

feeding and related behavioural strategies among hybodonts might have been as diverse as among living sharks and rays.

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A pivotal Archaea group

Barns *et al.* have identified two novel Archaea sequence types in an analysis of organisms from a hot spring in Yellowstone National Park¹. These types are different from all known archaeal sequences, and seem to represent a pivotal group, Korarchaeota, in the phylogenetic tree². The ability to culture and to identify these organisms is critical for understanding the evolution of the Archaea and Eukarya.

To cultivate the organisms in the laboratory, we took anaerobic water and sediment samples (pH 5.5; 70–90 °C) from Obsidian Pool, a hot spring in the mud volcano area of Yellowstone National Park, Wyoming¹. We set up a continuous culture at 85 °C in the laboratory (Fig. 1). After about 1 week,

we found roughly 2×10^8 cells per ml of very complex community with heterogeneous morphologies (cocci, rods and filaments). We determined that the appearance of the community remained stable for more than a year, using phase-contrast microscopy. Whole-cell hybridization with fluorescently labelled oligonucleotide probes targeted to Archaea- and Bacteria-specific regions within the 16S ribosomal RNA^{3–5} reveals that most of the organisms (more than 90%) belong to the Archaea.

Korarchaeota have, so far, been detected only by analyses of 16S rDNA sequences obtained directly from the environment^{1,2}. To detect members of this new archaeal kingdom in the mixed culture, we isolated DNA twice monthly for more than a year, and used it in polymerase chain reactions with primers highly specific for the 'korarchaeal' 16S rRNA gene (Fig. 1). All isolated DNA produced sequences identical to the korarchaeal sequence type pJP27 (GenBank accession no. L25852).

We determined the morphology of the Korarchaeota by whole-cell hybridizations^{3,6,7} using different fluorescently labelled oligonucleotide probes complementary to three regions of the korarchaeal 16S rRNA sequence (Fig. 1). We checked the sequence specificity of the probes by comparison with all 16S rRNA sequences available in databases, including the sequences obtained by *in situ* analyses of the Yellowstone hot spring^{1,2}. We also evaluated the specificity of the probes by hybridizations of pure cultures from known hyper-

thermophilic Archaea and Bacteria. Only those cells reacting to two differently labelled korarchaeal probes in a single hybridization experiment were identified as Korarchaeota.

As a positive control for the other organisms present in the culture, we used a universal probe in hybridization experiments. Very few cells (about 10^4 – 10^5 cells per ml) gave a positive hybridization signal with the korarchaeal probes. The organism corresponding to the sequence type pJP27 is rod-shaped, variable in length (between 5 and 10 μm) and most of the cells are slightly curved (Fig. 1). The diameter of the cells is about 0.5 μm . We detected dividing, actively growing cells. The number of these cells within the 85 °C mixed culture was stable for more than a year, indicating that this member of the Korarchaeota is a hyperthermophile.

To characterize the Korarchaeota, it is crucial to grow these organisms in the laboratory. Here we have shown that they can be cultivated as a stable population in a mixed laboratory culture. Isolation of the Korarchaeota into a pure culture for study of their biochemical and physiological properties in detail should improve our understanding of phylogenetic relationships during the early stages in the evolution of life.

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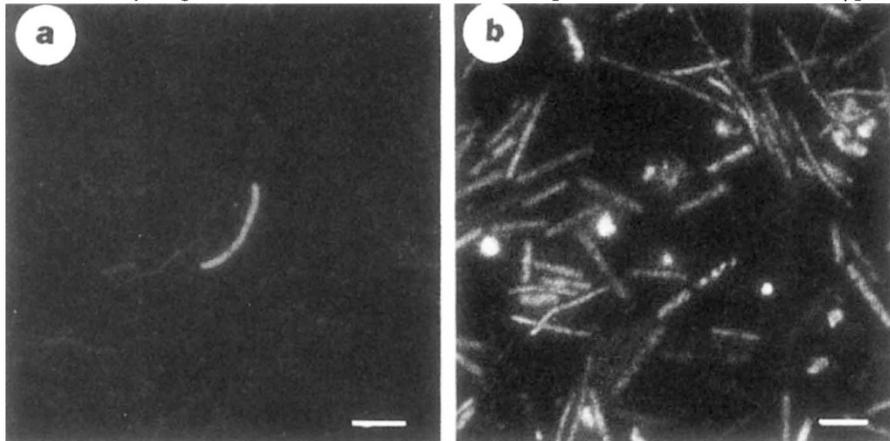


Figure 1 Micrographs of the mixed culture at 85 °C, taken on a Nikon Microphot EPI-FL microscope. Scale bars, 5 μm . a, Whole-cell hybridization with the fluorescently labelled probe 1135R (5'-GTTG-CCCGGCCAGCCGTAA-3') specific for Korarchaeota. Similar results were obtained with the probes 604R (5'-TGTCTTCAGGCGGATTAAC-3') and 546R (5'-AGTATGCGTGGGAACCCCTC-3') and combinations of these probes labelled with different fluorescence dyes. b, Diaminophenylindole-stained cells, showing the different morphotypes present in the culture. The primers used in the polymerase chain reaction to amplify 'korarchaeal' 16S rDNA specifically were 5'-GAGGCCCCAG-GRTGGGACCG-3' (236F) and 5'-GTTGCCCCGCCAGCCGTAA-3' (1135R). For cultivation, we placed ~400 ml of anaerobic water and sediment sample and ~400 ml anaerobic original spring water, supplemented with 0.001% yeast extract, 0.005% peptone and 0.005% $\text{Na}_2\text{S}_2\text{O}_3$ into a two-walled glass vessel (chemostat). We heated the sample to 85 °C by pumping heated glycerol between the two walls of the chemostat. The chemostat had a total volume of ~800 ml and was 'aerated' with N_2 and CO_2 (80/20 v/v, 20 ml min^{-1}). Later we substituted the original spring water for an anaerobic, low-salt medium (dilution rate, 20 ml h^{-1}) containing the same nutrients⁸.

Fullerene 'crop circles'

While examining laser-grown single-wall carbon nanotube (SWNT) material^{1,2} by scanning force and transmission electron microscopy, we regularly observed circular formations of SWNT ropes (Fig. 1). These ropes consist of between 10 and 100 individual nanotubes aligned over their entire length, packed in a two-dimensional crystalline array. Sceptical that these objects might be perfectly seamless toroidal nanotubes, we named them 'crop circles', but we are now convinced that many, perhaps most, of the individual tubes in these circular ropes are indeed perfect tori.

We estimate that between 0.01 and 1%