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Phylogenetic analysis of aerobic anoxygenic phototrophic bacteria and their relatives based on farnesyl pyrophosphate synthase gene

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Abstract
The study aims to reveal phylogenetic and evolutionary relationship between aerobic anoxygenic phototrophic bacteria (AAnPB) and their relatives, anaerobic anoxygenic phototrophic bacteria (AnAnPB) and nonphototrophic bacteria (NPB, which had high homology of 16S rDNA gene with AAnPB and fell into the same genus), and validate reliability and usefulness of farnesyl pyrophosphate synthase (FPPS) gene for the phylogenetic determination. FPPS genes with our modified primers and 16S rDNA genes with general primers, were amplified and sequenced or retrieved from GenBank database. In contrast to 16S rDNA gene phylogenetic tree, AAnPB were grouped into two clusters and one branch alone with no intermingling with NPB and AnAnPB in the tree constructed on FPPS. One branch of AAnPB, in both trees, was located closer to outgroup species than AnAnPB, which implicated that some AAnPB would be diverged earlier in FPPS evolutionary history than AnAnPB and NPB. Some AAnPB and NPB were closer located in both trees and this suggested that they were the closer relatives than AnAnPB. Combination codon usage in FPPS with phylogenetic analysis, the results indicates that FPPS gene and 16S rRNA gene have similar evolutionary pattern but the former seems to be more reliable and useful in determining the phylogenic and evolutionary relationship between AAnPB and their relatives. This is the first attempt to use a molecular marker beside 16S rRNA gene for studying the phylogeny of AAnPB, and the study may also be helpful in understanding the evolutionary relationship among phototrophic microbes and the trends of photosynthetic genes transfer.

Key words: aerobic anoxygenic phototrophic bacteria, farnesyl pyrophosphate synthase, phylogeny, anaerobic anoxygenic phototrophic bacteria, nonphototrophic bacteria

1 Introduction

Aerobic anoxygenic phototrophic bacteria (AAnPB) containing bacteriochlorophyll a (Bchl a), are widely distributed in the euphotic zone and play a significant role in biogeochemical cycle (Zeng et al., 2009; Salka et al., 2008; Jiao et al., 2003; Béjà et al., 2002; Kolber et al., 2001).

AAnPB consisted of diverse members interspersed predominantly throughout the alphaproteobacteria, and they appeared to be closely related to anaerobic anoxygenic phototrophic (AnAnPB) as well as nonphototrophic bacteria (NPB) from phylogenetic analysis based on 16S rRNA (Beatty, 2002; Yurkov and Beatty, 1998). Here, these AnAnPB and NPB which had high similarities of 16S rRNA with AAnPB were called the relatives of the latter. In 16S rRNA phylogeny, some NPB were heavily intermingled with AAnPB and their number is now increasing. However these NPB were evidently absent of *Bchl* and *puf* genes for being really phototrophic bacteria (Yurkov and Cstonyi, 2009; Rathgeber et al., 2004), which raised the question of the understanding of the intermingling of AAnPB and NPB. Despites of full use of 16S rRNA in bacterial phylogeny, only 16S rRNA sequences analysis could not resolve the confused problem of the intermingling of AAnPB with NPB. Moreover the intermingling would lead to the misunderstanding of the biodiversity and significance of bacteria, especially heterotrophic-and-phototrophic AAnPB in environmental samples, which were studied on the basis of 16S rRNA solely. Thus other phylogenetic molecule marker was necessary to lend credence to the 16S rRNA phylogeny of AAnPB.

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To our knowledge, little report was about the subject, particularly about phylogenetic and evolutionary relationship between AAnPB and NPB. Obviously none of photosynthetic genes could directly be served to expose the truth since the absence of these genes in NPB. Gupta (2003) proposed that the analysis of the shared conserved insert or deletions (indels) in protein sequences would be powerful to understand how the main groups within bacteria have evolved from a common ancestor. Besides the indels sequences mentioned by Gupta (2003), Farnesyl diphosphate synthase (FPPS, EC 2.5.1.10/ EC 2.5.1.1) was also showed to be reliable and useful in phylogenetic and revolutionary studies (Wang and Ohnuma, 1999; Chen and Poulter, 1993). The previous work (Cantera et al., 2002a, b) unexpectedly revealed that *Rps. palustris* ATCC 17001, the representative of subclass α-2 of alphaproteobacteria of AnAnPB, was not intermingled with other α-2 members; this was the remarkable incongruence between farnesyl diphosphate synthase and 16S rRNA gene sequences phylogenies.

