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ORIGINAL PAPER

Anaerobic transformation of carbon monoxide by microbial communities of Kamchatka hot springs

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Abstract Carbon monoxide (CO) is one of the common gaseous compounds found in hot volcanic environments. It is known to serve as the growth substrate for a number of thermophilic prokaryotes, both aerobic and anaerobic. The goal of this work was to study the process of anaerobic transformation of CO by microbial communities inhabiting natural thermal environments: hot springs of Uzon Caldera, Kamchatka. The anaerobic microbial community of Treshchinny Spring (80°C, pH 6.5) was found to exhibit two peaks of affinity for CO ($K_{S1} = 54$ nM and $K_{S2} = 1$ μM). The actual rate of anaerobic CO transformation by the microbial community of this spring, calculated after obtaining the concentration dependence curve and extrapolated to the natural concentration of CO dissolved in the hot spring water (20 nM), was found to be 120 μmol l^{-1} of sediment day^{-1}. In all the hot springs studied, more than 90% of the carbon of ^{14}CO upon anaerobic incubation was recovered as $^{14}CO_2$. From 1 to 5% of ^{14}CO was transformed to volatile fatty acids (VFA). The number of microorganisms capable of anaerobic CO oxidation determined by dilution-to-extinction method reached 10^6 cells ml^{-1} of sediment. CO-transforming anaerobic thermophilic microorganisms isolated from the springs under study exhibited hydrogenogenic type of CO oxidation and belonged to the bacterial genera *Carboxydocella* and *Dictyoglomus.* These data suggest a significant role of hydrogenogenic carboxydotrophic prokaryotes in anaerobic CO transformation in Uzon Caldera hot springs.

Keywords Hydrothermal springs · Anaerobic CO oxidation · Thermophilic hydrogenogenic carboxydotrophic prokaryotes · *Dictyoglomus* · *Carboxydocella*

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Introduction

Thermophilic microbial communities inhabiting hot volcanic environments are to a significant extent fueled by reduced inorganic compounds arriving with volcanic exhalations. H_2 and reduced sulfur compounds are considered to be the main inorganic sources of energy for thermophiles inhabiting hot volcanic environments (Amend and Shock 2001). Another possible inorganic substrate for thermophilic prokaryotes, carbon monoxide, is rarely mentioned although it is present in volcanic exhalations. The reported concentration of CO in volcanic gases varies from 0.6 to 5540 ppm (for references, see Sokolova et al. 2009). Apart from arriving with volcanic exhalations, CO in hot springs may be produced during thermal and photochemical decomposition of organic matter or as a side product of some of thermophilic anaerobes (for references, see Sokolova et al. 2009). CO is a potent electron donor ($E^{0'}_{CO/CO_2} = -520$ mV); however, its utilization by microorganisms may be restricted by its high toxicity to metal-containing enzymes (Thauer et al. 1974; Fauque et al. 1988; Adams 1990).

A number of thermophilic prokaryotes able to utilize CO have been isolated to date (King and Weber 2007;

Sokolova et al. 2009). However, the rate of microbial CO transformation in natural hot environments, the dominant processes of CO transformation, and the numbers of microorganisms capable of it are still unknown. The goal of this work was to estimate the rate of anaerobic microbial CO transformation and its products, as well as the number and phylogenetic position of anaerobic CO-oxidizing microorganisms in terrestrial hot springs of Kamchatka. We also studied the kinetics of microbial anaerobic CO transformation by a microbial community inhabiting a Kamchatka hot spring.

Materials and methods

Characterization of sampling sites

Uzon (N 54°30′; E 159°58′) is a 9 by 12 km volcanic caldera located in the eastern part of Kamchatka Peninsula, Russia. The bottom of the caldera is a float depression 650 m above sea level. In the northern sector of the caldera several detached thermal fields are distinguished. For a brief review on Uzon geology, see Kyle et al. 2007. The largest in size and maximum in the evacuation of heat is the Eastern Thermal Field. Three springs located at the Eastern Thermal Field were chosen for detailed analysis (Table 1). Temperature, pH and Eh were determined using a pH 300i WTW device (Germany) directly in the sampling point. The concentration of dissolved CO was determined by the headspace method based on the equilibrium degassing principle (Slepova et al. 2007).

