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REVIEW

Thermophilic Microbial Metal Reduction

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Abstract—Thermophilic microorganisms can reduce Fe(III), Mn(IV), Cr(VI), U(VI), Tc(VII), Co(III), Mo(VI), Au(I, III), and Hg(II). Ferric iron and Mn(IV) can be used as electron acceptors during growth; the physiological role of the reduction of the other metals is unclear. The process of microbial dissimilatory reduction of Fe(III) is the most thoroughly studied. Iron-reducing prokaryotes have been found in virtually all of the recognized types of terrestrial ecosystems, from hot continental springs to geothermally heated subsurface sediments. Thermophilic iron reducers do not belong to a phylogenetically homogenous group and include representatives of many bacterial and archaeal taxa. Iron reducing thermophiles can couple Fe(III) reduction with oxidation of a wide spectrum of organic and inorganic compounds. In the thermophilic microbial community, they can fulfil both degradative and productive functions. Thermophilic prokaryotes probably carried out global reduction of metals on Earth in ancient times, and, at the same time, they are promising candidates for use in modern biotechnological processes.

Key words: thermophilic microorganisms, microbial metal reduction, dissimilatory Fe(III) reduction, iron-reducing prokaryotes.

Microbial reduction of metals plays an important role in the biogeochemical cycles of the modern biosphere. The microbial processes of the reduction of iron, manganese, chromium, and uranium are interrelated with the cycles of carbon, oxygen, and sulfur and can exert a considerable ecological effect on the modern environment [1–3]. In certain marine, freshwater, and soil ecosystems, the reduction of Fe(III) and Mn(IV) by microorganisms is the main process providing for the oxidation of organic matter [4–6]. A still greater role may have been played by the reduction of ferric iron in the ancient biosphere, where Fe(III) was probably evolutionarily the first and, for a certain period, major oxidant of organic carbon [7]. Microbial reduction of metals considerably influences human activity. It suffices to mention biocorrosion, gley formation in soils, contamination of the environment with heavy metals and radionuclides, etc. [8–10]. The ability of microorganisms to use metal-containing compounds in their metabolic processes is a fact that has attracted the attention of astrobiologists [11]. Of great interest is the reduction of metals by thermophilic prokaryotes. Modern hydrothermal ecosystems are often considered to be relicts of the ancient Earth biosphere, and the processes that occur in them may serve as models for reconstruction of ancient biocenoses [12]. The hypothesis of the existence of a hot subsurface biosphere whose total biomass exceeds that of the surface one suggests active involvement of thermophilic microorganisms in modern global biogeochemical processes [13]. Thermophilic microorganisms have long been known to use many inorganic electron acceptors; however, it was only a short time ago that the ability of thermophiles to reduce metals was demonstrated [14].

TYPES OF MICROBIAL METAL REDUCTION

The reduction of metals can fulfil various functions in cell metabolism: the function of energy generation (dissimilatory reduction), a biosynthetic function (assimilatory reduction), or a detoxification function; it can also lack a definite function (nonspecific reduction). Dissimilatory reduction may be obligatory (anaerobic respiration) or facultative (fermentation facilitated by an exogenous acceptor of electrons). Thus, microorganisms carrying out dissimilatory metal reduction include not only metal-respiring organisms but also fermenters that discharge part of the reducing equivalents to a metal and thus gain an additional yield of energy. Some detoxication reactions can also be considered as dissimilatory processes; for example, a discharge of part of the electrons to an exogenous acceptor may relieve the inhibitory effect of the product otherwise formed (often hydrogen) and, thus, make resumption of energy generation possible. It is commonly believed that, during dissimilatory metal reduction, the acceptor is not transported into the cell. However, it has recently been found that bacteria carrying out dissimilatory reduction of Fe(III) and Mn(IV) can form intracellular inclusions containing iron and manganese [15, 16]. The quantity of metals involved in the dissimilatory

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processes is several orders of magnitude greater than that of metals involved in assimilatory processes.

Assimilatory reduction of metals has mainly been studied using the example of iron, which is a vitally important macroelement for most organisms. Reduction of chelated Fe(III) is carried out by iron reductases and may occur either prior to or after its transport into the cell [17]. The formation of magnetosomes by magnetotactic bacteria is also an assimilative process [18]. Other metals for which enzymatically catalyzed redox reactions are known (Mn, Mo, Co, Ni, V, W, are Cu) are present in cells in trace amounts [19]. Metal reduction by microorganisms may be a detoxication process, which has been studied in detail for Hg(II) and Cr(VI) compounds [20, 21]. In some cases, the reduction of metals may occur as a result of the nonspecific activity of certain enzymes, e.g., flavin reductases [17].

Many metal-containing compounds are insoluble, and microorganisms have elaborated different strategies for their reduction: direct contact, use of chelating compounds, employment of endo- and exogenous electron carriers, and interaction with the electron sphere [22–24]. Microbial reduction of metals may lead to the formation of minerals. It is believed that, during dissimilatory reduction, only the transfer of electrons to the metal is an enzymatic step, and the subsequent formation of minerals occurs without the involvement of enzymes under the action of physicochemical factors [25]; i.e., the process occurs as a biologically induced mineralization. In contrast, magnetotactic bacteria form intracellular crystals of magnetite or greigite in a process classified as a biotically controlled mineralization [18].

REDUCTION OF FE(III) BY THERMOPHILIC MICROORGANISMS

Fe(III) + e^- ⟶ Fe(II). The ability of thermophilic microorganisms to reduce trivalent iron was first demonstrated for *Sulfolobus acidocaldarius* in 1976 by Brock and Gustafson [26]. Intense research into thermophilic iron reduction was started in the mid-1990s with the obtaining of enrichment and pure cultures of thermophiles carrying out dissimilatory reduction of Fe(III) [27–31].

Natural Habitats and Cell Numbers of Thermophilic Iron Reducers

Microorganisms capable of dissimilatory reduction of Fe(III) have been found in virtually all the known types of thermal ecosystems, including terrestrial and marine hydrothermal vents and geothermally heated subsurface waters and sediments (see table).

