

論文の内容の要旨

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論文題目 **Phylogenetic studies on marine bacteria within the phylum ‘*Verrucomicrobia*’**
(‘*Verrucomicrobia*’門に属する海洋細菌の系統分類学的研究)

Introduction

The phylum ‘*Verrucomicrobia*’ (Hedlund *et al.*, 1997) is one of the primary lineages within the domain *Bacteria*. A lot of molecular phylogenetic approaches and culture-independent studies based on 16S rRNA gene sequences revealed that the members of this phylogenetic group have been detected in a very wide range of quite different habitats within the global ecosystem. At present, these lineages have been informally classified into five subdivisions numbered 1 to 5 and have also been classified into six monophyletic subdivisions numbered 1 to 6 of which three are recognized in the second edition of *Bergey’s Manual of Systematic Bacteriology* as the families *Verrucomicrobiaceae* (subdivision 1), *Opitutaceae* (subdivision 4) and ‘*Xiphinematobacteriaceae*’ (subdivision 2). Since the six informal monophyletic subdivisions of this phylogenetic group were first proposed, the names of only a few species belonging to subdivisions 1 and 4 have been validly published. The class *Opitutae*, comprising two orders: *Puniceicoccales* containing the family *Puniceicoccaceae* and *Opitutaes* containing the family *Opitutaceae*, was formally proposed recently for the classification of subdivision 4. However, in spite of wide ecological distribution of representatives of the phylum ‘*Verrucomicrobia*’ in nature, owing to relatively few pure cultivated and characterized species, the classification of this phylum is still ambiguous and informal. For this reason, for formal classification of the phylum ‘*Verrucomicrobia*’, it is recommended that many verrucomicrobia that thrives in a wide range of terrestrial, aquatic and marine habitats should be isolated and taxonomically investigated. In the present study, we attempted to elucidate the phylogenetic relationships of strains isolated from various marine environments using a polyphasic taxonomic approach including 16S rRNA and *gyrB* gene sequence analysis, together with molecular, physiological, biochemical and chemotaxonomic analyses to characterize the novel isolates.

Phylogenetic studies and analysis of the cell wall components on the strains of the class *Opitutae* (Subdivision 4)

Phylogenetic studies were performed using polyphasic taxonomic approaches on eleven chemoheterotrophic bacteria which were isolated from various marine environments. 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 that these isolates were clustered within the family *Puniceococcaceae* of the class *Opitutae* (subdivision 4) (Fig.). Eleven novel strains represented three independent monophyletic lineages that were distinct from species with validly published genera with high bootstrap confidence values. Furthermore, molecular (DNA-DNA hybridization test and determination of the DNA G+C content), physiological (API 20E, 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. In addition, increasing concentrations of the β -lactam antibiotic susceptibility tests and amino acid analysis of the cell wall hydrolysates indicated the absence of muramic acid and *meso*-diaminopimelic acid and the presence of relatively low molar ratios of alanine and glutamate, which suggested that these strains lack an ordinary Gram negative type of peptidoglycan in the cell wall. Based on the results of phylogenetic analysis and its polyphasic taxonomic evidences, the eleven isolates were considered to represent three novel genera and eight new species belonging to the family *Puniceococcaceae* within the class *Opitutae* (subdivision 4), for which the names *Coralimargarita akajimensis* gen. nov., sp. nov. (strain 04OKA010-24^T; Yoon *et al.*, 2007a), *Pelagicoccus mobilis* gen. nov., sp. nov. (strain 02PA-Ca-133^T; Yoon *et al.*, 2007b), *Pelagicoccus albus* sp. nov. (strain YM14-201^T; Yoon *et al.*, 2007b), *Pelagicoccus litoralis* sp. nov. (strains H-MN57^T, H-MN48 and MN1-156; Yoon *et al.*, 2007b), *Pelagicoccus croceus* sp. nov. (strain N5FB36-5^T; Yoon *et al.*, 2007d), *Cerasicoccus arenae* gen. nov., sp. nov. (strain YM26-026^T; Yoon *et al.*, 2007c), *Cerasicoccus maritimus* sp. nov. (strain YM31-114^T) and *Cerasicoccus frondicus* sp. nov. (strains YM31-066^T and YM31-067) are proposed.

