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EXPERIMENTAL ARTICLES

Detection of Hyperthermophilic *Archaea* of the Genus *Desulfurococcus* by Hybridization with Oligonucleotide Probes

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Abstract—Based on the analysis of nucleotide sequences of 16S rRNA, oligonucleotide probes were designed for the detection and identification of representatives of the genus *Desulfurococcus* (kingdom *Crenarchaeota* of the domain *Archaea*). The detection procedure included obtaining PCR products on DNA isolated from pure cultures, enrichments, or natural samples with a designed *Crenarchaeota*-specific primer pair: Cren 7F (5'-TTCCGGTTGATCCYGCCGGACC-3') and Cren 518R (5'-GCTGGTWTTACCGCGGCGGCTGA-3'). The PCR products were hybridized with Dig-11-dUTP–labeled oligonucleotide probes targeting the genus *Desulfurococcus* (Dco 198, 5'-CGTTAACYCCYGCCACACC-3') and its species *D. mobilis* (Dco_mob 198, 5'-CGTTAACCCCTGCCACACC-3') and *D. amylolyticus* (Dco_amy 198, 5'-CGTTAACCCCCGCCACACC-3'). With the use of these primers and probes, four new strains isolated from hydrotherms of Kamchatka and Kunashir Island were identified as members of the species *Desulfurococcus amylolyticus*. *Desulfurococcus* representatives were detected in several natural samples, including a sample taken from a marine hydrotherm at Kunashir Island; this demonstrates that representatives of this genus occur not only in terrestrial but also in marine environments.

Key words: Archaea, Crenarchaeota, Desulfurococcus, hyperthermophiles, oligonucleotide probes, primers, detection, identification.

Hyperthermophilic organotrophic archaea of the genus *Desulfurococcus* phylogenetically represent kingdom *Crenarchaeota* of the domain *Archaea*. The genus *Desulfurococcus* comprises three valid species: *D. mucosus* [1], *D. mobilis* [1], and *D. amylolyticus* [2]. Desulfurococci grow in the temperature range of 75–95°C, with an optimum at 85–92°C, and in the pH range of 4.5–7.0, with an optimum at 6.0. They are strict anaerobes that grow heterotrophically at the expense of proteins, peptides, or carbohydrates.

Investigation of the physiology of desulfurococci showed that they are incapable of complete oxidation of organic compounds. This is in agreement with data on the absence of acetate oxidation enzymes in desulfurococci [3]. The final products of peptone fermentation by desulfurococci in the presence of sulfur are acetate, isobutyrate, isovalerate, and hydrogen sulfide. In the absence of sulfur, desulfurococci carry out fermentation with the formation of molecular hydrogen, which inhibits their growth. Reduction of elemental sulfur is apparently a process relieving the inhibitory effect of hydrogen; moreover, it is probably coupled to the operation of an electron transport chain and synthesis of additional ATP [4].

Archaea of the genus *Desulfurococcus* are widespread in terrestrial hydrotherms of Kamchatka and the Kurile Islands [5]; their high population density has been recorded in many hot springs and thermal groundwaters, including those lacking elemental sulfur. In natural ecosystems, removal of hydrogen may occur via physical or biological processes, promoting the development of desulfurococci and degradation of complex organic substrates under extremely thermophilic conditions [4].

Research interest in *Desulfurococcus* representatives is mainly due to the presence in these microorganisms of thermostable enzymes that are or may be applied in various fields of biotechnology. These are amylases, xylanases, proteases, etc. [6].

The goal of this study was the development of a method for the detection and identification of *Desulfurococcus* representatives in pure cultures, enrichments, and natural samples by means of hybridization with oligonucleotide probes.

MATERIALS AND METHODS

Subjects of study. Cultivation of microorganisms. This work used the type strains *Desulfurococcus mobilis* DSMZ 2161, *D. mucosus* DSMZ 2162, and *D. amylolyticus* DSMZ 3822, as well as strain 1312, isolated by V. Svetlitchnyi from a Kamchatka hydro-

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therm and, according to its 16S rDNA sequence[1] and phenotypic properties, assigned by us to the genus *Desulfurococcus*.

