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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 to 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 monomethylester 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^{o}C in PYS or RCV 2/3PY medium (Bollivar et al., 1994). *Escherichia coli* strains were grown at 37^{o}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 *Sau*3A I restriction enzyme and ligating the restriction fragments into the *Bam*H 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^{o}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-

Strain	Relevant characteristics
Blastochloris viridis	
DSM1003	Wild type
Escherichia coli	
XLI-Blue MR	*Δ(mcrA)183Δ(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac*
Rhodobacter capsulatus	
DB171	*orf171* :: *Km*^{r} *rif-10*, 3-vinyl-bacteriochlorophyllide hydratase (BchF) mutant
DB304	*orf304* :: *Km*^{r} *rif-10*, Geranylgeranyl bacteriochlorophyll synthase (BchG) mutant
DB350	*orf350* :: *Km*^{r} *rif-10*, Mg protoporphyrin IX chelatase (BchI) mutant
DB561	*orf561* :: *Km*^{r} *rif-10*, Mg protoporphyrin IX chelatase (BchD) mutant
DB575	*orf575* :: *Km*^{r} *rif-10*, Mg protoporphyrin IX monomethyl ester oxidative cyclase (BchE) mutant
JDA	*orf513* :: *Km*^{r} *rif-10*, Protochlorophyllide reductase (BchB) mutant
JDB	*orf464* :: *Km*^{r} *rif-10*, Protochlorophyllide reductase (BchN) mutant
ZY4	*orf224* :: *Km*^{r} *rif-10*, Mg protoporphyrin methyl transferase oxidative cyclase (BchM) mutant
ZY5	*orf304* :: *Km*^{r} *rif-10*, Protochlorophyllide reductase (BchL) mutant
ZY6	*orf1195* :: *Km*^{r} *rif-10*, Mg protoporphyrin IX chelatase (BchH) mutant
SB1003	*Rif-10*

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 *bchH*, *bchI*, *bchD*, *bchM*, *bchE*, *bchB*, *bchL*, *bchF*, and *bchG* complemented *R. capsulatus* shows the same pattern as the *bchN* complemented spectrum (data not shown). These results indicate that all complemented strains synthesize BChl *a* and form light harvesting pigment protein complexes in the membranes.

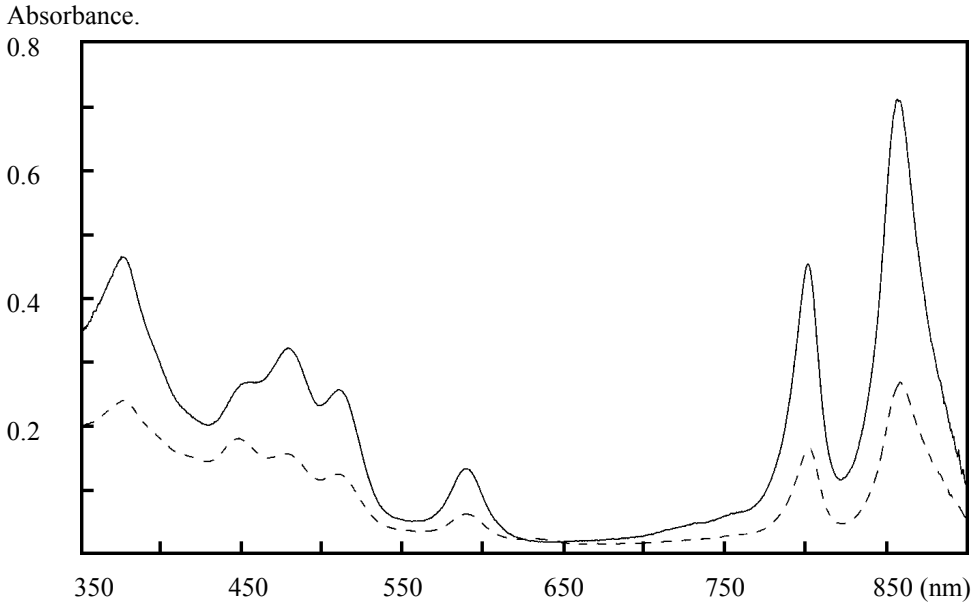

Fig 1. Absorption spectra of crude membrane fraction from *R. capsulatus* strains.
Solid line indicate wild type strain SB 1003. Dotted line indicate a *bchN* complemented *R. capsulatus* (JDB/pBvN). *R. capsulatus* strains were grown anaerobically in RCV2/3 medium at 34 ^{o}C for 2 days. Cells were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

cosmid	*Rhodobacter capsulatus* mutant strain									
	bchH (ZY6)	*bchD* (DB561)	*bchI* (DB350)	*bchM* (ZY4)	*bchE* (DB575)	*bchB* (JDA)	*bchL* (ZY5)	*bchN* (JDB)	*bchF* (DB171)	*bchG* (DB304)
pBvH	+									
pBvD		+	+							
pBvI			+							
pBvM				+						
pBvE					+					
pBvB						+		+		+
pBvL							+			
pBvN						+		+	+	+
pBvF						+		+	+	+
pBvG									+	+

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

Isolation of cosmids from complemented R. capsulatus mutants

The isolated cosmids from complemented strains were reintroduced to *R. capsulatus* strains that have mutation in BChl biosynthesis gene. Table 2 shows the results of cosmids complementation experiments for *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* 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.

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