Sample collection

Samples of mud, cyanobacterial mats (if present), and water were taken from selected hot springs in August 2005 and 2006. All the samples were collected into sterile 0.5-l serum bottles. The bottles were sealed with rubber stoppers and screw caps and transported at ambient temperature to the field laboratory, where the samples were immediately used.

Radioisotopic tracing experiments

Slurries for radioisotopic tracing experiments were prepared from sediment and water of the same hot spring mixed in a 1:8 ratio under an oxygen-free nitrogen flow. The slurries were placed in nitrogen-filled 18-ml Hungate tubes with no headspace left, and 1 ml of a $^{14}CO/N_2$ gas mixture containing 0.23 μmol of ^{14}CO (0.5% in the gas phase; 0.172 MBq; Isotope, Saint Petersburg, Russia) was injected into each tube. The excess of slurry was simultaneously removed through another syringe stabbed into the stopper. Tubes were incubated in situ (i.e., at the sampling site). The time course of ^{14}CO transformation was determined using several tubes containing portions of the same slurry and incubated for 3, 6, 9, 12, or 24 h. After incubation, the process was terminated by the addition of glutaraldehyde to a final concentration of 2.5% (vol/vol). Control samples were fixed with glutaraldehyde before incubation with ^{14}CO, as described previously (Slepova et al. 2007).

Radioactivity of the products of ^{14}CO transformation ($^{14}CH_4$, $^{14}CO_2$, microbial cells, dissolved organic matter (DOM) and volatile fatty acids (VFAs)) was measured in a RackBeta liquid scintillation counter (LKB, Sweden). Fractionation was done as described before (Slepova et al. 2007). All experiments were performed in duplicates.

Kinetics studies

Samples of sediments (2 ml) from Treshchinny Spring were dispensed into 60-ml flasks under an N_2 flow. The flasks were closed with rubber stoppers and screw caps and incubated at 80°C for 5 min. After that, different CO volumes were injected into the gas phase of flasks to achieve concentrations of CO from 0.2 to 155 μmol l^{-1} in the gas phase, or from 0.003 to 2.2 μmol l^{-1} in the liquid

Table 1 Characteristics of the samples collected in Uzon Caldera

Spring name	GPS coordinates	T (°C)	pH	Eh (mV)	C_{COdis} (nM)	Description of a spring	Sample type
Zavarzin	N 54°49′81.0″ E 160°01′46.0″	60	6.2	−96	20	2.5 × 7 m funnel covered with thick layered cyanobacterial mat with a thick elemental sulfur layer on the top; gases are issued as huge bubbles	Cyanobacterial mat
Treshchinny	N 54°29′56.3″ E 160°00′55.9″	80	6.5	−70	20	1-m long crack, edges are covered with gray silt	Gray silt
Burlyashchy	N 54°29′98.1″ E 160°00′11.3″	90	6.5	−90	33	A funnel 15 m in diameter, with a permanent intense pulsing gas effluent in the middle of the funnel	Gray filaments

phase as calculated using the concentrational form of Henry's law $C_{aq} = k_{H,cc} \times C_{gas}$ and a tabulated value (0.0143) of the correspondent constant for CO at the given temperature and mineralization (Gas–liquid equilibrium 1964). The flasks were incubated at 80°C in a Thermo-Haake SWB25 water bath-shaker (Germany) operated at 80 rpm. The shaking rate was chosen experimentally, for the dissolution rate of CO not to limit the rate of its oxidation. At regular time intervals, gas phase samples (0.5 ml) were taken, and the residual concentration of CO was determined using a Kristall 5000 gas chromatograph (Khromatek, Russia) equipped with a flame-ionization detector and a methanizer. The experiments were run in two replicates.

