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# Role of OrfQ in Formation of Light-Harvesting Complex of *Rhodobacter sphaeroides* under Light-Limiting Photoheterotrophic Conditions

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**Abstract** A *puc*-deleted cell of *Rhodobacter sphaeroides* grows with a doubling time longer than 160 h under light-limiting photoheterotrophic (3 Watts [W]/m$^2$) conditions due to an absence of the peripheral light-harvesting B800-850 complex. A spontaneous fast-growing mutant, *R. sphaeroides* SK101, was isolated from the *puc*-deleted cells cultured photoheterotrophically at 3 W/m$^2$. This mutant grew with an approximately 40-h doubling time. The growth of the mutant, however, was indistinguishable from its parental strain during photoheterotrophic growth at 10 W/m$^2$ as well as during aerobic growth. The membrane of SK101 grown aerobically did not reveal the presence of any spectral complex, while the amounts of the B875 complex and photosynthetic pigments of SK101 grown anaerobically in the dark with dimethylsulfoxide (DMSO) were the same as those of the parental cell. These results indicate that the oxygen control of the photosynthetic complex formation remained unaltered in the mutant. The B875 complex of SK101 under light-limiting conditions was elevated by 20% to 30% compared with that of the parental cell, which reflected the parallel increase of the bacteriochlorophyll and carotenoid contents of the mutant. When the *puc* was restored in SK101, the B875 complex level remained unchanged, but that of the B800-850 complex increased. The mutated phenotype of SK101 was complemented with *orfQ* encoding a putative bacteriochlorophyll-mobilizing protein. Accordingly, it is proposed that the mutated OrfQ of SK101 should have an altered affinity towards the assembly factor specific to the most peripheral light-harvesting complex, which could be either the B875 or the B800-850 complex.

**Key words:** *Rhodobacter sphaeroides*, OrfQ, bacteriochlorophyll-mobilizing protein, light-harvesting complex formation, light-limiting conditions

*Rhodobacter sphaeroides*, a purple nonsulfur photosynthetic bacterium, has been used as a model organism for studying bacterial photosynthesis and membrane development [17]. When the oxygen tension drops below threshold levels of approximately 2.5%, *R. sphaeroides* synthesizes a photosynthetic membrane system referred to as an intracytoplasmic membrane (ICM), in addition to the normal gram-negative membrane found during aerobic growth. The ICM abundance is inversely related to the light intensity [4], and it comprises the structural and functional components associated with photosynthesis [4, 17]. The ICM contains three distinct bacteriochlorophyll-protein complexes: the reaction center (RC) complex and two light-harvesting complexes, B875 and B800-850. The light-harvesting complexes absorb light energy and the RC complex transforms it into chemical energy [17, 22, 25]. The ratio of B875 to RC complexes within the ICM is fixed at approximately 12:1 to 15:1, irrespective of the light intensity, to comprise the fixed photosynthetic unit [17, 18]. The B800-850 complex formation is regulated inversely to the light intensity and, together with the fixed photosynthetic unit, it is referred to as the variable photosynthetic unit [16, 17].

The genes encoding the structural polypeptides of the RC, B800-850, and B875 complexes have been previously identified together with the genes coding for complex-specific assembly factors (CSAFs) and *trans*-acting factors involving the transcriptional expression of the photosynthesis genes [17]. The regulations controlling the photosynthetic complex formation by oxygen and light have been elucidated in great depth at the molecular level (for review, see reference 29). Special attention has been paid to the role of the *orfQ* located upstream of the *puf* operon encoding structural polypeptides of the B875 and RC complexes [2, 13]. It has been proposed that this OrfQ mobilizes bacteriochlorophyll into developing light-harvesting complexes and the RC complex by hierarchically interacting with complex-specific assembly factors (CSAFs); the highest affinity for the CSAF of RC, the next for the CSAF of B875, and the lowest affinity for the B800-850-specific CSAF [13]. The CSAF of the B875 complex is encoded by the *orf1696* located immediately upstream of

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the *puhA* coding for the H polypeptide of the RC complex [14]. The distal gene (*C*) of the *pucBAC* operon coding for the structural polypeptides of the B800-850 complex encodes a CSAF to assemble the B800-850 complex [21]. The CSAF for the RC complex has as yet not been identified.

Most studies concerning the light control of spectral complex formation have been performed with a light intensity that is sufficient enough to support cell growth at a maximum rate of 3-h doubling time. In this work, however, a *puc*-deleted cell capable of poor photoheterotrophic growth at 3 W/m$^2$ was utilized to isolate a spontaneous fast-growing mutant, and the mutation was then analyzed to understand the regulation of the photosynthetic complex formation under the light-limiting conditions. It was found that the mutation was responsible for an increased formation of the light-harvesting complexes and was complemented with the wild-type *orfQ* gene, thereby rehighlighting the importance of the OrfQ.

