Chapter 7
CO-Oxidizing Anaerobic Thermophilic Prokaryotes

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Abstract Being a potent electron donor ($E^{0'}_{CO/CO2} = -520$ mV), CO may serve as an energy source for anaerobic prokaryotes. The main sources of CO in hot environments inhabited by anaerobic thermophiles are volcanic exhalations and thermal degradation of organic matter. A number of phylogenetically diverse anaerobic prokaryotes, both Bacteria and Archaea, are known to metabolize CO. CO transformation may be coupled to methanogenesis, acetogenesis, hydrogenogenesis, and sulfate or ferric iron reduction. This review will mainly focus on the diversity, ecology, physiology, and certain genomic features of the hydrogenogenic species, which are most numerous among the currently recognized thermophilic anaerobic carboxydotrophs and many of which were isolated and described in recent years. Among them are diverse Firmicutes, Dictyoglomi, and Eury- and Crenarchaeota. Despite their phylogenetic diversity, they employ similar enzymatic mechanisms of the $CO + H_2O \rightarrow CO_2 + H_2$ process. The key enzyme of anaerobic CO utilization, the Ni-containing CO dehydrogenase, forms in hydrogenogens an enzymatic complex with the energy-converting hydrogenase, and genomic analysis shows this enzymatic complex to be encoded by a single-gene cluster.

Keywords CO • Carboxydotrophs • CO dehydrogenase • CO transformation • Hydrothermal environments • Volcanic gases

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7.1 Introduction

CO is a possible substrate for growth of thermophilic prokaryotes in their natural habitats – hot volcanic environments. The reported concentration of CO in volcanic gases varies from 0.6 to 5540 ppm (for references, see Sokolova et al. 2009). The main sources of CO in hydrothermal environments are eruptive (Menyailov and Nikitina 1980; Greenland 1986; Symonds et al. 1994; Allard and Barton 2004) or fumarolic volcanic gases (Garofalo et al. 2007; Tassi et al. 2003, 2005). A minor source of CO in hot volcanic environments may be its production in the course of thermochemical or photochemical degradation of organic matter (Conrad and Seiler 1985; Schade et al. 1999; Hellebrand and Schade 2008). One more minor source of CO is its formation as a side product by some thermophilic anaerobes (Conrad and Thauer 1983; Diekert et al. 1984). However, sources of CO mentioned as minor in hot volcanic environments, i.e., thermochemical degradation of organic matter and CO production by some thermophilic anaerobes as a side product, probably play a more important role in other thermophilic habitats, such as bioreactors or composts.

7.2 CO Transformation in Hot Volcanic Environments

CO is a potent electron donor ($E^{0\prime}_{CO/CO2} = -520$ mV); thus, it is an energy-rich substrate that can serve as an electron donor for the growth of lithotrophic microorganisms; however, its utilization by microorganisms is restricted because of its high toxicity to metal-containing enzymes (Thauer et al. 1974; Fauque et al. 1988; Adams 1990). However, a number of anaerobic thermophilic prokaryotes, among which there are carboxydotrophic hydrogenogens, methanogens, acetogens, and sulfate and ferric iron reducers, can use CO as a source of energy for growth; some organisms use CO as a cell carbon source as well. The concentration of CO dissolved in hot spring water is usually rather low (Shock et al. 2005; Kochetkova et al. 2011). An active process of CO transformation by thermophilic anaerobic community was demonstrated in several hot springs of Uzon Caldera (Kamchatka Peninsula) (Kochetkova et al. 2011). The actual rate of anaerobic CO transformation by the microbial community was determined for one of studied springs to be 120 μmol l^{-1} of sediment day^{-1}. For all the hot springs studied, the results of radioisotopic tracing experiments showed that active anaerobic transformation of CO took place and that the major part of CO (89–99%) was converted to CO_2. Production of ^{14}C-labeled methane was not detected, and the production of ^{14}C-labeled volatile fatty acids, presumably acetate, was comparatively low: it did not exceed 4.8%. These data indicate that either hydrogenogenic carboxydotrophy or CO oxidation in the course of anaerobic respiration are dominant processes of CO transformation in the habitats studied. However, methanogenesis from CO could not be completely excluded, as it proceeds through formation of CO_2 as an intermediate (Stupperich and Fuchs 1984), and thus, significant dilution of isotope with the HCO_3^- ions present in hydrothermal water should reduce significantly the sensitivity of the method. Anaerobic

respiration processes, such as sulfate reduction (Parshina et al. 2005a, b) or Fe(III) reduction (Slobodkin et al. 2006), could also be responsible for anaerobic CO oxidation in these thermophilic environments.

Thus, it was demonstrated that CO is a substrate that is present and is utilized in natural hot environments under conditions suitable for moderately, extremely, and hyperthermophilic prokaryotes (Kochetkova et al. 2011).

7.3 Hydrogenogenic Carboxydotrophic Thermophilic Anaerobes

The main feature of hydrogenogenic carboxydotrophic prokaryotes is their ability to grow producing hydrogen as the only reduced product of the oxidation of carbon monoxide in the reaction with water:

$$CO + H_2O = CO_2 + H_2\,\Delta G^{0'} = -20\ \text{kJ/mol CO}.$$

The representatives of thermophilic hydrogenogenic carboxydotrophic bacteria were first found by V. A. Svetlichny in hot springs of Kunashir Island (Kurils) (Svelichny et al. 1991b). Currently, there are 17 hydrogenogenic carboxydotrophic species belonging to Firmicutes, Dictyoglomi, Euryarchaeota, and Crenarchaeota (Table 7.1).

7.3.1 Genus Carboxydothermus

This genus belongs to the family *Thermoanaerobacteraceae* in the class *Clostridia* (Ludwig et al. 2009) and is the taxon containing first described thermophilic hydrogenogenic carboxydotrophic species (Svetlichny et al. 1991a, b; Slobodkin et al. 2006; Wiegel 2009). Cells of *Carboxydothermus* species are straight to slightly curved rods, 0.3–0.5 × 1.3–2.7 μm; they have cell wall of the Gram-positive structure. All *Carboxydothermus* species are obligate anaerobes, extreme thermophiles, and neutrophiles. The optimal temperature for growth is in the range of 65–70°C. All *Carboxydothermus* species can utilize CO, but they are facultative carboxydotrophs and can grow chemolithotrophically on CO or on other substrates. Genus *Carboxydothermus* currently contains four species (Table 7.1): the three hydrogenogenic species, *C. hydrogenoformans* (Svetlichny et al. 1991a, b), *C. siderophilus* (Slepova et al. 2009), and *C. islandicus* (Novikov et al. 2011), and one nonhydrogenogenic carboxydotrophic species, *C. ferrireducens* (Slobodkin et al. 1997; Gavrilov et al. 2003; Slobodkin et al. 2006), which grows on CO only in the presence of ferric iron hydromorphic oxide, without production of hydrogen. The species of *Carboxydothermus* were isolated from

Table 7.1 Hydrogenogenic CO-oxidizing prokaryotes

Organism	CO-trophy	Autotrophy/ organotrophy	Acceptors	Acceptors during growth on CO	Optimal temperature (°C)/optimal pH of growth	Reference
Domain *Bacteria*						
Phylum *Firmicutes*, Class *Clostridia*, Order *Thermoanaerobacterales*, Family *Thermoanaerobacteraceae*						
Carboxydothermus hydrogenoformans	Facultative	+/+	AQDS, S^0, SO_3^{2-}, $S_2O_3^{2-}$, fumarate, nitrate	AQDS, fumarate	70–72/7.0	Svetlichny et al. (1991b), Henstra and Stams (2004)
Carboxydothermus siderophilus	Facultative	–/–	Fe(III), AQDS	Fe(III), AQDS	70/7.0	Slepova et al. (2009)
Caldanaerobacter subterraneus subsp. *pacificus*	Facultative	–/+	$S_2O_3^{2-}$	–	70/6.8–7.1	Sokolova et al. (2001), Fardeau et al. (2004)
Caldanaerobacter strain 2707	Facultative	–/+	$S_2O_3^{2-}$	–	75/7.0	
T. hydrosulfuricus subsp. *carboxydivorans*	Facultative	–/+	AQDS, Fe(III), S^0, SO_3^{2-}, $S_2O_3^{2-}$, MnO_2	ND	70/6.3–6.8	Balk et al. (2009)
Phylum *Firmicutes*, Class *Clostridia*, Order *Clostridiales*, Family *Peptococcaceae*						
Thermincola carboxydiphila	Obligate	–/–	–	–	55/8.0	Sokolova et al. (2005)
Thermincola ferriacetica	Facultative	–/+	Fe(III) oxide, AQDS, MnO_2, $S_2O_3^{2}$	ND	57–60/7.0–7.1	Zavarzina et al. (2007)
Thermincola potens	Facultative	ND/+	Fe(III) oxide, MFC anodes	ND	55/6.8	Byrne-Bailey et al. (2010), Wrighton et al. (2008)
Desulfotomaculum carboxydivorans	Facultative	+/–	SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$	SO_4^{2-a}	55/6.8–7.2	Parshina et al. (2005b)

