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Regulation of aerobic photosystem synthesis in the purple bacterium *Rhodospirillum centenum* by CrtJ and AerR†

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Abstract

Genes coding for putative CrtJ and AerR homologs were identified and characterized in the purple photosynthetic bacterium *Rhodospirillum centenum* (also known as *Rhodocista centenaria*), an organism that synthesizes photopigments even under highly aerated conditions. Mutational analysis indicated that in *Rsp. centenum*, gene *crtJ* codes for a repressor for photosynthesis gene expression as in *Rhodobacter capsulatus*, which exhibits a high level of oxygen repression of photosystem synthesis. In contrast to *Rba. capsulatus*, AerR in *Rsp. centenum* appears to be an aerobic activator; an *aerR* mutation resulted in significantly reduced levels of photopigment synthesis. Both *aerR* and *crtJ* mutants retained essentially normal levels of photosystem synthesis under anaerobic conditions, indicating that their activities are specific for aerobic photosystem synthesis. The readthrough transcript from *crtE* promoter, which is regulated by AerR and CrtJ, seems to be significant in maintaining the expression levels of the light harvesting I (*puf*) genes in *Rsp. centenum.* We suggest that AerR and CrtJ regulate aerobic photosystem synthesis primarily through controlling activity of the transcriptional readthrough.

Introduction

Photosystem synthesis in non-sulfur purple bacteria is regulated by oxygen tension and light intensity. Many studies on the regulatory system have been conducted using *Rhodobacter capsulatus* and *Rhodobacter sphaeroides*, in which photosystem synthesis is almost completely repressed under aerobic growth conditions.[1,2] However, it is now known that there is a wide range of responses to oxygen among different species of purple bacteria. In contrast to *Rhodobacter* species, in *Rhodospirillum centenum* and *Rhodovulum sulfidophilum* photosystem synthesis is repressed only slightly when these organisms are grown aerobically.[3,4] Previous studies with *Rdv. sulfidophilum* indicated that the mRNA of some photosynthesis genes are kept at high levels even under aerobic conditions, indicating that oxygen may differentially regulate the transcription of photosynthesis genes depending on the species of purple bacteria.[5,6] However, the mechanism(s) of how the oxygen-insensitive photosystem synthesis is achieved is not fully understood.

The key regulators of photosystem synthesis under aerobic conditions are CrtJ and AerR in *Rba. capsulatus* and *Rba. sphaeroides* (called PpsR and PpaA, respectively, in *Rba. sphaeroides*).[7–10] Biochemical and molecular genetic analyses have indicated that CrtJ binds

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under aerobic conditions to the conserved palindromes (TGT-N12-ACA) which are positioned in the promoters of bacteriochlorophyll (*bch*), carotenoid (*crt*), and light harvesting II (*puc*) genes.[8,11–13] AerR also binds to the promoter regions, and co-operatively interacts with CrtJ.[9] The homologous genes of *crtJ* (*ppsR*) and *aerR* (*ppaA*) have been identified in other purple bacteria, including *Rubrivivax gelatinosus* and *Bradyrhizobium* ORS278.[14–16] This suggests that CrtJ and AerR are widely conserved in purple bacteria, although they exhibit different regulatory features in each bacterium: they can activate and/or repress the transcription of photosynthesis genes, and their activities are modulated or not by the redox conditions.[17,18] Thus, characterization of *crtJ* and *aerR* in bacteria that show different features in photopigment synthesis seem to be useful to elucidate the regulatory mechanisms.

In this study, we identified the homologous genes of *crtJ* and *aerR* in *Rsp. centenum.* Mutational analyses indicated that CrtJ and AerR act as aerobic repressor and activator, respectively, in this bacterium. Our results also suggest that the readthrough transcript from *crtEF* genes into the *puf* operon is significant in maintaining the mRNA levels of the light harvesting I (*pufB* and *pufA*) genes.

Materials and methods

Bacterial strains and growth conditions

The wild-type strain of *Rsp. centenum* (ATCC 43720), the *aerR*-disrupted strain AERR1, and the *crtJ*-disrupted strain CRTJ1 were grown at 42 °C in CENS medium as described previously.[19] For anaerobic growth, cultures were incubated in screw-cap tubes filled with CENS medium, and routinely illuminated by banks of 60 W incandescent Lumiline lamps. For aerobic growth, 15 mL cultures were incubated in 250 mL sidearm flasks with shaking (500 rpm). Cell growth was monitored by measuring turbidity with a Klett-Sumerson spectrophotometer. *Escherichia coli* strains DH5α and DH10B (Bethesda Research Laboratories) were used as host strains for routine cloning procedures, and the strain S17-1[20] was used as a mobilization strain. They were grown in Luria broth at 37 °C. Gentamycin, ampicillin, and spectinomycin were used for *E. coli* at 10, 150, and 50 μg mL^{-1}, respectively. Gentamycin and spectinomycin were used for *Rsp. centenum* at concentration of 10 μg mL^{-1} each.

Modified mini-Tn5 mediated mutagenesis

Wild type *Rsp. centenum* was mutagenized using a modified mini-Tn5 with an ΩSp^r cassette replacing the Km^r gene. Mutagenized culture was spread onto agar-solidified CENS media and grown aerobically in the dark. Pigment mutants were isolated based on colony color. The disrupted genes were cloned by isolating genomic DNA from each mutant strain and digesting the genomic DNA with various restriction enzymes in separate reactions. Digested DNA was ligated into pBluescript and transformed into *E. coli* strain DH10B. Strains containing the pBluescript plasmid with the appropriate insert were selected for by plating on LB medium containing both ampicillin and spectinomycin. The regions flanking the transposon were sequenced using primers to the spectinomycin gene. Sequence analysis was performed using the program, GCG.