FPPS is a key enzyme in the biosynthesis of isoprenoids, through which supplies precursors for several classes of important metabolites including sterols, (bacterio)chlorophyll, carotenoids, and ubiquinones (Ogura et al., 1997). Isoprenoids are known as the most ancient (Summons et al., 1999) and diverse class of natural product (Lange et al., 2000) and many of them are general biomarkers for bacterial grouping.

In this study, we compared the phylogeny of FPPS and 16S rRNA genes with the aim to validate the reliability and usefulness of FPPS sequence for phylogenetic and evolutionary analysis of the AAnPB (including type strains and our own isolates of *Erythrobacter* and *Roseobacter*), and their relatives AnAnPB and NPB. This may provide a solution key to the intermingling of AAnPB and their relatives, and help to understand the origin and evolution of photosynthesis.

2 Materials and methods

2.1 *Bacterial strains*

The strains used in this study are listed in Table 1. Our isolates (name containing JL-) were isolated by the method described previously (Koblizek et al., 2003) from the South China Sea (18.50–23.86°N, 111.45–117.45°E) during February and July in 2004

Table 1. Strains and GenBank accession numbers of farnesyl pyrophosphate synthase gene and 16S rRNA/rDNA used in this study

Strain	Phylogenetic group	Phototrophic characteristics	Accession no.	
			FPPS/GGPP	16S rRNA
Roseobacter denitrificans DSM 7001	α-3	aerobic anoxygenic phototrophic	AY998051	L01784
Roseobacter sp. JL-129	α-3	aerobic nonphotosynthetic	AY998067	AY646161
Sagittula sp. JL-351	α-3	aerobic anoxygenic phototrophic	AY998079	DQ104407
Roseobacter sp. JL-135	α-3	aerobic nonphototrophic	AY998068	AY745857
Rhodobacter blasticus ATCC 33485	α-3	anaerobic anoxygenic phototrophic	AB053173	D16429
Rhodobacter sp. AP-10	α-3	anaerobic anoxygenic phototrophic	AB062882	AB079681
Rhodobacter azotoformans JCM 9340	α-3	anaerobic anoxygenic phototrophic	AB053174	D70846
Rhodobacter sphaeroides ATCC 1167	α-3	anaerobic anoxygenic phototrophic	AB028044	D16425
Rhodobacter capsulatus ATCC 11166	α-3	anaerobic anoxygenic phototrophic	AB028046	D13474
Rhodovulum sulfidophilum DSM 1374	α-3	anaerobic anoxygenic phototrophic	AB028047	D13475
Rhodovulum strictum JCM 9220	α-3	anaerobic anoxygenic phototrophic	AB053176	AB079682
Rhodovulum sp. CP-10	α-3	anaerobic anoxygenic phototrophic	AB062884	AB079682
Rhodopseudomonas palustris ATCC 17001	α-3	anaerobic anoxygenic phototrophic	AB062883	D25312
Erythrobacter litoralis DSM 8509	α-4	aerobic anoxygenic phototrophic	AY998049	AF465836
Citromicrobium sp. JL-354	α-4	aerobic anoxygenic phototrophic	AY998080	DQ104408
Erythrobacter litoralis HTCC2594	α-4	aerobic anoxygenic phototrophic	AAGG01000005.1	NZ_AAGG01000008
Erythrobacter sp. JL-475	α-4	aerobic anoxygenic phototrophic	AY998082	DQ104409
Erythrobacter longus DSM 6997	α-4	aerobic anoxygenic phototrophic	AY998050	AF465835
Erythrobacter sp. JL-316	α-4	aerobic nonphotosynthetic	AY998058	AY646157
Erythrobacter sp. JL-310	α-4	aerobic nonphotosynthetic	AY998065	AY646156
Erythrobacter sp. JL-435	α-4	aerobic nonphototrophic	AY998081	DQ285071
Erythrobacter sp. JL-378	α-4	aerobic nonphototrophic	DQ104395	DQ285076
Bradyrhizobium japonicum USDA 110	α-2	aerobic nonphototrophic	U12678	D13430
Sinorhizobium sp. NGR 234	α-2	aerobic nonphototrophic	AE000082	AJ004860
Mesorhizobium loti MAFF 303099	α-2	aerobic nonphototrophic	NC_002678	AP003001
Thermoplasma volcanium GSS1	archaea	nonphototrophic	NC_002689 (GGPP)	NC_002689

