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REVIEWS

Thermophilic Prokaryotes from Deep Subterranean Habitats

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Abstract—The deep continental biosphere consists of geologically isolated ecosystems differing in their physicochemical, geological, and trophic parameters. Most of the deep ecosystems exist at elevated temperatures (50–120°C), which favor the development of thermophilic microorganisms. In many cases, the indigenous nature of subsurface microorganisms is questionable due to problems of collecting representative and non-contaminated samples. In spite of the numerous studies on the deep biosphere microbial communities, the number of cultivated thermophiles isolated from subsurface environments not associated with petroleum deposits does not exceed 30 species. More than half of the thermophilic species isolated from deep subsurface belong to the *Firmicutes*. The majority of the underground thermophiles are represented by strict or facultative anaerobes, with capacity for sulfate and iron reduction notably widespread. Most thermophilic subsurface microorganisms are organotrophs, although chemolithoautotrophic thermophiles also have been reported. This review deals with the phylogenetic diversity and physiological properties of the cultivated thermophilic prokaryotes isolated from various deep subterranean habitats.

Keywords: thermophilic microorganisms, deep biosphere, subsurface environments

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GENERAL CHARACTERIZATION OF DEEP SUBTERRANEAN HABITATS

It was not until relatively recently that the scientific community acknowledged the existence of the deep underground biosphere based on prokaryotic organisms [1, 2]. Deep continental ecotopes are composed of underground water and the enclosing rock formations. Groundwater can be formed by infiltration of atmospheric precipitation and surface water or by condensation of atmospheric vapors (vadose water), as well as in the course of magma pocket differentiation (juvenile water). By their mode of occurrence, underground water bodies can be classified as pore, stratal, or fissure water [3]. The underground biosphere is not a single entity (since it is comprised of numerous fragments which are spatially and geologically separated from each other) and isolated bodies of pore and fissure water may exist for as long as dozens of millions of years [4]. There is no single opinion as to what the boundaries of the underground biosphere are. The upper border of the deep continental biosphere should probably be defined as the zone that does not directly depend on the inflow of fresh organic matter of photosynthetic origin and from soil processes (i.e., below 50 m, depending on the rock type), whereas its lower border is the zone where liquid water has the temperatures of 100 to 120°C (at the average depth of 4 km) [5]. Due to the vast volume of the underground hydrosphere, the total biomass of subsurface microorganisms is sometimes estimated as comparable to the total biomass of the Earth's surface flora and fauna [1, 6]. The average geothermal gradient of the Earth crust is 3°C/100 m; therefore, environmental conditions in most of the underground biosphere favor the development of thermophilic microorganisms (optimal growth temperatures >50°C). Elevated temperatures, in combination with the presence of reduced gaseous compounds (H_2, CH_4, H_2S), supports anaerobic conditions in many deep ecosystems. The carbon sources available to indigenous subsurface microorganisms may include dispersed organic matter of rock formations (kerogen), organic compounds of petroleum and natural gas, abiogenic carbon dioxide, as well as organic compounds (methane, hydrocarbons, etc.) chemically formed from inorganic compounds at high temperatures (the Fisher–Tropsch process). From the point of view of organic matter concentration, stratal waters of oil reservoirs are clearly different from other subsurface environments: they contain significant amounts of hydrocarbons and products of hydrocarbon degradation, whereas underground waters in general have low concentrations of carbon compounds, sometimes below 1 ppm. Anthropogenic storage facilities for natural gas and radioactive waste represent another type of underground habitats rich in organic compounds. A surprising discovery of nematodes in deep habitats of South Africa demonstrated the scarcity of the currently available information on carbon amounts and flows in underground ecosystems [7]. The fact that deep continental ecosystems include

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multicellular consumers that feed presumably on lithoautotrophic microorganisms suggests that considerable microbial biomass is produced using some currently unknown sources of carbon and energy; it also implies the existence of a detritus food chain based on organotrophic decomposing microorganisms. Molecular hydrogen, which may be generated by serpentinization, thermal degradation of alkanes, or water radiolysis, is generally considered the most common inorganic electron donor capable of supporting the existence of autonomous subterranean microbial communities [8–10]. The concentrations of radiolytic hydrogen produced by radioactive decay of U, Th, and K in interstitial water may reach the molar level and greatly exceed hydrogen concentrations in surface ecosystems [11, 12]. In laboratory experiments, hydrogen produced from crushed basalt and anaerobic water was shown to support the growth of methanogenic archaea [13]. Hydrogen-based mesophilic microbial ecosystems comprising methanogenic and acetogenic microorganisms were discovered in underground water of basalt and granite rocks [13–15]. The number of possible electron acceptors available to the microorganisms of deep ecosystems includes, in addition to organic compounds and CO_2, oxygen, nitrate, sulfate, thiosulfate, elemental sulfur, and ferric iron [14, 16, 17].