Construction of *aerR*-disrupted strain, AERR1, from *Rsp. centenum*

Two 500-bp DNA fragments consisted of N-terminal and C-terminal regions of AerR were amplified by PCR using *pfu* DNA polymerase. Two sets of primers were used. One of the two is a forward primer AerRsacIfor (5′-GGGAGCTCTTCCGGGAAG-GCTAGACGTG-3′) and a reverse primer AerRecoVrev (5′-GG-GATATCGAATCAGGGACGCGAACCGG-3′). The other set is a forward primer AerRecoVfor (5′-GGGATATCCCGGCCA-GATCTGATCAAGAG-3′) and a reverse primer AerRxbaIrev (5′-GGTCTAGACGCGGACCCGGTTTGTCAAG-3′). *Sac*I, *Eco*RV, and *Xba*I restriction sites

were designed at additional polynucleotides of AerRsacIfor, AerRecoVrev and AerRecoVfor, and AerRxbaIrev primers, respectively (underlined). The first PCR fragment was digested with *Sac*I and *Eco*RV. The second PCR fragment was digested with *Xba*I and *Eco*RV. After the digestion, these two fragments were mixed and ligated together into *Sac*I-*Xba*I-cut pUC18, resulting in pUCAerR. The pUCAerR and the Gm^r-suicide vector pZJD29A[21] were digested with *Xba*I at their unique sites, and ligated together to construct a plasmid pAerRGm. The plasmid pZJD29A has a *sacB* gene encoding the levansucrase of *Bacillus subtilis.* The expression of *sacB* in the presence of sucrose is lethal for many Gram negative bacteria.[22] The resulting plasmid, pAerRGm was transferred into *Rsp. centenum* cells by conjugation with the mobilizing strain S17-1, and cells undergoing single crossover events were selected by plating exconjugants on CENS plates containing Gm. The streak-purified Gm^r cells were grown for over night in liquid medium without Gm, and then these cultures were plated onto CENS plates containing 5% sucrose. Several single colonies were picked up, and AerR deletion was confirmed by PCR (about half of these colonies were wild type as expected). The resulting *aerR*-deleted strain was named AERR1. The predicted amino acid sequence for AERR1 is 14 amino acids long (216 amino acids for the native protein).

Construction of *crtJ*-disrupted strain, CRTJ1, from *Rsp. centenum*

Two 500-bp DNA fragments consisted of N-terminal and C-terminal regions of CrtJ were amplified by PCR using *pfu* DNA polymerase. Two sets of primers were used. One of the two sets is a forward primer CrtJsacIfor (5′-GGGAGCTCGCAT-GCGCCAGCCTTAACGC-3′) and a reverse primer CrtJecoVrev (5′-GGGATATCGACTGCTGCGCTTCCACCAG-3′). The other set is a forward primer CrtJecoVfor (5′-GGGATATC-GCTCTATGCCACCACCCTGC-3′) and a reverse primer CrtJxbaIrev (5′-GGTCTAGACGGGCATCGCTCACCTGC-TG-3′). *Sac*I, *Eco*RV, and *Xba*I restriction sites were designed at additional polynucleotides of AerRsacIfor, AerRecoVrev and AerRecoVfor, and AerRxbaIrev primers, respectively (underlined). The first fragment was digested with *Sac*I and *Eco*RV, and the second fragment was digested with *Xba*I and *Eco*RV. After the digestion, these two fragments were mixed and ligated into *Sac*I-*Xba*I-cut pUC18, resulting in pUCCrtJ. Further procedure is as same as that for *aerR*-disrupted strain construction (described above). The resulting *crtJ*-deleted strain, named CRTJ1, has the 201 amino acids in-frame deletion at *crtJ* locus (the native protein has a predicted length of 468 amino acids).

Construction of *crtE::lacZ* and *puf::lacZ* fusions, and β-galactosidase assay

A DNA fragment consisted of 342-bp upstream and 57-bp downstream from *crtE* start codon was amplified from *Rsp. centenum* genomic DNA by PCR using *pfu* DNA polymerase. For the PCR reaction, forward and reverse primers (crtE-F: 5′-TCCA-GCCCCGGGAGCATG-3′ and crtE-R: 5′-AGACCGAATATC-GCATCGCC-3′, respectively) were designed. The amplified DNA fragment was cloned into the promoter testing vector, pCF1010,[23] at the *StuI* site, resulting in *crtE::lacZ* transcriptional fusion named pRC200. A 1-kbp *Rsp. centenum puf* upstream region was amplified by PCR from the plasmid pRCPUF8 (kindly provided by Joann Williams, Arizona State University) with *pfu* DNA polymerase. The forward and reverse primers (M13-M4: 5′-GTTTTCCCAGTCACGAC-3′ and RcenPuf-R: 5′-GAGGTATTTTCAGGCATTGG-3′, respectively) were designed for the PCR reaction. The amplified DNA fragment was cloned into a promoter testing vector, pCF1010, at the *StuI* site, resulting in *puf::lacZ* transcriptional fusion named pRC100. These plasmids were mobilized into *Rsp. centenum* cells by congugation with the mobilizing strain *E. coli* S17-1. The β-galactosidase activity was determined as essentially described.[24] Cell growth was monitored by measuring the optical density (OD) of the culture at 660 nm with a Klett-Summerson photometer. Final results were obtained as the amount of *o*-nitrophenyl-β-galactoside (ONPG) hydrolyzed per min per OD_{660}.