Phylum *Firmicutes*, Class *Clostridia*, Order *Clostridiales*, Unclassified *Clostridiales*						
Carboxydocella thermautotrophica	Obligate	+/–	–	–	58/7.0	Sokolova et al. (2002)
Carboxydocella sporoproducens	Facultative	+/+	–	–	60/6.8	Slepova et al. (2006)
Carboxydocella ferrireducens	Facultative	–/+	Fe(III), AQDS	Fe(III)	60/6.8	
Phylum *Firmicutes*, Class *Negativicutes*, Order *Selenomonadales*, Family *Veillonellaceae*						
Thermosinus carboxydivorans	Facultative	–/+	Fe(III), SeO_3^{2-}, $S_2O_3^{2-}$	Fe(III), SeO_3^{2}	60/6.8–7.0	Sokolova et al. (2004a)
Phylum *Firmicutes*, Class *Thermolithobacteria*, Order *Thermolithobacterales*, Family *Thermolithobacteraceae*						
Thermolithobacter carboxydivorans	Facultative	+/+	–	–	70/6.8–7.0	Sokolova et al. (2007)
Phylum *Dictyoglomi*, Class *Dictyoglomia*, Order *Dictyoglomales*, Family *Dictyoglomaceae* *Dictyoglomales*; *Dictyoglomaceae* Family *Dictyoglomaceae*						
Dictyoglomus carboxydivorans	ND	–/ND	ND	ND	75/ND	Slepova et al. (2007)
Domain *Archaea*						
Phylum *Euryarchaeota*, Class *Thermococci* or *Protoarchaea*, Order *Thermococcales*, Family *Thermococcaceae*						
Thermococcus AM4	Facultative	–/–	S^0	S^0	85/7.0	Sokolova et al. (2004b)
Thermococcus onnurineus	Facultative	–/+	S^0	ND	80/8.5	Lee et al. (2008)
Thermococcus barophilus	Facultative	–/+	S^0	S^0	85/6.0	
Phylum *Crenarchaeota*, Class *Thermoprotei* or *Crenarchaeota*, Order *Thermoproteales*, Family *Thermofilaceae*						
Thermofilum carboxydotrophus	Facultative	–/ND	ND	ND	92/ND	Kochetkova et al. (2011)

The hierarchical classification is according to the J. P. Euzéby's site http://www.bacterio.cict.fr/classification.html

geographically distant hot springs characterized by pH values close to neutral and temperatures in the range of 60–80°C. *C. hydrogenoformans, C. siderophilus,* and *C. islandicus* were isolated from hot springs of Kunashir Island (Kurils), Geyser Valley (Kamchatka), and Iceland, respectively. *C. ferrireducens* was isolated from a hot spring in Yellowstone National Park, USA (Slobodkin et al. 1997). All *Carboxydothermus* species grow well on high CO concentrations (100% v/v in the gas phase). The type species is *Carboxydothermus hydrogenoformans* (Svetlichny et al. 1991a). Currently, *C. hydrogenoformans* is the best-studied thermophilic hydrogenogen. *C. hydrogenoformans* enzymes involved in hydrogenogenic CO oxidation were thoroughly studied (Svetlitchnyi et al. 2001, 2004a, b; Soboh et al. 2002; Dobbek et al. 2001, 2004). The complete genome of *C. hydrogenoformans* was sequenced (Wu et al. 2005). *C. islandicus,* as well as *C. hydrogenoformans,* grows on CO chemolithoautotrophically. Two other species require yeast extract for growth (0.2 g l^{-1}). *C. hydrogenoformans* performs fermentative growth on pyruvate accompanied by hydrogen, acetate and CO_2 formation, or nonhydrogenogenic growth with various electron donors and electron acceptors: it reduces AQDS (9,10-anthraquinone-2,6-disulfonate) with CO, H_2, glycerol, lactate, or formate or it reduces sulfite, thiosulfate, sulfur, nitrate, and fumarate with lactate (Henstra and Stams 2004). *C. siderophilus*, in contrast to other *Carboxydothermus* species, can grow only in the presence of hydromorphic ferric iron oxide or AQDS in the medium during the chemolithotrophic growth on CO or chemoheterotrophic growth on other substrates, and it does not use other electron acceptors like sulfite, thiosulfate, sulfur, nitrate, and fumarate (Slepova et al. 2009). *C. islandicus* does not reduce any of acceptors tested, i.e., hydromorphic ferric iron oxide, AQDS, sulfate, sulfite, thiosulfate, sulfur, nitrate, or fumarate. In addition to chemolithotrophic growth on CO, it performs fermentative growth on pyruvate (Novikov et al. 2011).

7.3.2 Genus Thermincola (Sokolova et al. 2005)

Genus *Thermincola* (Table 7.1) is currently classified as a member of family *Peptococcaceae*, although rRNA analysis suggests only a weak relationship to other *Peptococcaceae* members (Ludwig et al. 2009). The genus contains two validly described species – *Thermincola carboxydiphila* (Sokolova et al. 2005) and *T. ferriacetica* (Zavarzina et al. 2007) – and one not validated species *Thermincola potens,* the complete genome of which has been sequenced (Byrne-Bailey et al. 2010). Cells of all species are straight rods with cell wall of the Gram-positive-type structure. *T. ferriacetica* cells are sporeforming. Representatives of *Thermincola* species are strict anaerobes and moderate thermophiles. *T. carboxydiphila* was isolated from the sample of cyanobacterial mat, mud, and hot water from slightly alkaline hot spring at Baikal Lake rift zone. *T. ferriacetica* was isolated from ochre deposits in a hot spring at Kunashir Island. *T. carboxydiphila* is alkalitolerant, the pH optimum for growth is 8.0, and *T. ferriacetica* is neutrophilic.

Both species grow on 100% CO hydrogenogenically; during chemolithotrophic growth on CO, both species require additional sources of cell carbon in the form of acetate or yeast extract. They do not grow on fermentable substrates. *T. carboxydiphila* is an obligate carboxydotroph. In addition to growth on CO, *T. ferriacetica* can grow at the expense of ferric iron reduction with hydrogen or acetate or some other organic substrates.

Thermincola potens strain JR (Byrne-Bailey et al. 2010) has been isolated from a biofilm that originated from an anoxic marine marsh sediment and developing at the anode of a thermophilic microbial fuel cell. It was the dominant member of the current-producing bacterial community (Wrighton et al. 2008; Mathis et al. 2008). The isolate shares 99% 16S rRNA gene sequence identity with *T. carboxydiphila* and *T. ferriacetica*. This strain couples acetate oxidation to the reduction of insoluble electron acceptors: microbial fuel cell (MFC) anodes and hydrous ferric oxide (Wrighton et al. 2008). It is also capable of growth with CO as the sole electron donor and carbon source (Byrne-Bailey et al. 2010).

7.3.3 Genus *Carboxydocella* (Sokolova et al. 2002)

Genus *Carboxydocella* is classified as a member of Family XVI *Incertae sedis* within *Thermoanaerobacterales* (Ludwig et al. 2009). The genus contains four species: the three thermophilic hydrogenogenic carboxydotrophic species, *Carboxydocella thermautotrophica* (Sokolova et al. 2002), *C. sporoproducens* (Slepova et al. 2006), and *C. ferrireducens* (our unpublished data), and one non-carboxydotrophic species, *C. manganica* (Slobodkina et al. 2012). Cells of *Carboxydocella* species are rods of various lengths with cell wall of Gram-positive-type structure. *C. thermautotrophica* and *C. ferrireducens* cells are motile due to peritrichous flagella. *C. sporoproducens* cells are nonmotile and spore-forming. Representatives of the genus *Carboxydocella* are strict anaerobes, moderate thermophiles, and neutrophiles. All *Carboxydocella* species were isolated from hot springs at Kamchatka Peninsula: *C. thermautotrophica,* from hot spring at Geyser Valley; *C. sporoproducens,* from the bottom hot spring in Karymsky Lake; and *C. ferrireducens,* from a hot ground layer at a thermal field of Uzon Caldera. All *Carboxydocella* species except *C. manganica* grow on 100% CO hydrogenogenically. *C. manganica* does not grow not only at 100% CO in the gas phase but also at lower concentrations (Slobodkina et al. 2012). *C. thermautotrophica* and *C. sporoproducens* grow on CO chemolithoautotrophically; *C. ferrireducens* requires an additional source of cell carbon in the form of acetate or lactate or pyruvate or yeast extract. *C. ferrireducens* can reduce Fe(III) during the growth on CO in the presence of hydromorphic ferric iron oxide, although hydrogen remains the main reduced metabolic product. *C. thermautotrophica* cannot reduce Fe(III) or Mn(IV) with CO, lactate, or molecular hydrogen. In contrast, *C. sporoproducens* can grow and reduce Fe(III) and Mn(IV) with lactate as electron donor at least in three culture transfers. Among *Carboxydocella* species,

C. thermautotrophica is the only obligate CO-troph. Two other hydrogenogenic species can grow organotrophically on several substrates.

