Evolutionary relationship of phototrophic bacteria in the α -*Proteobacteria* based on farnesyl diphosphate synthase

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Partial sequences of farnesyl diphosphate (FPP) synthase genes derived from the *Rhodobacter*–*Rhodovulum* group and from the *Rhodopseudomonas palustris*–*Bradyrhizobium japonicum* group of the α -*Proteobacteria* were subjected to phylogenetic analysis to investigate the relationships of phototrophic and non-phototrophic bacteria in the α -*Proteobacteria*. The four *Rhodovulum* species formed a monophyletic group within the *Rhodobacter* cluster, and *Agrobacterium ferrugineum* IAM 12616^T intermingled with the *Rhodobacter* species. This topology is in good agreement with the 16S rRNA phylogeny, although the FPP synthase gene was more divergent than the 16S rRNA. On the other hand, strains of the phototrophic *Rps. palustris* formed a cluster far from that of the non-phototrophic *Bradyrhizobium japonicum* strains. Moreover, *Rps. palustris* strains were differentiated from the nodule-forming *B. japonicum*, *Mezorhizobium loti* MAFF 303099 and *Sinorhizobium* sp. NGR 234 in the FPP synthase phylogeny. This relationship does not agree with the 16S rRNA phylogeny, wherein *Rps. palustris* was more closely related to *B. japonicum* than to strains of the *Rhodobacter*–*Rhodovulum* group. These results suggest that the FPP synthase gene of *Rps. palustris* diverged from that of *B. japonicum*.

Keywords: *Rhodobacter*, *Rhodopseudomonas*, nodule-forming bacteria, phylogeny, isoprenyl diphosphate synthase

INTRODUCTION

Phototrophic bacteria are distinguished from other *Proteobacteria* by their photosynthetic ability. They do not, however, constitute a phylogenetically homogeneous taxon based on 16S rRNA sequences, and are found in three of the five *Proteobacteria* classes, namely α -, β - and γ -*Proteobacteria* (Stackebrandt *et al.*, 1996), intermingling with non-phototrophic bacteria. For example, *Rhodopseudomonas palustris*, a phototrophic purple non-sulfur bacterium belonging to the α -2 *Proteobacteria*, is phylogenetically close to the nodule-forming, non-phototrophic *Bradyrhizobium japonicum* (Inui *et al.*, 2001; Fleischman *et al.*, 1995; Wong *et al.*, 1994; Young *et al.*, 1991). Moreover, *Rhodobacter* species in

the α -3 *Proteobacteria* are closely related to the non-phototrophic marine *Agrobacterium ferrugineum* (Uchino *et al.*, 1998). This type of relationship requires further investigation, since the evolution of phototrophic ability among the phototrophic members of *Proteobacteria* has not been accurately described. Studies on the evolution of photosynthesis have dwelt only on the evolution of the photosynthetic apparatus among the phototrophic species (Xiong *et al.*, 1998, 2000; Nagashima *et al.*, 1997); the diversification of phototrophic bacteria in the *Proteobacteria* has not been addressed in such studies, and remains unclear.

Farnesyl diphosphate (FPP) synthase, a key enzyme involved in the synthesis of isoprenoid compounds (Ogura *et al.*, 1997), is well conserved among microorganisms (Fujisaki *et al.*, 1990; Koyama *et al.*, 1993; Cunillera *et al.*, 1996; Song & Poulter, 1994; Anderson *et al.*, 1989). This enzyme plays an important role in photosynthesis as well as in the synthesis of other physiologically important compounds. Construction of the FPP synthase gene tree showed similar groupings as

Abbreviation: FPP, farnesyl diphosphate.