Enumeration of CO-utilizing prokaryotes

The cell numbers of anaerobic CO-utilizing prokaryotes were determined by serial tenfold dilutions of sediments in the anaerobic mineral Medium 1 under 15% CO mixed with N_2 or 100% CO gas phase. The Medium 1 was of the following composition (g l^{-1}): NH_4Cl (1); $MgCl_26H_2O$ (0.33); $CaCl_26H_2O$ (0.1); KCl (0.33); KH_2PO_4 (0.5); resazurin (0.001); 1 ml of trace element solution (Kevbrin and Zavarzin 1992); and 1 ml of vitamin solution (Wolin et al. 1963). After boiling, the medium was cooled under nitrogen flow. Then, $NaHCO_3$ (0.5 g l^{-1}) and $Na_2S{\cdot}9H_2O$ (1.0 g l^{-1}) were added and the pH was adjusted to 6.8 with 0.5 N HCl. Ten-ml portions of the medium were placed into 50-ml bottles and the head space was filled with 100% CO at atmospheric pressure. The medium was sterilized at 120°C for 0.5 h. Dilutions were incubated for 2 weeks at temperatures of the sampling sites (Table 1). The growth of CO-utilizing prokaryotes was followed using light microscopy (MIKMED-1 microscope, LOMO, Russia) and chromatographically, by estimating CO consumption and products' (H_2 and CH_4) formation (GLC-Chrom 5; Laboratorni Pristroje Praha). Total cell numbers in the sediment samples were determined by direct cell count using diamidino-4′,6-phenyl-2-indole (DAPI) staining (Huber et al. 1985), under a fluorescent microscope (Axio Imager D1, Germany).

Enrichment and isolation of anaerobic thermophilic carboxydotrophic prokaryotes

Laboratory enrichments of CO-utilizing anaerobes were obtained by the inoculation of 1 ml of sediment into 10 ml of Medium 1 in 60-ml flasks. To obtain the gas phase containing 45 or 15% CO, the required portion of CO was injected with syringe into flasks filled with oxygen-free nitrogen. Enrichments were incubated at temperatures of the sampling sites.

Anaerobic CO-oxidizing prokaryotes were isolated by repeated serial dilutions, subsequent transfers to medium 1 solidified with 3% agar (Difco), and isolation of single colonies.

16S rRNA gene sequence analysis

10 ml portions of the cultures of new isolates were centrifuged at 12000*g* for 15 min, and DNA was isolated from the cell precipitate by the modified alkaline Birnboim–Doly method (Boulygina et al. 2002) using Wizard-technology ("Wizard MaxiPreps DNA Purification Rezin", Promega, USA). PCR amplification of the 16S rRNA genes, performed using Bact8-27F and Univ1492R primers (Lane 1991), and sequencing of the products were performed as described by Subbotina et al. (2003). The sequences were compared with those in the GenBank database using the NCBI BLAST tool (http://www.ncbi.nlm.nih.gov/blast).

Results

Characteristics of sampling sites

Three hot springs with temperatures of 60, 80, and 90°C and with pH values close to neutral, located at Uzon Caldera, were chosen for detailed analysis (Table 1). Zavarzin Pool (60°C) was covered with dense brown cyanobacterial mats and thick layer of sulfur. In two other sites, Treshchinny Spring (80°C) and Burlyashchy Pool (90°C), no visible growth of phototrophic microorganisms was observed.

Concentration of dissolved CO in the hot spring water was 20 nM in Zavarzin Pool and Treshchinny Spring, and 33 nM in Burlyashchy Pool (Table 1).

Rates and products of CO transformation by microbial communities of Uzon hot springs

Radioisotopic tracing showed that the upper layers of sediments rapidly transformed CO in the hot springs studied (Table 2). More than 90% of ^{14}CO was consumed by samples from Zavarzin Pool (60°C) over 9 h of incubation; the activity of the microbial community of Treshchinny Spring (80°C) was even higher: 98% of ^{14}CO was utilized over 3 h. In samples from Bourlyashchy Pool (90°C), 80% of ^{14}CO was consumed within 12 h of incubation (Fig. 1).