7.3.4 Genus Thermosinus

The genus belongs to the family Veillonellaceae within the phylum Firmicutes, class *Negativicutes*, and order Selenomonadales (Ludwig et al. 2009). The genus is currently represented by a single species *T. carboxydivorans* (Sokolova et al. 2004b). Cells are curved rods motile by means of lateral flagella. Cell wall structure is of the Gram-negative type. *T. carboxydivorans* grows on CO both in the presence and absence of sodium sulfide in the medium (E_h − 250 mV or + 50 mV, respectively). It grows chemolithotrophically on CO forming equimolar amounts of H_2 and CO_2. *T. carboxydivorans* grows organotrophically on some carbohydrates or on pyruvate. During growth on CO, sucrose, or lactose, it reduces ferric iron. Selenite is reduced to elemental selenium during *T. carboxydivorans* growth on CO. Neither ferric iron nor selenite causes a significant shift in the ratio of H_2 and CO_2 produced. During the growth on CO, elemental sulfur, thiosulfate, sulfate, and nitrate do not stimulate growth and are not reduced. However, thiosulfate enhances growth rate and cell yield during growth on glucose, sucrose, or lactose; in this case, the fermentation products are acetate, H_2S, and CO_2. During glucose fermentation, acetate, H_2, and CO_2 are produced. Lactate, acetate, formate, and H_2 are not utilized, either in the absence or presence of ferric iron, thiosulfate, sulfate, sulfite, elemental sulfur, or nitrate. Growth is completely inhibited by penicillin, ampicillin, streptomycin, kanamycin, and neomycin. Draft genome sequence is available from NCBI (NZ_AAWL00000000).

7.3.5 Genus Caldanaerobacter

The genus belongs to the family *Thermoanaerobacteraceae* within the order *Thermoanaerobacterales*. It contains a few CO-trophic hydrogenogenic strains. *C. subterraneus* subsp. *pacificus* JM (Fardeau et al. 2004), formerly *Carboxydobrachium pacificum* (Sokolova et al. 2001), was the first hydrogenogenic CO-trophic thermophile isolated from deep-sea hot environment (Okinawa Trough). The species *Caldanaerobacter subterraneus* also contains another hydrogenogenic CO-trophic strain 2707 isolated from freshwater hot spring at Kunashir Island. Cells of both strains are very thin long rods. Cells of *C. subterraneus* subsp. *pacificus* JM sometimes are branching, and strain 2707 cells are sporeforming. Both strains are strict anaerobes, extreme thermophiles, and neutrophiles. In addition to lithotrophic hydrogenogenic growth on CO, they grow organotrophically on some fermentable substrates. Both strains reduce thiosulfate during organotrophic growth. Strain 2707 reduces hydromorphic ferric oxide during the growth on peptone. Draft genome sequence is available from NCBI for JM (NZ_ABXP00000000).

7.3.6 Genus Thermoanaerobacter

Representatives of this genus are widely distributed in various thermal environments. *Thermoanaerobacter* species are strictly anaerobic, thermophilic, rod-shaped bacteria, growing between 55 and 75°C, and most of them form round to oval terminal spores. Although the end products are mainly acetate, lactate, ethanol, H_2, and CO_2, the most abundant end product depends on the species and the growth conditions. Generally, thiosulfate can be used as electron acceptor in anaerobic respiration. There are 13 validly described species belonging to the genus (Onyenwoke and Wiegel 2009). The only hydrogenogenic carboxydotrophic representative of the genus, *T. hydrosulfuricus* subsp. *carboxydivorans,* has been isolated from a geothermal spring in Ayas, Turkey (Balk et al. 2009). The isolate cells are straight to curved rods which stain Gram-positively. They are sporeforming with terminal, round, heat-resistant endospores. Cells are 0.3–0.4 μm in diameter and 3.5–10 μm in length, occurred singly, in pairs, or in long chains. *T. hydrosulfuricus* subsp. c*arboxydivorans* grows both in the presence and absence of thiosulfate on a number of fermentable substrates including peptone, various sugars, xylan, starch, pectin, inulin, and cellobiose. End products of sugar fermentation and thiosulfate reduction are dependent on growth conditions, particularly on pH or thiosulfate presence. The main products of sugar fermentation are lactate, acetate, ethanol, alanine, H_2, and CO_2. In addition to thiosulfate, elemental sulfur, sodium sulfite, ferric iron, MnO_2, anthraquinone-2,6-disulfonate (AQDS), and arsenate can serve as electron acceptors. Sulfate, nitrate, and selenite cannot be utilized. *T. hydrosulfuricus* subsp. c*arboxydivorans* grows in an atmosphere containing up to 25% of CO. CO oxidation is coupled to equimolar H_2 and CO_2 formation. Cell-free extracts exhibit CO dehydrogenase activity (Balk et al. 2009).

7.3.7 Thermolithobacter carboxydivorans

Hydrogenogenic carboxydotrophic species *Thermolithobacter carboxydivorans* is a representative of the recently established class *Thermolithobacteria*, affiliated to Firmicutes (Sokolova et al. 2007). The single hydrogenogenic carboxydotrophic strain of this species was isolated from a freshwater hot spring at Raoul Island, Kermadec Archipelago. The initial description assigned this strain to *Carboxydothermus restrictus* on the basis of phenotypic similarity to *Carboxydothermus hydrogenoformans* (Svetlichnyi et al. 1994). Later, 99% similarity was revealed of the 16S rRNA gene sequences of this isolate and three strains of ferric iron-reducing chemolithoautotrophic bacteria *Ferribacter thermautotrophicus* isolated by J. Wiegel from hot springs of Yellowstone National Park and Fiji Island. DNA–DNA hybridization level between the carboxydotrophic and ferric iron-reducing strains was 35%. Surprisingly, strains of *Carboxydothermus restrictus* and *Ferribacter thermautotrophicus* are hardly similar physiologically. Strain R1 grows on CO hydrogenogenically, and it does not reduce Fe(III), does not grow on hydrogen at the excess of AQDS and does not reduce it, and does not reduce AQDS during the growth on CO. Strain R1 does not grow on CO in

the presence of NO_3^-, Fe(III) oxide/hydroxide, Fe(III) citrate, or SO_3^{2-}. Strain R1 grows on CO in the presence of SO_4^{2-}, $S_2O_3^{2-}$, or fumarate, but the presence of electron acceptors does not stimulate growth. R1 does not reduce SO_4^{2-}, $S_2O_3^{2-}$, or elemental sulfur in media supplemented with yeast extract, formate, acetate, pyruvate, citrate, succinate, lactate, or H_2:CO_2. Strain R1 also does not grow on the H_2:CO_2 mixture with NO_3^-, SO_3^{2-}, or fumarate. Physiological characteristics and the 16S rDNA sequence analysis indicated that *Carboxydothermus restrictus* and *Ferribacter thermautotrophicus* strains represent two novel species of the novel genus *Thermolithobacter* within the *Firmicutes* branch. Levels of 16S rRNA gene sequence similarity between the lineage containing *Thermolithobacter* and members of the other classes of the *Firmicutes* were less than 85%, and *Thermolithobacter* representatives appeared to form their own lineage. It was therefore proposed that the *Thermolithobacter* represent a new class within phylum BXIII *Firmicutes* (Gibbons and Murray 1978; Garrity et al. 2002), Thermolithobacteria. Class Thermolithobacteria was described to contain the order Thermolithobacterales ord. nov., the family Thermolithobacteraceae fam. nov., and the genus *Thermolithobacter* gen. nov.

