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SEQUENCE ANALYSIS OF 16SrRNA, *rpo*B, *rpo*C AND *rpo*D GENES FROM THE GENUS *RHODOBACTER*

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ABSTRACT

Molecular sequence data analysis become important tool to inferred evolutionary relationship and systematic ordering among among organism. It complementing traditional method such as comparison of morpholocical and biochemichal characteristics. In this article, three genes sequnces encoding RNA polymerase subunits and 16S rRNA gene sequence are discussed for their evolutionary relationship among species of *Rhodobacter*, a genus of phototrophic α-*proteobacteria*, and between *Rhodobacter* species and several reference microorganism. Level of sequence identity among gene sequence showed the lowest variation was in 16SrRNA gene sequences, and the the highest was in rpoD gene sequence. Phylogenetic tree constructions were done using Neighbour joining method and Kimura – 2 Parameter model to measure genetic distance. Bootstrap analysis was also applied with 1000 repeats. Members of *Rhodobacter* genus were divided into two major clusters based on phylognetic analysis of 16S rRNA gene sequence. There is ambiguity of *Pararhodobacter sp*. CCB-MM2 position between anlysis based on 16SrRNA gene sequnce and *rpo* gene sequences where analysis based on *rpo* genes sequences able to locate *Pararhodobacter sp*. CCB-MM2 on its own phylognetic tree branch, separated from cluster of *Rhodobacter* species.

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INTRODUCTION

DNA sequence analysis of evolutionary stable marker genes susch as the small-subunit (16S) rRNA gene is used in genotypic identification, clssification of prokaryotes as alternative or complement to phenotypic methods and phylogenetic study [1 – 3]. Because of its important role in protein synthesis, 16SrRNA gene is highly conserved among bacteria and other kingdoms [4]. Comparison of 16SrRNA gene sequence alows differentiation of bacteria at the genus level and classification at species and subspecies level, however its presences as multiple copies in many bacteria became its downside [5 – 6]. Researchers were looking for other alternative molecules, for example; GroEL [7], EF-Tu [8] and RNA polymerase subunit B [9]. Bacterial DNA polymerase is an enzyme with function to catalyze transcription process. It consist of 2 subunit α, sub unit β, subunit β’ and subunit ω, and also need σ factor for promotor recognition [10]. Subunit β and β’ are encoded by rpoB and rpoC genes, while σ^{70} factor is encoded by rpoD gene. *Rhodobacter* genus has characteristics of gram negative bacteria with rod shaped morphology, able to form vesicular intrasitoplasmic membrane as well as ability to grow anaerobically and photoheterotrophically using sulfide as electron donor [11]. Some members of the genus have ability to fix nitrogen and have been used as model system to study photosynthesis and membrane development [12]. *Rhodobacter* genus is a genus of phototrophic purple nonsulfur α-*proteobacteria*,a class in the phylum of *Proteobacteria.* Close phylogenetic relationship between phototrophic and non-phototropic members of *Proteobacteria* made communities analysis of *Rhodobacter*base solely on 16SrRNA gene become complicated [13].

The aim of this study was to investigate benefit of sequence analysis of 16SrRNA, *rpo*B, *rpo*C and *rpo*D genes for infering phylogeny and identification tools of *Rhodobacter* genus.

METHODS

Nucleotide sequences of *16SrRNA* genes, *rpoB* genes, *rpoC* genes and *rpo*D genes were retrieved from National Center for Biotechnology Information (NCBI) Data Bank, United States. The source of these genes sequences were *Rhodobacter megalophilus* DSM 18937, *Rhodobacter capsulatus* SB 1003, *Rhodobacter sphaeroides* 2.4.1, *Rhodobacter johrii* JA 192T, *Rhodobacter ovatus* JA 234, *Rhodobacter azotoformans* KA 25, *Rhodobacter blasticus* 28/5, *Rhodobacter veldkampii* DSM 11550, *Rhodobacter aestuarii* DSM 19945, *Rhodobacter vinaykumarii* DSM 18714, *Rhodobacter maris* JA 276, *Rhodobacter viridis* JA 737, *Rhodobacter sp*. LPB0142, *Pararhodobacter sp*. CCB-MM2, *Rhodovulum sulfidophilum* DSM 2351, *Rhodopseudomonas palustris*HaA2, *Escherichia coli* str. K-12 substr. MG1655, *Paracoccus denitrificans*, *Roseobacter denitrificans*. Percentage of identity between sequences was calculated as percentage number of identical nucleotides in a sequence relative to total number of nucleotides. Phylogenetic analysis was conducted using MEGA 5 software [14] with Neighbor-Joining Method [15] and Kimura 2-Parameter model to built the tree [16]. Multiple sequence alignments were obtained using Clustal W [17]. Topology of the phylogenetic tree was evaluated by performing a bootstrap analysis using 1000 bootstrapped trials.

