

論文の内容の要旨

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論文題目

Phylogenetic studies on marine bacteria within the phylum *Proteobacteria* and *Bacteroidetes*

(*Proteobacteria* 門および *Bacteroidetes* 門に属する海洋細菌の系統分類に関する研究)

The oceans occupy 71% of earth's surface, with a volume of 1.46×10^9 Km³ and average depth of 4,000 m, and maximal depth of approximately 11,000 m.. The main problem in studying species distribution of on marine bacteria lies in the methods used to obtain viable cultures. Whether the isolates obtained by current culture methods represent indigenous population is unknown. Determining the representative physiological studies that can be performed on the vast numbers of marine bacteria that have yet to be cultured is still a matter of guesswork (Schute et al.,1993). Marine sponges are the most ancient multicellular animals, dating back to the Precambrian period, more than 635 million years ago. Their natural product diversity is among the highest found in nature. There is growing evidence that bacterial symbionts play a crucial role as producers of sponge-derived metabolites. In many sponge species, termed "high microbial abundance" (HMA) sponges, up to half the biomass can consist of microbial symbionts, whereas "low microbial abundance" (LMA) species, which live in the same habitats, contain much fewer symbionts. Often,

distantly related HMA sponges from different oceans share remarkably similar microbial communities.

In this study we attempted to elucidate the taxonomic position of nine novel marine bacterial strains that were isolated from marine seawater and marine sponges within the phylum *Proteobacteria* and *Bacteroidetes* by using polyphasic taxonomic approach.

The phylogenetic trees based on the neighbour-joining (NJ), maximum-parsimony (MP) and maximum-likelihood (ML) generated comparisons of the 16S rRNA gene sequences revealed individual taxonomic positions. Also molecular (DNA-DNA hybridization test and determination of the DNA G+C content), physiological (API 20E, API 20NE, API 50CH and API ZYM tests) and biochemical and chemotaxonomic analyses (determination of respiratory quinone system and cellular fatty acid content) were investigated on the novel isolates.

As a results, six of them were determined to be four new species of three novel genera (*Oceanicoccus*, *Psychrosphaera*, *Halicoccus*) in the *Gammaproteobacteria*. Individually, it was concluded that strains SA4-31, SA4-46 and SA4-48^T should be classified as representing a new genus and species of the family *Pseudoalteromonadaceae*, for which the name *Psychrosphaera saromensis* gen. nov., sp. nov. is proposed. Strain PZ-5^T represents a novel genus and species, for which the name *Oceanicoccus sagamiensis* gen. nov., sp. nov., is proposed. Two strains S1-36^T and S1-72^T present a novel genus and two species as *Halicoccus marinus* and *H. pacifica* were proposed. The genus *Oceanicoccus* and the genus *Halicoccus* formed a cluster with genera *Spongiibacter*, *Melitea*, *Dasania*, *Haliea* and *Congregibacter* at a family level, for which the name *Dasaniaceae* the Class *Gammaproteobacteria*, is proposed (Fig. 1). Two strains SG-29^T and S1-66^T were determined to be two species of two novel genera (*Rubricoccus*, *Aureimonas*) belonging to the phylum *Bacteroidetes*. Also phylogenetic position based on 16S rRNA gene sequence, molecular, physiological and biochemical and chemotaxonomic analyses and cellular fatty acid content) were investigated. Strain SG-29^T was classified as a novel genus and species, for which the name *Rubricoccus marinus* gen. nov., sp. nov., within family '*Rhodothermaceae*', is proposed. Strain S1-66^T represents a species of novel genus as *Aureimonas marinu* was proposed.