7.3.8 Genus Dictyoglomus

The hydrogenogenic representative of phylum "Dictyoglomi," class Dictyoglomia, order *Dictyoglomales*, and family *Dictyoglomaceae, Dictyoglomus* sp. 1512 (Kochetkova et al. 2011), is currently the single thermophilic hydrogenogenic bacterium not affiliated to "Firmicutes." It was isolated from a hot spring at Uzon Caldera, Kamchatka, in which active process of CO transformation was demonstrated using radioisotopic tracing. The isolate is neutrophilic extremely thermophilic anaerobic bacterium, which grows on CO if its concentration in the gas phase does not exceed 15%. The isolate oxidizes CO to CO_2, producing equimolar quantities of H_2 from water. The optimum CO concentration in the gas phase is 5%. The isolate grows on CO significantly slower than other thermophilic hydrogenogens. The generation time of growth on 5% of CO is about 60 h. It grows on CO chemolithoheterotrophically but requires 0.2 g l^{-1} of yeast extract for growth. Cells of the isolate are long thin filaments. Analysis of 16S rDNA sequence revealed that the novel isolate belongs to Dictyoglomi. The highest similarity of 16S rDNA sequence (98.6%) was found with 16S rRNA of *Dictyoglomus thermophilum* (Saiki et al. 1985).

7.3.9 Hydrogenogenic Carboxydotrophic Representatives of Thermococcus

Thermococcus sp. AM4 was isolated from 1 of 13 enrichments of coccoid cells growing on CO with H_2 and CO_2 production at 80°C obtained from 24 samples of hydrothermal venting structures collected at East Pacific Rise 13 °N (Sokolova et al. 2004b). It was described as the first hydrogenogenic carboxydotrophic representative

of Archaea. Cells of strain AM4 are cocci 1–1.5 μm in diameter. Cells are motile by means of tuft of flagella. The isolate grows at 60–90°C and has a broad optimum temperature interval of 70–80°C. The pH range for growth is 5.5–8.5, with an optimum pH 6.5–7.0. Strain AM4 grows on 100% of CO in the gas phase producing equimolar quantities of H_2 and CO_2, similarly to all other hydrogenogenic carboxydotrophs. In the absence of CO, strain AM4 grows on some peptide substrates with elemental sulfur as electron acceptor under a nitrogen atmosphere. In the presence of elemental sulfur, *Thermococcus* sp. AM4 grows on CO with the production of hydrogen sulfide but not the hydrogen (our unpublished data). 16S rRNA gene analysis revealed high similarity of strain AM4 with *T. gammatolerans* (99.5%), T. kodakarensis (99.2%), *T. peptonophilus* (99.2%), *T. guaymasensis* (99.2%), and *T. fumicolans* (99.1%).

In silico hybridization of the genomes of strain AM4 and *T. gammatolerans* EJ3 yielded an ANI value (average nucleotide identity of shared protein-coding genes) of 87% (about 80% of genes shared). This ANI value is lower than the 95–96% value shown to correspond to the 70% DNA–DNA hybridization level accepted to delimit microbial species (Goris et al. 2007; Richter and Rosselló-Móra 2009; Tindall et al. 2010). Thus, AM4 phylogenetically represents a close to *T. gammatolerans* but distinct species.

Another hydrogenogenic species of the genus, *Thermococcus onnurineus* NA1 (Lee et al. 2008), was isolated from PACMANUS thermal field at the East Manus Basin in heterotrophic conditions on peptone with elemental sulfur. The analysis of whole genome sequence revealed the presence of a gene cluster containing genes for carbon monoxide dehydrogenase and energy-converting hydrogenase (Lee et al. 2008). Indeed, it was demonstrated that the strain NA1 can grow hydrogenogenically in the atmosphere of 100% CO (Lee et al. 2008).

From the deep-sea hydrothermal vents of Mid-Atlantic Ridge and Lau Spreading Center, we isolated six strains of CO-trophic hydrogenogenic hyperthermophilic Archaea. 16S rRNA gene sequence analysis revealed that new isolates are close to *Thermococcus barophilus* MP^T (nearly 100% identity). We also demonstrated hydrogenogenic growth on CO for the type strain *Thermococcus barophilus* MP^T.

The capacity for hydrogenogenic carboxydotrophy was tested for some other representatives of *Thermococcales*: *Pyrococcus furiosus, Thermococcus peptonophilus, T. profundus, T. chitonophagus, T. stetteri, T. gorgonarius, T. litoralis* and *T. pacificus.* The tested strains did not oxidize CO (100% in the gas phase) (Sokolova et al. 2004a).

Thus, it may be inferred that hydrogenogenic carboxydotrophic hyperthermophiles, particularly phylogenetically diverse thermococci capable of this process, may be abundant members of the microbial communities inhabiting deep-sea hot vents.

7.3.10 Hydrogenogenic Representative of Thermofilum

Strain 1505 was isolated from a hot spring at the slope of Mutnovsky volcano (Kamchatka Peninsula) (Kochetkova et al. 2011). Cells of the isolate are very thin long rods 15–50 μm in length. The isolate grows chemolithotrophically on

45% CO in the gas phase at 92°C producing equimolar quantities of H_2 and CO_2. Yeast extract (0.2 g l^{-1}) is required for growth. Strain 1505 can also grow fermentatively on yeast extract. 16S rRNA gene sequence analysis revealed that the closest relative of strain 1505 is *Thermofilum pendens* Hrk-5T (Zillig et al. 1983) with sequence similarity 97.8%. Based on 16S rRNA gene sequence analysis and physiological features, it was suggested to assign strain 1505 to a new species of the genus *Thermofilum, T. carboxydotrophus,* sp. nov. 16S rRNA gene sequence analysis revealed 100% sequence identity of strain 1505 to environmental clones obtained from samples taken from different hot springs of Yellowstone National Park (USA) (Barns et al. 1994; Reysenbach et al. 2000). Thus, it is highly possible that representatives of this species are widespread in terrestrial hot springs.

7.4 CO-Oxidizing Dissimilatory Fe(III)-Reducing Thermophiles

Few strains are known to be capable of Fe(III) reduction during the growth on CO. Among them are the hydrogenogenic carboxydotrophs *Carboxydothermus siderophilus, Carboxydothermus islandicus, Thermincola ferriacetica, Thermosinus carboxydivorans, and Carboxydocella ferrireducens.* These species grow on CO reducing ferric iron but still producing hydrogen as a main product. The only true dissimilatory Fe(III)-reducing CO-oxidizing thermophile is *Carboxydothermus ferrireducens* (Slobodkin et al. 2006), formerly *Thermoterrabacterium ferrireducens,* which was isolated from a hot spring in Yellowstone National Park (Slobodkin et al. 1997). This organism can grow by utilizing organic substrates or H_2 as electron donors, and, apart from Fe(III) or AQDS, it can also reduce sulfite, thiosulfate, elemental sulfur, nitrate, and fumarate (Slobodkin et al. 1997; Henstra and Stams 2004) during organotrophic growth. *Carboxydothermus ferrireducens* grows on CO with ferrihydrite as electron acceptor, forming magnetite precipitate and not producing hydrogen or acetate (Slobodkin et al. 2006), or with AQDS or fumarate as electron acceptors (Henstra and Stams 2004). Unlike *C. hydrogenoformans*, this bacterium cannot grow on CO without electron acceptors.

7.5 CO-Oxidizing Hydrogen Sulfide Producing Bacteria and Archaea

7.5.1 CO-Utilizing Sulfate-Reducing Bacteria and Archaea

Few thermophilic sulfate-reducing bacteria, *Desulfotomaculum nigrificans*, *D. kuznetsovii* and *D. thermobenzoicum* subsp. *thermosyntrophicum,* and *Desulfotomaculum carboxydivorans,* were found to be capable of anaerobic CO oxidation (Parshina et al.

2005a, b). These bacteria have been isolated from bioreactors. The capacity for anaerobic CO oxidation was not demonstrated for sulfate-reducing bacterial strains isolated from hot volcanic environments.

It was shown that the anaerobic extremely thermophilic euryarchaeote *Archaeoglobus fulgidus* VC-16 is capable of autotrophic growth with CO. *A. fulgidus* VC-16 was isolated from submarine solfataric fields near Vulcano Island, Italy (Stetter et al. 1987). *Archaeoglobus* species occur in both shallow and abyssal marine hydrothermal systems (Burggraf et al. 1990). *A. fulgidus* strains were also isolated from hot oil field waters (Beeder et al. 1994; Stetter 1988, 1993). *Archaeoglobus fulgidus* VC-16 can oxidize CO both in the presence and absence of sulfate. In the first case, the oxidation of CO to CO_2 is coupled to sulfate reduction; acetate and formate are formed as minor products. In the absence of sulfate, the only products of CO metabolism are acetate, formed via the reductive acetyl-CoA pathway with formyl-methanofuran as intermediate, and formate (Henstra et al. 2007). *A. fulgidus* can also completely oxidize various organic compounds in the presence of sulfate or grow chemolithoautotrophically on H_2 with thiosulfate but not with sulfate as electron acceptor (Zellner et al. 1989).

