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Picrophilus oshimae and *Picrophilus torridus* fam. nov., gen. nov., sp. nov., Two Species of Hyperacidophilic, Thermophilic, Heterotrophic, Aerobic Archaea

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We describe two species of hyperacidophilic, thermophilic, heterotrophic, aerobic archaea that were isolated from solfataric hydrothermal areas in Hokkaido, Japan. These organisms, *Picrophilus oshimae* and *Picrophilus torridus*, represent a novel genus and a novel family, the *Picrophilaceae*, in the kingdom *Euryarchaeota* and the order *Thermoplasmales*. Both of these bacteria are more acidophilic than the genus *Thermoplasma* since they are able to grow at about pH 0.

The moderately thermophilic, hyperacidophilic, aerobic archaea (archaebacteria) (7) *Picrophilus oshimae* and *Picrophilus torridus*, which have been described previously (4, 5), were isolated from moderately hot hydrothermal areas in solfataric fields in Hokkaido, Japan. One of the sources of isolation was a solfataric spring which had a temperature of 53°C and a pH of 2.2, and the other was a rather dry hot soil which had a pH of <0.5. Pure strains (strains DSM 9789^T [T = type strain] and DSM 9790, respectively) were obtained from single colonies that grew in 4 days at 60°C on 12.5% starch gels containing 2 g of yeast extract per liter at pH 1.

Both organisms utilize 1 to 5 g of yeast extract per liter as a carbon source by respiration and appear to be unable to grow by fermentation or chemolithoautotrophically by formation of H_2S from sulfur and hydrogen or by sulfur respiration. They grow at about pH 0 and optimally at pH 0.7; and they grow at 47 to 65°C and optimally at 60°C.

As determined by an analysis of 16S rRNA sequences, the closest relative of these organisms is *Thermoplasma acidophilum*, but *P. oshimae* and *P. torridus* are sufficiently distinct to be considered representatives of a different family, the *Picrophilaceae*, in the order *Thermoplasmales*. These bacteria can be distinguished by their growth characteristics, especially their hyperacidophilia, by the presence of a filigree tetragonal S layer, and by the presence of an RNA polymerase that has a different subunit pattern and does not cross-react with an antibody against the RNA polymerase of *T. acidophilum* in the immunodiffusion assay of Ouchterlony (8). On a phylogenetic tree based on 16S rRNA sequences in which transitions are neglected, members of the order *Thermoplasmales*, including the genus *Picrophilus*, form a deep branch in the kingdom *Euryarchaeota* between the *Thermococcales* and the methanogens, which is consistent with the position of the genus *Thermoplasma* on phylogenetic trees based on the large components of DNA-dependent RNA polymerase.

A detailed description of the genus *Picrophilus*, including the phylogeny of this taxon and its growth characteristics, is given elsewhere.

Description of *Picrophilaceae* Schleper, Zillig, and Pühler fam. nov. *Picrophilaceae* (Pi.cro.phi.la' ce.ae. Gr. adj. *pikros*, sour, acidic, sharp; Gr. adj., *philos*, loving; L. ending *aceae*, family; *Picrophilaceae*, acid-loving family) is another family belonging to the order *Thermoplasmales* besides the *Thermoplasmaceae* which comprises acid-loving (i.e., hyperacidophilic) organisms. Separation of these taxa is justified by their phylogenetic distance, (9.5% difference in the 16S rRNA sequences of members of the *Picrophilaceae* and *T. acidophilum*), by the lack of immunochemical cross-reactions in Ouchterlony immunodiffusion assays between the RNA polymerases of *P. oshimae* and *T. acidophilum*, which also do not occur between members of the closely related families *Thermoproteaceae* and *Thermophilaceae*, and by the presence of an S layer in the genus *Picrophilus* but not in the genus *Thermoplasma*.

