JOURNAL OF BACTERIOLOGY, Feb. 2007, p. 683–690 Vol. 189, No. 3
0021-9193/07/$08.00+0 doi:10.1128/JB.01390-06

The Complete Genome Sequence of *Roseobacter denitrificans* Reveals a Mixotrophic Rather than Photosynthetic Metabolism▽†

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Received 31 August 2006/Accepted 31 October 2006

Purple aerobic anoxygenic phototrophs (AAPs) are the only organisms known to capture light energy to enhance growth only in the presence of oxygen but do not produce oxygen. The highly adaptive AAPs compose more than 10% of the microbial community in some euphotic upper ocean waters and are potentially major contributors to the fixation of the greenhouse gas CO_2. We present the complete genomic sequence and feature analysis of the AAP *Roseobacter denitrificans*, which reveal clues to its physiology. The genome lacks genes that code for known photosynthetic carbon fixation pathways, and most notably missing are genes for the Calvin cycle enzymes ribulose bisphosphate carboxylase (RuBisCO) and phosphoribulokinase. Phylogenetic evidence implies that this absence could be due to a gene loss from a RuBisCO-containing α-proteobacterial ancestor. We describe the potential importance of mixotrophic rather than autotrophic CO_2 fixation pathways in these organisms and suggest that these pathways function to fix CO_2 for the formation of cellular components but do not permit autotrophic growth. While some genes that code for the redox-dependent regulation of photosynthetic machinery are present, many light sensors and transcriptional regulatory motifs found in purple photosynthetic bacteria are absent.

Among the five major phyla containing phototrophic prokaryotes, the purple proteobacteria are the most metabolically diverse. The anaerobic purple photosynthetic bacteria grow photoautotrophically only at low oxygen levels, while at higher oxygen levels, the photosynthetic apparatus is down-regulated, resulting in chemotrophic growth using organic carbon (3). In contrast, some related marine α-proteobacteria produce bacteriochlorophyll (BChl) *a* only in the presence of oxygen in the dark (39). These aerobic anoxygenic phototrophs (AAPs) (also known as AAnP or APB for aerobic phototrophic bacteria) have long been overlooked in oceanic studies, but recent data indicate that their contribution to the global carbon cycle could be significant (22).

Typically, AAPs grow photoheterotrophically by respiration of organic substrates (39), resulting in the release of CO_2, counter to the traditional "CO_2 sink" of the upper ocean. However, light-stimulated CO_2 uptake has been seen in some AAP species (22, 39), and several members of the *Roseobacter* clade were shown to oxidize CO to CO_2 (6).

The marine AAP species *Roseobacter denitrificans* grows not only photoheterotrophically in the presence of oxygen and light but also anaerobically in the dark using nitrate or trimethylamine *N*-oxide as an electron acceptor (39). This adaptability has made *R. denitrificans* the most studied AAP and a model organism for this group.

Our study of the *R. denitrificans* genome reveals a variety of metabolic options available to make this species successful in a competitive oligotrophic marine environment. We also investigated the complement of transcriptional regulators in *R. denitrificans* that are thought to be responsible for aerobic regulation of photosynthesis genes in phototrophic purple bacteria.

MATERIALS AND METHODS

A single colony of *Roseobacter denitrificans* strain OCh 114 was grown heterotrophically as described previously (8), and total DNA was isolated using proteinase K treatment followed by phenol extraction. The DNA was fragmented by kinetic shearing, and three shotgun libraries were generated: small- and medium-insert libraries in pOTWI3 (using size fractions of 2 to 3 kb and 6 to 8 kb, respectively) and a large-insert fosmid library in pEpiFOS-5 (insert sizes ranging from 28 to 47 kb), which was used as a scaffold. The relative amounts of sequence coverage obtained from the small-, medium-, and large-insert libraries were 8×, 1×, and 1×, respectively. The whole genome sequence was established from 55,081 end sequences (giving 9.6× coverage) derived from these libraries using dye terminator chemistry on ABI 3730xl automated sequencers. The sequence was assembled and finished as described previously (37). Pseudogenes contained one or more mutations that would ablate expression; each inactivating mutation was subsequently checked against the original sequencing data.

Initial annotation data were built using the Annotation Engine at The Institute for Genomic Research (http://www.tigr.org/edutraining/training/annotation_engine.shtml), followed by comprehensive manual inspection and editing of each feature by

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▽ Published ahead of print on 10 November 2006.

TABLE 1. General features of the *Roseobacter denitrificans* genome

Feature	Copy no.[a]	Total size (bp)	% G+C content	No. of:		Coding density (%)	Avg gene length (bp)	No. of:	
				Genes	Pseudogenes			rRNAs	tRNAs
Chromosome		4,133,097	58	4,007	20	89	888	3	38
pTB1	3.1	106,469	56	114	4	84.1	785		
pTB2	2	69,269	59	57	3	87.2	1,059		
pTB3	2.3	16,575	55	16	1	81.1	839		
pTB4	2.9	5,824	56	6	0	53.9	523		

[a] The copy number of the plasmids was estimated based on the number of reads per kilobase compared with the chromosome.

using Manatee (http://manatee.sourceforge.net/). The circular genome map was created using the program CGView (35).

Phylogenetic comparisons were performed using clustalW and MEGA (http://www.megasoftware.net/). *Jannaschia* sp. strain CCS1 (http://genome.jgi-psf.org/draft_microbes/jan_c/jan_c.home.html), *Roseobacter* sp. strain MED193 (http://www.moore.org/), and other draft genomes were analyzed and compared to their current genomes found in NCBI genome databases (http://www.ncbi.nlm.nih.gov/).

Nucleotide sequence accession numbers. The complete and annotated genome sequences of *Roseobacter denitrificans* strain OCh 114 and its associated plasmids have been deposited in the DDBJ/EMBL/GenBank database under accession numbers CP000362, CP000464, CP000465, CP000466, and CP000467.