Strains of *Desulfurococcus* spp. were grown in anaerobically prepared medium described by Prokofeva *et al.* [7], supplemented with 0.2 g/l of yeast extract, 3 g/l of peptone, and 10 g/l of elemental sulfur (pH 6.0–6.5), in 15-ml Hungate tubes; the medium volume was 10 ml; the gas phase was an N_2 –CO_2 (80 : 20) mixture.

The unidentified strains 204, 313, 603, and 2601, isolated from terrestrial hydrotherms of Kamchatka and Kunashir Island in 1988–1990 and stored at the Laboratory of Hyperthermophilic Microbial Communities at the Institute of Microbiology, Russian Academy of Sciences, were cultivated under the same conditions as the type strains of *Desulfurococcus* spp. In both cases, cultivation was performed at 82°C for 1–3 days.

The tests of primer and probe specificity employed, as reference strains, the following type strains of microbial species, either obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) or stored in the collection of the Laboratory of Hyperthermophilic of Microbial Communities at the Institute of Microbiology, Russian Academy of Sciences: *Staphylothermus marinus* DSMZ 3639, *Pyrodictium abyssi* DSMZ 6158, *Acidilobus aceticus* DSMZ 11585, *Methonosarcina barkeri* DSMZ 10343, *Thermococcus peptonophilus* DSMZ 10343, and *Thermoanaerobacter siderophilus* DSMZ 12299. Their cultivation was carried out on media and under conditions recommended by DSMZ.

In addition to pure cultures, this work also used natural samples taken from terrestrial hydrotherms of Kamchatka and a marine hydrotherm near Kunashir Island.

Isolation of DNA. Cells were harvested by centrifugation at 12000 *g* for 10 min and disintegrated by boiling for 5 min in D buffer (4 M guanidine isothionate, 2.5 mM Na citrate, 0.1 M β-mercaptoethanol, 0.5% sarcosyl, pH 7.0) [8]. DNA was extracted from supernatant with a phenol–chloroform (1 : 1) mixture and then twice with chloroform and precipitated with ethanol. The precipitate was dissolved in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0). The DNA isolated was used as the template for PCR.

PCR. For amplification of fragments of 16S rRNA genes, a *Crenarchaeota*-specific primer pair was used: Cren 7F (5'-TTCCGGTTGATCCYGCCGGACC-3') and Cren 518R (5'-GCTGGTWTTACCGCGGCG-GCTGA-3'). Detailed characterization of this primer pair will be given in a separate publication (Lebedinsky *et al.*, manuscript in preparation).

The reaction mixture for PCR (20 μl) contained PCR buffer (Fermentas, Lithuania, 1×; 75 mM Tris–HCl, pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.01 vol % Tween 20); 1.5 mM $MgCl_2$; dNTP, 200 μM each; primers, 0,5 μM each; 1 U of Taq polymerase (Fermentas, Lithuania); and 1 μl of template DNA solution (1–10 ng DNA). The temperature program of the reaction, adjusted experimentally, was as follows: 3 min at 95°C; then 32 cycles of denaturation at 94°C for 7 s, annealing and extension at 72°C for 2 min, and final extension at 72°C for 10 min. In all reactions, a reaction mixture without DNA was one of the negative controls. PCR was run in a Tertsik multichannel DNA amlifier (DNK-Tekhnologiya, Russia).

The PCR products (10 μl) were analyzed by electrophoresis run at 4 V/cm in 1.5–2.0% agarose gel (Tris–acetate–EDTA buffer) stained with ethidium bromide (0.5 μg/ml). For signal visualization, a UV transilluminator with a wavelength of 310 nm was used.

Design of oligonucleotide probes. The consensus sequence of the 16S rRNAs of the members of the target group was found using MultAlin software ([9]; http://prodes.toulouse.inra.fr/multalin/), and the search for probes was performed with the use of an original software package, ProbeDesigner, compatible with a DOS or Windows environment (Lebedinsky, manuscript in preparation; see also Subbotina *et al.* [10] for brief description of the algorithms of the component programs). During the search for probes, consensus sequence fragments of a certain length taken with a step of one nucleotide were checked for their specificity for the target group with the help of oligonucleotide vocabularies that were created in advance proceeding from the 18331 sequences of 16S and 18S rRNAs from the files SSU_Prok.gb and SSU_Euk.gb of the RDP release 8.0 database ([11]; http: //rdp.cme.msu.edu/html/). The probes complementary to the specific sites found were ranged according to certain parameters such as percent hit of target sequences, minimal number of mismatches with nontarget sequences, minimal difference between the melting temperature of the perfect duplex and the duplexes formed with nontarget sequences (the melting temperatures and their difference were calculated according to two models), and the tendency to form hairpins and self-dimers.