The determination of radioactivity of the possible products of CO transformation revealed that 95, 99, and 91% of the consumed ^{14}CO was oxidized to $^{14}CO_2$ in Zavarzin Pool, Treshchinny Spring and Burlyashchy Pool, respectively (Table 2). The microbial community of

Table 2 Anaerobic CO transformation by thermophilic microbial communities of Uzon Caldera hot springs

Spring	Potential activity (mmol $^{14}CO\ l^{-1}\ day^{-1}$)	Distribution of ^{14}C in products of ^{14}CO transformation[a] (% of ^{14}C of the utilized ^{14}CO)			
		$^{14}CO_2$	^{14}C-VFAs	^{14}C-DOM	^{14}C-cell biomass
Zavarzin	0.09	95.0	4.8	0	0.1
Treshchinny	0.48	99.2	0.8	0	0
Burlyashchy	0.13	90.4	0.8	8.3	0.5

[a] After 6 h of incubation

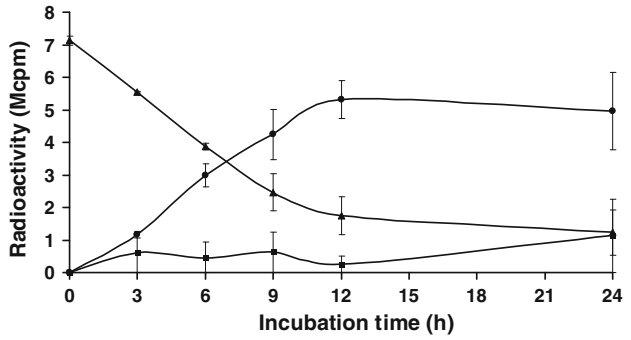

Fig. 1 Conversion of ^{14}CO (*filled triangles*) to $^{14}CO_2$ (*filled circles*) and ^{14}C-organic matter (*filled squares*), which includes ^{14}C-VFAs, ^{14}C-DOM and ^{14}C-cell biomass, by microbial communities of Burlyashchy spring (90°C) in the course of 24-h incubation. The experiment was performed in two replicates. *Vertical bars* indicate standard deviations

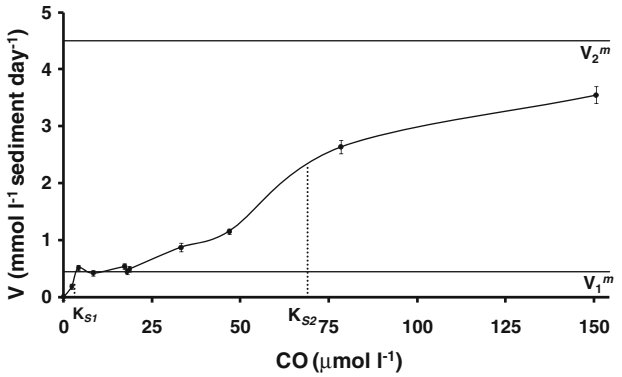

Fig. 2 The rate of CO consumption by the microbial community of Treshchinny Spring (80°C) as a function of its concentration in the gas phase. The experiment was performed in two replicates. *Vertical bars* indicate standard deviations

Zavarzin Pool transformed 4.8% of the consumed ^{14}CO to VFA; in Bourlyashchy Pool and Treshchinny Spring the production of VFA from CO accounted for only 0.8% of ^{14}CO consumed. Not more than 0.75% of the consumed ^{14}CO was transformed to cell material in any of the springs (Table 2). $^{14}CH_4$ was never detected.