The enrichment culture method has shown the presence of thermophilic iron reducers in sediments from hot freshwater springs, brooks, and geothermally heated soils in geographically diverse regions [27, 29]. New taxa of iron-reducing thermophiles have been isolated from the continental hot springs of Kamchatka and Yellowstone National Park [31–34]. Iron-reducing microorganisms have been found not only in thermal systems with neutral pH values but also in acidic and alkaline environments [35–37]. In terrestrial hot springs, the cell numbers of iron reducers may reach 10^7 cells/ml [38]. In these habitats, ferric iron originates from deposits of weakly crystalline iron oxides (either abiogenic or biogenic), which are formed at the sites of discharge of Fe(II)-containing hydrothermal waters. In the subsurface waters of the hydrothermal regions of Kamchatka, the total concentration of dissolved iron reaches tens of milligrams per liter for acid springs and usually does not exceed 1 mg/l in waters with pH values close to neutral [39]. The content of Fe in heated soils near solfataras reaches tens of grams per kilogram [40].

Thermophilic microorganisms capable of dissimilatory reduction of Fe(III) have been found in the formation waters of high-temperature oil fields, both marine ones and those located within continents [30, 41]. The cell numbers of iron reducers in these ecosystems is estimated to be 10–100 cells/ml [41]. Iron oxides may occur in the composition of oil-bearing rocks, and the concentration of dissolved iron in formation waters sometimes reaches tens of milligrams per liter. Thermophilic iron-reducing bacteria have been isolated from aquifers occurring under gold mines at depths varying from hundreds of meters to 3.2 km [42, 43]. Iron-reducing thermophiles were also found in sedimentary basins that had been isolated from the geological and hydrological processes occurring at the surface for millions of years [28, 44].

Deep-sea hydrothermal vents are also inhabited by thermophilic iron-reducing microorganisms; they have been detected on the outer surface and inside sulfide chimneys (*black smokers*) and in the hydrothermal fluid [45]. In different parts of the sulfide chimneys, the cell number of cultivable iron reducers varies from 10 to 10^7 cells/ml [45]. Iron-reducing thermophiles have been isolated from hydrothermal vents of the East Pacific Rise, Mid-Atlantic Ridge, Juan de Fuca Ridge, and Guaymas hydrothermal system [45–48]. The surface of the black smokers is often covered by iron oxide deposits that vary in color from black to light brown, and the content of iron in the hydrothermal fluid reaches molar values [49]. The formation of Fe(III) in these systems may occur as a result of oxidation of hydrothermally leached Fe(II) by cold oxygenated water or at the expense of the reduction of seawater sulfates by the ferrous iron present in basalts (this reaction occurs at temperatures above 300°C) [50].

The processes of microbial iron reduction occurring in shallow-water marine hydrothermal vents are less studied. Enrichment cultures of thermophilic iron reducers have been obtained from coastal marine hydrotherms near the Kuril Islands [51]. In some microorganisms that had been isolated from shallow-water hydrothermal vents at an earlier date, the capacity

Thermophilic microorganisms capable of dissimilatory reduction of Fe(III) during growth

Organism*	Isolation source	Growth temperature, °C, min–opt–max	Isolation with Fe(III)**	Fermentation	Lithoautotrophic growth***	Electron donors for Fe(III) reduction	Electron acceptors other than Fe(III)	Year and reference
Bacillus infernus TH-23^T	Sedimentary rock from a depth of 2700 m, Virginia, USA; 60°C	40–61–65	–	+	–	Formate, lactate	Mn(IV), NO_3^-, trimethylamine oxide	1995 [28]
Deferribacter thermophilus BMA^T	Formation water from an oil Field in the North Sea; depth of 2000 m; 40–110°C	50–60–65	–	–	ND	Yeast extract, peptone, tryptone, casamino acids, acetate, malate, citrate, pyruvate, lactate, succinate, valerate, H_2	Mn(IV), NO_3^-	1997 [30]
Thermoterrabacterium ferrireducens JW/AS-Y7^T	Hot brook, Yellowstone National Park, USA; 40–85°C	50–65–74	+	+	+	Glycerol, lactate, 1,2-propanediol, glycerate, pyruvate, yeast extract, H_2	$S_2O_3^{2-}$, fumarate, anthraquinone disulfonate	1997 [31, 69]
Sulfobacillus acidophilus ALV, THWX, YTF1	Self-heated coal pile, UK	45	–	–	ND	Glycerol, tetrathionate	O_2	1998 [55]
Sulfobacillus thermosulfidooxidans TH1	Hot brook, Iceland	45	–	–	ND	Glycerol, tetrathionate	O_2	1998 [55]
Acidimicrobium ferrooxidans TH3	Leached copper ore pile, USA	45	–	–	ND	Glycerol	O_2	1998 [55]
Pyrobaculum islandicum GEO3^T	Geothermal waters, Iceland	74–100–102	–	–	–	H_2, peptone	SO_3^{2-}, $S_2O_3^{2-}$, S^0, cystine	1998 [52, 77]
Thermotoga maritima MSB8^T (and strain M12597)	Hot marine sediments, Italy, and formation waters from an oil field, western Siberia	55–80–90	–	+	–	H_2	$S_2O_3^{2-}$, S^0	1998 [52, 41]
Thermus scotoductus SA-01, (and six other strains, including the type strain)	Aquifer of a gold ore deposit, South Africa; depth of 3200 m	65	+	–	ND	Lactate, acetate, formate	O_2, NO_3^-, S^0	1999 [42, 63]
Thermoanaerobacter siderophilus SR4^T	Hot spring near the Karymskii Volcano, Kamchatka; 94°C	39–70–78	+	+	–	Yeast extract, meat extract, casein, starch, glycerol, pyruvate, H_2	Mn(IV), SO_3^{2-}, $S_2O_3^{2-}$, S^0, anthraquinone disulfonate	1999 [32]
Thermoanaerobacter wiegelii Rt8.B1^T	Hot spring, New Zealand; 56–69°C	38–67–78	–	+	ND	Peptone	None	1999 [32]
Thermoanaerobacter sulfurophilus L-64^T	Cyanobacterial mat, Uzon Caldera, Kamchatka; 53–58°C	44–60–75	–	+	ND	Peptone	$S_2O_3^{2-}$, S^0	1999 [32]

Table. (Contd.)