The GenBank accession numbers for the FPP synthase gene sequences reported in this paper are AB053173–AB053178, AB053180 and AB062882–AB062884.

in the 16S rRNA tree at the suprageneric level within the eubacterial branch; the FPP synthase gene thus appeared to behave in the manner of the 16S rRNA gene (Cantera *et al.*, 2002). Here, we extended our study on the phylogenetic relationship of the FPP synthase genes among phototrophic and non-phototrophic bacteria belonging to the α -*Proteobacteria*. We also compared the phylogeny of the FPP synthase genes with that of the 16S rRNA, and showed that the FPP synthase genes of some species from the α -2 *Proteobacteria* may have been acquired by gene transfer.

METHODS

Organisms and growth conditions. *Rps. palustris* ATCC 17001^T, *Rhodobacter blasticus* NCIMB 11576^T, *Rps. palustris* VA2-2, *Rhodobacter* sp. AP-10 and *Rhodovulum* sp. CP-10 were grown as described previously (Kawasaki *et al.*, 1993). *Rhodobacter azotoformans* JCM 9340^T was cultured in medium 200 (Nakase, 1999) while *Rhodovulum strictum* JCM 9220^T and JCM 9221 were grown in medium 194 (Nakase, 1999). All phototrophic cultures were grown under light illumination (2000 lx) at 30 °C for 3–7 days (Kawasaki *et al.*, 1993). *A. ferrugineum* IAM 12616^T was grown aerobically in marine broth (Difco) at 28 °C for 2 days, and *B. japonicum* IAM 12608^T in yeast extract-mannitol (YM) medium for 5–7 days with shaking at 28 °C. *Escherichia coli* strains used for recombinant DNA manipulations were cultured overnight in Luria–Bertani (LB) medium at 37 °C.

DNA preparation and amplification of FPP synthase genes. Chromosomal DNA was prepared as described by Ausubel *et al.* (1995). Degenerate PCR primer combinations, which are bound between nucleotide positions 730 and 1170 (*E. coli* FPP synthase gene sequence numbering; Fujisaki *et al.*, 1990), were FPP α F3 (5'-CAY GAC GAY MTG CCC KSV ATG GA-3') and either FPP α R1 (5'-CYK YSR CRT CSA GAA TGT CGT C-3') or FPP α R3 (5'-CYT CGA CRT CSA GAA TRT CRT C-3'). They were designed from the highly conserved regions (regions II and VI) in FPP synthase genes of *Rhodobacter sphaeroides* ATCC 11167^T (accession no. AB028044), *Rhodobacter capsulatus* ATCC 11166^T (AB028046), *Rhodovulum sulfidophilum* W⁴ (AB028047) and *B. japonicum* USDA 110 (U12678) to amplify an approximately 420 bp product. Additional primers with lesser degeneracy and more specific for *Rhodobacter* and *Rhodovulum* species, FPPRbaF1 (5'-CAT GAC GAY ATG CCC TGY ATG GA-3') and FPP α R4 (5'-TCG TCS GCG AYC TGG AAS GCM AGS C-3'), were later designed from almost exactly the same conserved regions as more sequences became available. The 25 μ l reaction mixture consisted of bacterial DNA as the template (10–100 ng), 2.5 mM dNTP mix, 25 pmol of each primer and 1.25 U *Taq* polymerase (TaKaRa Shuzo). The reaction conditions for the primer pair FPP α F1/FPP α R1 were 94 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min, with a final extension at 72 °C for 7 min. For the primer pairs FPP α F3/FPP α R3 and FPPRbaF1/FPP α R4, initial denaturation was followed by either 6 or 10 cycles of 96 °C for 1 min, 37 °C for 2 min and 72 °C for 1 min, followed by 25 cycles of 96 °C for 1 min, 2 min at either 55 or 57 °C, and 72 °C for 1 min before the final elongation step. This step-up PCR modification was done to increase the number of putative FPP synthase gene products at low annealing temperature, followed by a high annealing temperature to specifically amplify the gene. PCR reaction was carried out using the GeneAmp PCR system 9700 (PE Applied Biosystems).