## MATERIALS AND METHODS

### Bacterial Strains, Plasmids, and Growth Conditions

The bacterial strains and plasmids used in this study are described in Table 1. *Escherichia coli* strains were grown

**Table 1.** Bacterial strains and plasmids used in this study.

| Strains or plasmids | Relevant characteristic(s) | Source or references |
|---|---|---|
| *E. coli* | | |
| DH5α *phe* | F$^-$ ϕ80d*lacZ*ΔM15Δ(*lacZYA-argF*)U169 *recA1 endA1 supE44 hsdR17*($r_K^- m_K^+$) λ$^-$ *thi-1 gyrA relA1 phe*::Tn*10dCm* | [9] |
| *R. sphaeroides* | | |
| 2.4.1 | Wild type | W. Sistrom |
| PUC-ZWT | *lacZY*::Ω*Sm*$^r$/*Sp*$^r$*A* inserted at the *Xmn*I site within *pucB* of wild type; B800-850$^-$ | [20] |
| SK101 | Spontaneous mutant derived from PUC-ZWT; high level accumulation of spectral complexes at low light | This study |
| WT-KDC | Km$^r$ cartridge inserted at the *Xmn*I site within *pucB* of wild type; B800-850$^-$ | This study |
| SK101-KDC | Km$^r$ cartridge inserted at the *Xmn*I site within *pucB* of SK101; B800-850$^-$ | This study |
| ZWT-DKSC | *puc* restored in the genetic background of PUC-ZWT by homologous recombination using plasmid pSUPPUCΩKm200 | This study |
| SK101-DKSC | *puc* restored in the genetic background of SK101 by homologous recombination using plasmid pSUPPUCΩKm200 | This study |
| Plasmids | | |
| pRK415 | RK2 derivative; Tc$^r$ | [15] |
| pSUP202 | pBR325-Mob$^+$; Ap$^r$, Cm$^r$, Tc$^r$ | [27] |
| pWS2 | R; R68.45 containing ~109-kb photosynthetic gene cluster of *R. sphaeroides* WS2 | [28] |
| pUI601 | pUC19/*Pst*I, 2.5-kb *Pst*I fragment containing *pucBAC* of wild type | [16] |
| pSUPPUCK200 | pSUP202/*Pst*I, 4.0-kb *Pst*I fragment of *pucBAC*::*aph*; Km$^r$, Cm$^r$, Tc$^r$ | This study |
| pSUPPUCΩKm200 | pSUP202/ 2.2-kb ΩKm$^r$ cartridge inserted at the 2.5-kb *puc* upstream; Km$^r$, Cm$^r$, Tc$^r$ | This study |
| pCF200 | *pucB-lacZ* transcriptional fusion; Sm$^r$/Sp$^r$, Tc$^r$ | [20] |
| pUI1830 | *pufB-lacZ* transcriptional fusion; Sm$^r$/Sp$^r$, Tc$^r$ | [12] |
| pHZ300 | *puhA-lacZ* transcriptional fusion; Sm$^r$/Sp$^r$, Tc$^r$ | This study |
| pLX200 | *bchF-lacZ* transcriptional fusion; Sm$^r$/Sp$^r$, Tc$^r$ | [11] |
| pLA2917 | IncP1 cosmid vector; Tc$^r$, Km$^r$ | [1] |
| pUI8487 | pLA2917-derived cosmid containing *puf*; Tc$^r$ | [8] |
| pQXX2 | pRK415/*Bam*HI, 13-kb *Bam*HI fragment of wild-type DNA from pUI8487; Tc$^r$ | This study |
| pRQX200 | pRK415/*Hin*dIII-*Eco*RI, ~4-kb *Hin*dIII-*Eco*RI fragment containing 4.0-kb *Sph*I fragment of *puf* from pUI8487; Tc$^r$ | This study |
| pQ200 | pRK415/*Eco*RI, 1.8-kb *Eco*RI fragment of wild-type DNA containing the *pufKBAL*; Tc$^r$ | This study |
| pQ200 | pQ200 derivative, 2-kb ΩSm$^r$/Sp$^r$ cartridge at the *Hin*cII site within *orfQ*; Tc$^r$ | This study |
| pUFX | pRK415/ *Hin*dIII-*Eco*RI, ~0.5-kb *Hin*dIII-*Eco*RI fragment containing 488-bp of *pufX* from pUI8487; Tc$^r$ | This study |