THERMOPHILIC MICROORGANISMS OF CONTINENTAL DEEP HABITATS

The phylogenetic diversity of microorganisms of deep subsurface habitats has been investigated using both cultural and molecular ecological methods, and is frequently discussed in reviews [18, 19]. Molecular techniques cannot provide reliable information on the range of temperatures supporting the growth of the microorganisms detected. Microbial diversity depends on the physicochemical, geological, and trophic features of each particular deep ecotope, and may range from several dozens of phylogenetic types to two or even a single species [20–22]. Deep habitats have been reported to contain microorganisms of nearly all currently identified bacterial and archaeal phyla [20, 23–26]. The *Firmicutes* are the predominating phylum in many subterranean communities: the most common findings are sulfate-reducing spore-formers of the genus *Desulfotomaculum*, which includes both mesophilic and thermophilic species [23, 27–29]. However, in many cases, the indigenous nature of microorganisms isolated from subterranean habitats is dubious, because of sample representativeness and contamination issues. From the microbiologist's point of view, it is reasonable to classify the microorganisms from deep habitats according to the natural site and the type of sample they were isolated from, since it provides information concerning the method of sample collection and allows for estimation of the possibility of indigenous origin of the isolates.

Formation Waters of Oil Fields

High concentrations of organic compounds make petroleum and natural gas deposits drastically different from all other subterranean ecosystems. Formation waters of petroleum deposits are the best-studied deep underground habitats [30, 31]. However, it is difficult to establish whether the prokaryotes found in oil reservoirs are genuinely indigenous, since sampling is very seldom performed as a sterile procedure, whereas drilling and subsequent exploitation (sometimes involving water injection from the surface) bring formation water in contact with the surface environment. However, the microorganisms detected in samples from boreholes that had not been water-flooded, in particular, from high-temperature reservoirs, may probably be considered autochthonous. High-temperature oil reservoirs were found to contain aerobic, sulfate-reducing, iron-reducing, and methanogenic prokaryotes, as well as the microorganisms with fermentative metabolism; for some deposits, data have been reported on their numbers and biogeochemical activity [32, 33]. The phylogenetic diversity of microorganisms inhabiting these high-temperature environments is quite high and includes a number of various phyla of bacteria and archaea [34–39]. An interesting example is the hyperthermophilic archaeon *Thermococcus sibiricus* isolated from the Samotlor oil field; its recently published complete genome sequence data indicates its deep subterranean origin [40]. Culturable thermophilic microorganisms of oil reservoirs have been extensively studied for a long time, which resulted in both isolation of previously known thermophiles and identification of over 40 novel species. The physicochemical conditions of high-temperature deposits favor the growth of anaerobic microorganisms. Table 1 provides the list of new taxa of anaerobic thermophilic prokaryotes isolated from petroleum deposits, and their phylogenetic affiliation. Most of the newly discovered species belong to the phylum *Thermotogae*; apart from the previously known *Thermotoga* and *Thermosipho*, four novel genera of this phylum have been described: *Geotoga, Petrotoga, Kosmotoga*, and *Oceanotoga* [42–44].

