Short Communication

Isolation of Strictly Thermophilic and Obligately Autotrophic Hydrogen Bacteria

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Various kinds of hydrogen bacteria, mainly mesophiles,¹⁾ some thermophiles^{2,3)} and a strictly thermophile,^{4,5)} have been isolated and reported on their taxonomy, physiology, and application. However, obligately autotrophic hydrogen bacterium is nowhere to be found up to date. We isolated a new type of hydrogen bacteria, strict thermophile and obligate autotroph, and described the characteristics of the strain.

Isolation medium had the following composition: 1 g NH_4NO_3 , 4.5 g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 1.5 g KH_2PO_4 , 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 10 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 10 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.0 g or 4.0 g NaCl , 0.5 ml trace element solution, 1000 ml deionized water. Trace element solution contained (per liter of deionized water): 4 mg MoO_3 , 28 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 2 mg $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 4 mg H_3BO_3 , 4 mg $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$ and 4 mg $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$.

Samples of thermal water and soils around hot springs in Izu Peninsula and Tochigi Prefecture were used as the isolation source.

Each sample was spread on the plates of isolation medium solidified by adding 1.5% Bacto-Agar (DIFCO). The plates were placed in desiccators containing the gas mixture ($\text{H}_2:\text{O}_2:\text{CO}_2=85:5:10$) and incubated at 65°C. After 2 to 4 weeks, one loop each of colonies developed on plates was picked up and inoculated into 40 ml L-tubes containing 7.5 ml of the same liquid medium and gas

mixture as above. These were shaken on a reciprocating shaker at 70°C. After one or two days cultivation, 0.5 ml of cultured broth was transferred to 7.5 ml of the new medium.

Four strains of thermophilic hydrogen bacteria were enriched by repeating this procedure and isolated by using the dilution method. The strain TK-6, which showed the most rapid growth among the four strains isolated, was used in the subsequent investigations.

TK-6 is Gram-negative, non-spore forming, non-motile, long straight rods. Cell size is 0.2 to 0.3×2.0 to $3.0 \mu\text{m}$. (Fig. 1) The optimal temperature for autotrophic growth is between 70 and 75°C. Specific growth rate at this temperature range is about $0.33 \text{ (hr}^{-1}\text{)}$. No growth occurs at 80°C and 37°C. No growth factor is required. GC-content of DNA of TK-6 is 43.7% (Tm method).⁶⁾ TK-6 had much lower GC-content of DNA compared with the other hydrogen bacteria so far isolated.

FIG. 1. Electron Micrograph of Strain TK-6.
Bar represents 1 μm .

The abilities to utilize various organic compounds were examined. The strain TK-6 showed heterotrophic growth on none of organic substrates shown in Table I. Ten of them (glucose, fructose, pyruvate, citrate, α -KG, succinate, fumarate, malate, acetate, and ethanol) were further studied for the effect of

TABLE I. LIST OF ORGANIC COMPOUNDS AND MEDIA TESTED FOR HETEROTROPHIC GROWTH OF TK-6

glucose*, fructose*, xylose*, sucrose, maltose, starch, L-rhamnose, D-trehalose, raffinose, D-mannose, mannitol
acetate, pyruvate, succinate, citrate, malate, fumarate, maleate, propionate, glycollate, gluconate, formate, DL-lactate, α -ketoglutarate, <i>p</i> -hydroxybenzoate, DL- β -hydroxybutyrate, betaine, methanol, ethanol, methane
glycine, L-glutamate, L-aspartate, L-serine, L-leucine, L-valine, L-tryptophan, L-histidine, L-alanine, L-lysine, L-proline, DL-arginine, nutrient broth, yeast extract-malt extract medium, Bacto-Brain Heart Infusion (DIFCO) medium

Each compound was added to autotrophic medium at the concentration of 0.1% (w/v). (* 0.2%)

addition of yeast extract or carbon dioxide, and the alteration of incubation temperature to 50°C. Effect of their concentration [0.02, 0.05 and 0.1% (w/v)] was also examined. In none of these cases, heterotrophic growth was observed at all.

The effect of various organic compounds on autotrophic growth was also studied. Pyruvate, fructose, citrate and acetate added at a concentration of 1.0% (w/v) markedly inhibited the autotrophic growth, while glucose, α -ketoglutarate and succinate added at the same concentration were not inhibitory. This inhibitory effect of organic compounds on autotrophic growth was common to such an obligately autotrophic bacterium as *Thiobacillus*,⁷⁾ and this similarity is of great interest.

All hydrogen bacteria so far isolated to date are known to be facultative chemolithoautotrophs and it has been believed that no obligately chemolithotrophic hydrogen bacterium exists on the world.⁸⁾ Davis *et al.*⁹⁾ regarded the ability to grow on hydrogen and carbon dioxide as only one of the physiological features and they reclassified all hydrogen bacteria, which had been classified to genus *Hydrogenomonas* till then, into various kinds

of existing genera on the basis of their general morphological and physiological characteristics other than hydrogen utilization.

In the 8th edition, the Bergey's Manual was revised according to this point of view, so the strain TK-6 could be classified into none of genera listed in the Manual. In this respect, our findings of obligately autotrophic hydrogen bacteria or so to call "obligate hydrogenotroph" requires new understandings to the hydrogen bacteria.

Other physiological characterization of the strain TK-6 and taxonomic studies on the other strictly thermophilic hydrogen bacteria obtained here are now in progress.

At present, physiological studies on the autotrophic bacteria in thermophilic environments are extensively being pursued by many investigators. Our isolates are very unique and attractive in this respect too, especially the ecological meaning or explanation of "obligate hydrogenotrophy" is the most interesting subject.

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