Organism*	Isolation source	Growth temperature, °C, min–opt–max	Isolation with Fe(III)**	Fermentation	Lithoautotrophic growth***	Electron donors for Fe(III) reduction	Electron acceptors other than Fe(III)	Year and reference
Thermoanaerobacter acetoethylicus SL 26, S128	Formation waters from an oil field, western Siberia; depth of 1700–2500 m; 60–84°C	40–65–80	–	+	ND	Peptone, H_2	$S_2O_3^{2-}$	1999 [41]
Thermoanaerobacter brockii M739	Formation waters from an oil field, western Siberia; depth of 1700–2500 m; 60–84°C	35–65–85	–	+	ND	Peptone, H_2	$S_2O_3^{2-}$	1999 [41]
Thermotoga subterranea SL1^T	Formation waters from a continental oil field, France; depth of 1670 m; 65–70°C	50–70–75	–	+	ND	Peptone, H_2	$S_2O_3^{2-}$, cystine	1999 [41]
Thermococcus sibiricus MM 739^T (and strain MM 642)	Formation waters from an oil field, western Siberia; depth of 1700–2500 m; 60–84°C	40–81–88	–	+	ND	Peptone, H_2	S^0	1999 [41]
Pyrobaculum aerophilum	Shallow-water marine hydrothermal vent, Italy	75–100–104	–	–	ND	H_2, peptone	O_2	2000 [77]
Thermococcus sp. SN531	Deep-sea hydrothermal vent, East Pacific Rise; depth of 2650 m; 30–101°C	50–85–95	–	+	ND	Peptone, yeast extract	S^0	2001 [45]
Ferroglobus placidus AEDII12DO^T	Shallow-water marine hydrothermal vent, Italy; 95°C	65–85–95	–	–	ND	Acetate, benzoate, benzaldehyde, 4-hydroxybenzoate, *p*-hydroxybenzaldehyde	NO_3^-, $S_2O_3^{2-}$	2001 [53, 70]
Geoglobus ahangari 234^T	Deep-sea hydrothermal vent, Guaymas; depth of 2000 m	65–88–90	+	–	+	H_2, acetate, pyruvate, malate, succinate, formate, fumarate, peptone, yeast extract, glycerol, isoleucine, arginine, serine, glutamine, asparagine, stearate, palmitate, valerate, butyrate, propionate	None	2002 [46]
Geothermobacterium ferrireducens FW-1a^T	Hot brook, Yellowstone National Park, USA	65–85–100	+	–	+	H_2	None	2002 [34]
Thermodesulfobacterium commune YSRA-1^T	Hot brook, Yellowstone National Park, USA	45–70–85	–	–	+	H_2	SO_4^{2-}, $S_2O_3^{2-}$	2002 [34]
Thermotoga lettingae TMO^T	Thermophilic bioreactor; 65°C	50–65–75	–	+	ND	Methanol	$S_2O_3^{2-}$, S^0, anthraquinone disulfonate	2002 [56]

Table. (Contd.)

Organism*	Isolation source	Growth temperature, °C, min–opt–max	Isolation with Fe(III)**	Fermentation	Lithoautotrophic growth***	Electron donors for Fe(III) reduction	Electron acceptors other than Fe(III)	Year and reference
Thermovenabulum ferriorganovorum Z-9801^T	Hot spring, Uzon Caldera, Kamchatka; 63°C	45–64–76	+	+	–	Peptone, yeast extract, meat extract, casamino acids, starch, pyruvate, H_2	Mn(IV), NO_3^-, SO_3^{2-}, $S_2O_3^{2-}$, S^0, fumarate, anthraquinone disulfonate	2002 [33]
Thermoanaerobacter sp. X514, X513, X561	Deep-sea sediments, Colorado, USA	60	+	+	ND	Lactate, acetate, succinate, xylose, glucose, H_2	Mn(IV), Cr(VI), Co(III), U(VI)	2002 [60]
Geothermobacter ehrlichii SS015^T	Hydrothermal fluid, Juan de Fuk Ridge	35–55–65	+	–	–	Malate, acetate, pyruvate, formate, starch, peptone, tryptone, casamino acids, isoleucine, arginine, asparagine, glutamine, histidine, serine, butyrate, propionate, maltose, fructose, ethanol, methanol, isopropanol	NO_3^-, NO_2^-, dimethyl sulfoxide	2003 [48]
Deferribacter abyssi JR^T	Deep-sea hydrothermal vent, Mid-Atlantic Ridge; depth of 2400 m	45–60–65	–	–	–	Acetate, succinate, H_2	S^0, NO_3^-	2003 [47]
Sulfurihydrogenibium subterraneum HGM-K1^T	Aquifer of a gold ore deposit, Japan; depth of 320 m	40–65–70	–	–	+	H_2, $S_2O_3^{2-}$	O_2, NO_3^-, SeO_3^{2-}, SeO_4^{2-}, $HAsO_4^{2-}$	2003 [64]
Sulfurihydrogenibium azorenze Az-Fu1^T	Terrestrial hot brook, the Azores	50–68–73	–	–	+	H_2	O_2, S^0, HAs	2004 [65]
Anaerobranca californiensis PAOHA-1^T	Alkaline hot brook, Mono Lake, USA	45–58–70	–	+	ND	Peptone	S^0, $S_2O_3^{2-}$, SeO_4^{2-}	2004 [37]
Anaerobranca horikoshii JW/YL-138^T	Hot brook, Yellowstone National Park, USA	30–57–66	–	+	ND	Peptone	S^0, $S_2O_3^{2-}$, fumarate	2004 [37]
Anaerobranca gottschalkii LBS3^T	Hot spring, Lake Bogoria, Kenya	30–55–65	–	+	ND	Glucose	S^0, $S_2O_3^{2-}$, fumarate	2004 [37]
Thermosinus carboxydivorans Nor1^T	Hot spring, Lake Bogoria, Kenya	48–60–68	–	+	–	Carbon monoxide (CO), yeast extract	$S_2O_3^{2-}$, SeO_3^{2-}	2004 [62]

Note: ND stands for “no data”.

