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Detection of Bacteriochlorophyll-*c* Containing Species of Green Sulfur Photosynthetic Bacterium *Chlorobium vibrioforme*

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Bacteriochlorophyll(BChl)-*c* containing species of green sulfur photosynthetic bacterium *Chlorobium* (*Chl.*) *vibrioforme*, which has BChl-*d* mainly, was detected. We obtained colonies on agar plates by spreading the liquid culture of *Chl. vibrioforme* f. sp. *thiosulfatophilum* strain NCIB 8327 which contained the high ratio of BChl-*c*/BChl-*d*, and transferred each colony into a new liquid medium. These cultures after growing were found to be classified into two categories. One possessed BChl-*d* as a light-harvesting pigment and the other did BChl-*c*. No colonies examined here contained both BChls-*d* and *c*. Therefore, the presence of both BChls-*d* and *c* in our cultures of *Chl. vibrioforme* was ascribed to the coexistence of two different cells which had BChl-*d* and *c* as the chlorosomal pigment, respectively. The change of pigment composition observed in our liquid cultures can be thus explained by the difference of growth rates between two kinds of cells.

Key Words: Bacteriochlorophyll-*c*, Bacteriochlorophyll-*d*, *Chlorobium vibrioforme*, Chlorosome, Light-harvesting Pigment, Pigment Composition Change

INTRODUCTION

Green photosynthetic bacteria have a unique antenna complex called chlorosome. In chlorosomes, bacteriochlorophyll(BChl)s-*c*, *d*, or *e* self-assemble to unsubstituted in BChl-*d* (the molecular structures of BChl-*c* and *d* are shown in Fig. 1). This substitution causes the difference of spectral properties of both monomeric and aggregation forms between BChl-*c* and *d* [1].

Usually, one strain of green photosynthetic bacteria possesses one type of chlorosomal BChls. However, there have been several reports on the coexistence of

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BChl-*d* and *c* in some species of green sulfur bacteria [2–4]. It has not been clear whether a single cell of the green sulfur bacterium has both BChls-*d* and *c*, or just one of them. In the present study, we report the separation of BChl-*c* containing species from those possessing BChl-*d* by preparing colonies on solid agar plates.

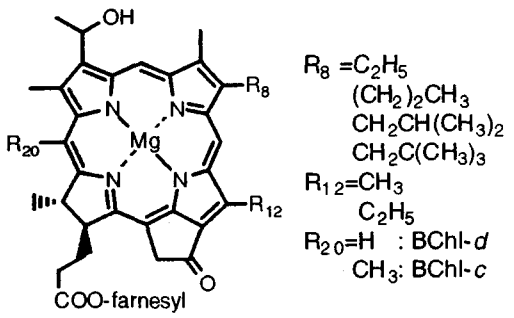

Figure 1 Molecular structures of BChl-*c* and *d*.

MATRIALS AND METHODS

Chl. vibrioforme f. sp. *thiosulfatophilum* strain NCIB 8327 was photoautographcally cultivated. Colonies were prepared on agar plates (Chlorobium medium containing 1.5% (w/v) agar) from liquid cultured cells which contained both BChls-*d* and *c*. Each colony was picked up and placed in the liquid medium.

Cultured cells were harvested by centrifugation and chlorosomal pigments were extracted from the cells with acetone/methanol (v/v=1/1). The extracted pigments were analyzed by reverse-phase HPLC. The dried chlorosomal pigments were dissolved in a HPLC eluent (methanol/water=9/1), and eluted on a reverse-phase column 5C18-AR-II (4.6 mmϕ × 150 mm, Nacalai Tesque) at a flow rate of 1.0 mL/min.

RESULTS AND DISCUSSION

Figure 2 shows visible absorption spectra of the initial culture of *Chl. vibrioforme* and cells obtained by transferring it repeatedly at reduced light conditions. The initial culture exhibited the absorption maxima around 450 and 735 nm at Soret and Q_y bands, respectively. When *Chl. vibrioforme* was grown by transferring the culture repeatedly at low light intensities, both Soret and Q_y bands of the cells were gradually shifted to longer wavelength. The cells obtained after 6-times transfers at low light intensities had the Soret and Q_y bands at 456 and 752 nm, respectively. HPLC analyses of chlorosomal chlorophyllous pigments revealed that the initial culture possessed BChl-*d*, and the cells obtained after 6-times transfers had BChl-*c* mainly.

We prepared colonies from the liquid culture of *Chl. vibrioforme* which had both BChls-*d* (55%) and *c* (45%).

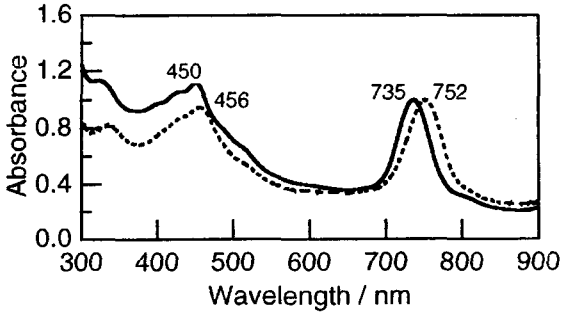

Figure 2 Visible absorption spectra of the initial culture (solid curve) and cells obtained by 6-times transfers at low light conditions (broken curve).

The formed colonies were transferred again to fresh liquid medium. The liquid culture grown from 14 colonies were classified to two patterns by means of visible absorption spectroscopy and HPLC analysis. One (9 colonies) exhibited the Soret and Q_y bands around 450 and 735 nm, respectively, and had BChl-*d* homologs as chlorosomal pigments. In contrast, the other (5 colonies) had the absorption bands around 460 and 750 nm, and possessed BChl-*c* homologs. No colonies examined in this study had both BChls-*d* and *c*. The ratio (5/14 = 36%) of colonies possessing BChl-*c* to all the colonies was similar to the ratio (45%) of BChl-*c* to the chlorosomal BChls in the liquid culture used for the preparation of colonies.

Genetic characterization of both the species containing BChl-*d* and *c* (A. Hiraishi and K. V. P. Nagashima, personal communication) indicates that both the species are phylogenetically the same strain and the BChl-*c* possessing species are not derived from contamination of any other species.

To summarize, we separated the BChl-*c* containing species of *Chl. vibrioforme* from the liquid culture. This suggests that the apparent change in chlorosomal pigment composition change in cultures of green sulfur photosynthetic bacteria might be ascribed to thepopulation change of two kinds of cells.

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