Underground Gas Storage Facilities

In most cases, underground gas storage consists of reservoir beds of geological structures and complex of technical constructions. With their total volume worldwide of hundreds of billions cubic meters, underground gas storages may be considered among the largest anthropogenic ecosystems. The first works on microbial diversity in the underground gas storage facilities appeared relatively recently [45–47]. So far, thermophilic prokaryotes have been isolated only from the Severo-Stavropolskoye underground gas storage, the largest one in Europe; its reservoir beds lie at the depth of 800–1000 m and have the temperature of 55–65°C. Three novel species of thermophilic anaerobic

Table 1. New species of thermophilic anaerobic microorganisms isolated from oil fields

Phylum	Genera	New species
	Bacteria	
Deferribacteres	*Deferribacter**	*Df. thermophilus*
Firmicutes	*Caldanaerobacter*, Desulfotomaculum, Garciella*, Mahella*, Thermoanaerobacter, Thermovirga**	*C. subterraneus, Dm. kuznetsovii, Dm. thermocisternum, Dm. salinum, G. nitratireducens, M. australiensis, Tb. brockii, Tv. lienii*
Proteobacteria	*Desulfacinum*, Desulfonauticus, Petrobacter**	*Dc. infernum, Dn. autotrophicus, Pb. succinatimandens*
Synergistetes	*Anaerobaculum**	*A. thermoterrenum*
Thermodesulfobacteria	*Thermodesulfobacterium*	*Td. thermophilum*
Thermotogae	*Geotoga*, Kosmotoga*, Oceanotoga*, Petrotoga*, Thermosipho, Thermotoga*	*Gt. petraea, Gt. subterranea, K. olearia, K. shengliensis, O. teriensis, Pt. miotherma, Pt. halophila, Pt. mexicana, Pt. mobilis, Pt. olearia, Pt. sibirica, Ts. geolei, Tt. elfii, Tt. hypogea, Tt. naphthophila, Tt. petrophila, Tt. subterranea*
	Archaea	
Euryarchaeota	*Methanoculleus, Methanothermobacter, Methermicoccus*, Thermococcus*	*Mc. receptaculi, Mt. crinale, Mm. shengliensis, Tc. sibiricus*

References to articles with description of new taxa presented in Table 1 can be found on internet-resource LPSN (http://www.bacterio.net) [41].
* Genera with the type strains isolated from oil fields.

organotrophic bacteria of the phylum *Firmicutes* were isolated from water samples collected in this gas storage: *Moorella perchloratireducens, Caloribacterium cisternae*, and *Tepidibacillus fermentans* (Table 2) [48–50].

Samples of Rock Formations Obtained by Drilling

Microorganisms isolated from freshly collected core samples are most likely to be genuinely autochthonous. Being highly expensive, drilling aimed specifically at obtaining microbiological samples is therefore extremely rarely performed. A study of high-temperature sandstones of Colorado (United States) indicated that hydrological connection to the surface, the maximal temperature, and the presence of fissures in the rocks were the major factors controlling the distribution of microorganisms. Thermophilic microorganisms were present in core samples with the temperatures of 65°C obtained at the depth of 856–862 m, but not in the deeper strata [51]. Culturable thermophiles isolated so far from deep rock formations are represented exclusively by anaerobic bacteria of the phylum *Firmicutes* (Table 3). *Bacillus infernus*, capable of reducing Fe(III) and Mn(IV), as well as sulfate-reducing *Desulfotomaculum putei*, were isolated from organic-rich fine-grained aleuric siltstone, a lake sediment dating back to 140 million years ago [52, 53]. Several strains of thermophilic anaerobes of the genus *Thermoanaerobacter* from Colorado high-temperature sandstones were isolated as pure cultures [54]. These bacteria were capable of reducing poorly crystalline iron (III) oxide to magnetite, using a range of organic compounds, as well as molecular hydrogen, as electron donors. In addition to ferric iron, *Thermoanaerobacter* strains were able to reduce Mn(IV), Cr(VI), Co(III), and U(VI). Several thermophilic Fe(III)-reducing strains of the *Firmicutes* were isolated from drilling fluids circulated at the depths of 2290–3350 m in the course of continental scientific drilling in China; however, the question concerning the proportion of indigenous and contaminant microorganisms in these samples remains unanswered [23].