RESULTS AND DISCUSSION

Genome properties. *R. denitrificans* contains a circular chromosome of 4,133,097 bp and four plasmids, yielding a total of 4,007 predicted genes (Tables 1 and 2 and Fig. 1; see Fig. S1 in the supplemental material). ABC-type transport proteins account for roughly 6.5 to 7.0% of all open reading frames, while tripartite ATP-independent periplasmic-type transporters are 10-fold less common. Major metabolic pathways for the tricarboxylic acid (TCA) cycle, amino acid and nucleotide biosynthesis, glycolysis, nitrogen metabolism, etc., fit the described lifestyle of *R. denitrificans* and are, in fact, quite similar to its well-studied anaerobic photosynthetic relative *Rhodobacter sphaeroides*. No extensive metabolic losses or gains indicate a change to aerobic phototrophic metabolism in *R. denitrificans*.

Nearly half of the genes in the largest plasmid (pTB1) share a high degree of similarity to genes in plasmid pSD25 from *Ruegeria* sp. isolate PR1b and the Ti plasmid from *Agrobacterium tumefaciens* strain C58 (16). *Agrobacterium* in particular is a well-studied plant pathogen known to transfer genetic elements into host genomes via plasmid-encoded integrases (41). The conservation of many DNA integration and transfer proteins, plasmid replication proteins, and inverted repeats imply that *R. denitrificans* plasmid pTB1 may also promote lateral gene transfer. This possibility is especially interesting considering the propensity of *R. denitrificans* to associate with higher organisms (39).

TABLE 2. Features of selected gene categories of the *Roseobacter denitrificans* genome

Selected gene category	No. of genes	% of genome
Energy, carbon, nitrogen, and other metabolism	658	16.4
Transport	548	13.7
Regulation and signal transduction	299	7.5
Protein synthesis, modification, folding, etc.	284	7.1
Cofactor biosynthesis	192	4.8
Amino acid synthesis	151	3.8
Envelope proteins	125	3.1
DNA metabolism	110	2.7
Transcription	62	1.5

Carbon fixation. *R. denitrificans* lacks the key Calvin cycle enzymes ribulose bisphosphate carboxylase (RuBisCO), phosphoribulokinase, and other proteins typically encoded by Calvin cycle operons in closely related anaerobic purple bacteria. Two strains closely related to *R. denitrificans*, *Jannaschia* sp. strain CCS1 and *Roseobacter* sp. strain MED193, both encode a putative form-IV RuBisCO-like protein, an enzyme that has not been found to fix CO_2 (25). These species also lack genes for phosphoribulokinase and other Calvin cycle-specific enzymes. As *R. denitrificans* is the first completely sequenced AAP, this is the first confirmation of the lack of these proteins. A full complement of Calvin cycle genes was found in the nonphotosynthetic plant symbiote *Bradyrhizobium japonicum* strain USDA110 (20), and our analyses also identified homologs in the genome assembly of the phototrophic plant symbiote *Bradyrhizobium* sp. strain BTAi1.

A phylogenetic tree of all α-proteobacteria with available genomic sequence data was constructed using the 16S small-subunit rRNA sequence (Fig. 2). Parasitic organisms were removed from the tree due to their loss of many metabolic pathways. The presence of RuBisCO across the represented α-proteobacterial lineages suggests that the ancestral species may have contained the enzyme (Fig. 2). Unfortunately, the gene tree for RuBisCO is riddled with lateral gene transfer, which leaves room for much debate (1). RuBisCO is not only missing in all AAPs, it is also missing from species that have a fermentative metabolism such as *Gluconobacter oxydans* and *Zymomonas mobilis*. These species and the AAPs are dependent on organic carbon compounds for growth. A combination of competitive RuBisCO inhibition by oxygen and the decreasing need for carbon assimilation could lead to a gene loss in these species. The ubiquity of AAPs worldwide suggests that the Calvin cycle is not an essential metabolic pathway for success in the open ocean.

Because *R. denitrificans* lacks a RuBisCO gene, the central question is how it might fix CO_2. Key genes involved in three anaerobic CO_2 fixation pathways, the reductive TCA cycle, the hydroxypropionate pathway, and the reductive acetyl coenzyme pathway (31), are absent from the genome. However, putative genes for pyruvate-orthophosphate dikinase and phosphoenolpyruvate (PEP) carboxylase, two proteins related to plant C_4 sequestration, crassulacean acid