and the East China Sea (31.0–32.0°N; 122.84–129.0°E) during August–October in 2002 and 2003. Some of NPB from our isolates, namely *Erythrobacter* sp. JL-378, *Erb.* sp. JL-435, *Erb.* sp. JL-310, *Erb.* sp. JL-316, *Roseobacter* sp. JL-129 and *Rsb.* sp. JL-135, were respectively assigned to the two major AAnPB genera of *Erythrobacter* and *Roseobacter* based on 16S rRNA relatedness, although they had no photosynthetic pigments/reaction center/light harvesting complexes and ability to utilizing light (data not shown). The selected strains of AAnPB were all proved to be distinguished from NPB by the presence of photosynthetic gene *puf* and Bchl-a. Each strain with prefix of JL- was grown aerobically in Media 2216, with shaking at 18 °C, for 24–48 h.

2.2 *Genome DNA extraction and 16S rRNA sequences*

Genomic DNAs were extracted from bacteria cultures as described by Wisotzkey et al. (1990). The 16S rRNA gene was amplified using universal primer pair for eubacteria, SSEub27F (5′-AGAGTTTGATCATGGCTCAG-3′) and SS1492R (5′-GGTACCTTGTTACGACTT-3′), in a T3 thermocycler (Biometra Co., Germany). The PCR condition setting was as follows: initial denaturation at 94 °C for 4 min, followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min, finally with extension at 72 °C for 10 min. The PCR products were gel-purified using Gel Extraction Kit (TaKaRa Biotech., China) according to the manufacture's protocols. Ligation into pMD18-T vector (TaKaRa Biotech., China) and transformation into *E. Coli* DH5α were performed by standard procedures. The positive ampicillin-resistant clones were screened through PCR using M13 primer pair of RV-M (5′-AGCGGATAACAATTTCACACAGG -3′) and M13-47 (5′-CGCCAGGGTTTTCCCAGTCACGAC -3′). The recombinant plasmid DNA was extracted by standard procedures and sequenced on ABI 377A automated sequencer (Applied Biosystems) using sequencing primer of M13-47.

2.3 *FPPS gene amplification and sequencing*

The following primers were used to amplify partial FPPS gene: FPP-F1, 5′-CAYGAYGAYHTGCCMKS VATGGA-3′; FPP-R3, 5′-CYTCGACRTCSAGAATR TCRTC-3′, with an expected product about 537 bp, as described by Cantera et al. (2002b), minor modifications on forward primer (FPP-F1) were made based on FPPS sequences of some marine proteobacteria. The reaction mixture (20 μl) contains 10 mmol/dm^3 Tris/HCl (pH8.3), 2.5 mmol/dm^3 $MgCl_2$, 0.2 mmol/dm^3 each dNTP (TaKaRa Biotech., China), and 10 pmol each primer. PCR amplification was carried out in a T3 thermocycler (Biometra Co., Germany). To elevate the specificity of the PCR product, HotStart and TouchDown PCR method was applied as follows: 95 °C for 5 min, then pause at 80 °C and add 0.15 U *Ex*Taq (TaKaRa) into each tube; then 94 °C for 1 min, 60 °C for 1 min, 72 °C 1 min, for 15 cycles and decrease annealing temperature of 0.5 °C at every cycle; then 15 cycles with 94 °C for 1 min, 52 °C for 1 min, 72 °C 1 min; finally with extension at 72 °C for 8 min. Subsequent PCR product purification, ligation, transformation and sequencing referred to the above.

2.4 *Phylogenetic data analysis*

The Cluster X Version 1.8 was applied to make alignments with default penalties. After that, phylogenetic trees were constructed respectively by the method of Neighbor-joining, Maximum-parsimony and Maximum-likehood through using the PHYLIP version 3.6 software package (Felsenstein, 1989). The topology of tree was evaluated by the bootstrap analysis with 100 trials. Neighbor-joining distance analyses used Jukes-Cantor genetic distances (Jukes and Cantor, 1969). Maximum-likehood method was performed without a gamma distribution for rate variation among sites (Olsen et al., 1994).

