APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1990, p. 2922–2923
0099-2240/90/092922-02$02.00/0

Vol. 56, No. 9

Thiobacillus ferrooxidans, a Facultative Hydrogen Oxidizer

ELISABETH DROBNER, HARALD HUBER, AND KARL O. STETTER*

Lehrstuhl für Mikrobiologie, Universität Regensburg, D-8400 Regensburg, Federal Republic of Germany

Received 24 April 1990/Accepted 14 June 1990

The type strain (ATCC 23270) and two other strains of *Thiobacillus ferrooxidans* were able to grow by hydrogen oxidation, a feature not recognized before. When cultivated on H_2, a hydrogenase was induced and the strains were less extremely acidophilic than during growth on sulfidic ores. Cells of *T. ferrooxidans* grown on H_2 and on ferrous iron showed 100% DNA homology. Hydrogen oxidation was not observed in eight other species of the genus *Thiobacillus* and in *Leptospirillum ferrooxidans*.

Members of the eubacterial genus *Thiobacillus* are presently characterized by their ability to oxidize elemental sulfur and other sulfur compounds (7). *T. ferrooxidans* is able to grow by oxidation of ferrous iron or sulfidic ores. As a result of these properties, it is the most important bacterium in bioleaching (6). Novel rod-shaped isolates are able to grow by oxidation of galena (PbS). Alternatively, they are oxidizers of molecular hydrogen (E. Drobner, H. Huber, and K. O. Stetter, unpublished data). In this study, we examined members of the genus *Thiobacillus* and *Leptospirillum ferrooxidans* for their ability to grow chemolithotrophically by hydrogen oxidation.

All strains were grown under shaking (100 rpm) in 100-ml Erlenmeyer flasks containing 30 ml of the corresponding medium (Table 1). H_2 oxidation was examined in 100-ml stoppered serum bottles (type III glass) (13) containing 20 ml of basal medium in the absence of the usual substrates (Table 1). The bottles were pressurized with H_2-CO_2 (80:20, 250 kPa). Finally, 4 ml of air (250 kPa) was added. In large scale, cells of *T. ferrooxidans* (ATCC 23270^T) were cultivated in an 85-liter enamel-protected fermentor (HTE Bioengineering, Wald, Switzerland) either on ferrous iron (500 g/50 liters; gassing by 2 liters of air per min) or on a mixture of H_2-CO_2-air (80:20:3, 200 kPa). Bacterial growth was followed by direct cell counting in a Thoma chamber (depth, 0.02 mm). Hydrogen was analyzed by gas chromatography. The G+C content of the DNAs was determined by the melting temperature (T_m) method in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (10, 15). DNA-DNA hybridization was performed (9) after radioactive in vitro labeling of the DNAs by nick translation (8) by the filter technique (1, 3). Hybridization lasted for 18 h in 3× SSC at 77°C (25°C below T_m = "optimal conditions" [10]).

Members of different *Thiobacillus* species and *L. ferrooxidans* were precultivated on different substrates (Table 1). During the exponential growth phase, the cultures were transferred (5% inoculum) into basal media and were incubated in the presence of a mixture of H_2, O_2, and CO_2. Surprisingly, all three strains of *T. ferrooxidans* (Table 1) grew under these conditions (final cell concentrations about 1×10^8 to 3×10^8/ml). This indicated that *T. ferrooxidans* was able to utilize molecular H_2 as the sole energy source. During growth on H_2, an increase of cell density was correlated to a decrease of H_2 (Fig. 1). In contrast, *T. acidophilus*, *T. cuprinus*, *T. neapolitanus*, *T. perometabolis*, *T. prosperus*, *T. thioparus*, *T. thiooxidans*, *T. versutus*, and *L. ferrooxidans* did not grow and were therefore unable to use H_2 as an energy source. Cultures of the three strains of *T. ferrooxidans* grown on H_2 could be successfully transferred into fresh medium. After 10 transfers in sequence on H_2, the cultures were still able to grow alternatively on iron, sulfur, and sulfidic ores. Therefore, these cultures still exhibited properties recognized for *T. ferrooxidans*. Identity of cultures of *T. ferrooxidans* on H_2 and on $FeSO_4$ was evident from the following results. (i) Cells were short gram-negative rods, about 0.5 μm in diameter, which are typical for *T. ferrooxidans* (7). (ii) DNA prepared from both cultures exhibited a G+C content of 60 mol%. (iii) The DNA-DNA hybridization between both cultures demonstrated 98 and 100% homology, respectively (Table 2).