7.5.2 *Thermococci Growing at the Expense of Sulfidogenic Oxidation of CO*

Recently, we have demonstrated the ability of some *Thermococcus* species to grow at the expense of CO oxidation with elemental sulfur. In this case, hydrogen production is very low or negligible. This ability was shown for *T. barophilus* strains MP^T and Ch5 and for the type strains of *T. chitonophagus*, *T. gammatolerans,* and *T. profundus.*

7.6 CO-Oxidizing Thermophilic Methanogenic Archaea

The only thermophilic methanogen demonstrated to be able to grow on CO is *Methanothermobacter thermautotrophicus* ΔH (Zeikus and Wolfe 1972). The strain was isolated from sewage sludge. *M. thermautotrophicus* has been found in various hot environments, including hot springs in Yellowstone National Park and Iceland (Sandbeck and Ward 1982) and biogas reactors. It converts CO according to the equation $4CO + 2H_2O = CH_4 + 3CO_2$. The growth rate on CO was only 1% of that on CO_2/H_2 (Daniels et al. 1977). The ability to grow on CO was more studied for mesophilic methanogens. Among them, six representatives of the genera *Methanosarcina, Methanobacterium,* and *Methanobrevibacter* were reported to grow on CO (Daniels et al. 1977; O'Brien et al. 1984; Rother and Metcalf 2004). It may be expected that the capacity for growth on CO can also be found in other thermophilic methanogens.

7.7 CO-Oxidizing Thermophilic Homoacetogenic Bacteria

Many homoacetogens can grow on CO. CO conversion has been documented for ten homoacetogens, four moderate thermophiles among them: *Moorella thermoacetica, Moorella thermoautotrophica, Thermoanaerobacter kivui,* and *Moorella perchloratireducens,* recently described by Balk et al. (2008). *M. thermoautotrophica* and *M. thermoacetica* can grow on CO at high partial pressures as sole energy source (Savage et al. 1987; Diekert and Thauer 1978; Daniel et al. 1990). *M. thermoacetica* and *T. kivui* can oxidize CO during growth on other substrates (Diekert and Thauer 1978; Yang and Drake 1990). Most *M. thermoacetica* strains (11 of 13 tested) possess this ability (Daniel et al. 1990). Homoacetogens grow on CO according to the equation (Drake and Daniel 2004)

$$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2 \left(\Delta G = -41.4 \text{ kJ / mol CO}\right).$$

M. thermoautotrophica, originally isolated from a hot spring (Wiegel et al. 1981), was found in temporarily heated soils (see Drake and Daniel 2004) and cyanobacterial mats in hot springs (Bateson et al. 1989). As shown by blastn at the NCBI site (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi), 16S rRNA genes exhibiting 99% similarity with *Moorella thermoacetica* have been obtained from bioreactors, hot springs, and oil reservoirs. The diverse metabolic capacities of this organism make it highly competitive. It grows both autotrophically and heterotrophically, fermentatively, or by respiration using various electron donors and acceptors (Drake and Daniel 2004). In the presence of nitrate, *M. thermoacetica* and *M. thermoautotrophica* can grow on CO if O-methyl groups (of vanillate or syringate) are provided. In these organisms, CO_2 reduction both to THF-CH_3 and to CO is blocked by nitrate (Drake and Daniel 2004).

7.8 Occurrence of Genetic Determinants of Anaerobic Carboxydotrophy in the Genomes of Thermophiles

The diversity of anaerobic carboxydotrophs can be estimated not only from isolation and cultivation data but also from data of genomics.

7.8.1 Distribution of Genetic Determinants of Carboxydotrophy

Utilization of CO by anaerobes is catalyzed by Ni-containing CO dehydrogenases (Ni-CODHs) and acetyl-CoA synthases (ACSs) (Ragsdale 2004). Ni-CODHs and Ni-CODH–ACS complexes are widespread among anaerobes and are found in methanogens, acetogens, sulfate reducers, and iron reducers. Ni-CODH–ACS

complexes catalyze catabolic and anabolic acetyl-CoA synthesis and catabolic acetyl-CoA cleavage. In these reactions, CO is an intermediate that travels along a hydrophobic channel between the Ni-CODH and ACS active sites (Ragsdale 2004). The capacity to use exogenous CO by Ni-CODH–ACS complexes is a debatable topic. Many relevant papers infer direct and preferential incorporation of CO into the acetate carboxyl group (Stupperich and Fuchs 1984; Martin et al. 1985; Henstra et al. 2007), but preferential incorporation of CO_2-derived CO has also been reported (Ragsdale 2004), and most probably this is the case at ecologically relevant CO concentrations.

It may be expected that the main enzymes responsible for the utilization of exogenous CO by anaerobes are the Ni-CODHs that are not part of the Ni-CODH–ACS complex. Our analysis at the "BLAST with microbial genomes" site (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi) of 1590 microbial species with available genomes (about 150 thermophiles) revealed 131 species (Table 7.2), including 35 thermophiles (Table 7.3) that contained Ni-CODH genes (Techtmann et al. 2012). Note that these values are considerably higher than King and Weber (2007) estimated the frequency of occurrence of the aerobic CO-oxidizing capacity (8 of 330 genomes). Of the 131 species whose genomes contained one or more Ni-CODH genes, 20, including six thermophiles (Table 7.2), encoded only Ni-CODHs as part of Ni-CODH–ACS gene clusters. It may be speculated that these organisms are mainly capable of dealing with endogenous CO. The remaining genomes contained Ni-CODH genes additional to those in Ni-CODH–ACS gene clusters or lacked ACS genes (Tables 7.2 and 7.3). It may be speculated that these organisms are adapted to deal with exogenous CO. Several organisms, including the thermophile *Thermodesulfovibrio yellowstonii*, harbor only ACS genes but not Ni-CODH genes, which is a somewhat puzzling occurrence pattern. It has been shown by Svetlitchnyi et al. (2004a, b) for the autotroph *Carboxydothermus hydrogenoformans* that, at high CO concentrations, acetate synthesis involves ACS but not Ni-CODH. However, *Thermodesulfovibrio yellowstonii* has been shown by Parshina et al. (2005) to be inhibited by CO concentrations as low as 2% during growth on pyruvate.

Ni-CODH genes occurred in the genomes of *Archaea* and *Bacteria* belonging to ten phyla. As far as thermophiles are concerned, Ni-CODH genes occurred in the genomes of *Archaea* and *Bacteria* from five phyla (Table 7.3).

Among the species whose genomes encode Ni-CODHs, there are obligate and facultative anaerobes and an obligate aerobe (*Azotobacter vinelandii*). Among thermophiles, facultatively anaerobic are *Geobacillus thermoglucosidasius, Geobacillus* sp. Y4.1MC1, and *Persephonella marina;* others are obligate anaerobes.

Whereas the presence of a Ni-CODH–ACS complex appears to be a genus-level character (not to mention autotrophic methanogens, among which this trait is ubiquitous), the presence of additional or sole Ni-CODH(s) is most often a species-level character. However, in a few genera (e.g., *Carboxydothermus, Thermincola*), all known representatives are so far carboxydotrophic (judging from cultivation and/or genomic data). On the other hand, in a few species, the

Table 7.2 Numbers of microbial species whose genomes exhibit a particular occurrence pattern of Ni-CODH and ACS genes

Total		With Ni-CODH and/or ACS genes	With only ACS gene(s)	With more ACS genes	With Ni-CODH and ACS gene(s) in equal proportion	With more Ni-CODH genes	With only Ni-CODH genes(s)
Entire NCBI database for blast with prokaryotic genomes							
Species	1,590	138	7	3	20	33	75
Thermophiles and hyperthermophiles							
Species	ca. 150	36	1	2	8	8	15

Note: The table is based on our analysis performed in January 2011 at the NCBI "BLAST with microbial genomes" site (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi)

Table 7.3 Numbers of genes encoding Ni-CODHs and ACSs and numbers of Ni-CODH–ACS, ECH, and Ni-CODH–ECH gene clusters in the genomes of thermophiles that contain at least one Ni-CODH or ACS gene