Additionally, the probes designed were analyzed with respect to their specificity using the BLASTN program ([12]; http://www.ncbi.nih.gov/BLAST/) and with respect to their tendency toward hairpin and self-dimer formation using the OligoAnalyzer 2.5 program (http://www. idtdna.com).

The oligonucleotides were synthesized by the Syntol company (Russia; http://www.syntol.ru), with purification in polyacrylamide gel. The probes were labeled with Dig-11-dUTP, which was introduced by the 3'-tailing method in an enzymatic reaction catalyzed by terminal deoxynucleotidyl transferase (Fermentas, Lithuania, #EP0162) and carried out according to the manufacturer's recommendations [13]: the probes were incubated for 15 min at 37°C in 20 μl of a mixture con-

[1] T.P. Tourova, unpublished data; the sequence has been deposited in GenBank under accession number AY264344.

Dco 198, probe	5'-CGTTAACYCCYGCCACACC-3'
Staphylothermus marinus, site	3'-GC**t**ATTGGGGG**t**GG**c**GTGG-5'
Dco_mob 198, probe	5'-CGTTAACCCCTGCCACACC-3'
D. amylolyticus	3'-GCAATTGGGG**g**CGGTGTGG-5'
Desulfurococcus sp. 1312 , site	3'-GCAATTG**a**GGACGGTGTGG-5'
Dco_amy 198, probe	5'-CGTTAACCCCCGCCACACC-3'
D. mobilis	3'-GCAATTGGGG**a**CGGTGTGG-5'
Desulfurococcus sp. 1312, site	3'-GCAATTG**a**GG**a**CGGTGTGG-5'

Fig. 1. The probes designed and the target sites of the most appropriate negative controls. Mismatches are lowercased.

taining 1× buffer for transferase (200 mM Ca-cacodylate, pH 7.2, 1 mM $CoCl_2$, 0.1 mM DTT, and 0.01 vol % Triton X-100), 0.05 mM DIG-11-dUTP, 5 pmol/μl of probe, 0.5 mM dATP, and 2.5 U/μl of deoxynucleotidyl transferase. The reaction was terminated by adding 2 μl of 0.5 M EDTA (pH 8.0), and the probes were stored at –20°C.

