ISSN : 2350-0743

www.ijramr.com

International Journal of Recent Advances in Multidisciplinary Research

Vol. 08, Issue 04, pp. 6732-6734, April, 2021

RESEARCH ARTICLE

ANALYSISOF MICROBIALCOMMUNITYINPETROLEUM-CONTAMINATEDSEAWATER AND SEDIMENTFROM XIUYINGPORT, HAIKOU

Yingying He[1], Xiuhong Wang[1], Shaobai Wen[1*], Qiongyin Zhang[1], Yan Liu[1] and Jianbo Liu[2*]

[1]School of Tropical Medicine and Laboratory Medicine, Hainan Medical University, Haikou 571199, P.R. China
[2]Hainan Nachuan Energy Environmental Protection Technology Co., Ltd, Haikou 571199, P.R. China

ARTICLE INFO

Article History:
Received 20th January, 2021
Received in revised form
19h February, 2021
Accepted 15th March, 2021
Published online 30th April, 2021

Keywords:

High throughput Sequencing
Petroleum Contaminated
Bacterial Community

ABSTRACT

Changes of microbial community in the petroleum-contaminated area may have a critical effect on the process of bioremediation. In the study, high-throughput sequencing was used to study the stucture of microbial community in the petroleum-contaminated area of Xiuying port, Haikou. In results, there showed differences in microbial communities between seawater and sediment at the same site. Base on the metagemone analysis, both bacteria and archaea were frequentuly found in seawater, whereas bacteria was the dominant microorganism in sediment. Based on category analysis, the seawater samples mainly include *Chloroflexi*(18%), *Proteobacteria*(18%), *Planctomycetes*(12%), The sediment samples were mainly include *Bacteroidetes* (29%), *Proteobacteria* (29%), *Firmicutes* (19%) and *Actinobacteria* (14%). In conlusion, the study is of great significance to the study of microbial remediation of petroleum contaminated marine waters.

INTRODUCTION

In recent decades, petroleum hydrocarbons have been frequently released into aquatic environments by anthropogenic activities such as extraction, transportation, or storage of petroleum-based products [1]. Recent studies have shown that microorganisms might play a crucial role in regulating the biodegradation of petroleum hydrocarbons [2,3], However, little is known about the mechanism of biodegradation. Nevertheless, understanding of the microbial community composition in remediation systems may provide in formations to study the mechanism biodegradation in remediation [4]. Thus, in the study, the structure and composition of microbial colonies in petroleum contaminated seawater and sediment from Xiuying port was analyzed by high-throughput sequencing.

MATERIAL AND METHODS

Sample collection and DNA isolation: The sampling site located at Xiuying harbor, Haikou City, Hainan Province (110°17'31.54, 20°1'38.81). One kilogram (kg) of sediment was collected by the mud grab bucket and transferred into a sterile bag. One liter (L) of seawater samples above the site of collected sediment and was filtered with 0.22μm mixed cellulose ester microporous membrane.

Corresponding author:** ***Shaobai Wen,
School of Tropical Medicine and Laboratory Medicine, Hainan Medical University, Haikou 571199, P.R.China.

After filtration, the samples were collected in a sterilized centrifuge tube and stored at -70 . Sample collecting was carried out three times to avoid the sampling biasis. DNA was extracted and purified from sediment and seawater samples according to the instruction of DNA extraction kit (MOBIO Laboratories, Inc., American).DNA extracted from each sample with three replicates were mixed for subsequent analysis. The purity and concentration of DNA were detected by UV spectrophotometer (NanoDrop, Japan).The extracted DNA samples were stored at -20 .The qualified DNA samples were subjected to library construction, which was further sequenced by Illumina PE150. The Raw Data obtained from DNA sequencing will be used for information analysis.

Information analysis: Data quality control: Raw data obtained from DNA sequencing contains a certain proportion of low-quality data. In order to ensure the accuracy and reliability of information analysis results, the quality control and host filtering of raw data were conducted and the Clean Data was harvested.

Metagenome assembly: Metagenome was assembled with the clean data of each sample, and the redundant reads of each sample were mixed and assembled to find the information of low abundance species in the sample.

Species note: Based on the gene catalogue, the species annotation information of each gene (Unigene) was obtained by comparing with the microNR, and the species abundance

tables of different classification levels were obtained by combining with the gene abundance table.