* Microorganisms are ranged according to the order of publication dates. Represented are strains identified at least at a generic level.

** Fe(III) was employed during isolation at the enrichment stage.

*** With molecular hydrogen as an electron donor and Fe(III) as an electron acceptor.

for dissimilatory reduction of Fe(III) was discovered [52, 53].

The thermophilic microorganisms inhabiting anthropogenic ecosystems are also able to use Fe(III) as an electron acceptor. Thermophilic iron reducers have been found in an amount of up to thousands of cells per cm^3 in water and deposits of corrosion products in heat supply pipelines [54]. Self-heated coal and ore piles are inhabited by moderately thermophilic acidophilic bacteria that can reduce Fe(III) under anaerobic conditions [55]. Thermophilic iron-reducing microorganisms have been found in an anaerobic bioreactor and in a system of nitric oxide removal from flue gas by absorption with Fe(II)–EDTA [56–58]. An enrichment culture of iron-reducing hyperthermophiles has been obtained from samples of the digested sludge of an anaerobic digestor [59].

Phylogenetic Diversity of Thermophilic Prokaryotes Carrying out Dissimilatory Reduction of Fe(III)

The thermophilic microorganisms capable of dissimilatory reduction of Fe(III) do not form a specific phylogenetic group. Representatives of both prokaryotic domains, *Bacteria* and *Archaea*, can use iron as an electron acceptor. Currently, about 30 species of iron-reducing thermophiles, representing 19 genera, are known (table) (to compare, 60 species of mesophilic iron reducers, representing 26 genera, are recognized [3]).

Iron-reducing thermophilic bacteria belong to phylogenetically diverse taxa. *Bacillus infernus, Thermoterrabacterium ferrireducens, Thermovenabulum ferriorganovorum, Thermosinus carboxydovorans*, and species of the genera *Thermoanaerobacter, Anaerobranca*, and *Sulfobacillus* represent the low G+C lineage of gram-positive bacteria [28, 31–33, 37, 41, 55, 60–62]. The moderately thermophilic *Acidimicrobium ferrooxidans* belongs to the class *Actinobacteria* [55]. *Geothermobacter ehrlichii* is a deltaproteobacterium [48]. *Deferribacter thermophilus* and *Deferribacter abyssi* represent a special class, namely, *Deferribacteres* [30, 47]. The following thermophilic iron reducers also represent separate phylogenetic high-level groups: *Thermus scotoductus* (the *Thermus/Deinococcus* group), *Sulfurihydrogenibium subterraneum* and *Sulfurihydrogenibium azorense* (the class *Aquificae*), *Thermotoga* species (the class *Thermotogae*), and *Geothermobacterium ferrireducens* and *Thermodesulfobacterium commune* (the class *Thermodesulfobacteria*) [34, 41, 52, 56, 63–65]. On the basis of results of molecular–ecological studies, it is assumed that thermophiles phylogenetically close to the mesophilic Fe(III)-reducing genus *Shewanella* (gammaproteobacteria) are also capable of iron reduction [66].

The archaea shown to be capable of dissimilatory reduction of Fe(III) belong to the kingdoms *Crenarchaeota* (the order *Thermoproteales: Pyrobaculum islandicum*) and *Euryarchaeota* (the order *Archaeoglobales: Ferroglobus placidus* and *Geoglobus ahangari* and the order *Thermococcales: Thermococcus* species) [41, 45, 46, 52, 56]. The highest temperature organism currently known (strain 121 of "*Geogemma barosii*") is also an iron-reducing archaeon and belongs to *Crenarchaeota* [3, 67].

Possession of the capacity for iron reduction does not correlate with the generic and, sometimes, even species affiliation of a microorganism and seems to be a strain-specific property. Thus, none of the four *Bacillus s*pecies phylogenetically close to *B. infernus* is capable of anaerobic growth with Fe(III) [28]. One of the three currently recognized species of *Deferribacter, D. desulfuricans*, is incapable of iron reduction [68]. *Thermodesulfobacterium commune* reduces Fe(III), and *Thermodesulfobacterium hveragerdense* does not [34]. Out of the five strains of *Thermus scotoductus* studied, four were capable of ion reduction, and one was not; the other three species of the genus *Thermus* do not include iron-reducing representatives [63]. Nevertheless, genera are known in which all of the representatives tested proved to be capable of dissimilatory reduction of Fe(III) (*Thermoanaerobacter, Thermotoga*, and *Anaerobranca*) [32, 37, 41].

Physiology of Thermophilic Iron Reducers

Temperature and pH ranges. The highest temperature at which microbial reduction of Fe(III) has been recorded is 121°C; currently, it is also the upper known limit of life [67]. The highest temperature eubacterium (*Geothermobacterium ferrireducens*, with a maximal growth temperature of 100°C) is also a dissimilatory iron reducer [34]. Many thermophilic iron-reducing prokaryotes have their lower growth limits at 30–40°C (table). Iron reduction has mainly been studied at optimal growth temperatures; however, for some microorganisms, temperature dependence curves have been obtained both for growth and iron reduction, and they invariably coincide [31, 42, 60]. Although most of the currently known iron reducers are neutrophilic microorganisms, the pH range of thermophilic iron reduction is quite broad. Representatives of the genera *Sulfolobus, Sulfobacillus*, and *Acidimicrobium* reduce ferric iron at pH 1.5–2.0 [26, 55]. In the alkaline zone, species of the genus *Anaerobranca* can grow at pH 10–10.5; the reduction of Fe(III) by these microorganisms has been studied at pH 9.5 [37].

