Persephonella hydrogeniphila sp. nov., a novel thermophilic, hydrogen-oxidizing bacterium from a deep-sea hydrothermal vent chimney

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A novel thermophilic, hydrogen-oxidizing bacterium, designated 29W^T, was isolated from a deep-sea hydrothermal vent chimney sample collected from the Suiyo Seamount in the Izu-Bonin Arc, Japan, at a depth of 1385 m. The cells were cocci (0.9–1.0 µm in diameter) and straight rods (2.3–2.7 µm long) under static and agitated culture conditions, respectively. The new isolate was an obligate chemolithoautotroph growing by respiratory nitrate reduction with H₂, forming N₂ as a final product. A very low concentration of O₂ (optimum 0.6–0.8%, v/v) was also used as an alternative electron acceptor while reduced sulfur compounds did not serve as electron donors. Anoxic hydrogen-oxidizing growth with nitrate was observed between 50 and 72.5 °C (optimum 70 °C; 40 min doubling time), pH 5.5 and 7.6 (optimum pH 7.2), and in the presence of 1.5 and 5.0% NaCl (optimum 2.5%). The G + C content of the genomic DNA was 37.3 mol%. Phylogenetic analysis based on 16S rDNA sequences indicated that the isolate was a member of the recently described genus *Persephonella* in a potential new family within the order *Aquificales*. On the basis of the physiological and molecular properties of the new isolate, the name *Persephonella hydrogeniphila* sp. nov. is proposed. The type strain is strain 29W^T (=JCM 11663^T = DSM 15103^T).

Thermophilic hydrogen- and/or sulfur-oxidizing bacteria of the order *Aquificales* are widely distributed in diverse hot environments such as terrestrial geothermal fields, shallow- and deep-sea hydrothermal vent systems and deep sub-surface hot aquifer environments (Kawasumi *et al.*, 1984; Kryukov *et al.*, 1984; Huber *et al.*, 1992, 1998; Shima & Suzuki, 1993; Hugenholtz *et al.*, 1998; L'Haridon *et al.*, 1998; Reysenbach *et al.*, 2000a, b, c; Marteinsson *et al.*, 2001; Takacs *et al.*, 2001; Takai *et al.*, 2001, 2002). Members of the *Aquificales* are thought to have an impact on biogeochemical processes in these ecosystems (Kristjansson *et al.*, 1985; Harmsen *et al.*, 1997; Götz *et al.*, 2002). In addition, the energy conversion systems and metabolism of *Aquificales* are interesting in relation to their antagonistic evolutionary traits inferred from rRNA gene trees (Burggraf *et al.*, 1992) and from comparison of the genome sequences (Deckert *et al.*, 1998).

A common property displayed by all of the cultivated strains of *Aquificales* is obligately or facultatively chemolithoautotrophic growth by oxidation of H₂ or reduced sulfur compounds. The most recently constructed phylogenetic tree including the cultivated strains and environmental rDNA clones clearly indicated three distinct lineages (probably classified into *Desulfurobacteriaceae*, *Aquificaceae* and *Hydrogenothermaceae*) within the order *Aquificales* (Takai *et al.*, 2002; Eder & Huber, 2002). The relatives of *Desulfurobacterium* are strictly anaerobic H₂-oxidizing autotrophs using elemental sulfur, thiosulfate, polysulfide, sulfite or nitrate as electron acceptors (L'Haridon *et al.*, 1998; Huber *et al.*, 2002). Members of the genera *Hydrogenobaculum* (Shima & Suzuki, 1993; Stöhr *et al.*, 2001), *Aquifex* (Huber *et al.*, 1992), *Thermocrinis* (Huber *et al.*, 1998) and *Hydrogenobacter* (Kawasumi *et al.*, 1984) have been the most extensively studied. Although *Thermocrinis ruber* (Huber *et al.*, 1998) is capable of chemo-organotrophic growth and *Hydrogenobacter subterraneus* (Takai *et al.*, 2001) is an obligate aerobic heterotroph, all other members are strict chemolithoautotrophs using H₂ and reduced sulfur compounds as electron donors, and O₂ and nitrate as electron acceptors.

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Supplementary data are available in IJSEM Online.

