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# Source Environment Feature Related Phylogenetic Distribution Pattern of Anoxygenic Photosynthetic Bacteria as Revealed by *pufM* Analysis

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**Anoxygenic photosynthesis, performed primarily by anoxygenic photosynthetic bacteria (APB), has been supposed to arise on Earth more than 3 billion years ago. The long established APB are distributed in almost every corner where light can reach. However, the relationship between APB phylogeny and source environments has been largely unexplored. Here we retrieved the *pufM* sequences and related source information of 89 *pufM* containing species from the public database. Phylogenetic analysis revealed that horizontal gene transfer (HGT) most likely occurred within 11 out of a total 21 *pufM* subgroups, not only among species within the same class but also among species of different phyla or subphyla. A clear source environment feature related phylogenetic distribution pattern was observed, with all species from oxic habitats and those from anoxic habitats clustering into independent subgroups, respectively. HGT among ancient APB and subsequent long term evolution and adaptation to separated niches may have contributed to the coupling of environment and *pufM* phylogeny.**

***Keywords***: anoxygenic photosynthesis, anoxygenic photosynthetic bacteria (APB), *pufM*, phylogenetic analysis, source environment, horizontal gene transfer (HGT)

Anoxygenic photosynthesis is widely distributed in the environments and plays an important role in the conversion process of solar energy into chemical energy, as well as in the elemental biogeochemical cycles, such as carbon and sulfur. Anoxygenic photosynthesis is mainly performed by 5 major anoxygenic photosynthetic bacterial (APB) groups, i.e. green sulfur bacteria (*Chlorobi*), green non sulfur bacteria (*Chloroflexi*), purple sulfur bacteria (*γ-Proteobacteria*), purple non sulfur bacteria (*α-Proteobacteria*), low G+C Gram-positive heliobacteria (*Firmicutes*) and the obligately aerobic APB (*a, β-Proteobacteria*) (Yurkov and Beatty, 1998). Due to the great phylogenetic diversity and long evolutionary history of the APB group on Earth, great attention has long been paid to the origin and evolution of anoxygenic photosynthesis (Nagashima *et al.*, 1997; Xiong *et al.*, 2000; Igarashi *et al.*, 2001; Raymond *et al.*, 2002).

Phylogenetic analysis of APB based on 16S rRNA genes (Woese, 1987; Olsen *et al.*, 1994), house keeping genes, such as Hsp60 and Hsp70 (Gupta *et al.*, 1999), genes involved in the bacteriochlorophyll biosynthesis pathway (Xiong *et al.*, 2000) or even whole genome analysis (Raymond *et al.*, 2002) has revealed many different phylogenetic patterns and prposed hypotheses concerning the evolution of APB. All these analyses were conducted from the viewpoint of molecular evolution, largely based on the inherent differences in nucleotide or amino acid sequences to deduce the evolutionary relationship among APB or between APB and other photosynthetic organisms. However, bacteria and the Earth environment are always interacting and inter- shaping especially with respect to APB, which was supposed to evolve as early as 3 billion years ago. This interaction would result in the adaptation of these bacteria and Earth to each other. The emergence of oxygenic photosynthetic bacteria is such a case, eventually transforming the ocean from anoxic to oxygenated status. With increasing diversity of niches, adaptation would furthermore result in increasing APB diversity. Intensive isolation and culture work confirmed this point and revealed that APB habitats covered soil, sewage, freshwater and brackish water, sediments and even hot springs and thermal vents on the sea floor (Overmann and Garcia-Pichel, 2003; Beatty *et al.*, 2005), that is, nearly every corner of the Earth where light radiation can reach.

This series of highly diverse habitats raises a fundamental issue as to whether APB or anoxygenic photosynthesis phylogeny would be imprinted by the source environment features. This issue has remained largely unexplored due to the insufficiency of data. A few studies based on 16S rRNA gene sequences revealed the compact clustering of APB in the phylogenetic trees (Woese, 1987; Olsen *et al.*, 1994), irrespective of isolated environmental features. This phylogenetic distribution pattern may largely be due to the high evolutionary pressure of the 16S rRNA gene as one of the most conserved genes in the bacterial genome. Other studies related to the molecular evolution of APB that were based on functional genes or the genome have never correlated the phylogeny and source environments (Nagashima *et al.*, 1997; Xiong *et al.*, 2000; Igarashi *et al.*, 2001; Raymond *et al.*, 2002).