Description of *Picrophilus*, Schleper, Zillig, and Pühler gen. nov. *Picrophilus* (Pi.cro.phi' lus. Gr. adj. *pikros*, acidic; Gr. adj. *philos*, loving; N. L. masc. n. *Picrophilus*, acid-loving organism). Obligately aerobic, heterotrophic, moderately thermophilic, hyperacidophilic, irregular cocci. Different dividing states occur beside each other, as determined by microscopic examination (Fig. 1). Grows at temperatures between 47 and 65°C and optimally at 60°C. Grows at pH values between about 0 and 3.5 and optimally at pH 0.7. Grows in the presence of 0.1 to 0.5% yeast extract and optimally in the presence of 0.2% yeast extract; a lag phase occurs in the presence of 0.5% yeast extract. Under optimal conditions, the generation time is 6 h. Slow

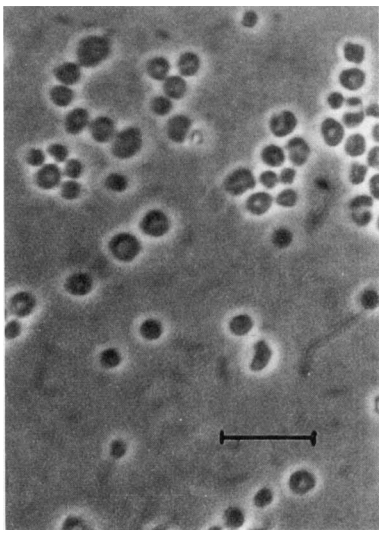

FIG. 1. Phase-contrast micrograph of *P. oshimae*. Bar = 5 μm.

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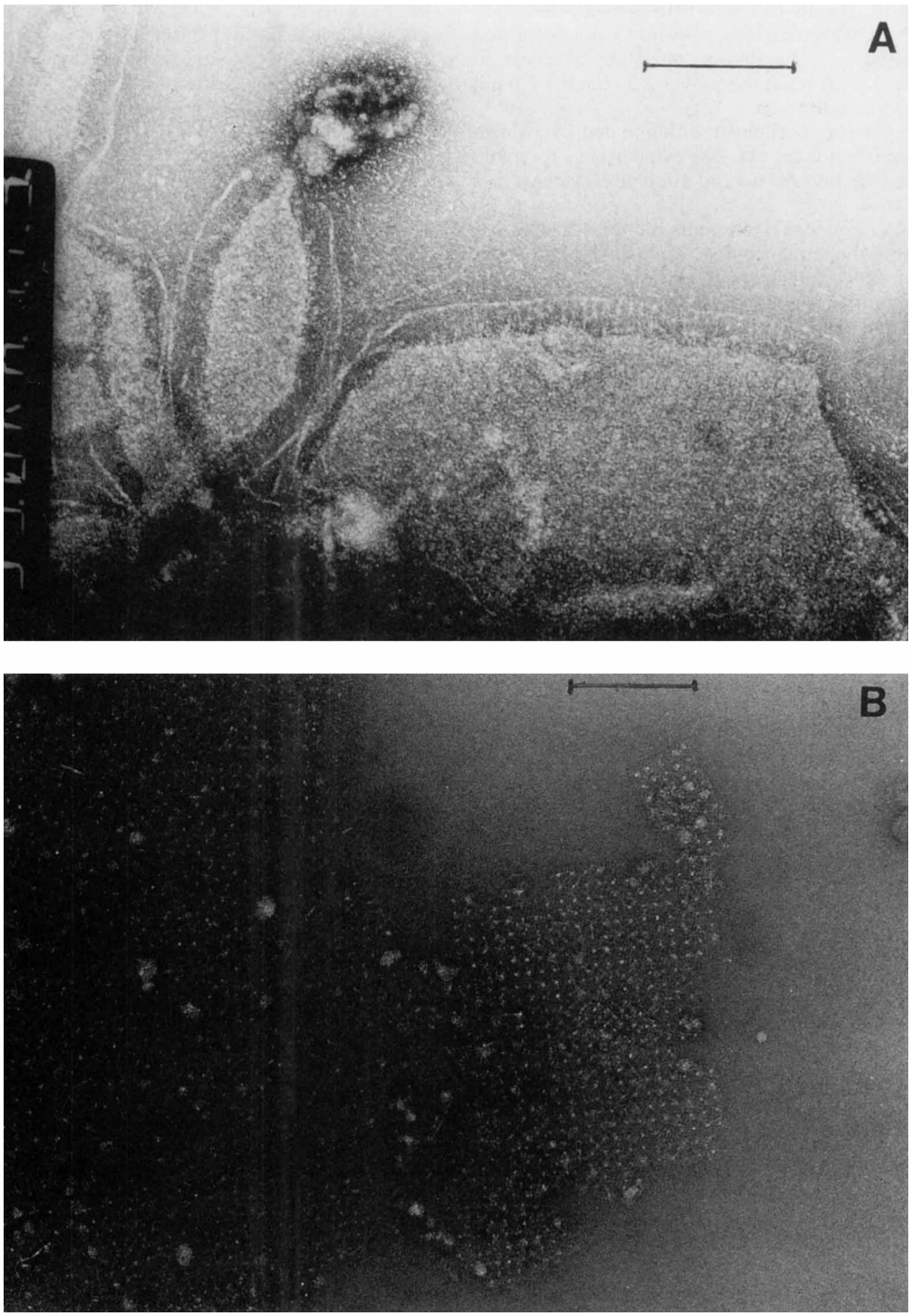

