J. Gen. Appl. Microbiol., **45**, 129–134 (1999)

Short Communication

Photosynthetic regulatory gene cluster in an aerobic photosynthetic bacterium, *Roseobacter denitrificans*[1]

Kohji Nishimura,[†] Hiroshi Shimada, Takashi Shinmen, Takeshi Obayashi, Tatsuru Masuda, Hiroyuki Ohta, and Ken-ichiro Takamiya*

Department of Biological Sciences, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama 226–8501, Japan

(Received February 4, 1999; Accepted July 2, 1999)

Key Words——aerobic photosynthetic bacteria; bacteriochlorophyll; photosynthetic genes; photosynthetic regulatory genes; *Roseobacter denitrificans*; signal transduction; two-component regulatory system

Aerobic photosynthetic bacteria such as *Roseobacter denitrificans* grow chemoheterotrophically under aerobic dark conditions; however, unlike purple nonsulfur bacteria such as *Rhodobacter sphaeroides* and *Rhodobacter capsulatus*, aerobic photosynthetic bacteria do not grow phototrophically under anaerobic conditions (Shiba and Simidu, 1982). Nevertheless, *Ros. denitrificans* forms almost the same photosynthetic apparatus as those of purple nonsulfur bacteria even under highly aerobic conditions in darkness (Harashima et al., 1980; Iba et al., 1988). In fact, we demonstrated that in *Ros. denitrificans*, the levels of photopigments (bacteriochlorophyll and carotenoids) and mRNA levels of the *puf* operon encoding the pigment-binding polypeptides, both of the reaction center and light-harvesting complexes I, were very high even at nearly oxygen saturation of the growth culture (Nishimura et al., 1996). In purple nonsulfur bacteria, the photopigment formation and the expression of the *puf* operon is repressed under high oxygen tension (for reviews, Bauer, 1995; Drews and Golecki, 1995). These results suggest that the mechanism for regulation by oxygen of the expression of the *puf* operon and of photopigment formation differs between aerobic photosynthetic bacteria and purple nonsulfur bacteria.

It is now widely accepted that in *Rba. capsulatus* and *Rba. sphaeroides*, a "two-component regulatory system" is involved in the signal transduction for the regulation of gene expression (e.g., *puf* operon) by oxygen tension (Eraso and Kaplan, 1994, 1995; Mosley et al., 1994; Sganga and Bauer, 1992). The regulatory system mainly consists of a sensor kinase (RegB or PrrB) and a response regulator (RegA or PrrA), and they are highly homologous between the two bacteria, probably reflecting a very similar response of both bacteria to oxygen tension. Downstream of the two-component regulatory gene cluster are several genes (*spb*, *hvrA*, *hvrB*, *orf5*, *orf318*, and *ahcY*), presumably involved in the signal transduction of oxygen or light (Buggy et al., 1994a, b; Mizoguchi et al., 1997; Sganga et al., 1992; Shimada et al., 1996). These additional genes are not always homologous in both bacteria, probably reflecting minor differences in the response to external environmental changes. Since the effect of oxygen tension on the formation of the photosynthetic apparatus is quite different between aerobic photosynthetic bacteria and purple nonsulfur bacteria as described above, it is anticipated that component(s) or their properties in the signal transduction of *Ros. denitrificans* differs from those of the purple nonsulfur bacteria. However, little is known about the regulatory components and their properties in *Ros. denitrificans*.

[1] The nucleotide sequence reported in this paper has been deposited in the DDBJ data base under the accession number AB020211.

* Address reprint requests to: Dr. Ken-ichiro Takamiya, Department of Biological Sciences, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 226–8501, Japan.

E-mail: ktakamiy@bio.titech.ac.jp

[†] Present address: Department of Life Science and Biotechnology, Faculty of Life and Environmental Science, Shimane University, Nishikawatsu-cho, Matsue 690–8504, Japan.

On the occasion of his retirement, this paper is dedicated to Professor Keiji Harashima who accomplished an epoch-making contribution to the research field of aerobic photosynthetic bacteria.

Table 1. Identities of regulatory genes among photosynthetic bacteria.