Types of metabolism. The ability to use Fe(III) as an exogenous electron acceptor has been found in thermophilic microorganisms with different types of metabolism. This capacity is displayed by fermenters and obligately respiring anaerobes with different types of respiration (sulfur, manganese, and nitrate reducers), as well as by aerobic microorganisms in the absence of oxygen (table). For most of these microorganisms, Fe(III) is not the sole electron acceptor used. *Geothermobacterium ferrireducens* and *Geoglobus ahangari* are an exception; moreover, *Geothermobacterium ferrireducens* can grow only with weakly crystalline iron

oxide and does not reduce soluble ferric compounds [34, 46]. Many microorganisms with a fermentative type of metabolism (representatives of the genera *Thermotoga, Thermoanaerobacter, Thermoterrabacterium*, and *Thermococcus*) are capable not only of fermentation with ferric iron reduction but also of lithotrophic growth with Fe(III) as an electron acceptor and molecular hydrogen as an electron donor [41, 52, 60, 69]. Quantitative data on the effect of Fe(III) reduction on the energy metabolism of fermentative microorganisms are scarce. It has been shown that, during the organotrophic growth of *Thermoterrabacterium ferrireducens* and *Thermoanaerobacter siderophilus* in the presence of ferric iron, the maximum cell yield increases three- and twofold, respectively, and the ratio of metabolic products changes. For *Thermoanaerobacter siderophilus*, growth stimulation by Fe(III) is due to elimination of the inhibitory effect of molecular hydrogen, whereas, for *Thermoterrabacterium ferrireducens*, it is evidently due to generation of additional energy in the electron transport chain [69].

Cell suspensions of some hyperthermophilic bacteria and archaea belonging to different physiological groups of prokaryotes (fermentative microorganisms, sulfate reducers, methanogens, and sulfur reducers) proved to be able to reduce Fe(III) with molecular hydrogen, and the iron-reducing activity was found to be constitutive [52]. This wide distribution of iron-reducing capacity among organisms that are commonly considered to be close to the last common ancestor of all living organisms suggests that the reduction of Fe(III) may be the most ancient type of metabolism.

Electron donors. Thermophiles can use a wide spectrum of organic compounds for the reduction of Fe(III) (table). Acetate, the key intermediate in the decomposition of organic matter under anaerobic conditions, is completely metabolized by iron reducers within the entire range of their growth temperatures. An important finding was the discovery of the capacity for acetate utilization coupled with Fe(III) reduction in hyperthermophilic archaea [53]; thus far, microbial acetate oxidation at temperatures above 80°C has been demonstrated only for iron reducers and has not been found in any other microbial group. Interestingly, some strains of the genus *Thermoanaerobacter* shown to be capable of acetate utilization in the presence of Fe(III) [60]; before this result was obtained, thermoanaerobacters were not known to grow by oxidation of nonfermentable organic compounds. Hyperthermophilic iron reducers have two more unique physiological properties, unknown in other hyperthermophilic prokaryotes: the capacities for anaerobic oxidation of aromatic compounds (*Ferroglobus placidus*) [70] and long-chain fatty acids (*Geoglobus ahangari*) [46]. The results of studies on the utilization of reducing sugars as substrates for iron reduction should be considered taking into account adequate abiotic controls, since, at elevated temperatures, these compounds chemically reduce ferric iron [71]. Among inorganic compounds, molecular hydrogen, thiosulfate, tetrathionate, elemental sulfur, and carbon monoxide can be electron donors for thermophilic iron reduction (table).

Autotrophic growth. Many iron-reducing thermophiles can use molecular hydrogen; however, the capacity for autotrophic growth has been studied in a limited number of these organisms (table). *Geoglobus ahangari, Geothermobacterium ferrireducens, Thermodesulfobacterium commune, Thermoterrabacterium ferrireducens, Sulfurihydrogenibium subterraneum*, and *Sulfurihydrogenibium azorense* [34, 46, 64, 65, 69] have proved capable of growth with H_2 and Fe(III) in the absence of organic carbon sources. *T. ferrireducens* could grow without any organic compounds (the cultivation medium was even devoid of vitamins). The H_2 concentration threshold for H_2 consumption by this organism is 3 ppmv (3×10^{-5} vol %). The presence of CO-dehydrogenase activity suggests that CO_2 fixation by *T. ferrireducens* occurs via the anaerobic acetyl-CoA pathway [69]. Among the mesophiles capable of dissimilatory reduction of Fe(III), the capacity for autotrophic growth has been shown only for the acidophile *Acidithiobacillus ferrooxidans*, which uses molecular hydrogen or elemental sulfur as electron donors during iron reduction [72]. In none of the mesophilic iron reducers growing at pH values close to neutral ones has the capacity for autotrophic growth been found.

Biochemistry of Fe(III) Reduction by Thermophiles

Information on the biochemical mechanisms of Fe(III) reduction by thermophilic prokaryotes is scattered in a few individual reports. Nothing is known about the assimilatory iron reductases of thermophilic bacteria, although assimilation of iron by mesophiles has been well studied [17]. The only iron reductase that has been purified from a thermophile, crystalized, and thoroughly characterized is the enzyme from the hyperthermophilic archaeon *Archaeoglobus fulgidus* [73, 74]. This enzyme consists of one 18-kDA subunit and occurs in the soluble cell fraction, accounting for 0.75% of the total soluble cell proteins. It can use both NADH and NADPH for iron reduction, and the presence of FMN or FAD is necessary for its catalytic activity. It remains unknown whether this iron reductase performs an assimilatory or a dissimilatory function. Although iron-reducing activity has been shown in *A. fulgidus* cell suspensions, this organism is incapable of using Fe(III) as an electron acceptor for growth [46, 52, 75]. Studies of another hyperthermophile, *Pyrobaculum islandicum*, grown on an Fe(III)-containing medium showed that, in this organism, the NADP-dependent iron reductase activity is localized in the cytoplasm and is not associated with *c*-type cytochromes, which are characteristic of mesophilic microorganisms capable of dissimilatory iron reduction [76]. In the gram-positive Fe(III)-reducing bacterium *Thermoterrabacterium ferrireducens*, the main part of the

iron reductase activity is localized in the cell membrane fraction. Preparations of partially purified membrane-bound iron reductase were found to contain *c*-type cytochromes and catalyze the reduction of soluble and insoluble forms of Fe(III), with NAD or NADPH as the electron donors [77].