The last lineage largely consisted of a number of previously uncultivated environmental rDNA clones obtained from global terrestrial hot-spring environments such as in Yellowstone National Park (Hugenholtz *et al.*, 1998; Reysenbach *et al.*, 2000a), Iceland (Skirnisdottir *et al.*, 2000; Takacs *et al.*, 2001) and Japan (Yamamoto *et al.*, 1998), and in subterranean hot springs (Marteinsson *et al.*, 2001), and even in deep-sea hydrothermal vent systems (Reysenbach *et al.*, 2000b, c). Recently, however, several representative strains of this lineage such as *Hydrogenothermus* (Stöhr *et al.*, 2001), *Persephonella* (Reysenbach *et al.*, 2000a; Götz *et al.*, 2002) and a potentially new genus of strains (Takai *et al.*, 2002) have been successfully isolated from shallow and deep marine hydrothermal vent environments and from a subsurface hot aquifer environment. Based on their characterization, the newly identified lineage of *Aquificales* shared similar physiological and metabolic properties with the members of the family *Aquificaceae*. However, many phylotypes of bacteria within this lineage remain uncultivated and unidentified, and further exploration of uncultivated members will provide important insights. In this study, we sought to cultivate anoxic H₂-oxidizing thermophiles using nitrate as an electron acceptor from a deep-sea hydrothermal vent chimney at the Suiyo Seamount in the Izu-Bonin Arc, Japan.

Sample collection, enrichment and purification

Samples from black smoker vents were obtained from the hydrothermal field at the Suiyo Seamount in the Izu-Bonin Arc, Japan (28° 34' 287" N, 140° 38' 663" E), at a depth of 1385 m, by means of the manned submersible *Shinkai 2000* in a dive (dive no. 1237) performed in November 2000. A bulk of chimney having 310.8 °C of vent emission was brought to the sea surface in a sample box supplied with the submersible and immediately subsampled into four different parts (top part, surface layer, inside structure and vent surface) as described by Takai *et al.* (2001). The chimney was mainly formed from anhydrite (top part), barite (surface layer), pyrite and chalcopyrite (inside structure), and pyrite or chalcopyrite crystals were observed at the passage of hot fluid (vent surface). Each of the subsamples (approx. 10 g) was suspended with 20 ml sterilized MJ synthetic seawater (Sako *et al.*, 1996) containing 0.05 % (w/v) sodium sulfide in a 100 ml glass bottle (Schott Glaswerke) tightly sealed with a butyl rubber cap under a N₂ atmosphere. These suspended portions of the subsamples were used to inoculate a series of media including HNW medium (described below) on board.

The enrichment was performed in test tubes (Pyrex; 180 × 18 mm) containing 5 ml of the medium with 80 % H₂ + 20 % CO₂ (300 kPa) tightly sealed with butyl rubber caps and the cultures were incubated at 70 and 85 °C. The tubes of HNW medium inoculated only with a subsample of surface layer became turbid after 1 day incubation at 70 °C and the other tubes did not provide positive enrichments after 5 days incubation at 70 and 85 °C. The enrichment cultures grown at 70 °C contained highly motile cocci. To

obtain a pure culture of cocci grown at 70 °C, an extinction-to-dilution method was employed and repeated at least five times. The first pure culture was designated strain 29W^T (=DSM 15103^T=JCM 11663^T) and investigated in detail. The purity was confirmed routinely by microscopic examination and by repeated partial sequencing of the 16S rRNA gene using several PCR primers.

Morphology

Cells were routinely observed with a differential interference microscope (UFX; Nikon). Negative staining of cells for electron microscopy was done with 2 % (v/v) phosphotungstic acid. The cells grown under static culture were Gram-negative cocci about 0.9–1.0 µm in diameter (Fig. 1a). Despite a body of reports on the isolation and characterization of hydrogen-oxidizing thermophiles within the order *Aquificales* including *Persephonella* species (Kawasumi *et al.*, 1984; Huber *et al.*, 1992, 1998; Shima & Suzuki, 1993; L'Haridon *et al.*, 1998; Takai *et al.*, 2001, 2002; Götz *et al.*, 2002; Huber *et al.*, 2002), the coccoid morphology is first described here. When grown with agitation, each cell exhibited a straight rod shape with a mean length of 2.3–2.7 µm and a width of approximately 0.4–0.5 µm (Fig. 1b). Both coccoid and rod types of the cells appeared to have a single polar flagellum as observed

Fig. 1. Electron micrographs of negatively stained *Persephonella hydrogeniphila* cells in the exponential growth phase. (a) Under static culture conditions; (b) under agitated culture conditions. Bars, 1 µm.

by electron microscopy and to be highly motile by observation using light microscopy. Pili-like filaments and internal stacked membranes reported in *Persephonella marina* (Götz *et al.*, 2002) were not observed in thin sections (data not shown). Cells occurred singly or in pairs in all phases of growth and no sporulation was observed.