Sequencing of FPP synthase genes. Purified PCR fragments approximately 400–450 bases long were cloned using a TA cloning kit (Invitrogen) and transformed into competent cells according to the manufacturer's protocol. DNA sequences were determined by the dideoxy chain-termination method (Sanger *et al.*, 1977) with a BigDye Terminator cycle sequencing kit (PE Applied Biosystems) using an ABI PRISM 310 Genetic analyser (PE Applied Biosystems). Sequence data were collected by means of the program ABI PRISM (Perkin-Elmer) and assembled with an ABI Auto Assembler (Perkin-Elmer).

In silico and phylogenetic analyses. Nucleotide sequences obtained in this work were converted to amino acid sequences after removing the gap-containing and the primer-annealing regions using Genetix-Win software (version 3.1.0). Seven FPP synthase gene sequences obtained from the National Center for Biotechnology Information (NCBI) databases (<http://www.ncbi.nlm.nih.gov/>) were combined with the partial sequences for sequence and phylogenetic analyses. A total of 93 amino acids (excluding gaps) were used to generate phylogenetic trees by the neighbour-joining method with CLUSTAL X (Thompson *et al.*, 1997; Jeanmougin *et al.*, 1998) (with 1000 bootstrap replicates using default parameters) and by the maximum-likelihood method (100 replicates using the Jones, Taylor and Thornton method) with MOLPHY version 2.3 (Adachi & Hasegawa, 1996). NJPlot (Perrière & Gouy, 1996) and TreeView (Page, 1996) were used to analyse the phylogenetic relationships of FPP synthase genes from various species. *E. coli ispA* (the FPP synthase gene in *E. coli*) was included in the analyses and used as an outgroup.

Nucleotide sequence accession numbers. The sequences determined in this study were submitted to the DDBJ/EMBL/GenBank databases and assigned accession numbers AB053173–AB053178, AB053180 and AB062882–AB062884 (Table 1).

RESULTS AND DISCUSSION

Comparison of the FPP synthase gene sequences of members of the α -*Proteobacteria*

BLAST searching revealed homologies of as high as 92 % between our partial sequences and the FPP synthases from *Rba. capsulatus* ATCC 11166^T, *Rba. sphaeroides* ATCC 11167^T and *Rdv. sulfidophilum* W4^T. The sequences also shared high homology with the FPP synthases (geranyltranstransferase) of *Zymomonas mobilis* and *B. japonicum* USDA 110, thus confirming that our sequences corresponded to those of FPP synthase. Alignment of the partial FPP synthase amino acid sequences with other FPP synthase sequences from several members of the α -2 and α -3 *Proteobacteria* available in the database showed three of the seven conserved regions (excluding the primer regions) common to all prenyltransferases (Koyama *et al.*, 1993). The resulting alignment produced a high overall amino acid sequence similarity between species of *Rhodobacter* (75.4–93.9 %) and *Rhodovulum* (86.8 % to as high as 100 %) (Table 2). These results suggest that the FPP synthase genes of members of the *Rhodobacter*–*Rhodovulum* species are related, and arose from the same ancestral lineage. The non-phototrophic marine *A. ferrugineum* IAM 12616^T on the other hand, is related to the *Rhodobacter* and *Rhodovulum* strains, having a mean sequence homology of 69.3 $\pm$ 3.5 % and 63.0 $\pm$

Table 1. List of strains and GenBank accession numbers of FPP synthase and 16S rRNA/DNA sequences used in this study