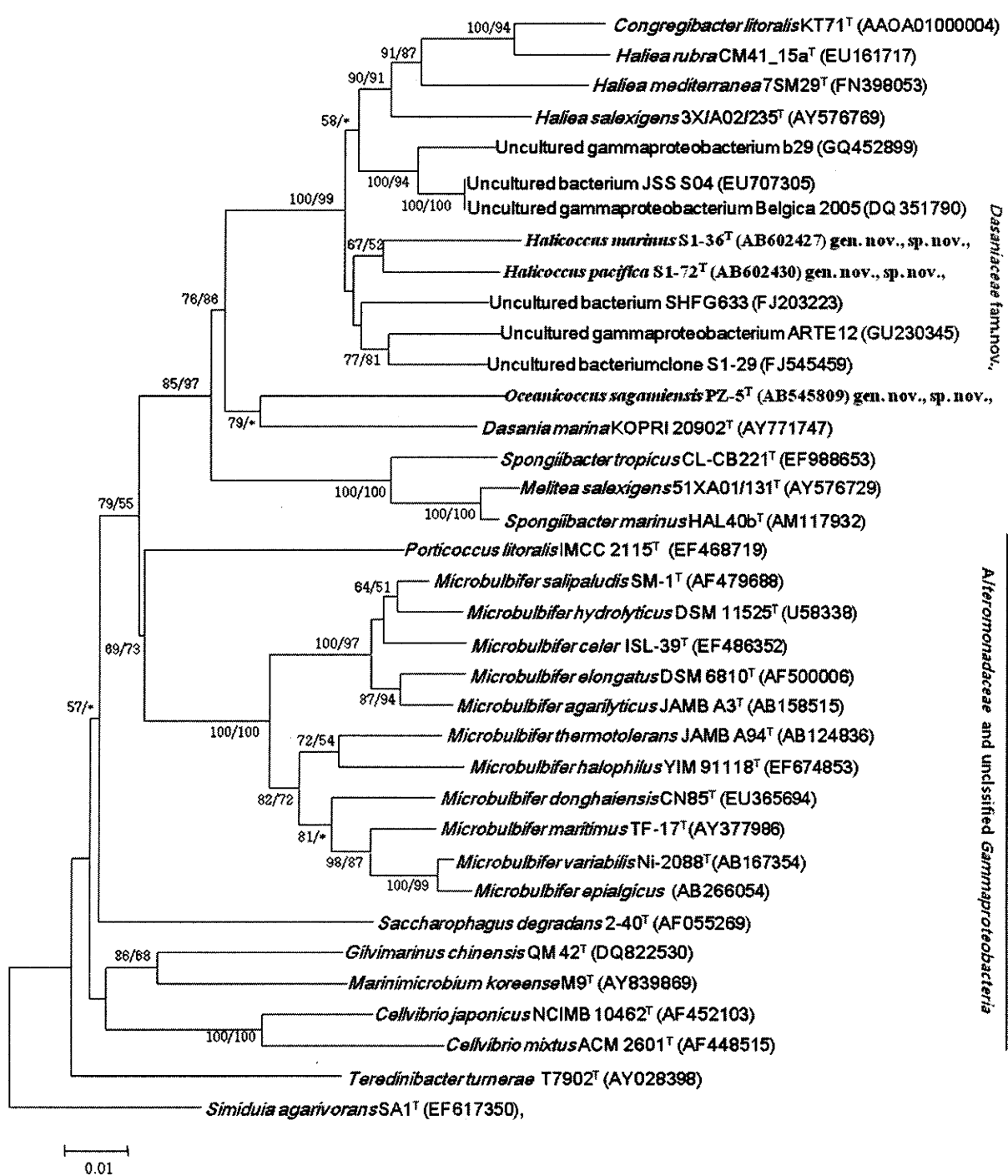


Figure 1. Neighbour-joining (NJ) phylogenetic tree based on the 16S rRNA gene sequences showing the position of strain S1-36^T among the currently known and related species of the class *Gammaproteobacteria*. Numbers at nodes are bootstrap percentages derived from 1000 replications (NJ/ML). Sequences of *Simidiua agarivorans* SA1^T (EF617350) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position. “ * ” indicates recovered nodes with <50% bootstrap values in the Maximum-likelihood (ML) tree.

The other hands, about 300 colonies were isolated from about 20 species of marine sponge were investigated to find novel bacterial strains using medium P and SN. On the medium P screen results (107 colonies), 27 genera in the 5 classes were isolated from marine sponge. On the medium SN screen results (132 colonies), 29 genera in the 5 classes were obtained. Interestingly we didn't find any strain belong to the phylum *Bacteroidetes* and the other class that previously reported using culture independent methods. But we obtained some 6 candidates showed =96% partial 16s rRNA gene sequences sequence similarity were re-sequenced of full 16S rRNA gene sequences and compared to data base information (Genbank) also respectively phylogenetic tree of six candidates were constructed. Finally strain MS-31^T isolated from marine sponge *Hymenicacidon flavia* was classified as *Sphingomonas Jejuensis* sp. nov., in the *Alphaproteobacteria*.

We attempted to elucidate the phylogenetic position of nine novel isolates using a polyphasic taxonomic approaches 16s rRNA gene sequence, physiological, biochemical and chemotaxonomic analyses to characterize the novel strains. Based on the data it is present that these isolates represent 7 species of the 5 genera in the phylum *Proteobacteria* and *Bacteroidetes*