Original article

Seasonal changes in the microbial communities of the Tama River at suburban Tokyo revealed by megatenomic analysis

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Introduction

The Tama River belongs to Class A river in Japan, running through suburban and urban areas in the border of Tokyo Metropolitan and Kanagawa Prefecture. Earlier studies suggested that the rapid urbanization around this area during the high economic growth period in 1950s - 1970s in Japan led to pollution of the Tama River ecosystems [1,2]. River restoration programs started in 1970's, leading to some progress including the recovery of endangered species [3].

The microbial community plays a pivotal role in the ecosystem in aquatic environments. The field survey on bacteria in the Tama River has been performed essentially based on the methods using conventional culture or denaturing gradient gel electrophoresis [4,5], which is considered to have not provided enough information, because most bacteria are now known to be unculturable. Thus, it is likely that the bacterial community in the Tama River has remained largely unknown. Metagenomic studies have overcome such problems encountered in ecological survey on the bacterial communities and enabled us to elucidate their ecological functions comprehensively [6].

In this study, we employed metagenomic analysis on the bacterial communities in a non-tidal, urban-impacted part of the Tama River to better understand their ecological significance.

Materials and methods

Water samples (~2 L) were collected every month from September 2015 to December 2016 with sterile beakers from the surface to 12.5 cm depth at a middle reach of the Tama River in Tama City, Tokyo, following the guidelines of the Bureau of Sewage, Tokyo Metropolitan Government (2012). The water temperature and levels of salinity and dissolved oxygen (DO) were measured with a portable meter (HQ40d, HACH). The samples were stored at 4ºC for a maximum of 24 h before subsequent treatments to DNA extraction. Of the collected water, 500 mL were filtered sequentially through Isopore™ membranes with a 45 mm diameter each of 5, 0.8 and 0.2-µm pore sizes. As we targeted free-living bacteria, DNA was extracted only from the 0.2-µm filters and subjected to the construction of shotgun metanogemic libraries using Nextera XT library preparation kit (Illumina, San Diego, CA, USA). The libraries were then sequenced with an Illumina MiSeq using MiSeq Reagent Kit v3. Acquired Illumina reads were first joined by overlapping forward and reverse reads of the same DNA fragments, using the software FLASH [7], and then assigned to their taxonomic affiliations by BLASTN comparisons to the NCBI-nt database using a cut-off E-value of 1e-10. These data were visualized on MEGAN v 6.0 (MetaGenome Analyzer software) with the least ancestor algorism (LCA) [8]. The correlations of bacterial abundances with measured environmental parameters were calculated using Spearman's rank correlation coefficient. The shotgun sequence data have been deposited into the DDBJ Sequence Read Archive under the accession DRA006047.

Results and discussion

Temperature and DO increased from spring to summer and decreased from autumn to winter (Fig. 1). The salinity level was sustained at a very low level (0.11 to 0.16 psu) and did not change much through the year except for September 2016. A marked decrease of salinity observed in September 2016 (0.07 psu) likely occurred due to a heavy rainfall by the 10th typhoon.

The JSFS 85th Anniversary-Commemorative International Symposium
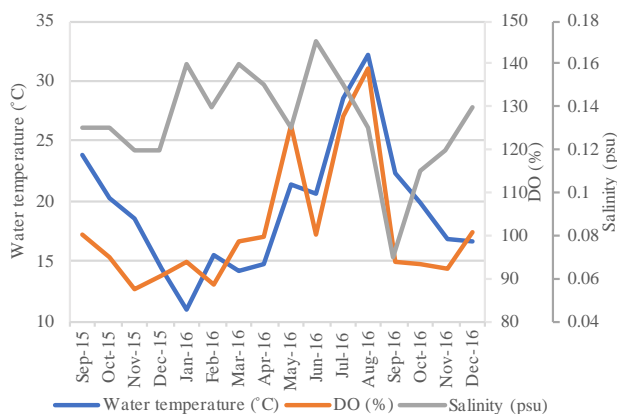"Fisheries Science for Future Generations"

Fig. 1. The seasonal changes in water temperature, DO saturation and salinity in the Tama River.

