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Mutational analyses of two unique genes contained in the *puf* operon of the purple bacterium, *Rubrivivax gelatinosus*

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Introduction

The reaction center (RC) complex of the β -purple photosynthetic bacterium, *Rubrivivax gelatinosus*, consists of the L, M, H and cytochrome subunits. The RC complex is surrounded by the light-harvesting 1 (LH1) complex, which has an absorption maximum at 880 nm in the infrared region. The other antenna complex, the light-harvesting 2 (LH2) complex, shows two large absorption bands peaked at 800 and 850 nm. Both of the LH complexes contain two small proteins spanning the membrane, α and β subunits.

The genes coding for the subunits of the RC and the LH1 complexes form an operon called *puf*. The structure of the *puf* operon is well conserved among purple bacteria, in which the genes are ordered from the upstream as *pufB*, *pufA*, *pufL*, *pufM* and *pufC* coding for the β and α subunits of the LH1 and the L, M and cytochrome subunits of the RC, respectively. Under anaerobic conditions, a large amount of short mRNA containing *pufB* and *pufA* and a small amount of long mRNA containing all of *puf* genes are accumulated.

Some additional genes have been reported in the *puf* operon of several species. In *Rhodobacter sphaeroides* and *Rhodobacter capsulatus*, *pufQ* and *pufX* are present in the regions upstream of *pufB* and the downstream of *pufM*, respectively. These genes have been suggested to code the proteins affecting in the process of bacteriochlorophyll biosynthesis and the effective electron transfer from the RC to the cytochrome *bc*₁ complex, respectively. In *R. sphaeroides* a presence of a gene coding for only 20 amino acids, *pufK*, located between *pufQ* and *pufB* has also been suggested, which seemed to control the translation of *pufB* (Gong and Kaplan 1996). *Rvi. gelatinosus* *puf* operon contains two open reading frames (ORFs) without known functions. One is the ORF48 located at the upstream of *pufB* with an overlap of 4 nucleotides, which possibly codes for a small soluble protein with a high isoelectric point (pH 11.92). The other is the ORF74 situated between *pufB* and *pufA* genes and codes for a possible membrane protein showing a significant sequence identity (24%) to the β subunit of the LH1 (Nagashima et al. 1994). The ORF48 showed no sequence similarities to the *puf* genes of other species. In this study, we constructed *Rvi. gelatinosus* mutants deleted in these two ORFs to examine their functions.

Materials and methods

Rvi. gelatinosus strain IL144 (WT) and its spontaneous mutant, IL144RL2, were used as parent strains to construct mutants. The strain IL144RL2 shows significantly reduced production of the LH2 complex but constant synthesis of the RC-LH1 complex even under aerobic conditions. The 4.6 kb DNA fragment containing the whole *puf* operon of *Rvi. gelatinosus* has been cloned (Nagashima et al. 1994). A region flanked by *NotI* restriction sites on this fragment (containing almost whole *puf* operon, see Fig. 1) was replaced by an