Species	Ni-CODH genes	ACS genes	Ni-CODH–ACS gene clusters	ECH gene clusters	Ni-CODH–ECH gene clusters		Growth on CO	Production of H_2 from CO
					"Classic" *coo* type	Other		
Domain *Bacteria*								
Phylum *Aquificae* Class *Aquificae* Order *Aquificales* Family *Hydrogenothermaceae*								
Persephonella marina	1	0	0	0	0	0	ND	ND
Phylum *Nitrospirae* Class "*Nitrospira*" Order "*Nitrospirales*" Family "*Nitrospiraceae*"								
Thermodesulfovibrio yellowstonii	0	1	0	1	0	0	–	–
Phylum *Firmicutes* Class *Bacilli* Order *Bacillales* Family *Bacillaceae*								
Geobacillus thermoglucosidasius	1	0	0	1	0	1	+[a]	+[a]
Geobacillus sp. Y4.1MC1	1	0	0	1	0	1	ND	ND
Phylum *Firmicutes* Class *Clostridia* Order *Clostridiales* Family *Clostridiaceae*								
Clostridium thermocellum	1	0	0	1	0	0	ND	ND
Phylum *Firmicutes* Class *Clostridia* Order *Clostridiales* Family *Peptococcaceae*								
Thermincola potens	4	1	1	1	1	0	+	ND
Phylum *Firmicutes* Class *Clostridia* Order *Natranaerobiales* Family *Natranaerobiaceae*								
Natranaerobius thermophilus	1	0	0	0	0	0	ND	ND
Phylum *Firmicutes* Class *Clostridia* Order *Thermoanaerobacterales* Family *Thermoanaerobacteraceae*								
Ammonifex degensii	1	1	1	1	0	0	ND	ND

(continued)

Table 7.3 (continued)

Species	Ni-CODH genes	ACS genes	Ni-CODH–ACS gene clusters	ECH gene clusters	Ni-CODH–ECH gene clusters		Growth on CO	Production of H_2 from CO
					"Classic" *coo* type	Other		
Caldanaerobacter subterraneus subsp. *pacificus*	1	0	0	2	0	1	+	+
Caldanaerobacter subterraneus ssp. *tengcongensis*	1	0	0	2	0	1	ND	ND
Carboxydothermus hydrogenoformans	5	1	1	1	1	0	+	+
Moorella thermoacetica	2	1	1	1	0	0	+	–
Phylum *Firmicutes* Class *Clostridia* Order *Thermoanaerobacterales* Unclassified *Thermoanaerobacterales*								
Caldicellulosiruptor hydrothermalis	1	0	0	1	0	0	ND	ND
Caldicellulosiruptor kristjanssonii	1	0	0	1	0	0	ND	ND
Caldicellulosiruptor lactoaceticus	1	0	0	1	0	0	ND	ND
Caldicellulosiruptor saccharolyticus	1	0	0	1	0	0	ND	ND
Thermosediminibacter oceani	3	1	1	0	0	0	ND	ND
Phylum *Firmicutes* Class *Negativicutes* Order *Selenomonadales* Family *Veillonellaceae*								
Thermosinus carboxydivorans	3	0	0	1	1	0	+	+

Domain *Archaea*								
Phylum *Crenarchaeota* Class *Thermoprotei* Order *Thermoproteales* Family *Thermofilaceae*								
Thermofilum carboxydotrophus[b]	1	0	0	2	0	1	+	+
Phylum *Euryarchaeota* Class *Archaeoglobi* Order *Archaeoglobales* Family *Archaeoglobaceae*								
Archaeoglobus fulgidus	3	1	0	0	0	0	+	–
Ferroglobus placidus	3	1	1	0	0	0	ND	ND
Phylum *Euryarchaeota* Class *Methanobacteria* Order *Methanobacteriales* Family *Methanobacteriaceae*								
Methanothermobacter marburgensis	1	1	1	2	0	0	ND	ND
Methanothermobacter thermautotrophicus	1	1	1	2	0	0	+	–
Phylum *Euryarchaeota* Class *Methanobacteria* Order *Methanobacteriales* Family *Methanothermaceae*								
Methanothermus fervidus	1	1	1	1	0	0	ND	ND
Phylum *Euryarchaeota* Class *Methanococci* Order *Methanococcales* Family *Methanocaldococcaceae*								
Methanocaldococcus fervens	1	1	1	2	0	0	ND	ND
Methanocaldococcus infernus	1	2	0	2	0	0	ND	ND
Methanocaldococcus jannaschii	2	2	1	2	0	0	ND	ND
Methanocaldococcus vulcanius	2	2	1	2	0	0	ND	ND
Methanocaldococcus sp. FS406-22	2	2	1	2	0	0	ND	ND
Phylum *Euryarchaeota* Class *Methanococci* Order *Methanococcales* Family *Methanococcaceae*								
Methanothermococcus okinawensis	1	2	1	2	0	0	ND	ND

(continued)

Table 7.3 (continued)

Species	Ni-CODH genes	ACS genes	Ni-CODH–ACS gene clusters	ECH gene clusters	Ni-CODH–ECH gene clusters		Growth on CO	Production of H_2 from CO
					"Classic" *coo* type	Other		
Phylum *Euryarchaeota* Class "*Methanomicrobia*" Order *Methanosarcinales* Family *Methanosaetaceae*								
Methanosaeta thermophila	3	1	1	0	0	0	ND	ND
Phylum *Euryarchaeota* Class *Methanopyri* Order *Methanopyrales* Family *Methanopyraceae*								
Methanopyrus kandleri	2	1	1	1	0	0	ND	ND
Phylum *Euryarchaeota* Class *Thermococci* Order *Thermococcales* Family *Thermococcaceae*								
Thermococcus barophilus	1	0	0	3	0	1	+[a]	+[a]
Thermococcus gammatolerans	1	0	0	3	0	0	+[a]	–[a]
Thermococcus onnurineus	1	0	0	4	0	1	+	+
Thermococcus sp. AM4	2	0	0	3	0	1	+	+
Phylum *Korarchaeota*								
"*Candidatus* Korarchaeum cryptofilum"[c]	2	0	0	0	0	0	ND	ND

Notes: The table is based on our analysis performed in January 2011 at the NCBI "BLAST with microbial genomes" site (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi). The hierarchical classification is according to the J. P. Euzéby's site (http://www.bacterio.cict.fr/classifphyla.html) as of August 28, 2011
ND no data

[a]According to our unpublished data

[b]The genome was sequenced at the Centre "Bioengineering," Russian Academy of Sciences and analyzed using the AutoFACT annotation tool (Koski et al. 2005), followed by manual curation

[c]The genome was analyzed at the NCBI BLAST site (http://blast.ncbi.nlm.nih.gov/Blast.cgi)

presence of Ni-CODH gene(s) is a strain-level character (among thermophiles, currently no examples are known).

In no case did our analysis reveal plasmid localization of Ni-CODH (in case of the molybdenum-containing CODHs of aerobes, plasmid localization has been shown for the genes encoding the enzyme of *Oligotropha carboxidovorans* (Fuhrmann et al. 2003)).

In the metabolism of many microorganisms possessing Ni-CODHs, the so-called energy-converting hydrogenases (ECHs, Table 7.2) play an important role. ECHs form a subclass within the class of [NiFe]-hydrogenases (Hedderich 2004; Vignais and Billoud 2007). ECHs are multisubunit membrane-bound enzyme complexes able to pump ions out of cells at the expense of proton reduction with low-potential electrons, including those accepted by Ni-CODH from CO ($E^{0'}$ is −520 mV for the CO/CO_2 couple and −414 mV for the H_2/H^+ couple). ECHs can also mediate the reverse transfer of electrons from hydrogen to low-potential electron carriers at the expense of the transmembrane ion gradient; these electrons can then be used for the reduction of CO_2 to CO, used further in acetate synthesis (Meuer et al. 2002; Hedderich 2004). The role of Ni-CODH–ECH gene clusters is discussed in the subsection devoted to hydrogenogenic carboxydotrophs.

7.8.2 Genetic Determinants of Carboxydotrophy in Thermophilic Methanogens

Methanothermobacter thermautotrophicus DH, which, as discussed above, is capable of weak growth on CO, contains in its genome a single Ni-CODH gene, and, judging from its genomic environment, this Ni-CODH is part of a Ni-CODH–ACS complex (Table 7.3). *Methanopyrus kandleri* and *Methanosaeta thermophila* contain in their genomes additional Ni-CODH genes beyond Ni-CODH–ACS gene clusters and thus seem to be better able to use exogenous CO than *M. thermautotrophicus* (Table 7.3). However, their ability to grow on CO has not been tested.

7.8.3 Genetic Determinants of Carboxydotrophy in Thermophilic Acetogens

The genome of *Moorella thermoacetica* contains two Ni-CODH genes (Table 7.3). Only one of them is part of a Ni-CODH–ACS gene cluster. Thus, *M. thermoacetica* is among those rather numerous organisms in which CO oxidation is not just a side effect of the capacity for acetate synthesis or cleavage. *Archaeoglobus fulgidus* VC-16, which can oxidize CO both in the presence of sulfate sulfidogenically and in the absence of sulfate acetogenically, contains in its genome three Ni-CODH genes and one ACS gene (Table 7.3).

