Investigation of bacteriochlorophyll biosynthesis genes in
Blastochloris viridis by functional complementation of mutants of
Rhodobacter capsulatus

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Introduction

Blastochloris (formerly Rhodopseudomonas) viridis produces bacteriochlorophyll (BChl) b which is distinguished from BChl a by the possession of an ethylidene group at C8 instead of an ethyl (Eimhjellen et al., 1963). This chemical structure enables BChl b to absorb longer wavelength light (~1100 nm) than BChl a. To investigate genes involved in the BChl b biosynthesis, we have constructed genomic DNA libraries from B. viridis and introduced them to mutants of the nonsulfur purple bacterium Rhodobacter capsulatus which is deficient in BChl a biosynthesis. Total ten kinds of R. capsulatus mutants, three Mg-chelatase (BchD, H, I) mutants, three protochlorophyllide reductase (BchB, L, N) mutants, Mg-protoporphyrin IX methyltransferase (BchM) mutant, Mg-protoporphyrin IX monomethyl ester oxidative cyclase (BchE) mutant, bacteriochlorophyll synthase (BchG) mutant, and 3-vinyl-bacteriochlorophyllide hydratase (BchF) mutant, were functionally complemented by B. viridis library DNA. The complemented R. capsulatus strains were demonstrated to be capable of synthesizing BChl a under photoautotrophic growth conditions.

Materials and methods

Bacterial strains and culture conditions

Wild type R. capsulatus strain SB1003 and ten kinds of BChl biosynthesis deficient R. capsulatus strains (cf. Table 1) that have disruption in ORF which are involved in BChl biosynthesis by insertion of kanamycin resistance gene cassette, respectively, were used. There R. capsulatus strains were grown chemoheterotrophically or photoautotrophically at 34°C in PYS or RCV 2/3PY medium (Bollivar et al., 1994). Escherichia coli strains were grown at 37°C in Luria broth medium. Ampicillin and kanamycin were used at concentration of 100 µg/ml and at 50 µg/ml, respectively. For R. capsulatus strains, kanamycin was used at 10 µg/ml. B. viridis strain (DSM1003) was grown photoautotrophically as described (Lang and Oesterhelt, 1989).

Construction of genomic DNA library and plasmids transfer by bipartite mating

The B. viridis cells were harvested and chromosomal DNA was isolated according to the method as described (Wilson, 1994). Genomic library of B. viridis was constructed by partial digestion of genomic DNA with Sau3A I restriction enzyme and ligating the restriction fragments into the BamH I site of pJRD215 cosmid vector.
(Davison et al., 1987). The cosmids were transformed into an E. coli XL-1 blue MR strain containing the mobilizing element pDPT51 (Taylor et al., 1983). The resulting transformants were pooled and used to complement BChl biosynthesis deficient R. capsulatus mutant strains. Bipartite mating was performed by mixing a small portion of a culture of the library-containing E. coli cells with the same volume of R. capsulatus mutant strains and by plating on RCV agar plates. The plates were incubated in an anaerobic Gas-PAK jar (BBL) and illuminated by 60 W incandescent lamp at 34°C.

Plasmids isolation and absorption spectra

Plasmids were isolated as described by Sambrook et al. (1989). Absorption spectra were measured with a spectrophotometer (Shimadzu UV-2500PC).

Results and Discussion

Functional complementation of genes involved in BChl biosynthesis

Because the RCV agar plate is a minimal medium for photosynthetic growth of R. capsulatus (Young et al., 1989), only R. capsulatus cells, of which mutated gene was functionally complemented with genomic DNA library of B. viridis, were rescued under these experimental conditions. After a few days, several colonies came up on the mating plates for three kinds of protochlorophyllide reductase mutants (bchB-, bchN- and bchL-), 3-vinyl-bacteriochlorophyllide hydratase mutant (bchF), Mg-

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant characteristics</th>
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<tbody>
<tr>
<td>Blastochloris viridis</td>
<td>Wild type</td>
</tr>
<tr>
<td>DSM1003</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td></td>
</tr>
<tr>
<td>XLI-Blue MR</td>
<td>Δ(mcrA)183Δ(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac</td>
</tr>
<tr>
<td>Rhodobacter capsulatus</td>
<td></td>
</tr>
<tr>
<td>DB711</td>
<td>orf171::Km’ rif-10, 3-vinyl-bacteriochlorophyllide hydratase (BchF) mutant</td>
</tr>
<tr>
<td>DB304</td>
<td>orf304::Km’ rif-10, Geranylgeranyl bacteriochlorophyll synthase (BchG) mutant</td>
</tr>
<tr>
<td>DB350</td>
<td>orf350::Km’ rif-10, Mg protoporphyrin IX chelatase (BchH) mutant</td>
</tr>
<tr>
<td>DB561</td>
<td>orf561::Km’ rif-10, Mg protoporphyrin IX chelatase (BchD) mutant</td>
</tr>
<tr>
<td>DB575</td>
<td>orf575::Km’ rif-10, Mg protoporphyrin IX monomethyl ester oxidative cyclase (BchE) mutant</td>
</tr>
<tr>
<td>JDA</td>
<td>orf513::Km’ rif-10, Protochlorophyllide reductase (BchB) mutant</td>
</tr>
<tr>
<td>JDB</td>
<td>orf464::Km’ rif-10, Protochlorophyllide reductase (BchN) mutant</td>
</tr>
<tr>
<td>ZY4</td>
<td>orf224::Km’ rif-10, Mg protoporphyrin methyl transferase oxidative cyclase (BchM) mutant</td>
</tr>
<tr>
<td>ZY5</td>
<td>orf304::Km’ rif-10, Protochlorophyllide reductase (BchL) mutant</td>
</tr>
<tr>
<td>ZY6</td>
<td>orf1195::Km’ rif-10, Mg protoporphyrin IX chelatase (BchH) mutant</td>
</tr>
<tr>
<td>SB1003</td>
<td>Rif-10</td>
</tr>
</tbody>
</table>