Deep Mines

For microbiological research, ultradeep mines represent unique sites providing access to the underground environments: they are considered "windows to subsurface biosphere." Some of the deepest gold mines reach the depth of 4.0 km. Microorganisms present in these deep habitats fall into two groups. One of them includes prokaryotes of the sediments, biofilms, and microbial mats growing on the walls and bottom of mine tunnels, as well as in subsurface water flowing along the underground roadways. Such habitats are not subjected to geostatic pressure and have contact to atmospheric oxygen and sometimes to technical water injected from the surface. The second group includes the microorganisms found in groundwater samples obtained from boreholes drilled at particular depths within the tunnels. Such water has no direct contact to underground roadways or to the

Table 2. Thermophilic microorganisms isolated from underground gas storages

Microorganism, strain	Phylum	Geographical location and characteristics of the sampling site	Sample description	Temperature range of growth min–(opt)–max	Relation to oxygen/ Type of metabolism/ Electron acceptors	Reference
Caloribacterium cisternae SGL43^T	*Firmicutes*	Severo-Stavropolskoye underground gas storage (Russia). The gas-bearing strata containing aleurolites, aleurites, and clay varieties. Depth 800–1000 m. *T* 55–65°C	Water from observation wells taken by sludge pump	26–(50)–65	Anaerobic/Organotrophic, fermentative/$S_2O_3^{2-}$	[49]
Moorella perchloratireducens An10^T	*Firmicutes*	"	"	40–(55–60)–70	Anaerobic/Organotrophic, fermentative/(Per)chlorate, NO_3^-, $S_2O_3^{2-}$, Fe(III), anthraquinone-disulfonate	[48]
Tepidibacillus fermentans STGH^T	*Firmicutes*	"	"	36–(50)–65	Facultative anaerobic, microaerophilic/Organotrophic, fermentative/O_2, NO_3^-, $S_2O_3^{2-}$, S^0, anthraquinone-disulfonate	[50]

Table 3. Thermophilic microorganisms isolated from subsurface rock samples obtained by deep-drilling

Microorganism, strain	Phylum	Geographical location and characteristics of the sampling site	Sample description	Temperature range of growth min–(opt)–max	Relation to oxygen/ Type of metabolism/ Electron acceptors	Reference
Bacillus infernus TH-23^T	*Firmicutes*	Taylorsville Triassic Basin, Virginia, United States. Depth 2700 m *T* 60°C	Side wall core samples	40–(61)–65	Strictly anaerobic/Organotrophic, fermentative, iron-reducing/Fe(III), Mn(IV), NO_3^-, trimethylamine oxide	[52]
Desulfotomaculum putei TH-11^T	*Firmicutes*	"	"	22–(64)–65	Strictly anaerobic/Facultatively lithotrophic (H_2), sulfate-reducing/SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$	[53]
Thermoanaerobacter sp. X513, X514, X521, X524, X561	*Firmicutes*	Piceance Basin, Colorado, United States. Depth 860–2000 m *T* 81°C	Core sediment samples and drilling fluids	45–(60)–75	Anaerobic/Facultatively lithoheterotrophic (H_2), iron-reducing/Fe(III), Mn(IV), Cr(VI), Co(III), U(VI)	[54]

Earth's surface; however, fissure water samples are often obtained using metal pipes and valves that remain installed for a long time and can become sites of contaminant microbial growth. Thus, microorganisms of both groups may be either of indigenous or of external origin. The extent of sample contamination can be evaluated using tracer techniques, such as solute tracers or fluorescent microspheres [55]. Important information on microbial communities of deep mines can be obtained using the methods of molecular biology and metagenomic analysis. For instance, unique nucleotide sequences of the 16S rRNA genes, which apparently belong to new phylogenetic lineages of archaea, were detected in subsurface waters of South African gold mines [20, 25]. Sequencing of the DNA isolated from fissure water samples obtained at the depth of 2.8 km showed that this particular habitat contained a single bacterial species: a sulfate-reducing lithoautotrophic spore-forming thermophile, *Candidatus* "Desulforudis audaxviator," which has been surviving and proliferating in this completely isolated ecosystem for millions of years [22, 56]. Genomes of two uncultured, presumably thermophilic prokaryotes: an archaeon *Candidatus* "Caldiarchaeum subterraneum" and a bacterium *Candidatus* "Acetothermum autotrophicum," which probably represent previously unknown phyla, were characterized by means of a metagenomic analysis of a microbial mat growing in a geothermal water flow in a Japanese gold mine [57, 58]. The range of culturable microorganisms obtained from the samples collected on tunnel walls includes mainly anaerobic and aerobic *Firmicutes* (Table 4). Noteworthy, such samples contain sulfate-reducing bacteria (e.g., *Desulfotomaculum thermosubterraneum, Desulfovirgula thermocuniculi*), which suggests that anaerobic conditions exist relatively close to the site of the contact of rock formations with atmospheric air [59, 60]. An obligate methanotroph *Methylothermus subterraneus* was isolated from a microbial mat sample [61]. Phylogenetic and physiological diversity of microorganisms isolated from borehole samples was considerably larger, including representatives of *Aquificae, Deinococcus-Thermus, Proteobacteria*, and *Planctomycetes*. Interestingly, all thermophilic microorganisms isolated from this group of samples were facultative anaerobes, possibly because some parts of the aquifers locally contained oxygen. The presence of obligate chemolithoautotrophs, *Sulfurihydrogenibium subterraneum* and *Thiobacter subterraneus*, which may function as primary producers of organic compounds in underground ecosystems, is especially remarkable [62–64]. *S. subterraneum* can utilize an exceptionally wide range of inorganic electron donors and acceptors, including insoluble Fe(III) oxides. *Thermus scotoductus* SA-01, isolated from the depth of 3.2 km, is also an Fe(III)-reducing species [65]. Fissure water samples were also found to contain an obligate organotroph, planctomycetes '*Thermogutta hypogea*' SBP2, consuming mono-, di-, and polysaccharides, which indicates the presence of complex organic compounds in this biotope, apparently, as the products of biomass degradation [66].