7.8.4 Genetic Determinants of Thermophilic CO Oxidation with Yet-Unknown Acceptors

Ferroglobus placidus and *Thermosediminibacter oceani,* the ability of which to grow on CO has not been tested, contain in their genomes three Ni-CODH genes and one ACS gene (Table 7.3). Thus, these organisms are likely to grow at the expense of CO oxidation with some (or probably all) of the electron acceptors that they can utilize: for *F. placidus,* these are nitrate, thiosulfate (Hafenbradl et al. 1996), and Fe(III) (Tor et al. 2001) and for *Thermosediminibacter oceani,* thiosulfate, elemental sulfur, and Mn(IV) (Lee et al. 2005).

A single Ni-CODH gene is present in the genomes of the following organisms with untested ability to grow on CO: the facultative anaerobe *Persephonella marina*, able to use as acceptors O_2, nitrate, thiosulfate, and Fe(III) (Götz et al. 2002); the anaerobic alkalithermophile *Natranaerobius thermophilus*, using fumarate, thiosulfate, nitrate, and Fe(III) (Mesbah et al. 2007); and, paradoxically, the fermenters *Clostridium thermocellum* and *Caldicellulosiruptor* spp., for which no electron acceptors have been reported (Rainey et al. 2009; Rainey 2009).

7.8.5 Genetic Determinants of Carboxydotrophy in Thermophilic Hydrogenogens

The key enzymes of the process $CO + H_2O \rightarrow CO_2 + H_2$ are the enzymes already considered above: the Ni-containing CO dehydrogenase (Ni-CODH) (Svetlitchnyi et al. 2001) and the energy-converting hydrogenase (ECH), which form the CO-oxidizing H_2-producing enzymatic complex, studied in the thermophile *Carboxydothermus hydrogenoformans* (Soboh et al. 2002). The gene cluster encoding this complex was also studied in the mesophile *Rhodospirillum rubrum* (Fox et al. 1996; Kerby et al. 1997). The remarkable similarity of the two gene clusters, called *coo* gene clusters, was noted (Soboh et al. 2002). Comparison of the *coo* gene clusters of *C. hydrogenoformans* and *R. rubrum* is presented in Fig. 7.1a. A fact deserving attention is the close disposition in both gene clusters of the Ni-CODH gene *cooS* and the gene *cooH* of the ECH catalytic subunit.

With the aim to search for the *coo* gene cluster in phylogenetically diverse thermophilic hydrogenogenic carboxydotrophs, we designed primers proceeding from the consensus sequence of the Ni-CODH genes of *R. rubrum* (*cooS*) and *C. hydrogenoformans* (*cooS-I*) and from the consensus sequence of the ECH gene *cooH* of these two organisms (Fig. 7.1b). With the use of these primers, the presence of rather closely related genes (72–89% identity of nucleotide sequences with the corresponding genes of *C. hydrogenoformans*) and their localization within a single-gene cluster was demonstrated in *Thermosinus carboxydivorans, Carboxydocella thermautotrophica, Thermincola carboxydiphila*, *Thermolithobacter carboxydivorans,* and *Desulfotomaculum carboxydivorans* (Fig. 7.1c; Lebedinsky et al. 2005; Techtmann et al. 2012).

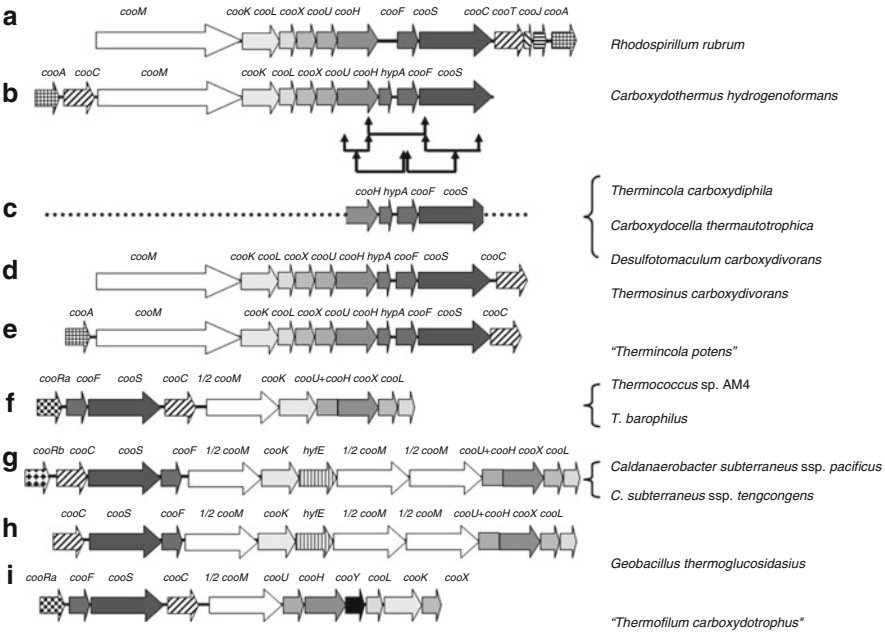

Fig. 7.1 (**a**) The *coo* gene clusters of *Rhodospirillum rubrum* and *Carboxydothermus hydrogenoformans* include the Ni-CODH gene (*cooS*), a ferredoxin-like protein gene (*cooF*), genes of a six-subunit ECH (*cooM–cooH*), and genes of Ni-CODH and hydrogenase maturation proteins. (**b**) Primer systems designed by us proceeding from the consensus sequences of the Ni-CODH genes *cooS* of *R. rubrum* and *cooS1* of *C. hydrogenoformans,* genes of the ECH catalytic subunits (*cooH)* of these organisms, and genes of ferredoxin-like proteins (*cooF*). (**c**) The fragment of the *coo* gene cluster of diverse thermophilic hydrogenogenic carboxydotrophs, amplified with the primers designed. (**d**) The *coo* gene cluster of *Thermosinus carboxydivorans* (based on our analysis of the genome shotgun sequence available from NCBI (NZ_AAWL00000000)). The transcriptional regulator gene *cooA* is located in the *Thermosinus* genome apart from the *coo* gene cluster. (**e**) The *coo* gene cluster of *Thermincola potens* (based on our analysis of the genome (Byrne-Bailey et al. 2010) available from NCBI (NC_014152)). (**f**) The Ni-CODH–ECH gene cluster in carboxydotrophic thermococci. *cooRa* is our name for the gene of a transcriptional regulator absent from the "classical" *coo* cluster, where the regulator gene is *cooA*. The adjoining Na+/H+ antiporter genes located downstream (see Lim et al. 2010) are not shown. (**g**) The Ni-CODH–ECH gene cluster in *C. subterraneus* ssp. *pacificus* and *C. subterraneus* ssp. *tengcongensis. cooRb* is our designation for a transcriptional regulator gene unrelated to *cooRa* or *cooA*. (**h**) The Ni-CODH–ECH gene cluster in *Geobacillus thermoglucosidasius*. (**i**) The Ni-CODH–ECH gene cluster in *Thermofilum carboxydotrophus. cooY* is our designation for a gene absent in other *coo* clusters

Thus, it was demonstrated that the coo enzyme complex, encoded by the *coo* gene cluster, plays a key role in hydrogenogenic carboxydotrophy in phylogenetically diverse bacteria. However, the primers that we designed failed to produce positive PCR result with the hydrogenogenic carboxydotrophs *Thermococcus* sp. AM4 and *Caldanaerobacter subterraneus* subsp. *pacificus.* Analysis of the genomes of these organisms, shotgun sequenced at J. Craig Venter Institute, showed that both these genomes contain a Ni-CODH gene clustered with ECH genes (Lebedinsky et al. 2008; Techtmann et al. 2012), but these gene clusters considerably differ from the

"classical" *coo* gene cluster in terms of the gene primary structure and order (Fig. 7.1f, g). A Ni-CODH–ECH gene cluster very similar to that present in the genome of *Thermococcus* sp. AM4 was found in the genome of *Thermococcus onnurineus* (Lee et al. 2008; Lim et al. 2010; Fig. 7.1f); this thermococcus was tested for the ability to grow on CO and proved to be capable of hydrogenogenic carboxydotrophy (Lee et al. 2008). Our analysis of the genome of the crenarchaeote *Thermofilum carboxydotrophus,* capable of hydrogenogenic carboxydotrophy, showed that it contains a Ni-CODH–ECH gene cluster (Fig. 7.1i) whose dehydrogenase module (the transcriptional regulator gene *cooRa*, the electron transfer protein gene *cooF*, the Ni-CODH gene *cooS*, and the nickel-insertion protein gene *cooC*) is similar to that of the thermococcal gene cluster, while the hydrogenase module (*cooM–cooL* genes) differs from those of other known Ni-CODH–ECH gene clusters in terms of the gene primary structure and order.