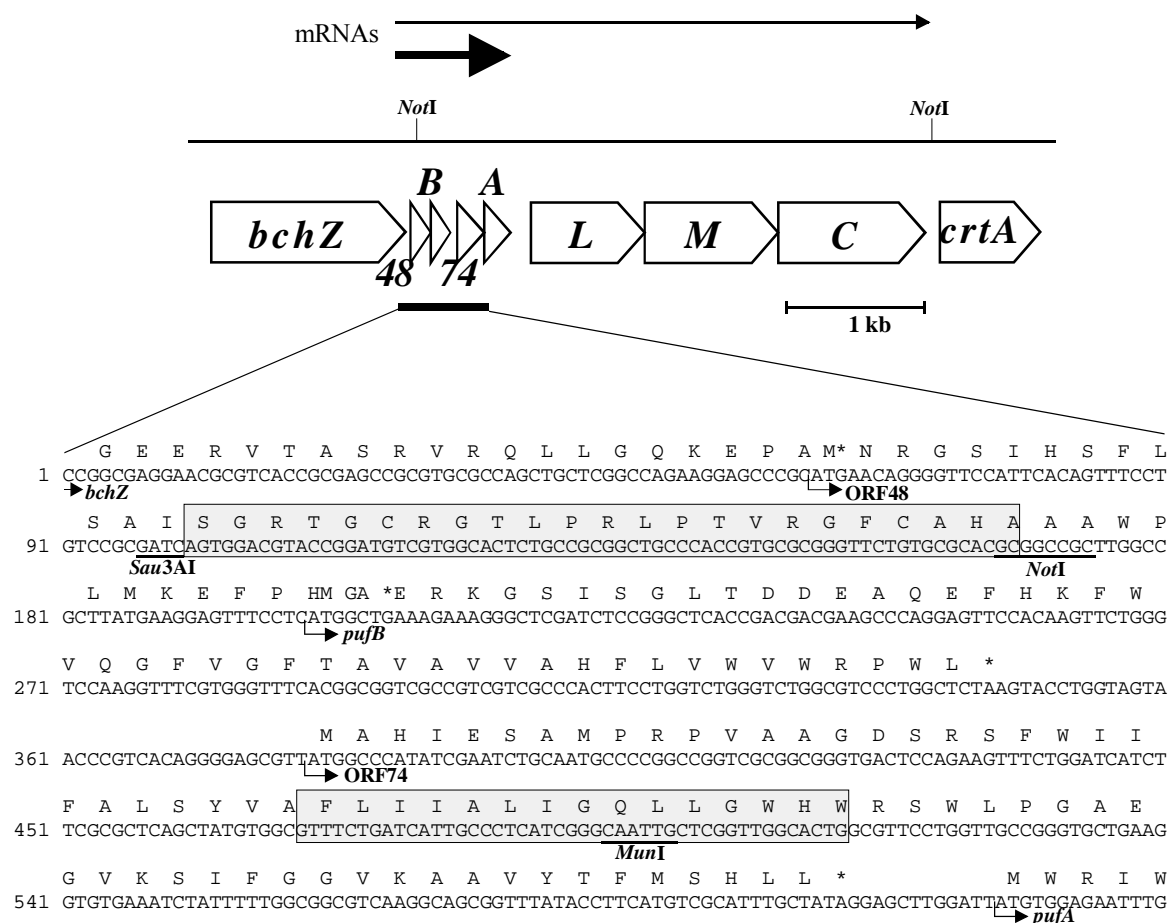


Fig. 1. Structure of *Rvi. gelatinosus* *puf* operon. DNA regions deleted to construct mutants in this study were boxed and shaded.

ampicillin resistance gene or a kanamycin resistance gene. This DNA construct was introduced into cells of *Rvi. gelatinosus*. The cells showing the antibiotics resistance and no production of the RC-LH1 complex (no growth under the photosynthetic conditions) were picked up and named strain WDP1 (derived from the strain IL144, Km^r) and strain DP2 (derived from the strain IL144RL2, Amp^r). Southern hybridization analyses and DNA sequencing verified the lack of the *puf* operon in the genomes of these strains. Plasmids containing a *puf* operon in which a 68 nucleotides flanked by *Sau3AI* and *NotI* restriction sites on ORF48 was replaced by a 6 nucleotides, 5'-CTCTAG, were introduced into the cells of the strain WDP1 by electroporation according to Nagashima et al. (1996). Plasmids containing the *puf* operon which lacks 45 nucleotides within ORF74 by a digestion with a *MunI* restriction endonuclease followed by treatment with Exonuclease III were introduced into the strain DP2 cells. Cells recovering photosynthetic abilities were selected and named

strain Δorf48 in the former case and strain Δorf74 in the latter case. Southern hybridization and DNA sequencing confirmed an incorporation of the modified *puf* operons into the genome of the mutants through double crossover recombination. In the Δorf48 mutant, the possible translation of ORF48 was terminated after the 14th amino acid residue. The Δorf74 mutant lacks 15 amino acids of the ORF74 product which corresponds possible spanning region to the membrane.

Results

The mutant strains of *Rvi. gelatinosus*, Δorf48 and Δorf74 , can grow under photosynthetic conditions. Measurements monitored by the optical density at 660 nm showed that the photosynthetic growth rates of the Δorf48 and Δorf74 cells were 3.4 and 1.4 times slower than those of the parent strains, respectively, although their growth rates under respiratory

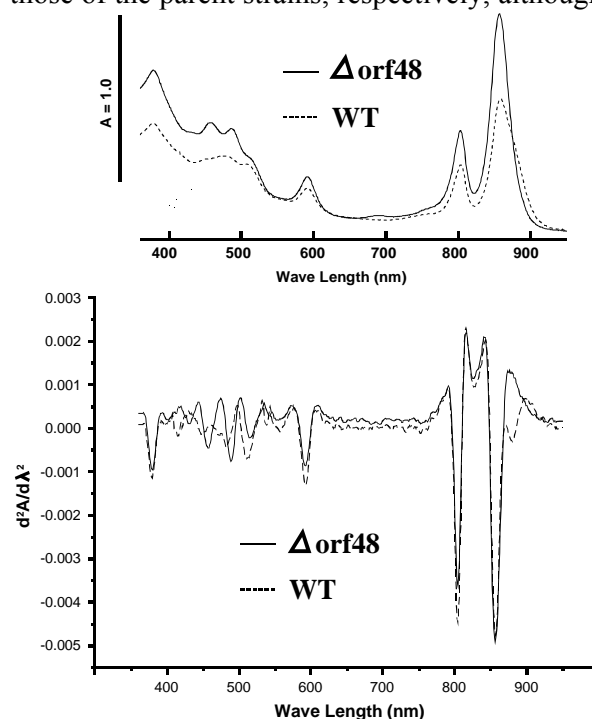


Fig. 2. Absorption spectra (upper) and their 2nd derivatives (bottom) of membranes prepared from Δorf48 and IL144 cells of *Rvi. gelatinosus*.