Underground Water Collected at the Surface

Physicochemical conditions within a borehole sometimes differ significantly from those in the underground reservoir itself, which may favor selective growth of either contaminant or indigenous microorganisms on the interior surfaces of casing pipes due to the processes uncharacteristic of deep ecosystems. A well-known example is the growth of microorganisms utilizing cathodic hydrogen produced in the course of steel pipe corrosion [67]. Borehole cleaning and disinfection are rarely performed prior to microbiological sample collection, although these procedures considerably reduce the numbers of contaminant microorganisms [68]. Geothermal water samples collected on nearly all continents of the Earth were found to contain thermophilic microorganisms of different phylogenetic groups (Table 5). Most of them are *Firmicutes*, although representatives of *Aquificae, Chloroflexi, Deinococcus-Thermus*, and *Thermotoga* have also been reported. *Archaeoglobus fulgidus* L3 has so far been the only culturable hyperthermophilic archaeon isolated from subsurface habitats not associated with oil deposits [69]. Most isolates are organotrophic anaerobes, although aerobic *Thermus* and *Hydrogenobacter* species have also been described [70, 71]. Sulfate-reducing bacteria of the genus *Desulfotomaculum* have been found in water samples collected in Europe, North America, and Australia [72–74]. *A. fulgidus* L3 and *Thermotoga elfii* G1 are representatives of iron-reducing microorganisms [69]. The sulfate-reducing species *A. fulgidus* L3 and *Desulfotomaculum geothermicum* are capable of lithoautotrophic growth using molecular hydrogen as an electron donor [69, 73].

Run-off Channels of Geothermal Boreholes

Locations of geothermal water discharge are commonly characterized by microbial mat development and sediment deposition. Obviously, these are not subsurface ecosystems, but they are related to the latter through water composition. Some microorganisms of these mats and sediments may probably be of deep underground origin. The overwhelming majority of currently known thermophilic species were isolated from geothermal waters of the Great Artesian Basin, Australia (Table 6). Most of them are anaerobic *Firmicutes* species, able to ferment organic compounds. Many of them can reduce Fe(III), Mn(IV), and other metals [75–80]. Bacteria of four new genera (*Fervidicella, Fervidicola, Sporolituus*, and *Thermotalea*) were isolated from the run-off channel of a single bore [76, 78–80]. The *Thermus* and *Thermaerobacter* species isolated from the same habitats were aerobes [70, 81]. *Melioribacter roseus*, which represents a new phylum