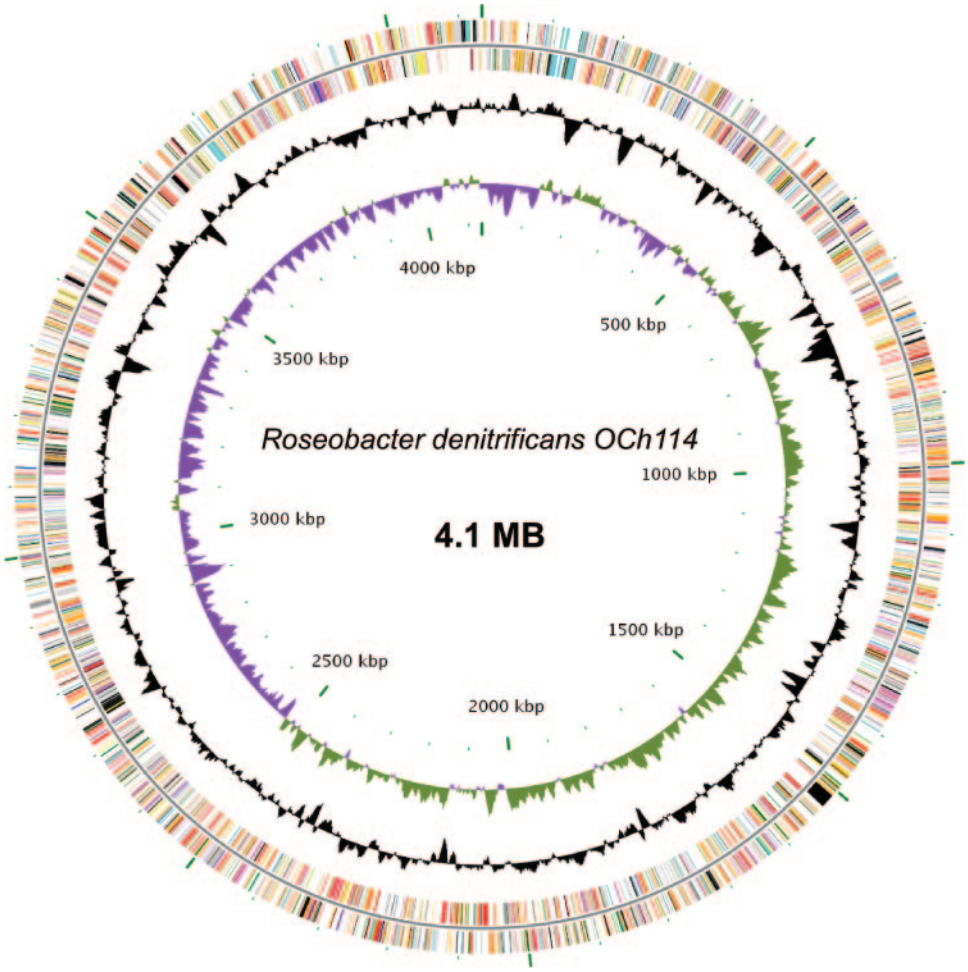

FIG. 1. Circular representation of the *Roseobacter denitrificans* OCh 114 chromosome (maps of plasmids pTB1, pTB2, pTB3, and pTB4 are found in Fig. S1 in the supplemental material). The different rings represent (from outer to inner) all genes and insertion elements, which are color coded by functional category (rings 1 and 2), deviation from average G+C content (ring 3), and GC skew (ring 4). Color codes are as follows: turquoise indicates small-molecule biosynthesis, yellow indicates central or intermediary metabolism, black indicates energy metabolism, red indicates signal transduction, lavender indicates DNA metabolism, blue indicates transcription, purple indicates protein synthesis/fate, dark green indicates surface-associated features, gray indicates miscellaneous features, pink indicates phage and insertion elements, light green indicates unknown function, orange indicates hypothetical proteins, and brown indicates pseudogenes.

metabolism, and anaplerotic carbon fixation enzymes, are present in *R. denitrificans* (loci RD1_1948 and RD1_14248, respectively). In a mechanism first described by Wood et al. (38), PEP carboxylase attaches CO_2 to PEP to form oxaloacetate (OAA) (Fig. 3). This two-step reaction can also be performed in a single step by pyruvate carboxylase. In C_4 and crassulacean acid metabolism plants, a similar process provides high amounts of CO_2 substrate to RuBisCO. In heterotrophic bacteria, this is primarily an anaplerotic reaction that feeds oxaloacetate into the TCA cycle (32). We suggest that this pathway, coupled with photoheterotrophic energy metabolism, is responsible for the observed mixotrophic CO_2 assimilation in *R. denitrificans* and other AAPs (22, 39).

Two key enzymes of the heterotrophic carbon fixation pathway, pyruvate-orthophosphate dikinase and pyruvate carboxylase, form monophyletic clusters distinct from closely related species (see Fig. S2 and S3, respectively, in the supplemental material). Their sequences may have diverged to accommodate a CO_2 fixation role larger than purely anaplerotic assimilation. Other carboxylases within *R. denitrificans* could also have functionally diverged to further catalyze CO_2 fixation, and these concerted carboxylase activities could account for the AAP CO_2 assimilation seen previously (6, 39).

The global carbon cycle may also be influenced by members of the widespread *Roseobacter* lineage that were shown to metabolize CO (6). All other aerobic organisms that oxidize CO to CO_2 for energy grow autotrophically using the Calvin cycle (21). Although *R. denitrificans* has not been shown to oxidize CO in culture, it contains the necessary genes (*coxG* and *coxSML*) (Table 3) and likely performs the reaction under proper conditions. Along with phototrophy and denitrification, this further mechanism for energy metabolism, as well as a full complement of genes for the transport and metabolism of inorganic sulfur (*sox*) and phosphonate (*phn*) (discussed below), indicates the great

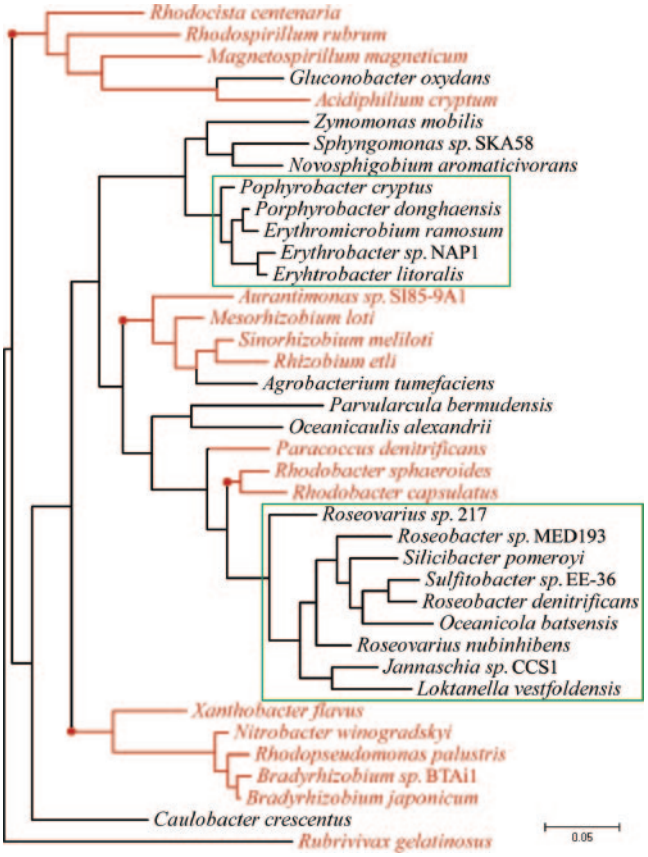

FIG. 2. 16S ribosomal tree for the α-proteobacterial lineage using genomes available in the NCBI database. The tree was constructed using maximum likelihood methods. RuBisCO-containing taxa are indicated in red text, with putative RuBisCO-containing nodes highlighted with red dots. Both clades of aerobic phototrophic bacteria (AAP) are highlighted within green boxes. This tree illustrated the widespread character of RuBisCO among α-proteobacteria as well as its conspicuous absence within the AAPs. Note that without the vast oversampling among AAP lineages, a higher percentage of sequenced α-proteobacteria contain RuBisCO than do not.

metabolic versatility of *R. denitrificans* and possibly other AAPs.

Based on the genome sequence of *R. denitrificans*, we suggest that they fix CO_2 mixotrophically by using a sequestration pathway supplemented with additional CO_2 provided by CO oxidation and heterotrophic respiration. Further studies will show whether this metabolic activity is conserved within all AAPs. The diverse array of energy-yielding metabolic pathways in this organism likely helps provide additional energy, in the form of ATP, for the formation of OAA from CO_2 and metabolic intermediates.

