Characterization and Complementation of a Mutant of *Rhodobacter sphaeroides* with a Chromosomal Deletion in the Light-harvesting (LH2) Genes

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An LH2⁻ strain of *Rhodobacter sphaeroides*, DBC1, has been constructed by deleting the *puc* operon, which encodes the LH2 α and β polypeptides, from the chromosome and replacing it with a kanamycin resistance gene. Southern blot analysis indicates that the 950 bp *Bam*HI restriction fragment which contains the *puc* operon has been lost and has been replaced by the 1.25 kb Km^R cassette derived from Tn903. Strain DBC1 lacked the LH2 complex, as shown by loss of the characteristic absorbance bands at 800 and 850 nm. The LH2 polypeptides were also found to be absent after SDS-PAGE. The wild-type phenotype was restored to DBC1 by the transfer of a 3.8 kb *Bse*I fragment containing the *puc* operon in plasmid pMA81. Transconjugants possessed a wild-type absorbance spectrum and LH2 polypeptides.

INTRODUCTION

The photosynthetic bacterium *Rhodobacter sphaeroides* has a relatively simple photosystem, consisting of the reaction centre and LH1 antenna complexes surrounded by a peripheral light-harvesting system, LH2 (Vos *et al.*, 1986). These antenna polypeptides bind bacteriochlorophyll (bchl) and are interconnected to form highly efficient domains for light capture and energy transfer. Excitation energy is transferred to the 'special pair' bchls of the reaction centre, where the primary photochemical events occur. The genes encoding the reaction centre L and M, and the LH1 α and LH1 β polypeptides are encoded on the *puf* operon, and their nucleotide sequences have been determined (Williams *et al.*, 1983, 1984; Kiley *et al.*, 1987). The reaction centre H gene is encoded on a separate operon, *puh* (Donohue *et al.*, 1986). The LH2 α and LH2 β polypeptides are encoded on the *puc* operon and their DNA sequences are also known (Ashby *et al.*, 1987; Kiley & Kaplan, 1987). The amount of LH1 relative to the reaction centre complex remains relatively constant with changes in growth conditions, whereas the amount of LH2 complex increases when light intensity and oxygen levels change (Aagaard & Sistrom, 1972; Niederman *et al.*, 1976). This phenomenon also occurs in *Rhodobacter capsulatus* (Schumacher & Drews, 1979). Zucconi & Beatty (1988) have proposed a post-transcriptional mechanism for the regulation of steady-state levels of mature LH2 complexes in *Rb. capsulatus*. The regulation of the *puc* operon in *Rb. sphaeroides* is less well understood and may be affected indirectly by a number of factors (see Kiley & Kaplan, 1988). Clearly, strains with mutant light-harvesting systems, or strains with mutations in regulatory regions of the *puc* operon, will be useful in probing the function and regulation of these antenna complexes.

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Abbreviations: bchl, bacteriochlorophyll; RC, reaction centre; LH1, B875 antenna complex; LH2, B800–850 antenna complex.

The use of light-harvesting mutants for the study of excitation energy transfer has so far proved valuable for the detailed analysis of the energetic interactions between the three photosynthetic components *in vivo* (Hunter & van Grondelle, 1988; van Dorssen *et al.*, 1988; Vos *et al.*, 1988). The LH2 complex may be assembled from units consisting of three α and three β polypeptides, together with six 850 nm bchls, three 800 nm bchls and six carotenoid molecules (Hunter *et al.*, 1989). LH2 is the major light-harvesting pigment-protein complex in the membrane and has been well characterized biophysically (Kramer *et al.*, 1984; van Dorssen *et al.*, 1988). It would, therefore, be an ideal model system for more detailed mutational analysis of the structure and function of the LH2 antenna, particularly with regard to the importance of residues conserved in the antenna polypeptides of a number of photosynthetic bacteria (Brunisholz & Zuber, 1988). It is with this purpose in mind that an LH2 deletion/insertion mutant of *Rb. sphaeroides* has been constructed, since such a mutant provides a null genetic background against which altered complexes may be analysed *in vivo*. Youvan *et al.* (1985) have also constructed a *puc* deletion strain in *Rb. capsulatus* which is spectrally similar to the previously described LH2⁻ *Rb. sphaeroides* mutant M21 (Ashby *et al.*, 1987). One valuable aspect of such an LH2⁻ strain is that it permits a systematic investigation into the properties of the LH1/RC core complex in the photosynthetic membrane (van Dorssen *et al.*, 1988; Hunter & van Grondelle, 1988). The reversion rate of M21 (about 10⁻⁶), and the lack of information concerning the precise nature of the mutation, provide reasons for constructing a stable, genetically well-defined LH2⁻ mutant in *Rb. sphaeroides*.

