Note

Production of Molecular Hydrogen
by Photosynthetic Bacteria
with Raw StarchLerluck BURANAKARL,* FAN Cheng-Ying,**
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We are interested in the production of molecular hydrogen by photosynthetic bacteria because this process will show the most vigorous evolution of hydrogen among known biological systems. Usually, we used DL-malate or DL-lactate as an electron donor.^{1,2)} Recently, various agricultural products and wastes have been used as substrates for photosynthetic bacteria. For example, Zürrer and Bachofen reported hydrogen production by *Rhodospirillum rubrum* with lactate containing wastes, including waste from lactic acid bacteria production, yogurt waste and whey.³⁾ Sasaki and Nagai used soluble starch for the growth of *Rhodopseudomonas gelatinosa* to produce vitamin B₁₂ and ubiquinone.⁴⁾ Noparatnaraporn *et al.* used cooked cassava starch to produce single cell protein with *R. gelatinosa*.^{5,6)}

In this paper, we report that two strains of non-sulfur purple photosynthetic bacteria produced hydrogen with raw starch from corn, potato or cassava as well as with soluble starch under phototrophic conditions.

Two pure strains of non-sulfur purple photosynthetic bacteria designated as T-14 and T-20 were used. They were isolated in the Bangkok area, Thailand. The bacteria were grown at 30°C in the modified Ormerod medium (basal medium)⁷⁾ supplemented with an electron donor and a nitrogen source. The pH of the medium was adjusted to 6.8 unless otherwise stated.

The two strains, T-14 and T-20, grew well in the basal medium containing 30 mM DL-malate and 10 mM ammonium sulfate under anaerobic photosynthetic conditions. They grew under dark and aerobic conditions in the same medium as well. Under anaerobic-light conditions, the color of the cell suspension of T-14 was pinkish to red while that of T-20 was pinkish to purple red. When cultures of both strains were shifted from anaerobic to aerobic conditions, there was no change in color. Moreover, neither of these strains was able

to utilize thiosulfate or sulfide as an electron donor under anaerobic-light conditions. On electron microscopic observation, T-14 and T-20 were found to be short rods (0.6~0.7 nm wide and 1.85~2.3 nm long), each with a polar flagellum. These results indicated that both strains were non-sulfur purple photosynthetic bacteria of the genus *Rhodopseudomonas*. Both were capable of gelatin liquefaction. No growth was observed in a medium of pH 5.0. Molecular hydrogen could be used as an electron donor. A small amount of yeast extract added to the medium stimulated the growth of the two strains. Both strains required thiamine for growth but not biotin, *p*-aminobenzoic acid or nicotinic acid. Malate, lactate, citrate, formate, L-glutamate, glucose and fructose supported the growth of both strains but not propionate, tartrate, glutarate, butyrate, benzoate, L-leucine, mannitol, sorbitol, ethanol or glycerol. The morphological and physiological properties, especially the capability of gelatin liquefaction, suggested that these two strains resembled *Rhodopseudomonas gelatinosa*, but these two strains did not require biotin for growth under anaerobic-light conditions. This is in contrast to the description for *R. gelatinosa* in Bergey's Manual.⁸⁾

Cells were grown photosynthetically under anaerobic conditions to the logarithmic phase in the basal medium containing 1% soluble starch and 5 mM sodium L-glutamate. Cells were harvested and washed once with the basal medium supplemented with 5 mM glutamate. Then the cells were resuspended heavily in the same medium as above. The concentrated cell suspensions were inoculated into five different media at the cell density of OD₆₆₀=0.3 (determined with a photoelectric colorimeter EPO-B, Hiranuma Sangyo). The following media were used; the basal medium supplemented with 5 mM glutamate (control), and the control medium containing 1% each of soluble starch (from potato), raw potato starch, raw corn starch or raw cassava starch. The media without raw starch were sterilized by autoclaving. The raw starch was sterilized separately with ethylene oxide and added aseptically to the media. Each culture (5 ml) was kept in a gas exchange tube and incubated under an argon atmosphere and illumination. Cultures were stirred continuously during the incubation. The rates of hydrogen evolution were measured with a gas chromatograph as described previously.⁷⁾

Figure 1 shows the hydrogen production activity during incubation for 30 hr of strain T-14. This strain produced molecular hydrogen when the cells utilized either soluble starch or raw starch from potato, corn or cassava under anaerobic conditions. Raw corn starch was the best substrate among the four carbohydrates, and the total hydrogen produced was about 35 ml while that with the raw potato starch and raw cassava starch media was about 23 ml and 18.5 ml, respectively. Although the utilization of

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FIG. 1. Total Hydrogen Production per Culture of Strain T-14 during 30 hr Incubation in Media Containing Soluble Starch (●—●), Raw Corn Starch (□—□), Raw Potato Starch (△—△) and Raw Cassava Starch (▽—▽), and without Starch (○—○).

soluble starch at the early phase of the incubation was slow, it became a good electron donor after 10 hr. The rates of hydrogen production with raw corn starch and soluble starch were almost identical in the latter part of the incubation. Strain T-14 also produced hydrogen in the control medium without starch, the total hydrogen produced being about 3.7 ml. However, the activity became almost negligible after 16 hr incubation probably due to the disappearance of glutamate in the medium.

In the case of strain T-20, essentially similar results were obtained (Fig. 2). Raw corn starch was also shown to be the best electron donor for hydrogen production among the four carbohydrates. The total hydrogen produced in the medium containing raw corn starch was 37 ml, while that in the media containing raw potato starch and cassava starch was 35 ml and 16 ml, respectively. In the latter part of the incubation, raw corn starch and raw potato starch showed almost identical rates of hydrogen production. The hydrogen production activity with raw cassava starch was much less compared with that with the above substrates. Again, a very long lag-time was observed with soluble starch. However, the final rate of hydrogen production with soluble starch was almost identical to those with raw corn starch and raw potato starch. A small but significant amount of hydrogen was produced in the control medium without starch. However, the amount of hydrogen produced by T-20 was much less compared to that by T-14.

To our knowledge, this is the first report showing that non-sulfur purple photosynthetic bacteria produce mo-

FIG. 2. Total Hydrogen Production per Culture of Strain T-20 during 30 hr Incubation in Media Containing Soluble Starch (●—●), Raw Corn Starch (□—□), Raw Potato Starch (△—△) and Raw Cassava Starch (▽—▽), and without Starch (○—○).

lecular hydrogen with raw starch as an electron donor. It is noteworthy that the hydrolysis of raw starch by fungal amylase became poorer in the order of corn, cassava and potato.⁹⁾ As shown above, the order was different with respect to hydrogen production. The purification of the amylase responsible for the digestion of raw starch as well as the cloning of its gene in *Escherichia coli* are in progress in this laboratory.

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