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METAGENOMICS AND CONVENTIONAL ANALYSES OF BACTERIAL DIVERSITY IN SELECTED SITES OF PUTRAJAYA LAKE, MALAYSIA

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By

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March 2020

Chairman: Shahrizim Bin Zulkifly, PhD
Faculty: Science

The anthropogenic activities will give impact to the water quality and bacterial diversity in a freshwater lake. The abundance and diversity of bacteria were determined through Illumina Miseq Sequencing as well as through bacterial identification on enrichment media. The physico-chemical parameters were analyzed to determine the water quality and to assess their relation to the bacterial community. Triplicate water samples were collected from Putrajaya Lake at three different sites monthly from November 2017 to January 2018 and from May 2018 to August 2018. The metagenomics analysis revealed that a total of 46 phyla, 276 classes, 460 order, 460 families, 765 genera and selected bacteria species were identified. Proteobacteria represented most abundant Phylum (6.49%) throughout the dry and wet seasons, followed by Actinobacteria (2.95%), Bacteroidetes (2.86%), Verrucomicrobia (1.74%), Cyanobacteria (1.48%), Planctomycetes (1.34%), Firmicutes (0.52%), Chloroflexi (0.34%), Chlamydiae (0.14%) and Acidobacteria (0.09%). Through culture studies, a limited number of bacteria were identified which include these 6 genera; *Acinetobacter*, *Enterobacter*, *Bacillus*, *Pseudomonas*, *Lysinibacillus* and *Staphylococcus*. Phylogenetic analysis using clustered Neighbour Joining method showed that there were 23 phylotypes which were classified into two phyla; Firmicutes and Proteobacteria. The Pearson Correlation Coefficient and Canonical Correspondence Analysis showed that the species composition of bacteria at each site was influenced by physico-chemical parameters (e.g., phosphate, nitrate, ammonia-nitrogen, pH) and tropical climate conditions of the lake. The study demonstrates that the lake has a multitude of bacterial communities shown at three different sampling sites that are associated with physico-chemical parameters. The dominant phyla; Proteobacteria are important decomposers in lake sediments. The wetlands of Putrajaya plays a great role as a filtration system to remediate the chemical content of water into Putrajaya Lake as well as acts as an important source of influence to a balanced bacterial diversity of the lake.

Keywords: bacteria, water quality, physico-chemical, Pearson Correlation Coefficient, Canonical Correspondence Analysis

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Master Sains

ANALISIS METAGENOMIK DAN KONVENSIONAL KEPELBAGAIAN BAKTERIA DI TAPAK TERPILIH TASIK PUTRAJAYA, MALAYSIA

Oleh

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Aktiviti antropogenik dijangka memberi kesan kepada kualiti air dan kepelbagaian bakteria di tasik air tawar. Kelimpahan dan kepelbagaian bakteria ditentukan oleh *Illumina Miseq Sequencing* dan serta identifikasi bakteria melalui media pengayaan. Parameter fiziko-kimia dianalisis untuk menentukan kualiti air dan menilai hubungan mereka dengan komuniti bakteria. Tiga sampel air diambil dari Tasik Putrajaya di tiga tapak berbeza setiap bulan dari November 2017 hingga Januari 2018 dan dari Mei 2018 hingga Ogos 2018. Analisis metagenomik menunjukkan sejumlah 46 filum, 276 kelas, 460 order, 460 keluarga, 765 genus dan beberapa spesies bakteria telah dikenalpasti. Filum Proteobakteria mewakili bakteria yang paling banyak (6.49%) sepanjang musim persampelan, diikuti oleh Aktinobakteria (2.95%), Bakteroidetes (2.86%), Verrucomikrobia (1.74%), Cyanobakteria (1.48%), Planctomycetes (1.34%), Firmikutes (0.52%), Chloroflexi (0.34%), Chlamydiae (0.14%) dan Acidobakteria (0.09%). Melalui kajian tradisional, bilangan bakteria yang terhad telah dikenal pasti termasuk 6 genus; *Acinetobacter*, *Enterobacter*, *Bacillus*, *Pseudomonas*, *Lysinibacillus* dan *Staphylococcus*. Analisis filogenetik menggunakan kaedah *clustered Neighbor joining* menunjukkan bahawa terdapat 23 *phylotypes* diklasifikasikan kepada dua phyla; Firmikutes dan Proteobakteria. Koefisien korelasi Pearson dan Analisis Kaitan Canonical menunjukkan bahawa kehadiran bakteria di setiap tapak dipengaruhi oleh parameter fiziko-kimia dan keadaan iklim tropika tasik. Kajian ini menunjukkan bahawa tasik mempunyai pelbagai komuniti bakteria yang dikaitkan dengan parameter fiziko-kimia. Filum dominan; Proteobakteria adalah penting kerana mereka boleh berfungsi sebagai pengurai di sedimen tasik. Wetland Putrajaya memainkan peranan yang besar sebagai sistem penapisan untuk menyusun semula keseimbangan kimia air ke dalam Tasik Putrajaya serta bertindak sebagai sumber pengaruh penting kepada kepelbagaian bakteria yang seimbang di tasik.

