Hydrogenophilus thermoluteolus gen. nov., sp. nov., a thermophilic, facultatively chemolithoautotrophic, hydrogen-oxidizing bacterium

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The taxonomic positions of 'Pseudomonas hydrogenothermophila' strain TH-1 and 'Flavobacterium autothermophilum' strain TH-4 were studied by 16S rDNA sequencing. These organisms are Gram-negative, strictly aerobic, thermophilic, facultatively chemolithoautotrophic hydrogen-oxidizing rods and have a DNA G+C content of 63-65 mol%. The major isoprenoid quinone is ubiquinone-8 and 3-hydroxy decanoic acid (3-OH C10:0) is the major 3-hydroxy cellular fatty acid. A phylogenetic analysis based on 16S rDNA sequences placed strains TH-1T and TH-4 in the β-subclass of the Proteobacteria. The taxonomic characteristics of these organisms are different from those of previously described aerobic, facultatively chemolithoautotrophic, hydrogen-oxidizing bacteria that belong to the β-subclass of Proteobacteria. On the basis of the information described above, a new genus and species, Hydrogenophilus thermoluteolus gen. nov., sp. nov., is described to include both strains. The type strain is strain TH-1T (= IFO 14978T).

Keywords: Hydrogenophilus thermoluteolus gen. nov., sp. nov., hydrogen-oxidizing bacterium, thermophile, 16S rRNA

INTRODUCTION

Many aerobic, hydrogen-oxidizing (Knallgas) bacteria have been isolated and identified as Gram-negative or Gram-positive organisms (Schlegel, 1989). These include unique genera, Hydrogenobacter (Kawasumi et al., 1984; Shima & Suzuki, 1993), Calderobacterium (Kryukov et al., 1983), Aquific (Huber et al., 1992) and Hydrogenobacterium (Nishihara et al., 1991). Five facultatively chemolithoautotrophic, hydrogen-oxidizing Pseudomonas species have been reclassified as members of the genus Hydrogenophaga (Willems et al., 1989).

Two aerobic, hydrogen-oxidizing bacteria, strains TH-lT and TH-4, were isolated from the soil around a hot spring (Goto et al., 1977). These strains are facultative chemolithoautotrophs that are able to grow not only autotrophically using H2, O2 and CO2, but also heterotrophically in organic media. The optimum temperatures for the growth of strains TH-1T and TH-4 are 52 and 50 °C, respectively. The properties of both strains have been described previously. In early taxonomic studies, strains TH-1T and TH-4 were identified as 'Pseudomonas hydrogenothermophila' and 'Flavobacterium autothermophilum', respectively (Goto et al., 1978). However, more recent studies on their cellular fatty acid composition and isoprenoid quinone composition revealed that both strains TH-1T and TH-4 belonged to 'P. hydrogenothermophilica' (Takeuchi & Yokota, 1991). In the present study, the 16S rDNA sequences of the two strains have been determined and we propose a new genus and species, Hydrogenophilus thermoluteolus gen. nov., sp. nov., for the strains TH-1T and TH-4 on the basis of the phylogenetic and chemotaxonomic results.

METHODS

Bacterial strains. Strains TH-1T (= IFO 14978T) and TH-4 (= IFO 14593) were isolated from soil around a hot spring in Izu peninsula, Shizuoka Prefecture, Japan (Goto et al.,

The EMBL/DBJ/GenBank accession numbers for the sequences reported in this paper are AB009828 (strain TH-1T) and AB009829 (strain TH-4).
1977). *Alcaligenes faecalis* strain IAM 12369T, used as a negative control for DNA–DNA hybridization, was obtained from the IAM culture collection.

**Morphological characteristics.** The cell morphology of strain TH-1T was studied by using cells grown under heterotrophic conditions with malate (Goto et al., 1978). The sample used for scanning electron microscopy was prepared by fixing cells with 1% glutaraldehyde, dehydrating the cells with a graded acetone series, freeze-drying and finally sputter-coating the preparation with platinum under a vacuum (E-1030 Ion sputter; Hitachi). Electron microscopy was performed with a Hitachi S-4500 scanning electron microscope.

**Isolation of DNA and sequencing.** The methods used for the isolation of DNA for 16S rDNA cloning. DNA sequencing and sequence data analysis were described previously (Ishida et al., 1997). Strains TH-1T and TH-4 and *A. faecalis* were cultivated in 2× YT broth (Sambrook et al., 1989) and DNA was isolated as described previously (Yokoyama et al., 1995) for DNA–DNA hybridization. The primers used for PCR amplification of 16S rDNA were: forward primer, 5' AGAGTTTGATCCTGGCTCAG 3' (Escherichia coli numbering system); and reverse primer, 1512R (5' ACGGCTACCTTGTTACGACT 3'). Recombinant DNA manipulation was carried out as described by Sambrook et al. (1989).

**DNA–DNA hybridization.** Levels of DNA relatedness were determined by the method of Ezaki et al. (1989).

**Phylogenetic analysis.** The new 16S rDNA sequences of the two strains were multiple aligned using CLUSTAL W version 1.6 (Thompson et al., 1994) with a selection of reference sequences of Proteobacteria obtained from the DDBJ, EMBL, GenBank and Ribosomal Database Project (RDP) databases. The multiple sequence alignment was then corrected manually as necessary. A phylogenetic tree was constructed from evolutionary distance data by applying the algorithm of the neighbour-joining method (Saitou & Nei, 1987) to *K* values (Kimura, 1980). To evaluate the robustness of the branches of the inferred tree, the bootstrap resampling method of Felsenstein (1985) was used with 1000 replicates.