at 37°C in Luria medium supplemented, when required, with the following antibiotics; tetracycline (Tc), 20 μg/ml; ampicillin (Ap), 50 μg/ml; chloramphenicol (Cm), 35 μg/ml; kanamycin (Km), 25 μmg/ml; streptomycin (Sm) and spectinomycin (Sp), 50 μg/ml each. *R. sphaeroides* strains were grown at 28°C in Sistrom's minimal medium [22]. The dark anaerobic growth of *R. sphaeroides* was performed using 75 mM DMSO as a terminal electron acceptor. Photoheterotrophic conditions were achieved by illuminating either culture plates maintained anaerobically in vinyl bags (Bio-Bag Environmental Chamber Type A, Becton Dickinson, Cockeysville, U.S.A.) or broth-filled culture tubes with incandescent lamps. Light intensities of 10 W/m$^2$ and 3 W/m$^2$ were regarded as either sufficient or limiting for a cell growth of 3-h doubling time, respectively. Antibiotics were used, when appropriate, at the following concentrations; Tc, 1 μg/ml; Km, 25 μg/ml; Sm and Sp, 50 μg/ml each. The *R. sphaeroides* growth was monitored with a Klett-Summerson colorimeter (No. 66 filter); 1 Klett Unit (KU) corresponds to approximately $10^7$ cells/ml.

### DNA Manipulation and Conjugation Techniques

Plasmid DNA was prepared as previously described [23] and the DNA was treated with restriction enzymes and other nucleic acid-modifying enzymes in accordance with the manufacturers' specifications. Conjugation was performed as described elsewhere [6].

### Southern Hybridization Analysis

*R. sphaeroides* chromosomal DNA digested to completion with appropriate restriction enzymes was electrophoresed on 0.8% agarose gel. Blotting to Hybond-N membrane (Amersham, U.K.) was performed as previously described [7]. Hybridization and detection using the Fluorescein Gene Image Labelling and Detection system (Amersham, U.K.) were carried out by following the manufacturer's instructions.

### β-Galactosidase Assays

*R. sphaeroides* cultures used for the measurement of the β-galactosidase activities were grown aerobically or photoheterotrophically. These aerobically- and photoheterotrophically-grown cells were harvested at cell densities of 20-40 and 50-100 KU, respectively. β-Galactosidase assays were performed as described previously [20]. All experiments involving β-galactosidase assays were performed in at least two separate experiments.

### Spectrophotometric Assays

The absorption spectra of the membrane fraction extracted from *R. sphaeroides* were analyzed as previously described [19] with a UV2041-PC spectrophotometer (Shimadzu, Japan). The same concentration of protein was used for every spectra profile of each *R. sphaeroides* strain examined. The amount of protein was determined using a modified Lowry method [24] with bovine serum albumin as the standard (Sigma, St. Louis, U.S.A). The level of the B800-850 complex was calculated from the spectral data by using $A_{849}$-$A_{900}$ with an extinction coefficient of $\varepsilon$=96 mM$^{-1}$cm$^{-1}$, normalized for three molecules of bacteriochlorophyll *a* per complex. The amount of the B875 complex was measured from $A_{875}$-$A_{820}$ with an extinction coefficient of $\varepsilon$=73 mM$^{-1}$cm$^{-1}$, normalized for two molecules of bacteriochlorophyll *a* per complex [17]. The photopigments of the whole cells were determined after extraction into acetone-methanol (7:2, vol/vol), as described previously [5].

## RESULTS

### Isolation of *R. sphaeroides* Mutant Showing Increased Growth Rate under Light-Limiting Conditions

The B800-850 complex formation of *R. sphaeroides* was the highest compared with that of the fixed photosynthetic unit comprised of the B875 and RC complex when the light intensity was reduced [17]. An increased amount of the peripheral antenna complex may explain the more effective light energy absorption with a dim light. At light intensities below ~5 W/m$^2$, however, the light becomes too limited for cell growth to show a 3-h doubling time even with an increased level of the B800-850 complex formation. A *puc*-deleted cell of *R. sphaeroides* grows with a doubling time that is longer than 160 h at 3 W/m$^2$ due to the absence of the peripheral B800-850 complex (Figs. 1B and 2B). Accordingly, the *puc*-deleted cell was used to obtain a mutant exhibiting faster growth, possibly due to the elevated formation of the fixed photosynthetic unit under light-limiting conditions. The analysis of the mutation was aimed at understanding the regulation control of the formation of spectral complexes by light, especially at low intensities.

The spontaneous fast-growing mutant, SK101, was isolated from the B800-850$^-$ derivative, PUC-ZWT, of *R. sphaeroides* (Fig. 1B) after three consecutive enrichments of a culture grown photoheterotrophically at 3 W/m$^2$. The mutant SK101 exhibited a growth rate of ~40-h doubling at 3 W/m$^2$, whereas its growth was indistinguishable from its parental strain under both photoheterotrophic conditions at 10 W/m$^2$ as well as aerobic conditions (Fig. 2). Thus, only SK101 showed a higher growth rate than its parental cell with dim light.