Materials

Restriction endonucleases and T4 ligase were purchased from New England Biolabs. Synthetic oligonucleotide primers were purchased from MWG Biotech, Inc.

Accession numbers

The nucleotide sequence data reported in this study are available in the DDBJ, EMBL, and GenBank nucleotide sequence databases under accession no. AB369262.

Results and discussion

Identification of *crtJ* and *aerR* genes in *Rsp. centenum*

Rsp. centenum is one of a few photosynthetic bacteria that express photosynthetic pigments under aerobic conditions. To determine the regulatory elements that control expression of photosynthetic pigments in this organism, transposon mutagenesis with a modified mini-Tn5 was utilised to isolate mutants with altered expression patterns. Since *Rsp. centenum* is able to express its photosystem under aerobic conditions, we could directly screen for photosystem mutants based on colony color, under conditions where expression of the photosystem is not required for growth. Colonies with decreased photopigment expression (pink or yellow) were isolated, as well as colonies with increased photopigments (dark red). Out of 10 000 colonies screened, 20 mutants showed a yellow or pink phenotype and 2 mutants showed a dark-red phenotype. In this report, two of these mutants are discussed as follows.

Strain JBC003 forms colonies with a dark-red color indicative of derepression of the photosystem. The disruption in this mutant was cloned and sequenced and mapped to an open reading frame of 1404 base pairs. A search of the BLAST database with the predicated amino acid sequence of this gene showed similarity to CrtJ and PpsR from *Rba. capsulatus* and *Rba. sphaeroides*, respectively (Fig. 1), which are known to be aerobic repressors for photosynthesis gene transcription.[8,11–13] We also found that CrtJ/PpsR homolog of *Rsp. centenum* show much higher similarity to those of *Thiocapsa roseopersicina* and *Rhodopseudomonas palustris* (~55% identity) than to those of *Rhodobacter* species (~30% identity). This is in agreement with the phylogenetic distances between each bacterium based on the 16S rRNA sequences that showed that *Rsp. centenum*, *Tca. roseoperisicina* and *Rps. palustris* belong to α1-subgroup of proteobacteria; whereas, *Rba. capsulatus* and *Rba. sphaeroides* belong to α3-subgroup of proteobacteria.[25] *Tca. roseoperisicina* and *Rps. palustris* contain two CrtJ/PpsR homologs, [18,26] suggesting that *Rsp. centenum* possesses also two CrtJ/PpsR homologs, although, at present, no additional *crtJ/ppsR* gene has been found on the partially sequenced genome of *Rsp. centenum* (available at http://genomes.tgen.org/index.html) (data not shown).

It was shown that *Rba. capsulatus* CrtJ makes an intramolecular disulfide bond under aerobic growth conditions, which results in stimulation of DNA-binding activity for target promoters. [12] Sequence alignment of the *Rsp. centenum crtJ* gene product with the CrtJ (PpsR) proteins from other species reveals the presence of several conserved regions including the highly conserved C-terminal helix-turn-helix DNA-binding domain (Fig. 1). Also of note is the conserved cysteine residue at position 426, which has been shown to be essential for formation of an intramolecular disulfide bond in *Rba. capsulatus.* Interestingly, the other cysteine residue implicated in disulfide bond formation with cysteine 426 in *Rba. capsulatus* CrtJ[12] is not conserved in *Rsp. centenum* at the corresponding position (valine 255), suggesting a different oxygen-sensitivity of this protein in this bacterium. If the function of the *Rsp. centenum* CrtJ protein requires the formation of an intramolecular disulfide bond, it must occur at the only other cysteine residue in the predicted amino acid sequence at position 373 (Fig. 1).

Strain JBC315 forms pale colonies indicative of decreased expression of photosynthetic pigments. The disruption in this mutant was cloned and sequenced and mapped to an open reading frame of 648 base pairs. A search of the BLAST database with the predicated amino acid sequence of this gene showed similarity to AerR from *Rba. capsulatus* and PpaA from *Rba. sphaeroides* respectively. AerR/PpaA functions as a repressor in *Rba. capsulatus* and an activator in *Rba. sphaeroides* for transcription of photosynthetic genes.[9,10] To investigate the roles of AerR as well as CrtJ in controlling aerobic photosystem synthesis in *Rsp. centenum*, we constructed *aerR*- and *crtJ*-deletion strains of this bacterium as follows.