In this study we report the sequence of a 1078 bp *Xho*I-*Pvu*II restriction fragment which appears to contain only two genes, *pucA* and *B*. The deletion of the LH2 genes and subsequent insertion of the Km^R cassette to form the mutant DBC1, together with its genetic and biochemical characterization, are presented. We have restored this mutant to its parent phenotype by transfer of the cloned genes, demonstrating its suitability as a background for mutational analysis of regulatory or structural genes for LH2 synthesis. We envisage that such a mutant will be essential for testing functional models that describe the assembly, regulation and energy transfer properties of the LH2 antenna.

Table 1. *Bacterial strains and plasmids*

Strain or plasmid	Relevant characteristics	Source*	Reference
<i>E. coli</i>			
DH5	A derivative of DH1 (which is F ⁻ <i>recA endA gyrA thi-1 hsdR supE</i>)	V.S.	Hanahan (1985)
Sm10	<i>recA thi thr leu</i> ; RP4-2-Tc::Mu integrated into the chromosome; Tra ⁺ Km ^R	R.S.	Simon <i>et al.</i> (1983)
S17-1	RP4-2 (Tc::Mu, Km::Tn7) integrated into the chromosome; <i>thi pro hsdR hsdM⁺ redA Tpr Sm^R</i>	R.S.	Simon <i>et al.</i> (1983)
JM105	Δ (<i>lac-proAB</i>) <i>thi gyrA96 endA1 hsdR17 relA1 supE44</i> (r _K ⁻ , m _K ⁻) [F' <i>traD36 proAB lacI^qZ</i> Δ M15]	S.A.C.	Messing <i>et al.</i> (1981)
<i>Rb. sphaeroides</i>			
NCIB 8253	Wild-type	R.A.N.	
DBC1	Km ^R , LH2 ⁻	This work	
DBC1.81	Sm ^R Km ^R , LH2 ⁺	This work	
Plasmids			
pSUP202	Ap ^R Tc ^R Cm ^R ; Mob ⁺ Tra ⁻ ; ColE1 replicon	R.S.	Simon <i>et al.</i> (1983)
pMA81	Sm ^R ; Mob ⁺ ; 3.8 kb <i>Bsc</i> I chromosomal fragment from <i>Rb. sphaeroides</i> containing <i>pucAB</i> cloned into pNH2	M.K.A.	Ashby <i>et al.</i> (1987)
pMAT81	Ap ^R Tc ^S ; 3.8 kb <i>Bsc</i> I chromosomal fragment from <i>Rb. sphaeroides</i> containing <i>pucAB</i> cloned into pAT153	This work	
pMSK11	Ap ^R Cm ^R Km ^R Tc ^S ; Mob ⁺	This work	

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      10              30              50
CTCGAGCGGTTTCGAGTCGGTGCTCGCGGGCCCTGTCGATAGTAGCGGGCTGTATTGAGG