### Increased Level of Spectral Complexes and Photopigments of *R. sphaeroides* SK101 under Photoheterotrophic Conditions

To identify whether the increased growth rate of SK101 at 3 W/m$^2$ is associated with an elevated level of the B875 complex, the absorption spectra of SK101 were determined and compared with those of the *puc*-deleted cell, PUC-

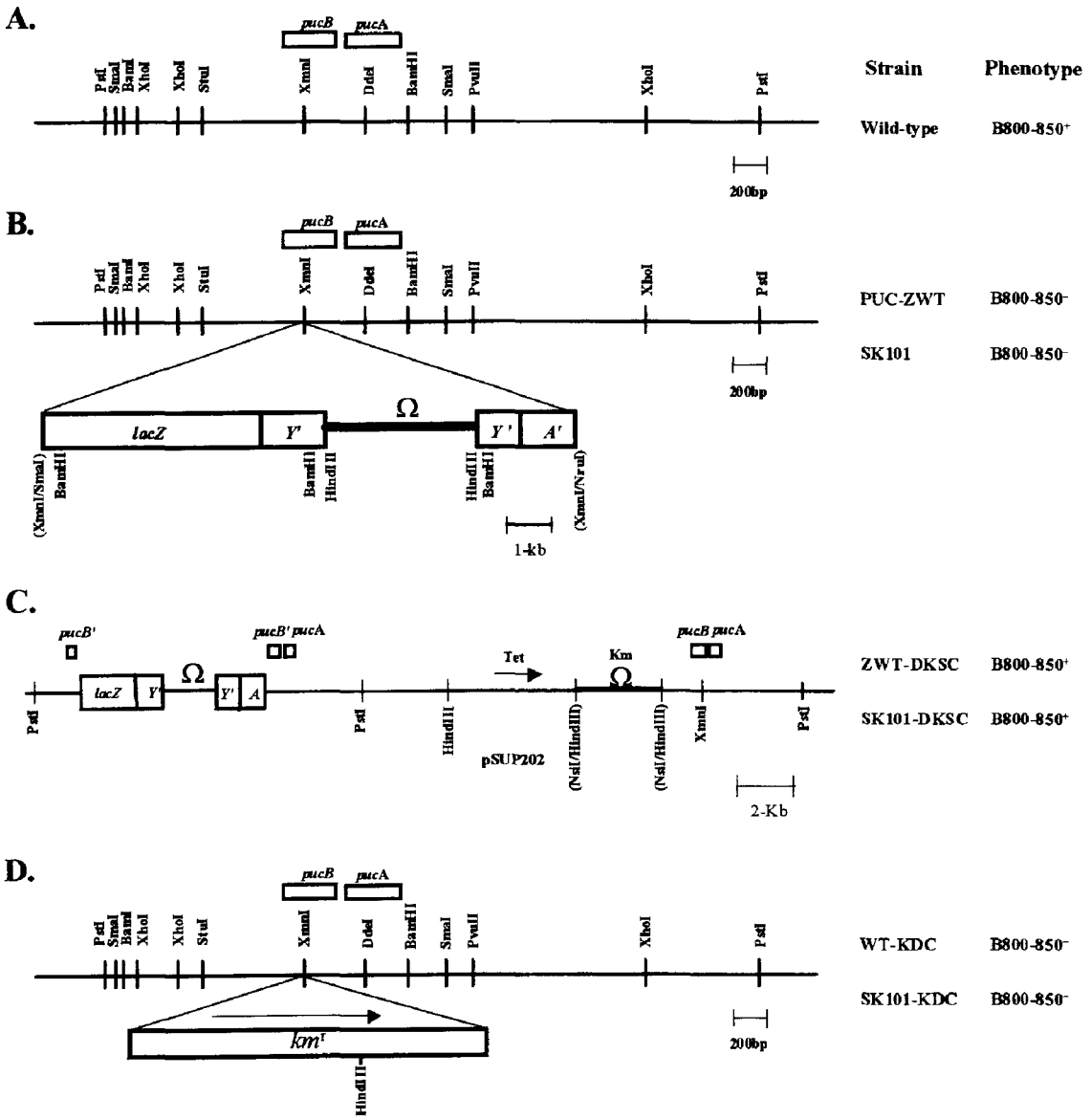


**Fig. 1.** Genomic structures of the *R. sphaeroides* strains used in this work.
PUC-ZWT, ZWT-DKSC, and WT-KDC were constructed in a wild-type genetic background, while both SK101-DKSC and SK101-KDC were derived from SK101.

ZWT. The aerobic cells of SK101 showed no detectable spectral complexes as observed in PUC-ZWT (Fig. 3C). In addition, the levels of both the B875 complex and the photopigments (bacteriochlorophyll and carotenoid) of SK101 under anaerobic dark conditions with DMSO were the same as those of the *puc*-deleted cells (Table 2). These results indicate that the oxygen control for the formation of the spectral complex and photopigments was not altered in SK101. The B875 complex of SK101, however, increased by 20 to 30% compared with that of the PUC-ZWT at both 10 and 3 W/m$^2$ (Figs. 3A, 3B and Table 2). The parallel increase of the bacteriochlorophyll and carotenoid contents of SK101 by ~60% and 40%, respectively, was observed especially at 3 W/m$^2$ (Table 2). Accordingly, under photoheterotrophic conditions, the mutation of SK101 resulted in an altered formation of the B875 complex and photopigments. The higher growth rate of SK101 compared to PUC-ZWT at 3 W/m$^2$ was thus deemed to be related to an increased level of the B875 complex.