As expected, no *T. ferrooxidans* strains were able to grow in the absence of O_2. With S^0 or ferrous iron as the energy source, they grew between pH 1 and 6 with an optimum around pH 2 (doubling time, 4.5 h). With H_2, however, they grew between pH 2.5 and 6 with a relatively broad optimum

TABLE 1. Sources and culture conditions of the strains

Strain[a]	Medium (reference)	pH	Temp (°C)	Substrate[b]
T. acidophilus (DSM 700^T)	12	3.5	30	0.3% glucose
T. cuprinus (DSM 5495^T)	4	3.5	37	3.3% ore mixture G1 (5)
T. ferrooxidans (ATCC 23270^T)	4	2.5, 3.5	30	4.0% $FeSO_4$
T. ferrooxidans (ATCC 19859)	4	2.5, 3.5	30	4.0% $FeSO_4$
T. ferrooxidans (isolate Hv9)	4	2.5, 3.5	30	4.0% $FeSO_4$
T. neapolitanus (DSM 581^T)	14	3.5	30	0.1% $Na_2S_2O_3$
T. perometabolis (ATCC 23370^T)	12	2.5, 3.5	30	0.1% $Na_2S_2O_3$ + 0.1% yeast extract
T. prosperus (DSM 5130^T)	4	2.5, 3.5	37	3.3% ore mixture G1 (5)
T. thiooxidans (ATCC 19377^T)	4	2.5, 3.5	30	0.1% sulfur
T. thioparus (DSM 505^T)	14	6.0	30	0.1% $Na_2S_2O_3$
T. versutus (DSM 582^T)	2	3.5	30	0.1% $Na_2S_2O_3$ + 0.1% yeast extract
L. ferrooxidans (DSM 2705)	12	2.5, 3.5	30	1.7% pyrite (5)

[a] DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Federal Republic of Germany; ATCC, American Type Culture Collection, Rockville, Md.
[b] In the absence of H_2.

* Corresponding author.

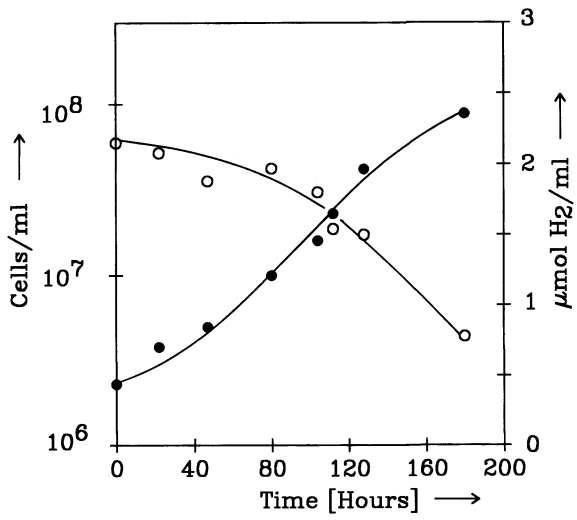

FIG. 1. Growth of *T. ferrooxidans* (ATCC 23270^T) by hydrogen oxidation. Symbols: ●, cell concentration; ○, H_2 concentration.

between pH 3.0 and 5.8 (doubling time around 5 h [data not shown]). No growth occurred on H_2 at pH 2.2 and 6.5. Therefore, the *T. ferrooxidans* strains were less acidophilic when grown on H_2 instead of sulfurous compounds or ferrous iron. In the presence of H_2, cells grown on H_2 were able to reduce methylene blue (11; A. Segerer, personal communication), while they were unable to do this when grown on ferrous iron. This is evidence for the presence of an inducible hydrogenase. When cells of *T. ferrooxidans* were cultivated in the presence of H_2 and S^0 or Fe^{2+} or (sulfidic) ore mixture G1 (5) at the same time, no hydrogenase activity was detected. Therefore, H_2 oxidation appeared to be repressed by the presence of S^0, Fe^{2+}, and sulfidic ores.

Our experiments demonstrate for the first time H_2 oxidation within a member of the genus *Thiobacillus*. This novel feature of *T. ferrooxidans* may be of great interest for metabolic, genetic, and ecologic studies of this organism important for ecology and biotechnology.

