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Short Communication

Bacterial community shift revealed *Chromatiaceae* and *Alcaligenaceae* as potential bioindicators in the receiving river due to palm oil mill effluent final discharge

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ABSTRACT

A thorough outlook on the effect of palm oil mill effluent (POME) final discharge towards bacterial community dynamics in the receiving river is provided in this study by using a high-throughput MiSeq. The shift of bacterial composition could be used to determine the potential bacterial indicators to indicate contamination caused by POME. This study showed that the POME final discharge did not only alter the natural physicochemical properties of the river water but also caused the reduction of bacterial diversity in the receiving river. The *Chromatiaceae* and *Alcaligenaceae* which were not detected in the upstream but were detected in the downstream part of the river are proposed as the indicator bacteria to indicate the river water contamination caused by POME final discharge. The emergence of either one or both bacteria in the downstream part of the river were shown to be carried over by the effluent. Therefore, an accurate pollution monitoring approach using bacterial indicator is expected to complement the conventional POME pollution assessment method which is currently dependent on the physicochemical properties of the final discharge. This is the first study that reported on the potential indicator bacteria for the assessment of river water contamination caused by POME final discharge.

1. Introduction

As the demands for palm oil outstripped the production rate, the rapid increase of production caused the generation of the enormous amount of effluents which led to improper waste treatment (Rupani et al., 2010). Palm oil mill effluent (POME) is known as one of the high strength wastewater generated from palm oil extraction process which could create potential hazards to the environment (Bala et al., 2015). Thus, the biological monitoring of the river water is crucial to ensure a thorough understanding of the effect of POME final discharge to the receiving river water.

The idea of using microorganisms to provide an indication of the quality of a particular environment has been widely used and understood in different ways (Ahmed et al., 2016; Guo et al., 2016). The used of biological monitor is one of the promising approaches to determine the impact of a pollution to the environment since they are continuously exposed to the perturbations and variations in the natural ecosystem; thus their response reflects the ecological consequences of the environmental variability (Shade et al., 2012). The initial effort needed to establish a potential bacterial indicator would be defining the community present and identifying their functional contributions with regards to particular nutrients present. We are hypothesising that the changes of the receiving river quality may exceed the tolerance of the certain taxa in the community which consequently cause the bacterial community shift. The emergence of a community that will multiply when the environmental condition is favorable can be further explored to determine the potential bacterial indicators to indicate the contamination caused by POME final discharge.

Hence, the present study is focused on the assessment of bacterial community composition in the receiving river water due to POME final discharge which are potential bacterial indicators by using high-throughput sequencing, MiSeq, and advanced bioinformatics analysis.

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2. Materials and methods

2.1. Sampling sites

POME final discharge was collected from the typical palm oil mill in Malaysia which implemented the secondary (aerobic and anaerobic) and tertiary (biopolishing plant) treatment system. The POME samples were collected after the effluent released from the secondary and tertiary treatment stage (final discharge). Water samples were also collected from the plantation channel where the POME final discharge flowed through before entering the receiving river water located approximately 3 km from the mill. The water sample from downstream part of the river was identified as the receiver point of the POME final discharge, whereas the sample from the upstream part of the river is taken as a control which considered as unpolluted due to POME final discharge. The grab samples of 2 L were collected monthly in a pre-cleaned plastic container for one whole year period starting from March 2015 to February 2016 (n = 35) to comprehend variability due to the unpredictable weather in a tropical climate.

2.2. Physicochemical and nutrient analyses

The pH and temperature values were recorded *in situ* using the portable meter. Biochemical oxygen demand (BOD_5) test was conducted according to the procedure in Standard Method APHA 5210-B (APHA, 2002) and chemical oxygen demand (COD) was measured by using reactor digestion method (HACH method 8000). The determination of total organic carbon (TOC) was conducted by using Shimadzu TOC-V_{CSH} Analyzer (Tokyo, Japan) according to TOC Analyzer Manual (Annual Book of ASTM Standard, Standard D 7573-09).

2.3. DNA extraction

DNA extraction from water sample was done by filtering the sample (1 L) with Sterivex™ filter units. DNA extraction was carried out using the PowerWater® Sterivex™ DNA Isolation Kit following manufacturer's instructions (Mo Bio Laboratories, Carlsbad, CA, USA).