Kata kunci: bakteria, kualiti air, fiziko-kimia, Koefisien Korelasi Pearson, AnalisisKorespondensi Kanonikal

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS AND SYMBOLS

°C	Degree Celsius
µm	Micrometer
AN	Ammoniacal nitrogen
BBDuk	Bestus Bioinformatics Decontamination Using Kmers
BLAST	Basic Local Alignment Search Tool
BOD	Biological oxygen demand
bp	Base pair
CCA	Canonical correspondence analysis
Chla	Chlorophyll-a
COD	Chemical oxygen demand
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DOE	Department of Environment
INWQS	Interim National Water Quality Standard for Malaysia
LDA	Linear discriminant analysis
mA	Milliampere
MARDI	Malaysian Agricultural Research and Development Institute
ml	Milliliter
mm	Millimeter
NAHRIM	Malaysian National Hydraulic Research
NGS	Next-generation sequencing
NMDS	Non-Metric multi-dimensional scaling
OTU	Operational Taxonomic Unit

PCA	Pearson correlation analysis
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PES	Polyethersulfone
PLWQS	Putrajaya Lake Water Quality Standard
QIIME	Quantitative Insights Into Microbial Ecology
rRNA	Ribosomal RNA
SD	Secchi Disc depth
TAE	Tris-acetate-EDTA
TP	Total Phosphorus
TSA	Tryptic soy agar
TSI	Trophic State Index
TSS	Total suspended solids
UPM	Universiti Putra Malaysia
V	Voltage
VSEARCH	Victorized search
WQI	Water Quality Index
wqp	Water quality parameters

CHAPTER 1

INTRODUCTION

1.1 Background of study

Environmental problems as a result of anthropogenic activities such as water pollution, have directly or indirectly impacted the water quality and diversity of aquatic ecosystems in Putrajaya Lake. Putrajaya Lake is one of the prides of Putrajaya, being the centre of administration of the Malaysian Government that also serves as a site for tourism, recreation and sports activities. Putrajaya Holdings is entrusted to manage and maintain the Putrajaya Lake by evaluating and monitoring the water quality according to the National Water Quality Standards (Abdullah, 2013).

Unfortunately, some parts of Putrajaya Lake are experiencing steady deterioration on the water quality with numerous inputs entering the lake (Siong, 2006). The water body has shown early signs of deteriorative conditions demonstrated by the presence of algal bloom, cloudy water and dead fishes in some parts of the lake (Zulzaha, 2016). Putrajaya Lake is a diverse ecosystem for research. Microorganisms such as bacteria are good indicators of the aquatic ecosystem of the water body and provide the current status and key information needed for the management of Putrajaya Lake. In addition to the current monitoring of the water quality parameters, there is a need to understand Putrajaya Lake in a more holistic approach such as in biodiversity studies. Biodiversity is one of the key components that determine the wellbeing of the lake as it can be used as an indicator to assess the health of the lake. The bacteria population in the lake forms a very important niche in nutrient cycling, decomposition and remediation. Bacteria are essential for all life in the ecosystem as they play the role in maintaining the structure, function, and sustainability of the ecosystem (Briones & Raskin, 2003).

It is evident that bacteria have a significant role to regenerate and mobilize the nutrients in freshwater food webs and are important in recycling of most naturally dynamic components in the ecosystem (Newton, Jones, Eiler, McMahon & Bertilsson, 2011). Bacteria collectively, are responsible for the movement of substances and conditions of water body (Pernthaler & Amann, 2005) as a result of their biomass creation and trophic coupling to eukaryotic predators as well as the foremost important degraders and converters of an organic compound into inorganic material (Cotner & Biddanda, 2002).

In this study, the bacteria population of Putrajaya Lake is sampled periodically to determine species composition based on seasonal influence. The identification and classification of ubiquitous prokaryotes are widely investigated (Gupta, Ratha, Sood, Chaudhary & Prasanna, 2013). Bacteria can be identified based on morphology, biochemical tests, and culturing with selective media. However, not all environmental bacteria can be cultured with artificial laboratory conditions. The most current and effective approach to bacterial identification is through molecular methods such as metagenomics. Metagenomics are the study of the direct genetic material of samples from the environment, bypassing the culturing process.

Next-generation sequencing (NGS) is one of the new technologies in the field of genomic analysis for DNA sequencing. It is a persuasive tool for demonstrating the diversity of various samples and studying metabolic pathways (Al-Sulaiman, 2012). The application of NGS leads to a high-throughput accurate sequence read length and has enabled investigation of a huge number of samples at a greater depth (Fadrosh et al., 2014). By using the Illumina Miseq platform, the community composition of the samples from clinical and the environment can be examined (Fadrosh et al., 2014).

1.2 Problem Statement

Seasonal anthropogenic activities surrounding the Putrajaya Lake are major contributors to the changes of water quality in the lake; which include the release of effluents from the point and non-point sources, sewage from residential houses and commercial areas, the use of fertilizers for farming and rubbish littering. The identification of bacteria community is important as bacteria can be an environmental bioindicator to detect changes in the water quality of the lake. Does the bacterial community of the lake serve as an important indicator of the health of the lake? Does season and nutrient content due to anthropogenic activities influence the bacteria diversity and water quality of the lake?

Thus, this study determined the bacteria diversity in Putrajaya Lake by using both advanced and conventional methods. Physico-chemical parameters of the lake were also observed that might have effects on bacterial dynamics. It was expected that there will be seasonal shapes of bacterial diversity and abundance due to changes of the environmental parameters of the lake.

1.3 Objectives

The objectives of this research are;

1. To utilize 16S ribosomal RNA (rRNA) gene for the identification of bacteria species in next generation sequencing.
2. To identify bacteria species using culturing technique and conventional (Sanger) sequencing.
3. To determine the correlation between physico-chemical properties on the bacteria diversity

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