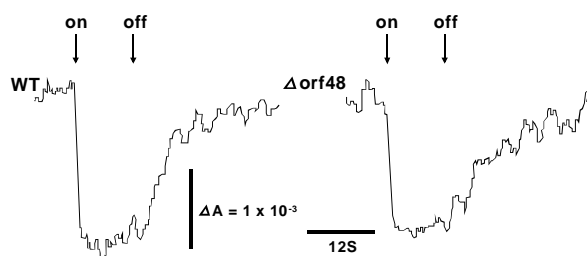


Fig. 3. Light-induced redox changes of the RCs in the membranes of the WT and Δorf48 of *Rvi. gelatinosus*. Each sample contained 0.4 mg protein ml^{-1} . The kinetics were measured at 603-minus-573 nm.

conditions are nearly identical. The absorption spectrum and the RC content of the Δorf74 membrane were nearly identical to those of the strain IL144RL2 (data not shown). The results indicate that the ORF48 and ORF74 are not essential but have some roles for photosynthesis of *Rvi. gelatinosus*. Figure 2 shows absorption spectra of the membranes prepared from the cells of the Δorf48 mutant and the wild type strain IL144. In the spectrum of the wild type membrane an absorption by the LH1 was observed as a shoulder in the longer wavelength region of a 850 nm band, which was distinguished as a negative peak at 880 nm in the second derivative of the spectrum. This component was not detected in the spectrum of the Δorf48 mutant membrane (solid line). This shows that the synthesis of the LH1 complex in the Δorf48 mutant was significantly reduced. Kinetics of light-induced redox change of the RC monitored at 603-minus-575 nm showed, however, that the RC content in the Δorf48 mutant membrane was comparable to that in the wild type membrane (Fig. 3).

Figure 4 shows a result of the Northern hybridization analysis using a DNA fragment containing the four genes, ORF48, *pufB*, ORF74 and *pufA* as a probe.

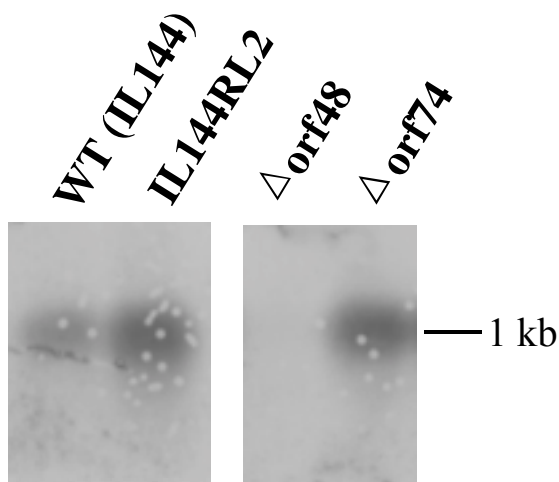


Fig. 4. Northern hybridization of total RNA from *Rvi. gelatinosus* strains with a probe specific for the *pufBA* region. Each lane contained 0.5 μ g of RNA. A DIG-labeled DNA fragment was used as a probe.

A 1 kb mRNA was detected in the wild type but not in the Δ orf48 mutant. The 1 kb mRNA was detected also in the IL144RL2 and Δ orf74, although their signal intensities were comparable and were stronger than that detected in the wild type.

Discussion

The phenotype of the *Rvi. gelatinosus* Δ orf48 mutant was similar to that of the *pufK*-depleted mutant of *Rba. sphaeroides* in terms that the synthesis of the LH1 is greatly depressed without clear effects on the expression of RC (Gong and Kaplan 1996). The enhanced synthesis of the LH2 complex shown in the *pufK* mutant was also observed in the Δ orf48 mutant. In *Rba. sphaeroides* it has been shown that the mutant deleted in *pufK* accumulates lesser amount of but more stable *pufBA* mRNA than the wild type. The repression of the LH1 synthesis was ascribed to a disruption of the translational coupling from *pufK* to *pufB* and/or inaccessibility of ribosomes to *pufB*. However, *Rvi. gelatinosus* Δ orf48 mutant showed no detectable accumulation of the short mRNA containing *pufBA*. The regulatory mechanisms for the LH1 synthesis affected by ORF48 in *Rvi. gelatinosus* seem to be different from those by *Rba. sphaeroides pufK*. One possibility is that the product of ORF48 is necessary to protect the short *puf* mRNA against the digestion by RNases. Or, a region within ORF48 may contribute to form a stable secondary structure of the short mRNA to avoid the digestion. Another possibility is that the ORF48 product is a factor controlling the transcription of the *puf* operon.

The Δ orf74 mutant showed no significant differences from the parent strain except that the growth rate of the mutant cells was slightly slower than that of the parent cells under the photosynthetic conditions. Active RC content and the rate of electron transfer from the RC to the cytochrome *bc*₁ in Δ orf74 cells were almost identical to those in the parent strain IL144RL2 (data not shown). It was suggested that ORF74 is not a homologue of neither *pufQ* nor *pufX* found in *Rhodobacter* species.

References

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