Table 4. Thermophilic microorganisms isolated from mines

Microorganism, strain	Phylum	Mine	Sample description	Temperature range of growth min–(opt)–max	Relation to oxygen/Type of metabolism/Electron acceptors	Reference
Geobacillus thermoleovorans GE-7	*Firmicutes*	Driefontein, Witwatersrand, South Africa	Water from a dripping fracture near the top of an exploration tunnel. Depth 3.2 km, *T* 50°C	47–(65)–70	Facultative anaerobic/ Organotrophic/NO_3^-	[83]
Desulfotomaculum thermosubterraneum RL50JIII^T	*Firmicutes*	Underground mine in a geothermally active area, Japan	Sediment layer on a tunnel wall. Depth 250 m, *T* 70–80°C	50–(61–66)–72	Anaerobic/Facultatively lithotrophic (H_2/SO_4^{2-}) sulfate-reducing/SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$ S^0	[59]
Desulfovirgula thermocuniculi RL80JIV^T	*Firmicutes*	"	"	61–(69–72)–80	"	[60]
Thermovorax subterraneus 70B^T	*Firmicutes*	"	"	50–(71)–81	Anaerobic/Organotrophic, fermentative/$S_2O_3^{2-}$	[84]
Methylothermus subterraneus HTM55^T	*Proteobacteria*	Hishikari, Kagoshima prefecture, Japan	Microbial mat in the geothermal water on the tunnel floor. Depth 320 m, *T* 69°C	37–(55–60)–65	Aerobic/ Obligately methanotrophic	[61]
Thermanaeromonas toyohensis ToBE^T	*Firmicutes*	Toyoha Mine, Hokkaido, Japan	Geothermal water seeped from the wall. Depth 550 m, *T* 71°C	55–(70)–73	Strictly anaerobic/ Organotrophic, fermentative/$S_2O_3^{2-}$, NO_3^-, NO_2^-	[85]
Sulfurihydrogenibium subterraneum HGMK-1^T	*Aquificae*	"	Hot aquifer water from the borehole drilled at the depth of 320 m, *T* 70°C	40–(65)–70	Facultative anaerobic, microaerophilic/Lithoautotrophic (H_2, $S_2O_3^{2-}$, S^0)/O_2, NO_3^-, Fe(III), $HAsO_4^{2-}$, SeO_4^{2-}, SeO_3^{2-}	[63]
Thiobacter subterraneus C55^T	*Proteobacteria*	"	"	35–(50–55)–62	Microaerobic/Obligately chemolithoautotrophic ($S_2O_3^{2-}$, S^{2-}, S^0)	[64]
'*Thermogutta hypogea*' SBP2	*Planctomycetes*	Beatrix, Witwatersrand, South Africa	Fracture water from a borehole at 1375 m below the shaft collar	36–(52)–60	Facultative anaerobic/ Organotrophic, fermentative/O_2, NO_3^-, NO_2^-, S^0	[66]
Thermus scotoductus SA-01	*Deinococcus-Thermus*	Western Deep Levels, Inc,. Witwatersrand, South Africa	Freshly mined rock surface and a water-producing borehole that penetrated 121 m horizontally into the formation at a depth of 3198 m *T* 60°C	45–(65)–75	Facultative anaerobic/ Organotrophic, fermentative/ O_2, Fe(III), NO_3^-, S^0	[65]

Table 5. Thermophilic microorganisms isolated from subterranean waters collected on land surface

Microorganism, strain	Phylum	Geographical location and characteristics of the sampling site	Sample description	Temperature range of growth min–(opt)–max	Relation to oxygen/ Type of metabolism/ Electron acceptors	Reference
Archaeoglobus fulgidus L3	*Euryarchaeota*	Melun-L'Almont, Paris Basin, France	Artesian geothermal water from the depth of 1900 m, *T* 70°C	55–(75)–85	Strictly anaerobic/Facultatively lithoautotrophic (H_2/Fe(III)), sulfate-reducing, iron-reducing/ SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, Fe(III)	[69]
Caldilinea tarbellica D1-25-10-4^T	*Chloroflexi*	Dax, St Paul-le`s-Dax Aquitaine Basin, France	Water collected from the head pump at a depth of 149 m, *T* 60°C	43–(55)–65	Anaerobic/Organotrophic, fermentative	[86]
Caloramator indicus IndiB4^T	*Firmicutes*	Surat District, Gujarat State, India	Artesian geothermal water, *T* 60–65°C	37–(60–65)–75	Strictly anaerobic/ Organotrophic, fermentative	[87]
Caloramator mitchellensis VF08^T	*Firmicutes*	Great Artesian Basin, Mitchell, Queensland, Australia	Free-flowing water from a bore. Depth 403 m, *T* 46°C	37–(55)–60	Strictly anaerobic/ Organotrophic, fermentative	[88]
Desulfotomaculum australicum AB33^T	*Firmicutes*	Great Artesian Basin, Mitchell, Queensland, Australia	Free-flowing water from a bore. Depth 914 m, *T* 60°C	40–(68)–74	Anaerobic/Sulfate-reducing/SO_4^{2-}	[72]
Desulfotomaculum geothermicum BSD^T	*Firmicutes*	Paris Basin, France	Geothermal ground-water at the discharge pipe. Depth 2500 m. *T* 58°C	37–(54)–56	Anaerobic/Facultatively lithoautotrophic (H_2/SO_4^{2-}), sulfate-reducing/SO_4^{2-}, SO_3^{2-}	[73]

