Hydrogenophilus

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2. **KEYWORDS:** *Hydrogenophilus*, *Hydrogenophilus thermoluteolus*, *Hydrogenophilus hirschii*, *Hydrogenophilus islandicus*, facultative chemolithoautotrophs, thermophile

3. ABSTRACT:

Straight rods 0.4–0.8 × 1.0–3.0 µm during exponential growth. Occur singly or in pairs (in *H. islandicus* and *H. thermoluteolus*). Motile or nonmotile. Gram negative. Nonsporulating. Aerobic or microaerobic, having a strictly respiratory type of metabolism, with oxygen or nitrate as the terminal electron acceptor. **Colonies are yellow or greyish**. **Thermophilic**; two species grow optimally at 50–55°C; and another at 63°C. **Facultatively chemolithoautotrophic**; can use H_2 as an electron donor and CO_2 as a carbon source. CO_2 is fixed via the Calvin-Benson cycle. Acetate, pyruvate, DL-lactate, and DL-malate can be used as electron donors and carbon sources. Ammonium can be used as a nitrogen source. The major quinone system is ubiquinone 8. Isolated from hot springs and surrounding soil.

The mol% G + C of the DNA is: 61–65.

Type species: ***Hydrogenophilus thermoluteolus*** Hayashi, Ishida, Yokota, Kodama and Igarashi 1999, 785.[VP]

4. DEFINING PUBLICATION:

Hydrogenophilus, Hayashi, Ishida, Yokota, Kodama and Igarashi 1999, 785[*VP*]

5. ETYMOLOGY:

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

6. GENERIC DEFINITION:

Straight rods 0.4–0.8 × 1.0–3.0 µm during exponential growth. Occur singly or in pairs (in *H. islandicus* and *H. thermoluteolus*). Motile or nonmotile. Gram negative. Nonsporulating. Aerobic or microaerobic, having a strictly respiratory type of metabolism, with oxygen or nitrate as the terminal electron acceptor. **Colonies are yellow or greyish**. **Thermophilic**; two species grow optimally at 50–55°C; and another, at 63°C. **Facultatively chemolithoautotrophic**; can use H_2 as an electron donor and CO_2

as a carbon source. CO_2 is fixed via the Calvin-Benson cycle. Acetate, pyruvate, DL-lactate, succinate, and DL-malate can be used as electron donors and carbon sources. Ammonium can be used as a nitrogen source. The major quinone system is ubiquinone 8. Isolated from hot springs and surrounding soil.

The mol% G + C of the DNA is: 61–65.

Type species: ***Hydrogenophilus thermoluteolus*** Hayashi, Ishida, Yokota, Kodama and Igarashi 1999, 785.[VP]

Number of species with validated names: 3

7. FAMILY CLASSIFICATION:

Hydrogenophilaceae (fbm00184)

8. FURTHER DESCRIPTIVE INFORMATION

8.1. Cell morphology

The genus *Hydrogenophilus* currently harbors the species *H. thermoluteolus*, *H. hirschii* and *H. islandicus* (Hayashi et al., 1999; Stöhr et al., 2001; Vésteinsdóttir et al., 2011). Cells of all species are Gram-staining negative, non-spore forming straight rods. Cells occur mostly single but can also occur in pairs (in *H. islandicus* and *H. thermoluteolus*). Concerning motility, Hayashi et al. (1999) found the type strain (TH-1) of *H. thermoluteolus* to be nonmotile; however, Goto et al. (1978) reported the same strain to be motile. Reference strain TH-4 is nonmotile (Hayashi et al., 1999). The type strain of *H. hirschii* is motile by a single polar flagellum; also the type strain of *H. islandicus* is described as being motile. *H. thermoluteolus* cells are 0.5-0.6 µm wide and 2.0-3.0 µm long, *H. hirschii* are 0.6-0.8 µm wide and 1.0-1.5 µm long, and *H. islandicus* cells are 0.4 µm wide and 2.6 µm long.