In contrast to conserved house keeping genes, the functional genes involved in anoxygenic photosynthesis have re-

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**Table 1.** Taxonomic affiliation and source information of the 89 *pufM* containing species analyzed in this work

| Phylum Class /Order | Species | Source environment | Oxic status | *Subgroup |
|---|---|---|---|---|
| **Proteobacteria** | *Acidiphilium acidophilum* | culture of iron-grown *T. ferrooxidans* | + | A |
| Alpha I | *Acidiphilium angustum* | acidic coal mine drainage | + | A |
| | *Acidiphilium cryptum* | acid mine water | + | A |
| | *Acidiphilium multivorum* | acid mine water | + | A |
| | *Rhodospirillum molischianum* | mud from a ditch | - | F |
| | *Rhodospirillum photometricum* | freshwater pond | - | P |
| | *Rhodospirillum rubrum* | mud from pasture land | - | P |
| | *Rhodospirillum rubrum* ATCC11170 | NA | NA | P |
| | *Roseococcus thiosulfatophilus* | cyanobacterial mat from hot spring | + | J |
| | *Rubritepida flocculans* | hot spring | + | J |
| Alpha III | *Jannaschia* sp. CCS1 | Pacific coastal waters | + | C |
| | *Loktanella vestfoldensis* SKA53 | microbial mats in Antarctic lakes | + | L |
| | *Rhodobacter azotoformans* | photosynthetic sludge | - | G |
| | *Rhodobacter blasticus* | eutrophic pond | - | G |
| | *Rhodobacter capsulatus* B10 | duck farm outlet/sewage/ditch | - | G |
| | *Rhodobacter capsulatus* | duck farm outlet/sewage/ditch | - | G |
| | *Rhodobacter sphaeroides* | small river/pond waters | - | G |
| | *Rhodobacter sphaeroides* ATCC17023, 17029, 17025 | NA | NA | G |
| | *Rhodobacter veldkampii* | freshwater pond | - | G |
| | *Rhodovulum sulfidophilum* | mud from intertidal flats | - | F |
| | *Roseobacter denitrificans* MBIC2684 | Japan coastal sediment | +/- | D |
| | *Roseobacter denitrificans* OCh 114 | marine sediment | +/- | D |
| | *Roseobacter litoralis* | marine algae | + | D |
| | *Roseobacter* sp. BS90 | northwest shelf of the Black Sea | + | E |
| | *Roseobacter* sp. SO3 | Southern Ocean | + | I |
| | *Roseobacter* sp. SYOP2 | Sydney beach | + | E |
| | *Roseobacter* sp. BS110 | deep Mediterranean waters | + | I |
| | *Roseobacter denitrificans* | marine sediment | +/- | D |
| | *Roseovarius* sp. 217 | UK.Coastal seawater | + | C |
| Alpha IV | *Blastomonas* sp. NT12 | freshwater | + | K |
| | *Blastomonas natatoria* | freshwater swimming pool | + | K |
| | *Blastomonas ursincola* | freshwater cyanobacterial mat of thermal springs | + | K |
| | *Citromicrobium* sp. CV44 | Mediterranean or Red seas | + | B |
| | *Erythrobacter litoralis* | Cyanobacterial mat | + | L |
| | *Erythrobacter longus* | seaweed | + | L |
| | *Erythrobacter* sp. BA13 | Northern East Pacific ocean | + | L |
| | *Erythrobacter* sp. COL13 | French Mediterranean coast | + | L |
| | *Erythrobacter* sp. MBIC3019 | Japan coastal seawater | + | L |
| | *Erythrobacter* sp. MBIC3031 | Palau, Pacific Ocean | + | L |
| | *Erythrobacter* sp. MBIC3361 | Ishigaki Island Seawater, Japan | + | L |
| | *Erythrobacter* sp. MBIC3960 | Hamadume sea coast | + | L |
| | *Erythrobacter* sp. MG3 | Southern East Atlantic Ocean | + | L |
| | *Erythrobacter* sp. NAP1 | Northern West Atlantic Ocean | + | L |
| | *Erythrobacter* sp. MBIC3017 | Pacific Ocean, Nansei Islands, Japan | + | L |
| | *Erythromicrobium ramosum* | Cyanobacterial mat from hot spring | + | L |
| | *Agrobacterium sanguineum* | Baltic Sea | + | L |
| | *Porphyrobacter neustonensis* | eutrophic freshwater pond | + | L |
| | *Porphyrobacter tepidarius* MBIC3363 | thermophilic.brackish hot spring, Japan | + | L |
| | *Sphingomonas* sp. PB180, PB229, PB56 | NA | + | K |