Metabolic versatility. The ability of *Roseobacter* clade members to metabolize sulfur compounds has been well studied (15). Although *R. denitrificans* has not been studied in the same thorough manner, its genome codes for many of the same conserved sulfur metabolic pathways (Table 3). Inorganic sulfur oxidation, encoded by the large *soxWXYZABCDEF* cluster, is a likely source for extra energy production yielding sulfate as environmental output. The presence of *dms* and *dmd* genes indicates that dimethyl sulfoxide reduction and the breakdown of the biological by-product dimethyl sulfoniopropionate could be useful for assimilating additional sulfur into cellular material or providing sulfide for oxidation (15).

One of the major limiting growth factors in the open ocean is phosphorous availability (primarily as organic phosphates). *R. denitrificans* has the ability to process organic phosphates as well as degrade the chemically stable C-P bond-containing phosphonates (Table 3). While the concentration of natural phosphonates in seawater is likely low, it has been shown to be an important alternative phosphorous source during phosphate starvation (17, 36).

The reduction of oxidized nitrogen compounds is a significant aspect of the lifestyle of *R. denitrificans*, and it has a large complement of genes to account for this (Table 3). All nitrogen metabolism genes are located in three gene clusters, the largest of which codes for nitrite, nitrous oxide, and nitric oxide reduction pathways. Because *R. denitrificans* cannot fix dinitrogen, the presence of *nirBD*, coding for assimilatory nitrite reductase, shows that *R. denitrificans* can acquire nitrogen through means other than recycling.

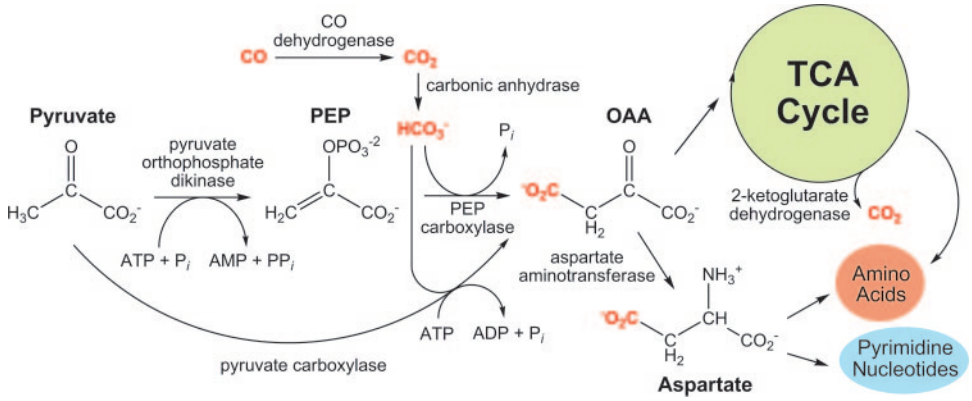

FIG. 3. Proposed mixotrophic CO_2 assimilation pathway in *Roseobacter denitrificans*. CO_2 is taken up from the environment or supplied by the action of CO dehydrogenase. Carbonic anhydrase converts CO_2 to HCO_3^-, which is then used by PEP carboxylase and pyruvate carboxylase. OAA is either entered into the TCA cycle or converted to aspartate, which can be converted to several amino acids and pyrimidine nucleotides. Although the assimilated carbon can be lost by the action of 2-ketoglutarate dehydrogenase, it will be maintained in TCA cycle intermediates that are recruited into other pathways.

TABLE 3. Genes involved in major metabolic pathways in *Roseobacter denitrificans*

Metabolic Function	Gene(s)	Locus tag(s)
Carbon monoxide dehydrogenation	*coxG*, *coxSLM*	RD1_0532, RD1_2972–2974
Inorganic sulfur oxidation	*soxWXYZABCDEF*	RD1_1510–1519
Dimethyl sulfoxide reduction	*dmsA*, *dmsBC*	RD1_3664, RD1_0973–0974
Dimethyl sulfoniopropionate methyltransfer	*dmdA*	RD1_2288
Phosphate transport and metabolism	*phoR*, *pstSCAB*, *phoUB*	RD1_2641–2648
Phosphonate metabolism	*phnF*, *phnGHIJKLNM*	RD1_2387–2395
Phosphonate transport	*phnDCE(D)*	RD1_1054–1057
Nitrous oxide reduction	*nosRZDFYLX*, *nosR*	
Nitric oxide reduction	*norCBQDEF*	RD1_1546–1573
Nitrite reduction	*nirSCFDGHJN*	
Nitrate reduction	*napA*, *narKGHJI*	RD1_2440–2445
Nitrate/nitrite reduction and transport (assimilatory)	*nasTSFED*, *nirBD*, *nasA*	RD1_4166–4174

Like the metabolically diverse anaerobic purple photosynthetic bacterium *Rhodopseudomonas palustris* (24), *R. denitrificans* dedicates nearly 15% of its genome to the construction of transport proteins (Table 2). Among these transporters are more than 120 genes coding for peptide and amino acid transporters (many branched chain), more than 110 genes coding for sugar and carbohydrate transporters, and more than 70 cation and iron transporters. The number of iron transport mechanisms, while not as high as that in *R. palustris* (24), should still be sufficient to supply *R. denitrificans* with iron. The high number of peptide and sugar transporters suggests that *R. denitrificans* and perhaps other non-Calvin-cycle AAPs rely on available sources of organic carbon compounds. However, the library of diverse channel proteins should allow *R. denitrificans* to adapt to the uptake of a variety of available carbon sources.

PGC. Figure 4 shows the photosynthesis gene cluster (PGC) organization in *R. denitrificans*, spanning between loci RD1_0102 and RD1_0147. The organization of genes within purple bacterial PGCs was proposed to reflect regulatory mechanisms, evolutionary history, and relationships between species (18, 40).