Strain	Identity of amino acid sequence (%)	
	Ros. denitrificans	*Rba. sphaeroides*
PrrA (RegA)		
Rba. sphaeroides	83	—
Rba. capsulatus	79	83
PrrB (RegB)		
Rba. sphaeroides	62	—
Rba. capsulatus	57	60
PrrC (SenC)		
Rba. sphaeroides	54	—
Rba. capsulatus	48	49
Orf5		
Rba. sphaeroides	73	—
Rba. capsulatus	71	74
Orf329		
Rba. sphaeroides	55	—
AhcY		
Rba. sphaeroides	84	—
Rba. capsulatus	84	92

In this study, we identify the photosynthetic regulatory gene cluster in *Ros. denitrificans* and reveal that the organization of genes in the cluster is similar to that of *Rba. sphaeroides* and *Rba. capsulatus*. Furthermore, we investigate the expression of these genes under various growth conditions and discuss the possible mechanism for regulating the pigmentation and the expression of *puf* operon in *Ros. denitrificans*.

An aerobic photosynthetic bacterium *Ros. denitrificans* (ATCC 33942) was cultured as described previously (Nishimura et al., 1996, 1998). A genomic library was constructed with *Bam*HI-digested genomic DNAs and screened for *prrA* of *Ros. denitrificans* by using a *Rba. sphaeroides prrA*-specific probe. The preparation of RNA and Northern hybridization analysis was performed by using specific probes for each gene and 16S rRNA as described by Nishimura et al. (1996, 1998).

Ros. denitrificans had homologs of *prrA*, *prrB*, and *prrC* as reported by Matsumoto et al. (1997). As shown in Table 1, the identity of PrrAs (RegA) among these three bacteria was about 80%; for PrrBs (RegB), about 60%; and for PrrCs (SenC), about 50%, indicating that these three genes of *Ros. denitrificans* do not especially differ from the corresponding genes of *Rba. sphaeroides* and *Rba. capsulatus*. As with PrrA (or RegA) of *Rba. sphaeroides* (Eraso and Kaplan, 1994) and *Rba. capsulatus* (Du et al., 1998), a putative DNA binding domain, helix-turn-helix, was in the C-terminal region of PrrA of *Ros. denitrificans* (data not shown, Matsumoto et al., 1997), suggesting that PrrA of *Ros. denitrificans* was also a *trans*-factor. As in purple nonsulfur bacteria, no distinct oxygen-binding domain or redox-sensing domain was found in PrrB of *Ros. denitrificans*, suggesting that PrrB of this bacterium did not also directly sense molecular oxygen or the redox state of the environment.

In purple nonsulfur bacteria, other additional regulator genes are in the flanking region downstream of *prrA*, such as *spb* and *orf318* in *Rba. sphaeroides*, *hvrA* and *hvrB* in *Rba. capsulatus*, and *orf5* and *ahcY* in both bacteria (Buggy et al., 1994a, b; Mizoguchi et al., 1997; Shimada et al., 1996). We therefore further analyzed the flanking region downstream of *prrA* of *Ros. denitrificans* and determined nucleotide and amino acid sequences of *orf5*, *orf318*, and *ahcY* homologs of *Rba. sphaeroides* (Mizoguchi et al., 1997) (Fig. 1).

Orf5 of 195 amino acid residues exhibited 73 and 71% amino acid identity to those in *Rba. sphaeroides* and *Rba. capsulatus*, respectively (Fig. 1; Table 1). No potential Shine-Dalgarno sequence was found upstream of *orf5* in *Ros. denitrificans*, as in *Rba. sphaeroides* (Mizoguchi et al., 1997). Orf329 of 329 amino acid residues exhibited 55% amino acid identity to Orf318 in *Rba. sphaeroides* (Fig. 1; Table 1). Orf329 also exhibited 27 and 22% amino acid identities to RfaI and RfaJ of *E. coli*, respectively, which are involved in the biosynthesis of lipopolysaccharide (Pradel et al., 1992), as Orf318 of *Rba. sphaeroides* (Mizoguchi et al., 1997). Although the structure of lipopolysaccharide moiety of *Ros. denitrificans* has not been reported, Orf329 is possibly responsible for the biosynthesis of lipopolysaccharide or involved in the transcriptional regulation of photosynthesis genes, since the *orf318* is found in the regulatory gene cluster of *Rba. sphaeroides*. The potential Shine-Dalgarno sequence upstream of *orf329* is underlined in Fig. 1.

AhcY of 462 amino acid residues exhibited 84% amino acid identity to AchYs in *Rba. sphaeroides* and *Rba. capsulatus* (Fig. 1; Table 1). *ahcY*, which encodes S-adenosyl-L-homocysteine hydrolase, is thought to be involved in the regulation of bacteriochlorophyll biosynthesis (Sganga et al., 1992). The putative NAD^{+}-binding domain (Fig. 1, boxed amino acids) was highly conserved among these bacteria (data not shown). The potential Shine-Dalgarno sequence upstream of *ahcY* is underlined in Fig. 1. As shown in Table 1, the identities of Orf5 and AhcY among the three bacteria are 71–74% and 84–92%, respectively, indicating that these genes of *Ros. denitrificans* do not especially differ from the corresponding genes of the other two bacteria.