TABLE 2. DNA-DNA homology between *T. ferrooxidans* (ATCC 23270^T) cells grown on H_2 and those grown on $FeSO_4$

Filter-bound DNA from *T. ferrooxidans* grown on:	% Homology with ^{32}P-labeled DNA from *T. ferrooxidans* grown on:	
	H_2	$FeSO_4$
H_2	(100)[a]	98
$FeSO_4$	100	(100)
Calf thymus (control)	3	4

[a] 150,000 cpm per filter.

The excellent technical assistance of Lucia Schwarzfischer and Silvia Frank is greatly appreciated. We thank Andreas Segerer for the determination of hydrogenase activity.

This work was supported by grants of the Bundesministerium für Forschung und Technologie (BMFT, Projektleitung Rohstofforschung, FKZ 03 R 085 A) and the Fonds der Chemischen Industrie.

LITERATURE CITED

1. **Birnstiel, M. L., B. H. Sells, and I. F. Purdom.** 1972. Kinetic complexity of RNA molecules. J. Mol. Evol. **63**:21–39.
2. **Brock, T. D., K. M. Brock, R. T. Belley, and R. L. Weiss.** 1972. *Sulfolobus*: a new genus of sulfur-oxidizing bacteria living at low pH and high temperature. Arch. Microbiol. **84**:54–68.
3. **Gillespie, S., and G. D. Gillespie.** 1971. Ribonucleic acid-deoxyribonucleic acid hybridization in aqueous solutions and in solutions containing formamide. Biochem. J. **125**:481–487.
4. **Huber, G., H. Huber, and K. O. Stetter.** 1986. Isolation and characterization of new metal-mobilizing bacteria. Biotechnol. Bioeng. Symp. **16**:239–251.
5. **Huber, H., and K. O. Stetter.** 1989. *Thiobacillus prosperus* sp. nov., represents a new group of halotolerant metal-mobilizing bacteria isolated from a marine geothermal field. Arch. Microbiol. **151**:479–485.
6. **Kelly, D. P.** 1988. Evolution of the understanding of the microbiology and biochemistry of the mineral leaching habitat, p. 3–14. *In* P. R. Norris and D. P. Kelly (ed.), Biohydrometallurgy. Science and Technology Letters, Kew, Surrey, England.
7. **Kelly, D. P., and A. P. Harrison.** 1989. Genus *Thiobacillus*, p. 1842–1858. *In* J. T. Staley, M. P. Bryant, N. Pfennig, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 3. The Williams & Wilkins Co., Baltimore.
8. **Kelly, R. B., N. R. Cozzarelli, M. P. Deutscher, J. R. Lehmann, and A. Kornberg.** 1970. Enzymatic synthesis of deoxynucleic acid by polymerase at a single strand break. J. Biol. Chem. **245**:39–45.
9. **König, H.** 1984. Isolation and characterization of *Methanobacterium uliginosum* sp. nov. from a marshy soil. Can. J. Microbiol. **30**:1477–1481.
10. **Marmur, J., and P. Doty.** 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. **5**:109–118.
11. **Schink, B., and H. G. Schlegel.** 1979. The membrane-bound hydrogenase of *Alcaligenes eutrophus*. I. Solubilization, purification, and biochemical properties. Biochim. Biophys. Acta **567**:315–324.
12. **Silverman, M. P., and D. G. Lundgren.** 1959. Studies on the chemolithoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. I. An improved medium and harvesting procedure for securing high cell yields. J. Bacteriol. **77**:642–647.
13. **Stetter, K. O., M. Thomm, J. Winter, G. Wildgruber, H. Huber, W. Zillig, D. Janécovic, H. König, P. Palm, and S. Wunderl.** 1981. *Methanothermus fervidus*, sp. nov., a novel extremely thermophilic methanogen isolated from an Icelandic hot spring. Zentralbl. Bakteriol. Mikrobiol. Hyg. I Abt. Orig. C **2**:166–178.
14. **Vishniac, W. V., and M. Santer.** 1957. The *Thiobacilli*. Bacteriol. Rev. **21**:195–213.
15. **Wildgruber, G., M. Thomm, H. König, K. Ober, T. Ricchiuto, and K. O. Stetter.** 1982. *Methanoplanus limicola*, a plate-shaped methanogen representing a novel family, the *Methanoplanaceae*. Arch. Microbiol. **132**:31–36.