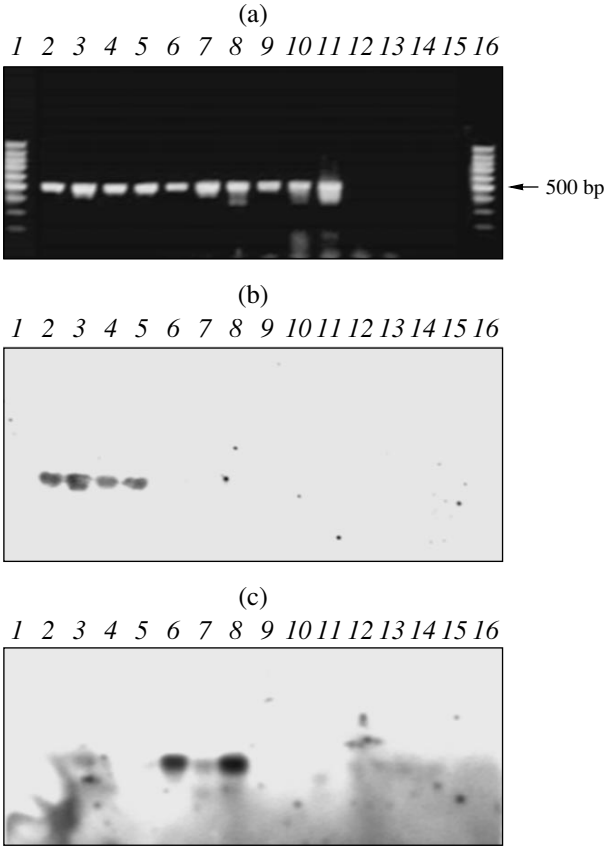

Fig. 2. Identification of members of the species *D. amylolyticus* and *D. mobilis*: (a) electrophoresis of PCR products obtained with *Crenarchaeota*-specific primers Cren 7F–Cren 518R and subsequent hybridization with the species specific probes (b) Dco_amy 198 and (c) Dco_mob 198. Lanes: (*1, 16*) molecular weight marker GeneRuler^{TM} 100 bp DNA Ladder(Fermentas, Lithuania); (*2*) strain 204; (*3*) strain 313; (*4*) strain 603; (*5*) *D. amylolyticus*; (*6*) *D. mucosus*; (*7*) *Desulfurococcus* sp. 1312; (*8*) *D. mobilis*; (*9*) *Staphylothermus marinus*; (*10*) *Pyrodictium abyssi*; (*11*) *Acidilobus aceticus*; (*12*) *Thermococcus peptonophilus*; (*13*) *Methanosarcina barkeri*; (*14*) *Thermoanaerobacter siderophilus*; (*15*) H_2O.

Hybridization of oligonucleotide probes and signal detection. PCR products obtained on the studied and control DNAs were transferred to a positively charged nylon membrane by the Southern method [14]. Further steps were performed according to protocols from a Boehringer Manheim laboratory manual ([15]; http://www.roche-applied-science.com/fst/products.htm?/prod_inf/manuals/dig_man/dig_toc.htm) with slight modifications; all reagents were from Boehringer Manheim. The DNA samples were fixed on the membrane by UV light for 3–4 min and kept for 2 min in 6× SSC (1× SSC contains 150 mM NaCl and 15 mM Na citrate, pH 7.0). After that, membrane prehybridization was performed to prevent nonspecific sorption of the oligonucleotide probe: membranes were incubated in prehybridization buffer (20 ml per 100 cm^2 of the membrane; 5× SSC, 0.1% *N*-lauroyl sarcosine, 0.02% SDS, 1% Blocking reagent, and 100 μg/ml poly(A) or pancreatic DNA). Prehybridization was carried out for 2 h at a temperature equal to the melting temperature of the probe, T_m, which was calculated according to the formula $T_m = 81.5 + 16.6\log M + 0.41[\%(G+C)] - 820/n$.

Then, the prehybridization solution was replaced with an analogous solution that contained, instead of poly(A) or pancreatic DNA, 5 pmol/ml of labeled probe (the probe stock solution was preheated for 2–3 min at 70°C to eliminate possible self-dimers). The hybridization solution was applied in an amount of 3 ml per 100 cm^2 of the membrane. Hybridization was carried out for 12–15 h at a temperature calculated by the formula $T_{hyb} = T_m - 12$°C (for species-specific probes) or $T_{hyb} = T_m - 14$°C (for genus-specific probe).

Then, the membrane was washed to remove unbound or nonspecifically bound probe. The optimal temperature of washing and composition of the washing solutions were found experimentally. To wash off the probe of negative controls, the membrane was first washed under low-stringency conditions: at room temperature, first in 2× SSC + 0.5% SDS for 5 min and then in 2× SSC + 0.1% SDS for 15 min (20 ml of each solution per 100 cm^2).

Hybridization of oligonucleotide probes with PCR products obtained with *Crenarchaeota*-specific primers Cren 7F–Cren 518R on DNA isolated from pure cultures and natural samples