The organization of the regulatory gene cluster of *Ros. denitrificans*, *prrB-prrC-prrA-orf5-orf329-ahcY* was somewhat simpler than that of the purple nonsulfur bacteria because *spb*, *hvrA*, and *hvrB* are lacking

```
     aagcttgctg atcttgcaga cgaaaggtcg gctttgaaaa cgctgacctg aggcgcggtgc gtggctactg cgtgccggct tggcccgtg      90
195      * P   M A A   L L E   N H R A   T F A   D R T E   A P P   R L T   I E L   169
     aggaTCACGG CATGGCAGCC AGCAATTCGT TGTGACGCGC GGTAAAGGCG TCGCGCGTCTC AGCCGGGGGC CGCAGGGTAA TTTCCAGCC      180
168  G Q M I   K H D   P T G   F F K S   A E T   E S F G   A I Q   T A E   M W A   139
     CTTGCATGAT CTTGTGATCC GGTGTGCCAA AGAACTTGCT GGCCTCGGTT TCTGAAAAACC GGCGATCTGC GTGGCTTCCA TCCATGCGC      270
138  S I K D   A K K   I Q R   K V A V   P I A   A P L G   F R L   H I A   A T L   109
     TGATTTTATC GGCCTTCTTG ATCTGACGCT TCACGGCGAC AGGAATGGCA GCAGGTAGACC AAACCGCAGG TGGATGGCCG CCGTCAGTC      360
108  R E D L   A E Y   G P G   V A A K   V P S   I M D G   I V Y   E P A   D H L    79
     GCTCATCCAG CGCCTCATAC CCCGGCCCGA CAGCGGCCTT TACCGGGGAG ATCATATCGCC GATCACGTAT TCCGGGGCGT CATGCAAAA      450
78   L A A L   R W K   A P S   K D A I   R T F   L T E V   L L S   H E A   V S Y    49
     GGGCTGCCAA TCGCCATTTC GCGGGTGATT TATCAGCGAT GCGGGTAAAA AGCGTCTCTAC CAAAAGCGAA TGTTCCGCAA CTGAATAGG      540
48   A H A G   S T Q   G N W   R A V F   A L G   R A I D   E I E   V D V   P T P    19
     CATGCGCGCC ACTGGTCTGG CCATTCCAGC GCGCCACAAA GGCAAGACCG CGCGCGATATC TTCAATTTCT ACGTCAACCG GAGTCGGAT      630
18   D L L D   L R R   G S L   M R Q W   A R K   M                                       1    orf5
329                                                 * R H V   T L E   N P F R   A L Y 317
     CCAGCAAATC CAGCCTGCGC CCCGATAGCA TTCGCTGCCA GGCCCGCTTC ATCGGTGGACC GTCAATTCAT TAGGAAATCT CGCGAGATA      720
316   H A I   K R A   T I L H   K I L   M K T   M K M S   D P A   L R R G   G T E 287
     GTGCGCTATT TTCCGGGCTG TTATCAGATG TTTGATCAGC ATTTTCGTCA TTTTCATACTG TCCGGCGCGA GTCTTCTACC CCCCGTTTC      810
286   V Q R   S G S   F H T R   I F R   D F S   D A F R   P E F   Q G S E   D K W 257
     CACTTGTCTG GAACCTGAAA AGTGTGTCCT GATGAAACGA TCAAATGAGT CAGCAAAGCGC GGTTCGAACT GTCCGCTCTC GTCTTTCCA      900
256   P K A   P G I   F H I I   N P Y   A F V   A F L R   T S W   S Y Q W   N W V 227
     TGGTTTCGCG GGCCCGATGA AATGTATGAT GTTCGGATAT GCGAAAACTG CAAAAAGCCGC GTGGACCACG AATATTGCCA GTTCCAGAC      990
226   P S I   E A W   D N Q L   V A N   Y L N   Q D H R   K L D   R R R A   R G F 197
     GGGGCTTATT TCGGCCCAGT CGTTTTGCAG CACCGCATTG TAAAGGTTCT GATCATGCCGT TTCAAATCCC GACGGCGTGC GCGGCCAAA     1080
196   E V C   R R M   L E Q E   T Y A   Q V D   M L M V   G A N   F Y A S   P P I 167
     TTCGACGCAA CGTCGCATCA GCTCCTGTTC GGTATAGGCC TGCACATCCA TTAGCATTACT CCTGCGTTGA AGTATGCAGA TGGGGGTAT     1170
166   G K I   T N R   K N Q R   K P T   R W Q   V N D R   V S A   I C H P   A V D 137
     CCCTTTGATC GTATTCCGCT TGTTTTGCCG TTTTGGTGTG CGCCATTGCA CGTTGTCGCGA ACCGATGCGA TGCAATGCGG TGCTACATC     1260
136   I D F   L A N   F D G G   Q V F   I D S   D L Y L   I K D   Y E G A   F A T 107
     AATATCAAAA AGCGCGTTGA AATCGCCACC TTGAACGAAA ATATCCGAAT CCAGATAGAGA ATTTTGTCAT ATTCACCGGC AAAAGCGGT     1350
106   P L A   I R L   Y V D H   T K G   K D L   R L G E   F V D   G V D V   H C L  77
     CGGCAAGGCG ATGCGCAAAT ATACGTCATG CGTTTTGCCC TTATCCAAGC GCAGGCCTTCA AAGACGTCGC CGACATCTAC GTGACAAAG     1440
76    R I G   L G A   L S D P   L V V   A Q H   G Y C I   C I D   F E P K   E V L  47
     CCGTATGCCC AATCCGGCAA GGCTGTCCGG AAGCACAACT GCCTGATGAC CGTAACAGATA CAGATGTCGA ATTCCGGCTT TTCCACAAG     1530
46    S A I   Q A A   A H A A   F V L   Y N Q   D C C F   V I A   R K H R   A P R  17
