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SHORT COMMUNICATIONS

Organotrophic Activity in Kamchatka Hot Springs with Low pH

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Thermoacidophilic prokaryotes inhabiting acidic hot springs are represented by archaea and bacteria with either respiratory [1] or anaerobic fermentative [2, 5] metabolism. Thermoacidophilic microorganisms available in laboratory cultures grow at temperatures ranging from 50 to 92°C and at pH 0–5.5. The goal of this study was to determine the activities of organotrophic thermoacidophilic prokaryotes directly in their natural habitats, acidic hot springs in Uzon caldera, Kamchatka (Table 1).

Samples of sediment (5 ml) and water (10 ml) from each hot spring were placed in 25-ml penicillin bottles and sealed. The organic substrate was added to each bottle as 0.2 ml of a sterile water solution of uniformly labeled ^{14}C sucrose (0.63 μg, total activity 1 μCi, Amersham, England). The specific activity of sucrose was 540 mCi/mol, and its final content was 42.1 μg/l.

The bottles to be used in anaerobic incubation tests were first blown with nitrogen; then after introducing the samples, $Na_2S \cdot 9H_2O$ (500 mg/l) and cysteine chloride (500 mg/l) were added to each bottle. For aerobic incubation, the bottle head space was filled with air. Each test was replicated three times. All bottles were incubated for 24 h at temperatures close to those in sampling sites (Table 2). Upon incubation, concentrated solution of KOH (0.5 ml) was introduced to each bottle to stop biological processes and fix sucrose oxidation products. In control runs, concentrated KOH (0.5 ml) was added to parallel samples, and they were allowed to stand for 12 h and then, upon the addition of labeled sucrose, were incubated together with the test bottles.

The amount of completely oxidized sucrose was determined from the quantity of the labeled carbon dioxide formed. Carbon dioxide was distilled upon return to Moscow in an apparatus equipped with a reflux condenser [3]. The sample was placed in a flask where tap water (100–150 ml) and orthophosphoric acid (a few drops) were added; CO_2 was distilled in an argon flow under continuous boiling for 1 h. Carbon dioxide was captured in a scintillation mixture composed of LS-8, 2-phenylethylamine and 96% ethanol (7 : 1 : 2). The radioactivity of the mixture was determined using a Rack-Beta 1291 liquid scintillation counter (LKB, Sweden).

The radioactivity of volatile fatty acids was determined by the following method. Upon the separation of sediment by centrifugation (10 min at 4000 *g*), a solution (0.5 ml) containing 10 μg of sodium salts of formate, propionate, and butyrate was added to the supernatant (5 ml), and the mixture was acidified to a pH below 2 and distilled with water vapor. The obtained condensate sample (0.1 ml) was immediately assayed on a Biotronic ion chromatograph (Germany). Different fractions of the solution passing through the column were collected in scintillation vials to which LS-8 was added (10 ml), and the radioactivity of the obtained solution was determined using the same scintillation counter.

The obtained results are presented in Table 2. It can be seen that sucrose is actively oxidized in the springs studied. Up to 43.5% of the introduced sucrose was oxidized to CO_2 within 24 h. This process is of a biological nature, because its rate in abiotic controls never exceeded 7.04% of that observed in the experimental runs.

The most vigorous oxidation of sucrose (17.5–18.4 μg/l per day) was observed at 81°C. Under these conditions, the influence of pH and aeration on the process rate was insignificant. In hot springs with a water temperature of about 70°C, the most active oxidation of sucrose was noted under aerobic conditions at low pH values. The least active oxidation of sucrose was observed in springs with a temperature of 60°C. Its rate, on average, was a factor of 2.5 lower than at 81°C. In the spring with the lowest pH value, the utilization of sucrose under anaerobic conditions was an order of magnitude lower than in the same sample incubated under aerobic conditions. This spring was different from other ones in being totally devoid of dissolved sulfide and, supposedly, of sulfide oxidation products able to act as electron acceptors in anaerobic oxidation of sucrose or products of its fermentation. Another explanation could lie in the absence of anaerobic microflora

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Table 1. Characteristics of sampling sites in the Uzon caldera, Kamchatka

