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The reaction center complex and photosynthesis genes in the green filamentous bacterium lacking chlorosomes, *Roseiflexus castenholzii*

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Introduction

Green filamentous bacteria, including *Chloroflexus aurantiacus*, have been placed on the deepest branch among all photosynthetic bacteria in the phylogenetic tree based on 16S-rDNA sequence data (Oyaizu et al., 1987). On the other hand, in the phylogenetic trees based on the genes for bacteriochlorophyll (BChl) biosynthesis enzymes, purple bacteria showed the deepest branch (Xiong et al., 2000).

The photochemical reaction center (RC) complex of *C. aurantiacus* has transmembrane L and M subunits similar to those of purple bacteria (Shiozawa et al. 1989). The RC of *C. aurantiacus* also has a cytochrome subunit, which contains four *c*-type hemes and donates electrons to the photo-oxidized special pair of BChls, as in many purple bacteria.

A newly described filamentous photosynthetic bacterium, *Roseiflexus castenholzii*, has been isolated from pink-colored biomat in a hot spring. *R. castenholzii* belongs to the group of green filamentous bacteria, based on the phylogenetic analysis of 16S-rDNA sequence (Hanada et al. 2001). Chlorosomes, which are light-harvesting apparatus characteristic of *Chloroflexus* species, are absent in this bacterium (Hanada et al. 2001). *R. castenholzii* looks like purple bacteria in terms of BChl species and the color of cells. In the present study, we analyzed properties of the RC complex and

photosynthetic genes in *R. castenholzii*, concerning the early evolution of photosynthesis.

Materials and Methods

The cells of *R. castenholzii* were grown anaerobically at 50˚C in the 02YE medium (pH 7.2) (Hanada et al. 2001) in a 4-l fermenter (MBF-500M, EYLA, Japan) illuminated by a tungsten lamp (100 W) placed 5-cm from the vessel.

The membrane preparations were obtained by disruption of the cells using a French press and differential centrifugation. Membranes were solubilized in pre-cooled 10 mM Tris-HCl buffer (pH 8.7) with 2% n-octyl β-D-glucopyranoside. The solubilized membranes were subjected to a DEAE-Sepharose Fast Flow column equilibrated with a 10 mM Tris-HCl buffer (pH 8.0) containing 0.05% Triton X-100. Using a stepwise salt gradient, RC was eluted from the column at 125 mM NaCl. The eluted RC fraction was applied to the preparative polyacrylamide gel electrophoresis. After electrophoresis, the highest-mobility band among four major-pigmented bands was extracted as the RC fraction. The same electrophoresis was repeated for further purification.

To obtain the RC genes, a cosmid library of the *R. castenholzii* genome was screened by DIG-labeled DNA fragments containing a part of *pufM* gene, which were amplified by PCR using primers designed from the comparison of the *pufM* sequences of purple bacteria and *Chloroflexus*. The cosmid DNA was purified from positive cells by secium chloride/ethidium bromide method. The purified cosmid DNA was partially digested with *Sau*3A1, subcloned into puc19 plasmid, and sequenced.

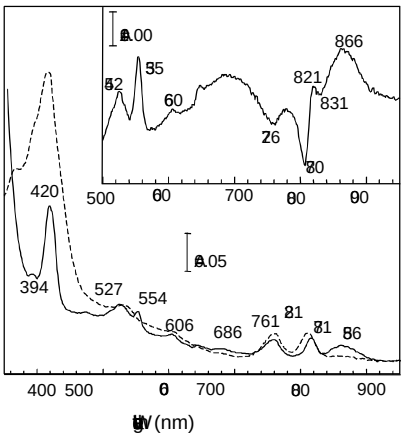

Fig. 1. Absorption spectra of reduced (solid line) and oxidized (dotted line) RCs from *R. castenholzii*.
The *inset* shows the reduced-*minus*-oxidized difference spectrum of RCs from *R. castenholzii*.

Results and Discussion

Figure 1 shows the absorption spectra of the purified *R. castenholzii* RC in the reduced and oxidized states. Peaks in the near infrared region were found at 865, 817 and

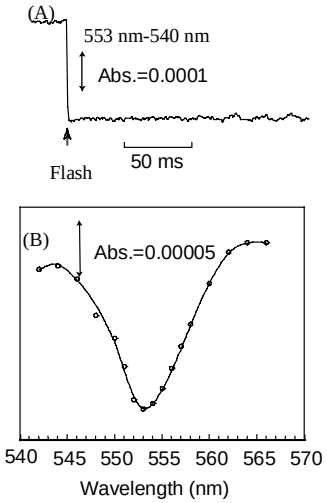

Fig. 2. Flash-induced absorbance and spectral changes in the RCs from *R. castenholzii*. (A), the kinetic trace showing the flash-induced absorbance changes at 553 nm-*minus*-540 nm. (B), the corresponding transient spectra 20 ms after the actinic flash.