Mutational analyses of AerR and CrtJ in *Rsp. centenum*

The *aerR* and *crtJ* deletion strains were constructed by site-specific recombination (designed as AERR1 and CRTJ1, respectively). To avoid the complicating effects of expression of downstream or upstream genes, no antibiotic-resistance cassette was employed for the construction (see Materials and methods). Phenotypic differences between aerobically grown colonies of these mutants and wild type were striking. The AERR1 strain exhibits poorly pigmented (pale pink) colonies whereas CRTJ1 strain makes colonies that are more pigmented (dark red) than observed with wild type cells (data not shown). Fig. 2A and B show the spectral analyses of *Rsp. centenum* wild-type (solid lines), AERR1 (short-dashed lines), and CRTJ1 (long-dashed line) strains grown under anaerobic light and aerobic dark conditions, respectively. CRTJ1 cells grown under aerobic conditions synthesized higher levels of photopigments than the wild type strain whereas aerobically grown AERR1 cells synthesized significant reduced amounts of photopigments (Fig. 2B). Spectral analysis of phosynthetically grown cells indicated that CRTJ1 had the same amounts of photopigments as the wild type strain (data not shown). Similarly, AERR1 showed just slightly reduced levels of photopigment synthesis (Fig. 2A). These results indicated that CrtJ represses and AerR activates photosystem synthesis only under aerobic conditions.

We next tested the effect of *aerR* and *crtJ* mutations on gene expression by measuring β-galactosidase activities in strains that harbored the *crtE::lacZ* or *puf::lacZ* reporter plasmids. As indicated in Fig. 3, disruption of *aerR* results in lower (0.7 fold) *crtE* expression than that of wild type when grown aerobically. In contrast, CRTJ1 showed high levels (1.7 fold) of activity. There is no significant difference in the *crtE* expression between these mutant strains and wild type when grown photosynthetically. These results indicated that AerR and CrtJ act as activator and repressor, respectively, for the *crtE* transcription under aerobic conditions. The *aerR* mutation, but not *crtJ* mutation, affected the *puf* operon expression. The AERR1 cells grown aerobically demonstrated 0.4-fold levels of *puf* expression, but anaerobically grown cells showed 1.7-fold expression levels. These results indicated that AerR is a bifunctional regulator for *puf* operon expression which activates transcription under aerobic conditions, but represses under anaerobic conditions.

Identification of CrtJ binding site in the *Rsp. centenum crt* promoters

As shown in Fig. 4, a CrtJ-recognition site (TGT-N12-ACA) with a −35 σ70-recognition motif (TTGACA) which was well matched with the consensus sequence shown in the *Rhodobacter* species, were detected in the promoter regions of *Rsp. centenum crtE* and *crtD* genes. This observation suggests that *Rsp. centenum* CrtJ binds to the same consensus motifs shown in *Rba. capsulatus* for its repressor activity. The CrtJ-repressed promoters often contain two CrtJ-recognition sequences in tandem as observed in *Rba capsulatus bchC* promoter (Fig. 4). The two palindromic sites were critical for cooperative binding of CrtJ, because CrtJ works as tetramer (dimmer of dimmer).[27] However, *Rsp. centenum crtE* and *crtD* promoters contain only one CrtJ-recognition site as shown in *Rba. capsulatus crtE, crtI* and *bchF* promoters (Fig. 4). In *Rba. capsulatus*, CrtJ still binds to the promoters in a cooperative manner with a distantly separated palindrome located in a different promoter through forming a looped DNA structure.

[28] Given that *Rsp. centenum crtE* and *crtD* genes adjoin on the genome (Fig. 5) and *crtE* promoter activity is influenced by the *crtJ* mutation (Fig. 3), *Rsp. centenum* CrtJ may bind to the *crtE* promoter region cooperatively with the *crtD* promoter.

No CrtJ binding motif was found within 1-kbp upstream of *Rsp. centenum puf* operon (data not shown). These observations are consistent with the *lacZ* reporter experiments, described above, in the sense that *crtJ* mutation affects the expression of *crtE*, but not of the *puf* operon. A previous study indicated that *Rba. capsulatus* AerR binds to the promoter regions of some photosynthesis genes including *puf*.[9] Both *puf* and *crtE* expression are regulated by AerR in *Rsp. centenum* (Fig. 3), suggesting the same binding pattern in *Rsp. centenum.* However, any AerR recognition sequences have not been clear yet even in *Rba. capsulatus.*[9]

Readthrough transcript for the *Rsp. centenum puf* operon

In *Rba. capsulatus*, the *puf* operon is co-transcribed with the upstream *crtEF* and *bchCXYZ* genes, although a large amount of *puf* mRNA are also transcribed by its own promoter[24,29] (Fig. 5). Specifically, based on the reporter gene fusion analysis, about 83% and 50% *puf* transcription come from *bchC* promoter under high- and low-oxygen conditions, respectively.[30] The similar polycistronic transcription for *puf* was also reported in another purple bacterium *Rbv. gelatinosus.*[31] A previous genetic study suggested the same gene arrangement in the *Rsp. centenum puf* upstream region (*crtEF-bchCXYZ-puf*)[32] (Fig. 5). Sequence analysis indicated that the *bchZ* gene locates in the upstream of the *Rsp. centenum puf* operon, supporting the data (data not shown). The superoperonal structure (*crtEF-bchCXYZ-puf*) has been observed in all purple bacteria studied to date including *Rba. spheroides, Rps. palustris* and *Bradyrhizobium* ORS278,[15,18,31,33] suggesting the importance of the gene arrangement for their regulated expressions. Interestingly, expression of *Rsp. centenum crtE* gene is at much higher levels than that of the *puf* specific promoter (15-fold higher than that of *puf* operon) (Fig. 3). In *Rba. capsulatus, crtE* expression is significantly lower (30-fold) than that of the *puf* specific promoter.[24] These results suggest that most of *puf* transcription under aerobic conditions in *Rsp. centenum* is derived from the readthrough as observed in *Rba. capsulatus* (Fig. 5), although detailed mRNA analyses have not been performed. Given that the *crtE* expression is weakly repressed by oxygen in *Rsp. centenum* (about 3-fold) (Fig. 3), *puf* mRNA could be maintained at high levels even under aerobic conditions in this bacterium.