2.5 *Codon usage analysis*

The G+C nucleotides composition and relative synonymous codon usage (RSCU) values of the FPPS genes were calculated using online CodonW (written by Hohn Peden) analysis (http://bioweb.pasteur.fr/seqanal/interfaces/codonw.html). The RSCU value was calculated by dividing the observed frequency of that codon by the frequency that would be expected if each codon encoding a particular amino acid was used equally (Sharp and Li, 1987). On the RSCU data, correspondence analysis was conducted to investigate the major trends in codon usage in different species. The pattern of codon space resulting from correspondence analysis of RSCU suggested that codon usage bias.

2.6 *Nucleotide sequence accession numbers*

The sequences obtained through amplification in the study were submitted to GenBank databases

and assigned accession numbers AY998049-AY998052, AY998058, AY998065, AY998067, AY998068, AY998079-AY998082 and DQ104395.

3 Results and discussion

3.1 *Sequence analysis of the FPPS gene*

The products of expected length were obtained in PCR of FPPS genes with our modified primers, further BLAST searching revealed that these partial sequences were most similar to the FPPS genes deposited in GenBank. Alignment of amino acid sequences of selected bacterial strains also showed three of seven conserved regions (excluding the primer regions) common to all prenyltransferases (Liang et al., 2002). Thus the primers and their PCR products in this study were confirmed. Table 2 showed the similarity percentages of deduced amino acid sequences of bacterial FPPS in this study. In all FPPS amino acids, the Ala (10%–21%), Gly (6%–10%), Leu (10%–13%) and Val (2%–11%) were the richest. Our sequences shared homologies with reported sequences (Cantera et al., 2002a, b) of nucleotide and amino acid as high as 55.6%–74.2% and 38.1%–77.7%, respectively.

Table 2. Farnesyl pyrophosphate synthase gene induced amino acid sequence (lower triangle) and 16S rRAN gene sequence (upper triangle) similarities (%)

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. *Roseobacter denitrificans* DSM 7001[1)]	–	83.5	83.9	84.0	90.6	83.3	83.5	84.3	92.0	84.0	88.5	55.6	82.2	86.0
2.*Erythrobacter litoralis* DSM 8509[1)]	96.6	–	97.4	98.7	86.4	95.2	98.5	97.5	86.4	96.9	87.9	57.3	86.3	89.0
3.*Erythrobacter longus* DSM 6997[1)]	45.5	45.5	–	97.7	86.4	95.0	97.7	97.3	87.2	96.7	87.9	68.7	85.7	88.2
4.*Erythrobacter* sp. JL-475[1)]	47.6	49.7	87.8	–	87.1	95.9	98.2	98.3	87.1	97.6	87.5	68.3	86.4	88.8
5. *Sagittula* sp. JL-351[1)]	97.9	98.6	46.2	50.3	–	86.0	86.7	86.8	94.6	86.9	90.0	56.6	83.8	87.1
6.*Citromicrobium* sp. JL-354[1)]	98.6	97.9	46.9	49.7	99.3	–	94.8	96.2	85.6	95.5	85.9	56.6	84.8	86.5
7.*Erythrobacter litoralis* HTCC2594[1)]	45.5	45.5	75.2	77.9	45.5	46.2	–	97.6	87.0	97.5	88.3	65.4	85.9	89.1
8. *Erythrobacter* sp. JL-316[2)]	44.1	46.2	74.5	79.3	46.2	45.5	75.2	–	87.0	97.9	87.5	66.6	86.2	87.9
9.*Roseobacter* sp. JL-135[2)]	44.4	45.1	50.7	54.2	45.1	45.8	52.1	51.4	–	86.9	92.3	74.2	83.9	88.1
10. *Erythrobacter* sp. JL-378[2)]	44.1	44.1	76.6	77.9	44.1	44.8	76.6	93.8	50.0	–	87.3	66.1	85.9	89.1
11. *Rhodobacter capsulatus* ATCC 11166[3)]	44.9	46.4	52.9	55.1	46.4	45.7	54.3	51.4	69.6	52.2	–	76.2	86.5	88.8
12. *Rhodovulum sulfidophilum* DSM 1374[3)]	38.1	40.3	51.8	54.7	39.6	38.8	51.8	53.2	77.7	51.8	69.6	–	86.2	89.9
13. *Rhodopseudomonas palustris* ATCC 17001[3)]	47.6	48.3	48.3	51.0	48.3	47.6	52.4	53.8	52.8	52.4	50.7	49.6	–	88.2
14. *Mesorhizobium loti* MAFF 303099[2)]	44.8	43.4	43.5	44.9	44.1	44.8	44.1	43.4	47.9	44.8	47.8	46.8	40.1	–

Notes: [1)]AAnPB, [2)]NPB and [3)]AnAnPB.