Igarashi et al. (18) previously described large-scale gene rearrangements in PGCs between the α-proteobacteria *Rhodobacter capsulatus* and *R. sphaeroides* and the β-proteobacterium *Rubrivivax gelatinosus*. The PGC organization in *R. denitrificans*, although unique, is similar to both PGCs (Fig. 4). The gene arrangement of *idi-bchFNBHLM-lhaA-puhA* in *R. denitrificans* matches that seen in *R. gelatinosus* (18), including the segregation of *bchE* and *bchJ* to distant parts of the chromosome. However, the *crtEF-bchCXYZ-puf* cluster resembles *Rhodobacter* rather than *Rubrivivax* organization, including the transposition of *crtI* and *crtA*. The central region of the PGC in *R. denitrificans* also includes the additional BChl/heme biosynthesis genes *acsF* and *puhE* (as in *R. gelatinosus*), *hemA*, and the gene encoding cytochrome c_2, *cycA*.

The phylogenetic position of *R. denitrificans*, nested within

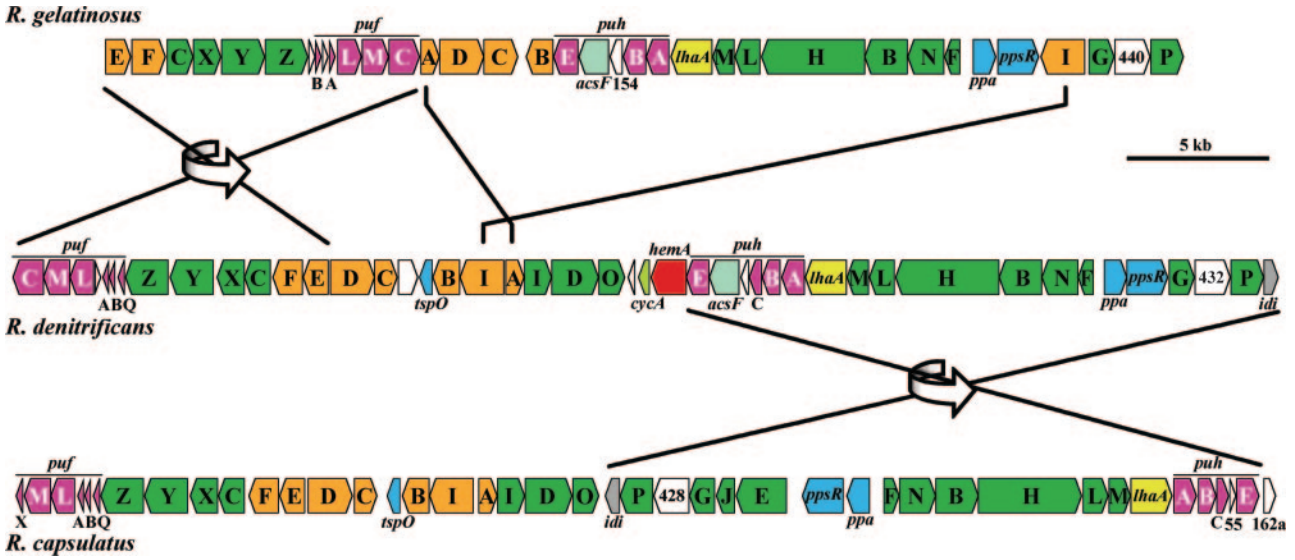

FIG. 4. Photosynthesis gene cluster arrangement in the purple bacteria *Rubrivivax gelatinosus*, *Roseobacter denitrificans*, and *Rhodobacter capsulatus*. Genes are presented as arrows indicating their direction of transcription. Genes are colored as listed: chlorophyll biosynthesis (*bch*) in green, carotenoid biosynthesis (*crt*) in orange, reaction centers and light-harvesting complexes (*puf* and *puh*) in purple, regulatory proteins in blue, and uncharacterized genes in white. All other gene colors are unique for clarity. Lines illustrate gene rearrangement, and arrows illustrate the reversal of large superoperonal clusters.

the *Rhodobacteraceae* (Fig. 2), invites speculation on the relationship of its PGC to that of *R. gelatinosus*. Previous work proposed a lateral transfer of the PGC from an *R. palustris* ancestor into *R. gelatinosus* (18) due to the high BLAST similarity between PGC proteins in these species. However, the *R. denitrificans* cluster also shares a large degree of synteny with that of *R. gelatinosus* (as described above). Given only these few species, these two lines of conflicting evidence might imply a lateral genetic relationship between *R. denitrificans*, *R. palustris*, and *R. gelatinosus* clusters. However, our further analysis of purple bacterial PGCs revealed far more synteny between the *Roseobacter* cluster and the *Rhodopseudomonas*/*Rubrivivax* PGC than that from *Rhodobacter* species (the presence of *acsF* and the translocation of *bchE* and *bchJ*). It seems likely that evolutionary differentiation in the *Rhodobacter* species after the divergence of *Roseobacter* has led to its current PGC organization.

Our investigation of PGCs from all phototrophic α-proteobacteria with sequence data available at the time of submission of this paper revealed numerous gene arrangements with very few exact matches (data not shown). However, the two major superoperons found in *Rhodobacter* species, *bchFNBHLM-lhaA-puh* and *crtEF-bchCXYZ-puf* (4), were not disrupted in any species. A similar PGC operon structure in aerobic and anaerobic species suggests that PGC organization has no clear relationship to aerobic regulation of photosynthesis genes in *R. denitrificans*.

Regulation of gene transcription. All phototrophic organisms contain mechanisms to sense environmental changes and appropriately regulate the transcription of genes encoding vital metabolic pathways. In photosynthetic purple bacteria, oxygen and light levels are known to regulate photosynthesis (2, 3). Characterizing these sensory pathways in *R. denitrificans* and other AAPs is key to understanding their unique pattern of gene regulation.

The primary cellular redox sensors RegA and RegB from *R. denitrificans* share a high degree of similarity with those in other purple bacteria (27). These proteins have been characterized as key activators of anaerobic photosynthesis gene expression as well as other cellular processes (3).

The global anaerobic activator FnrL in *R. denitrificans* (RD1_1268) is also quite similar to that studied in *Rhodobacter* species. FnrL not only is responsible for primary oxygen sensing but is also a DNA-binding transcription factor (33). Consensus recognition motifs for this protein have been identified in the promoter regions of *Rhodobacter* heme biosynthesis (*hem*) genes (33). This may regulate BChl levels via the shared biosynthesis pathway with heme. Of all *hem* promoters thought to be regulated by FnrL, only one of three *hemN* promoters in *R. denitrificans* (RD1_1267) contained a clear consensus binding motif.