Thus, all hydrogenogenic carboxydotrophs studied in this respect contain a gene cluster encoding Ni-CODH–ECH enzymatic complexes. On the other hand, we found that the occurrence of Ni-CODH–ECH gene clusters is most probably restricted to hydrogenogenic carboxydotrophs. Our analysis at the "BLAST with microbial genomes" site (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi) of 1590 microbial species with available genomes (about 150 thermophiles, Table 7.2) revealed five cases of the occurrence of a Ni-CODH–ECH gene cluster in organisms whose ability to grow on CO has not been tested: these are the mesophile *Rhodopseudomonas palustris* BisB18 and the thermophiles *Caldanaerobacter subterraneus* subsp. *tengcongensis, Geobacillus thermoglucosidasius, Geobacillus* sp. Y4.1MC1, and *Thermococcus barophilus* (Fig. 7.1f, g, and h). Most probably, these are actually not exceptions: for *Thermococcus barophilus* and *G. thermoglucosidasius*, our tests already demonstrated the capacity for hydrogenogenic growth on CO (Techtmann et al. 2012). In organisms in which the Ni-CODH–ECH interplay results in the reverse reaction, i.e., in CO_2 reduction by H_2 to CO at the expense of transmembrane ion gradient (this interplay of Ni-CODH and ECH, important for autotrophic CO_2 fixation via Wood–Ljungdahl pathway (Ragsdale 2004) and proven for at least *Methanosarcina barkeri* (Meuer et al. 2002), is probably widespread), the Ni-CODH and ECH genes do not cluster. Thus, the presence of a Ni-CODH–ECH gene cluster can be used as a marker of hydrogenogenic carboxydotrophy when interpreting genomics and metagenomics data. Our primers specific for the *coo* gene cluster may also be an informative tool for molecular ecological studies, since they reveal many (although not all) hydrogenogenic carboxydotrophs and do not produce false-positive results concerning their presence.

Taking into account that the Ni-CODH–ECH complex is an efficient, compact, and "self-sufficient" enzymatic machine, is encoded by a common gene cluster, and is scattered over the phylogenetic tree, it could be expected that, in its evolution, interspecies horizontal transfer has played an important role. However, the topology of the relatedness dendrograms constructed for the proteins of the Ni-CODH–ECH enzymatic complexes shows that the main way of evolutionary inheritance of the discussed gene clusters is vertical inheritance. Figure 7.2 presents comparison of 16S-rRNA-based dendrograms of relatedness of hydrogenogenic carboxydotrophs

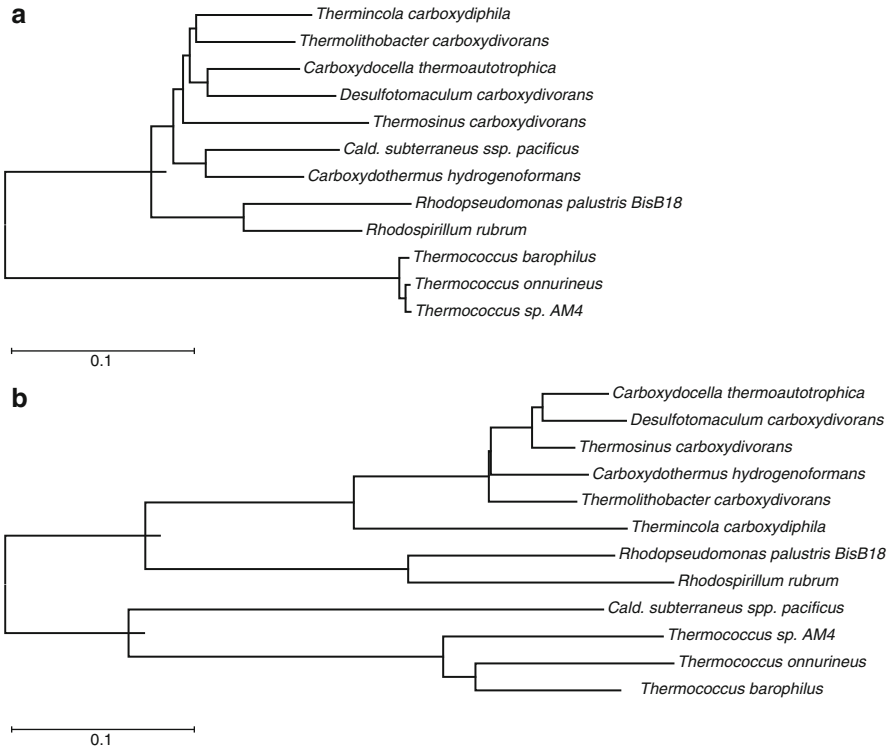

Fig. 7.2 (**a**) 16S-rRNA-based dendrogram of relatedness of hydrogenogenic carboxydotrophs and (**b**) dendrograms of relatedness of the Ni-CODHs encoded by the Ni-CODH–ECH gene clusters. The sequences were aligned by MAFFT v. 6 (Katoh and Toh (2008); http://align.bmr.kyushu-u.ac.jp/mafft/online/server/). Dendrograms were constructed by the neighbor-joining method after calculation of evolutionary distances with Jukes and Cantor and Poisson corrections, respectively (the methods are implemented in the TREECONW v. 1.3b software package (Van de Peer and De Wachter (1994); http://bioinformatics.psb.ugent.be/software/details/TREECON))

and dendrograms of similarity of the Ni-CODHs encoded by the Ni-CODH–ECH gene clusters. The similarity dendrograms that we constructed for the ECH subunits encoded by the discussed cluster are not presented; they are on the whole congruent to the Ni-CODH dendrogram shown in Fig. 7.2b. The few exceptions (*C. subterraneus* subsp. *tengcongensis,* Geobacilli, and *Thermofilum carboxydotrophus*) were deliberately omitted when constructing the trees in Fig. 7.2 to allow it to demonstrate the general tendency. Comparison of the dendrograms in Fig. 7.2a, b leads to a conclusion that interspecies horizontal transfer of the discussed gene cluster is, in any case, not frequent. No recent events of horizontal transfer can be deduced from comparison of Figs. 7.2a, b. However, the gene cluster of *Caldanaerobacter subterraneus* ssp. *tengcongensis* has experienced a recent event of xenologous replacement of *cooS* and *cooF* genes, which is evident from their distinct positions in the relatedness trees (not shown in Fig. 7.2) and from their G+C content 20 points

higher than that of the genome; the donor of the genes is unknown. More ancient occurrence of horizontal transfer events requires special analysis. The understanding of the evolution of hydrogenogenic carboxydotrophy needs further studies, including those that will involve sequence data from new microorganisms.

7.9 Conclusions

Being a potent electron donor ($E^{0'}_{CO/CO2} = -520$ mV), CO serves as an energy source for various anaerobic prokaryotes. The main sources of CO in hot environments inhabited by anaerobic thermophiles are volcanic exhalations and thermal degradation of organic matter. Anaerobic transformation of CO by microbial communities of hot volcanic environments has been demonstrated. A number of phylogenetically diverse anaerobic prokaryotes, both *Bacteria* and *Archaea*, are known to metabolize CO. CO transformation can be coupled to methanogenesis, acetogenesis, hydrogenogenesis, sulfur, sulfate, or ferric iron reduction. Genomic analysis gives indications that anaerobic carboxydotrophy may be inherent in a wider range of organisms than it is currently recognized. Most numerous among the currently known thermophilic anaerobic carboxydotrophs are hydrogenogens. Among them are diverse *Firmicutes*, *Dictyoglomi*, and *Eury-* and *Crenarchaeota*. Despite their phylogenetic diversity, they employ similar enzymatic mechanisms of the $CO + H_2O \rightarrow CO_2 + H_2$ process. The key enzyme of anaerobic CO utilization, the Ni-CODH, forms in hydrogenogens an enzymatic complex with the energy-converting hydrogenase (ECH), and genomic analysis shows this enzymatic complex to be encoded by a single-gene cluster, whose presence can be used as a marker of hydrogenogenic carboxydotrophy when interpreting genomics and metagenomics data. Notably, most of the currently known species of hydrogenogens were isolated under 100% CO, but some species grow only at low CO concentrations in the gas phase. This calls for studies aimed to explain mechanisms of high resistance to CO and the evolutionary significance of their conservation and opens prospects for expanding the diversity of anaerobic carboxydotrophs by using low CO concentration in cultivation tests and isolation procedures.

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