Transformation of Iron Compounds

Most of the studies on thermophilic microbial reduction of iron have been performed with the use of insoluble amorphous Fe(III) oxide or soluble ferric iron citrate playing the role of electron acceptors. In the published studies, amorphous Fe(III) oxide is also termed weakly crystalline Fe(III) oxide, Fe(III) hydroxyhydroxide, hydrated Fe(III) oxide, Fe(III) hydroxide, amorphous Fe(III) hydroxide, or ferrihydrite. These terminological differences seem to be due both to differences in the extent of crystallization of the oxides of ferric iron synthesized in different laboratories and to subjective preferences. There are virtually no data on the reduction of Fe(III) oxides other than the amorphous oxide. Three hyperthermophilic iron reducers investigated in this respect did not reduce hematite or goethite [34, 46, 78]. Under acidic conditions, moderately thermophilic iron-reducing bacteria reduce goethite and jarosite, bringing about dissolution of these minerals [55]. The soluble Fe(III) forms reduced by thermophiles include, in addition to Fe(III) citrate, Fe(III) complexes with EDTA and nitrilotriacetate, as well as Fe(III) sulfate, which is employed at low pH values [42, 55, 73]. Some organisms are unable to reduce soluble Fe(III) compounds and reduce only amorphous oxide [34, 48]. Thermophiles and hyperthermophiles have been shown to reduce humic acids and their analogue anthraquinone disulfonate [31, 79]. In the presence of micromolar concentrations of the latter compound, hyperthermophilic archaea can reduce crystalline iron oxides [79]. Most probably, in some ecosystems, humic compounds are involved in iron reduction as mediators of electron transfer.

During the reduction of amorphous Fe(III) oxide, laboratory cultures of thermophilic iron reducers form magnetite (Fe_3O_4) and siderite ($FeCO_3$) [27, 80]. Extracellular magnetite crystals formed under different conditions measure from 0.01 to 40 μm. They have an octahedral or prismatic shape and may contain a single magnetic domain [27, 60, 81]. Moessbauer and diffraction studies have shown that the newly formed magnetite crystals are not perfectly ordered [27, 82, 83]. Data from thermodynamic modeling and laboratory experiments indicate that the magnetite/siderite ratio depends on a number of parameters, including pH, partial pressure of CO_2, Fe^{2+} concentration, the amount of amorphous Fe(III), and the presence of inert organic matter [27, 83]. Magnetite can also be formed in cultures of hyperthermophilic sulfate-reducing microorganisms at the expense of the chemical interactions occurring at elevated temperatures between H_2, amorphous Fe(III) oxide, and the sulfide formed enzymatically as a result of dissimilatory reduction of sulfate [75]. Under certain conditions, the formation of magnetite films by thermophilic iron reducers might decrease the corrosion of steel [54]. However, data are also available on the activation of corrosive processes in the presence of iron-reducing bacteria [84]. A study of the fractionation of oxygen and carbon isotopes in siderite formed as a result of the activity of thermophilic iron reducers showed that it is mainly dependent on the temperature and bicarbonate concentration; under the conditions studied, the microbial fractionation was indistinguishable from the abiotic one [85].

REDUCTION OF OTHER METALS BY THERMOPHILIC MICROORGANISMS

As a rule, hydrothermal fluids are enriched with ions of various metals. In marine hydrothermal fluids, the concentration of manganese may be as high as several moles, and the concentrations of cobalt and molybdenum may reach several micromoles per liter [49]. Certain terrestrial hydrothermal waters are characterized by chromium and uranium contents of several micrograms per liter [39]. Insoluble iron oxides are also components of hydrothermal systems.

As compared to ferric iron reduction, microbial reduction of other metals has been less thoroughly studied. Apart from Fe(III), only Mn(IV) and Mo(VI) have been shown to be used by thermophilic prokaryotes for energy generation during growth [30, 86]. Chromium, uranium, technetium, gold, and mercury compounds are toxic for microorganisms, and this hinders performing experiments with growing cultures. The available data allow a conclusion to be made that no correlation exists between the capacity of a microorganism to reduce Fe(III) and its ability to reduce other metals (table). The fact that not all of the iron reducers investigated in this respect could reduce other metals indicates that the biochemical mechanisms of reduction of different metals are different. Experiments on metal reduction commonly use microorganisms for which the capacity for iron reduction has earlier been shown. Tests of the ability of thermophiles belonging to other physiological groups to reduce toxic metals and the employment of metal-containing media to obtain new enrichment and pure cultures should extend the list of known metal reducers. The processes of vanadium(V) and copper(II) reduction, demonstrated for mesophilic prokaryotes, are unknown in thermophiles. Neither are any data available on the reduction by thermophilic prokaryotes of actinides such as neptunium(V) and plutonium(IV), although mesophilic microbial processes of their reduction have been demonstrated [87].

Since the reduced forms of uranium, technetium, cobalt, and chromium are less soluble than their oxidized forms, thermophilic microbial metal reduction can be used for immobilization of toxic metals and radionuclides, e.g., in the biotechnological processes of hot

wastewater treatment or for bioremediation of disposal sites of radioactive wastes, where the temperature near the gradually cooling radioactive masses remains within the range favorable for thermophiles for a long period of time.