Kinetics of anaerobic CO transformation by the microbial community of Treshchinny Spring

Kinetic analysis carried out with a sample from Treshchinny Spring, showed a two-step dependence of the CO transformation rate on the CO concentration (Fig. 2). By the nonlinear regression method, the following values of kinetic parameters were calculated: $K_{S1} = 54$ nM (nmol CO l^{-1} sediment; the corresponding equilibrium concentration in the gas phase is 3.75 μmol CO l^{-1}, or 84 ppm); $V_1^m =$ 0.45 mmol CO l^{-1} sediment day^{-1}; $K_{S2} = 1$ μM (the equilibrium concentration in the gas phase, 69 μmol CO l^{-1}, or 1,568 ppm,); $V_2^m = 4.5$ mmol CO l^{-1} sediment day^{-1}. The graph of the rate of CO consumption by sediments versus CO concentration is a superposition of two saturation curves related to at least two individual enzymatic systems.

The actual rate of anaerobic CO transformation by the microbial community of Treshchinny Spring, calculated proceeding from the natural concentration of CO dissolved in the hot spring water (20 nM) and the concentration dependence curve that we had obtained, was estimated to be 120 μmol of CO per 1 liter of sediment per day.

Cell numbers of CO-utilizing anaerobes and their ratios to total cell numbers

In Zavarzin and Bourlyashchy Pools, the cell numbers of CO-utilizing anaerobes were estimated to be 10^6 cells cm^{-3} sediment, both at 15 and 100% CO in the gas phase (Table 3). CO consumption was recorded in all growth-positive dilutions. Microscopy of last positive dilutions revealed that short rods were dominant in those from Zavarzin Pool and cocci were dominant in cultures from Bourlyashchy Pool. No production of methane was detected. In Treshchinny Spring no CO-trophic anaerobes able to grow at 100% CO in the gas phase were found. The number of CO-utilizing anaerobes able to grow under 15% CO did not exceed 10 cells per cm^3 at this site. The total cell count values were 2.3×10^8, 0.7×10^8, and 1.4×10^8 cells per ml sediment in Zavarzin Pool, Treshchinny Spring, and Bourlyashchy Pool, respectively (Table 3). Thus, the quota

Table 3 Cell numbers of anaerobic CO-trophs and the total cell numbers in Uzon Caldera hot springs

Spring	Cell number (cells ml^{-1})		Total
	Anaerobic CO-trophs		
	Enriched at 15% CO (dominant cell forms[a])	Enriched at 100% CO (dominant cell forms[a])	
Zavarzin	10^6 (short rods)	10^6 (short rods)	2.3×10^8
Treshchinny	10 (thick and thin rods, filaments)	0	0.7×10^8
Burlyashchy	10^6 (cocci)	10^6 (cocci)	1.4×10^8

[a] In the final dilution

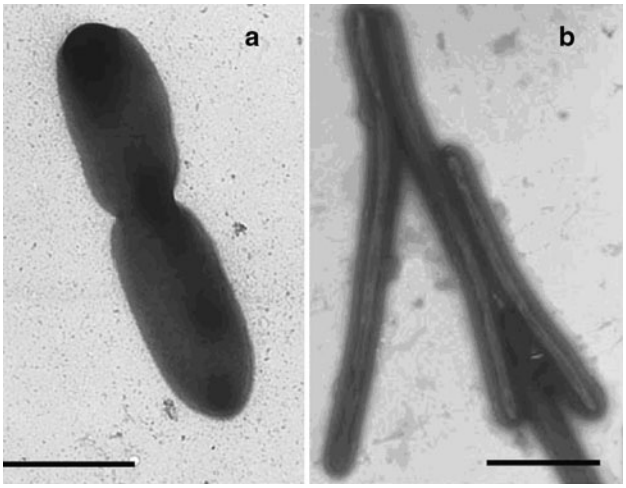

Fig. 3 Electron micrographs of new thermophilic carboxydotrophic isolates (negatively stained whole cells). *Bar* 1 μm. **a** *Carboxydocella* sp. 1244, **b** *Dictyoglomus* sp. 1512

of anaerobic microorganisms capable of CO utilization was up to 1% of the total cell number of prokaryotes.