Although both photosynthetic purple bacteria and AAPs repress photosynthetic apparatus production in high light, AAPs respond much more sensitively (39). One major feature implicated in light sensing in anaerobic purple bacteria is the BLUF (blue-light-using flavin adenine dinucleotide) domain (14), which has been studied in the *R. sphaeroides* AppA protein (5, 26). While the BLUF domain proteins found in other organisms and *R. denitrificans* (RD1_1653) are likely light sensors, they bear very little similarity to *R. sphaeroides* AppA. No genes coding for homologs to other major light-sensing proteins in purple bacteria, bacteriophytochromes (10, 11), photoactive yellow proteins (23), and HvrA/Spb (7) are found in the genome of *R. denitrificans.* This suggests the presence of a novel sensory mechanism that is responsible for the sensitive light response in *R. denitrificans* and other AAPs.

In anaerobic purple bacteria, several global transcription factors are involved in oxygen and light regulation. The most studied of these proteins is PpsR (CrtJ in *R. capsulatus*), a DNA-binding protein that attaches to (usually) two tandem $TGTN_{12}ACA$ motifs upstream from promoters to block RNA polymerase sigma-subunit binding and repress transcription (9, 29). Although *R. denitrificans* encodes a homolog of PpsR (RD1_0143), aerobic gene repression would be counterproductive to the observed aerobic photosynthetic expression. Interestingly, PpsR in *R. gelatinosus* and PpsR1 in *Bradyrhizobium* strain ORS278 have been proposed to activate photosynthesis gene transcription under aerobiosis (19, 34).

The *R. denitrificans* PGC contains at least 22 putative PpsR-binding sites, compared to 13 in *R. sphaeroides*, 12 in *R. capsulatus*, and 9 in *R. gelatinosus*. This surprisingly large number of PpsR motifs implies that this protein is a transcriptional modulator in this species. It has also been shown that the spacing between tandem PpsR-binding motifs can drastically affect transcriptional regulation (30). In *R. denitrificans*, only *crtE*, *crtI*, and *bchG* have PpsR-binding motifs that would fit the previously described functional profile, overlapping the −10 or −35 promoter regions with the tandem motifs separated by 6 to 11 bases. The tandem PpsR-binding motifs at the major control sites of *bchF* and *bchC* (13, 30) are spaced too far apart and may be too far upstream in *R. denitrificans*, 39 and 57 bp, respectively, to properly regulate RNA polymerase activity. Many of the PpsR-binding motifs in *R. denitrificans* are also downstream of the promoter-binding site, which may also decrease their interaction with RNA polymerase (9). Future analyses of PpsR regulation in both aerobic and anaerobic species will clarify the importance of the conservation of the binding motif in the transcriptional regulation of photosynthesis.

The role of the transcriptional regulator PpaA is also unclear. PpaA overexpression in *R. sphaeroides* has been shown to activate the transcription of photosynthetic proteins only under aerobic conditions (12). This is contrary to the fact that this organism maximally produces these protein products under low oxygen tension. PpsR-binding sites in the *R. sphaeroides ppaA* promoter region are likely to repress transcription aerobically, negating its activation role. Since *ppaA* promoter regions in both *R. denitrificans* and *R. sphaeroides* contain similar PpsR consensus-binding motifs, *ppaA* transcription in *R. denitrificans* is likely to be activated by PpsR in the presence of oxygen.

The complement of sensory and regulatory proteins in *R. denitrificans* does not give a clear indication of its aerobic lifestyle. The roles of PpsR and PpaA have not been clearly defined (9, 12, 19, 34), and biochemical studies of *R. denitrificans* and other AAPs are needed to clarify their function. Furthermore, AAP genome sequences may reveal patterns in regulatory proteins that could lead to a better understanding of aerobic phototrophy. Of special interest is the genome of the "crossover" species *Rhodocista centenaria*, which photosynthe-

sizes anaerobically but still produces pigments aerobically (http://genomes.tgen.org).

Conclusions. The genome analysis of *R. denitrificans*, the first AAP sequenced, has opened the door to understanding their physiological capacity to fill a broad niche in the ocean ecosystem. The numerous mechanisms that members of the *Roseobacter* clade have evolved for chemotrophic and phototrophic energy metabolism may explain their wide dispersal and high abundance in the biosphere, by allowing them to adapt to changing conditions and outcompete organisms with more conventional lifestyles (6, 28). Because of this "jack-of-all-trades" approach, their contribution to the global carbon cycle is likely to be complex. Analyses of additional AAP genomes in combination with biochemical studies should clarify the information that we have presented regarding the role of the transcriptional regulators PpsR and PpaA, the photosynthesis gene cluster, and the details of the CO_2 fixation pathway.

ACKNOWLEDGMENTS

We thank Paul Stothard and Jason Touchman for assistance with the generation of the circular genome and plasmid maps and Carl Bauer, Gerald Edwards, and Shinji Masuda for thoughtful discussion.

This work was supported by the U.S. National Science Foundation Phototrophic Prokaryotes Sequencing Project, grant number 0412824, and a grant to J.T.B. from the Natural Sciences and Engineering Research Council, Canada.