| Phylum Class /Order | Species | Source environment | Oxic status | *Subgroup |
|---|---|---|---|---|
| Alpha VI | *Roseospirillum parvum* | microbial mat covering intertidal sandy sediments | - | P |
| | *Blastochloris sulfoviridis* DSM729 | sulfur spring | +/- | B |
| | *Bradyrhizobium denitrificans* USDA 4427, 4391, 4393, 4403, 4440 | rhizobia.symbiont | + | M |
| | *Bradyrhizobium* sp. ORS278 | plant nodules | + | M |
| | *Methylobacterium extorquens* ATCC14718 | soils/plant surface | + | S |
| | *Methylobacterium extorquens* ATCC8457 | soils/plant surface | + | S |
| | *Methylobacterium radiotolerans* ATCC27329 | unhulled rice | + | S |
| | *Methylobacterium rhodinum* ATCC14821 | Alnus rhizosphere | + | S |
| | *Rhodomicrobium vannielii* MBIC2956 | NA | - | P |
| | *Rhodomicrobium vannielii* | NA | - | P |
| | *Rhodoplanes elegans* | activated sludge | - | N |
| | *Rhodopseudomonas palustris* BisB18, BisA53, BisB5 | temperate soil/water environments | +/- | O |
| Beta | *Rhodocyclus tenuis* | forest pond | - | P |
| | *Rhodoferax antarcticus* | Antarctic microbial mat | - | R |
| | *Rhodoferax fermentans* | Sewage ditch | - | R |
| | *Roseateles depolymerans* | Hanamuro river, Japan | + | Q |
| | *Rubrivivax gelatinosus* | Fresh water pond | - | H |
| Gamma | *Allochromatium vinosum* strain D | ditch water | - | P |
| | *Allochromatium vinosum* | ditch water | - | P |
| | *Congregibacter litoralis* KT71 | North sea coastal surface water | + | Q |
| | *Ectothiorhodospira shaposhnikovii* | salt flat of soda lake | - | P |
| | *Lamprocystis purpureus* | stratified freshwater | - | H |
| | *Thiocapsa roseopersicina* | Microbial mats on the Orkney Islands | - | P |
| | *Thiocystis gelatinosa* | meromictic lake | - | O |
| **Bacteroidetes** Flavobacteria | Psychroflexus torquis ATCC700755 | Antarctic sea ice | + | T |
| **Chloroflexi** Chloroflexales | *Chloroflexus aurantiacus* | hot spring | - | U |
| | *Chloroflexus aurantiacus* J-10-fl | hot spring | - | U |
| | *Chloroflexus aggregans* DSM 9485 | hot spring | - | U |
| | *Roseiflexus castenholzii* | hot spring | - | U |

*Subgroup is based on phylogenetic analysis of *pufM* sequences.
NA, Not available

ceived much less evolutionary pressure and are easily prone to HGT (Xiong *et al.*, 2000; Raymond *et al.*, 2002). Therefore, anoxygenic photosynthesis genes are most likely to show an environmental feature related phylogenetic distribution pattern. To test this hypothesis, we targeted a marker gene of anoxygenic photosynthesis, *pufM*, which encodes the photosynthetic reaction center M subunit and has been widely used to study APB diversity or evolution (Nagashima *et al.*, 1997; Igarashi *et al.*, 2001; Beja *et al.*, 2002; Allgaier *et al.*, 2003; Schwalbach and Fuhrman, 2005; Yutin *et al.*, 2005; Du *et al.*, 2006; Hu *et al.*, 2006; Okubo *et al.*, 2006; Ranchou-Peyruse *et al.*, 2006). The large data set of *pufM* produced from bacterial cultures provided a good opportunity to perform an analysis of the HGT potential of *pufM*, and the relationship between *pufM* phylogenetic distribution pattern and source environment features. In this work, the *pufM* sequences currently available in the public database and related source information concerning *pufM* containing bacteria were carefully collected and thoroughly analyzed.