Growth characteristics

The new isolate was routinely cultivated in HNW medium, which contained 1 g NaNO₃, 1 g NaHCO₃, 0.1 mg Na₂WO₄·2H₂O, 0.5 g Na₂S·9H₂O and 10 ml trace vitamin solution (Balch *et al.*, 1979) per litre of DMJ synthetic seawater (2/3-fold-diluted MJ synthetic seawater; Sako *et al.*, 1996). To prepare HNW medium, all the components of DMJ seawater were dissolved in 1 litre distilled deionized water, and the pH was adjusted to around 7.0 with NaOH at room temperature prior to autoclaving, unless otherwise noted. After autoclaving, filter-sterilized NaNO₃ solution (100 g l⁻¹), NaHCO₃ solution (100 g l⁻¹), Na₂S solution (100 g l⁻¹; pH 7.5) and trace vitamin solution were added. The tubes were then tightly sealed with butyl rubber stoppers under a gas phase of 80 % H₂ + 20 % CO₂ (300 kPa).

Growth of the new isolate under varying conditions was determined by direct cell counts after staining with 4',6-diamidino-2-phenylindole (DAPI) (Porter & Feig, 1980) using a Nikon Eclipse E800 microscope equipped with colour chilled 3 CCD camera system (C5810; Hamamatsu Hokutokusu). To determine the temperature, pH and NaCl range for growth, duplicate cultures were grown in 100 ml glass bottles (Schott Glaswerke) containing 20 ml medium in an air-batch rotary shaker (RGS-32.TT; Sanki Seiki) and were shaken at 100 r.p.m. in all cases. The isolate grew over a temperature range of about 50–72.5 °C, showing optimum growth at 70 °C, and the generation time at 70 °C was about 40 min. No growth was observed at 75 and 45 °C (supplementary Fig. 1a in IJSEM Online). Effects of pH and NaCl concentration on growth of the isolate were determined at 70 °C. When the pH optimum was determined, the pH of the pre-heated medium was readjusted immediately before inoculation with H₂SO₄ or NaOH by using a compact pH meter (Horiba B-212). The pH was found to be stable during the cultivation period. Growth of the new isolate occurred between pH 5.5 and 7.6, with optimum growth at about pH 7.2. No growth was detected below pH 5.5 or above pH 7.6 (supplementary Fig. 1b in IJSEM Online). NaCl requirements were determined with varying concentrations (from 0 to 6 %, w/v) of NaCl in DMJ synthetic seawater. The isolate required NaCl for growth, and grew over a concentration range of about 1.5–5.0 % NaCl, with optimum growth at around 2.5 % NaCl. No growth was observed below 1.5 % NaCl or above 5.0 % NaCl (supplementary Fig. 1c in IJSEM Online).

O₂ tolerance and requirements were determined by injecting defined volumes of O₂ into culture tubes of HNW medium without Na₂S and NaNO₃, and O₂ final concentrations from

0 to 5 % (v/v) were tested. A very limited amount of O₂ (below 1.2 %; optimum 0.6–0.8 %, v/v) supported growth in place of nitrate (supplementary Fig. 2 in IJSEM Online). However, microaerobic growth with the optimal O₂ concentration produced a lower growth rate and yield than anaerobic growth with nitrate.

The following analytical procedures were used for testing the change of inorganic substrates during bacterial growth in HNW medium. Gas composition was measured by the gas chromatograph Micro GC CP2002 (GL Sciences). Anion samples were analysed by ion chromatography using a Shim-pack IC column (Shimadzu). The diazotization method was employed to determine the concentration of nitrate/nitrite (Matsunaga & Nishimura, 1969) in addition to ion chromatography, and Nessler's reagent was employed to measure the ammonia concentration in the medium (Allen *et al.*, 1974). The concentration of N₂ in the gas phase was increased with decreasing concentration of nitrate during growth (supplementary Fig. 3 in IJSEM Online). The accumulation of potential end products such as nitrite, N₂O and ammonium ions was not detected. These results suggest that the new isolate was a hydrogen-oxidizing chemolithoautotroph preferring anaerobic denitrifying growth to microaerobic growth.

