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博 士 学 位 论 文

产蛋白酶极地海洋浮游细菌的系统发育

多样性及生物地理学研究

**Phylogenetic diversity and biogeography of protease-producing  
bacterioplankton in polar marine environments**

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厦门大学博硕士学位论文摘要

## 摘 要

浮游细菌是海洋生态系统的主要成员之一并且具有重要的生态学意义。胞外蛋白酶是这些细菌发挥特定生态功能、表征生理活性的一个重要标志。在极地低温海洋环境中同样分布着数量众多的浮游细菌，它们在极地海洋生态系统的物质循环与能量流动中扮演着极其重要的角色。而两极地区独特的地理位置及气候环境特征，是否会导致生存其中的浮游细菌群落组成存在差异，是一个广受关注的科学问题。此外，相距遥远的两极之间是否存在相同种属的海洋细菌，也是一个值得探讨的问题。

本研究采用 16S rRNA 基因文库与 ARDRA 分析相结合的分子生物学手段，对白令海北部海域的浮游细菌群落组成进行了研究。结果表明，检测到的细菌序列包括  $\alpha$ -变形细菌纲 (Alphaproteobacteria)、 $\beta$ -变形细菌纲 (Betaproteobacteria)、 $\gamma$ -变形细菌纲 (Gammaproteobacteria)、 $\delta$ -变形细菌纲 (Deltaproteobacteria)、拟杆菌门 (Bacteroidetes)、放线菌门 (Actinobacteria)、厚壁菌门 (Firmicutes)、疣微菌门 (Verrucomicrobia)、浮霉菌门 (Planctomycetes)、酸杆菌门 (Acidobacteria)、梭杆菌门 (Fusobacteria)、衣原体门 (Chlamydiae)、绿菌门 (Chlorobi)、绿弯菌门 (Chloroflexi)、螺旋体门 (Spirochaetes) 以及候选类群 TM6、TM7、OP8、OP11 和 WS3 等 20 个类群，另外还有微藻（或蓝细菌）序列。底层水体中的细菌类群多样性高于表层水体。放线菌在整个浮游细菌群落中占据明显优势，在表层及底层水样文库中的比例分别为 25.9% 和 26.4%。 $\alpha$ -变形细菌是表层水体中另一优势类群 (20.9%)，它与微藻（或蓝细菌）在表层水样文库中的比例远高于底层水样文库。这与春夏季期间藻类及光合细菌等光合微生物在上层水体中大量生长繁殖的现象相吻合。而  $\beta$ -及  $\delta$ -变形细菌、厚壁菌在底层水样文库中的比例远高于表层水样文库，其中大多数  $\delta$ -变形细菌序列与脱硫线菌属 (*Desulfonema*)、脱硫管状菌属 (*Desulforhopalus*) 具有亲缘关系。在该海域获得的克隆序列与已报道序列的相似性为 79.9–100%，其中表层、底层水样文库中分别有 18.3%、21.5% 的序列与已知序列的相似性低于 97%，这说明在白令海北部海域可能蕴藏着较丰富的、新颖的微生物资源。

在北极王湾地区，PCR-DGGE 指纹图谱显示从海湾湾口至湾内的浮游细菌群落组成没有明显差异。但在海湾中部站位 stn. 3 的底层水体中，观测到相对更高的细菌多样性。基于 16S rRNA 基因文库与 ARDRA 的分析结果表明，在王湾海水中检测到的序列包括变形细菌（含  $\alpha$ 、 $\beta$ 、 $\gamma$  及  $\delta$  亚群）、拟杆菌、放线菌、疣微菌、浮霉菌以及部

分分类地位不明确的细菌, 另外还有微藻叶绿体(或蓝细菌)。α-变形细菌(43.6%)与微藻(27.7%)在表层水样文库中占优势, γ-变形细菌(36.5%)与α-变形细菌(29.4%)在底层水样文库中为主要细菌类群。这与放线菌在白令海北部海域的浮游细菌群落中占明显优势的情况不一致。在王湾海域获得的克隆序列与已报道的序列具有82.1-100%的相似性, 其中表层与底层水样文库中分别有29.2%、11.8%的序列与已知序列相似性低于97%。包括冰川融水在内的淡水输入可能给王湾浮游细菌群落带来大量的新颖物种。由于受洋流及夏季期间淡水输入的双重影响, 王湾中的浮游细菌群落可能是由分布于海洋中的广布性物种与来源于冰川等陆地环境的区域性物种共同组成的。

采用ARDRA与16S rRNA基因测序相结合的方法, 本研究对分离自南极普里兹湾、北极王湾、楚科奇海-加拿大海盆以及白令海北部海域的533株浮游细菌进行了分子鉴定。结果表明, 这些可培养细菌的多样性集中在α-变形细菌、γ-变形细菌、拟杆菌、放线菌以及厚壁菌等类群上。尽管从不同极地海域中分离到的浮游细菌在具体的类群组成以及属组成上存在一定差异, 但γ-变形细菌在所有调查海域的分离菌中都占绝对优势(87.4-98.9%), 其中又以假交替单胞菌属(*Pseudoalteromonas*)为优势属, 它在两极不同海域的分离菌株中所占比例为42.3-84.5%。胞外蛋白酶筛选结果显示, 这些细菌普遍具有蛋白酶活性, 产酶菌株在总分离菌株中所占比例为57.1-88.4%。γ-变形细菌在产蛋白酶菌株中所占比例为95.7-100%, 其中假交替单胞菌属仍是优势属, 在两极不同海域的产蛋白酶菌株中所占比例为47.8-87.5%。