Species	Phylogenetic group	Strain designation*†	Accession/Protein ID no.	
			FPP synthase	16S rDNA
<i>Rhodobacter blasticus</i>	α -3	NCIB 11576 ^T /ATCC 33485 ^T	AB053173‡	D16429
<i>Rhodobacter</i> sp.	α -3	AP-10§	AB062882‡	AB079681
<i>Rhodobacter azotoformans</i>	α -3	JCM 9340 ^T /KA25 ^T	AB053174‡	D70846
<i>Rhodobacter sphaeroides</i>	α -3	ATCC 11167 ^T /IFO 12203 ^T	AB028044	D16425
<i>Rhodobacter capsulatus</i>	α -3	ATCC 11166 ^T	AB028046	D13474
<i>Rhodovulum sulfidophilum</i>	α -3	W4 ^T /DSM 1374 ^T	AB028047	D13475
<i>Rhodovulum strictum</i>	α -3	JCM 9220 ^T /MB-G2 ^T	AB053177‡	D16419
		JCM 9221	AB053176‡	AB079635
<i>Rhodovulum</i> sp.	α -3	CP-10§	AB062884‡	AB079682
<i>Agrobacterium ferrugineum</i>	α -3	IAM 12616 ^T	AB053175‡	D88522
<i>Rhodopseudomonas palustris</i>	α -2	ATCC 17001 ^T	AB062883‡	D25312
		VA2-2	AB053180‡	AB079634
<i>Bradyrhizobium japonicum</i>	α -2	USDA 110	U12678	D13430
	α -2	IAM 12608 ^T	AB053178‡	D11345
<i>Sinorhizobium</i> sp.	α -2	NGR 234/MSMC 310	AE000082	AJ004860
<i>Mezorhizobium loti</i>	α -2	MAFF 303099	vNP106892	AP003001
<i>Escherichia coli</i>	γ	K-12 ^T	D00694	AE000474

* Type strain designation (^T) may have differed between the FPP synthase DNA and 16S rRNA/rDNA depending on the culture collection from which the strains were acquired.

† Culture collections: ATCC, American Type Culture Collection, Manassas, VA, USA; USDA, United States Department of Agriculture, Washington DC, USA; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; IFO, Institute for Fermentation, Osaka, Japan; IAM, Institute of Applied Microbiology, Univ of Tokyo, Tokyo, Japan; NCDO, National Collection of Dairy Organisms, Aberdeen, UK; JCM, Japan Collection of Microorganisms, Saitama, Japan.

‡ Determined in this study.

§ Isolated and characterized by Kawasaki (1997).

|| Isolated and characterized by H. Kawasaki (unpublished results); this strain shares a high DNA–DNA homology (> 70%) with *Rps. palustris* ATCC 17001^T.

0.4%, respectively. Although the gene exhibited high amino acid sequence similarities among species of *Rhodobacter* and *Rhodovulum*, the amino acid sequence of FPP synthase is likely to be appropriate for comparisons at the genus and species levels due to the high sequence variation among species of both the *Rhodobacter* genus and the *Rhodovulum* genus (ranging from an approximately 5.4% difference between *Rba. sphaeroides* ATCC 11167^T and *Rba. azotoformans* JCM 9340^T to as high as 19.4% between *Rba. blasticus* NCIMB 11576^T and *Rhodobacter* sp. AP-10). Moreover, the FPP synthase sequence analysed here is not the entire sequence of the gene, and thus it is possible that additional nucleotide bases may be different in the FPP synthase sequence.

The partial FPP synthase sequence of *Rps. palustris* ATCC 17001^T determined in this study was identical with the FPP synthase (*ispA* gene, encoding FPP synthase/geranyltransferase) sequence available in the *Rps. palustris* genome sequence (<http://spider.jgi-psf.org/>), confirming that the sequence we amplified encodes FPP synthase. The same sequence differed from that of *Rps. palustris* VA2-2 by at least 3 amino acids,

suggesting that FPP synthase is highly conserved among these strains of *Rps. palustris*. Nevertheless, the three FPP synthases from *Rhodopseudomonas* species showed a very low sequence similarity (only 36.8%) with the closest relatives of this species, *B. japonicum* IAM 12608^T and USDA 110, whereas the FPP synthases of the nodule-forming rhizobia (*B. japonicum* strains IAM 12608^T and USDA 110, *M. loti* MAFF 303099 and *Sinorhizobium* sp. NGR 234) shared a high degree of homology with each other, ranging from 93.9% to 100%. In fact, the FPP synthase of the rhizobial strains had the highest homology values among the strains examined. The very high FPP synthase sequence similarity among the rhizobial strains suggests that the FPP synthase genes of these rhizobia are highly conserved; the FPP synthase gene is not necessarily appropriate for differentiating the nodule-forming species.