Phylogenetic studies on the strains of the family *Verrucomicrobiaceae* and reclassification of the class *Verrucomicrobiae*, the order *Verrucomicrobiales* and the family *Verrucomicrobiaceae* (Subdivision 1)

Nineteen chemoheterotrophic bacterial strains were isolated from various marine environments and organisms, and were subjected to phylogenetic investigations using polyphasic taxonomic methods. Phylogenetic analyses based on the neighbour-joining (NJ) and maximum-parsimony (MP) generated comparisons of the 16S rRNA gene sequences indicated that these isolates were affiliated to the family *Verrucomicrobiaceae* (subdivision 1) within the phylum '*Verrucomicrobia*' (Fig.). Among them, three novel strains had sequences that were related to the genus *Rubritalea*. Sixteen isolates studied form four different monophyletic clades that were distinct from species with validly published genera with high bootstrap confidence values. Furthermore, molecular (DNA-DNA hybridization test and determination of the DNA G+C content), physiological (API 20NE, API ZYM and Biolog tests) and biochemical and chemotaxonomic analyses (determination of respiratory quinone system and cellular fatty acid content) were investigated on the novel isolates. Some of the novel strains produced carotenoid pigments and squalene. Additionally, with these isolates, we proposed emended descriptions of the class

Verrucomicrobiae, the order *Verrucomicrobiales* and the family *Verrucomicrobiaceae* for formal classification of the phylum '*Verrucomicrobia*'. Analysis of the nearly complete 16S rRNA gene sequences from these strains indicated that the *Rubritalea* strains formed a robust clade having only limited resolution for this very tight group of species. However, the interrelationships deduced from the *gyrB* gene-based phylogeny showed better resolution than those based on their 16S rRNA gene sequences for the differentiation of strains at the species level. The genomic DNA-DNA relatedness values, biochemical and chemotaxonomic data (proportions of cellular fatty acids and menaquinones) also supported the results. On the basis of polyphasic evidences and phylogenetic characteristics, it was concluded that these strains should be classified as four novel genera and fourteen new species of the family *Verrucomicrobiaceae* within the class *Verrucomicrobiae* (subdivision 1), for which the names *Rubritalea spongiae* sp. nov. (strain YM21-132^T; Yoon *et al.*, 2007e), *Rubritalea tangerina* sp. nov. (strain YM27-005^T; Yoon *et al.*, 2007e), *Rubritalea sabuli* sp. nov. (strain YM29-052^T; Yoon *et al.*, 2007f), *Persicirhabdus sediminis* gen. nov., sp. nov. (strains YM20-087^T and YM21-151; Yoon *et al.*, 2007g), *Roseibacillus ishigakijimensis* gen. nov., sp. nov. (strain MN1-741^T; Yoon *et al.*, 2007g), *Roseibacillus ponti* sp. nov. (strain YM27-120^T; Yoon *et al.*, 2007g), *Roseibacillus persicicus* sp. nov. (strains YM26-010^T, YM24-184 and YM20-122; Yoon *et al.*, 2007g), *Luteolibacter pohnpeiensis* gen. nov., sp. nov. (strain A4T-83^T; Yoon *et al.*, 2007g), *Luteolibacter algae* sp. nov. (strains A5J-41-2^T and A5J-40; Yoon *et al.*, 2007g), *Haliferula harenosa* gen. nov., sp. nov. (strain YM23-227^T), *Haliferula pudorinus* sp. nov. (strain 06SJR1-1^T), *Haliferula phycoensis* sp. nov. (strain AK18-024^T), *Haliferula helveola* sp. nov. (strain 05IJR53-1^T) and *Haliferula sargassicola* sp. nov. (strains MN1-1037^T and MN1-1047) are proposed.

Conclusion

We attempted to elucidate the phylogenetic relationships of the thirty novel isolates using a polyphasic taxonomic approaches including 16S rRNA and *gyrB* gene sequence analysis, together with molecular, physiological, biochemical and chemotaxonomic analyses to characterize the novel strains. Based on these data, it is proposed that these isolates represent seven novel genera and twenty-two new species within the phylum '*Verrucomicrobia*'.