     CGATGCAATC TGCGCCGCGG CATGTGCCGC AAAAACAAGG TAATTCTGGT CGCAACAAAAG ACGATTGCCC TTTTGTGGCG TGCCGGGCG     1620
16    S A T   L R V   H F S E   A P A   T S M                                          1  orf329
     CGATGCCGTC AACCTGACGT GAAATGACTC TGCGGGTGCT GTGGACATag ccgggcgtttg ctcgttgcgt tctgtaaatg tgtcgctgg     1710
     cggggctgta ctcaagacac gcccacttct agagaactga aagatattgt ccagcactcaa cttttggag tcacacatgc agggcaaac      1800
     ccaatacgat tacgcttgta aagaggatgt ggacaattgc tggcattcgt tctgttgcagc gccctgcaac gggtggtaga gcagcggca      1890
     aagatcattc agagcctcgg agtgcacgcG TGACCAAAGA CTTCATCGTA AAAGATATTGC CCTTGCCGAG TTTGGCCGCA AAGAGCTTG     1980
1                               M   T K D   F I V   K D I A   L A E   F G R K   E L D  21  ahcY
     ATATTGCCGA AACCGAAATG CCGGGGCTGA TGGCCTTGCG CGCGGAATAT GGCGACAGCAA GCCGCTGGCC GGTGCGCGCA TTGTCGGCT     2070
22    I A E   T E M   P G L M   A L R   A E Y   G D S K   P L A   G A R I   V G S  51
     CGCTGCACAT GACGATCCAG ACGGCAGTTC TGATCGAAAC GCTTGTTGCC TTGGGTGCCGA TGTCCGCTGG GCGTCCTGCA ATATCTTTT     2160
52    L H M   T I Q   T A V L   I E T   L V A   L G A D   V R W   A S C N   I F S  81
     CGACGCAGGA CCACGCGGCG GCGGCCATCG CGGCGGGCGG GACGCCTGTT TTCGCGATCAA GGGTCAGTCG CTGGAAGAGC ATTGGGATT     2250
82    T Q D   H A A   A A I A   A G G   T P V   F A I K   G Q S   L E E H   W D Y 111
     ACCTCGACCG GTCGTTCATG TTTGAGGATG GTCCCAACCT TATCCTTGAC GATGGCGGTGA TGCGACCCTC TATGTTCTGC TTGGTGCCC     2340
112   L D R   S F M   F E D G   P N L   I L D   D G G D   A T L   Y V L L   G A R 141
     GTGCCGAGGC GGGTGAAGAG ATTATCCCCG TGCCCACATC CGAAGAGGAA GAGGCCATCAA GGCGCAGATC AAGAAGCGCA TGGCGGCGT     2430
142   A E A   G E E   I I P V   P T S   E E E   E A I K   A Q I   K K R M   A A S 171
     CTCCCGGTTG GTTCACGAAA GTGCGTGATC AGATCAAAGG TGTCAGCGAA GAGACGACAAC TGGCGTTCAC CGTCTCTATG ACCTCGTCA     2520
172   P G W   F T K   V R D Q   I K G   V S E   E T T T   G V H   R L Y D   L V K 201
     AGCAGGGGCA ATTGCCATTC CCTGCGATCA ATGTGAATGA TTCTGTCACC AAGTCGAAGTT TGACAACAAA TACGGCTGCA AGGAATCGC     2610
202   Q G Q   L P F   P A I N   V N D   S V T   K S K F   D N K   Y G C K   E S L 231
     TGGTGGATGG TATCCGCCGC GCCACGGATA CGATGATGGC GGGCAAGGTT GCCGTGGTAAT GGGCTACGGC GATGTGGGCA AAGGCTCCG     2700
232   V D G   I R R   A T D T   M M A   G K V   A V V M   G Y G   D V G K   G S A 261
     CTGCCAGTCT GCGCGGTGCC GGTGCCCGCG TAAAAGTCAC CGAAGTGGAC CCGATTTGCGC GCTGCAAGCC GCCATGGACG GTTTCGAGG     2790
262   A S L   R G A   G A R V   K V T   E V D   P I C A   L Q A   A M D G   F E V 291
     TTGTCCTGCT CGAAGATGTG GTGGGATCGG CGGATATCTT TATCACGACG ACGGGCAACAA GGACGTGATC CGCATCGAGC ATATGCGTG     2880
292   V L L   E D V   V G S A   D I F   I T T   T G N K   D V I   R I E H   M R A 321
     CGATGAAAGA CATGGCGATC GTTGGCAACA TTGGTCACTT TGACAATGAA ATTCAGGTTGC TGCCCTCAAG AACCACAAGT GGACCAATA     2970
322   M K D   M A I   V G N I   G H F   D N E   I Q V A   A L K   N H K W   T N I 351
     TCAAAGAACA GGTAGACATG ATCGAGATGC CGAATGGCAA TCGTCTCATT CTGCTTTCTGA GGGGCGCTTG CTGAACCTCG GGAACGCGA     3060
352   K E Q   V D M   I E M P   N G N   R L I   L L S E   G R L   L N L G   N A T 381
     CCGGGCACCC GTCCTTCGTG ATGTCCGCGT CCTTTACCAA TCAGGTGCTG GCGCAGATCGA ACTGTGGACC CGTGCGGATG CGTATGACA     3150
382   G H P   S F V   M S A S   F T N   Q V L   A Q I E   L W T   R A D A   Y D N 411
     ACGAGGTCTA CATTCTGCCC AAACATCTGG ATGAAAAGGT CGCACGTTTG CACCTTGACCG GATCGGTGTG AAACTGACCC CGCTTGACC     3240
412   E V Y   I L P   K H L D   E K V   A R L   H L D R   I G V   K L T P   L D P 441
     CGGAGCAAGC GGCATATATC GGCGTGAAAC CCGAAGGTCC CTTCAAGCCG GAACACTACCG GTACTGAtta aaaaccggta tgcgaccag     3330
442   E Q A   A Y I   G V K P   E G P   F K P   E H Y R   Y *                          462
```