3.2 *Codon usage*

Correspondence analysis results (Fig. 1) showed the major trends in codon usage of FPPS: the horizontal axis is associated with frequencies of codons ending in C or U versus A or G (Fennoy and Bailey-Serres, 1993), the vertical axis is correlated with GCIII (G+C frequency at third position of codon) (Musto et al., 1998). AAnPB were clustered into three groups which distributed respectively in three regions, I (*Erb. litoralis* HTTC 2594), II (*Erb.* sp. JL-475 and *Erb. Longus* DSM 6997), and III (*Citromicrobium.* sp. JL-354, *Sagittula.* sp. JL-351, *Erb. litoralis* DSM 8509, and *Rsb. denitrificans* DSM 7001). Region III contained only one AAnPB group, which was far from others. Although members of the group belonged to different genera including one species of *Erythrobacter* genus, they were positioned tightly. Members of the other two AAnPB groups all came from the genus *Erythrobacter*. This suggested that most of AAnPB genera used similar codon in FPPS and they related

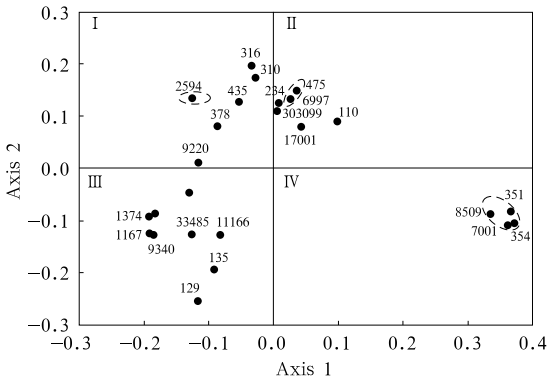

Fig.1. Correspondence analysis of RSCU of aerobic anoxygenic bacteria and their relatives. Axis 1 associated with frequencies of codons ending in C or U versus A or G, Axis 2 is correlated with GCIII (G+C frequency at third position of codon). The numbers in figure (not on axis) denote the bacteria whose name containing them and the numbers in bold and italic, italic and regular fonts represent AAnPB, NPB and AnAnPB, respectively.

closely, but the exception of *Erythrobacter*. Species of *Erythobacter* used variable codon and their classification deserved more attention.

3.3 *Phylogenetic analysis*

In the construction of phylogenetic trees, Neighbor-joining, maximum-likehood and maximum-parsimony methods were applied and archaeal geranylgeranyl pyrophosphate synthase (GGPPS) of *Thermoplasma volcanium* GSS1 was used as outgroup species in FPPS tree. Because archaeal GGPPS was more ancient than FPPS and other prenyltransferases gene, it could predict the divergence of bacterial FPPS (Chen et al., 1994). Finally, very similar topologies were observed of the trees constructed by three methods respectively.

Figure 2 showed the phylogenetic trees based on FPPS nucleic acid sequences (left) and 16S rRNA gene sequences (right) of AAnPB and their relatives. Comparative analysis of the trees of FPPS and 16S rRNA revealed that the two trees' topologies were basically in a good agreement. For instance, both FPPS and 16S rRNA phylogenies showed, the grouping of alphaproteobacteria subgroup α-4 included NPB *Erb.* sp. JL-378, JL-435, JL-310 and JL-316, as well as the grouping of subgroup α-3 included *Rsb.* sp. JL-129 and JL-135, and these groupings were both supported with high bootstrap values. This result indicated that FPPS tree well supported NPB grouping of α-3 and α-4. As to some AnAnPB, the overall topologies of both phylogenetic trees were also principally consistent as reported by Cantera et al. (2002b). The similarity of both phylogenies of NPB and AnAnPB between FPPS and 16S rRNA trees suggested that FPPS might have a similar evolutionary pattern as the 16S rRNA. FPPS could be useful for bacterial phylogenetic researching because it was more divergent than 16S rRNA.