Taxonomic profiles were determined at the domain level by MEGAN6, revealing that the assigned reads for Bacteria, Eukaryota, Archaea, and viruses were 96.8 - 98.9%, 0.9 - 2.0%, 0.1 - 0.7%, and 0.0 - 1.0%, respectively. The major bacteria at the phylum level were Proteobacteria (the relative abundance of 21.5 - 63.6%), Bacteroidetes (16.4 - 73.9%), Actinobacteria (0.9 - 14.4%), and Firmicutes (0.0 - 8.3%) (Fig. 2). This relative abundance was similar to those as reported previously for field survey on freshwater bacterial communities in foreign countries [9-12].
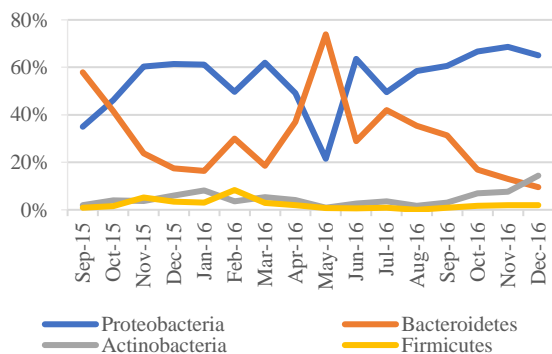

Fig. 2. The relative abundance of the major bacteria at the phylum level in the Tama River revealed by metagenomics shotgun sequencing.

The relative abundance of the major bacteria at the class level is shown in Fig. 3. Betaproteobacteria and Flavobacteriia were the most abundant in the Tama River. It has been reported that Betaproteobacteria is dominant in freshwater environments, while Flavobacteriia is rare [8-10]. The present study revealed that the relative abundance of Flavobacteriia decreased from 72.5% in May to 5.3% in December, while that of Gammaproteobacteria increased gradually from 2.4% in May to 12.4% in November. A marked increase of Bacteroidia was observed in February. Flavobacteriia is known to contain commensal bacteria and opportunistic pathogens of fish. It has been reported that the cold

water disease caused by *Flavobacterium psychrophilum* is seasonally prevalent in the Tama River [12]. Thus it seems that the outbreaks of Flavobacteriia from April to September is reflected by the occurrence of such fish disease.
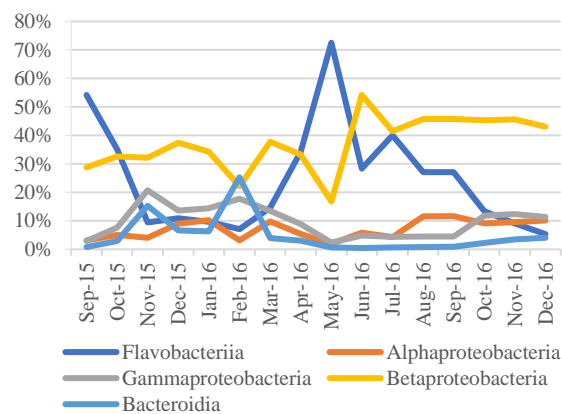

Fig. 3. The relative abundance of major bacteria at the class level in the Tama River revealed by the metagenomics shotgun sequencing.

Environmental factors significantly influenced the bacterial communities in the Tama River. The negative correlation was observed against water temperature with the relative abundances of Gammaproteobacteria, Bacilli, Mollicutes, Chlamydiia and Chlorobia, while a negative correlation observed with Flavobacteriia. A positive correlation against dissolved oxygen was observed with Flavobacteriia, while a negative correlation observed with Gammaproteobacteria. These results suggest that seasonal changes in the environmental factors contribute to dynamic changes in the bacterial communities in the Tama River. It is interesting to reveal the origins of bacteria which change the bacterial communities in the river water.

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