Fig. 1. Nucleotide and amino acid sequences of *orf5*, *orf329*, and *ahcY*.

Putative coding sequences are indicated in capital letters. Arrows indicate the direction of transcription of these genes. A putative NAD^+ binding site in *ahcY* is boxed. Nucleotide sequences underlined indicate potential Shine-Dalgarno sequences of each open reading frame.

in *Ros. denitrificans* (Fig. 2A). Moreover, in the region upstream of *ahcY* were σ^{70}-type promoter sequence and photopigment-like palindrome sequence (TGTGT-N_{6-10}-ACACA) (Fig. 2B), instead of 26 bp-dyad sequence in the region upstream of *ahcY* in *Rba. capsulatus* (Buggy et al., 1994a).

As shown in Fig. 3, *prrA*, *prrB*, and *prrC* were constitutively transcribed under semiaerobic darkness and light, and aerobic light conditions as in *Rba. sphaeroides* (Mizoguchi et al., 1997), although the mRNA levels of *prrB* were very low. By using the *prrA*- and *prrC*-specific probes, a similar size of transcripts (1.1 and 1.2 kb) was detected. In the *prrCA*-specific probe, the 1.2 kb transcript was also detected (data not shown). Since the predicted transcripts of *prrA* and *prrC* are 0.6 kb and 0.5 kb, respectively, *prrA* and