RESULTS AND DISCUSSION

Analysis of sequencing results: In the study, Illumina HiSeq sequencing platform was applied for metagenome sequencing, and a total of 12,911.39 Mbp of Raw Data was obtained（the average data was 6,455.69 Mbp）. Then, theclean data of 12,895.48 Mbp were obtained (the average data was 6,447.74 Mbp) after quality control. After single sample assembly and mixed assembly, a total of 440,693,361 bp of Scaftigs were obtained. Meta Gene Mark software was used to predict the results of each sample and mixed assembly, and 720,948 ORFs (240316 on average) were obtained by the analysis. After redundancy elimination, a total of 692,427 ORFs, a total length of 377.14 Mbp, were obtained. The number of complete genes was 176,935, accounting for 25.55% of total ORFs. Based on Blastp comparison between non-redundant gene sets and microNR and species annotated by LCA algorithm, the proportion of annotated genera and phyla was 44.63% and 64.94% respectively. According to the non-redundant gene sets annotated by DIAMOND software (E-value $\leq 10^{-5}$),0 (0.00%), 452992 (65.42%), and 437719 (63.22%) ORFs were classified in the CAZy database, KEGG database, and eggNOG database, respectively.

Relative abundance of species in seawater and sediment samples: The relative abundance of microbial species in seawater and sediment samples are shown in Figure 1.The seawater samples mainly contain bacteria and archaea, in which bacteria and archaea account for 66% and 11%, respectively. The bacteria mainly include *Chloroflexi* (18%), *Proteobacteria* (18%), *Planctomycetes* (12%) and *Bacteroidetes* (2%).The archaea mainly include *Candida bathyarchaeota* (4%), *Euryarchaeota* (4%) and *Candida thorachaeota* (1%). In contrast, in the sediment samples, bacteria accounts for 96% and no genes from archaea was detected. The types of bacteria in sediment mainly include *Bacteroidetes* (29%), *Proteobacteria* (29%), *Firmicutes* (19%) and *actinobacteria* (14%).The result showed that the common dominant species in petroleum contaminated water and sediment were *Proteobacteria* and *Bacteroidetes*, which indicated that those had a wide range of adaptability each other and could be further used to screen petroleum degrading bacteria and select functional genes.

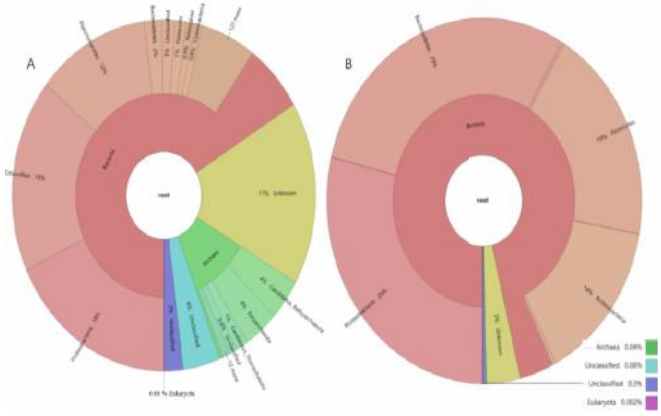

Figure 1 Relative abundancy of species at different taxonomic levels A and B was seawater and sediment sample, respectively

The top ten species with the largest relative abundance: Based on the classification analysis of relative abundance, the top 10 species with the largest relative abundance in each sample were selected, and the rest species were set as others (Fig. 2). As shown in Fig. 2A, the composition of seawater samples was richer than the sediment samples according to the analysis of the phylum-level diversity, and *Chloroflexi* and *Proteobacteria* account for the highest proportion at the phylum level in seawater. Among the sediment samples, *Bacteroidetes* and *Proteobacteria* had the highest proportion at the phylum level. Fig. 2B shows the relative abundance of genera in seawater and sediment samples, the dominant bacteria in sediment samples were *glycomyces*, the relative abundance of the top 10 species in sediment was significantly higher than that in seawater. It may be that the condition in sediment is more stable than that in seawater, so the composition of dominant community is more stable.