采用包括形态学观测、表型检测、基因型分析等在内的多相分类学手段, 对两极不同海域浮游细菌的研究结果显示, 假交替单胞菌属与嗜冷杆菌属(*Psychrobacter*)这2个属细菌在两极海洋环境中普遍存在。此外, 本研究证实了来自南极普里兹湾、北极加拿大海盆以及格陵兰海的3株细菌属于同一个种冷海希瓦氏菌(*Shewanella frigidimarina*)。这些分布于两极海洋中的同属甚至同种细菌普遍具有耐冷、耐盐等特性, 说明耐冷、耐盐能力对于浮游细菌在两极甚至全球海洋环境中的广泛分布具有重要意义。

**关键词:** 极地海洋; 浮游细菌; 多样性与两极分布

## Abstract

Being major components of food webs, bacterioplankton play key roles in marine ecosystems. Extracellular proteases are one of important symbols showing the specific ecological function and physiological activity of planktonic bacteria. Playing important roles in biogeochemical cycles and energy flow in cold marine ecosystems, bacterioplankton have been observed in high abundance in polar marine environments. For the unique geographic location, and special climatic and environmental characteristics of the Arctic and the Antarctic, a related question concerning bacteria living in the polar marine environments is whether bacterioplankton community composition at both poles are the same or different. At the same time, another question whether there exist the same planktonic bacteria at the species level both in the Arctic and Antarctic marine environments is also interesting to people, although it has been accepted that bacteria genera are widely distributed.

Phylogenetic diversity of the marine bacterioplankton in the Northern Bering Sea was investigated by a combination of 16S rRNA gene clone library and ARDRA (Amplified ribosomal DNA restriction analysis). Sequences detected fell into 20 major lineages of the domain bacteria, including Proteobacteria (Alpha, Beta, Gamma and Delta), Bacteroidetes, Actinobacteria, Firmicutes, Acidobacteria, Planctomycetes, Verrucomicrobia, Fusobacteria, Chlamydiae, Chloroflexi, Chlorobi, Spirochaetes and candidate divisions OP8, OP11, TM6, TM7 and WS3, in addition to chloroplasts of algae (or Cyanobacteria). At the division level, higher bacterial diversity was observed in the bottom water than that in surface water. Accounting for 25.9 and 26.4% of the total clones in clone library of the surface water and bottom water, respectively, Actinobacteria dominated in the bacterioplankton community of the Northern Bering Sea. Alphaproteobacteria was another dominant fraction (20.9%) in the surface water. Percentages of Alphaproteobacteria and algae (or Cyanobacteria) in clone library of the surface water were much higher than

those in bottom water, consistent with the phenomena that phototrophic microorganisms, including algae and phototrophic bacteria, usually distribute in upper water layers in spring and summer. On the contrary, Beta- and Deltaproteobacteria, and Firmicutes possessed a higher percentage in the bottom water than them in surface water. Most cloned sequences within the Deltaproteobacteria showed close relationships to genera *Desulfonema* and *Desulforhopalus*. Cloned sequences of the Northern Bering Sea showed 79.9–100% similarity to those described sequences. Among them, there were 18.3 and 21.5% of sequences from the surface water and bottom water, respectively, had similarity values lower than 97% to reported sequences. It indicates a relatively abundant but unknown bacteria resource in the Northern Bering Sea. Community fingerprint analysis by PCR-DGGE (denaturing gradient gel electrophoresis) revealed that there was no apparent difference of bacterioplankton community composition between sampling locations in Kongsfjorden, an arctic fjord in Spitsbergen. However, a higher biodiversity was observed in the bottom water of station Stn.3 in the central part of the fjord. By a combination of 16S rRNA gene clone library and ARDRA, sequences detected fell into 9 putative divisions, including Proteobacteria (Alpha, Beta, Gamma and Delta), Bacteroidetes, Actinobacteria, Verrucomicrobia, Planctomycetes and unidentified bacteria, in addition to chloroplasts of algae (or Cyanobacteria). Compared to the preponderance of clones representing Gammaproteobacteria (36.5%) and Alphaproteobacteria (29.4%) in the bottom water, Alphaproteobacteria (43.6%) and algae (27.7%) constituted two dominant fractions in the surface water, showing a difference from the Northern Bering Sea where the Actinobacteria dominated in the bacterioplankton community. Cloned sequences showed 82.1–100% similarity to those described sequences. Among them, there were 29.2 and 11.8% of sequences from the surface water and bottom water, respectively, had similarity values lower than 97% to reported sequences, suggesting that the freshwater input including the glacial meltwater provides a possibility to carry novel



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