Hydrogenovibrio marinus gen. nov., sp. nov., a Marine Obligately Chemolithoautotrophic Hydrogen-Oxidizing Bacterium

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The name Hydrogenovibrio marinus gen. nov., sp. nov. is proposed for an obligately chemolithoautotrophic, mesophilic, gram-negative, motile, comma-shaped, aerobic, hydrogen-oxidizing bacterium that was isolated from seawater. The optimum temperature and NaCl concentration for growth are 37°C and 0.5 M, respectively. The guanine-plus-cytosine content of the DNA is 44.1 mol%. The ubiquinone is ubiquinone-8, and the major cellular fatty acids are C16:0, C18:1ω9c, and C16:1ω7c. The type strain of this species is strain MH-110T (= JCM 7688).

Hydrogen supersaturation of oceanic surface waters with respect to atmospheric equilibrium can be widely observed throughout the world (2), and some hypotheses to explain this phenomenon have been presented (3, 12, 18-21). On the other hand, hydrogen undersaturation has also been found (4). Herr et al. (4) suggested that the dissolved hydrogen concentration should reflect the balance between biological production and consumption. These authors also suggested that a major decrease in concentration might be due to chemolithotrophic consumption by aerobic hydrogen-oxidizing bacteria because of their high hydrogen oxidation activities.

An aerobic hydrogen-oxidizing bacterium, strain MH-110T (T = type strain), was recently isolated from seawater for the first time. The properties of this organism have been described previously (14). Strain MH-110T cells are gram-negative, comma-shaped rods and are motile by means of a polar flagellum (Fig. 1). The guanine-plus-cytosine (G+C) content of the DNA is 44.1 mol%. The optimum temperature and NaCl concentration for growth are 37°C and 0.5 M, respectively. Strain MH-110T is the first obligately chemolithoautotrophic, mesophilic, aerobic, hydrogen-oxidizing bacterium that has been described. Aerobic, hydrogen-oxidizing bacteria can be isolated from almost every soil and water sample (1). However, it is interesting that strain MH-110T has unique properties compared with strains that are isolated from soil, especially its obligately autotrophic characteristic. Obligate autotrophism might reflect an important role in the hydrogen and carbon dioxide cycle in the marine environment. In this work, further studies were performed to clarify the taxonomic position of strain MH-110T. We propose creation of a new genus and species, Hydrogenovibrio marinus, for strain MH-110T.

MATERIALS AND METHODS

Strain. Strain MH-110T was isolated from seawater from the Shonan Coast, Kanagawa Prefecture, Japan (14).

Culture methods. Strain MH-110T was cultivated in shaking flasks on basal medium as described previously (14). A 100-ml portion of a 14-h broth culture was inoculated into a 2-liter jar fermentor (Labotec Co.) containing a working volume of 1.1 liters, and this preparation was cultivated at 37°C by using a continuous flow system (17). The gas mixture, which contained H2, O2, and CO2 (8:1:1), was prepared by using thermal mass flow meters (Ueshima Seisakusho Ltd., Tokyo, Japan) and was supplied to the fermentor constantly at a flow rate of 1 liter/min. The fermentor was agitated at 1,000 rpm.

Maintenance of strain MH-110T. The liquid cultures were stored at 4°C after renewal of the gas phase containing H2, O2, and CO2 (7:1:1). A freeze-drying method was also used for long-term preservation; 50 mM phosphate buffer (pH 7.0) containing 2% sodium glutamate was used as the suspending medium.

Cellular fatty acid composition. Cells cultivated at 37°C in a jar fermentor were used for the analysis of cellular fatty acids. Fatty acid methyl esters were liberated from 40 mg of lyophilized cells by methanalysis at 100°C for 3 h with 3 ml of 5% anhydrous metanolic HCl, extracted three times with 3 ml of petroleum ether, and washed with water. Polar and nonpolar fatty acid methyl esters were separated and identified by thin-layer chromatography of silica gel plates (Kieselgel 60; E. Merck, Darmstadt, Federal Republic of Germany), which were developed by using n-hexane-diethyl ether (1:1, vol/vol). Spots were visualized by spraying the plates with 50% H2SO4 and charring them at 150°C for 10 min. A gas chromatograph equipped with a glass column (3 mm by 2 m) packed with 10% diethylene glycol succinate and 2% OV-1 on Chromosorb W (80/100 mesh) was used to separate the fatty acid methyl esters. Unsaturated fatty acids were detected by the disappearance of their gas chromatogram peaks and increases in the peaks of the corresponding saturated fatty acids after saturation of the double bonds by using a palladium black catalyst and hydrogen gas.