RESULTS

We have analysed fourteen species of *Rhodobacter* and five reference organisms in this study. The level of genes sequences identity among *Rhodobacter* species were 93.4 – 99 % (16SrRNA), 81.7 – 99 % (*rpo*B), 81.8 – 99 % (*rpo*C) and 80.2 – 99.1 % (*rpo*D). The level of genes sequences identity between *Rhodobacter* species and and reference organisms were 77.8 – 94,8 % (16SrRNA), 64.5 – 87.5 % (*rpo*B), 63.8 – 86 % (*rpo*C), 62.5 – 86.4 % (*rpo*D). The range between the highest and lowest level of sequence identity for each gene was calculated and the data is presented in figure 1.

Figure 1 show that the range of level identity among 16SrRNA gene sequence of *Rhodobacter* species is the smallest and on the other side, range of level identity among *rpo*D gene sequence is the bigest compare to other gene sequncesanalysed. The same result is also shown for comparison of the level identity range of genes sequences between *Rhodobacter* species to reference organisms.

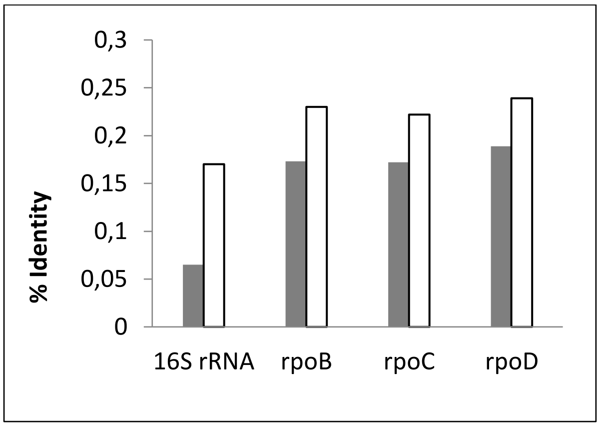

Figure 1. Range between highest and lowest percentage identity for each gene tested, measure among *Rhodobacter* species (filled bars), between *Rhodobacter*species and reference organisms (open bars).

Phylogenetic based on 16SrRNA sequence showed that all species members of*Rhodobacter* genus are separated into two major clusters (Figure 2). First cluster of *Rhodobacter* genus consist of *R. megalophilus*, *R. sphaeroides*, *R. johrii*, *R. ovatus*, *R. azotoformans*, *R. blasticus* and the second cluster consist of *R.veldkampii*, *R. aestuarii*, *R. vinaykumarii*, *R. maris*, *R. capsulatus*, *R. viridis* and *Rhodobacter sp*. LPB0142. *Pararhodobacter sp*. CCB – MM2 is placed in the first cluster of *Rhodobacter* genus eventhough it is not the member of this genus. *Roseobacter denitrifican* and *Paracoccus denitrificans* are clustered together. *Rhodovulum sulfidophilum* and *Rhodopseudomonas palustris,* two other phototrophic *Proteobacteria* are placed on their own tree branch. Gene sequence of *Escherichia coli* was used as an outlier to root the tree.*rpo*B sequence based phylogenetic tree showed that topology of the tree has high similarity to topology of tree based on 16 S rRNA gene sequence,except for *Paracoccusdenitrifican*is movedin second cluster of *Rhodobacter* genus and *Pararhodobacter sp*. CCB – MM2 is placed on its own tree branch separated from clusters of *Rhodobacter* (Figure 3).. *Pararhodobacter sp*. CCB – MM2 is also placed at its own branch in phylogenetic tree based on *rpo*C gene sequence. *Paracoccus denitrificans* together with *R. veldkampii* and *R. vinaykumarii* are shifted to first cluster of *Rhodobacter* (Figure 4). Each of *Pararhodobactersp*.CCB – MM2 and *Paracoccus denitrificans* are placed at their own branch on the phylogenetic tree based on *rpo*D gene sequence however *Rhodovulum sulfidophilum* and *Rhodobacter vinaykumarii* are placed together in one cluster (Figure 5).