**Altered Level of Spectral Complex Located at Extreme Periphery of Photosynthetic Unit in *R. sphaeroides* SK101**

The B800-850 complex was restored in SK101 to determine whether the mutation was specific to an increased formation of the B875 complex. A 2.5-kb *Pst*I fragment of pUI601 [16], containing *pucBA* and part of *pucC*, was

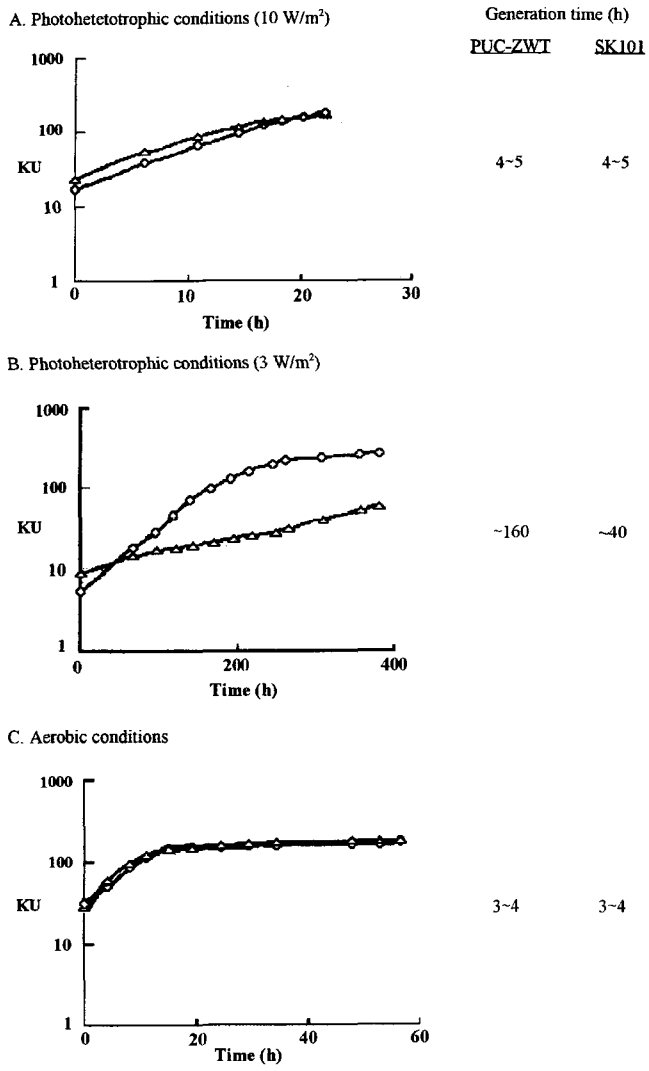


**Fig. 2.** Growth of PUC-ZWT and SK101 under various conditions.
△, PUC-ZWT; ○, SK101.

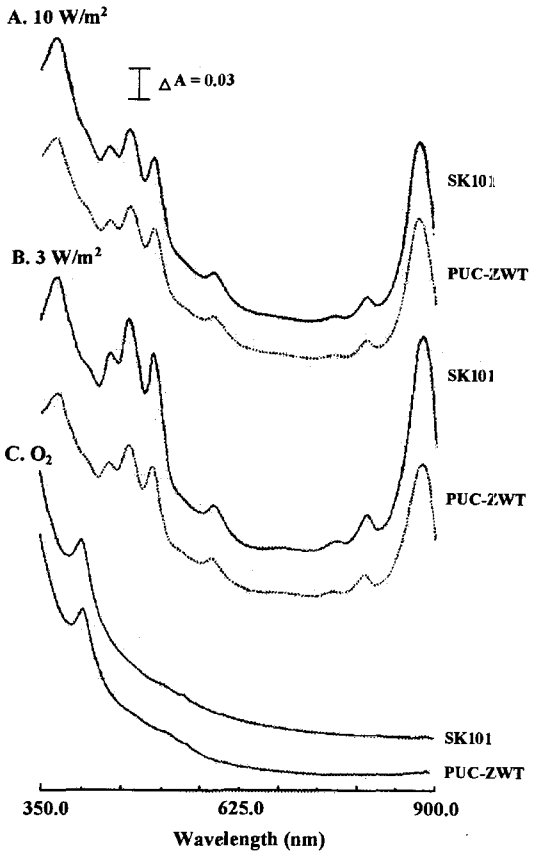


**Fig. 3.** Absorption spectra of membranes derived from PUC-ZWT and SK101.
Cells were grown photoheterotrophically at 10 W/m² (A), 3 W/m² (B), or aerobically (C). The bar represents an absorbance value of 0.03.