Purification and identification of the quinone. Crude lipid was extracted from lyophilized cells by shaking them vigorously in ether-ethanol (2:1, vol/vol) for 1 h at room temperature. This extraction procedure was repeated once. The combined extract was evaporated in vacuo, and the residue was dissolved in a small amount of acetone. The resulting solution was subjected to thin-layer chromatography on a silica gel plate (Kieselgel 60; Merck) for purification. Mass spectrometry was used to identify the quinone from strain MH-110T as described by Yamada et al. (24). Authentic vitamin K1 and crystalline ubiquinone-7 were spotted onto the thin-layer chromatography plate as reference standards. Ubiquinone-7 was kindly donated by K. Suzuki, RIKEN Institute, Wako-shi, Japan.

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RESULTS AND DISCUSSION

Strain MH-110T, which was isolated from seawater, is the first mesophilic, aerobic, hydrogen-oxidizing bacterium which is obligately autotrophic. Only one genus, Hydrogenobacter (8–10, 15), has been described previously as consisting of obligately chemolithoautotrophic, aerobic, hydrogen-oxidizing bacteria. Differences between strain MH-110T and members of the genus Hydrogenobacter have been found in morphology, optimum growth temperature, requirement for NaCl for growth, CO₂-fixing pathway, and habitat, as described previously (14). To clarify in more detail the relationship between strain MH-110T and the genus Hydrogenobacter, cellular fatty acid composition and quinone systems were investigated.

The main fatty acids of strain MH-110T were straight-chain saturated C₁₆:₀ acid (25.3%) and C₁₈:₀ acid (15.8%) and straight-chain unsaturated C₁₆:₁ acid (26.0%). The minor fatty acids identified (less than 10% of the total acids) were C₁₀:₀, C₁₂:₀, C₁₄:₀, C₁₂:₁, C₁₈:₁, 3-ΟH C₈:₀, 3-ΟH C₁₀:₀, 3-ΟH C₁₂:₀, 3-ΟH C₁₄:₀, and 3-ΟH C₁₄:₁ acids. This composition is the same as the compositions found in many bacterial groups that have fatty acid patterns comprised mainly of C₁₀ and C₁₂ acids (5, 7, 16, 23), but is clearly different from the composition of members of the genus Hydrogenobacter, which have C₁₈:₀ and C₂₀:₁ acids as their major fatty acids (10).

The R₂ value (0.20) of the quinone on thin-layer chromatograms was identical to that of the standard ubiquinone, and the mass spectrum showed that the molecular ion peak was at m/z 726. These results suggest that the quinone of strain MH-110T is ubiquinone-8. On the other hand, the quinone found in members of the genus Hydrogenobacter is methionquinone [MTK-7(H₄)], a new type of sulfur-containing quinone that was first discovered in Hydrogenobacter thermophilus cells (6). As described above, the taxonomic position of strain MH-110T is clearly distinct from that of the genus Hydrogenobacter. Major properties of these two obligately chemolithoautotrophic, aerobic, hydrogen-oxidizing bacteria are shown in Table 1.

Strain MH-110T is also clearly differentiated from other facultatively chemolithoautotrophic, aerobic, hydrogen-oxidizing bacteria by its obligate autotrophism, vibrioid-shaped cells, requirement for NaCl for growth, DNA base composition, and habitat. The DNA base composition of strain MH-110T is 44.1 mol% G+C (14), whereas the G+C contents of all of the facultatively chemolithoautotrophic, aerobic, hydrogen-oxidizing bacteria described previously are more than 60 mol%.

