Taxonomic Diversity of the Marine Bacteria of the Family Flavobacteriaceae Inhabiting the Far-Eastern Seas

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The taxonomic composition of microbial population is one of the most important characteristics of the natural marine ecosystems. Molecular techniques used to study complex biological formations have essentially enriched our knowledge of the taxonomic diversity and structure of the microbial communities in marine environments. The gliding Gram-negative heterotrophic bacteria are widely distributed in marine coastal ecosystems, composing from 6 to 72% of total microbial population.

Historically the bacteria belonging to the Cytophaga-Flavobacterium-Bacteroides (CFB) complex were weakly investigated from a phylogenetic view. In the recent years, isolation procedures supported by molecular methods have led to the description representatives of such readily culturable bacterial lineages as Gelidibacter, Psychroserpens, Polaribacter, Cellulophaga, Zobellia, Tenacibaculum, Muricauda, Psychroflexus, Salegentibacter, Muricauda, Kordia, Gillisia and Aequorivita, belonging to the family Flavobacteriaceae, that was proposed by Jooste in 1985. During 1992 Reichenbach has described validly the family Flavobacteriaceae, in which he included aerobic, Gram-negative, non-motile bacteria, the majority of them forms the color colonies due to production of the carotenoid or flexirubin type pigments. Later Bernardet et al. (1996, 2002) included in this taxonomic group closely related genera, represented by motile by gliding or non-gliding bacteria. The genus Flavobacterium Bernardet et al. 1996 was proposed to be the type genus of the family Flavobacteriaceae. The main or single respiratory quinone is menaquinone 6. Homospermidin is the major polyamine. The predominant cellular fatty acids are strait- and branched-chain saturated or unsaturated fatty acids namely 15:0, iso-15:0 or iso-17:0-3OH. G + C composition of the DNA ranging from 29 to 56 mol%. The majority of the flavobacteria are saprophitic organisms, some of them are the animal pathogens. Currently, the family Flavobacteriaceae includes 31 genera validly described, 17 from them are obligately marine microorganisms. Two marine bacteria Cytophaga latercula and Cytophaga marinoflava, those are close relatives of the members of the family Flavobacteriaceae, are formally included in the genus Cytophaga.

In course of the study of the microbial communities of the sea water, the bottom sediments, the edible holothurian *Apostichopus japonicus*, the sea urchin *Strongylocentrotus intermedius*, the soft corals *Paragoria arborea* and *Paragoria* sp., the green algae *Ulva fenestrata* and *Acrosiphonia sonderi*, the red alga *Polysiphonia japonica*, the brown algae *Chorda filum y Laminaria japonica*,

collected in the Sea of Okhotsk, at the Marine Experimental Station of PIBOC FEB RAS, located in Troitsa Bay, and Amyrsky Bay of the Gulf of Peter the Great of the Sea of Japan 371 strains of marine heterotrophic Gram-negative bacteria were isolated.

Using the polyphasic method including analysis of morphological, phisiological or biochemical properties, cellular fatty acid or respiratory quinone comopositions, the determination of G + C content of the DNA, performance of DNA-DNA hybridization experiments, sequencing of 16S ribosomal gene and phylogenetic analysis, allow to consider the strains studied to be the bacteria of the CFB complex. Among 372 isolates 13 strains were Cytophaga-like bacteria, sharing menaquinone 7 as the major respiratory quinone, that included the representives of the genera Algoriphagus (9), Reichenbachia (1) and Cyclobacterium-like bacteria (3). 273 strains were belonged to the 15 currently described genera of the family Flavobacteriaceae: Zobellia (70), Cellulophaga (60), Maribacter (50), Arenibacter (16), Formosa (15), Salegentibacter (12), 'Algibacter' (11), Mesonia (7), Ulvibacter (6), Tenacibaculum (6), Gelidibacter (3), Gillisia (2), Polaribacter (2) and Vitellibacter (1). It should be noted that such genera as Arenibacter, Maribacter, 'Algibacter', Mesonia, Ulvibacter and Vitellibacter were first described. Also, phylogenetic analysis showed that sequences of 49 strains formed the some separate lineage and were not affiliated with any previously documented phylotypes. Consequently, above mentioned strains are considering to be described as the members of six novel genera of the family Flavobacteriaceae, and 37 strains remain to be known. It should be noted isolation sources are characterized by significant diversity. For example, the strains of the genus Arenibacter, comprising three species A. latericius, A. troitsensis and A. certesii, were isolated from the bottom sediment samples, brown alga Chorda filum and holothurian Apostichopus japonicus, collected in the Okhotsk Sea and the Sea of Japan. Four species of the genus Maribacter, M. aquivivus, M. orientalis, M. sedimenticola and M. ulvicola, were recovered from seawater, bottom sediment and green alga Ulva fenestrata. The representatives of the genera Algibacter, Mesonia and Ulvibacter were isolated from the algal fronds. The single strain of Vitellibacter vladivostokensis was isolated from the holothurian Apostichopus japonicus. Three novel species of the genus Zobellia, Z. amurskyensis, Z. laminariae and Z. russelii, isolated from the sea water, the brown alga Laminaria japonica and the green alga Acrosiphonia sonderi, respectively, were described by us. Also we isolated from the sea water sample the novel species of the genera Cellulophaga and Gillisia, C. pacifica and 'G. mitskevishiae', respectively. The members of the novel Salegentibacter species, S. holothuriorum KMM 3524^T and 'S. mishustinae' KMM 6049^T were associated with the holothurian Apostichopus japonicus and the sea urchin Strongylocentrotus intermedius. Wide distribution of the family Flavobacteriaceae representatives in different marine environments probably may be explained by their broad adaptation spectrum to habitat conditions.

Thus, based on data presented in this work, we reported a large taxonomic diversity of marine bacteria of the family *Flavobacteriaceae*, isolated and classified in this study, and dominance of this taxonomic group among other *Cytophaga*-like bacteria. Our investigations of taxonomy, physiology and ecology showed a significant biotechnological potential of the marine flavobacteria as a perspective source of biologically active substances.

References

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