8.2. Colonial and cultural characteristics:

On solid culture medium, *H. thermoluteolus* and *H. hirschii* form dull yellow and yellow colonies, respectively, while *H. islandicus* form greyish colonies.

8.3. Nutrition and growth conditions:

Hydrogenophilus can grow under aerobic or microaerobic conditions, on the presence of H_2. *H. thermoluteolus* revealed a remarkably growth improvement in the presence of a gas mixture of H_2:O_2:CO_2 = 7:2:1 (Goto et al., 1978). *H. hirschii* growth is inhibited by O_2 concentrations of 5% or more. *H. thermoluteolus* was the only species able to grow with thiosulphate. All the species have optimum growth temperatures equal or above the

50 ºC: 50-52 ºC for *H. thermoluteolus*, 63 ºC for *H. hirschii*, and 55 ºC for *H. islandicus*. The optimum pH is 7 for *H. thermoluteolus* and *H. islandicus*, and 6.5 for *H. hirschii*. *H. hirschii* growth is inhibited in salt concentrations of 2% or higher.

Members of the three species can use acetate, pyruvate, DL-lactate, and DL-malate, but not D-xylose, D-galactose, sucrose or formate as carbon source. Growth factors are not required by *H. hirschii* or *H. islandicus*. *H. islandicus* is able to use D-fructose or D-glucose in the presence of 2 g/L yeast extract.

8.4. Metabolism and metabolic pathways:

Hydrogenophilus are facultative chemolithoautotrophs, able to use H_2 as electron donor, O_2 as electron acceptor and CO_2 as carbon source. Carbon dioxide is fixed via the Calvin-Benson cycle. Heterotrophic growth may occur at expenses of organic substrates. Ammonium can be used as sole nitrogen source by *H. hirschii* and *H. thermoluteolus*, which can also use nitrate or urea for this purpose. *H. hirschii* can use nitrate as final electron acceptor.

8.5. Chemotaxonomic characteristics:

The major quinone is ubiquinone 8. The fatty acid $C_{16:0}$ is the most abundant in all *Hydrogenophilus* species. The species differ in the relative abundance of other fatty acid components, with $C_{18:0}$ among the most abundant in *H. thermoluteolus,* cyclo $C_{17:0}$ and cyclo $C_{19:0}$ in *H. hirschii*, and $C_{18:1}$ *ω7c,* cyclo $C_{17:0}$ and $C_{19:0}$ *ω8c* in *H. islandicus*.

8.6. Genome

From the three species validly described only the genome of the *H. thermoluteolus* type species TH-1 is sequenced (Arai et al., 2018). It comprises a circular chromosome of 2.2 Mbp, with a 61.7% GC content, and a plasmid (pTH1) of approximately 66 Kbp, with a 58.3% GC content. Three rRNA operons and 2077 predicted protein-coding genes were identified.

8.7. Ecology and Habitat:

The members of the three *Hydrogenophilus* species were isolated from hot springs or surrounding soil in Japan (*H. thermoluteolus*), USA (*H. hirschii*) and Iceland (*H. islandicus*).

9. ENRICHMENT AND ISOLATION PROCEDURES

Isolation and culture conditions for *H. thermoluteolus* have been described by Goto et al. (1977). The isolation of *H. hirschii* - a microaerophilic organism - has been described by Stöhr et al. (2001); and of *H. islandicus* by Vésteinsdótiir et al. (2011). *H. hirschii* was isolated in the autotrophic medium of Huber et al. (1992), lacking NaCl and containing 0.02% $CaCl_2 \cdot 2H_2O$. The medium was deoxygenated with N_2 and dispensed in 20 ml portions into 120 ml serum bottles under N_2. The gas phase in the bottles was changed to H_2:CO_2 (80:20, v/v), and the medium was sterilized at 100 °C for 90 min and cooled. To each bottle was added 100 µl of a 10% sterile solution of $CaCO_3$ and 20 ml of filter-