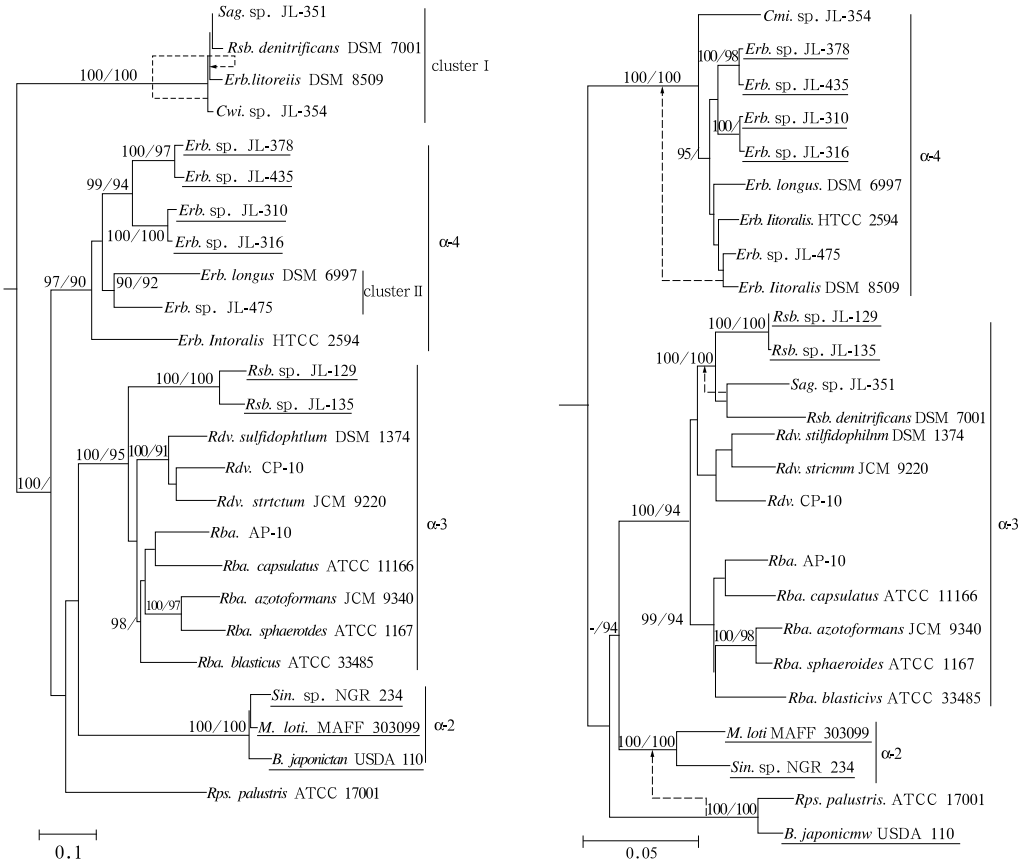

Fig.2. Neighbor-joining phylogenetic tree obtained from FPPS (left) and 16S rRNA (right) gene sequences. The scale bar represents an estimated two nucleotides substitution per 100 nucleotides. Percentages refer to significant bootstrap values of 100 calculated trees. Bootstrap values greater than 90% are shown above the branch (neighbor-joining/ maximum-likehood). Tree is rooted with *Thermoplasma volcanium* GSS1 as outgroup. The broken lines with an arrowhead show the altered position of correspondent strains in the trees constructed using maximum-likehood. AAnPB are marked in bold, NPB in underline and AnAnPB in regular.