## Materials and Methods

### *Collection of pufM sequences and source environment information of APB strains*

All *pufM* sequences from bacterial pure cultures were retrieved from the GenBank database (up to March 2007) through gene name search or using the BLAST tool (http://www.ncbi.nih.gov/blast). The taxonomic affiliation of *pufM*-containing species was confirmed through the 16S rRNA gene sequence classifier tool in Ribosomal Database Project II website (RDP II; http://rdp.cme.msu.edu/). Those *pufM*-containing species having no corresponding 16S rRNA gene sequence recorded in the database were omitted from fur-

ther analysis. Source environment information of related strains was obtained from GenBank records or descriptions in the linked references. APB habitat information was complemented from references (Blankenship *et al.*, 1995; Overmann and Garcia-Pichel, 2003) where needed.

***Phylogenetic analysis of pufM sequences***

All *pufM* sequences were multi-aligned using the CLUSTAL X 1.81 program (Thompson *et al.*, 1997). The common sequence region was retained for subsequent phylogenetic analysis. The neighbor joining (NJ), minimum evolution (ME) and maximum parsimony (MP) phylogenetic trees were inferred using the molecular evolution software package Mega 3.0 (Kumar *et al.*, 2004), and the MrBayes program (ver. 3.1.2; http://morphbank.ebc.uu.se/mrbayes) was used to infer the Bayesian tree. Only the first and second codon positions of the *pufM* open reading frame were involved for phylogenetic analysis in order to avoid potential substitution saturation and compositional bias at the third position. The NJ, ME, and MP trees were bootstrapped by 1,000 replications with correction for multiple substitutions. The Bayesian tree was generated using a general time-reversible model plus gamma distribution and the invariant sites model of molecular evolution. Bayesian trees were sampled every 100 generations, and 2,000 "burn-in" trees were excluded from the consensus tree. Topology of all four types of tree was further assessed manually to search the most stable nodes. To increase the resolution of closely related species, all three codon positions of closely clustered *pufM* sequences in the same subgroup were re-subjected to phylogenetic analysis, and the sub-trees yielded were merged into a whole *pufM* tree.

## Results and Discussion

***Inference of pufM phylogenetic tree and topological analysis***

Records of 89 species that have both *pufM* and 16S rRNA gene sequence were found in the GenBank database. The corresponding full-length or partial *pufM* sequences and source environments' information were retrieved. These species were classified into 3 phyla and 8 classes (Table 1), covering 4 major APB groups, i.e. green non sulfur bacteria, purple sulfur bacteria, purple non sulfur bacteria and aerobic anoxygenic photosynthetic bacteria. Preliminary multi-alignment analysis of retrieved *pufM* sequences indicated that the shared region by all *pufM* fragments was about 190-200 bp, right corresponding to the most targeted region for amplification of *pufM* partial sequences from environmental community DNA using previously successfully designed *pufM* primers (Beja *et al.*, 2002). Previous environmental *pufM* survey and phylogenetic analysis also confirmed this region's adequacy of information for phylogenetic analysis (Schwalbach and Fuhrman, 2005; Du *et al.*, 2006; Hu *et al.*, 2006; Okubo *et al.*, 2006; Ranchou-Peyruse *et al.*, 2006). In this work, a phylogenetic tree was also inferred based on this region with the exclusion of the third codon position (Fig. 1). For the tree's subgroup, phylogenetic analysis based on all three codon positions showed generally consistent tree topology with that inferred from only the first and second codon positions of *pufM* partial sequence (data not shown), indicating that the sequence information of the first two codon positions is enough for phylogenetic inference of the whole *pufM* partial sequences. The third codon position, however, would provide higher resolution if more *pufM* fragments of closely related species are sequenced for phylogenetic analysis in the future.

Based on the tree's overall topology and node reliability, all sequences were further classified into 21 subgroups (Fig. 1). Only the clustering of 9 subgroups (Subgroups A, D, E, G, I, M, O, R, and S) was consistent with their taxonomical affiliation determined by the 16S rRNA genes, whereas the other 12 subgroups contained species from different taxonomic units, such as family or genus, indicating that HGT likely occurred among these APB groups. Potential HGT among APB was also proposed previously (Nagashima *et al.*, 1997; Xiong *et al.*, 2000; Raymond *et al.*, 2002), based mainly on the observation of deviant nucleotide composition and anomalous phylogenetic distribution. Ochman *et al.* (2000) suggested that a large proportion of bacterial genes (up to 20% in the genome) can be rapidly exchanged. Raymond *et al.* (2002) further conducted whole genome analysis of phototrophic bacteria, and revealed that horizontal gene flow has played a major role in the evolution of bacterial phototrophs, and that many of the essential components of photosynthesis have been among these horizontally transferred genes. Although there is no direct experimental evidence that *pufM* was involved in this horizontal gene flow, given the long history and diverse niches of APB, potential HGT among APB most likely occurred as suggested by the interlaced tree topology in this work.