Phylogenetic analysis of the FPP synthase sequence

The results of the phylogenetic tree inference analysis by the neighbour joining method are shown in Fig. 1. The relationship among species in the α -3 *Proteobacteria*

Table 2. Amino acid sequence similarity (%) of the FPP synthase gene of selected members of α -*Proteobacteria* used in this study

Species: 1, *Rba. blasticus* NCIB 11576^T; 2, *Rba. capsulatus* ATCC 11166^T; 3, *Rhodobacter* sp. AP-10; 4, *Rba. sphaeroides* ATCC 11167^T; 5, *Rba. azotoformans* JCM 9340^T; 6, *A. ferrugineum* IAM 12616^T; 7, *Rdv. sulfidophilum* W4^T; 8, *Rhodovulum* sp. CP-10; 9, *Rdv. strictum* JCM 9221; 10, *Rdv. strictum* JCM 9220^T; 11, *Rps. palustris* ATCC 17001^T; 12, *Rps. palustris* VA2-; 13, *Sinorhizobium* sp. NGR 234; 14, *M. loti* MAFF 303099; 15, *B. japonicum* USDA 110; 16, *B. japonicum* IAM 12608^T; 17, *E. coli*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	100																
2	82.5	100															
3	84.2	78.1	100														
4	85.1	78.1	80.7	100													
5	83.3	75.4	79.8	93.9	100												
6	68.4	67.5	64.9	71.9	73.7	100											
7	75.4	66.7	73.7	75.4	73.7	62.3	100										
8	75.4	65.8	74.6	78.1	77.2	63.2	91.2	100									
9	75.4	68.4	73.7	77.2	77.2	63.2	86.8	91.2	100								
10	75.4	68.4	73.7	77.2	77.2	63.2	86.8	91.2	100	100							
11	47.4	46.5	44.7	46.5	47.4	42.1	44.7	46.5	44.7	44.7	100						
12	47.4	46.5	44.7	46.5	47.4	42.1	44.7	46.5	44.7	44.7	98.2	100					
13	50.0	44.7	43.0	45.6	44.7	37.7	44.7	43.9	44.7	44.7	37.7	37.7	100				
14	50.0	45.6	43.9	46.5	45.6	38.6	44.7	43.9	44.7	44.7	37.7	37.7	97.4	100			
15	49.1	43.9	42.1	44.7	43.9	36.8	43.9	43.0	43.9	43.9	36.8	36.8	94.7	93.9	100		
16	49.1	43.9	42.1	44.7	43.9	36.8	43.9	43.0	43.9	43.9	36.8	36.8	94.7	93.9	100	100	
17	51.8	50.9	50.9	50.0	49.1	46.5	48.2	47.4	49.1	49.1	41.2	41.2	57.9	57.9	55.3	55.3	100

Fig. 1. Phylogeny of the FPP synthase gene (a) and 16S rDNA (b) showing the relationships among the α -2 and α -3 *Proteobacteria* constructed using the neighbour-joining method. Branches supported by 90% or more using the neighbour-joining method are shown in bold; bootstrap values greater than 90% are shown above the branch. Bootstrap values greater than 90% from the maximum-likelihood method are shown below the branch. Arrows point to the positions of each group in the 16S rRNA tree. Bar, 10 nucleotide or amino acid substitutions per 100 nucleotides.

based on the FPP synthase amino acid sequences is consistent with the results obtained from 16S rRNA sequences.