Table 5. (Contd.)

Microorganism, strain	Phylum	Geographical location and characteristics of the sampling site	Sample description	Temperature range of growth min–(opt)–max	Relation to oxygen/ Type of metabolism/ Electron acceptors	Reference
*Desulfotomaculum nigrificans*** >3 strains	*Firmicutes*	Billings, Montana, United States	Water, 1264–1752 m, *T* 47–54°C	50	Anaerobic/Organotrophic, sulfate-reducing/SO_4^{2-}	[74]
Geosporobacter subterraneus VNs68^T	*Firmicutes*	Paris Basin, France	Water from the artesian well. Depth ca. 800 m, *T* 38°C*	30–(42)–55	Strictly anaerobic/ Organotrophic, fermentative	[89]
Hydrogenobacter subterraneus HGP1^T	*Aquificae*	Hacchoubaru geothermal plant, Oita Prefecture, Japan	Water obtained from 1500 m below the surface at the production well, *T* 96°C	60–(78)–85	Aerobic/Organotrophic/ ($S_2O_3^{2-}$, S^{2-}, S^0)	[71]
Thermotoga elfii G1	*Thermotoga*	Melun-L'Almont, Paris Basin, France	Artesian geothermal water from the depth of 1900 m, *T* 70°C	50–(65)–75	Strictly anaerobic/ Facultatively lithotrophic (H_2/Fe(III)) iron-reducing/ $S_2O_3^{2-}$, Fe(III)	[69]
*Thermus aquaticus*** 6 strains	*Deinococcus-Thermus*	Great Artesian Basin, Mitchell, Queensland, Australia	Artesian geothermal water from the depth of 1089–1268 m, *T* 63–75°C	45–(70)–78	Aerobic/Organotrophic	[70]
Thermoanaerobacter sp. ABI 1 Ad	*Firmicutes*	"	Artesian geothermal water, *T* 70°C	68	Aerobic/Organotrophic	[90]

* Well-cleaning procedure was applied before the sampling.

** Sequencing of 16S rRNA genes was not conducted.

Table 6. Thermophilic microorganisms isolated from run-off channels of geothermal wells