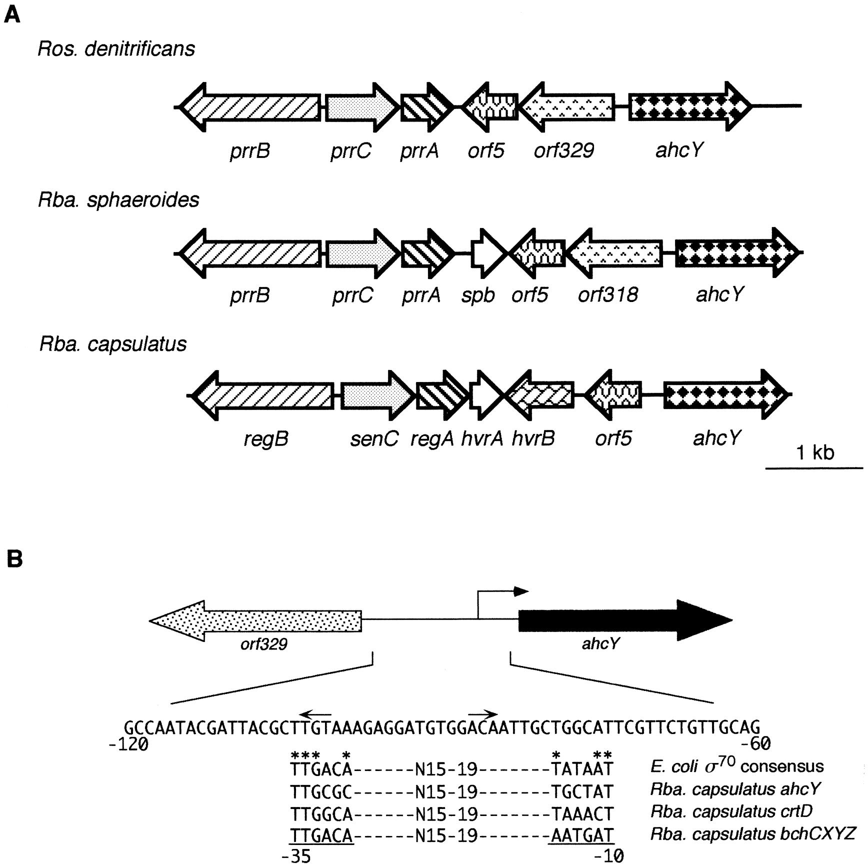

Fig. 2. Comparison of the photosynthetic regulatory gene clusters of photosynthetic bacteria (A) and nucleotide sequence for the *ahcY* promoter region (B).

(A) Arrows indicate the location of the genes and the direction of the transcription. (B) Asterisks indicate sequences identical to *E. coli* σ^{70} consensus promoter elements. Opposite arrows indicate a putative photopigment-like palindrome sequence (TGTGT-N_{6-10}-ACACA). −120 and −60 indicate the positions from the ATG initiation codon, and −35 and −10 indicate the positions from a putative transcriptional initiation site.

prrC may be transcribed polycistronically.

orf5, *orf329*, and *ahcY* were also constitutively transcribed under all the conditions tested as in *Rba. sphaeroides* (Mizoguchi et al., 1997), although the mRNA level of *orf5* was very low (Fig. 3). Two species of *orf329* transcripts were detected, and the longer one (1.1 kb) was close to the predicted 1.0 kb of *orf329*. Two species of transcript of *ahcY* also were detected, and the longer one (1.5 kb) was close to the predicted 1.4 kb of *ahcY*. The shorter transcripts may be caused by exo- or endonuclease digestion of the 1.5-kb transcript.

In *Rba. capsulatus*, the light-responding expression of both *orf5* and *ahcY* is thought to be regulated by the binding of HvrB to a 26-bp dyad symmetry region between *orf5* and *ahcY* (Buggy et al., 1994a). By contrast, in the region upstream of *ahcY* in *Ros. denitrificans*, as described above, were σ^{70}-type promoter sequence and a photopigment-like palindrome sequence instead of the 26-bp dyad sequence in *Rba. capsulatus* (Buggy et al., 1994a) (Fig. 2B). Since no *hvrB* gene existed in the flanking region of the *orf5* gene of *Ros. denitrificans*, it is possible that another transcriptional factor binds to this promoter region in *Ros. denitrificans*.