On the other hand, 4 anoxic subgroups (Subgroups F, G, H, and P) showed unstable topology with low bootstrap value support. The instability of some peripheral or high-order parts or nodes of the tree were also observed. The instability indicated that many more *pufM* containing bacteria remain to be cultured from environments and sequenced to fill these gaps, given that the evolution of *pufM* containing bacteria is a continuing process. Sequencing *pufM* directly from environmental community DNA samples provided much information about the evolution of *pufM* in the environment (Beja *et al.*, 2002; Allgaier *et al.*, 2003; Oz *et al.*, 2005; Schwalbach and Fuhrman, 2005; Yutin *et al.*, 2005; Du *et al.*, 2006; Hu *et al.*, 2006; Okubo *et al.*, 2006; Ranchou-Peyruse *et al.*, 2006) and made it possible to depict the full *pufM* evolutionary picture. However, the absence of the corresponding 16S rRNA gene or other taxonomical information concerning uncultured *pufM* containing bacteria makes it impossible to assess the potential of HGT. Meanwhile, there is also another possibility to explain these gaps, i.e. that some transitional form of *pufM* has disappeared or been eliminated due to non-adaptation to the environment in the long evolutionary course of APB. Considering the great non-cultivability of bacteria in the environment (Keller and Zengler, 2004), the culturing of *pufM* containing bacteria should be our first step at present in order to provide more insight into the confused parts of the phylogenetic tree.

As seen from the phylogenetic tree, HGT likely occurred not only among the species within the same class but also among the species of different phyla or subphyla. Subgroups O and P contained both alpha and gamma; subgroups H

