Original Article

The implication of the datasets obtained from periodic surveys on the microbial community by metagenomic analysis in evaluating the marine ecosystem

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Introduction

The Ofunato Bay is a type of an enclosed bay in the Sanriku *Rias Coast* in the Pacific Ocean, where seawater environments rich in nutrients are affected by both warm and cold ocean currents along with river, providing good aquaculture fields for marine bivalves. Since microbes are critical players in the primary productivity and biogeochemical cycle, their community structure holds the key for sustainability of the bay. Thus we aimed to conduct metagenomic profiling of microbial communities in the Ofunato Bay using high throughput sequencing and compared their changes among three different sampling locations throughout the year as a King Abdullah University of Science and Technology (KAUST) project since 2014.

Here we report typical results that we obtained in 2014 and 2015.

Materials and methods

Details are described in this Proceedings [1]. Briefly, three sampling stations were selected along the length of the bay: KSt. 1 (avg. depth 10.3 m), KSt. 2 (avg. depth 25.3 m) and KSt. 3 (avg. depth 38.5 m). Seawaters were collected monthly from September 2014 to December 2015 using a vertical water sampler from two depths, 1 m and 8 or 10 m below the surface that were filtered sequentially through 20, 5, 0.8 and 0.2-µm filters. Only 0.2-µm filters were used targeting the free-living bacteria where extracted genomic DNA was used for shotgun sequencing with an Illumina MiSeq (Illumina Inc., CA, USA). The whole-genome sequence (WGS) datasets have been registered in the DDBJ Sequence Read Archive under the accession number DRA005744.

The acquired Illumina paired reads were first joined by overlapping the forward and reverse reads of the same DNA fragment (paired-end sequences) using the software FLASH [2] with the default parameters. Quality filtering of these WGS reads was then performed by removing the reads of <50 bp and quality trimmed to Phred 20 using Genomics Workbench (CLCbio, Cambridge, MA). BLASTn was performed locally using the quality- and size-filtered sequences against the NCBI-nt reference database. Taxonomic analysis was then performed using MEGAN ver 5.10.3after parsing the BLAST output, whereas comparative analysis in MEGAN was also performed after normalizing the counts [3].

Principal component analyses (PCA) of relative abundance of bacterial numbers at the genus level were carried out using R software [4].

Results and discussion

Changes in the microbial community
We investigated the changes in the bacterial community at the genus level in seawaters collected from September 2014 to December 2015, focusing on KSt. 2 (Fig. 1), where oyster aquaculture is flourishing in the bay. The most dominant genus was *Candidatus* Pelagibacter which is an abundant member of the SAR11 clade in the class Alphaproteobacteria of the phylum Proteobacteria. The second dominant genus was *Planktomarina* which also belongs to the class

Alphaproteobacteria (family *Rhodobacteraceae*). In our study on KSt. 2, these accounted for about 40% of the bacterial community in September at 1 m depth in the minimum case and over 90% at 10 m depth in the maximum case. *Candidatus* Pelagibacter increased in its community ratio in May and June and then decreased in July and August irrespective of 1 or 10 m depths. In contrast, *Planktomarina* increased in July and August for both 1 and 10 m depth. This genus markedly decreased in October at 10 m depth. Further investigation and analysis are now under progress in our project.
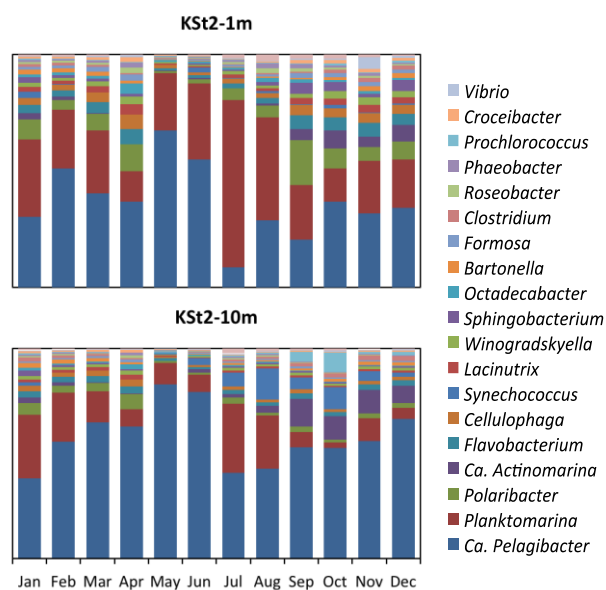

