Isolation of Denitrifying Photosynthetic Bacteria

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In spite of numerous works on the utilization of nitrate by microorganisms,¹⁾ little has been studied on the problem of nitrate reduction by photosynthetic bacteria. Katoh made a search in *Rhodospirillum (Rsp.) rubrum* for an alternative hydrogen acceptor which could take the place of oxygen, but without success.²⁾ Then he tried to adapt the organism to grow on a nitrate-medium, and could isolate the adapted cells which had capacity to grow either aerobically or anaerobically in the light with nitrate as the sole source of nitrogen.³⁾ Taniguchi and Kamen also showed that *Rsp. rubrum* assimilated nitrate for growth either aerobically or photosynthetically, but they concluded that photoheterotrophs were unable to adapt for heterotrophic growth by utilization of nitrate as electron acceptor other than oxygen.⁴⁾ Contrary to these reports, Ketchum recently reported that *Rsp. rubrum* was unable to grow on nitrate as the sole nitrogen source and that it had no nitrate-reductase activity in cell-free extracts of the strain.⁵⁾ As to the denitrification by photosynthetic bacteria, no information has been obtained so far.

In the course of study on the role of photosynthetic bacteria in purification of polluted water, we newly isolated denitrifying photosynthetic bacteria from polluted water of the lagoon pond of Asahimatsu Frozen Bean-Curd Co., Ltd., Iida. Photosynthetic bacteria were isolated as the purple colonies on 0.3% of yeast extract plus 0.2% of casamino acids (YC-medium) agar plate under aerobiosis. Three strains, IL-101, IL-103, and IL-106, were denitrifiers among eight strains isolated as photoheterotrophs. *Rhodopseudomonas (Rps.)*

spheroides S, which was isolated in our laboratory from trickling filter at the sewage treatment plant in Takada,⁶⁾ was also a denitrifier. The strain IL-106 was used for further study because of the strongest denitrifying activity. IL-106 was purified by the method of repeated colony selection.

When IL-106 was cultured anaerobically either with or without illumination in YC-liquid medium containing 0.2% of KNO₃ and 0.2% of malate, it evolved gas intensely. The gas was trapped and identified as nitrogen gas with gas chromatography (Fig. 1). The trapped gas is consisted mostly of nitrogen, but contained very small amount of unknown gas.

FIG. 1. Gas Chromatogram of the Gas Evolved by the Strain, IL-106.

Gas Chromatogram of the gas evolved by IL-106 was compared with that of air.

Instrument: Shimadzu GC-3AH; column: Molecular Sieve 5A, 1m; temperature: 40°C; carrier gas: He, 97.2 ml/min.

a: air, b: the gas of IL-106.

The cells of IL-106 are Gram-negative, ovoid or spherical, and 1 μ m wide and 1.3 μ m long. They appear to resemble to *Rps. spheroides* in morphology. The dimensions are rather in-

dependent of the composition of the growth medium. Cells are almost single or 2-celled. On agar the cells form lens-shaped colonies. The strain is motile by means of a polar flagellum (not shown).

The colour of photosynthetically grown culture is yellowish-green. Aerobically grown culture is brown-red. Under anaerobiosis with nitrate, it shows yellowish-brown. The absorption spectrum of intact cells of photosynthetically grown IL-106 shows the maxima characteristic of bacteriochlorophyll *a* (Fig. 2).

FIG. 2. *In vivo* Absorption Spectrum of the Strain, IL-106.

Cells were cultured photosynthetically on the YC-liquid medium, collected, suspended in 60% (w/w) sucrose solution, and observed for absorption.

The main near-infrared peak in the spectrum of the strain is situated at 850 nm. This absorption peak together with those at 800, 593, and 378 nm indicates the presence of bacteriochlorophyll *a*.⁷⁾ The position of the peaks between 450 and 550 nm is similar to those encountered in *Rps. spheroides* strain and indicates the presence of carotenoids of the spheroidene group.⁸⁾

The growth factor requirement can be satisfied by the addition of niacine, thiamine, and biotin. Gelatin is not liquefied. The strain is capable of photolithotrophic growth with molecular hydrogen and carbon dioxide. Thiosulfate and hydrogen sulfide are not utilized. It can grow using propionate as the sole carbon source, although slowly. It grows phototrophically using mannitol and sorbitol.

These characters are the same as those of *Rps. spheroides*. However, IL-106 can not utilize tartrate for growth unlike *Rps. spheroides*. The range of utilization of other organic substrates for phototrophic growth is the same as *Rps. spheroides*. IL-106 can grow well aerobically in the dark.

The most remarkable property of IL-106 is the ability to use nitrate as an electron acceptor, accompanying the evolution of nitrogen gas. The strain, therefore, can grow on a nitrate-containing medium anaerobically in the dark, though it can not grow on a nitrate-free medium as the ordinary *Athiorhodaceae*. IL-106 neither aerobically nor photosynthetically grows using nitrate as the sole source of nitrogen, showing that nitrate is not assimilated. The new isolate grows well photosynthetically by fixing molecular nitrogen as the sole source of nitrogen as the ordinary photosynthetic bacteria do.

With respect to the morphology, photopigments, requirement for growth factors, and the range of utilization of organic substrates, IL-106 might be named *Rps. spheroides*. In our experiments, however, *Rps. spheroides*, IFO 12203 (van Niel's collection), had not denitrifying activity and could not use nitrate as the terminal electron acceptor for growth. IL-106 is the first photosynthetic bacterium which has been isolated as a denitrifier. Therefore, the difference in the ability of denitrification between IL-106 and *Rps. spheroides*, IFO 12203, raises the question whether IL-106 could be identified as *Rps. spheroides* or as a new species. Similarly, the strain which was named *Rps. spheroides* S⁸⁾ is also questionable. Further experiments on the photosynthetic membrane system and the mode of multiplication are in progress.

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