cloned into the *Pst*I site of pSUP202, a suicide plasmid in *R. sphaeroides*. Then, the transcription and translation stop Ω*aph* cartridge conferring $Km^r$ was cloned between the vector and the upstream region of the *puc* to block any fortuitous transcriptional read-through from the vector DNA. The resultant plasmid, pSUPPUCΩKm200, was mobilized into SK101 and PUC-ZWT, and the exconjugants showing $Km^r$ $Tc^r$ were then selected. Among the exconjugants, the B800-850$^+$ cells amounted to ~50%, and were generated by a single crossover between the *puc* DNA downstream of the *lacZYA*::Ω on the chromosomes and in the homologous area of the suicide plasmid. The other 50% of the exconjugants came from the single crossover events between the homologous DNA regions

**Table 2.** Levels of bacteriochlorophyll (Bchl), carotenoids (Crt), and the B875 complex of PUC-ZWT and SK101.

| Growth conditions | Strains | Bchl (ng/KU) | Crt (ng/KU) | B875 complex (nmol/mg of proteins) |
|---|---|---|---|---|
| Photoheterotrophic | | | | |
| 3 W/m² | SK101 | 37.51 | 9.90 | 11.76 |
| | PUC-ZWT | 23.64 | 6.90 | 9.13 |
| 10 W/m² | SK101 | 26.10 | 8.53 | 11.37 |
| | PUC-ZWT | 25.65 | 7.02 | 9.33 |
| Anaerobic | SK101 | 10.66 | 3.06 | 4.40 |
| Dark/DMSO | PUC-ZWT | 10.00 | 3.02 | 4.20 |

**Table 3.** Levels of Bchl, Crt, and light-harvesting complexes of ZWT-DKSC and SK101-DKSC.

| Growth conditions | Strains | Bchl (ng/KU) | Crt (ng/KU) | B875 complex (nmol/mg of protein) | B800-850 complex (nmol/mg of proteins) |
|---|---|---|---|---|---|
| Photoheterotrophic | | | | | |
| 3 W/m$^2$ | SK101-DKSC | 91.82 | 13.52 | 12.23 | 22.61 |
| | ZWT-DKSC | 68.10 | 10.63 | 14.21 | 18.44 |
| 10 W/m$^2$ | SK101-DKSC | 39.06 | 8.30 | 8.87 | 7.50 |
| | ZWT-DKSC | 39.69 | 9.05 | 8.21 | 4.96 |
| Anaerobic | SK101-DKSC | 27.83 | 5.49 | 6.03 | 9.63 |
| Dark/DMSO | ZWT-DKSC | 30.30 | 6.07 | 5.71 | 9.08 |

upstream of the *lacZYA*::Ω, and contained incomplete *pucC* after recombination to reveal B800-850$^-$. The B800-850$^+$ exconjugants from SK101 and PUC-ZWT were designated SK101-DKSC and ZWT-DKSC, respectively, and their chromosomal structures, as depicted in Fig. 1C, were confirmed by genomic Southern hybridization analysis (data not shown).

Interestingly, it was not the level of the B875 complex but rather that of the B800-850 complex of the SK101-DKSC which was increased by 20 to 50% compared with that of the ZWT-DKSC at both 10 and 3 W/m$^2$ (Table 3). The B875 complex in both the mutant and its parental strain was observed at a similar level. The level increment of the bacteriochlorophyll and carotenoid of SK101-DKSC by ~30% was observed, especially at the low-light intensity. In addition, the relative amounts of light-harvesting complexes and photopigments between SK101-DKSC and ZWT-DKSC were not different from each other under anaerobic respiratory conditions (Table 3). These results indicate that the mutation of SK101 specifically alters the formation of the most peripheral light-harvesting complex under photoheterotrophic conditions.

## Transcription Analyses of Photosynthetic Genes of SK101

Since SK101 and PUC-ZWT contain the *puc-lacZ* fusion on their chromosomes (Fig. 1B), the transcriptional activities of the *puc* operon were analyzed by measuring the β-galactosidase activities. No significant difference in the *puc* transcription was observed between the two bacterial strains irrespective of the culture conditions (data not shown). To determine whether the transcriptional activities of other photosynthetic genes, including *puf*, *puh*, and *bchF*, were affected by the mutation of SK101, the *lacZY*::ΩSm$^r$/Sp$^r$*A*$'$ present on the SK101 and PUC-ZWT chromosomes were replaced with Km$^r$ cartridge to generate SK101-KDC and WT-KDC, respectively (Fig. 1D).

