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西太平洋微生物群落结构及其影响因子
的初步研究

A preliminary study on microbial community structures and
their influencing factors in the Western Pacific waters

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厦门大学博硕士学位论文摘要库

摘要

西太平洋, 尤其是“暖池”区, 常年温度高于 28 °C, 在全球气候变化中起着“引擎”作用。微生物在西太平洋“暖池”中类群多样、代谢旺盛、物质循环较快。同时, 微生物在微生物碳泵 (Microbial Carbon Pump, MCP) 中起着重要的作用, 驱动全球生物地球化学循环。此外, 西太平洋也是起源于高纬度海区的各种复杂水团的交汇区。本文采用纯培养法和 454 焦磷酸测序法对西太平洋微生物群落结构和多样性及其与环境因子的关系进行了调查研究, 扩展了微生物群落结构和多样性在该海区研究的广度和深度。

基于纯培养法对 2009 年大洋航次上 6 个站位表层海水中可培养细菌多样性进行了调查, 结果显示西太平洋的可培养细菌拥有较高的多样性。分离获得的 39 株细菌与已发表模式菌株的 16S rRNA 基因序列相似性在 96.6~100% 之间, 分布于 19 个属, 其中赤杆菌属 (*Erythrobacter*)、单胞菌属 (*Alteromonas*) 和 *Loktanella* 属为优势属。选取一株相似性最低的菌株 JLT2008^T 进行进一步的鉴定, 通过对其形态结构、细菌叶绿素、细胞脂肪酸、呼吸醌、极性脂、GC 含量、单碳源的利用和酶活性等生理生化特征的检测, 并与参考菌株作对比分析, 确定 JLT2008^T 为赤杆菌属的一个新种, 命名为 *Erythrobacter westpacificensis*。

基于流式细胞检测法和 GS FLX 454 测序法在 2011 年“科学一号”西太平洋航次上, 对 5~8 °N、127.5~135 °E 海域的 7 个站位垂直水柱 (0~2000 m) 中的微生物丰度、群落结构和多样性进行了调查, 并分析了影响细菌优势类群和不同水团中细菌群落群落组成的环境因素。分析结果如下:

1. 西太平洋“暖池”垂直水柱中, 海水层化现象明显, 根据位温-盐度 (Potential Temperature-Salinity, T-S) 图, 将海水分为 4 个不同性质的水团, 分别是表层水 (Surface Water, SW)、次表层水 (Subsurface Water, SSW)、中层水 (Intermediate Water, IW) 和深层水 (Deep Water, DW)。
2. 垂直水柱中, 异养细菌的丰度变化为 $0.8 \times 10^4 \sim 3 \times 10^5$ cells/mL, 5 °N 断面异养细菌丰度最大层较 7 °N 断面下沉约 50 m。从西到东 (130~135 °E), SW 中, 异养细菌丰度在 134 °E 最大, 两边较大; SSW 和 IW 中, 西高东低; 而 DW 中, 西低东高。

3. 454 测序后的序列经质量筛选后共获得 900,665 条高质量序列，其中 92.6% 的序列为细菌。在已知细菌类群中，变形菌门（*Proteobacteria*, 26.96±13.63%）、拟杆菌门（*Bacteroidetes*, 16.89±11.44%）和蓝细菌门（*Cyanobacteria*）是相对丰度最高的类群，占有所有已知细菌类群序列的 83%。所有样品中的主导优势类群分别是 SAR11-like 和鲁杰氏菌属（*Ruegeria*）（ α 变形菌纲）、盐单胞菌（*Halomonas*）、假单胞菌（*Pseudomonas*）和交替假单胞菌（*Pseudoalteromonas*）（ γ 变形菌纲）及黄杆菌属（*Flavobacterium*）和列文虎克菌属（*Leeuwenhoekiella*）（拟杆菌门）。
4. 细菌优势类群与深度、温度和营养盐均有显著相关性，而受盐度的影响不显著。SW、SSW 和 IW 3 个水团中优势类群随深度的变化快慢与 DW 中不同。
5. 不同的水团中细菌群落结构不同。SW 和 DW 中细菌群落结构相似性较 SSW 和 IW 中高。深度和温度是影响不同水团中细菌群落结构差异的最主要环境因素。不同水团中存在水团特异性的细菌类群，如分布在 SW 和 SSW 中的蓝细菌门、梭杆菌门（*Fusobacteria*）、黄杆菌属和弧菌（*Vibrio*），除 SW 以外的 3 个水团中特有的 δ 变形菌纲（*Deltaproteobacteria*）及 IW 和 DW 中特异性分布的食烷菌属（*Alcanivorax*）。

关键词：微型生物丰度；细菌群落组成；细菌多样性；水团；焦磷酸测序；西太平洋“暖池”

Abstract

The Western Pacific, especially the “Warm Pool” is regarded as one of “engine” of global climate system with the annual surface water temperature over 28 °C. High diversity of microbial, more active metabolism and more quick substance cycles happen in the region of the Western Pacific “Warm Pool”. Microbes, especially heterotrophic bacteria play important roles in the microbial carbon pump (MCP) and drive the global biogeochemical cycles. Simultaneously, various complex water masses originating from the higher latitude meet and mix in this region. In this study, we survey the microbial community and diversity in the Western Pacific based on both culture-dependent and culture-independent methods, and analyzed the relationship to environmental factors. Results of this study will expand the breath and depth of researches about microbial community and diversity in the Western Pacific.