Culture or sample	PCR with primers Cren 7F–Cren 518R	Hybridization with probes		
		Dco 198	Dco_amy 198	Dco_mob 198
Desulfurococcus amylolyticus, DSMZ 3822	+	+	+	–
Desulfurococcus mobilis, DSMZ 2161	+	+	–	+
Desulfurococcus mucosus, DSMZ 2162	+	+	–	+
Desulfurococcus sp. 1312	+	+	–	–
Staphylothermus marinus, DSMZ 3639	+	–	–	–
Pyrodictium abyssi, DSMZ 6158	+	–	–	–
Acidilobus aceticus, DSMZ 11585	+	–	–	–
Thermococcus peptonophilus, DSMZ 10343	–	–	–	–
Methanosarcina barkeri, DSMZ 800	–	–	–	–
Thermoanaerobacter siderophilus, DSMZ 12299	–	–	–	–
Stain 204	+	+	+	–
Strain 313	+	+	+	–
Strain 603	+	+	+	–
Strain 2601	+	+	+	–
Sample 801 (Geyser Valley, Kamchatka)	+	+	ND	ND
Sample 803 (Caldera Uzon, Kamchatka)	+	+	ND	ND
Sample 804 (Caldera Uzon, Kamchatka)	–	ND	ND	ND
Enrichment 804 (sample 804)	–	ND	ND	ND
Sample 805 (Caldera Uzon, Mutnovski volcano, Kamchatka)	–	ND	ND	ND
Sample 402 (Marine hydrotherm, Kunashir Island)	+	+	ND	ND

Note: "ND" stands for "not determined."

After that, signal detection was carried out as follows. The membrane was placed for several minutes into washing buffer (20 ml/100 cm^2; 100 mM disodium maleinate, 150 mM NaCl, pH 7.5, 0.3 vol % Tween 20). Then, the membrane was incubated for 30–60 min in blocking solution (20 ml/100 cm^2; 100 mM disodium maleinate, 1% Blocking reagent) to prevent nonspecific sorption of antibodies, after which this solution was removed and the membrane was incubated for 30–60 min in a solution (20 ml/100 cm^2) prepared by supplementing (in a ratio of 1 : 20000) the blocking solution with Fab fragments of antibodies against digoxygenin, bound with alkaline phosphatase (Anti-Dig-AP, Fab fragments). Following this, the membrane was incubated two times for 15 min in washing buffer (20 ml/100 cm^2) and placed for 3 min in detection buffer (20 ml/100 cm^2; 100 mM Tris–HCl, 100 mM NaCl, pH 9.5). Then, the membrane was placed in a plastic bag, and a 1% solution of chemiluminescent substrate (CDP-star) in detection buffer was applied to the membrane (0.5 ml/100 cm^2). To record the signal emitted by the labeled probe, the membrane was kept in the dark in contact with an X-ray film (RT-1V, Svema, Russia). After the exposition (4–12-h), the film was developed according to the manufacturer's recommendations.

Then, the same membranes were washed under high-stringency conditions: at a temperature equal to T_m – 12°C, first in 0.2× SSC + 0.1% SDS for 5 min and then in 0.2× SSC for 1 min (20 ml of each solution per 100 cm^2). Then, the detection procedure was repeated.

RESULTS AND DISCUSSION

Design of oligonucleotide probes. For the design of the genus-level probe specific for *Desulfurococcus* representatives (probe Dco 198), we used 16S rRNA nucleotide sequences of representatives of valid species of the genus: *D. mobilis* and *D. amylolyticus* (for the third valid species, *D. mucosus*, the 16S rRNA nucleotide sequence has not been determined). We also used the 16S rRNA sequence of *Desulfurococcus* sp. strain 1312, which, according to genotypic and phenotypic properties, represents a new species of the genus *Desulfurococcus* (Perevalova *et al.*, manuscript in preparation). We also designed probes Dco_mob 198 and Dco_amy 198 specific for two valid species of *Desulfurococcus*, *D. mobilis* and *D. amylolyticus*,