The *pucB* of pUI601 was interrupted at the *Xmn*I site with 1.5-kb *Hin*cII Km$^r$ DNA from Tn903, and the *Pst*I fragment containing *pucB*::*Km$^r$AC* was cloned into the *Pst*I site of pSUP202. The resulting plasmid, pSUPPUCK200, was mobilized into SK101 and PUC-ZWT to select the double-crossed exconjugants showing Km$^r$ Tc$^s$. The chromosomal structures of SK101-KDC and WT-KDC (Fig. 1D) were confirmed by genomic Southern hybridization analysis (data not shown).

The *puf* and *puh* operons encode the structural polypeptides of the B875 and RC complexes [17], whereas the *bchF* codes for the enzyme involved in bacteriochlorophyll biosynthesis [3]. The plasmids containing *lacZ* constructs that were transcriptionally fused to the photosynthetic genes were then mobilized and maintained *in trans* in each strain. The β-galactosidase activities of the *pucB-lacZ* plasmid in SK101-KDC and WT-KDC showed similar levels to each other as observed with SK101 and PUC-ZWT (Table 4). The *bchF* transcription of SK101-KDC was notably increased

**Table 4.** Transcriptional expression of photosynthetic genes of WT-KDC and SK101-KDC.

| | β-Galactosidase activity (MU) | | | | | |
|---|---|---|---|---|---|---|
| | Photoheterotrophic conditions | | | | Aerobic conditions | |
| | 3 W/m$^2$ | | 10 W/m$^2$ | | | |
| *lacZ* fusion | SK101-KDC | WT-KDC | SK101-KDC | WT-KDC | SK101-KDC | WT-KDC |
| pCF200 (*pucB*) | 243±19[a] | 244±9 | 552±24 | 543±38 | 122±24 | 102±15 |
| pUI1830 (*pufB*) | 231±6 | 304±10 | 762±42 | 489±49 | 151±1 | 160±2 |
| pHZ300 (*puhA* ) | 317±12 | 243±2 | 977±16 | 926±49 | 122±7 | 112±5 |
| pLX200 (*bchF* ) | 242±16 | 23±8 | 217±4 | 86±9 | 29±5 | 6.3±5 |

[a]Standard deviations of the β-galactosidase activities are indicated following ±.

by two- to ten-fold compared with that of WT-KDC. The *pufB* and *puhA* transcriptions were not significantly affected by the mutation of SK101. These results suggest that the increased level of the bacteriochlorophyll of SK101 under light-limiting conditions may be regulated at the step of the *bchF* transcription. However, the level of the B875 complex of SK101 appears to be regulated post-transcriptionally.

### Complementation of SK101 Mutation

To determine the location of the SK101 mutation(s), pWS2 containing ~109-kb DNA of the photosynthetic gene cluster of *R. sphaeroides* WS2 [28] was mobilized into SK101. The DNA complemented the mutated phenotype of the growth observed at 3 W/m$^2$ (Table 5). The genomic cosmid library of *R. sphaeroides* 2.4.1 was screened further and one cosmid, pUI8487, was isolated which complemented the SK101 mutation. The DNA insert of pUI8487 was confined within the DNA of the photosynthetic genes of pWS2. The complementing DNA was narrowed down to a 1.8-kb DNA containing *orfQ* and *pufKBA* as shown with pΩQ200, whereas the *pufX*, encoding a protein, the function of which has not yet been clearly established, although it is potentially involved in the correct assembly of the B875 complex [10], did not complement the mutation (Fig. 4). When the *orfQ* of pΩQ200 was interrupted with Sm$^r$/Sp$^r$ transcription translation stop DNA, the complementation was abolished (Fig. 4). From these results the SK101 mutation would appear to be exerted through the mutation of the *orfQ* gene encoding a putative bacteriochlorophyll-mobilizing protein.

## DISCUSSION

The light-harvesting B800-850 and B875 complexes of *R. sphaeroides* play an important role in capturing light energy during photosynthesis. The *puc*-deleted cell, PUC-ZWT, grows with a long doubling time of ~160 h under photoheterotrophic conditions at 3 W/m$^2$. Under the same conditions, a spontaneous mutant, SK101, was isolated, the growth rate for which was approximately four-fold higher than its parental strain. The higher growth rate of SK101 was related to an increased level of the B875 complex, which was increased by ~30% at 3 W/m$^2$. However, when the B800-850 complex was restored in SK101, it was not the level of B875 but rather that of the B800-850 complex

**Table 5.** Complementation of the mutated phenotype of SK101 with the R clone and cosmid library of *R. sphaeroides*.

| | SK101 (pLA2917) | PUC-ZWT (pLA2917) | SK101 (pWS2) | SK101 (pUI8487) |
|---|---|---|---|---|
| Doubling time (h) during photosynthetic growth at 3 W/m$^2$ | ~160 h | ~40 h | ~40 h | ~40 h |