      70              90              110
CAGAGGCCGCCAAGCCATCCTGAAATCTCGAAAAATCCCCGCAAGATGGGGTCCGGAAGG

      130             150             170
GCCGGGCGCAAGGCCTCGGACACCCTCGTTTTTGCAGCAGCGAGAGGCTGCGGGACGGCC

      190             210             230
CTGTGGGGCCGGGACAGCAGCGTCAATTTCCCGCGCGCTGCGGCAAAATTGTCCTTTT

      250             270             290
TCAAGCCGTTACGCAGGATTCCCGGCCGATCTGGCGGCCAATAAGTCGCACCCAAAACGG

      310             330             350
CCTTGTCAGCCAACAGTGACATTGAATCTGTCAGCGCAATGTGACACCCATAATGCGAGC

      370             390             410
CGGGGCGGATCAGAAATCGCCGACAAGGTGATCCAGGTCTCTCCGTCTCGTGAAGCCC

      430             450             470
CGGTGCAGGCCCTACACGCAAACCGTCGATTACCAGTTGGGAGACGACACAATGACTGA
                                     pucB MetThrAs

      490             510             530
CGATCTGAACAAAGTCTGGCCGAGCGGCCTGACCGTTGCCGAAGCCGAAGAAGTTCATAA
pAspLeuAsnLysValTrpProSerGlyLeuThrValAlaGluAlaGluValHisLy

      550             570             590
GCAACTCATCTCGGCACCGCGTCTTCGGTGGCATGGCGCTCATCGGCACTTCCTCGC
sGlnLeuIleLeuGlyThrArgValPheGlyGlyMetAlaLeuIleAlaHisPheLeuAl

      610             630             650
CGCCGCTGCGACCCGCTGGGCTCGGCTGATAGGAGAAGACTGACATGACCAACGGCAAAAT
aAlaAlaAlaThrProTrpLeuGly                                     pucA MetThrAsnGlyLysIl

      670             690             710
CTGGCTCGTGGTGAAACCGACCGTCCGGCTTCGGCTGTTCTCAGCGCTGCCGTCATCGC
eTrpLeuValValLysProThrValGlyValProLeuPheLeuSerAlaAlaValIleAl

      730             750             770
CTCCGTCGTTATCCAOGCTGCTGTGCTGACGACCACCACCTGGCTGCCCGCCTACTACCA
aSerValValIleHisAlaAlaValLeuThrThrThrThrTrpLeuProAlaTyrTyrGl

      790             810             830
AGGCTCGGCTGCGGTGCGGCCGAGTAATGCTGCGCAAGGCGGGGCTGCGGGCCCACG
nGlySerAlaAlaValAlaAlaGlu

      850             870             890
CCAGCCAGTCCGTGAGTTCGAGCAGGCCGGGATCCTTGGGTTCCGGCTGCTCGTCACAC

      910             930             950
CAGCGACGTGTTCTGTGCGCTTGCCCGTTGGGCTGTGTGCTGAAGCCCGAGATCCTT

      970             990             1010
TCCAGTCGCGCAGTCTGCGCCTACCCCGGGATTCTTCACGCATGAGCCGAATTGCCGAAC

      1030            1050            1070
ATCTGGTCCGCATCGGACCGGGTTTCTGCCATTTCCGACGCGGCCTCGGACCAGCTG

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Fig. 1. Sequence of a 1078 bp *XhoI*–*PvuII* fragment containing the *pucA* and *B* genes. Open reading frames corresponding to *pucA* and *pucB* are indicated by the gene products.

Fig. 2. Construction of DBC1, showing the restriction map of the cloned 3.8 kb *BscI* fragment, the *Km*^R cassette and the cassette integrated into the *Rb. sphaeroides* chromosome. Restriction enzymes: B, *Bam*HI; S, *Bsc*I; T, *Taq*I.

METHODS

Media and antibiotics. *Escherichia coli* strains were grown in LB medium. *Rb. sphaeroides* was grown in M22 + medium (Hunter & Turner, 1988) supplemented with 0.1% Casamino acids. Antibiotics were used at the following concentrations ($\mu\text{g mg}^{-1}$): *E. coli*, ampicillin 50, chloramphenicol 25, kanamycin 30, streptomycin 50; *Rb. sphaeroides*, kanamycin 20, streptomycin 50.