Microorganism, strain	Phylum	Geographical location and characteristics of the sampling site	Sample description	Temperature range of growth min–(opt)–max	Relation to oxygen/ Type of metabolism/ Electron acceptors	Reference
Caloramator australicus RC3^T	*Firmicutes*	Great Artesian Basin, Queensland, Australia / New Lorne bore, depth 1613 m, *T* 88°C	Red-coloured microbial mats, *T* 66°C	45–(60)–70	Strictly anaerobic/Organotrophic, fermentative, iron-reducing/Fe(III), Mn(IV), S^0	[75]
Desulfotomaculum varum RH04-3^T	*Firmicutes*	"	"	37–(50)–55	Strictly anaerobic/ Facultatively lithotrophic (H_2/SO_4^{2-}), sulfatereducing/ SO_4^{2-}, $S_2O_3^{2-}$, SO_3^{2-}, S^0	[92]
Fervidicella metallireducens AeB^T	*Firmicutes*	"	Brown-coloured microbial mats, *T* 52°C	37–(50)–55	Strictly anaerobic/ Organotrophic, fermentative, iron-reducing/ Fe(III), Mn(IV), V(V), Co(III)	[76]
Fervidicola ferrireducens Y170^T	*Firmicutes*	"	Grey microbial mat, *T* 75°C	55–(70)–80	Strictly anaerobic/ Organotrophic, fermentative, iron-reducing/ Fe(III), Mn(IV), S^0, $S_2O_3^{2-}$	[77]
Sporolituus thermophilus AeG^T	*Firmicutes*	"	Green microbial mat, *T* 57°C	45–(55)–60	Strictly anaerobic/ Organotrophic, fermentative/SO_3^{2-}, $S_2O_3^{2-}$	[78]
Thermovenabulum gondwanense R270^T	*Firmicutes*	"	Red-coloured microbial mats, *T* 66°C	50–(65)–70	Anaerobic/Organotrophic, fermentative, lithoheterotrophic (H_2)/Fe(III)), iron-reducing/Fe(III), Mn(IV), S^0, $S_2O_3^{2-}$	[79]
Thermotalea metallivorans B2-1^T	*Firmicutes*	"	Brown microbial mat, *T* 52°C	30–(50)–55	Strictly anaerobic/Organotrophic, fermentative, iron-reducing/Fe(III), Mn(IV), S^0	[80]
Thermaerobacter subterraneus C21^T	*Firmicutes*	"	Sediment and water from the run-off channel, *T* 66°C	55–(70)–80	Strictly aerobic/Organotrophic	[81]
Fervidobacterium gondwanense AB39^T	*Thermotoga*	Great Artesian Basin, Australia	Microbial mat, *T* 66°C	44–(65–68)–80	Strictly anaerobic/Organotrophic, fermentative/S^0	[91]
*Thermus aquaticus** AB17	*Deinococcus-Thermus*	"	Sediment and water from the run-off channel, *T* 53–76°C	45–(70)–78	Aerobic/Organotrophic	[70]
Melioribacter roseus P3M-2^T	*Ignavibacteriae*	Tomsk region, Western Siberia, Russia/Borehole P3, Parabel' district, artesian, thermal water, depth 2775 m	Microbial mat developing on the wooden surface of a chute, *T* 46°C	35–(55)–60	Facultative anaerobic/Organotrophic, fermentative/O_2, Fe(III), NO_2^-, AsO_4^{3-}	[82]

* Sequencing of 16S rRNA genes was not conducted.

Ignavibacteriae, was isolated from a microbial mat developing on a wooden chute collecting water from a geothermal borehole in Tomsk oblast, Russia [82]. This facultative anaerobe is capable of cellulose degradation and uses Fe(III) and As(V) compounds as electron acceptors.

CONCLUSION

The deep continental biosphere is not a single entity; it comprises a number of spatially and geologically isolated ecosystems differing in their physicochemical, geological, and trophic features. The common property of deep underground biotopes is their isolation from the sources of carbon and energy produced in the photosynthesis-based modern surface biosphere. Most deep ecosystems exist at elevated temperatures of 50–120°C, which favor the development of thermophilic and hyperthermophilic microorganisms. The existence of autonomous microbial communities comprised of subsurface thermophilic organisms that utilize sources of carbon and energy produced by geological processes alone remains a hypothesis. The indigenous nature of bacteria and archaea isolated from underground habitats is often questionable because of sample representativeness and sterility issues. Technical and financial problems associated with deep drilling limit the availability of ultradeep rock and water samples. Promising and convenient sites for investigation of deep continental ecosystems are gold mines of South Africa, which can provide recovery of natural samples from the depths of 3.5–4.0 km. Although the number of studies investigating the phylogenetic diversity of the deep biosphere has been considerable, no more than 30 culturable thermophilic species have so far been isolated from subsurface habitats not associated with petroleum deposits, and the number of indigenous thermophilic prokaryotes of definitely underground origin is even lower. More than a half of the thermophilic species isolated from underground habitats belong to the *Firmicutes*, which agrees with predominance of this phylogenetic group among the known thermophilic prokaryotes. Most subsurface thermophiles are strict or facultative anaerobes, although strict anaerobes represent a relatively small fraction; relatively frequent occurrence of sulfate and iron reduction is noteworthy. Most underground thermophilic microorganisms are organotrophic, although chemolithoautotrophic species have been also described. Thus, the currently available body of information on thermophilic microorganisms of deep subterranean habitats is scarce and provides only a very general understanding of their phylogenetic and physiologic diversity.

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