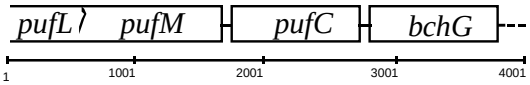

Fig. 3. Arrangement of sequenced *R. castenholzii* photosynthesis genes.

761 nm in the dithionite-reduced state. Each absorption band was assigned as the band of the special pair of BChls (865 nm), the accessory BChl (817 nm) or bacteriopheophytin (BPhe) (761 nm) based on the reported spectra of *C. aurantiacus* and purple bacteria. The absorbance ratio of accessory BChl to BPhe was almost 1:1 suggesting that *R. castenholzii* contains 1 molecule of the accessory BChl and 3 molecules of the BPhe per a special pair, as was in the RC from *C. aurantiacus* (Pierson et. al. 1983). The presence of three BPhe was also supported by the amino-acid sequence analysis of the M subunit of the RC. The histidine residue conserved in the M subunits of purple bacteria as a ligand to the Mg-atom of an accessary BChl was changed to an isoleucine in *R. castenholzii*. The dithionite-reduced *minus* ferricyanide-oxidized difference spectrum showed peaks at 866, 553 and 524 nm, indicating the presence of the special pair of BChl and cytochromes *c* (Figure 1 *inset*). The measurements of flash-induced absorption changes in the purified RC showed the fast oxidation of the RC-bound heme(s) *c* with the peak at 553 nm (Figure 2 (A), (B)). These results indicate that the RC of *R. castenholzii* was purified with the tightly-bound cytochrome subunit, which keeps the ability to donate electrons to the photooxidized special pair of BChl. Such a complex with the cytochrome subunit has not been isolated from *C. aurantiacus*, although the subunit has been assumed to be present in the whole cells and the membrane preparations. In the SDS-PAGE of the RC complex of *R. castenholzii*, the band of 35 kDa was identified as the tetra-heme cytochrome subunit, based on heme-staining data and the molecular mass number estimated from the gene sequence data. The molecular mass was significantly smaller than that of the cytochrome subunit of *C. aurantiacus*

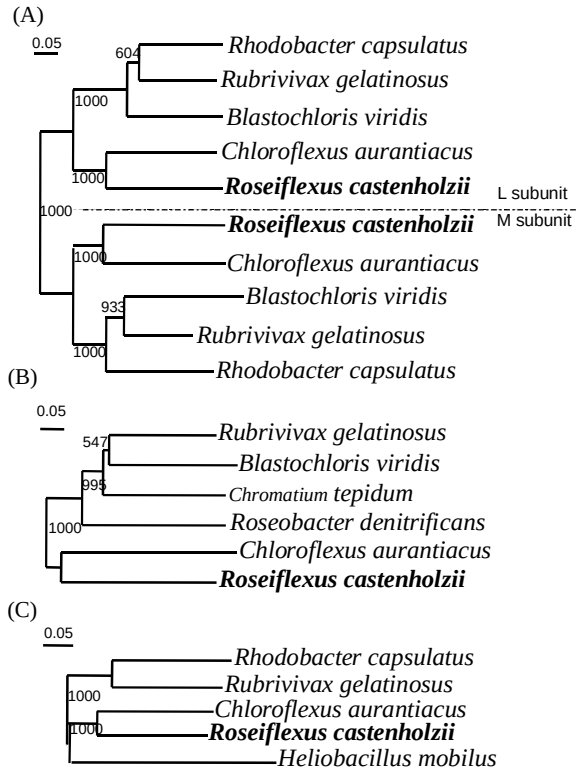

Fig. 4. Phylogenetic relationships between purple bacteria and green filamentous bacteria based on amino acid sequences deduced from *pufL/M* (A), *pufC* (B) and *bchG* (C).

(43 kDa).

Photosynthetic genes found in the sequenced DNA fragment are shown in Figure 3. They are a part of *pufL*, *pufM*, *pufC*, and *bchG* genes. The sequential arrangement of genes, *pufL*, *M*, and *C*, is the same as conserved in many purple bacteria. The gene for the cytochrome subunit of *C. aurantiacus*, on the other hand, is coded by another operon, *puf2C* (Watanabe et al. 1995). The *puf* gene arrangement in *R. castenholzii* is apparently close to those in purple bacteria, although the position of *bchG* after *pufC* is the same as that of *C. aurantiacus*.

Figure 4 shows phylogenetic relationships among green filamentous bacteria and purple bacteria, based on amino acid sequences deduced from *pufL/M* (A), *pufC* (B) and *bchG* (C). *R. castenholzii* gene products were the closest to *C. aurantiacus* in the all trees as has been shown in the 16S-rDNA analysis (Hanada et al. 2001). At the same time, *R. castenholzii* was branched from *C. aurantiacus* very early after the deviation from purple bacteria in all the trees. Thus, the analysis of photosynthesis genes in *R. castenholzii* should be very important to elucidate the ancestral characteristics of photosynthetic apparatus and genes among purple bacteria and green filamentous bacteria. The absence of chlorosomes and the gene arrangement of the *puf* operon may be examples of such ancestral characteristics.

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