2.4. High-throughput 16S rRNA sequencing

The extracted DNA samples were amplified with a set of primers targeting the hypervariable V4–V5 region of the 16S rRNA gene (Chen et al., 2016). The amplification of the 16S rRNA gene was performed using the forward and reverse primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′), respectively. The 25 μL PCR reactions contain 10 × *Taq* buffer and *Taq* polymerase (BioLabs), 20 μM each primer, 2 mM each dNTP, 25 mM $MgSO_4$ (Toyobo), and approximately 50 ng cDNA template. Samples were amplified using an initial denaturation at 94 °C for 3 min, followed by 35 cycles consisting denaturation at 94 °C for 45 s, 50 °C for 60 s, 72 °C for 90 s, and final extension at 72 °C for 10 min. Following the amplification, amplicons were run on a 2% agarose gel and purified using a NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel, Duren, Germany). The concentration of purified amplicons from each sample was determined using a Qubit dsDNA HS Assay Kit (Life Technologies, Oregon, USA). Amplicons were processed using Nextera XT DNA Library Preparation Kit according to Illumina's protocol (Illumina, San Diego, CA). The pooled samples were loaded onto the 500-cycle V2 MiSeq reagent cartridge (Illumina, San Diego, CA) and sequencing was performed for 251, 8, 8 and 251 cycles for forward, Index 1, Index 2 and reverse reads, respectively using the MiSeq Sequencing System (Illumina, San Diego, CA). The raw sequence data were deposited into the NCBI short reads archive database under accession number SRP106254.

2.5. Data analysis for bacterial community composition

The raw paired-end reads were assembled using a PAired-eND Assembler for Illumina sequences (PANDAseq) to improve the accuracy of the reads that were processed using QIIME v1.9.0 (Caporaso et al., 2010). A low quality sequence (quality score < 20) and any read containing ambiguous bases were discarded. The high-quality reads were clustered into operational taxonomic units (OTUs) with 97% sequence identities using the de novo OTU picking pipeline. The UCLUST consensus taxonomy classifier v1.2.22q was used to classify each representative sequence before querying the Greengenes database v13.8 (DeSantis et al., 2006) using the PyNAST program (Caporaso et al., 2010; DeSantis et al., 2006). The rarefied OTU tables were generated to calculate alpha diversity, and rarefaction curves were computed using the Shannon index.

2.6. Statistical analysis

The redundancy analysis (RDA) was performed to examine the relationship between bacterial communities and environmental variables (Liu et al., 2013). The data were centered and transformed before redundancy analysis.

3. Results and discussion

The MiSeq analysis of 16S rRNA gene amplicons from 35 samples produced a total of 1,218,503 sequences after quality filtering (Q20) and 690,893 of observed OTU. FastQC quality check tool in QIIME pipeline revealed that these libraries represented the majority of 16S rRNA sequences present in each sample, with values ranging from 90% to 99%. Taxonomically, the major phyla observed in all samples belong to *Proteobacteria* and *Bacteroidetes*. The *Proteobacteria* is the most predominant phylum as suggested by other studies which reported as the most frequently encountered taxa in the river at different locations (Cai et al., 2016; Chakraborty et al., 2013; Essahale et al., 2010). As shown in Fig. 1(A), the higher percentage of *Actinobacteria* found in the upstream part of the river showed a reduction as it flows through to the downstream part. This is in accordance with Lu and Lu (2014) who recorded a decrease in *Actinobacteria* in the downstream part of Nanxi River due to effluents from paper mill.

A thorough evaluation on the shift of bacterial community structure is carried out to search for the potential bacterial indicators to indicate contamination caused by POME final discharge. It was expected that the dynamic bacterial community residing in the river water is sensitive to the changes in their environment, hence the shift of bacterial community either increasing or decreasing in relative abundance is a direct consequence of the environmental alterations. From Fig. 1(B), several families including the *Rhodocyclaceae, Oxalobacteraceae, Caulobacteraceae* and *Chitinophagaceae* were reduced (57% − 85%) from upstream to the downstream part of the river as a result of the discharged effluent. The *Oxalobacteraceae* and *Caulobacteraceae* were considered to be oligotrophic-adapted as they are able to strive in low availability of metabolic substrate (Balmonte et al., 2016; Lu and Lu, 2014) which explained their presence in the upstream part with lower BOD_5, COD and TOC as compared to the downstream part of the river.

Interestingly, as *Chromatiaceae* and *Alcaligenaceae* can be detected in the downstream and not in the upstream part of the river, hence it is proposed that these bacteria as potential bioindicators to indicate contamination caused by POME final discharge. The presence of *Chromatiaceae* and *Alcaligenaceae* in the final discharge samples showed that they were carried over by the effluent to the downstream part of the river. Similarly, the RDA result (Fig. 2) demonstrated that the *Chromatiaceae* and *Alcaligenaceae* were positively related to the environmental factors including the BOD_5, COD, TOC and temperature. This is in accordance with the study conducted by Guo et al. (2016) which selected the potential indicator bacteria based on the increment