But predominant incongruence of the phylogenetic pattern of AAnPB existed between FPPS and 16S rRNA gene trees. As expected, selected AAnPB were intermingled with NPB species in 16S rRNA tree sustained by high repeat ratio of the tree topology reliability, though AAnPB possessed distinct phenotypic characteristic of photosynthesis from these NPB. Althorugh AAnPB *Erb.* sp. JL-475 and *Erb. litoralis* DSM 8509 were grouping together in 16S rRNA phylogenetic tree with Neighbor-joining method, the low bootstrap value (32%, not shown on branch) couldn't validate their grouping and moreover in the maximum-likehood applied tree they failed to be formed into a group. The similar result was obtained in the grouping of *Sag.* sp. JL-351 and *Rsb. denitrificans* DSM 7001 in the reconstruction of 16S rRNA phylogeny. So it could be seen that phylogenetic position of AAnPB was still confused only based on 16S rRNA phylogeny, as previously reported by Yurkov and Beatty (1998). In fact, the result of 16S rRNA phylogeny of selected strains abided by the grouping of α-2, α-3 and α-4 with much confidence for their high bootstrap values. Independent of the inference methods employed, however, AAnPB formed into monophylogenetic groups without intermingling with NPB or AnAnPB in FPPS tree, which was most notable deviation from the tree from 16S rRNA. In the FPPS gene tree, the AAnPB strains *Sag.* sp. JL-351, *Rsb. denitrificans* DSM 7001, *Cmi.* sp. JL-354 and *Erb. litoralis* DSM 8509 formed a highly supported (bootstrap value, 100%/100%) monophylogenetic cluster I and the group located deepest branch, whereas *Erb.* longus DSM 6997 and *Erb.* sp. JL-475 formed Cluster II with bootstrap values 90%/92% and *Erb. litoralis* HTCC 2594 fell into one branch alone. The distribution of AAnPB in FPPS phylogeny was clearly consistent with the result of codon usages among AAnPB FPPS genes of (Fig. 1). *Erb. longus* DSM 6997, *Erb.* sp. JL-475, *Erb. litoralis* HTCC 2594 and some NPB were contained in subgroup α-4 in both trees, which indicated that they had higher relatedness among them than with other bacteria, such as AnAnPB.

The phylogenetic analysis also revealed that Cluster I of AAnPB was closer to the outgroup species with archaeal GGPPS than AnAnPB, which was supported by significant bootstrap values. Interestingly, the AAnPB strains of subclass α-4 were also deeper located than AnAnPB in 16S rRNA phylogenic tree. These results implied that some AAnPB seemed to be diverged earlier than AnAnPB with FPPS in evolutionary history, yet it's generally proposed that AnAnPB should appear earlier than AAnPB for archaean non-oxidation condition (Beatty, 2002). The evolution of oxygenic photosynthetic cyanobacteria largely increased the oxygen in atmosphere about 2.3 billion years (Ga) ago and facilitated the developments of complex life forms depending on aerobic metabolism (Raymond and Segre, 2006). However the archaean atmosphere was not obligate non-oxygenation with the archaean oxygen on earth (Kaufman et al., 2007). Therefore we hypothesized that the earlier emergence of some AAnPB than AnAnPB could happen with the earliest appearing archaean oxygen and this also may be supported by the argument of Canfield et al. (2006), where an ecosystem based on oxygenic photosynthesis was also possible with complete removal of the oxygen by reaction with reduced species from the mantle. While *Erb. longus* DSM 6997, *Erb.* sp. JL-475 and *Erb. Litoralis* HTCC 2945 were not grouped together with other AAnPB, this may be the result of later acquisition (e.g. horizontal gene transfer). Among AAnPB, indirect evidence of lateral gene transfer was provided by the difference between the phylogeny of photosynthetic gene like *pufM* and *pufL* and the phylogeny of 16S rRNA (Igarashi et al., 2001). Photosynthetic genes may yet be lost in the evolution (Yurkov and Cstonyi, 2009), it may be one reason for the co-existence of some *Erythrobacter* spp. and some NPB.

Similar results also were obtained from the phylogenetic analysis based on FPPS protein sequence (Fig. 3 and the notes). The phylogenetic relationships determined by FPPS gene and FPPS protein sequence analysis were more consistent than those established by the 16S rRNA phylogenetic tree for AAnPB as well as for AnAnPB. Because of high variability, FPPS gene phylogenetic analysis was more reliable than 16S rRNA and FPPS protein analysis to infer intrageneric relationships of AAnPB and their relatives. Moreover, FPPS may be more competent for studying the phylogenetic relationship between photosynthetic and non-photosynthetic bacteria, not only because it was present both in photosynthetic bacteria and in NPB, but also because FPPS was related with carotinoids and (Bacterio) chlorophyll biosynthesis.