FPP synthase tree supports the grouping of the α -3 *Proteobacteria*

Representative members (*Rhodobacter* and *Rhodovulum* spp. and *A. ferrugineum* IAM 12616^T) of the α -3 subgroup of the *Proteobacteria* formed a highly supported phylogenetic cluster that is clearly distinct from other taxa. This suggests that the FPP synthase genes of members of the α -3 *Proteobacteria* have the same evolutionary origin. This was further supported by the high sequence similarity among the representative members, and is thus consistent with our initial analysis (to be published elsewhere) that the FPP synthase gene reflects the species phylogeny. Our phylogenetic analysis also showed a monophyletic grouping of the marine *Rhodovulum* spp. separated from the freshwater *Rhodobacter* spp. that confirms the result of the 16S rRNA phylogeny (Hiraishi & Ueda, 1994). The same tree also confirms the close association of *Rba. sphaeroides* and *Rba. azotoformans*, as well as *Rba. blasticus* and *Rba. capsulatus*.

One notable deviation of the FPP synthase phylogeny from the 16S rRNA phylogeny is the positioning of the non-phototrophic *A. ferrugineum* IAM 12616^T outside the *Rhodobacter* clade. This organism is closely related to the members of the phototrophic *Rhodobacter* genus, and its closest neighbours are *Rba. azotoformans* and *Rba. sphaeroides* (Uchino *et al.*, 1997) based on 16S rRNA phylogeny, although it differs from the species of *Rhodobacter* in its G + C content, and by the absence of photosynthetic abilities, bacteriochlorophyll *a* and intracytoplasmic membrane systems, which are defining characteristics of the genus *Rhodobacter* (Uchino *et al.*, 1998). The result of the analysis using the maximum-likelihood method, however, does not support the position of this species outside the *Rhodobacter* clade, instead placing *A. ferrugineum* closer to *Rba. azotoformans* and *Rba. sphaeroides*. This implies that the position of *A. ferrugineum* IAM 12616^T in the FPP synthase gene tree is uncertain. Nevertheless, our data demonstrate the phenotypic diversity of species in the *Rhodobacter*–*Rhodovulum* group, as exemplified by the close association of the non-phototrophic *A. ferrugineum* IAM 12616^T with phototrophic species of the *Rhodobacter* and *Rhodovulum* genera in the FPP synthase tree. It is imperative that more detailed taxonomic and phylogenetic studies using other molecular genes as markers, or comparison of the genome contents between this species and species of *Rhodobacter*, be performed in order to determine an adequate taxonomic position for *A. ferrugineum* IAM 12616^T. Our results clearly show that FPP synthase is a useful molecule not only for differentiating *Rhodobacter* species from *Rhodovulum* species but also for revealing variations between *A. ferrugineum* IAM 12616^T and the species of *Rhodobacter*.

Unusual clustering of representative members of the α -2 *Proteobacteria*

The FPP synthase gene of the phototrophic members of the α -2 subgroup, namely *Rps. palustris* ATCC 17001^T and *Rps. palustris* VA2-2, formed a separate cluster from the two strains of the non-phototrophic *B. japonicum*, i.e. strains IAM 12608^T and USDA 110. As supported by the high homology among the FPP synthase protein sequences, the distance analysis placed the two *Rps. palustris* strains far from these two strains of *B. japonicum* (Fig. 1a). This suggests that the FPP synthase genes of these two species are not closely related and probably evolved from different ancestors. Sequence analysis of 16S rRNA placed *Rps. palustris* and *B. japonicum* into a single group despite their differing phenotypes: *Rps. palustris* is phototrophic, whereas *B. japonicum* is a non-phototrophic, nodule-forming bacterium.