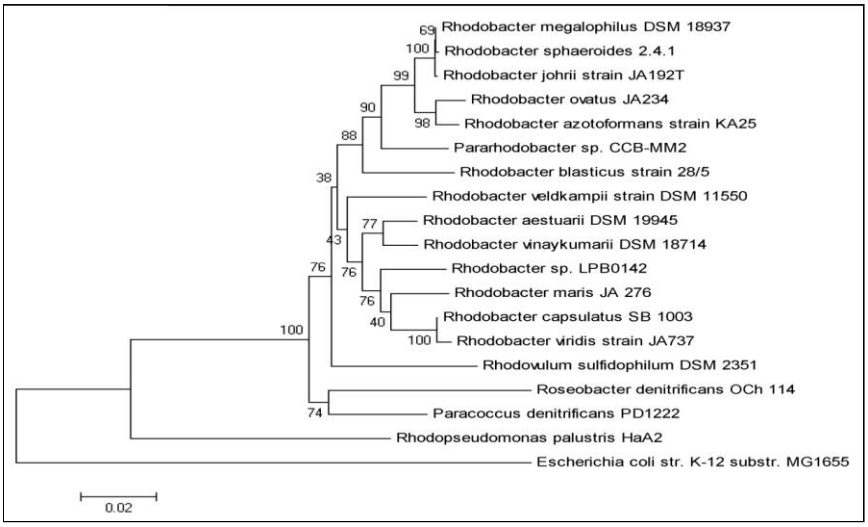

Figure 2. Phylogenetic tree based on analysis of 16SrRNA gene sequence. Genetic distance is indicated by scale.

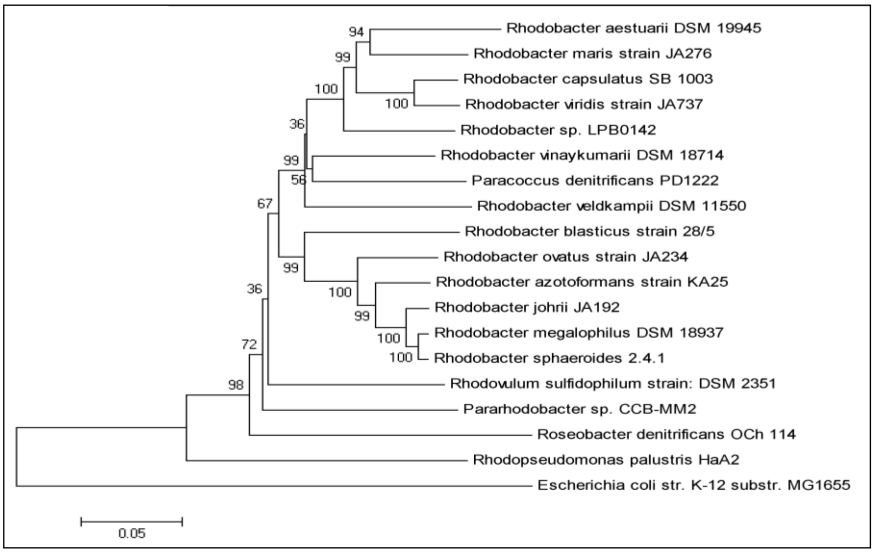

Figure 3. Phylogenetic tree based on analysis of *rpo*B gene sequence. Genetic distance is indicated by scale.

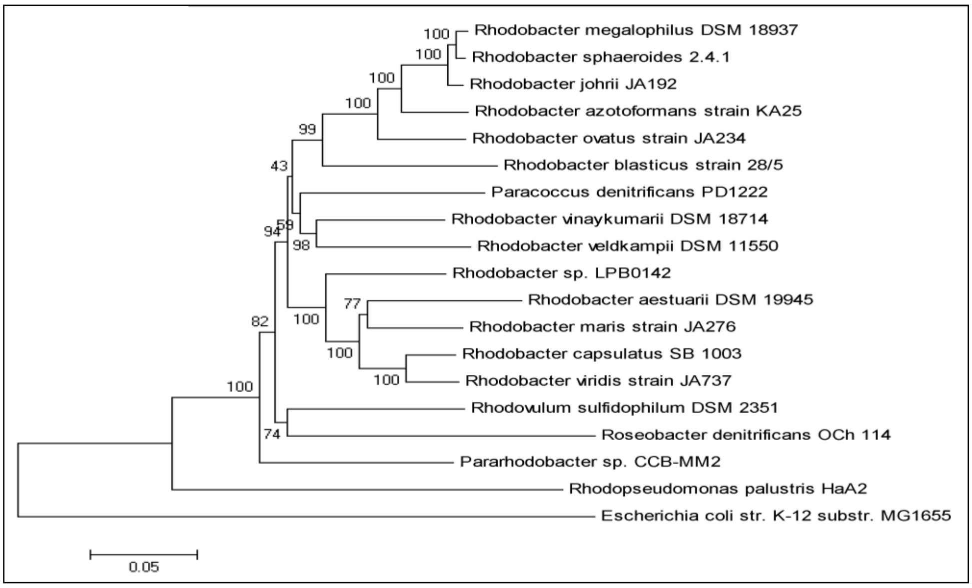

Figure 4. Phylogenetic tree based on analysis of *rpo*C gene sequence. Genetic distance is indicated by scale.