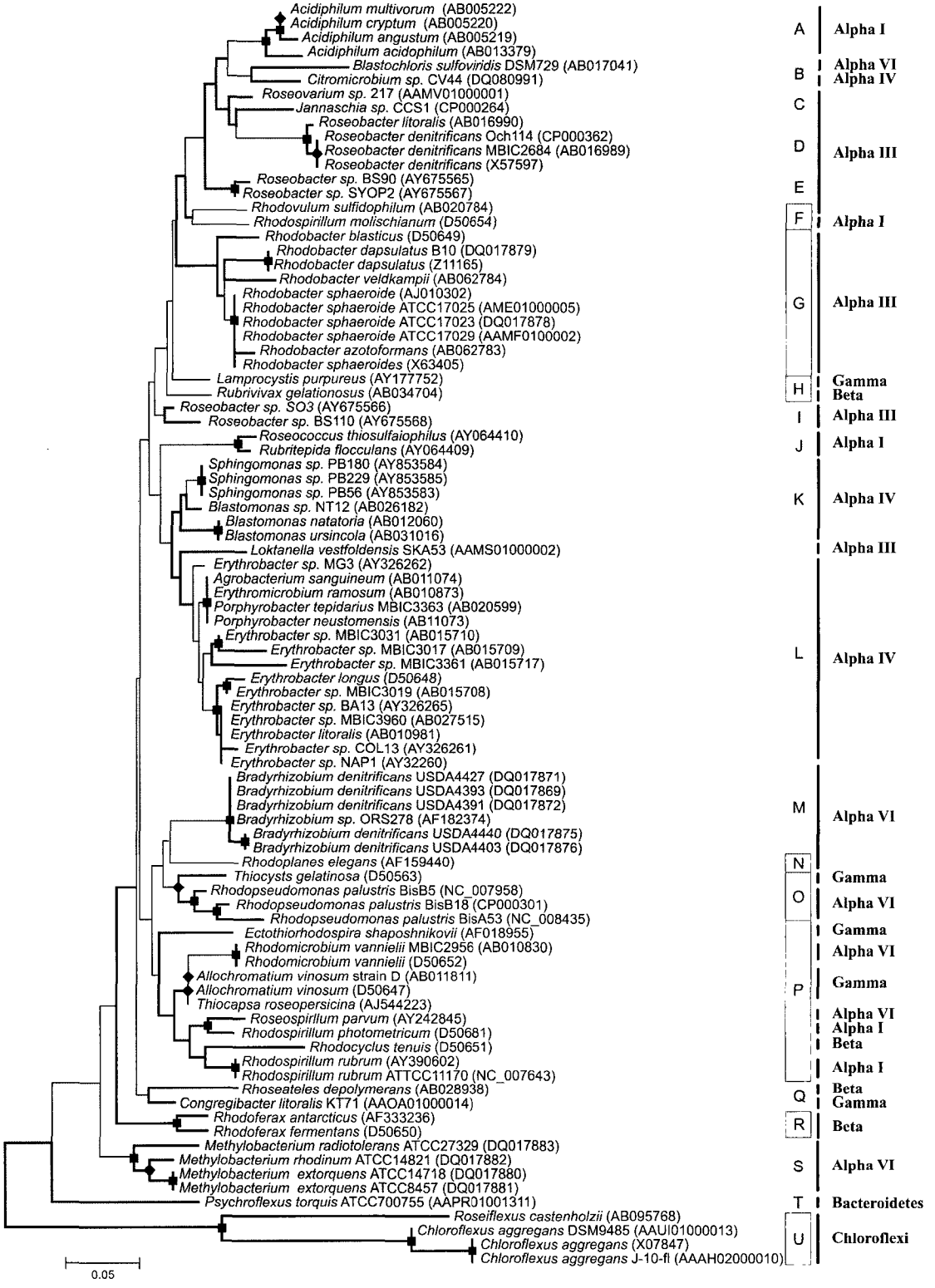


**Fig. 1.** Phylogenetic analysis of *pufM* gene partial sequences from 89 *pufM* containing species. Four algorithms, neighbor joining, minimum evolution, maximum parsimony and Bayesian, were used to infer trees and the consensus tree was manually created. The bold black branches represent the most stable topological structure that appeared in all four trees. The nodes with the symbol "■" have the bootstrap value higher than 50% in all four trees, and those with the symbol "◆" have the bootstrap value higher than 50% in three of the total four trees. The scale bar represents 5% nucleotide substitution. Species in the grey region were isolated from oxic environments. The right column lists the subgroup and taxonomic affiliation of each species. Alpha, Beta and Gamma represent alpha-, beta- and gamma-proteobacteria respectively.

and Q contained both beta and gamma. Furthermore, genome sequencing for the first time revealed that *Bacteriodetes* contained an analog of *pufM* (Bowman *et al.*, 2006). Phylogenetic analysis placed this sequence into an independent clade, suggesting a large gap in current knowledge about *pufM* evolution. Despite the deficiency of direct experimental data to confirm its photosynthetic ability at present, this broadened the potential category of bacteria with the ability of anoxygenic photosynthesis. Recently, a newly isolated marine *Flavobacteria* was also found to contain the proteorhodopsin (PR) gene and light can stimulate its growth (Gomez-Consarnau *et al.*, 2007). PR based photosynthesis is another special mechanism transforming the light energy into chemical energy, and is also prone to HGT (Frigaard *et al.*, 2006). These new findings suggest that evolution favors organisms with the potential to complement their chemotrophic life style with phototrophy.

Overall, subgroups of Alpha-proteobacteria, Beta-proteobacteria, Gamma-proteobacteria, Bacteroidetes, and Chloroflexi are interlacedly distributed in crossed subgroups. HGT most likely resulted in this interlacement and gave rise to great incongruence within the bacterial 16S rRNA gene phylogeny. From the ecological viewpoint, HGT would likely provide an advantage to the niche's extendedness and also preservation of the anoxygenic photosynthesis function. This also further revealed different evolutionary course of APB species and the physiological function of anoxygenic photosynthesis. The evolution of APB may be dominated by a vertical genetic process, while the evolution of anoxygenic photosynthesis may be more prone to the horizontal process, i.e. HGT. For mixotrophic APB, photosynthetic ability was dispensable to their growth, making the anoxygenic photosynthesis operon easily transferred among APB or between APB and non-photosynthetic bacteria, as with other metabolic traits among bacteria (see the review by Gogarten and Townsend, 2005). The species with anoxygenic photosynthesis dispensable to their growth, such as APB of the phylum Bactoroidetes and the genera *Roseobacter*, *Erythrobacter*, etc., can grow without the energy supplied by photosynthesis, likely resulting in the photosynthesis function being easily lost evolutionarily. As an important vehicle of HGT, viruses have been reported to be widely distributed in great abundance in natural environments (see reviews by Wommack and Colwell, 2000; Weinbauer, 2004) and could carry photosynthesis genes, such as *psb* (Mann *et al.*, 2003; Lindell *et al.*, 2004; Millard *et al.*, 2004). We suggest that viruses also play an important role in the *pufM*'s HGT. However, more work on the isolation of APB viruses needs to be carried out to provide direct evidence.