Reduction of manganese: Mn(IV) + 2e ⟶ Mn(II). Mn(IV) can be used as an electron acceptor by *Bacillus infernus, Deferribacter thermophilus, Thermoanaerobacter siderophilus, Thermovenabulum ferriorganovorum*, and *Thermoanaerobacter* spp. (table). The isolation of pure cultures of *B. infernus* and *D. thermophilus* has been performed with the employment of insoluble MnO_2, both at the enrichment stage and to obtain colonies on solid medium. Cell suspensions of *Thermus scotoductus* and *Pyrobaculum islandicum* can also reduce Mn(IV) [42, 78]. The product of Mn(IV) oxide reduction is rhodochrosite ($MnCO_3$) [60].

Reduction of chromium: Cr(VI) + 3e ⟶ Cr(III). Hexavalent chromium can be reduced by growing cultures of *Thermoanaerobacter* spp. (0.5 mM $KCrO_4$ and lactate or glucose as the electron donor) and cell suspensions of *Thermus scotoductus* (0.1 mM $KCrO_4$ and lactate), *Pyrobaculum islandicum* (0.4 mM $KCrO_4$ and H_2), and *Deinococcus geothermalis* [42, 60, 71, 78, 88].

Reduction of uranium: U(VI) + 2e ⟶ U(IV). U(VI) is reduced by growing cultures of *Thermoanaerobacter* spp. (with 1 mM uranyl carbonate and lactate as the electron donor) and cell suspensions of *Thermus scotoductus* and *Pyrobaculum islandicum* (0.3 mM uranyl acetate and H_2). The final product of U(VI) reduction has been identified as uraninite (UO_2), which is deposited extracellularly [42, 60, 78].

Reduction of Tc(IV). Cell suspensions of *Pyrobaculum islandicum* incubated with molecular hydrogen as the electron donor converted 0.25 mM ammonium pertechnetate into the insoluble reduced forms Tc(IV) or Tc(V) [78].

Reduction of cobalt: Co(III) + 2e ⟶ Co(II). Trivalent cobalt compounds occur rarely. All the investigations on microbial reduction have been carried out with artificially synthesized Co(III)–EDTA. Reduction of this compound to Co(II)–EDTA could be performed by growing cultures of *Thermoanaerobacter* spp. and cell suspensions of *Thermus scotoductus* and *Pyrobaculum islandicum* [42, 60, 71, 78].

Reduction of molybdenum: Mo(VI) + e ⟶ Mo(V). The reduction of Mo(VI) to ⟶ Mo(V) has been observed in cultures of *Sulfolobus acidocaldarius* and *Acidianus brierleyi* growing anaerobically with elemental sulfur as the electron donor at pH 1.5–2.5 and a temperature of 60°C. The use of Mo(VI) and an electron acceptor under these conditions can be inferred from the high initial concentrations of molybdate (5.2 mM) and lack of Mo(VI) reduction during growth on a fermentable organic substrate [86].

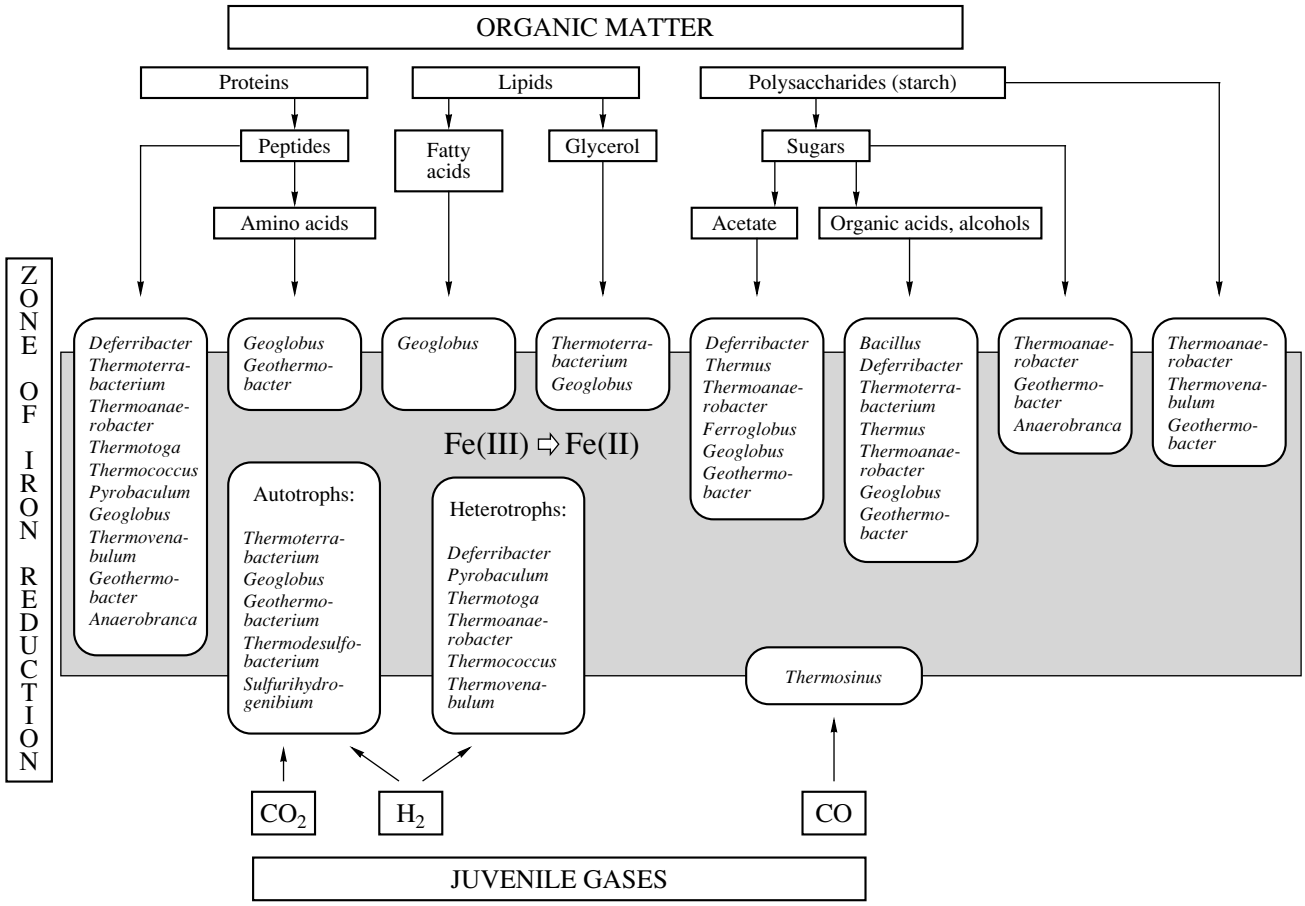
Reduction of gold. Cell suspensions of four out of the seven hyperthermophilic microorganisms tested (*Pyrobaculum islandicum, Geoglobus ahangari, Pyrococcus furiousus*, and *Thermotoga maritima*) have been found to reduce soluble Au(III) to insoluble Au(0), which was deposited in the medium or on the cell surface. Reduction of trivalent gold occurred only if H_2 was used as the electron donor. None of the organisms studied could grow with Au(III) as the electron acceptor [89]. The reduction of Au(I) to Au(0) has been observed during growth of *Thermoanaerobacter* spp.; however, the cultures that performed the process could not grow after transfers [60].