In an attempt to examine the alternative electron acceptors which could support growth, 0.1 % (w/v) NaNO₂, Na₂S₂O₃·5H₂O, Na₂S₄O₆·2H₂O and Na₂SO₃, 3 % (w/v) S⁰ and 100 mM ferrihydrite were tested in place of nitrate in HNW medium. At the time of analysing ferrihydrite as electron acceptor, Na₂S was omitted from the medium. None of these electron acceptors could support growth of the isolate. For the test of the alternative energy sources, 0.1 % (w/v) Na₂S₂O₃, 3 % (w/v) S⁰ and organic substrates (described below) were used instead of H₂ in HNW medium under a gas phase of 80 % N₂ + 20 % CO₂ (300 kPa). This test was also conducted under a gas phase of 80 % N₂ + 19 % CO₂ + 1 % O₂ (300 kPa) in HNW medium without NaNO₃ and Na₂S. The isolate could not utilize any of these alternative energy sources. To our knowledge, the isolate is the first example of a hydrogen-dependent, facultatively anaerobic, and chemolithoautotrophic thermophile isolated from deep-sea hydrothermal environments.

When growth of the new isolate was tested in HNW medium (nitrate provided as a sole electron acceptor) in the absence of NaHCO₃, no growth was observed at 70 °C even under 80 % H₂ + 20 % CO₂ (300 kPa). The results suggested that bicarbonate was the preferred carbon source for the autotrophic growth of the isolate as observed in a mesophilic hydrogen-oxidizer, *Ralstonia eutropha* (Repaske *et al.*, 1971).

In an attempt to examine organotrophic growth of 29W^T, experiments were conducted using HNW medium replacing NaHCO₃ and CO₂ with various organic carbon sources. Each of the following substrates was added at concentrations of 0.01 and 0.1 % (w/v): L-cystine, L-phenylalanine, L-proline, Casamino acids, D-(+)-glucose, lactose, maltose,

chitin, starch, cellulose, formate, acetate, citrate, pyruvate, propionate, 2-propanol, methanol, tryptone peptone (Difco) and yeast extract (Difco). Two gas phases (100 % H₂, and 80 % N₂ + 20 % CO₂; 300 kPa) were used. These tests were run in duplicate. The isolate was unable to grow under any of the organotrophic conditions tested in this study.

Cellular fatty acid composition

Cellular fatty acid composition was analysed using cells grown in HNW medium at 70 °C with agitation in the late-exponential growth phase. Cellular fatty acid composition analysis was performed at the NCIMB Japan Corporation, Shizuoka, Japan. Fatty acids were converted to methyl esters by treatment with anhydrous methanolic HCl. Fatty acid methyl esters (FAMES) were analysed by GC using a non-polar capillary column and flame-ionization detection. The major cellular fatty acids were C_{20:1} (40.8 %), C_{18:0} (24.7 %), C_{18:1} (23.8 %) and C_{16:0} (4.2 %). The presence of C_{18:0} and C_{20:1} as the major components is a common feature of members of the order *Aquificales*. The percentages of each fatty acid of the isolate are particularly similar to those of *Persephonella guaymasensis* and *Hydrogenothermus marinus* (Stöhr *et al.*, 2001; Götz *et al.*, 2002). However, the new isolate can be distinguished by the presence of a relatively high amount of C_{16:0}.

Isolation and base composition of DNA

Genomic DNA was prepared as described by Lauerer *et al.* (1986). The G + C content (mol%) of the genomic DNA was determined by direct analysis of the deoxyribonucleotides using HPLC with a DNA-GC kit (Yamasa Shouyu) after digestion of the DNA with nuclease P1 (Tamaoka &

Komagata, 1984). The G + C content of the genomic DNA of strain 29W^T was found to be 37.3 mol%, which was similar to that of *Persephonella* species (Table 1).