However, there are some conflicting observations. Specifically, the levels of light-harvesting complexes in AERR1 were about 20% of those in wild type under aerobic conditions (Fig. 2B); however, promoter activity of *crtE* in AERR1 was about 75% of that in wild type under the identical conditions (Fig. 3). These results suggest that there are still unknown mechanisms for controlling *puf* mRNA levels by AerR in *Rsp. centenum.* One possibility is that *bchC* promoter activity in *Rsp. centenum* is significant to influence *puf* transcription, as observed in *Rba. capsulatus*,[30] and that the activity is also regulated by AerR.

Conclusion

In this study, we show that aerobic photosystem synthesis in *Rsp. centenum* is regulated by CrtJ and AerR regulatory factors. AerR is necessary for the aerobic induction of photosynthesis gene expression in *Rsp. centenum.* CrtJ, on the other hand, is an aerobic repressor for photosynthesis genes, as in *Rhodobacter* species. The typical CrtJ-binding motifs of *Rhodobacter* species are observed at *Rsp. centenum crtE* and *crtD* promoter regions (Fig. 4), indicating that they may recognise the same motif. However, one of the two conserved cysteine residues in the *Rhodobacter* CrtJ which make an intramolecular disulfide bond is not conserved in *Rsp. centenum* (Fig. 1), suggesting that CrtJ sensitivity to oxygen is somehow different between these two species.

The *puf* specific promoter activity in *Rsp. centenum* is much lower than that of the *crtE* promoter, which makes a superoperonal structure with the *puf* operon. As a result, the readthrough transcript may be high enough to influence *puf* mRNA levels, especially under aerobic conditions. The importance of readthrough for aerobic induction of photosynthesis genes in another purple bacterium, *Rdv. sulfidophilum* was also suggested previously.[6] Perhaps, CrtJ and AerR are the main regulators for the aerobic synthesis of the photosynthetic apparatus through regulating superoperonal transcription in purple bacteria that aerobically synthesize photopigments. Finally, AerR works as an activator in *Rsp. centenum*, although it was reported to be a repressor in *Rba. capsulatus*.[9] Further studies of *Rsp. centenum* AerR should be useful in clarifying the exact mechanism of how AerR regulates photosystem synthesis with respect to the phenotypic difference observed in this bacterium.

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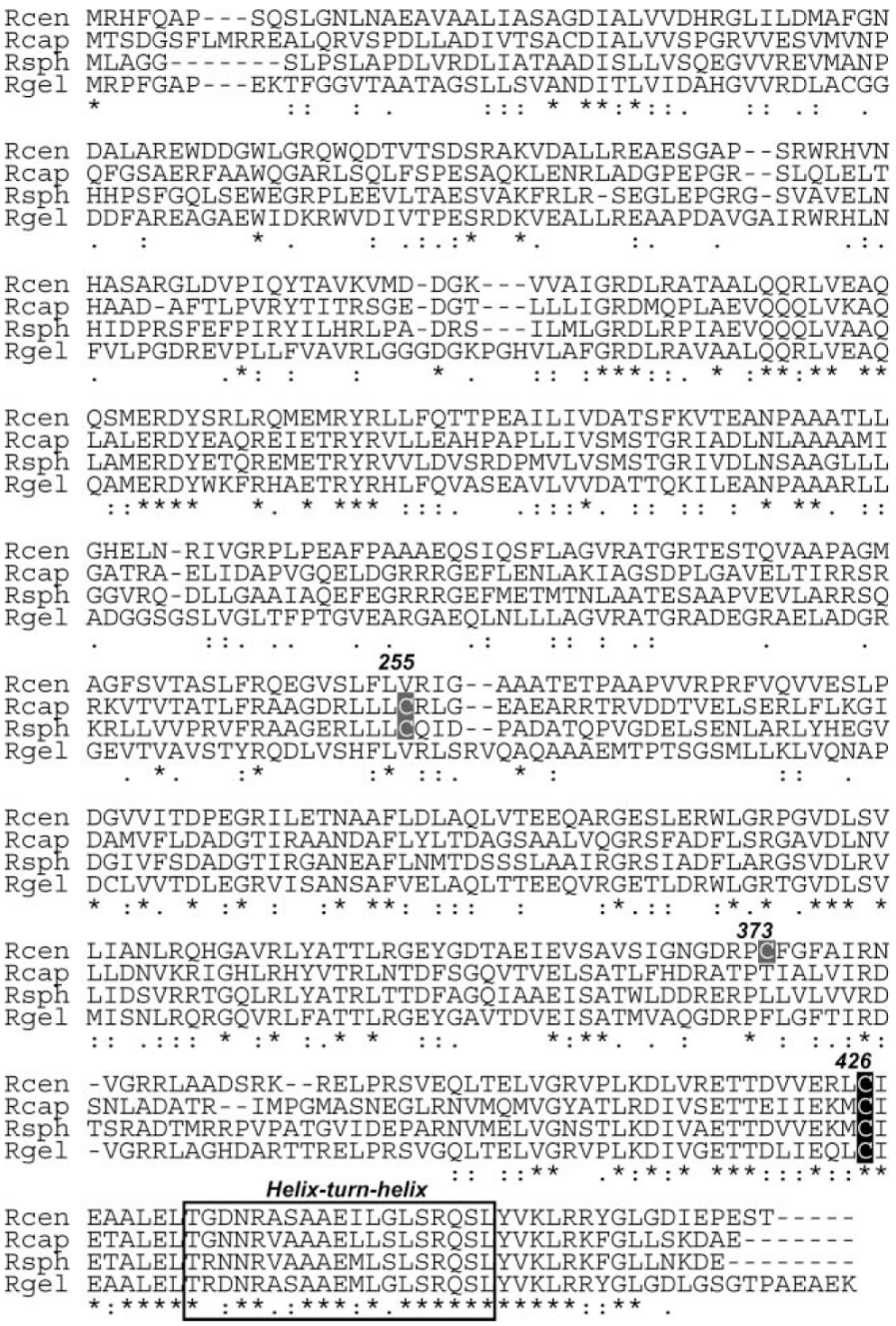