The two *B. japonicum* strains, on the other hand, tended to cluster and formed a monophyletic group together with the other non-phototrophic bacteria *M. loti* MAFF 303099 and *Sinorhizobium* sp. NGR 234, and this cluster was supported by a high bootstrap value of 100%. These organisms, collectively referred to as rhizobia (Van Rhijn & Vanderleyden, 1995), are capable of eliciting nodules on their leguminous hosts. Although these rhizobia shared several morphological and biochemical properties, particularly the ability to form nodules in their hosts, they differed from each other in terms of host specificity and did not form a monophyletic group in the 16S rRNA phylogeny, but rather were placed in several well-separated genera. Reconstruction of the evolutionary history from 16S rRNA gene sequence divergence showed that *B. japonicum* IAM 12608^T and *B. japonicum* USDA 110 are more related to *Rps. palustris* strains than to either *Sinorhizobium* sp. NGR234 or *M. loti* MAFF 303099 (Fig. 1b). In the FPP synthase tree, however, the positioning of *M. loti* MAFF 303099 closer to *Sinorhizobium* sp. NGR 234 than to the *B. japonicum* strains was similar to the positioning in the 16S rDNA phylogeny. Hence, it is interesting to note that in the FPP synthase gene tree, the non-phototrophic rhizobia (*B. japonicum* IAM 12608^T, *B. japonicum* USDA 110, *Sinorhizobium* sp. NGR234 and *M. loti* MAFF 303099) were all included in a single cluster supported by a significant bootstrap value of 100%, suggesting that their FPP synthase genes share a common ancestor. This was further supported by the high mean sequence similarity value of their FPP synthases. The longer branch length in the phylogenetic tree that supports the cluster of rhizobia, and the higher homology values of the FPP synthase genes as compared to the 16S rDNAs of rhizobia, are suggestive of a recent lateral gene transfer event before their diversification into their present genera. The lateral transfer of symbiotic genes mediated by plasmids and phages in rhizobia has been reported to be common (Sullivan *et al.*, 1995; Sullivan & Ronson, 1998), and it is not impossible that FPP synthase gene transfer occurred among these rhizobia. Analysis of the *M. loti* MAFF

303099 chromosome and the plasmid of *Sinorhizobium* sp. NGR 234 showed that the FPP synthase genes of these rhizobia are located in the symbiotic island region, flanked by highly conserved genes/ORFs (>90% similarity), and included in a cluster containing the nitrogen fixation and nodulation genes (data not shown). Based on the nucleotide sequence analyses, the FPP synthase gene of *B. japonicum* strains would have been transferred before the divergence of *M. loti* MAFF 303099 and *Sinorhizobium* sp. NGR 234. However, the mechanism of FPP synthase gene transfer in this case remains to be clarified. Alternatively, since the FPP synthase gene is within the symbiotic island region proximal to the nitrogen fixation and nodulation genes, the gene might have played a major role in nodulation; the high similarity of the FPP synthase gene could be due to high selection pressure restricting its divergence.

In summary, the overall FPP synthase tree topology is in good agreement with the 16S rRNA phylogeny, although the FPP synthase gene was more divergent than the 16S rRNA. The greater sequence variations of the FPP synthase gene than the 16S rRNA make this gene useful for indicating the phylogenetic relationships among species belonging to a genus, and relationships among several genera. The use of the FPP synthase gene to clarify phylogenetic relationships at the inter- and intragenic levels may not be appropriate for rhizobial strains due to the high homology among FPP synthases of rhizobia. Nevertheless, the topology of the FPP synthase tree is useful for identifying unusual evolutionary processes that probably occurred during the diversification of the *Proteobacteria*, and particularly in the case of the α -2 *Proteobacteria*, as observed from the FPP synthase genes of *Rps. palustris* and *B. japonicum*. The discrepancies detected between the FPP synthase phylogeny and the 16S rRNA phylogeny suggest that lateral gene transfer had occurred. FPP synthase sequences therefore offer one of the best opportunities to establish the relationships among the phototrophic *Rps. palustris* and the nodule-forming bacteria.

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