Culture-dependent method was used to survey the diversity of culturable bacteria in the Western Pacific of the surface water. Results indicated that high diverse culturable bacteria were present in the Western Pacific. All 39 isolates belonging to 19 genera possessed the highest 16S rRNA gene similarity 96.6~100% with the type strains. Among all identified genera, *Erythrobacter*, *Alteromonas* and *Loktanella* were dominant genera. We chose one strain (JLT2008^T) with the lowest similarity with published type strains to do further analysis. After determination of physiological and biochemical characteristics such as cell morphology, bacteriochlorophyll, cellular fatty acids, respiratory quinone, polar lipids, GC content, utilization of carbohydrate and activities of enzyme and contrast to the reference strain, we proposed the strain JLT2008^T as a new species of *Erythrobacter* genus, named *Erythrobacter westpacificensis*.

Culture-independent methods including flow cytometry and GS FLX barcode 454 pyrosequencing were used to investigate microbial abundance, community structure and diversity in the Western Pacific “Warm Pool”, and their relationships to environmental factors were also analyzed. Results were as followings:

1. In the vertical water column of the “Warm Pool”, sea water stratified significantly. Based on the potential temperature (T-S) diagram, four different hydrological properties water masses were divided which were surface water (SW), subsurface water (SSW), intermediate water (IW) and deep water (DW).

2. In the vertical water column (0~2000m), the abundance of heterotrophic bacteria ranged from 0.8×10^4 to 3×10^5 cells/mL. Generally, the maximal abundance layers of heterotrophic bacteria in transect 5 °N were about 50 m lower than those in transect 7 °N. On horizontal scale (130~135 °E), in the SW, the abundance of heterotrophic increased to the maximum in 134 °E and then decreased. For SSW and IW, it decreased from the west to the east, and opposite in the DW.
3. After quality screening, we totally obtained 900,665 valide pyrotags, 92.6% of which were bacteria. In the known bacterial groups, Proteobacteria ($26.96 \pm 13.63\%$), Bacteroidetes ($16.89 \pm 11.44\%$) and Cyanobacteria were the most abundant phyla, accounting 83% of all identified bacterial sequences. Alphaproteobacteria, Gammaproteobacteria (Proteobacteria) and Flavobacteria (Bacteroidetes) were the most abundant classes in all samples. SAR11-like and *Ruegeria* dominated in Alphaproteobacteria, and for Gammaproteobacteria, dominant groups were *Halomonas*, *Pseudomonas* and *Pseudoalteromonas*. *Flavobacterium* and *Leeuwenhoekiella* were the most abundant genera in Bacteroidetes.
4. In the vertical water columns, dominant bacterial groups were significantly related to depth, temperature and nutrients, while not significantly influenced by salinity. Variations of the relative abundance of dominant bacterial groups with depth were different in three water masses (including SW, SSW and IW) and DW.
5. The bacterial community structures were different in different water masses. The similarities of bacterial community structure in SW and DW were higher than those SSW and IW. Temperature and depth were the main significant environmental factors. Water specific bacterial groups were found in different water masses, such as Cyanobacteria, Fusobacteria, *Flavobacterium* and *vibrio* in the SW and SSW, Deltaproteobacteria in the SSW, IW and DW, and *Alcanivorax* in the IW and DW.

Key Words: Microbial abundance; Bacterial community; Bacterial diversity; Water mass; Pyrosequence; The Western Pacific “Warm Pool”

第 1 章 绪论

1.1 海洋微生物

1.1.1 海洋微生物在海洋储碳中的作用

海洋约占地球表面的 71%，是地球上决定气候发展的主要因素之一，它与大气之间的相互交换对气候的变化和发展有极大的影响。海洋微生物是海洋生态系统的重要组成成分，它遍布在整个海洋环境中，且具有丰度高、生物量大、种类复杂多样和代谢多样等特点，因此，在海洋生物地球化学循环中发挥着重要作用（Azam et al., 1998）。

经典海洋牧食食物链中，自养生物包括浮游植物、藻类和蓝细菌作为主要生产者利用光能将无机碳转化为有机碳，同时产生地球上生物所需的氧气。而异养细菌作为分解者，利用生产者产生的有机物进行生长代谢并将其还原为无机物。上世纪 80 年代以来的研究表明，细菌可以进行二次生产（bacterial secondary production）。初级生产的产物除了包括构成浮游植物自身生物量的颗粒有机物（Partical Organic Matter, POM），还包括释放到海水中的溶解有机物（Dissolved Organic Carbon, DOM）。这些 DOM 基本上不能被浮游植物吸收，但却可以被海洋细菌吸收利用，转化为自身生物量的增长。异养细菌可被鞭毛虫和纤毛虫等原生动物所捕食，而后者又可被小型浮游动物所捕食。基于以上的研究认识，Azam 于 1983 年将 DOM 通过细菌二次生产后形成的异养细菌-原生动物-后生动物的捕食关系称为微型食物环，简称微食物环（Microbial loop）（Azam et al., 1983）。微食物环中，异养细菌在有机碳的转换过程中起关键作用，它不仅是分解者，同时也是“生产者”。异养细菌可以利用生态系统中所有生物产生的 DOM，这使异养细菌生产力超过初级生产力成为可能（焦念志等，2006）。浮游病毒是海洋中数量最多的生命体，它可快速侵染宿主包括异养细菌、蓝细菌和其他微生物。病毒裂解宿主细胞产生 DOM 形成病毒回路（Virus shunt）或称病毒环（Virus loop）（Fuhrman, 1999），病毒环改变了海洋生态系统中物质循环和能量流动的途径，它裂解宿主产生的 DOM 被异养细菌再次吸收利用进入微食物环。

自工业革命以后，人类将大量的 CO₂ 排放到大气中，使得大气中 CO₂ 的含

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