Bacterial strains and plasmids. These are listed in Table 1.

Preparation and analysis of DNA. Plasmid and genomic DNA were prepared as described previously (Hunter & Turner, 1988). Restriction analysis, agarose gel electrophoresis and Southern hybridizations were performed as described in Maniatis *et al.* (1982). Radioactive DNA probes were made by the mixed oligonucleotide fragment labelling method of Feinberg & Vogelstein (1983). DNA sequencing was performed on M13mp18/19 clones using the dideoxy procedure of Sanger *et al.* (1977). Colony lysis and filter screening was by the method of Woods (1984).

Preparation of intracytoplasmic membranes. *Rb. sphaeroides* cells were harvested by centrifugation after photosynthetic growth and membranes were prepared as described by Hunter & Turner (1988).

Spectroscopy. Room-temperature absorbance spectra of whole cells and purified membranes were obtained using a Perkin Elmer 554 spectrophotometer.

SDS-PAGE. Purified membranes were analysed on polyacrylamide gradient gels using the buffer system described by Tadros *et al.* (1982) and an acrylamide gradient of 12–20% (w/v).

Plasmid transfer. The plasmids pMA81 and pMSK11 were transferred from *E. coli* strains Sm10 and S17-1, respectively, by a conjugative transfer procedure described by Hunter & Turner (1988).

RESULTS AND DISCUSSION

The sequence of a 1078 bp *Xho*I–*Pvu*II restriction fragment containing the *puc* operon is shown in Fig. 1. The *pucB* and *A* genes are encoded by open reading frames of 150 bp and 164 bp, respectively, and are separated by a 15 bp spacer region; no other reading frames are

Fig. 3. Southern hybridization of: tracks A, NCIB 8253 genomic DNA; tracks B, DBC1 genomic DNA; track C, pMSK11. Blots 1 and 2 were digested with *Bam*HI, blots 3 and 4 with *Bsc*I. Probes used: 1, 950 bp *Bam*HI *puc* fragment; 2, 1.25 kb *Bam*HI *Km*^R cassette; 3, 3.8 kb *Bsc*I *puc* fragment; 4, pSUP202.

present. A single transcript of 0.55 kb can be detected by Northern blot analysis using either the 950 bp *Bam*HI or the 1078 bp *Xho*I-*Pvu*II fragment as a probe. The sequence presented represents all but 60 bp of the 950 bp *Bam*HI fragment deleted from the chromosome to produce DBC1 (see Fig. 2), suggesting that only the *puc* genes are encoded in this region.

Deletion of the *puc* operon from plasmid pMA81 was accomplished by removing a 950 bp *Bam*HI fragment from the cloned 3.8 kb *Bsc*I fragment (Ashby *et al.*, 1987). This was replaced with a 2.25 kb *Bam*HI-ended kanamycin resistance gene from transposon Tn903. The 4.0 kb *Bsc*I fragment containing the *Km*^R cartridge was then cloned into the mobilizable suicide vector pSUP202 (Simon *et al.*, 1983). This construct, pMSK11, was transferred into wild-type *Rb. sphaeroides* NCIB 8253 by conjugative transfer from *E. coli* S17-1 (Simon *et al.*, 1983). Photosynthetic, kanamycin-resistant *Rb. sphaeroides* colonies were obtained, about half of which were smaller than the others. This was to be expected, since LH2-deficient strains would have a decreased light-harvesting capacity with only 330 bchls per photosynthetic domain, rather than the approximately 3000 bchls in wild-type cells (Vos *et al.*, 1988).