Fig. 1.

Sequence alignment of the predicted CrtJ proteins of *Rsp. centenum* to homologous proteins in other purple bacteria. Abbreviations and their meaning: Rcen; *Rsp. centenum*, Rcap; *Rba. capsulatus*, Rsph; *Rba. sphaeroides*, Rgel; *Rbv. gelatinosus.* Accession numbers of the sequences are AB369262, P26167, ABA79455 and BAA94062, respectively.

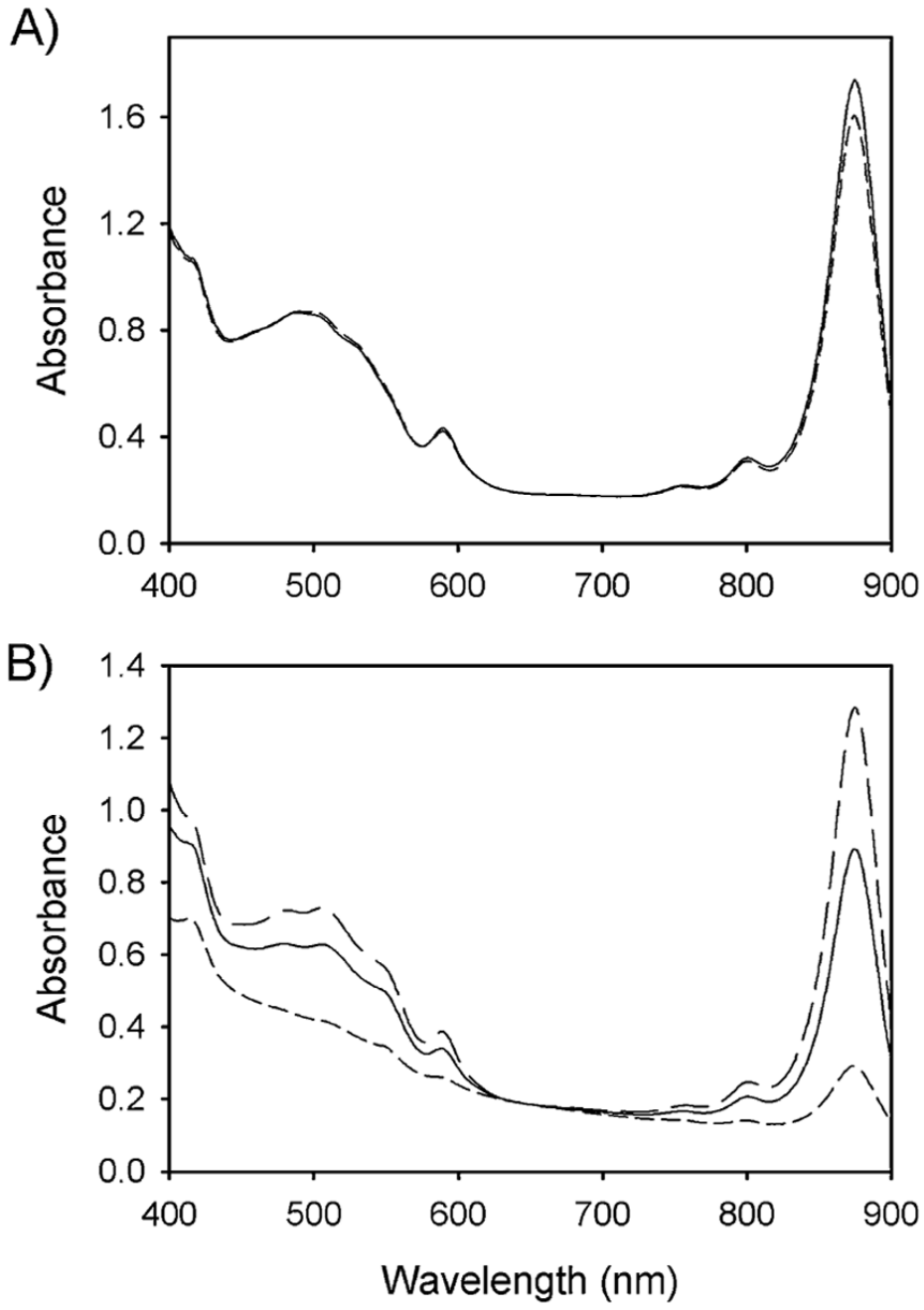

Fig. 2.