Phylogenetic analyses

The 16S rRNA gene (rDNA) was amplified by PCR using Eubac 27F and 1492R primers (DeLong, 1992). The sequence of the 1.5 kb PCR product was directly determined in both strands using the dideoxynucleotide chain-termination method with the ABI 373A DNA sequencer (Applied Biosystems). The almost complete sequence (1506 bp) of the 16S rDNA of strain 29W^T has been deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases under accession number AB086419. In order to determine the phylogenetic position of the isolate, the sequence was manually aligned with a subset of 16S rDNA sequences obtained from the DNA Database of Japan (DDBJ) and the Ribosomal Database Project II (RDP-II) (Maidak *et al.*, 2001) by using CLUSTAL X (Thompson *et al.*, 1997). Phylogenetic analyses were restricted to nucleotide positions that were unambiguously alignable in all sequences (Takai & Horikoshi, 1999; Takai & Sako, 1999). Neighbour-joining analysis (Saitou & Nei, 1987) of 914 bases of sequence from each organism was accomplished using CLUSTAL X software. Bootstrap analysis was used for 1000 trial replications to provide confidence estimates for the phylogenetic tree topologies. The phylogenetic tree demonstrated that the new isolate was a member of the genus *Persephonella* (Fig. 2). However, 16S rDNA sequence similarities between strain 29W^T and *P. guaymasensis*, *P. marina* and *Aquificales* strain CIR30126 were 95.6, 95.0 and 96.7 %, respectively. These similarity values indicated that the new isolate was a potentially new species of the

Table 1. Comparison of properties of *Persephonella hydrogeniphila* sp. nov. and related species

Strains (source): 1, *Persephonella hydrogeniphila* 29W^T (this study); 2, *Persephonella marina* EX-H1^T (Götz *et al.*, 2002); 3, *Persephonella guaymasensis* EX-H2^T (Götz *et al.*, 2002); 4, *Aquifex pyrophilus* Kol5a^T (Huber *et al.*, 1992); 5, *Hydrogenothermus marinus* VM1^T (Stöhr *et al.*, 2001). ND, No data.

Character	1	2	3	4	5
Temperature range (°C)	50–72.5	55–90	60–80	67–95	45–80
Temperature optimum (°C)	70	70	75	85	65
pH range	5.5–7.6	4.7–7.5	4.7–7.5	5.4–7.5	5.0–7.0
pH optimum	7.2	6.0	6.0	6.8	5.0–7.0
NaCl range (% w/v)	1.5–5.0	1.0–4.5	1.0–4.5	1.0–5.0	ND
NaCl optimum (% w/v)	2.5	2.5	2.5	3.0	ND
Electron donor	H ₂	H ₂ , S ₂ O ₃ ²⁻ *, S ⁰ *	H ₂ , S ₂ O ₃ ²⁻ *, S ⁰ *	H ₂ , S ₂ O ₃ ²⁻ , S ⁰	H ₂
Electron acceptor	NO ₃ ⁻ , O ₂	NO ₃ ⁻ , O ₂ , S ⁰ , SO ₄ ²⁻ †, acetate†	NO ₃ ⁻ , O ₂	NO ₃ ⁻ , O ₂	O ₂
O ₂ tolerance (% v/v)	<1.2	<9.0	<11.0	<0.5‡	<8.0
O ₂ optimum (% v/v)	0.6–0.8	2.0–3.0	2.0–3.0	ND	1.0–2.0
G + C content (mol%)	37.3	38.5	37.4	40	44

*Only with O₂ as an electron acceptor.

†Inconsistent growth.

‡Adaptation up to 6 % was observed.

Fig. 2. Phylogenetic tree of representative members and environmental rDNA clones of thermophilic hydrogen-oxidizing bacteria, inferred from 16S rDNA sequences by the neighbour-joining method using 914 homologous sequence positions for each organism. The numbers are the bootstrap values for the branches (based on 1000 replicates). The bar indicates two substitutions per 100 nucleotides. The EMBL/GenBank/DBJ database accession numbers are shown in parentheses.

genus *Persephonella* based on the evolutionary distance (97 %) of differentiation on the species level (Stackebrandt & Goebel, 1994).

DNA–DNA hybridization analysis was performed at 30 °C for 3 h and was measured fluorometrically using photobiotin according to the method of Ezaki *et al.* (1989). *P. marina* EX-H1^T (=DSM 14530^T = OCM 794^T), *P. guaymasensis* EX-H2^T (=DSM 14351^T = OCM 975^T) and *Aquificales* strain CIR30126 (Van Dover *et al.*, 2001) were used as reference strains. Although the phylogenetic analyses based on the 16S rRNA gene sequence indicated that the new isolate was closely related to two known species of the genus *Persephonella*, the mean hybridization value for the isolate and *P. guaymasensis* EX-H2^T was 5.4 %, the value for the new isolate and *P. marina* EX-H1^T was 4.0 %, and the value for the isolate and *Aquificales* strain CIR30126 was 10.3 %. These results indicated that the new isolate could be genotypically differentiated from previously described species of the genus *Persephonella*.