4 Conclusions

Our comparative phylogenetic analysis shows that the overall FPPS DNA tree topology is principally

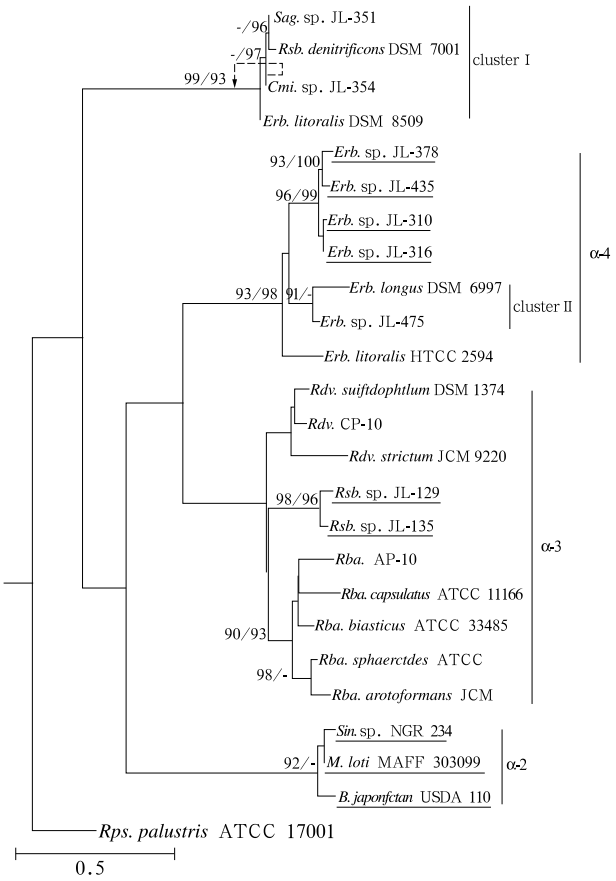

Fig.3. Neighbor-joining phylogenetic tree obtained from FPPS protein sequences. The scale bar represents an estimated number of amino acid replacements per position. Percentages refer to significant bootstrap values of 100 calculated trees. Bootstrap values greater than 90% are shown above the branch (neighbor-joining/ maximum-likehood). Tree is rooted with *Thermoplasma volcanium* GSS1 as outgroup. Bootstrap values greater than 50% obtained from maximum-likehood (front/) and maximum-parsimony (behind/) methods are shown below the branch. The broken lines with an arrowhead show the altered position of correspondent strains in the trees constructed using maximum-likehood. AAnPB are marked in bold, NPB in underline and AnAnPB in regular. One difference of the protein phylogeny from DNA phylogeny was that the group of α-4 containing *Erb. longus* DSM 6997 and *Erb.* sp. JL-475 was closer to the group of containing AnAnPB α-3. Another notable difference of FPPS protein tree from DNA tree was the position of *Rps. palustris* ATCC 17001. In protein tree, the deepest branch only contained *Rps. palustris* ATCC 17001 rather the aggregate of *Sag.* sp. JL-351, *Rsb. denitrificans* DSM 7001, *Cmi.* sp. JL-354 and *Erb. litoralis* DSM 8509 in DNA tree. Nevertheless, protein sequences phylogenetic grouping of *Rps. palustris* ATCC 17001 was uncertain for no a significant bootstrap value strong supported.

well consistent with one of 16S rRNA, although the FPPS gene was more divergent than the 16S rRNA. FPPS gene phylogeny clearly discriminated AAnPB from AnAnPB and related NPB, and could place them into different clusters or blanch without the intermingling with their relatives like in 16S rRNA phylogeny. This discrimination was in good agreement with the unique and important phenotypic characteristic of photosynthesis of AAnPB and thus it made the FPPS gene reliable for the study of phylogenetic relationship among AAnPB and their relatives. Also, our data suggest that AAnPB were evolutionarily closer to NPB than to AnAnPB, and moreover some AAnPB were diverged possibly earlier than AnAnPB. The greater variability of FPPS gene sequence than the 16S rRNA granted this gene to fully use in phylogenetic relationships among species of different bacteria genera and even same genus. Furthermore, with the increasing of available bacterial FPPS genes, FPPS was probably applied in diversity and phylogenetic analysis of aerobic anoxygenic phototrophic bacteria in environmental samples, because of the shortage of 16S rRNA

as noted before in related researches.

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