KSt2-1m

KSt2-10m

Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

Vibrio
Croceibacter
Prochlorococcus
Phaeobacter
Roseobacter
Clostridium
Formosa
Bartonella
Octadecabacter
Sphingobacterium
Winogradskyella
Lacinutrix
Synechococcus
Cellulophaga
Flavobacterium
Ca. Actinomarina
Polaribacter
Planktomarina
Ca. Pelagibacter

Fig. 1. Shotgun metagenomic sequence reads assigned to specific genera with bacteria genome sequence representation based on MEGAN analysis for seawaters from KSt. 2 at 1 and 2 depths. Reads assigned to bacteria were categorized by genera based on January to December 2015 shotgun metagenomic libraries. These are genera based on normalized hits, where only those genera that had >1% hits are shown for ease of data representation.

PCA was then employed to reveal site-specific differences and seasonal changes in the profiling of free-living bacteria at three sampling locations and two depths in the Ofunato Bay. As shown in Figure 2a, the dataset for seawater at 10 m depth from KSt. 2, where oyster aquaculture facilities are concentrated, apparently formed one cluster, suggesting that microflora in this seawater is very specific. When the datasets were compared focusing sampling months, those from winter months including November, December, January and February were clearly separated from those of other months (Fig. 2b). These results indicate that seawater temperature, possibly together with available nutrients in seawater, has a critical role in determining the profiling of bacterial community in the Ofunato Bay.
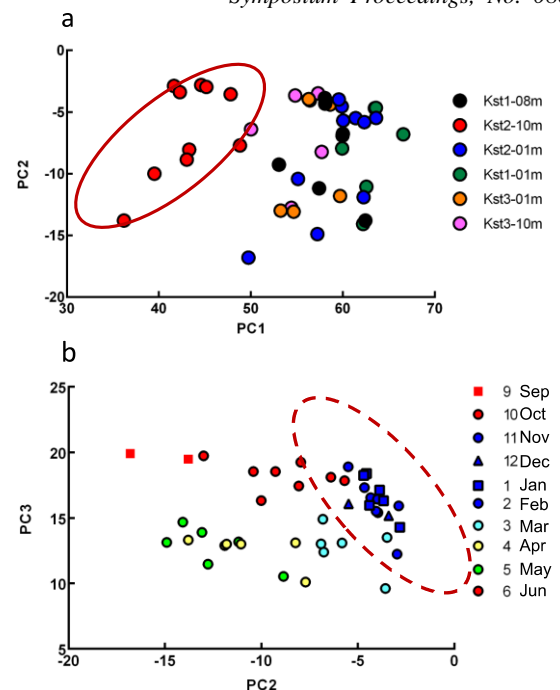

Fig. 2. Principal component analyses (PCA) of relative abundance of bacterial numbers at the genus level in seawaters collected from September 2014 to June 2015 at three sampling locations and two depths. Panel 'a' shows KSt. 2-10 m datasets derived from 10 m water layer were clustered (filled parabola) while Panel 'b' depicts datasets derived from colder period of the year were clustered together (dotted parabola).

Other analytical data

The abundance of the photosynthetic picoeukaryotes (PPEs) showed a clear seasonal pattern. It was implied that during winter and summer, when larger phytoplankton are not dominant, PPEs contribute an important part to the primary production.

Seasonal changes were observed with the abundance of bacterial genes (*dddP, dmdA*) in *Candidatus* Pelagibacter related to the catabolism of dimethylsulfoniopropionate (DMSP), one of globally the most abundant organosulfur molecules. This suggests that DMSP and its related molecules, dimethyl sulfide and methanethiol, are important in the possible formation of the scent of the tide or fish attractants.

Acknowledgement

References

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