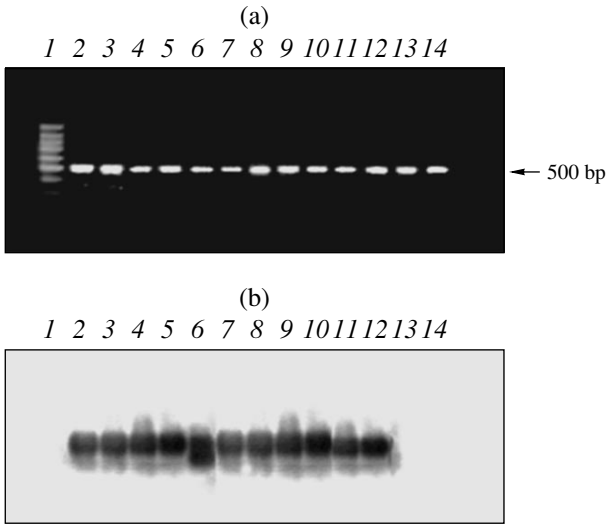

Fig. 3. Identification and detection of members of the genus *Desulfurococcus*: (a) electrophoresis of PCR products obtained with *Crenarchaeota*-specific primers Cren 7F–Cren 518R and (b) subsequent hybridization with the genus-specific probe Dco 198. Lanes: (*1*) molecular weight marker GeneRuler^{TM} 100 bp DNA Ladder (Fermentas, Lithuania); (*2*) strain 204; (*3*) strain 313; (*4*) strain 603; (*5*) strain 2601; (*6*) sample 803; (*7*) sample 801; (*8*) sample 402; (*9*) *D. amylolyticus*; (*10*) *D. mucosus*; (*11*) *Desulfurococcus* sp. 1312; (*12*) *D. mobilis*; (*13*) *Staphylothermus marinus*; (*14*) *Pyrodictium abyssi.*

respectively. In silico analysis showed that these probes had at least two nonterminal mismatches with 16S rRNAs (rDNAs) of nontarget organisms. There were only three exceptions: the probe targeting *D. mobilis* had a single mismatch with *D. amylolyticus* and with *Desulfurococcus* sp. 1312, and the probe specific for *D. amylolyticus* had a single mismatch with *D. mobilis.* The probes and the most adequate negative controls are shown in Fig. 1.

The following melting temperatures were calculated for the probes: 62°C for Dco 198, 64°C for Dco_mob 198, and 66°C for Dco_amy 198.

PCR with *Crenarchaeota*-specific primers. The DNA isolated from pure microbial cultures and natural samples was amplified using the primer pair Cren 7F–Cren 518R. As positive controls, we used DNA of desulfurococci and other representatives of *Crenarchaeota: Staphylothermus marinus, Pyrodictium abyssi,* and *Acidilobus aceticus.* DNA of the *Euryarchaeota* representatives *Thermococcus peptonophilus* and *Methanosarcina barkeri* and the bacterium *Thermoanaerobacter siderophilus* was used as negative controls (table, Fig. 2a). For positive controls, a single amplification product was obtained about 500 bp in size. For negative controls, no amplification products were recorded. For all of the four pure unidentified cultures of hypertheromophilic cocci (table, Figs. 2a, 3a) and for three environmental samples (table, Fig. 3a), formation of a single PCR product about 500 bp in size was observed. For the two other environmental samples, amplification products were not recorded.

The results of PCR with positive and negative controls illustrate the specificity of the Cren 7F–Cren 518R primer pair with respect to *Crenarchaeota* representatives. A detailed characterization of this primer pair will be given in a separate publication; here, we will only mention that its discriminating ability with respect to other organisms is based on the presence in crenarchaeotal 16S rRNA of very strong unique signatures in positions 27, 28, and 518 (*E. coli* numbering). As shown by our analysis, the specificity of these signatures, described, among other ones, by Winker and Woese in 1991 [16] on the basis of an analysis of only five crenarchaeotal (as well as 36 euryarchaeotal, 380 bacterial, and 50 eukaryal) sequences of SSU rRNA, has not been shaken over the last decade, despite the 50- to 100-fold increase in the database of aligned sequences. These signatures are adjoined by conserved sites of 16S rRNA. Due to these two factors, primers Cren 7F and Cren 518R form perfect duplexes with 16S rDNA of most *Crenarchaeota* and exhibit 3'-terminal mismatches with 16S rDNA of all other organisms (Lebedinsky *et al.*, manuscript in preparation). It should be mentioned here that one of our primers, Cren 7F, has an analogous predecessor [17].

Thus, taking into account the specificity of the primers Cren 7F–Cren 518R, the results of amplification of DNAs of the unidentified pure cultures allow their affiliation with *Crenarchaeota* to be inferred. The results of amplification of DNA isolated from natural samples show that, in three of them, *Crenarchaeota* were present in detectable amounts.