Reduction of mercury: Hg(II) + 2e ⟶ Hg(0). Reduction of divalent mercury to volatile metallic Hg^0 can be performed by *Deinococcus geothermalis*, which has the upper growth limit at 55°C. Genetic modification of this organism by its transformation with a plasmid bearing a Hg(II) resistance operon considerably improves the parameters of mercury reduction [88].

CONCLUSION

Thermophilic Fe(III)-reducing microorganisms belong to 15 bacterial and 4 archaeal genera. Like many other physiological groups of prokaryotes, the iron reducers are a group composed of microorganisms that are not united by common origin. Nor are iron-reducing organisms strictly specific in a physiological respect: all the known mesophilic and most of the thermophilic iron reducers, including Fe(III) respirers, can also grow at the expense of other metabolic processes. Several hyperthermophilic organisms that are obligately dependent on Fe(III) are an exception. Data on the ability of hyperthermophiles to reduce Fe(III) have been used as the basis of a hypothesis that views iron reduction as one of the most ancient metabolic capacities. However, the deficiency of data on the biochemical mechanisms of iron reduction does not allow an unambiguous conclusion to be made on whether the capacity for iron reduction has arisen only once or whether it originated many times independently in the course of microbial evolution. The distinctions between closely related organisms, which may belong to the same genus or even species, with respect to the presence of the iron-reducing ability may indicate both its acquisition via lateral gene transfer and loss as a result of mutation.

Iron-reducing prokaryotes have been found in virtually all the known types of thermal ecosystems, from hot continental springs to deep-sea hydrothermal vents and hydrothermally heated subsurface sediments. The cell number of Fe(III)-reducing microorganisms in different biotopes varies within a wide range and is evidently limited not only by the concentration of utilizable iron compounds but also by the presence of an electron donor. The amounts of iron reduced by microorganisms and the rates of this process in natural thermal ecosystems are unknown. Despite the fact that the ability to reduce Fe(III) has been found in such ubiqui-



Utilization of organic and inorganic compounds as electron donors during the reduction of Fe(III) by thermophilic prokaryotes.

tous genera as *Thermoanaerobacter* and *Thermococcus*, there are no data on the predominance of a particular taxonomic group of thermophilic iron reducers in natural biocenoses.

Iron-reducing thermophiles couple the reduction of Fe(III) with the oxidation of a wide range of organic and inorganic compounds. The discovery of the ability of hyperthermophilic iron reducers to utilize acetate, long-chain fatty acids, and aromatic compounds has changed the current concepts of fluxes of compounds in microbial ecosystems developing at temperatures above 80°C. Fe(III)-reducing thermophiles oxidize the main groups of compounds formed during decomposition of dead microbial mass. Thus, complete mineralization of organic matter may occur in zones of ferric iron deposits (see the figure). The presence, in many iron-reducing prokaryotes, of the capacity for lithoautotrophic growth, which has not been found in neutrophilic iron-reducing mesophiles, suggests that, in thermal ecosystems, iron reducers may be not only degraders but also producers of organic matter. Autotrophic iron reducers that utilize juvenile gases can be primary producers in autonomous microbial communities, e.g., those developing in the depth of the Earth's crust.

In addition to Fe(III), eight more metals have been shown to undergo microbial reduction under thermal conditions: Mn(IV), Cr(VI), U(VI), Tc(VII), Co(III), Mo(VI), Au(I, III), and Hg(II). With the exception of Mn(IV) and Mo(VI), which are used as electron acceptors during growth, the physiological role of the reduction of these metals is unknown. It is reasonable to assume the existence of nonspecific biochemical mechanisms of the reduction of some metals, e.g., technetium and other radionuclides, which never occur in natural environments in considerable concentrations. Single experimental studies on the reduction of toxic metals by thermophiles have mainly been performed with cell suspensions of iron-reducing organisms and soluble forms of metals.

The utilization of insoluble oxides of metals as electron acceptors is a unique feature of metal-reducing microorganisms. Thermophiles have been shown to enzymatically reduce amorphous and weakly crystalline oxides of Fe(III) and Mn(IV). The strategies of cell interaction with a solid phase need further biochemical and mineralogical studies.

The worldwide scientific community displays keen interest in the processes of microbial metal reduction. Thermophilic prokaryotes, which probably carried out

global reduction of metals on Earth in ancient times, are, simultaneously, promising candidates for modern biotechnological processes.

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