Southern blot analysis of *Rb. sphaeroides* strain DBC1 (Fig. 3) confirmed the presence of the *Km*^R gene in the chromosome and indicated deletion of the *puc* genes by a double recombination event (Niaudet *et al.*, 1982); a single event would have led to the presence of both pSUP202 and *puc* DNA in the chromosome. In Fig. 3, blot 1 shows the absence of *puc* genes in DBC1 compared with the wild-type while blot 2 confirms the concomitant insertion of the *Km*^R gene, which is not present in the wild-type. The *Km*^R gene from Tn903 was used in preference to that from Tn5 since this latter transposon may also confer resistance to streptomycin. The *puc* operon is encoded on a 15 kb *Bsc*I fragment as shown in Fig. 3, blot 3, track A. This region hybridizes with the 3.8 kb *Bsc*I fragment from pMAT81. Note that the two *Bsc*I sites in pMAT81 (Fig. 2)

Fig. 4

Fig. 4. Room-temperature absorbance spectra of membrane preparations from: A, DBC1; B, DBC1.81; and C, wild-type NCIB 8253.

Fig. 5

Fig. 5. SDS-PAGE of solubilized membranes. 1, Wild-type NCIB 8253; 2, DBC1; 3, DBC1.81.

have both arisen fortuitously as a result of the original cloning strategy in which partial *TaqI* genomic digests were cloned into the *BscI* site of pNH2 (Hunter & Turner, 1988). This 3.8 kb *BscI* fragment hybridizes to 10.5 kb and 4.5 kb *BscI* fragments from DBC1 (blot 3, track B), indicating that a 15 kb *BscI* fragment has been interrupted by the *BscI* site in the *Km^R* gene of Tn903. Fig. 3, blot 4 shows no hybridization between DBC1 genomic DNA and the plasmid pSUP202, indicating that this plasmid did not integrate into the chromosome, and that the deletion of the *puc* operon has occurred by a double recombination event which has replaced the 950 bp *Bam*HI fragment with the 1.25 kb *Bam*HI fragment containing the *Km^R* gene of Tn903.

The absorbance spectra of wild-type NCIB 8253, DBC1 and the complemented strain DBC1.81 are shown in Fig. 4. The wild-type shows the peaks at 800 nm and 850 nm characteristic of LH2 antenna complexes, as well as a shoulder at 875 nm due to the LH1 antenna. DBC1 lacks these 800 nm and 850 nm absorbance peaks and instead exhibits an absorbance maximum at 875 nm, which arises from the LH1 antenna, together with a small peak near 800 nm due to reaction centres. Thus, the absorbance spectra indicate that the LH2 complex is not present in the membrane of DBC1 but the LH1/RC core (Vos *et al.*, 1988) remains.

SDS-PAGE analysis of photosynthetic membranes is shown in Fig. 5. Track 1 shows the three low-molecular-mass bands that are observed in the wild-type; they arise from LH1 α , LH2 α and superimposed LH1 β and LH2 β , in decreasing molecular size (Broglie *et al.*, 1979). The upper

band LH α is 6809 daltons, the middle band LH2 α is 5599 daltons and the lower band arises from LH1 β (5441 daltons) and LH2 β (Theiler *et al.*, 1984, 1985) (5448 daltons). The LH2 α and β polypeptides are absent from DBC1 membranes because the *puc* gene has been lost (track 2). The remaining bands are LH1 α and β .

The *pucA* and *B* genes were transferred to DBC1 in an attempt to complement the mutation. The plasmid pMA81 (Ashby *et al.*, 1987), which contains the *puc* operon as a 3.8 kb *Bse*I fragment, was transferred to DBC1 by conjugative transfer from *E. coli* Sm10. Streptomycin-resistant, photosynthetic colonies were obtained. Colonies were cultured photosynthetically for spectral analysis. One transconjugant strain was found, DBC1.81, which exhibited the characteristic LH2 absorbance peaks (Fig. 4). SDS-PAGE analysis of membranes from DBC1.81 showed that the B800–850 polypeptides had been restored (Fig. 5, track 3).

In this work we have reported the construction of an *Rb. sphaeroides* chromosomal deletion mutant for the *puc* genes resulting in a mutant deficient in the secondary light-harvesting complex LH2. This complex has been successfully restored to the mutant by conjugative gene transfer, indicating that DBC1 is a suitable recipient for mutagenized LH2 genes.

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