Comparison with related species

Phylogenetic analysis based on the 16S rRNA gene sequence indicated that strain 29W^T is closely related to the genus *Persephonella*. Cellular fatty acid composition analysis also supports the new isolate being a member of the genus. However, the isolate differs from previously described *Persephonella* species in many physiological properties (Table 1). The isolate is coccoid when grown under static culture conditions. This morphological feature is for the first time observed within the order *Aquificales*. The isolate grows significantly faster (40 min) than *P. marina* (5.02 h) and *P. guaymasensis* (7.8 h) when compared under their optimum growth conditions. The temperature range for

growth of the new isolate is slightly lower than that of the two species of *Persephonella*. The new isolate is unable to utilize reduced sulfur compounds as energy sources (Table 1). In addition, DNA–DNA hybridization analysis clearly indicated that the new isolate could be genotypically differentiated from previously described species of the genus *Persephonella*. On the basis of these physiological and genetic properties, we propose a new species of the genus *Persephonella*, designated *Persephonella hydrogeniphila*; the type strain is strain 29W^T (=JCM 11663^T = DSM 15103^T).

Recovery of *Persephonella hydrogeniphila* only from the surface zone of the chimney structure provides an important clue into delineating the ecological niche of the facultatively anaerobic hydrogen-oxidizing thermophiles. Nitrate is known to be unstable under high temperature and highly reducing environments such as deep-sea hydrothermal vents (Blöchl *et al.*, 1992; Völkl *et al.*, 1993). However, nitrate can be supplied from seawater with oxygen. Accordingly it might be possible that a population of facultatively anaerobic hydrogen-oxidizing thermophiles such as *P. hydrogeniphila* occurs in the surface area of the chimney, where nitrate and oxygen can be present and hydrogen is provided both from geochemical (gas propagation from the superheated vent fluids) and microbiological (indigenous microbial H₂ production by abundant fermentative thermophiles such as *Thermococcales* and *Thermotogales* members) processes. In fact, the predominant occurrence of the *Thermococcales* population is specifically observed in the surface layer of the same chimney structure as observed in a black smoker chimney from the Manus Basin (Takai *et al.*, 2001; K. Takai and others, unpublished results). We also succeeded in isolating both the obligately aerobic thermophiles *Marinithermus hydrothermalis* and *Rhodothermus marinus* and the strictly

anaerobic nitrate- or sulfur-reducing thermophile *Deferribacter* sp. from the same subsample used in this study (Sako *et al.*, 2003; Takai *et al.*, 2003). The ecological potential of primary production by facultatively anaerobic, thermophilic chemolithoautotrophs in relatively low temperature and oxidative microhabitats in deep-sea hydrothermal vent environments is still unclear. This is a focus of our future investigations.

Description of *Persephonella hydrogeniphila* sp. nov.

Persephonella hydrogeniphila (hy.dro.ge.ni.phi'la. N.L. neut. n. *hydrogenum* hydrogen; Gr. adj. *philos* loving; N.L. fem. adj. *hydrogeniphila* hydrogen-loving, because growth depends on hydrogen).

Cells are motile cocci about 0.9–1.0 µm in diameter under static culture. Cells grown with agitation become straight rods 2.3–2.7 µm long and 0.4–0.5 µm wide. Gram-negative. Growth occurs at temperatures between 50 and 72.5 °C (optimum 70 °C), at pH 5.5–7.6 (optimum pH 7.2), and in the presence of 1.5–5.0 % NaCl (optimum 2.5 %). The optimum doubling time is about 40 min. The major cellular fatty acids are C_{20:1} (40.8 %), C_{18:0} (24.7 %), C_{18:1} (23.8 %) and C_{16:0} (4.2 %). Obligately chemolithoautotrophic. Utilizes only H₂ as electron donor and nitrate and O₂ as electron acceptors. Growth is inhibited by O₂ concentrations above 1.2 % (v/v). The G+C content is 37.3 mol% (HPLC). The DNA–DNA relatedness to *P. marina* EX-H1^T and *P. guaymasensis* EX-H2^T is low.

The type strain is 29W^T (=JCM 11663^T=DSM 15103^T), which was isolated from a deep-sea hydrothermal vent chimney at Suiyo Seamount in the Izu-Bonin Arc, Japan (28° 34' 287" N, 140° 38' 663" E; depth 1385 m).

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