The relationship between strain MH-110T and aerobic sulfur-oxidizing bacteria also had to be examined because strain MH-110T can use reduced sulfur compounds, such as elemental sulfur, thiosulfate, and tetrathionate, as well as molecular hydrogen, as sole sources of energy (14). Two species of the genus Thiomicrospira, Thiomicrospira pelophila and Thiomicrospira crunogena, are similar to strain MH-110T in morphology and DNA base composition according to Kuenen and Robertson (11). We checked the hydrogen autotrophy abilities of these organisms under various conditions (changing gas composition, temperature, and medium, including salinity and pH) and found that they did not grow on molecular hydrogen as a sole energy source under any of the culture conditions tested (data not shown). Unfortunately, there is no available description of the chemotaxonomic properties of the genus Thiomicrospira except the DNA G+C content. It is very difficult to obtain cells of Thiomicrospira spp. in quantity because of their poor growth on sulfur compounds, which causes severe difficulties in chemotaxonomic studies of the genus. The growth of strain MH-110T on sulfur compounds is also very poor, but strain MH-110T grows rapidly and vigorously in the presence of molecular hydrogen as a sole energy source. More than 20 (dry weight) of cells per liter can be obtained by cultivating the strain for 24 h (13). Strain MH-110T seems to be highly adapted to utilizing molecular hydrogen as an energy source, and hydrogen oxidation seems to be more significant than sulfur oxidation in the energy metabolism of the strain. We believe that the different behaviors on molecular hydrogen of members of the genus Thiomicrospira and strain MH-110T are distinctive and that hydrogen autotrophy is a definitive characteristic for strain MH-110T. Strain MH-110T can be differentiated from other aerobic sulfur-oxidizing bacteria by its vibrioid-shaped cells and the G+C content of its DNA.
As described above, strain MH-110\textsuperscript{T} has unique morphological and physiological properties compared with previously described genera of aerobic hydrogen- and sulfur-oxidizing bacteria. We propose that a new genus and species, \textit{Hydrogenovibrio marinus}, be created. Sequencing of the 5S and 16S rRNAs is now under way to clarify the phylogenetic or evolutionary position of \textit{Hydrogenovibrio marinus}.

**Description of \textit{Hydrogenovibrio marinus} gen. nov., sp. nov.**

\textit{Hydrogenovibrio marinus} (Hy.dro.ge.no.vib'ri.o. Gr. n. hy-
dro, water; Gr. n. genus, offspring; M.L. masc. n. hydroge-
num, hydrogen, that which produces water; M.L. masc. n.
\textit{Vibrio}, a generic name; M. L. masc. n. \textit{Hydrogenovibrio},
hydrogen vibrio. ma.ri'nus. L. adj. marinus, marine, of the
sea). Cells are comma-shaped rods (0.2 to 0.5 by 1 to 2 \textmu m)
that occur singly. Gram-negative. Nonsporulating. Motile by
means of a polar flagellum. Respiratory metabolism; mole-
cular oxygen is used as the electron acceptor. Obligately
chemolithoautotrophic, using molecular hydrogen or re-
duced sulfur compounds, such as elemental sulfur, thiosul-
fate, and tetrathionate, as electron donors and carbon diox-
ide as the carbon source. Hydrogenase is membrane bound
and does not reduce pyridine nucleotides. Type \textit{b, c, and}
\textit{u} cytochromes have been found. Carbon dioxide is fixed via
the Calvin-Benson cycle. Ammonium ions and urea are
utilized as sole nitrogen sources, but nitrate ions, nitrite
ions, and gaseous nitrogen are not. Nitrite inhibits growth.
The optimum temperature for growth is about 37°C. The
 optimum pH for growth is around 6.5. Halophilic, with an
 optimum NaCl concentration for growth of around 0.5 M.
No growth occurs in the absence of NaCl.

The \textit{G+C} content of the DNA is 44.1 mol\% (as determined
by high-performance liquid chromatography).

Straight-chain saturated \textit{C}_{16:0} and \textit{C}_{18:0} acids and a
straight-chain unsaturated \textit{C}_{16:1} acid are the major compo-
nents of the cellular fatty acids. Ubiquinone-8 is the major
component of the quinone system.

Isolated from seawater from the Shonan Coast, Kanagawa
Prefecture, Japan.

The type strain is strain MH-110 (= JCM 7688).

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