CHARACTERISATION OF METAGENOMIC BOUGAINVILLEA SOIL DNA THROUGH rDNA PCR AMPLIFICATION

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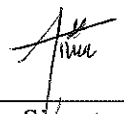
ARUNA GANESAN	
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ABSTRACT

Rhizosphere is an important part of soil ecosystem, being a habitat for a vast amount of bacteria that is influenced by root secretions, which contribute to the richness of bacteria in the soil. Conventional culturing often is not able to simulate the environmental settings microorganisms are adapted to, thus posing barrier for the advancement in environmental and clinical microbiology. Metagenomics evades this unculturability by analysing genomic variability of bacteria in environmental samples and this process starts off with DNA extraction. The aim of this research is to select several soil samples, extract the DNA from the microbiota within, and analysed. Soil samples were collected under bougainvillea shrubs for DNA isolation. All the samples were analysed using agarose gel electrophoresis, restriction digestion, polymerase chain reaction and spectrophotometer. CTAB DNA extraction produced intact high molecular weight band on agarose gel suggesting intact metagenomic DNA was isolated and had moderate A_{260}/A_{280} ratio indicating moderate purity from protein contamination. From the sequencing data, identification of plant growth promoting microorganisms can be done which are beneficial to the field of agriculture.

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LIST OF ABBREVIATIONS

×	dilution factor
×g	relative centrifugal force
%	percentage
°C	degrees Celsius
°F	degrees Fahrenheit
µg	microgram
µL	microlitre
A	absorbance
bp	basepair
cm	centimetre
CTAB	cetyl trimethylammonium bromide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
FDPM	Forestry Department of Peninsular Malaysia
ft	feet
HCl	hydrochloric acid
h	hour
ha	hectare
kb	kilobase
m	metre
M	molar concentration
mDNA	metagenomic DNA
min	minute
mg/mL	milligram per millilitre

mM	millimolar
mL	millilitre
$MgCl_2$	magnesium chloride
ng	nanogram
nm	nanometre
Na	sodium
NaCl	sodium chloride
NaOCl	sodium acetate
pH	potential hydrogen
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGPR	plant growth promoting rhizobacteria
rDNA	ribosomal DNA
rRNA	ribosomal RNA
rpm	revolutions per minute
s	seconds
SDS	sodium dodecyl sulfate
TBE	tris-boric-EDTA buffer
TE	tris and EDTA buffer
Tris-Cl	tris-chloride
Tris-HCl	tris-hydrochloride
U	enzyme unit
UV	ultraviolet
V	voltage
vol	volume
v/w	volume per weight

w/w	weight per weight

CHAPTER 1

INTRODUCTION

Malaysia is an equatorial country being hot and rainy throughout the year. This climate supports the existence of tropical rainforests in Malaysia. Forestry Department quoted 5.8 million ha or 44.0% of the total land area of Malaysia is covered with forest in 2014 (Forestry Department of Peninsular Malaysia [FDPM], 2014). Our tropical forests are rich in biodiversity and ecological functions consisting of 2,500 trees, 200 mammalians, 600 birds, 110 snakes, 80 lizards and thousands of spiders (FDPM, 2014). Up to 92% of various plant species from different families need association with mycorrhizae and rhizobacteria (Huynh, Thomson, McLean, & Lawrie, 2009) to get their essential nutrients for growth. Bougainvillea is a tropical and subtropical group of plants that need the association with mycorrhizae and rhizobacteria (Arteca, 2014). The symbiotic relationship with mycorrhizae and rhizobacteria affects the distribution of Bougainvillea (Swarts, Sinclair, Francis, & Dixon, 2010) around the globe.

There is a wide variety of microbes living in association with one another as a microbiota on leaves, barks, roots and soil. Soil in particular contains enormous amount of carbon, which contributes to microbial activity and therefore microbiotic composition (Shah, 2014). These microbes produce metabolites for their survival and also for the survival of their host plants. Many are saprotrophs where they endogenously have the ability to degrade organic compounds in soil for their growth while obtaining trace elements from the host plants. Some of them are able to gain carbon by decomposing dried leaves and gain nitrogen by digesting proteins from soil. Microbes produce metabolites to increase resource availability for them and their hosts to survive (Stumpf et al., 2016). Their symbiotic relationship with host plant or animal is beneficial most of the time, but they may be harmful to other living organisms. For example when a bark beetle make a hole in a tree, the ectopic and entopic bacteria from the beetle will colonise around the hole (Durand et al., 2015), secreting toxin causing harm to the trees.

We have been traditionally studying purely grown cultures in the laboratory in the field of microbiology. There are population of microorganisms which are uncultivable and ignored about their existence (Mohd Shaufi, Sieo, Chong, Gan, & Ho, 2015). This unawareness causes potentially important compounds to be undiscovered for development of pharmaceuticals in the medical field, for example, through bioprospecting for anticancer treatment (Stewart, 2012). Today the rising field of metagenomics puts its lights on the genome extraction of fastidious microorganism from environmental samples. The quest for prominent ecological roles of uncultivable microbes' genome sequence can be solved via advancement in recombinant DNA technology in the field of DNA isolation, rDNA amplification and sequencing, cloning and/or library screening. This broad field may branch out as environmental genomics, community genomics and eco-genomics. Researches on metagenomics had been done in Malaysia involving peat swamp soil, human gut, animal gut and a plant subject (Bunterngsok et al., 2010; Chan, Hong, Yin, & Chan, 2016; Mohd Shaufi et al., 2015; Yap et al., 2016) to name a few.

Changing weather pattern these days can increase crop's susceptibility to pest infestation, infection and weeds. Plant's altered vulnerability may affect largely developing countries like Malaysia in terms of food supply and public health (Rosenzweig, Iglesius, Epstein, & Chivian, 2001). In order to prevent or solve this problem, the agricultural field should be enriched with the knowledge from biotechnology and advancing technology. Today, plant growth promoting bacteria and arbuscular mycorrhizas are being used to stimulate the growth of plants which have been found effective on vegetative propagation of plants (Abdel-Rahman & El-Naggar, 2014). These are the microorganisms that are potential instrument (Egamberdieva, Shrivastava, & Varma, 2015) to sustain agriculture in Malaysia and it is suspected more of similar microorganisms can be hidden in the soil of our local plants waiting to be discovered.

It is impossible to study every environmental sample from Malaysia in sufficient detail. For example, there are so many different types of soils and water samples with different and diverse microbial community which may not be cultivable. Thus DNA approach is preferred to study them in detail. Soil samples, especially those from rhizosphere of local plants may contain such uncultivable microbes. The

objective of this research was to select several soil samples, extract the DNA from the microbiota within, and analyse the DNA by using spectrophotometer and agarose gel electrophoresis. PCR was performed and optimised on the isolated DNA using universal primers to amplify rDNA region in the microbial genome from at least three microbiota. This would assess the possibility of using isolated and/or amplified DNA for next generation sequencing as a platform to identify useful microorganisms in the field of agricultural development.