions

DB, AL, GC these authors made major contributions to the formatting and analysis of the literature data collected. DB, PJ, GC, SB, SR, FE, JL, RH, JU these authors made major contributions to the acquisition and interpretation of the literature data. All the authors proofread and approved the final manuscript.

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Declarations

Competing interests

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