Isolation and characterization of thermophilic carboxydotrophs from Uzon hot springs

Two strains of anaerobic thermophilic hydrogenogenic bacteria were isolated from the samples of sediments and water of Zavarzin Pool and Treshinny Spring. Strain 1244 was enriched from Zavarzin Pool on Medium 1 under 100% CO in the gas phase, at 60°C. Its cells were short rods 0.5 μm wide and from 1- to 3-μm long (Fig. 3a). It was found to be a strict anaerobe, growing optimally at 60°C and pH 6.8–7.0. Strain 1244 grew chemolithoautotrophically on 100% CO producing hydrogen and carbon dioxide. The generation time of growth on 100% CO under optimal conditions was 1 h. It could also grow by fermentation of few organic compounds: yeast extract, glucose, and sucrose. Analysis of the 16S rRNA gene sequence (GenBank accession number EU260048) revealed the highest homology (99.5% identity) with the 16S rRNA gene of *Carboxydocella thermautotrophica* (Sokolova et al. 2002), previously isolated from Geyser Valley, Kamchatka.

Strain 1512 was obtained from a sediment/water sample of Treshchinny spring. Initial enrichment cultures grew in Medium 1 supplemented with 0.2 g/l of yeast extract, under 5 or 15% CO in the gas phase. In both cultures, the growth of long, thin filaments and few cocci was observed. The organism with long, filamentous cells was isolated in Medium 1 supplemented with pyruvate (2 g/l) and yeast extract (0.2 g/l) and designated strain 1512 (Fig. 3b). The isolate was able to grow on CO producing CO_2 and H_2. Optimal CO concentration in the gas phase for growth of strain 1512 was 5%; no growth occurred at CO concentrations above 15%. Cells of strain 1512 were long, thin filaments, about 0.25 μm wide, and from 2 to 10 μm long. It was found to be an obligate anaerobe, extreme thermophile, growing optimally at 75°C, and neutrophile. The generation time under 5% CO was about 60 h; the minimum yeast extract concentration required for growth was 0.2 g/l. Analysis of the 16S rRNA gene sequence (GenBank accession number FJ626840) revealed the highest homology (100%) with the 16S rRNA gene of *Dictyoglomus turgidum* (Svetlichny and Svetlichnaya 1988), isolated previously from Uzon Caldera.

Discussion

CO, a common component of volcanic gases, is an energy-rich substrate that can serve as an electron donor for the growth of lithotrophic microorganisms, including anaerobic thermophilic prokaryotes, among which there are carboxydotrophic hydrogenogens, methanogens, acetogens, sulfate, and ferric iron reducers. Carboxydotrophic hydrogenogens, which perform the reaction: $CO + H_2O \rightarrow CO_2 + H_2$, rank first in the number of isolates and are phylogenetically diverse (Sokolova et al. 2009). Most of these organisms are bacteria of the phylum *Firmicutes*; however, the ability to grow at the expense of hydrogenogenic CO oxidation was also shown for some hyperthermophilic archaea of the genus *Thermococcus* (Sokolova et al. 2004; Lee et al. 2008). All hydrogenogenic carboxydotrophs known so far have been isolated and maintained at 100% CO in the headspace, far in excess of

natural CO concentrations. This work was aimed at the characterization of natural anaerobic thermophilic CO-oxidizing communities: their activity, affinity to CO, their composition, and abundance of their members. For this purpose, three hot springs of Uzon Caldera, Kamchatka, were chosen. The first, Zavarzin Pool, is characterized by lower water temperature and the presence of phototrophic communities, while two other sites are much hotter (Bourlyashchy Pool is the hottest hot spring of Uzon Caldera). Trace element and stable isotope composition of the water in the third chosen spring, Treshchiny, suggest a high contribution of magmatic fluid (Romanek et al. 2005). The highest concentration of dissolved CO (33 nM) was found in Bourlyashchy Pool; which is in agreement with the pool features, such as high temperature of its water, indicative of a high content of hydrothermal fluid, and grass-covered surrounding soil, which may be an additional source of CO originating from the thermal decomposition of organic matter. The results of radioisotopic tracing experiments showed that active anaerobic transformation of CO took place at all the three sites studied 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, 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 CO_2 formation as the 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. Sulfate reduction (Parshina et al. 2005) and Fe(III) reduction (Slobodkin et al. 2006) could be responsible for anaerobic CO oxidation in moderately thermophilic environments.