Absorption spectra of *Rsp. centenum* wild type (solid lines), AERR1 (short dashed lines) and CRTJ1 (long dashed line) grown anaerobically (panel A) or aerobically (panel B). AERR1 is the *aerR*-disrupted strain, and CRTJ1 is the *crtJ*-disrupted strain. The spectrum for CRTJ1 cells grown anaerobically is not shown, but identical to that of the wild type cells (panel A, solid line).

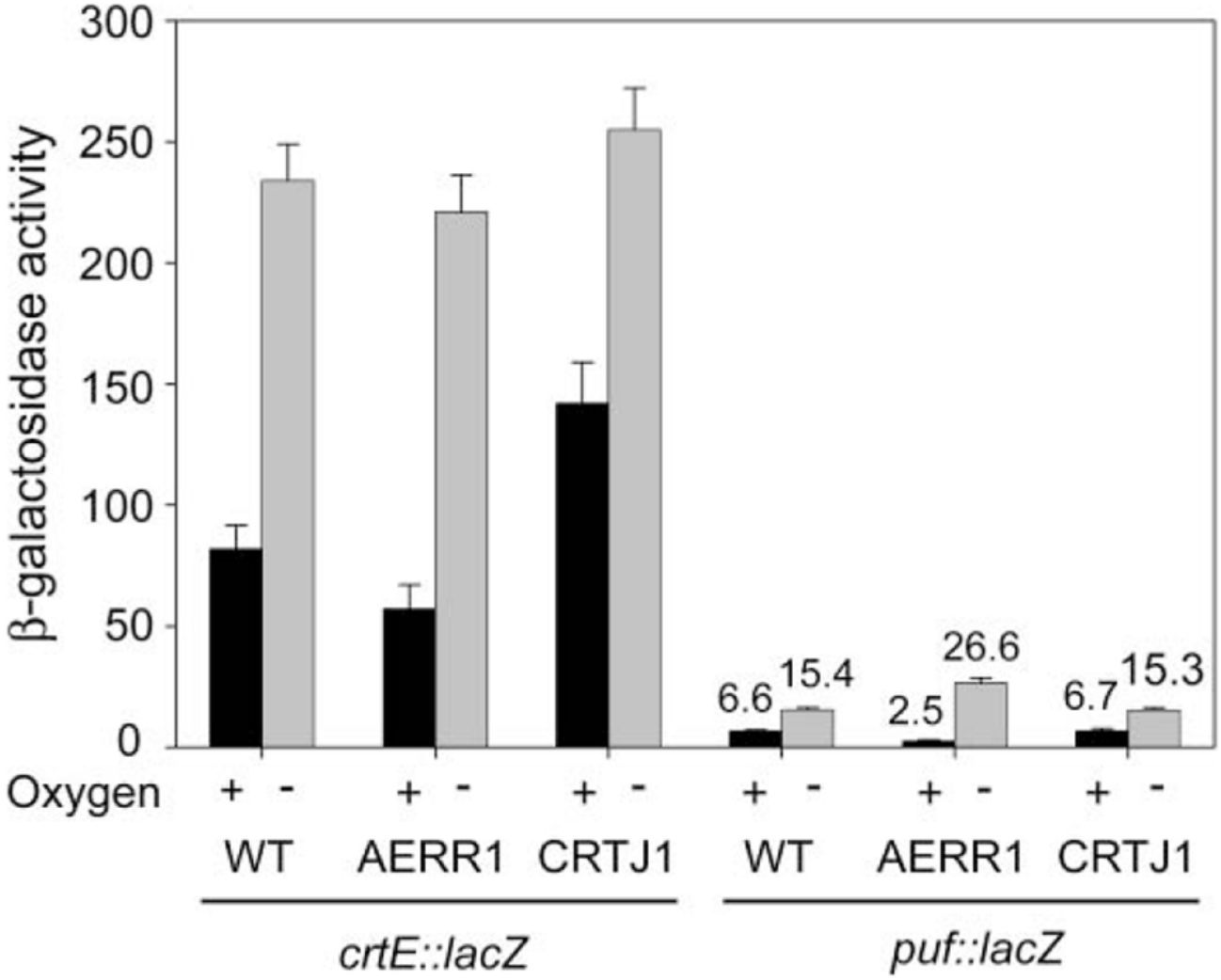

Fig. 3.

β-galactosidase activity measurements of the *crtE::lacZ* and *puf::lacZ* fusions in wild type, *ΔaerR* (AERR1) and *ΔcrtJ* (CRTJ1) strains. Cultures were grown either aerobically (black bars) or anaerobically (gray bars) in CENS medium and then assayed for β-galactosidase activity. Units of β-galactosidase activity represent μmol of ONPG (*o*-nitrophenyl-β-D-galactopyranoside) hydrolyzed/min/OD_{660}. The data represent the mean of at least three independent experiments (error bars indicate ±S.D.).