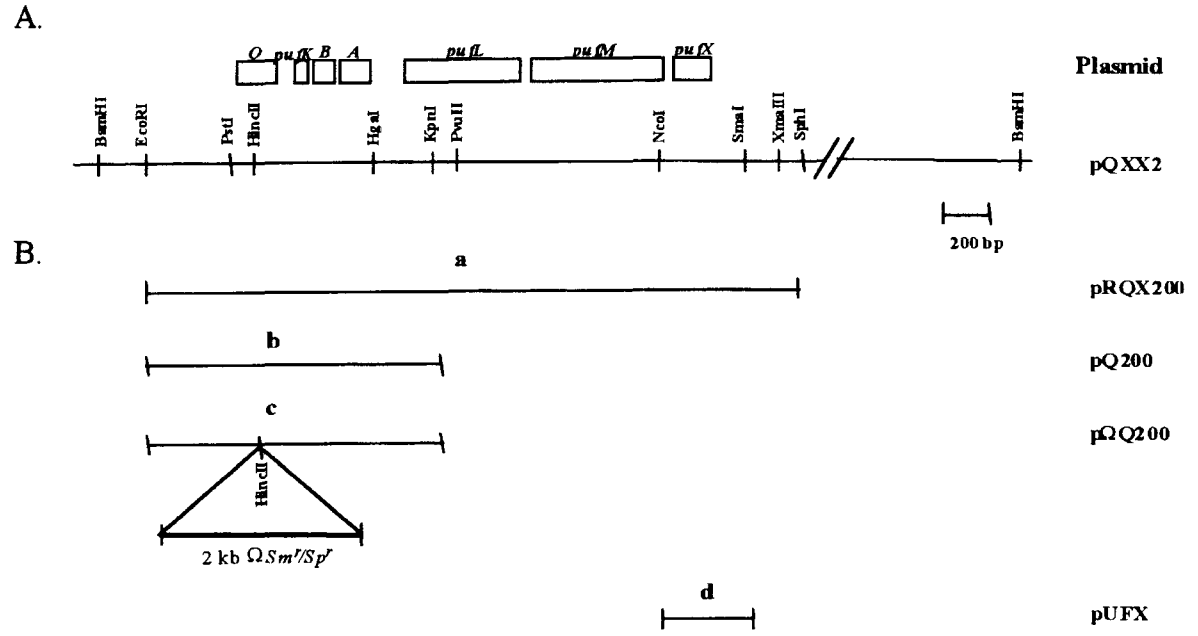


**Fig. 4.** Restriction map of the DNA region encompassing the *puf* operon and subclones used for the complementation of the mutated phenotype of SK101.

(A) An approximately 13-kb *Bam*HI restriction endonuclease fragment was generated from the cosmid pUI8487. (B) Subclones used for the complementation of SK101.

which was elevated compared with that of the parental strain at 3 W/m$^2$. Accordingly, these results indicate that the mutation(s) of SK101 affect the most peripheral spectral complex formation.

The transcriptional activities of the *bchF* of SK101 showed the most dramatic increase among photosynthetic genes whose expressions were examined. The transcriptional regulator, PpsR, is known as an aerobic repressor for the transcription of *bchF* as well as that of *puc* [11, 26]. However, the SK101 mutation did not seem to reside in the *ppsR*, because the *puc* transcription of the mutant was not derepressed aerobically. The mutation was mapped at the 1.8-kb DNA containing an *orfQ* and *pufKBA*. The interruption of the *orfQ* of the 1.8-kb DNA abolished the complementation of the mutated phenotype of the growth exhibited by SK101, suggesting that the mutation appeared to be exerted through the *orfQ* gene.

Recently, OrfQ has been proposed to insert bacteriochlorophyll into developing RC and light-harvesting complexes by hierarchically interacting with complex-specific assembly factors (CSAFs) [13]; the lowest affinity for the B800-850-specific factor ($CSAF_{800-850}$), a higher affinity for the B875-specific factor ($CSAF_{875}$), and the highest affinity for the presumed RC-specific factor(s) ($CSAF_{RC}$). The results shown in this work suggest that the OrfQ affinity for CSAFs may be changed by the mutation of the SK101 *orfQ* gene to have a higher affinity towards a CSAF specific to the most peripheral light-harvesting complex, which could be either the B800-850 or the B875 complex. Analysis of the *orfQ* of SK101 is underway to identify the mutation site, which will elucidate the structural basis of the OrfQ involved in the interaction with the CSAFs.

In this study, we have isolated and characterized a *trans*-acting mutant showing an increased level of spectral complex to result in a faster growth rate, especially at low-light intensity. This is the first report of an *R. sphaeroides* mutant isolated spontaneously under light-limiting conditions, where its mutation site possibly mapped at the *orfQ*. Additionally, the assembly control for the formation of photosynthetic complex located at the most periphery of the photosynthetic unit was altered by the mutation.

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