FIG. 2. (A) Electron micrograph of a cell of *P. oshimae*, showing a seam essentially representing a side view of the S layer. (B) Top view of a piece of an isolated S layer. Both preparations were negatively contrasted with uranyl acetate. Both photographs were supplied by Ute Santarius and Wolfgang Baumeister. Bars = 0.2 μm.

growth to a low density occurs on tryptone, and no growth occurs on many other carbon sources.

The diameter of the cells is about 1 μm. Division occurs by constriction. The division intermediates resemble those of *Thermococcus* strains. There is a regular filigree teragonal S layer, which probably has polysaccharide chains on the outside (Fig. 2).

Two species are known, type species *P. oshimae* and *P. torridus.*

Description of type species *Picrophilus oshimae* Schleper, Zillig, and Pühler sp. nov. *Picrophilus oshimae* (o.shi' mae. M.L. gen. n. *oshimae*, of Oshima, referring to Japanese biochemist Tairo Oshima, who furnished invaluable help in organizing our collecting trip).

Shape, size, and growth characteristics are as described above for the genus. Large cavities, apparently not separated from the cytoplasm by a membrane, often are observed in sections. The lattice constant of the S layer is 20 nm (6). No pili or flagellae are observed.

The bis-phytanyl tetraetherlipids are dominated by a single phosphoglycolipid component (1). The cytochromes resemble those of the genus *Thermoplasma* and are type *b* cytochromes (3).

The 16S rRNA sequence (accession number X84901) is 9.5% different from the sequence of *T. acidophilum* and about 3% different from the sequence of *P. torridus.*

The G+C content of the DNA is 36 mol%. The *Eco*RI restriction pattern of the DNA is similar to, but characteristically different from, that of *P. torridus*. Two different but partially homologous plasmids, one about 8.3 kbp long and the other about 8.8 kbp long, occur in some but not all strains. One strain contains both plasmids. The type strain is strain DSM 9789.

Description of *Picrophilus torridus* Zillig, Schleper, and Pühler sp. nov. *Picrophilus torridus* (tor' ri.dus. L. part. *torridus*, dried, burned, referring to the fact that the species was found in rather dry hot soil). The DNA restriction pattern resembles, but is clearly different from, that of *P. oshimae*; the 16S rRNA sequences of the two organisms differ by 3% at the 250 positions analyzed. At least as acidophilic as *P. oshimae*. Grows significantly faster than *P. oshimae*. No plasmid is present.

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