



Faculty of Resource Science and Technology

**Characterization of Bacteria Isolated from Water from Rivers of
Kampung Paku, Betong**

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Characterization of Bacteria Isolated from Waters from Rivers of Kampung Paku, Betong

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A thesis submitted in partial fulfilment of the Requirement of The Degree Bachelor of
Science with Honours
(Resource Biotechnology)

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Programme of Resource Biotechnology
Faculty of Resource Science and Technology
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2022

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ABSTRACT

Bacteria isolated from the water environment are mostly Gram-negative bacteria. These Bacteria can either be pathogenic or non-pathogenic bacteria. Considering that the water environment plays a major role for communities living in residential areas. It is important to ensure that the water quality in this area is not contaminated. This study aims to determine and characterize the bacteria in water samples from rivers within Paku village area in Betong, Sarawak. There were 15 samples cultured on Nutrient agar which were then identified by using 16S rDNA Sequencing. The bacteria isolates were characterized by using AGE and tested against eight types of antimicrobial agents. Based on the result, there were five family of bacteria identified, which were Enterobacteriaceae, Aeromonadaceae, Moraxellaceae, Bacillaceae, and Necessariaceae. Some of the bacteria identified were coliform bacteria, for instance *Escherichia coli*, which indicates that there might be contamination in the water samples. From the MAR index, only 38.46% of bacteria isolates have a value lower than 0.2, whereas 30.77% has a value higher than 0.2. This study reveals the possibility of contamination in the water sample.

Key words: Bacteria, isolation, identification, characterisation, MAR index

ABSTRAK

Kebanyakan bakteria yang diambil dari kawasan air adalah bakteria gram-negatif. Bacteria ini boleh menjadi patogen atau bukan patogen bakteria. Mengikut pertimbangan penduduk yang tinggal berdekatan dengan kawasan sungai, sungai ini memainkan peranan penting bagi mereka. Amatlah penting untuk memastikan kualiti air bagi kawasan ini adalah tidak tercemar. Kajian ini bertujuan untuk menentukan identiti bakteria, untuk mengasingkan berlainan jenis bakteria, dan untuk mencirikan jenis bakteria yang diambil daripada sampel air. Terdapat 15 sampel yang telah dikultur pada Nutrient Agar dan kemudian dikenalpasti menggunakan cara penjujukan 16S rDNA. Manakala, bakteria yang telah diasingkan akan dicirikan dengan menggunakan AGE dan diuji bersama lapan jenis antibiotik. Berdasarkan keputusan, terdapat lima jenis keluarga bakteria yang dikenal pasti iaitu Enterobacteriaceae, Aeromonadaceae, Moraxellaceae, Bacillaceae, dan Necessariaceae. Beberapa bakteria yang dikenal pasti juga tergolong dalam jenis bakteria koliform iaitu Escherichia coli yang menunjukkan bahawa terdapat pencemaran pada sampel air tersebut. Berdasarkan keputusan indeks MAR, hanya 38.46% bakteria yang diasingkan mempunyai nilai yang kurang daripada 0.2, manakala 30.77% mempunyai nilai lebih tinggi daripada 0.2. Kajian menunjukkan bahawa ada terdapat kemungkinan pencemaran pada sampel air tersebut.

Kata kunci: Bakteria, pengasingan, pengenalpastian, penyifatan, indeks MAR

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List of Abbreviation

16S	16 subunit
AGE	Agarose Gel Electrophoresis
AK	Amikacin
bp	Base pair
C	Chloramphenicol
CLSI	The Clinical Laboratory Standard Institute
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
<i>E. coli</i>	<i>Escherichia Coli</i>
EtBr	Ethidium Bromide
IPM	Imipenem
KM	Kilometre
L	Litre
mA	Milliamp
MAR	Multiple Antibiotic Resistance
MgCl ₂	Magnesium chloride
MHA	Mueller Hinton Agar
ml	Millilitre
mM	Millimolar
NA	Nutrient agar
OFX	Ofloxacin
P	Penicillin
PCR	Polymerase Chain reaction
rDNA	Ribosomal deoxyribonucleic acid

rpm	Rate per minute
S	Streptomycin
TBE	Tris-Borate-Ethylenediaminetetraacetic acid
TE	Tetracycline
UV	Ultra Violet
V	Volt
°C	Degree Celcius
μl	Microlitre

CHAPTER 1

INTRODUCTION

1.1 Study Background

Water is considered as one of the most important sources we need in our daily life. Most of our regular activities involve the use of water. For example bathing, cleaning, cooking, and washing our materials. However, not every source of water is clean. Especially important are river water sources. Some people, especially in rural areas, do not have the access to clean water due to contamination and pollution. Which is the reason why there is a shortage of clean water provided to them. This also makes it difficult for them to carry out certain activities that require the use of clean water.