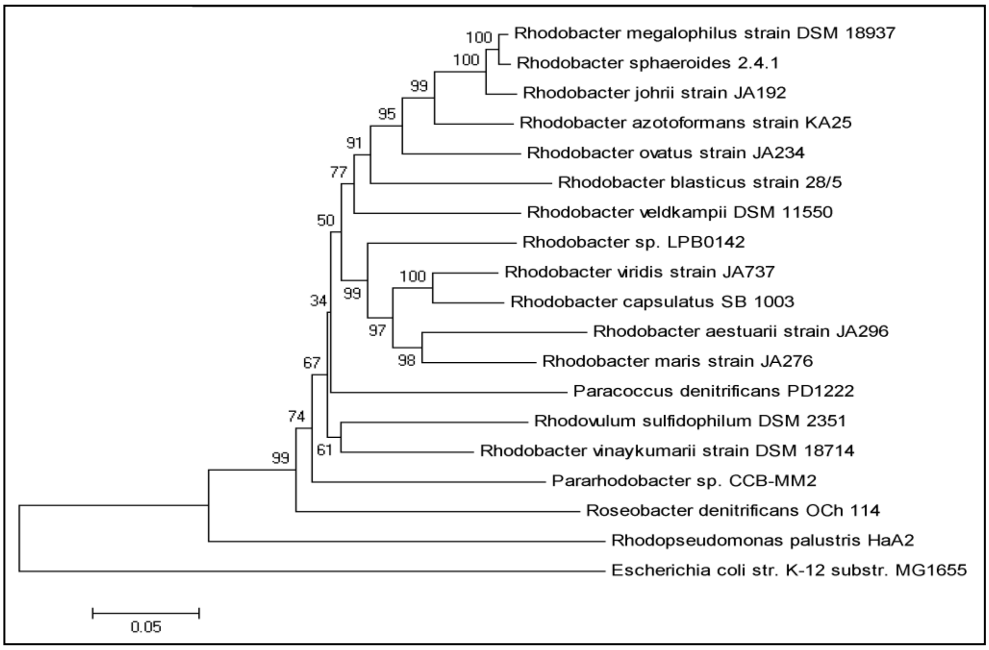

Figure 5. Phylogenetic tree based on analysis of *rpo*D gene sequence. Genetic distance is indicated by scale.

DISCUSSION

The result of phylogenetic analysis of *rpo*B, *rpo*C and *rpo*D genes sequences from *Rhodobacter* and some reference organisms largerly consistent with analysis based on 16SrRNA gene sequence eventhough some minor differences were exist, for example *Pararhodobactersp*.CCB-MM2 is placed in first cluster of *Rhodobacter* at tree based on 16SrRNA gene sequence but in anlysis based on *rpo*genes sequences, it is placed on its own tree branch. Position of *Parahodobacter sp*. CCB-MM2 on tree based on *rpoC* gene sequence analysis was supported by 100% boots replicates, exced 90%, 72%, 74% boots replicates for positions in tree based on 16SrRNA, *rpo*B and *rpo*D genes sequence. *Pararhodobactersp*.CCB-MM2 was previously known as *Rhodobacter sp*. CCB-MM2 before transfer *Pararhodobacter* genus. The only species of *Pararhodobacter* that have well caharacterized was *Pararhodobacter aggregans* [18]. Contradiction find in 16SrRNA gene based phylogenetic analysis have been report for *Hypomonas neptunium* which clasified as member of order *Rhodobacterales*, based on 16SrRNA gene but as member of order *Caulobacter* if analysis was based 23SrRNA, HSP 70 and EF-Tu genes [19]. The placement of gene sequences from different genus at same cluster in phylogenetic tree indicating that these genes maybe acquired through gene duplication or lateral gene transfer through their evolution history. There are reports of genes duplication in *Rhodobacter* *sphaeroides* 2.4.1 such as genes involved in Calvin - Benson – Bassham reductive pentosa-phosphate pathway; *cbb*AI and *cbb*AII [20 – 21], and three ribosomal RNA gene; *rrna* [22]. The example of lateral gene transfer between species of class *Proteobacteria* was *tfd*A gene encoding 2, 4 – Dichlorophenoxyacetic acid degraders [23].

CONCLUSION

Phylogeny of rpo genes especially *rpo*B largely consistent with phylogeny of 16SrRNA gene and can be used as additional marker genes.

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