sterilized air. After several passages of serially diluted cultures, the cultures were plated onto the isolation medium solidified with 1.5% agar to obtain yellow colonies consisting of motile rods. *H. islandicus* was isolated in the mineral medium for chemolithotrophic growth (H-3) (DSMZ medium 81), with 0.1 mg L^{-1} of resazurin, from a hot spring with temperature of 54 ºC and pH 6.75, under atmospheric air pressurized with 101 kPa (1 atm) of a 80:20 (v/v) mixture of hydrogen and carbon dioxide. The final gas phase of 202 kPa (2 atm) consisted of H_2:CO_2:N_2:O_2 (40 : 10 : 40 : 10, by vol.). Isolation resulted from repeated enrichments [10 % (v/v) inoculation] and a pure culture was recovered on the same medium supplemented with 1.5% agar (w/v). *H. thermoluteolus* was isolated from enrichment cultures in mineral medium (Goto et al., 1977) gassed with a gas mixture of H_2 : O_2 : CO_2 = 7 : 1: 1, incubated at 50 ºC at an initial pressure of 600 mmHg. After eight transfers the broth cultures were diluted and spread on the same culture medium supplemented with 2% (w/v) agar.

10. DIFFERENTIATION OF THE GENUS *HYDROGENOPHILUS* FROM OTHER GENERA

Within the family *Hydrogenophilaceae* that comprises moderate thermophiles, the major distinctive feature between the genus *Hydrogenophilus* and the genus *Tepidiphilus* (see gbm01831) is the fact that members of the latter are obligatory chemo-organotrophic, while members of the first are facultative hydrogen autotrophs. This feature also distinguishes the genus *Hydrogenophilus* from the genus *Hydrogenobacter* (see gbm00231), from the family *Aquificaceae,* comprising obligate hydrogen autotrophs.

Moreover, the optimal growth temperatures of *H. thermoluteolus, H. icelandicus* and *H. hirschii* (50–52°C, 55ºC and 63°C, respectively) are lower than that of *Hydrogenobacter* (70–75°C; Kawasumi et al., 1984). In addition, the cellular fatty acids and the mol% G + C content of DNA for *Hydrogenophylus* species are distinct of those of the genus *Hydrogenobacter*, of the phylum *Aquificae*.

11. TAXONOMIC COMMENTS

Based on DNA–DNA hybridization experiments, Hayashi et al. (1999) found strain TH-1 - the type strain of *H. thermoluteolus* - and reference strain TH-4 to have a hybridization value of 89%, indicating that the two strains belong to the same species. Moreover, 16S rRNA gene sequences analysis indicated that, although the two strains belonged to the class *Betaproteobacteria*, they were sufficiently unrelated to this class as to warrant their placement in a new genus, *Hydrogenophilus* (Hayashi et al., 1999). Analysis of 16S rRNA gene sequences by Stöhr et al. (2001) indicated that strain TH-1 - the type strain of *H. thermoluteolus* - has a phylogenetic distance of 0.0257 from the type strain of *H. hirschii* (Yel5a); thus, the two type strains should be placed within the same genus. The last species described, *H. islandicus*, has a 16S rRNA gene sequence similarity of 96.6% with *H. thermoluteolus* and of 95.4% with *H. hirschii*.

The genus *Hydrogenophilus* is classified in the Family *Hydrogenophilaceae*, Order *Hydrogenophilales*, within the Class *Betaproteobacteria*. The only other member of the

family is the genus *Tepidiphilus* (see gbm01831), which differs in its phenotypic properties from *Hydrogenophilus*. Other genera of negative Gram-staining, thermophilic, hydrogen chemolithotrophs include *Hydrogenobacter* (see gbm00231), which are classified in the Phylum *Aquificae,* Class *Aquificae*, Order *Aquificales*, and Family *Aquificaceae* (Kawasumi et al., 1984).