The presence of PrrA and PrrB and their constitutive expression (Figs. 2 and 3), the oxygen-insensitive transcription of the *puf* operon (Nishimura et al., 1996), the absence of a distinct oxygen-sensor domain in PrrBs (Matsumoto et al., 1997; this study), the putative DNA-binding activity of PrrA as an anaerobic activator for the *puf* and *puc* operons (Du et al., 1998), and the difference of the promoter region of the *puf* operon (Liebetantz et al., 1991; Zhu et al., 1986) all are clues to characterize and to elucidate the mechanism for the oxygen-insensitive expression of the *puf* operon in *Ros. denitrificans*. The cognate PrrBs/PrrAs/PrrCs is common among these bacteria, whereas the primary oxygen-sensor (or redox-sensor) of *Ros. denitrificans*, if any, may have a quite different affinity to molecular oxygen or the redox state of the environment from those of the latter two bacteria. Alterna-

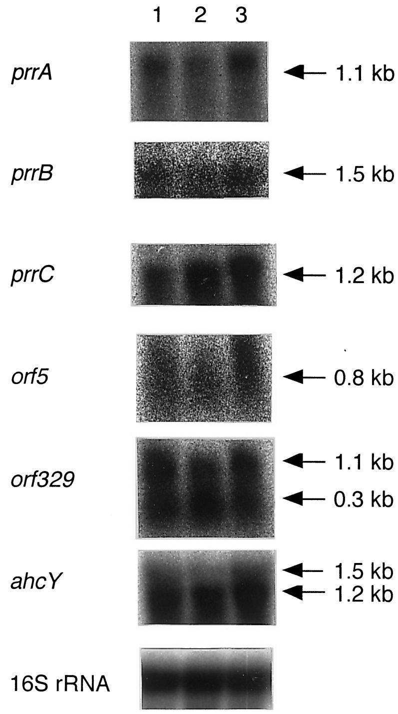

Fig. 3. Northern hybridization of *prrA*, *prrB*, *prrC*, *orf5*, *orf329*, and *ahcY* of *Ros. denitrificans* under various growth conditions.

Lane 1, aerobic (oxygen tension, 78% saturation) dark condition; Lane 2, semiaerobic (oxygen tension, 2% saturation) dark; Lane 3, semiaerobic (2.5×10^6 erg/cm$^2\cdot$s) light. Twenty micrograms of total RNA for mRNAs of *prrA*, *prrC*, and *ahcY*, 30 μg for *prrB*, *orf5*, and *orf329*, and 1 μg for 16S rRNA were loaded in each lane. Arrows on the right side indicate the size of transcripts. Analyses of the hybridization were performed in triplicated independent experiments.

tively, direct evidence for or against involvement of PrrB/PrrA in the oxygen insensitivity may be found by the analysis of PrrA- or PrrB-mutant of *Ros. denitrificans*. For example, it is possible that PrrA of *Ros. denitrificans* is always bound to a promoter region of the *puf* operon, resulting in the constitutive expression of the *puf* operon at any oxygen tension, as in a PrrA-mutant of *Rba. capsulatus* (Du et al., 1998).

This work was supported financially by a Grant for Scientific Research (no. 09440265 to K.T.) from the Ministry of Education, Science, Sports and Culture, Japan.

References

Bauer, C. E. (1995) Regulation of photosynthesis gene expression. *In* Anoxygenic Photosynthetic Bacteria, ed. by Blankenship, R. E., Madigan, M. T., and Bauer, C. E., Kluwer Academic Publishers, Dordreccht, The Netherlands, pp. 1121–1234.

Buggy, J. J., Sganga, M. W., and Bauer, C. E. (1994a) Nucleotide sequence and characterization of the *Rhodobacter capsulatus hvrB* gene: HvrB is an activator of *S*-adenosyl-L-homocysteine hydrolase expression and is a member of the LysR family. *J. Bacteriol.*, **176**, 61–69.

Buggy, J. J., Sganga, M. W., and Bauer, C. E. (1994b) Characterization of light-responding *trans*-activator responsible for differentially controlling reaction center and light-harvesting-I gene expression in *Rhodobacter capsulatus. J. Bacteriol.*, **176**, 6936–6943.

Drews, G. and Golecki, J. R. (1995) Structure, molecular organization, and biosynthesis of membranes of purple bacteria. *In* Anoxygenic Photosynthetic Bacteria, ed. by Blankenship, R. E., Madigan, M. T., and Bauer, C. E., Kluwer Academic Publishers, Dordreccht, The Netherlands, pp. 231–257.

Du, S., Bird, T. M., and Bauer, C. E. (1998) DNA binding characteristics of RegA. *J. Biol. Chem.*, **273**, 18509–18513.

Eraso, J. M. and Kaplan, S. (1994) *prrA*, a putative response regulator involved in oxygen regulation of photosynthesis gene expression in *Rhodobacter sphaeroides. J. Bacteriol.*, **176**, 32–43.