References

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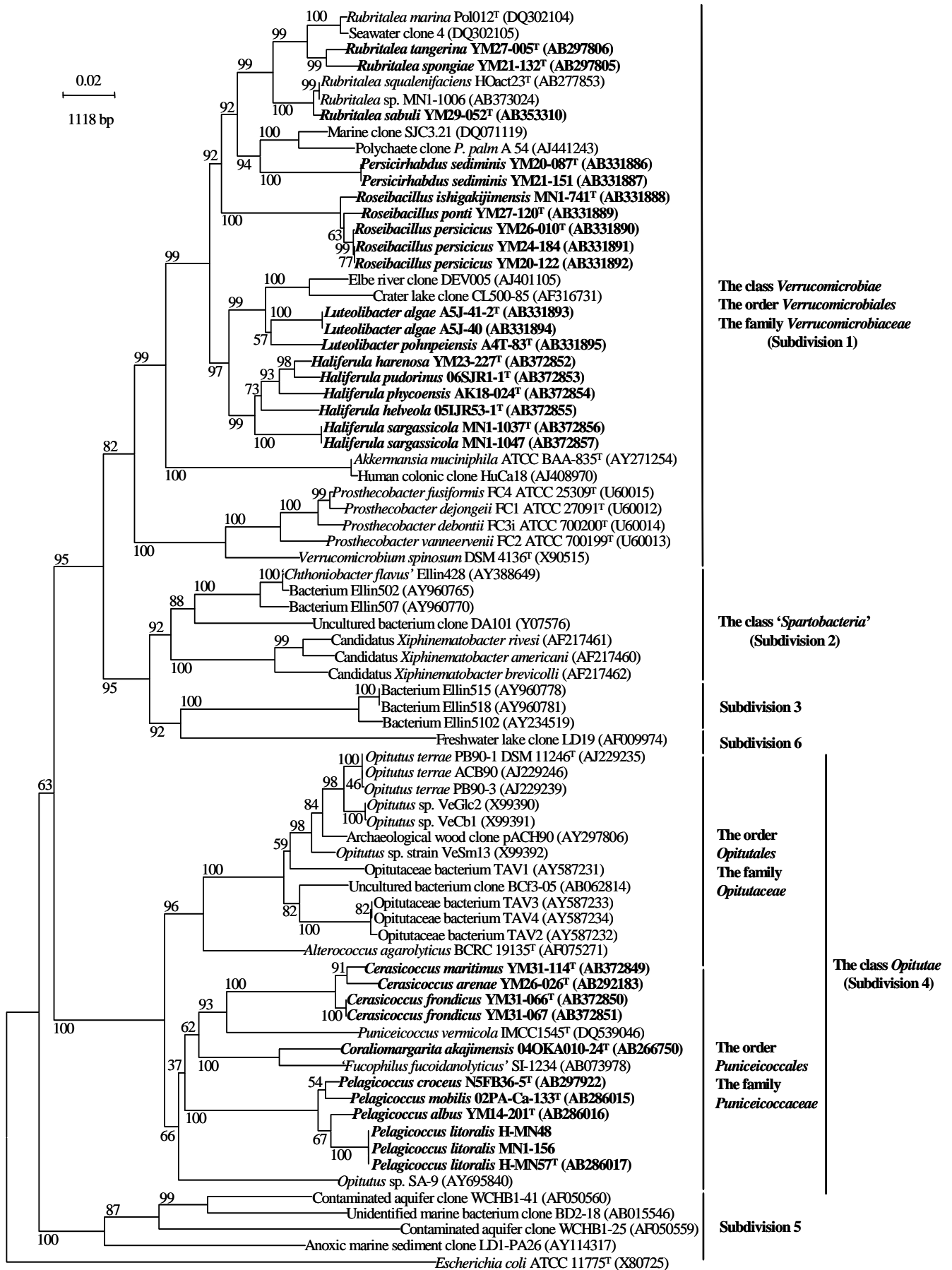


Fig. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relative positions of the novel strains described in this study among the currently known and cultivated species of the phylum '*Verrucomicrobia*'. Numbers at the nodes indicate the bootstrap value derived from 1000 replications. Sequences determined in this study are shown in bold. Bar, 2% sequence divergence.