12. DIFFERENTIATION OF THE SPECIES OF THE GENUS *HYDROGENOPHILUS*

Hydrogenophilus thermoluteolus grows optimally at 50–52°C, *H. icelandicus* grows best at 55ºC and *H. hirschii* at 63°C (Table 1). In regard to their relationship to oxygen, *H. thermoluteolus* grows best at 22% O_2, *H. hirschii* is a microaerophile that grows best at 2.5% O_2 and fails to grow at O_2 levels higher than 5%, and *H. icelandicus* grows under aerobic conditions. Other differentiating features among the three species refer to the nutritional profile. For example, *H. thermoluteolus* is the only species able to use thiosulfate as electron donor chemolithotrophically (Miyake et al., 2007), and *H. islandicus* is the only species described as being able to use the sugars glucose and fructose in presence of yeast extract (Véstidóttir et al., 2011).

<Table 1 near here>

13. LIST OF SPECIES OF THE GENUS *HYDROGENOPHILUS*

1. Hydrogenophilus thermoluteolus

Hayashi, Ishida, Yokota, Kodama and Igarashi 1999, 785^{VP}

ther.mo.lu.te'o.lus. Gr. adj. *thermos* hot; L. adj. n. *luteolus* light yellow; N.L. masc. adj. *thermoluteolus* hot and light yellow.

Exponentially growing cells are 0.5–0.6 × 2.0–3.0 µm. Optimal temperature, 50–52°C. Optimal pH, 7.0. Aerobic. Other characteristics are as given for the genus, with the following additional information. Carbon sources include acetate, propionate, butyrate, succinate, DL-lactate, DL-malate, fumarate, pyruvate and α-ketoglutarate. No growth occurs on lactose, D-glucose, D-galactose, sucrose, citrate, ethanol, benzoate, and *m*-hydroxybenzoate, among other. Ammonium, nitrate, and urea can be used as sole nitrogen sources; nitrite and N_2 are not used. Major fatty acids: $C_{16:0}$, $C_{18:0}$. The major 3–hydroxy cellular fatty acid is $C_{10:0}$ 3OH. Cyclic fatty acids have not been described in *H. thermoluteolus*. Isolated from soil around a hot spring in Izu peninsula, Shizuoka Prefecture, Japan (Goto et al., 1977).

The mol% G + C of the DNA is: 63–65 (T_m).

Type strain: TH-1, IFO (now NBRC) 14978, CIP 106958.

GenBank accession number (16S rRNA): AB009828.

GenBank accession number (genome bioproject): PRJDB6915.

2. Hydrogenophilus hirschii

Stöhr, Waberski, Liesack, Völker, Wehmeyer and Thomm 2001, 488[VP]

hir'schi.i. N.L. gen. n. *hirschii* in honor of Peter Hirsch, in recognition of his fundamental contributions to the taxonomy of unusual bacteria.

Cells are 0.6–0.8 × 1.0–1.5 µm. Motile by a single polar flagellum. Growth in the range 50-68 ºC, with optimal growth temperature at 63°C. Microaerophilic, growing best at 2.5% O_2 and failing to grow at O_2 levels higher than 5%. Other characteristics are as given for the genus, with the following additional information. Growth occurs anaerobically with nitrate. Growth occurs on yeast extract, peptone, meat peptone, and meat extract. Fumarate, glutamate, and gluconate can be used as carbon sources. No growth occurs on carbohydrates, aromatic compounds, L-alanine, L-proline, citric acid, methanol, or ethanol, among other. Neither thiosulfate nor sulfur is used as an electron donor. Major fatty acids: $C_{16:0}$, cyclo $C_{17:0}$, and cyclo $C_{19:0}$. Isolated from a water sample from Angel Terrace in Yellowstone National Park, U.S.A.

The mol% G + C of the DNA is: 61 (HPLC).

Type strain: Yel5a, DSM 11420, JCM 10831, CIP 107057.

GenBank accession number (16S rRNA): FR749905.

3. Hydrogenophilus islandicus

Vésteinsdóttir, Reynisdóttir and Örlygsson, 2011, 294[VP]

is.lan.di´cus. N.L. masc. adj. islandicus Icelandic, describing the place of its first isolation).