Though diverse and widely spread, hydrogenogenic CO-trophs were so far grown only at 100% CO content of the gas phase, i.e., in conditions which are very far from natural. Still, the numbers of organisms able to transform CO at such concentrations were comparatively high (10^6 cells cm^{-3} of sediment) at two of the three sites studied (Zavarzin and Bourlyashchy Pools). From Zavarzin Pool, a strain of *Carboxydocella thermautotrophica* was isolated. Growth characteristics of this bacterium are in good agreement with the conditions in Zavarzin Pool (62°C, pH 6.2). Being a lithoautotroph, *C. termautotrophica* makes its contribution to lithotrophic organic matter production in this spring. However, the low production of ^{14}C-labeled biomass (0.1% of utilized ^{14}CO) compared with the production of ^{14}C-labeled biomass by a pure culture of *Carboxydocella* sp. 1503 growing autotrophically on CO (0.6% of utilized ^{14}CO) (Slepova et al. 2007) suggests that nonautotrophic CO-oxidizing organisms are involved in the process of CO transformation in Zavarzin Pool. Most of currently known CO-oxidizing anaerobes use additional sources of cell carbon during growth on CO (Sokolova et al. 2009). The production of ^{14}C-labeled biomass from ^{14}CO by the microbial community of Bourlyashchy Pool (0.5% of the utilized ^{14}CO) indicates the possibility of the involvement of autotrophic CO-oxidizing organisms in the anaerobic CO transformation process. However, the phylogenetic position and physiological properties of the CO-utilizing microorganisms in Bourlyashchy Pool at 90°C remain unclear. In submarine hydrothermal ecosystems, anaerobic CO oxidation can be performed either by hyperthermophilic archaeal hydrogenogens (Sokolova et al. 2004), or by hyperthermophilic archaeal sulfate reducers of the genus *Archaeoglobus* (Henstra et al. 2007). However, our attempts to isolate hyperthermophilic carboxydotrophs from Bourlyashchy Pool were so far unsuccessful.

In Treshchinny Spring, the rate of CO oxidation revealed by radioisotopic tracing was maximal for the hot springs studied. At the same time, microorganisms able to grow at 100% CO in the gas phase were absent at this site, and those tolerating 15% CO were present in much lower numbers than CO-trophs in Zavarzin and Bourlyashchy Pools. This observations are in agreement with kinetic studies: the microorganisms of Treshchinny Spring exhibited an extremely high affinity to CO and were represented by two groups of organisms: those oxidizing CO with half of maximum rate at 54 nM of dissolved CO and at 1 μM of dissolved CO, which would correspond to 0.15% CO in the gas phase. We succeeded in isolating from Treshchinny spring the thermophilic bacterium *Dictyoglomus turgidum* strain 1512 that was capable of hydrogenogenic CO-trophy and grew optimally at 5% CO in the gas phase. *Dictyoglomus* sp. 1512 is the first representative of *Dictyoglomy* phylum capable of CO oxidation or of any type of lithotrophic growth.

Thus, in spite of the low dissolved CO concentrations in hot springs, the presence of populations of CO-oxidizing prokaryotes and active CO transformation in the springs studied were revealed. From two springs, hydrogenogenic CO-trophs were isolated. Our work also showed that a decrease in CO concentration during enrichment and isolation widens significantly the knowledge of the diversity of microorganisms participating in CO oxidation. In particular, a new phylogenetic group—microorganisms of *Dictyoglomy* phylum—was found to perform this reaction at CO concentration not exceeding 15%. It can be assumed that the group of thermophilic hydrogenogenic carboxydotrophs is still much more diverse, both phylogenetically and physiologically.

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