**RESULTS AND DISCUSSION**

**Morphology**

The morphologies of strains TH-1T and TH-4 have been described previously (Goto et al., 1978). The present study contributes the result of an electron micrograph to the morphological study of strain TH-1T. Cells of TH-1T showed a straight-rod form and were about 0.5 μm in diameter and 2.0 μm in length (Fig. 1). This result agrees with the previous report on its morphological characteristics (Goto et al., 1978). Previously, TH-1T was reported to be motile, but motility was not observed in cells of strain TH-1T in this study. Strain TH-4 was not motile.

**Phylogenetic analysis**

Almost complete 16S rDNA sequences of strains TH-1T and TH-4 (1461 nucleotides) were determined. The sequence of strain TH-1T showed high identity (99.5%) to that of strain TH-4. The phylogenetic tree constructed by the neighbour-joining method and *K* values showed that these strains belonged to the β-subclass of the Proteobacteria. These results agree with the studies of the polyamine distribution pattern (Hamana et al., 1991). Subsequently, the results of a comparison with the sequences of representative genera of the β-subclass showed that the sequences of the two strains were not closely related to those of members of any other genera. The tree clearly indicated that the two strains formed a novel lineage and a deep branch within the β-subclass of the Proteobacteria (Fig. 2). The two strains seemed to be a new genus among the members of β-subclass of the Proteobacteria.

DNA–DNA hybridization experiments demonstrated that strain TH-1T showed 89% DNA relatedness to strain TH-4. These results indicated that the strains belonged to the same genus and the same species.

Major properties of the two strains were compared with those of facultatively chemolithoautotrophic, hydrogen-oxidizing bacteria in the β-subclass of the Proteobacteria. The genus *Hydrogenophaga* includes mesophilic, facultatively chemolithoautotrophic, hydrogen-oxidizing bacteria, and four species are included in this genus, which were transferred from the genus *Pseudomonas* (*Hydrogenophaga flava*, formerly *Pseudomonas flava*; *Hydrogenophaga palleronii*, formerly *Pseudomonas palleronii*; *Hydrogenophaga pseudoflava*, formerly *Pseudomonas pseudoflava* and *Pseudomonas carboxydoflava*; and *Hydrogenophaga taeniospiralis*, formerly *Pseudomonas taeniospiralis*) (Willems et al., 1989). *Alcaligenes latus* and *Alcaligenes eutrophus* are aerobic, hydrogen-oxidizing bacteria (Aragno & Schlegel, 1992), although *Alcaligenes eutrophus* has been reclassified as *Ralstonia eutropha* (Yabuuchi et al., 1995). *Acidovorax delafeldii* and *Acidovorax facilis*, which formerly belonged to the genus *Pseudomonas*, are facultatively hydrogen-oxidizing bacteria (Willems et al., 1990). *Aquaspirillum autotrophicum* is also known to be a facultatively
Hydrogenophaga thermoluteolus gen. nov., sp. nov.

Hydrogenophaga (Hy.dro.ge.no'phi.lus. Gr. n. hydor water; Gr. v. genein to produce; M.L. neut. n. hydrogenum hydrogen (that which produces water); Gr. adj. philo loving, friendly to; M.L. masc. n. Hydrogenophilus hydrogen lover.

Cells are straight rods, Gram-negative and non-sporulating. Chemolithoautotrophic, using molecular hydrogen as an electron donor and carbon dioxide as a carbon source. Carbon dioxide is fixed via the Calvin–Benson cycle. The G+C content of the DNA is about 63–65 mol% (Tm). Straight-chain saturated C16:0 and C18:1 acids are the major components of the cellular fatty acids and 3-OH C19:0 is the major 3-hydroxy cellular fatty acid. Ubiquinone-8 is the major component of the quinone system.

Description of Hydrogenophilus thermoluteolus sp. nov.

Hydrogenophilus thermoluteolus (ther.mo.lu.te'o.lus. Gr. adj. thermos hot; L. adj. n. luteus light-yellow; M.L. masc. adj. thermoluteus hot and light-yellow).

Exponentially growing cells are 0.5–0.6 x 2.0–3.0 μm and occur singly. Colonies are dull yellow. Heterotrophic, using acetate, propionate, butyrate, succinate, DL-lactate, pyruvate and α-ketoglutarate as electron donors and carbon sources. Ammonium ions, nitrate ions and urea are utilized solely as nitrogen sources but nitrite ions and gaseous nitrogen are not. The optimum temperature for growth is about 50–52 °C. The optimum pH for growth is around 7.0. Isolated from soil around a hot spring in Izu peninsula, Shizuoka Prefecture, Japan. The type strain is strain TH-1T (=IIFO 14978T).

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At least five species, Bacillus schlegelii, Bacillus tusciae, Hydrogenobacter thermophilus, Calderobacterium hydrogenophilum and 'Pseudomonas thermophilta', have been reported to be thermophilic, aerobic, carbon-oxidizing bacteria (Aragno, 1992). Strains TH-1T and TH-4 have high optimum temperatures for growth but are taxonomically and phylogenetically quite different from these species.

On the basis of the findings described above, we propose that strains TH-1T and TH-4 should be placed in a new genus as Hydrogenophilus thermoluteolus gen. nov., sp. nov.

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REFERENCES