### *Relatedness analysis of pufM phylogeny and source environments*

Source environments of *pufM* containing bacteria studied in this work covered freshwater, seawater, hot spring, sludge, plant symbiosis, terrestrial soil, sediment or mud, sewage or ditch, pond and acid mine waters, etc. The versatility of habitats makes it difficult to classify for performing the correlation analysis with *pufM* phylogeny. However, the physiological status of APB is very different in aerobic and anaerobic conditions where APBs use aerobic and anaerobic electron transport chains respectively (Zannoni, 1995). To assess whether habitat's oxic status, one of the most important environmental variables for bacterial physiology, exhibit somewhat relatedness to *pufM* phylogeny, we classified the habitats into two groups, oxic and anoxic (Table 1). When combining information for the oxic status of habitat with *pufM* phylogeny (Fig. 1), a clear pattern was observed that phylogenetic clustering was closely related to the oxic status of the habitat of species within the cluster. All species from oxic habitats (in grey, Fig. 1) and those from anoxic habitats clustered into independent subgroups. A total of 13 oxic subgroups (subgroups A-E, I-M, Q, S, and T) and 8 anoxic groups (subgroups F-H, N-P, R, and U) were seen from Fig. 1. The biggest oxic group A-E contained species from Alpha I, III, IV, and VI, the sources of which covered acid mine water, sulfur springs, seawater, etc.; the biggest anoxic group I-M contained species from Alpha I and VI, Beta and Gamma with the habitats covering stratified lake, soda lake, ditch, pond, microbial mat, mud, etc. These environments are geographically almost completely isolated, making it a challenge to explain their tight clustering in *pufM* phylogeny. We suggest that, considering that all APB have been proposed to share a common ancestor that evolved in the early history of Earth (Woese, 1987), HGT among ancient *pufM* containing bacteria and their subsequent long term evolution and adaptation to separated niches may have contributed to the coupling of environment and *pufM* phylogeny.

The influence of the features of source environments on *pufM* phylogeny was also reflected in the fact that species from highly differentiated specific niches, such as acid mine water (subgroup A), plant symbiosis (subgroup M) and hot springs (subgroups J and U), clustered tightly, whereas clustering of these species with those from different niches had relatively low tree topological support. This also may suggest limited *pufM* horizontal transfer events between these differentiated niches and other more common environments, which could be explained by the facts that, 1) although microbes inhabited nearly every corner of the Earth and showed extremely high overall diversity, the diversity in specific restricted niches can be different to a great extent (Keller and Zengler, 2004); 2) highly differentiated niches often have low biodiversity, and heterogeneous living environments benefit bacterial diversification (Rainey and Travisano, 1998; Maharjan *et al.*, 2006); 3) HGT should be based on close contact and interaction among species in the same environments, and therefore gene flow always occurred locally. The accumulation of this type of region-restricted HGT and species vertical evolution could result in the environmental features dependent distribution pattern of *pufM* phylogeny.

Among the aerobic species, a large number come from marine or river systems, which are the two biggest oxic regions on Earth. In the marine environment, aerobic APB have attracted many attentions due to their high abundance and diversity since Kolber *et al.* (2000) for the first time revealed the common presence of anoxygenic photosynthesis in oxic seawaters. Beja *et al.* (2002) further revealed unexpected high diversity of aerobic APB in marine environments based on *pufM* and environmental genome analysis. Recently, Du *et al.* (2006) found abundant gamma-proteobacteria like *pufM* sequences in Pacific surface seawater

and Fuchs *et al.* (2007) further isolated the first gammaproteobacteria aerobic APB from North Sea. These reports revealed high HGT potential of *pufM* within oxic aquatic systems. Considering the vast area and diverse environments of the ocean, many more new *pufM* containing bacterial groups are ready to be discovered in the sea. More anoxygenic photosynthesis gene sequence data from pure cultures will undoubtedly contribute much to a better understanding of the co-evolution of anoxygenic photosynthesis and environments.

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