Sample no.	Site and characteristics	*T*, °C	pH	Eh	S^{2-}, mg/l*
908	Central Thermal Field; small mud pot	79	3.7	–85	109
966	Orange Field; small mud pot	69	3.6	ND**	170
967	Orange Field; big center lake	60	3.7	ND	272
968	Orange Field; mud pot	59	2.1	ND	0
969	Central Thermal Field, Izvilisty creek bank; small spring with gray sediment and decayed plant material	71	2.2	ND	204
970	Central Thermal Field, Izvilisty creek bank; small mud pot	82	2.6	–83.5	204

* The concentrations of hydrogen sulfide and sulfides in sampling sites were determined by iodometric titration[15].
** ND means "not determined".

Table 2. Formation of CO_2 from ^{14}C-sucrose in samples from Kamchatka hot springs

Sample (temperature, pH)	Incubation temperature, °C	Formation of CO_2 from sucrose			
		Aerobic conditions		Anaerobic conditions	
		rate, μg/(l day)	% of introduced C over 24 h	rate, μg/(l day)	% of introduced C over 24 h
967 (60°C, 3.7)	60	7.4*	17.5	6.2	14.65
968 (59°C, 2.1)		8.4	19.9	0.4	1.04
966 (69°C, 3.6)	71	4.8	11.53	9.5	22.36
969 (71°C, 2.2)		14.6	37.61	9.4	22.28
908 (79°C, 3.7)	81	18.4	43.53	17.7	42.05
970 (82°C, 2.6)		17.8	42.16	17.5	41.5

* The calculations account for the concentration of labeled sucrose introduced in the incubation mixture.

in this spring with insufficiently low reduced conditions.

The formation rate of ^{14}C-volatile fatty acids (VFAs) was determined in sample 970 (82°C, pH 2.6) with the most complete sucrose oxidation. The main half-oxidized product of sucrose degradation was acetate. Its production rate was as high as 0.066 and 0.1 μg/(l day) under aerobic and anaerobic conditions, respectively. Under aerobic conditions formate was also formed in small quantities, while other VFAs were present only in background concentrations.

The results of radioisotopic assays demonstrate that the hot acidic springs of Kamchatka contain active microbial communities capable of rapid oxidation of readily mobilized organic matter (sugars), under both aerobic and anaerobic conditions. Aerobic degradation of organic substrates at high temperatures and low pH can be accomplished by extremely acidophilic moderately thermophilic organotrophic organisms of the genera *Picrophilus* [4] and *Thermoplasma* [5], as well as by organisms of the genus *Sulfurococcus* [6, 7], which are known to grow in a wide range of low pH values and, in addition to lithotrophic growth, are capable of utilizing certain sugars and peptides. *Sulfurococcus* spp. were isolated from hydrothermal vents in the Uzon caldera, and *Thermoplasma* spp. were previously detected in the same habitats [2].

Anaerobic organotrophic activities at 80°C can be attributed to representatives of the genus *Acidilobus*, which were isolated from similar environments [8, 2]. Organisms of this genus can utilize sugars and polysaccharides, forming acetate and CO_2 as main products. Organic compounds in hot acidic springs could also be destroyed by representatives of the genera *Sulfurisphaera* [9], *Thermocladium* [10], *Caldivirga* [11], *Vulcanisaeta* [12], and *Caldisphaera* [13], but these have not yet been detected in the hot springs of the Uzon caldera.

At 60°C, anaerobic oxidation of sucrose could also be effected by representatives of the genus *Thermoplasma*, which are able to grow under both aerobic and anaerobic conditions and by the moderately thermoacidophilic anaerobic bacterium *"Thermoanaerobacterium acidotolerans,"* isolated from a similar habitat in the Uzon caldera. This is the only representative of the genus that can grow at pH values as low as those in the springs studied.

The anaerobic lithoautotrophic processes in the hot springs of Kamchatka were previously studied by radioisotopic methods [14]. Complete oxidation of

organic matter in hydrothermal springs requires the presence of a complex microbial community. However, currently there are no known microorganisms that can completely oxidize nonfermentable substrates, such as acetate, at high temperatures and low pH. The goal of future studies could be the detection and identification of this new physiological group by radioisotopic and cultural methods.

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