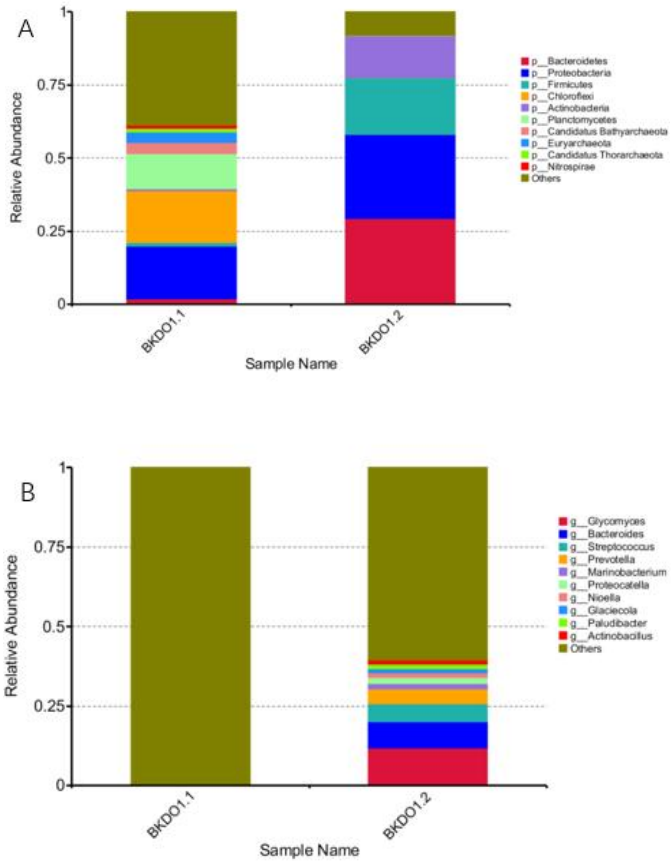

Figure 2. The top ten species with the largest relative abundance in the sample.A the histogram of the horizontal relative abundance of the phylum.B the histogram of relative abundance at genus level. BKDO1.1 and BKDO1.2 was seawater and sediment sample, respectively. The horizontal axis indicates the sample name, the vertical axis represents the relative proportion of species annotated to a certain type,the types of objects corresponding to each color block are shown in the legend on the right

Conclusion

The balance and diversity of the microbial community structure were beneficial to petroleum hydrocarbon decontamination [5]. The result showed that there were differences in phyla and genera of microbial communities in the petroleum contaminated seawater and sediment from Xiuying Port.

Bacteria and archaea were detected in seawater samples, while only bacteria were detected in sediment samples. At the phylum level, the abundance of the top 10 species in the seawater samples was higher than that in the sediment samples, while at the genus level, the abundance of the top 10 species in the sediment samples was higher than that in the seawater samples. The microbial community structure was different in different environmental media even in the same area. These results provide certain support for the study of microbial remediation of petroleum pollution in different environments.

Acknowledgment

This work was financially supported by the National Training Programs of Innovation and Entrepreneurship for Undergraduates (201911810031) and the Natural Science Foundation of Hainan Province (20164165).

REFERENCES

Li, X., Li, Y., Zhang, X., Zhao, X., Sun, Y., Weng, L., Li, Y. 2019. Long-term effect of biochar amendment on the biodegradation of petroleum hydrocarbons in soil microbial fuel cells. *Sci. Total Environ*., 651, 796–806.

Qian, J., Zhang, M.K., Pei, X.J., Zhang, Z., Niu, J.T., Liu, Y. 2018. A novel integrated thiosulfate-driven denitritation (TDD) and anaerobic ammonia oxidation (anammox) process for biological nitrogen removal. *Biochem. Eng. J*., 139, 68–73.

Wu M.L., Wu J.L., Zhang X.H., Ye X.Q. 2019. Effect of bioaugmentation and biostimulation on hydrocarbon degradation and microbial community composition in petroleum contaminated loessal soil. *Chemosphere*., 237, 124456

Xu, X.J., Liu, W.M., Wang, W., Tian, S.H., Jiang, P., Qi, Q.G., Li, F.J., Li, H.Y., Wang, Q.Y., Li, H., Yu, H.W. 2019. Potential biodegradation of phenanthrene by isolated halotolerant bacterial strains from petroleum oil polluted soil in Yellow River Delta. *Sci.Total Environ*., 664, 1030–1038.

Zhang, H.H., Feng, J., Chen, S.N., Zhao, Z.F., Li, B.Q., Wang, Y., Jia, J.Y., Li, S.L., Wang, Y.,Yan, M.M., Lu, K.Y., Hao, H.Y. 2018. Geographical patterns of nirS gene abundance and nirs-type denitrifying bacterial community associated with activated sludge from different wastewater treatment plants. Microb. Ecol., https://doi.org/10.1007/s00248-018-1236-7.

****