Table 1. Bacterial strains used in this study

protoporphyrin IX methyltransferase mutant (bchM), and bacteriochlorophyll synthase mutant (bchG), respectively. Three kinds of Mg-chelatase mutants (bchH, bchD, bchI), and Mg-protoporphyrin IX monomethylester oxidative cyclase mutant (bchE) were complemented after a week. These indicated the occurrence of functional complementation with cosmids from a B. viridis genomic library. A single colony was picked up from each plate and streaked out on new RCV plates for isolation. Fig. 1 shows absorption spectra of membrane preparation derived from wild type and bchN complemented R. capsulatus. Two prominent peaks at 800 and 850 nm were observed in both wild type and bchN complemented spectrum, which
can be attributed to the B800-850 antenna-pigment complex (Zuber and Cogdell, 1995). The spectrum for \textit{bchH}, \textit{bchI}, \textit{bchD}, \textit{bchM}, \textit{bchE}, \textit{bchB}, \textit{bchL}, \textit{bchF}, and \textit{bchG} complemented \textit{R. capsulatus} shows the same pattern as the \textit{bchN} complemented spectrum (data not shown). These results indicate that all complemented strains synthesize BChl \textit{a} and form light harvesting pigment protein complexes in the membranes.

\textbf{Fig 1.} Absorption spectra of crude membrane fraction from \textit{R. capsulatus} strains. Solid line indicate wild type strain SB 1003. Dotted line indicate a \textit{bchN} complemented \textit{R. capsulatus} (JDB/pBvN). \textit{R. capsulatus} strains were grown anaerobically in RCV2/3 medium at 34 °C for 2 days. Cells were disrupted by sonication and unbroken cells were removed by centrifugation.

\begin{table}
\begin{center}
\begin{tabular}{|c|cccccccccc|}
\hline
\textit{Rhodobacter capsulatus} mutant strain & \textit{bchH} & \textit{bchD} & \textit{bchI} & \textit{bchM} & \textit{bchE} & \textit{bchB} & \textit{bchL} & \textit{bchN} & \textit{bchF} & \textit{bchG} \\
\textit{cosmid} & \textit{bchH} & \textit{bchD} & \textit{bchI} & \textit{bchM} & \textit{bchE} & \textit{bchB} & \textit{bchL} & \textit{bchN} & \textit{bchF} & \textit{bchG} \\
pBvH & + & + & + & + & + & + & + & + & + & + \\
pBvD & + & + & + & + & + & + & + & + & + & + \\
pBvI & + & + & + & + & + & + & + & + & + & + \\
pBvM & + & + & + & + & + & + & + & + & + & + \\
pBvE & + & + & + & + & + & + & + & + & + & + \\
pBvB & + & + & + & + & + & + & + & + & + & + \\
pBvL & + & + & + & + & + & + & + & + & + & + \\
pBvN & + & + & + & + & + & + & + & + & + & + \\
pBvF & + & + & + & + & + & + & + & + & + & + \\
pBvG & + & + & + & + & + & + & + & + & + & + \\
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\end{tabular}
\end{center}
\end{table}

\textbf{Table 2.} Complementation of \textit{Rhodobacter capsulatus} bacteriochlorophyll \textit{a} biosynthesis-deficient mutants with cosmids from a \textit{Blastochloris viridis} genomic library. +; denotes complementation to photosynthetic competence.

\textit{Isolation of cosmids from complemented \textit{R. capsulatus} mutants}

The isolated cosmids from complemented strains were reintroduced to \textit{R. capsulatus} strains that have mutation in BChl biosynthesis gene. Table 2 shows the results of cosmids complementation experiments for \textit{R. capsulatus} BChl biosynthesis mutants.
Cosmids pBvN and pBvF, which isolated from *bchB* complemented and *bchF* complemented *R. capsulatus* strains respectively, show abilities of complementation for *bchB*, *bchN*, *bchF* and *bchG*. These results indicate that pBvN and pBvF contains for these four genes of *B. viridis*, respectively, suggesting these genes form a gene cluster. In photosynthesis gene cluster of *R. capsulatus*, *bchB*, *bchN*, *bchF* and *bchG* are present in this order and form an operon (Bollivar DW et al., 1994). Additional four ORF including *bchE* are present between *bchB*-*bchN*-*bchF* operon and *bchG* in *Rhodobacter*. Since neither pBvN nor pBvF have an ability complementation of *bchE*, the gene arrangement in photosynthesis gene cluster of *B. viridis* may be different from that of *Rhodobacter*. Cosmid pBvD, which has isolated from *bchD* complemented *R. capsulatus* strain, shows ability of complementation for *bchD* and *bchI*. These results indicate that pBvD contains, two Mg-chelatase genes, *bchD* and *bchI*, suggesting these two genes form a gene cluster.

**Acknowledgments**

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**References**