Tests of the specificity of oligonucleotide probes. To test the specificity of the oligonucleotide probes, we used DNA of the type strains of *Desulfurococcus* species (*D. amylolyticus, D. mobilis,* and *D. mucosus*), DNA of strain *Desulfurococcus* sp. 1312, and DNA of *Staphylothermus marinus, Pyrodictium abyssi,* and *Acidilobus aceticus,* which were the negative controls for the genus-level probe, as well as additional negative controls for species-level probes.

The probe Dco 198, targeting the genus *Desulfurococcus,* yielded positive reactions with PCR products obtained on the DNA of all representatives of this genus (table, Fig. 3), including *D. mucosus,* whose 16S rDNA has not yet been sequenced. No positive reaction was recorded after hybridization of this probe with PCR products obtained for *Staphylothermus marinus, Pyrodictium abyssi,* and *Acidilobus aceticus* (table, Fig. 3).

The species-level probe Dco_amy 198 yielded a positive signal with the amplicon of the type strain of its target species *D. amylolyticus* and no positive signals with PCR products obtained for other desulfurococci and other negative controls (Fig. 2b).

The species-level probe Dco_mob 198 gave a positive signal with the PCR product of the type strain of its target species *D. mobilis* and also with the PCR product

of *D. mucosus* (Fig. 2c). This result does not necessarily mean nospecificity of this probe because the 16S rDNA of *D. mucosus* has not been sequenced and it remains questionable to what extent its affiliation to a separate species is justified.

It should be mentioned that in the case of the probes Dco 198 and Dco_amy 198, washing under low-stringency conditions was sufficient to wash off the probe from negative controls, whereas the probe Dco_mob remained bound not only to the DNA of *D. mobilis*, but also to the DNA of *D. mucosus*, *D. amylolyticus*, and *Desulfurococcus* sp. 1312. Only under high-stringency conditions was Dco_mob 198 washed off from the DNA of *D. amylolyticus* and *Desulfurococcus* sp. 1312; this correlates with the fact that the probe Dco_mob 198 has a single mismatch with these negative controls (Fig. 1). The reaction of Dco_mob 198 with *D. mucosus* could be detected even after the high-stringency washing, and further increase in the washing temperature resulted in the disappearance of the signal from the positive control.

Identification of pure microbial cultures. The probes designed were used for identification of hyperthermophilic cocci isolated from terrestrial hydrotherms of Kamchatka and Kunashir Island. Hybridization of amplificates obtained for strains 204, 313, 603, and 2601 with the Dco 198 genus-level probe yielded positive results (Fig. 3b). Hybridization of all of these strains with the Dco_amy species-level probe also produced positive signals (Fig. 2b), whereas the results of their hybridization with the Dco_mob probe were negative (Fig. 2c). Thus, the four unidentified strains may be assigned to *Desulfurococcus amylolyticus.*

Detection of *Desulfurococcus* representatives in natural samples. The genus-level probe Dco 198 was used for the detection of *Desulfurococcus* representatives in freshwater hydrotherms of Kamchatka and a coastal marine hydrotherm at Kunashir Island; a total of five natural samples were investigated (table). In all cases where PCR with the *Crenarchaeota*-specific primers gave positive results, subsequent hybridization with the probe Dco 198 also yielded a positive signal (table, Fig. 3c).

Molecular biological methods making possible the investigation of the composition of microbial communities without cultivation methods have been rapidly developing over the last decade (see, e.g., [18, 19]). The molecular biological method for the detection of hyperthermophilic archaea of the genus *Desulfurococcus* that we propose in this work allows representatives of this genus to be rapidly and reliably screened for in pure cultures, enrichments, and natural samples. Thus, with the use of the species-level probe Dco_amy 198 we were able to identify four pure cultures of hyperthermophilic cocci as representatives of *D. amylolyticus.* We also managed to detect *Desulfurococcus* representatives in natural samples without cultivation. Two of the natural samples that gave a positive hybridization signal were taken from environments typically inhabited by desulfurococci (samples 801 and 803), whereas sample 402 was taken form a shallow-water marine hydrotherm near Kunashir Island. So far, there has been only one report on the presence of desulfurococci in marine environments, namely deep-sea hot vents [20]; however, these data were not supported by 16S rRNA analyses. Our data confirm the presence of archaea of genus *Desulfurococcus* not only in terrestrial, but also in shallow-water submarine habitats.

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