REFERENCES

1. **Ashida, H., A. Danchin, and A. Yokota.** 2005. Was photosynthetic RuBisCO recruited by acquisitive evolution from RuBisCO-like proteins involved in sulfur metabolism? Res. Microbiol. **156:**611–618.
2. **Bauer, C. E.** 2004. Regulation of photosystem synthesis in *Rhodobacter capsulatus*. Photosynth. Res. **80:**353–360.
3. **Bauer, C. E., S. Elsen, L. R. Swem, D. L. Swem, and S. Masuda.** 2003. Redox and light regulation of gene expression in photosynthetic prokaryotes. Philos. Trans. R. Soc. Lond. B **358:**147–154.
4. **Beatty, J. T.** 1995. Organization of photosynthesis gene transcripts, p. 1209–1219. *In* R. E. Blankenship, M. T. Madigan, and C. E. Bauer (ed.), Anoxygenic photosynthetic bacteria, vol. 2. Kluwer Academic Publishers, Dordrecht, The Netherlands.
5. **Braatsch, S., M. Gomelsky, S. Kuphal, and G. Klug.** 2002. A single flavoprotein, AppA, integrates both redox and light signals in *Rhodobacter sphaeroides*. Mol. Microbiol. **45:**827–836.
6. **Buchan, A., J. Gonzalez, and M. A. Moran.** 2005. Overview of the marine *Roseobacter* lineage. Appl. Environ. Microbiol. **71:**5665–5677.
7. **Buggy, J. J., M. W. Sganga, and C. E. Bauer.** 1994. Characterization of a light-responding *trans*-activator responsible for differentially controlling reaction center and light-harvesting-I gene expression in *Rhodobacter capsulatus*. J. Bacteriol. **176:**6936–6943.
8. **Candela, M., E. Zaccherini, and D. Zannoni.** 2001. Respiratory electron transport and light-induced energy transduction in membranes from the aerobic photosynthetic bacterium *Roseobacter denitrificans*. Arch. Microbiol. **175:**168–177.
9. **Elsen, S., M. Jaubert, D. Pignol, and E. Giraud.** 2005. PpsR: a multifaceted regulator of photosynthesis gene expression in purple bacteria. Mol. Microbiol. **57:**17–26.
10. **Giraud, E., J. Fardoux, N. Fourrier, L. Hannibal, B. Genty, P. Bouyer, B. Dreyfus, and A. Vermeglio.** 2002. Bacteriophytochrome controls photosystem synthesis in anoxygenic bacteria. Nature **417:**202–205.
11. **Giraud, E., S. Zappa, L. Vuillet, J. M. Adriano, L. Hannibal, J. Fardoux, C. Berthomieu, P. Bouyer, D. Pignol, and A. Vermeglio.** 2005. A new type of bacteriophytochrome acts in tandem with a classical bacteriophytochrome to control the antennae synthesis in *Rhodopseudomonas palustris*. J. Biol. Chem. **280:**32389–32397.
12. **Gomelsky, L., J. Sram, O. V. Moskvin, I. M. Horne, H. N. Dodd, J. M. Pemberton, A. G. McEwan, S. Kaplan, and M. Gomelsky.** 2003. Identification and *in vivo* characterization of PpaA, a regulator of photosystem formation in *Rhodobacter sphaeroides*. Microbiology **149:**377–388.
13. **Gomelsky, M., and S. Kaplan.** 1995. Genetic evidence that PpsR from *Rhodobacter sphaeroides* 2.4.1 functions as a repressor of *puc* and *bchF* expression. J. Bacteriol. **177:**1634–1637.
14. **Gomelsky, M., and G. Klug.** 2002. BLUF: a novel FAD-binding domain involved in sensory transduction in microorganisms. Trends Biochem. Sci. **27:**497–500.
15. **Gonzalez, J. M., R. P. Kiene, and M. A. Moran.** 1999. Transformation of sulfur compounds by an abundant lineage of marine bacteria in the α-subclass of the class *Proteobacteria*. Appl. Environ. Microbiol. **65:**3810–3819.
16. **Goodner, B., G. Hinkle, S. Gattung, N. Miller, M. Blanchard, B. Qurollo, B. S. Goldman, Y. Cao, M. Askenazi, C. Halling, L. Mullin, K. Houmiel, J. Gordon, M. Vaudin, O. Iartchouk, A. Epp, F. Liu, C. Wollam, M. Allinger, D. Doughty, C. Scott, C. Lappas, B. Markelz, C. Flanagan, C. Crowell, J. Gurson, C. Lomo, C. Sear, G. Strub, C. Cielo, and S. Slater.** 2001. Genome sequence of the plant pathogen and biotechnology agent *Agrobacterium tumefaciens* C58. Science **294:**2323–2328.
17. **Huang, J., Z. Su, and Y. Xu.** 2005. The evolution of microbial phosphonate degradative pathways. J. Mol. Evol. V **61:**682–690.
18. **Igarashi, N., J. Harada, S. Nagashima, K. Matsuura, K. Shimada, and K. V. P. Nagashima.** 2001. Horizontal transfer of the photosynthesis gene cluster and operon rearrangement in purple bacteria. J. Mol. Evol. **52:**333–341.
19. **Jaubert, M., S. Zappa, J. Fardoux, J.-M. Adriano, L. Hannibal, S. Elsen, J. Lavergne, A. Vermeglio, E. Giraud, and D. Pignol.** 2004. Light and redox control of photosynthesis gene expression in *Bradyrhizobium*. Dual roles of two PpsR. J. Biol. Chem. **279:**44407–44416.
20. **Kaneko, T., Y. Nakamura, S. Sato, K. Minamisawa, T. Uchiumi, S. Sasamoto, A. Watanabe, K. Idesawa, M. Iriguchi, K. Kawashima, M. Kohara, M. Matsumoto, S. Shimpo, H. Tsuruoka, T. Wada, M. Yamada, and S. Tabata.** 2002. Complete genomic sequence of nitrogen-fixing symbiotic bacterium *Bradyrhizobium japonicum* USDA110. DNA Res. **9:**189–197.
21. **King, G. M.** 2003. Molecular and culture-based analyses of aerobic carbon monoxide oxidizer diversity. Appl. Environ. Microbiol. **69:**7257–7265.
22. **Kolber, Z. S., F. G. Plumley, A. S. Lang, J. T. Beatty, R. E. Blankenship, C. L. VanDover, C. Vetriani, M. Koblizek, C. Rathgeber, and P. G. Falkowski.** 2001. Contribution of aerobic photoheterotrophic bacteria to the carbon cycle in the ocean. Science **292:**2492–2495.
23. **Kyndt, J. A., T. E. Meyer, and M. A. Cusanovich.** 2004. Photoactive yellow protein, bacteriophytochrome, and sensory rhodopsin in purple phototrophic bacteria. Photochem. Photobiol. Sci. **3:**519–530.
24. **Larimer, F. W., P. Chain, L. Hauser, J. Lamerdin, S. Malfatti, L. Do, M. L. Land, D. A. Pelletier, J. T. Beatty, A. S. Lang, F. R. Tabita, J. L. Gibson, T. E. Hanson, C. Bobst, J. L. Torres y Torres, C. Peres, F. H. Harrison, J. Gibson, and C. S. Harwood.** 2004. Complete genome sequence of the metabolically versatile photosynthetic bacterium *Rhodopseudomonas palustris*. Nat. Biotechnol. **22:**55–61.
25. **Li, H., M. R. Sawaya, F. R. Tabita, and D. Eisenberg.** 2005. Crystal structure of a RuBisCO-like protein from the green sulfur bacterium *Chlorobium tepidum*. Structure **13:**779–789.
26. **Masuda, S., and C. E. Bauer.** 2002. AppA is a blue light photoreceptor that antirepresses photosynthesis gene expression in *Rhodobacter sphaeroides*. Cell **110:**613–623.
27. **Masuda, S., Y. Matsumoto, K. V. P. Nagashima, K. Shimada, K. Inoue, C. E. Bauer, and K. Matsuura.** 1999. Structural and functional analyses of photosynthetic regulatory genes *regA* and *regB* from *Rhodovulum sulfidophilum*, *Roseobacter denitrificans*, and *Rhodobacter capsulatus*. J. Bacteriol. **181:**4205–4215.
28. **Moran, M. A., A. Buchan, J. M. Gonzalez, J. F. Heidelberg, W. B. Whitman, R. P. Kiene, J. R. Henriksen, G. M. King, R. Belas, C. Fuqua, L. Brinkac, M. Lewis, S. Johri, B. Weaver, G. Pai, J. A. Eisen, E. Rahe, W. M. Sheldon, W. Ye, T. R. Miller, J. Carlton, D. A. Rasko, I. T. Paulsen, Q. Ren, S. C. Daugherty, R. T. Deboy, R. J. Dodson, A. S. Durkin, R. Madupu, W. C. Nelson, S. A. Sullivan, M. J. Rosovitz, D. H. Haft, J. Selengut, and N. Ward.** 2004. Genome sequence of *Silicibacter pomeroyi* reveals adaptations to the marine environment. Nature **432:**910–913.
29. **Moskvin, O. V., L. Gomelsky, and M. Gomelsky.** 2005. Transcriptome analysis of the *Rhodobacter sphaeroides* PpsR regulon: PpsR as a master regulator of photosystem development. J. Bacteriol. **187:**2148–2156.
30. **Ponnampalam, S. N., J. J. Buggy, and C. E. Bauer.** 1995. Characterization of an aerobic repressor that coordinately regulates bacteriochlorophyll, carotenoid, and light harvesting-II expression in *Rhodobacter capsulatus*. J. Bacteriol. **177:**2990–2997.
31. **Ragsdale, S. W.** 1991. Enzymology of the acetyl-CoA pathway of CO_2 fixation. Crit. Rev. Biochem. Mol. Biol. **26:**261–300.
32. **Sauer, U., and B. J. Eikmanns.** 2005. The PEP-pyruvate-oxaloacetate node as the switch point for carbon flux distribution in bacteria. FEMS Microbiol. Rev. **29:**765–794.
33. **Smart, J. L., J. W. Willett, and C. E. Bauer.** 2004. Regulation of *hem* gene expression in *Rhodobacter capsulatus* by redox and photosystem regulators RegA, CrtJ, FnrL, and AerR. J. Mol. Biol. **342:**1171–1186.
34. **Steunou, A. S., C. Astier, and S. Ouchane.** 2004. Regulation of photosynthesis genes in *Rubrivivax gelatinosus*: transcription factor PpsR is involved in both negative and positive control. J. Bacteriol. **186:**3133–3142.
35. **Stothard, P., and D. S. Wishart.** 2005. Circular genome visualization and exploration using CGView. Bioinformatics **21:**537–539.
36. **Ternan, N. G., J. W. McGrath, G. McMullan, and J. P. Quinn.** 1998.