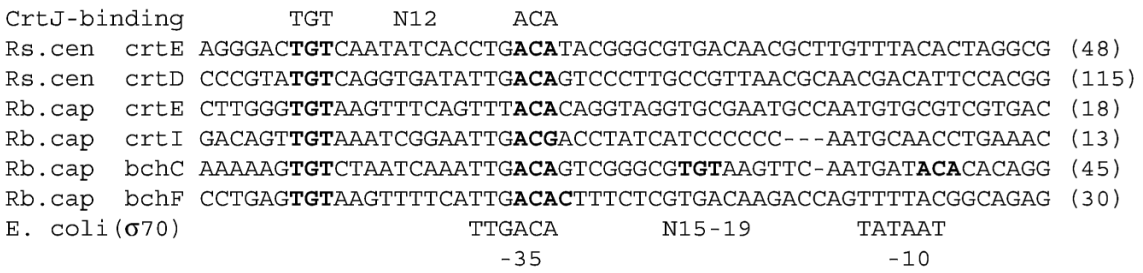

Fig. 4.

Comparison of possible promoter sequences found in *Rsp. centenum crtE* and *crtD* genes and those reported in *Rba. capsulatus* photosynthesis genes. The number in parentheses indicates the number of nucleotides between the start codon, ATG, and the right end of the sequence. The proposed CrtJ-binding sites (TGT-N12-ACA) are shown in bold. Nucleotide sequences of the consensus *E. coli* σ70 recognition site are shown in the bottom.

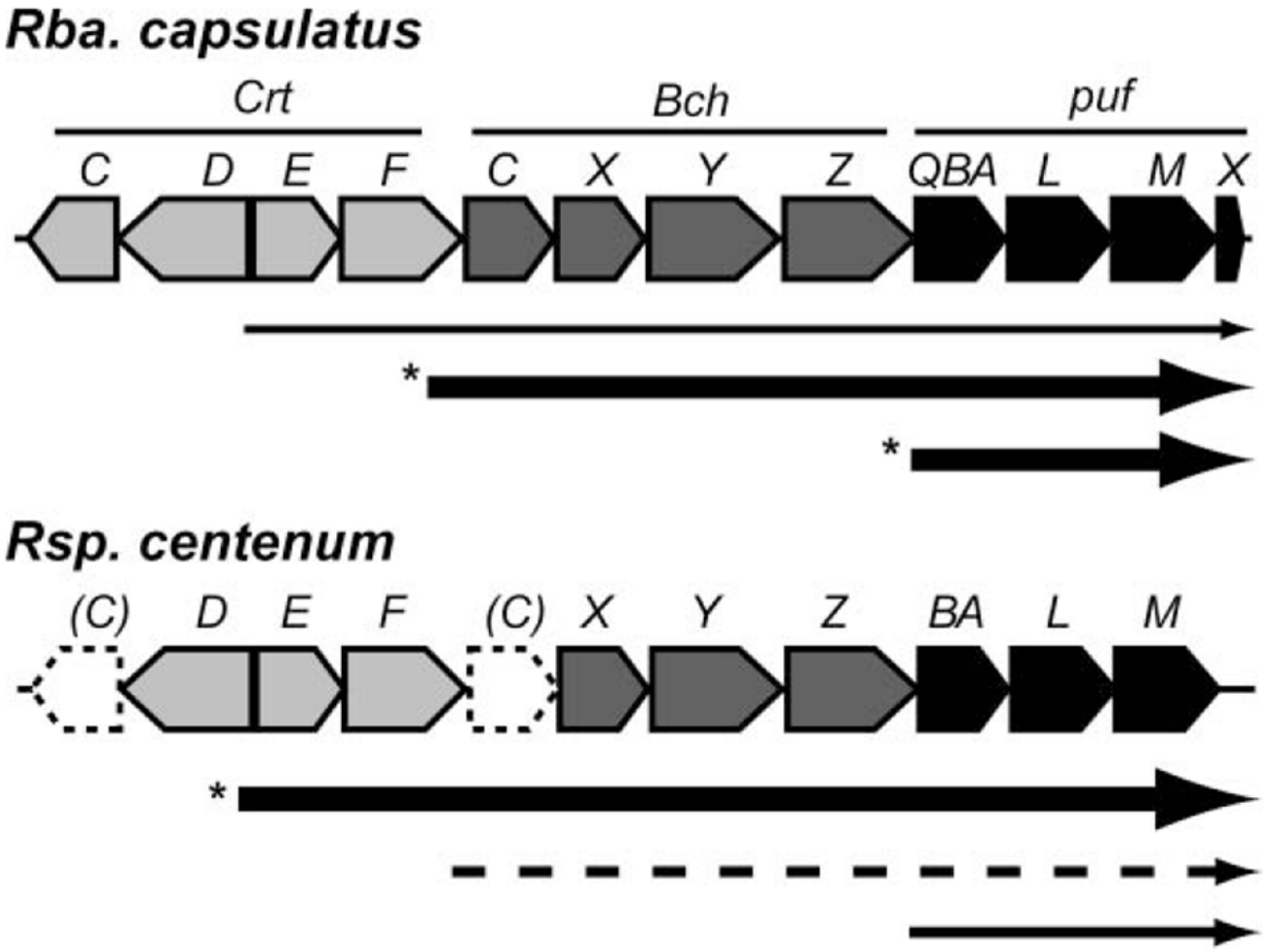

Fig. 5.

Comparison of readthrough transcription for *puf* operon between *Rba. capsulatus* and *Rsp. centenum*. Thickness of the arrows indicates the levels of transcription. The dotted lines donate possible gene locations and transcripts that have not been experimentally proven. Asterisks denote the major transcripts for *puf* operon.