

PAa-09**Snapshot analysis of phage metagenomics in algae-blooming freshwater environment of Hachiroko Lake, Akita Prefecture**

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秋田県八郎湖のアオコ発生環境でのファージメタゲノムスナップショット分析

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Key word : phage, metagenome, hachiroko, *microcystis*, *anabaena*

There are many problems of algal blooms caused by eutrophication in Hachiroko Lake of Akita Prefecture. In the course of the study on the mechanism of algal blooming, we intend to understand the relationships of environmental phages in the controlling of the blooms caused by blue-green algae in the lake. To understand the phage flora in the lake, the metagenomic approach and the next generation DNA sequencer (NGS) were used for analysis. Firstly, we have sampled a 200 L of surface water in September 2011, from the point of 39.95N and 140.06E in the Hachiroko Lake. The sampled water was filtered through 0.2 µm membrane and then precipitated by ultra-centrifugations to collect the virus fraction. DNA from virus fractions were extracted by Proteinase K followed by Phenol/Chloroform treatment and sequenced by the NGS (GS Junior, Roche) with shotgun library methods. We got 140,000 sequences from this analysis and were compared with the database sequences by Blastn and Blastx search. From the results of these experiments, about 49% of total phage sequences were classified in Myoviridae, 25% were Shiphoviridae, and 19% were Podoviridae, respectively. The Proteobacteria, *Synechococcus*, *Anabaena*, and *Microcystis* prophage sequences were found in the library. The analysis of the seasonal variation in phage flora should be required to understand the relationships with algal blooms.

PAa-11**Isolation and characterization of microorganisms in Beppu hot springs, Oita, Japan**

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別府温泉に生息する微生物の単離と特性

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Key word : hot spring, *B.licheniformis*, *Sphingomonas*, *Paenibacillus*, *Lysinibacillus*

Beppu spas are famous for their wide range and ample supply of thermal water. Previous research has been focused on the physicochemical properties of the water such as temperature, pH, minerals, salts and radioactivity of the hot springs. However, little is known about the biological composition of the water and roles that could explain the beneficial effects of the thermal water on human health and beauty. Here we show that both novel and known bacterial strains that inhabit Beppu hot springs. We successfully cultivated and isolated 45 strains of microorganisms from thermal water. The genetic codes of these organisms were analyzed by 16S rRNA gene sequencing/BLAST search. We also characterized the organisms from morphological, biochemical and physiological points of view. *Bacillus licheniformis*, *Sphingomonas* sp., *Chromobacterium* sp. as well as several green algae species were identified. Some of these microorganisms may contribute to maintaining the ecological systems or the properties of the thermal water and mud. The others could produce biomaterials that account for the health-promoting effects of hot springs. We therefore examined anti-inflammatory and anticarcinogenic activities of the bacterial extracts or purified materials, using cell-based assays. We will discuss possible applications of the identified microorganisms for use in the health and beauty industry.

PAa-10**Isolation and cultivation of heterotrophic nanoflagellate from anoxic layer of Lake Suigetsu**

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水月湖嫌気層からの従属栄養ナノ鞭毛虫の分離・培養

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Key word : anoxic environments, heterotrophic nanoflagellate, isolation, cultivation

Heterotrophic nanoflagellates (HNF) are known to play a major role in consumption of bacteria in the oxic aquatic microbial food web. In contrast, little is known about the ecology of HNF in the microbial food web of anoxic waters. More recently, we have shown that HNF are present and act as bacterial consumers with high potential bacterivory in sulfidogenic, anoxic layer of the meromictic Lake Suigetsu. However, their physiology remains obscure. The aim of this study was isolation and cultivation of anaerobic HNF from anoxic layer of Lake Suigetsu for understanding the microbial food web ecology in anoxic aquatic environments. To obtain an initial enrichment culture, water samples were taken from anoxic layers of Lake Suigetsu and were added to anaerobic test tubes containing one wheat grain or yeast extract using a needle and syringe. Headspace of the tube was replaced with N₂ and the tubes were incubated at 20°C in the dark. After initial enrichment, anaerobic HNF cultures were maintained by serial monthly 5% transfer to fresh media for anaerobic bacteria. Microscopic observation revealed the presence of at least three different morphological types of HNF in the enrichment cultures. HNF densities in enrichment culture increased from 3.5 × 10² to a maximum of 1.3 × 10⁴ cells ml⁻¹. An oval shaped HNF with one flagellum was isolated by serial dilutions of enrichment culture.

PAa-12**Evaluation of the filterability of bacteria isolated from ground water**

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地下水から単離された細菌のフィルター通過性評価

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Key word : ground water, bacteria, membrane filter

Generally, 0.2 µm membrane filters (MF) are utilized for processing sterile water, and their performance is confirmed by LRV>7 (Log Reduction Value) in validation study with *Brevundimonas diminuta* as a standard test strain. However, it has been reported that *Hylemonella gracilis* has an ability of passing through micropore filter(1), which led us to consider that unknown MF-penetrated bacterial species might exist in natural environments. In this study, we investigated the filterability of bacterial isolates from ground water in a deep well. Approximately 100 L of ground water sample were filtered through 0.2 µm MF to obtain aquatic bacteria from deep wells. These filters were incubated aerobically with sterile ground water at 30°C for 2 month. During the incubation, the water samples were filtered weekly by 0.45 µm MF and the filtrate was cultivated with R2A agar to isolate the bacteria passed through 0.45 µm MF. Then, in order to assess the LRV, these isolates were filtered through 0.2 µm or 0.1 µm MF by filterability test machine. Phylogenetic analysis based on 16S rRNA gene revealed that the 0.45 µm MF-penetrated bacteria belonged to 21 genera and 0.2 µm ones belonged to the genera *Curvibacter*.

1) Y. Wang et. Al., Influence of size, shape, and flexibility on bacterial passage through micropore membrane filters .Environ. Sci. Technol., 42, 6749-6754(2008)