Almost all the water in the river contains bacteria. These bacteria can either be pathogenic or non-pathogenic bacteria. Besides, water is one of the most common ways for disease to be transmitted. For instance cholera, and typhoid fever (Cabral, 2010). Some of the bacteria that can commonly be found in water from rivers came from the Enterobacteriaceae family such as *Escherichia Coli*, *Enterobacter*, *Klebsiella*, *Shigella* and *Citrobacter*.

Three rivers located in Kampung Paku were chosen as the study areas, namely Sungai Samu, Sungai Paku and Sungai Kaong. Owing to the fact that water is one of the essentialities in the resident's lives, all these rivers are being microbiologically assessed in order to identify and characterise the bacteria found in the water. Since water is an essential

for living. It is important for us to determine if the water in Kampung Paku is not contaminated with pathogenic bacteria as these bacteria can transmit disease.

The bacteria isolates found in the water samples from these location will be sequenced by using 16S rDNA sequencing. These bacteria were then identified and categorized based on their group. From here, we can identify which bacteria is pathogenic or non pathogenic bacteria that can cause contamination in water sample.

Antibiotic susceptibility test was also done to test against the isolates bacteria. There are eight types of antibiotic that were chosen to be tested in this study, consisting of Chloramphenicol (C), Penicillin (P), Amikacin (AK), Streptomycin (S), Tetracycline (TE), Nalidixic Acid (NA), Ofloxacin (OFX), and Imipenem (IPM). From the result, we will calculate the MAR index value and interpret the data accordingly.

1.4 Objectives

The objectives for this study are:

1. To determine the identity of isolates bacteria found in water sample from Kampung Paku, Betong
2. To characterise the isolates of bacteria found in water samples from the river in Kampung Paku, Betong

CHAPTER 2

LITERATURE REVIEW

2.1 Background of Kampung Paku

Kampung Paku is located in Betong, Sarawak. Betong is 245 kilometres from Kuching. Some of the nearby rivers located at Betong are Sungai Paku, Sungai Samu, and Sungai Kaong.

2.2 *Enterobacteriaceae*

Enterobacteria is a group of families consisting of mainly Gram-negative bacteria. The domain of *Enterobacteria* is bacteria. *Enterobacteria* fall under the phylum: *Proteobacteria*, class: *Gammaproteobacteria* and order: *Enterobacteriales* (Rock & Donnenberg, 2014). Most *Enterobacteria* are facultative anaerobes. *Enterobacteria* can be found in river water and used as measurement for faecal contamination. *Enterobacteria* mostly occupy the human and animal gastrointestinal tract (Rock & Donnenberg, 2014). Which is why this group of bacteria can survive in an environment where the energy sources are scarce. *Enterobacteria* can also be detected in soil and food, which can cause foodborne pathogens (D'Agostino & Cook, 2016)

Enterobacteria found in the environment can either be harmless or pathogens to humans. *Enterobacteria* that can cause disease is able to invade their hosts in variety of ways,

which is due to the bacteria having certain important characteristic such as motility, colonisation factors, endotoxin, and enterotoxin. Some examples of *Enterobacteriaceae* are *Escherichia Coli*, *Enterobacter*, *Plesiomonas*, and *Klebsiella*.

2.2.1 *Escherichia Coli*

Escherichia is a member of the *Enterobacteriaceae* family. The general characteristic of *Escherichia coli* cells is that this bacterium is a Gram-negative bacteria and can either be motile or non-motile. *Escherichia* is one of the bacteria commonly found in water and commonly found in human gastrointestinal tract (Rock & Donnenberg, 2014). The existence of *Escherichia coli* in the water may indicate that there is fecal contamination.

2.2.2 *Enterobacter*

Enterobacter is one of the bacteria in the family of *Enterobacteriaceae*, which is also a Gram-negative bacteria and an opportunistic pathogenic bacteria that usually infects humans, plants, and animals. *Enterobacter* is usually found in human or animal microbiota of the gut (Davin-Regli et al., 2019).

2.2.3 *Plesiomonas*

Plesiomonas shigelloides is a Gram-negative bacteria that belongs to *Enterobacteriaceae* family that can be found in freshwater (Levin, 2014). These bacteria can cause diseases such as diarrhea.