Organophosphonates: occurrence, synthesis and biodegradation by microorganisms. World J. Microbiol. Biotechnol. V **14:**635–647.

37. **Thomas, J. W., J. W. Touchman, R. W. Blakesley, G. G. Bouffard, S. M. Beckstrom-Sternberg, E. H. Margulies, M. Blanchette, A. C. Siepel, P. J. Thomas, J. C. McDowell, B. Maskeri, N. F. Hansen, M. S. Schwartz, R. J. Weber, W. J. Kent, D. Karolchik, T. C. Bruen, R. Bevan, D. J. Cutler, S. Schwartz, L. Elnitski, J. R. Idol, A. B. Prasad, S. Q. Lee-Lin, V. V. B. Maduro, T. J. Summers, M. E. Portnoy, N. L. Dietrich, N. Akhter, K. Ayele, B. Benjamin, K. Cariaga, C. P. Brinkley, S. Y. Brooks, S. Granite, X. Guan, J. Gupta, P. Haghighi, S. L. Ho, M. C. Huang, E. Karlins, P. L. Laric, R. Legaspi, M. J. Lim, Q. L. Maduro, C. A. Masiello, S. D. Mastrian, J. C. McCloskey, R. Pearson, S. Stantripop, E. E. Tiongson, J. T. Tran, C. Tsurgeon, J. L. Vogt, M. A. Walker, K. D. Wetherby, L. S. Wiggins, A. C. Young, L. H. Zhang, K. Osoegawa, B. Zhu, B. Zhao, C. L. Shu, P. J. De Jong, C. E. Lawrence, A. F. Smit, A. Chakravarti, D. Haussler, P. Green, W. Miller, and E. D. Green.** 2003. Comparative analyses of multi-species sequences from targeted genomic regions. Nature **424:**788–793.
38. **Wood, H. G., C. H. Werkman, A. Hemingway, and A. O. Nier.** 1941. The position of carbon dioxide in succinic acid synthesized by heterotrophic bacteria. J. Biol. Chem. **139:**377–381.
39. **Yurkov, V. V., and J. T. Beatty.** 1998. Aerobic anoxygenic phototrophic bacteria. Microbiol. Mol. Biol. Rev. **62:**695–724.
40. **Yutin, N., and O. Beja.** 2005. Putative novel photosynthetic reaction centre organizations in marine aerobic anoxygenic photosynthetic bacteria: insights from metagenomics and environmental genomics. Environ. Microbiol. **7:**2027–2033.
41. **Zupan, J., T. R. Muth, O. Draper, and P. Zambryski.** 2000. The transfer of DNA from *Agrobacterium tumefaciens* into plants: a feast of fundamental insights. Plant J. **23:**11–28.