Cells are 0.4 x 2.6 µm. Motile. Optimal growth temperature at 55°C with no growth above 60°C or below 35°C. Optimal pH growth is 7 and observed between 6 and 10. Growth is observed under aerobic conditions. Other characteristics are as given for the genus. Tryptone, beef extract, peptone, propionate, butyrate, lactate, crotonate, pyruvate or yeast extract support heterotrophic growth. Yeast extract enhances heterotrophic growth with most of those substrates and permits growth on fructose, glucose, acetate, and malate. Glycine, serine, threonine, alanine, histidine, glutamate, aspartate, oxalate, formate, sorbitol, mannose, xylose, succinate, α-ketoglutarate or galactose do not support growth. Chemolithotrophic growth at expenses of hydrogen does not require growth factors. Thiosulfate does not support chemolithotrophic growth. Doubling times at 50 ºC under mixotrophy conditions with hydrogen and butyrate, chemolithotrophy on hydrogen, heterotrophic conditions on butyrate were 18.2, 10.1 and 6.6 h, respectively. Major cellular fatty acids are $C_{16:0}$ and $C_{18:1}$ ω7c, followed by cyclo $C_{17:0}$, and $C_{19:0}$ ω8c. Isolated from a liquid and mud sample collected from the hot spring in Graendalur, south-west Iceland.

The mol% G + C of the DNA is: 63.9 (HPLC).

Type strain: 16C, DSM 21442, JCM 16106.

GenBank accession number (16S rRNA): EU625664

RELATED ARTICLES:

gbm01831

gbm00231

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Stöhr R, Waberski A, Liesack W, Völker H, Wehmeyer U, & Thomm M (2001) *Hydrogenophilus hirschii* sp. nov., a novel thermophilic hydrogen-oxidizing beta-proteobacterium isolated from Yellowstone National Park. *Int J Syst Evol Microbiol* **51**: 481–488.

Vésteinsdóttir H, Reynisdóttir DB, & Örlygsson J (2011) *Hydrogenophilus islandicus* sp. nov., a thermophilic hydrogen-oxidizing bacterium isolated from an Icelandic hot spring. *Int J Syst Evol Microbiol* **61**:290-294.

311 Table 1. The differential characteristics of the species of *Hydrogenophilus*

Characteristic	***H. thermoluteolus***	***H. hirschii***	***H. islandicus***
Colonies pigmentation	dull yellow	yellow	greyish
Motility	- [1]	+	+
Optimum growth temperature (ºC)	50–52	63	55
Optimum growth pH	7	6.5	7
Optimum O_2 concentration (%)	22	2.5	n.a.
Growth with 5% O_2	+	-	n.a.
Chemolithotrophic growth with thiosulfate	+	-	-
Heterotrophic growth			
Acetate	+	+	+*
Butyrate	+	-	+
D-Fructose	-	-	+*
D-Glucose	-	-	+*
Glutamate	n.a.	+	-
a-Ketoglutarate	+	-	-
DL-Malate	+	+	+*
Peptone	n.a.	+	$+^{w}$
Succinate	+	+	-
Major fatty acids	$C_{16:0}$ $C_{18:0}$	$C_{16:0}$ cyclo $C_{17:0}$ cyclo $C_{19:0}$	$C_{16:0}$ $C_{18:1}$ *ω7c* cyclo $C_{17:0}$ $C_{19:0}$ *ω8c*
DNA G+C content (mol %)	63–65 (Tm)	61 (HPLC)	63.9 (HPLC)
Isolation source	soil in a hot spring area	freshwater hot spring	hot spring

312 [1] Goto et al. (1978) described the strain TH-1 as motile.

313 n.a., not available; *positive in the presence of 2 g/L yeast extract; [w], weakly positive

314 HPLC, High-Performance Liquid Chromatography; Tm (determined by the thermal

315 melting point)