Eraso, J. M. and Kaplan, S. (1995) Oxygen-insensitive synthesis of the photosynthetic membranes of *Rhodobacter sphaeroides*: A mutant histidine kinase. *J. Bacteriol.*, **177**, 2695–2706.

Harashima, K., Hayasaki, J., Ikari, T., and Shiba, T. (1980) O_2-stimulated synthesis of bacteriochlorophyll and carotenoids in marine bacteria. *Plant Cell Physiol.*, **21**, 1283–1294.

Iba, K., Takamiya, K., Toh, Y., and Nishimura, M. (1988) Roles of bacteriochlorophyll and carotenoid synthesis in formation of intracytoplasmic membrane systems and pigment-protein complexes in an aerobic photosynthetic bacterium, *Erythrobacter* sp. strain OCh114. *J. Bacteriol.*, **170**, 1843–1847.

Liebetantz, R., Hornberger, U., and Drews, G. (1991) Organization of the genes coding for the reaction-centre L and M subunits and B870 antenna polypeptides α and β from the aerobic photosynthetic bacterium *Erythrobacter* species OCH114. *Mol. Microbiol.*, **5**, 1459–1468.

Matsumoto, Y., Nagashima, K. V. P., Inoue, K., Bauer, C. E., Shimada, K., and Matsuura, K. (1997) Determination of nucleotide sequences of putative RegA and RegB; oxygen sensitive regulatory components related to the expression of photosystem in *Rhodovulum sulfidophilum* and *Roseobacter denitrificans. Plant Cell Physiol.*, **38**, s13.

Mizoguchi, H., Masuda, T., Nishimura, K., Shimada, H., Ohta, H., Shioi, Y., and Takamiya, K. (1997) Nucleotide sequence and transcriptional analysis of the flanking region of the gene (*spb*) for the *trans*-acting factor that controls light-mediated expression of the *puf* operon in *Rhodobacter sphaeroides. Plant Cell Physiol.*, **38**, 558–567.

Mosley, C. S., Suzuki, J. Y., and Bauer, C. E. (1994) Identification and molecular genetic characterization of a sensor kinase responsible for coordinately regulating light harvesting and reaction center gene expression in response to anaerobiosis. *J. Bacteriol.*, **176**, 7566–7573.

Nishimura, K., Shimada, H., Hatanaka, S., Mizoguchi, H., Ohta, H., Masuda, T., and Takamiya, K. (1998) Growth, pigmentation, and expression of the *puf* and *puc* operons in a light-responding-repressor (SPB)-disrupted *Rhodobacter sphaeroides. Plant Cell Physiol.*, **39**, 411–417.

Nishimura, K., Shimada, H., Ohta, H., Masuda, T., Shioi, Y., and Takamiya, K. (1996) Expression of the *puf* operon in an aerobic photosynthetic bacterium, *Roseobacter denitrificans. Plant Cell Physiol.*, **37**, 153–159.

Pradel, E., Parker, C. T., and Schnaitman, C. A. (1992) Structures of the *rfaB*, *rfaI*, *rfaJ*, and *rfaS* genes in *Escherichia coli* K-12 and their roles in assembly of the lipopolysaccharide core. *J. Bacteriol.*, **174**, 4736–4745.

Sganga, M. W., Aksamit, R. R., Cantoni, G. L., and Bauer, C. E. (1992) Mutational and nucleotide sequence analysis of *S*-adenosyl-L-homocysteine hydrolase from *Rhodobacter capsulatus. Proc. Natl. Acad. Sci. U.S.A.*, **89**, 6328–6332.

Sganga, M. W. and Bauer, C. E. (1992) Regulatory factors controlling photosynthetic reaction center and light-harvesting gene expression in *Rhodobacter capsulatus. Cell*, **68**, 945–954.

Shiba, T. and Simidu, U. (1982) *Erythrobacter longus* gen. nov., sp. nov., an aerobic bacterium which contains bacteriochlorophyll *a*. *Int. J. Syst. Bacteriol.*, **32**, 211–217.

Shimada, H., Wada, T., Handa, H., Ohta, H., Mizoguchi, H., Nishimura, K., Masuda, T., Shioi, Y., and Takamiya, K. (1996) A transcription factor with a leucine-zipper motif involved in light-dependent inhibition of expression of the *puf* operon in the photosynthetic bacterium *Rhodobacter sphaeroides*. *Plant Cell Physiol.*, **37**, 515–522.

Zhu, Y. S., Kiley, P. J., Donohue, T. J., and Kaplan, S. (1986) Origin of mRNA stoichiometry of the *puf* operon in *Rhodobacter sphaeroides*. *J. Biol. Chem.*, **261**, 10366–10374.