2.2.4 *Klebsiella*

Klebsiella is also a bacteria that belongs to the Enterobacteriaceae family. *Klebsiella pneumoniae* is a non-motile Gram-negative bacterium. This bacterium can infect human with pneumonia by invading the mucosal surfaces of the oropharynx and gastrointestinal tract (Ashurst & Dawson, 2022).

2.3 *Aeromonadaceae*

Aeromonas is a type of Gram-negative bacteria from the family of *Aeromonadaceae*. Janda & Abbott (2010) stated that *Aeromonas* species can commonly be found in environmental niches such as aquatic environments and natural soils. Some of the *Aeromonas* species, for instance *Aeromonas caviae*, and *Aeromonas hydrophila*, are known to cause infection in humans. *Aeromonas caviae* and *Aeromonas hydrophila* are two pathogenic genomospecies (Borrell et al., 1998). This bacterial species can be found in the surface water of lakes and rivers.

2.4 Molecular Identification and Characterisation of Bacteria

2.4.1 16S rDNA Sequencing

16S rDNA Sequencing is a method used to discern the bacteria species found in the water samples for this study. This method is accurate method and can be used for the identification of bacteria isolates. This method aids in clinical microbiology, laboratories, especially in identifying rare species of bacteria and bacteria that are difficult to identify (Woo et al., 2008). Besides, this method also assists in finding the right antibiotic and treatment for certain bacterial infections. Although the reliability and performance of this

method have never been tested, there are quite a number of bacteria isolated are unidentifiable (Drancourt et al., 2000). However, this method is widely used for bacteria identification because of its accuracy.

2.4.2 Agarose Gel Electrophoresis

Agarose Gel Electrophoresis is a method used to separate DNA fragment according to their size. This method is used to ensure that there is DNA fragment in the DNA extraction before sending the sample for identification. In this procedure, the DNA fragment will move along the positively charge anode after being loaded into the wells (Lee et al., 2012). Ethidium Bromide (Promega, USA), which is also known as EtBr (Promega, USA) is used to stain the DNA in an agarose gel. The DNA band can be identified under the UV light (Maestrogen, TW) after staining it with EtBr (Promega, USA).

CHAPTER 3

MATERIALS AND METHOD

3.1 Study Area

The location of Station 1 where the study was done is Sungai Kaong, Betong Sarawak. For this sample the location is labelled as ST1



Figure 1: The sample for ST1 was taken from Sungai Kaong, Betong

The location for Station 2 where the study was done is Sungai Samu, Betong, Sarawak. For this sample the location is labelled as ST2



Figure 2: The sample for ST2 was taken from Sungai Samu, Betong

The location for station 3 where the study was done is Sungai Paku, Betong, Sarawak. For this location the sample is labelled as ST3



Figure 3: The sample for ST3 was taken from Sungai Paku, Betong

Table 1: The sample code for each of the station where the bacteria isolates were taken

Samples code	Source
ST1 C1	Water
ST1 C2	Water
ST1 C3	Water
ST1 C4	Water
ST1 C5	Water
ST2 C1	Water
ST2 C2	Water
ST2 C3	Water
ST2 C4	Water
ST2 C5	Water
ST3 C1	Water
ST3 C2	Water
ST3 C3	Water
ST3 C4	Water
ST3 C5	Water

3.2 Media preparation

3.2.1 Nutrient agar

To prepare Nutrient agar (NA) (Merck Millipore, UK) 28g of Nutrient agar powder was weighed and diluted with 1L of distilled water. The solution was stirred by using magnetic stirrer and autoclaved for 15 minutes at 121°C. The sterile NA was left to cool down before being poured into the petri dishes. Once it solidified, the petri dishes containing agar were stored in the refrigerator for further use.

3.2.2 Mueller Hinton Agar

The preparation of Mueller Hinton Agar (MHA) (Oxoid, UK) was done by diluting 38g of MHA with 1L of distilled water inside the Schott's bottle. Then mixed the solution thoroughly, utilising magnetic stirrer and autoclaved at 121°C minutes for 15 minutes. After the autoclave process was done, the sterilised MHA was left to cool down before being poured into the petri dishes. Once it solidified, the petri dishes containing agar were stored in the refrigerator for further use.

3.2.3 Muller Hinton Broth

To prepare Mueller Hinton Broth (Oxoid, UK), 4.2g of Mueller Hinton Broth (Oxoid, UK) powder was weighed and diluted with 200ml of distilled water inside the conical flask. Then, the solution was mixed thoroughly by shaking and boiling it. After that, the solution was sterilised by using an autoclave at 121°C for 15 minutes. After the autoclave process was