SCREENING AND CHARACTERIZATION OF THERMOPHILES FROM MALACCA HOT SPRING

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Specially dedicated to :

My Beloved Family

SSN

&

All my Fellow Friends

“THANKS FOR ALL THE GUIDANCE AND CARING”

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ABSTRACT

Seven strains of thermophilic bacteria (GD-1, GD-2, GD-3, GD-4, GD-5, GD-6 and GD-7) and three strains of thermophilic bacteria (JS-1, JS-2 and JS-3) were isolated and cultivated from samples collected from Kg Ganun Gadek, Alor Gajah and Kg Air Panas, Bemban hot springs respectively which both located at Malacca. The temperature and pH of the Kg Ganun Gadek sampling sites were 55℃ and 7.59 respectively while 52 ℃ and 7.7 for Kg Air Panas Sampling sites. *Thermus* medium was found to be the most suitable medium for the growth for the eight strains. Strains GD-1, GD-2, GD-3 and GD 5 formed yellow colonies while strains GD-4, GD-6, JS-1, JS-2 and JS-3 formed white colonies. Further studies were done for strains GD-1, GD-2, GD-3, GD-4, GD-5, JS-1, JS-2 and JS-3. Cells of these strains were rod-shaped and occurred in single and chain like arrangement. Only two strains were Gram-negative (JS-2 and JS-3). Strains GD-1, GD-2 and GD-6 were able to hydrolyse starch. Catalase test revealed that strains GD-1, GD-2, GD-3, GD-6, JS-1 and JS-2 able to degraded hydrogen peroxide (H_2O_2) to water and oxygen. Blast search results of partial 16S rRNA sequences revealed strains GD-1, GD-2 and GD-6 to be *Anoxybacillus gonesis* while strains GD-3 and GD-5 are *Anoxybacillus sp*. Strain GD-4 showed closest to *Bacillus licheniformis*. Strain JS-1 showed closest relationship to *Bacillus sp.* and *Geobacillus kautophilus* while the result showed strain JS-3 is the most similar to *Bacillus licheniformis* and *Bacillus sp*.. All result has 99% identity. The tentative identities of the strain were supported by the phenotypic and phylogenetic evidence.

ABSTRAK

Tujuh jenis termofilik bacteria (GD-1, GD-2, GD-3, GD-4, GD-5, GD-6 and GD-7) dan tiga jenis termofilik bacteria (JS-1, JS-2 dan JS-3) telah diperoleh and dipeliharakan dari mata air panas Kg Ganun Gadek, Alor Gajah dan Kg Air Panas, Bemban masing-masing. Suhu and pH bagi tapak penyampelan Kg Ganun Gadek, Alor Gajah adalah 55℃ dan 7.59 masing-masing manakala untuk tapak penyampelan Kg Air Panas, Bemban adalah 52 ℃ dan 7.7 masing-masing. Media *Thermus* merupakan media yang paling sesuai untuk pertumbuhan keempat-empat stran tersebut. Stran GD-1, GD-2, GD-3 dan GD 5 berwarna kuning manakala warna koloni bagi stran GD-4, GD-6, JS-1, JS-2 dan JS-3 adalah putih. Kajian lanjutan terhadap GD-1, GD-2, GD-3, GD-4, GD-5, JS-1, JS-2 dan JS-3 telah dilaksanakan. Semua sel bacteria berbentuk rod dan sebuah-sebuah berbentuk rantaian. Hanya dua bacteria adalah Gram-negative (JS-2 dan JS-3). GD-1, GD-2 dan GD-6 boleh menguraikan kanji. Kajian Katalase membuktikan bahawa strain GD-1, GD-2, GD-3, GD-6, JS-1 dan JS-2 dapat memecahkan hidrogen peroksida kepada air dan oksigen. Keputusan separa jujukan 16S rRNA mencadangkan GD-1, GD-2 and GD-6 mungkin adalah *Anoxybacillus gonesis* manakala stran GD-3 dan GD-5 adalah *Anoxybacillus sp*. Stran GD-4 menunjukkan persamaan rangkaian yang paling tinggi dengan *Bacillus licheniformis.* Stran JS-1 menunjukan hubungan rapat dengan *Bacillus sp.* and *Geobacillus kautophilus* manakala keputusan bagi stran JS-3 adalah *Bacillus licheniformis* and *Bacillus sp.*. Semua keputusan diperolehi pada 99% identity. Identiti bacteria disokong oleh bukti fenotipik dan filogenetik.

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LIST OF ABBREVIATIONS AND SYMBOLS

℃	-	Degree celcius
µg	-	Microgram
µL	-	Microliter
A.	-	*Anoxybacillus*
BLAST	-	Basic Local Alignment Search Tool
Blastn	-	Nucleotide BLAST
bp	-	Base pair
DNA	-	Deoxyribonucleic acid
dNTPs	-	Deoxynucleotide Triphosphates
et al.	-	And friends
G	-	Gram
G.	-	*Geobacillus*
g/L	-	Gram/liter
GD	-	Gadek

JS	-	Jasin
Kb	-	Kilobase pairs
Kg.	-	Kampung
kPa	-	Kilopascal
MEGA	-	Molecular Evolutionary Genetics Analysis
mL	-	Milliliter
mM	-	Millimeter
MW	-	Molecular weight
NCBI	-	National Center for Biotechnology Information
PCR	-	Polymerase chain reaction
RNA	-	Ribonucleic Acid
rpm	-	Revolutions per minute
sp.	-	Species
UV	-	Ultraviolet
V	-	Volts
v/v	-	Volume/volume
w/v	-	Weight/volume
x g	-	Times gravity

CHAPTER 1

INTRODUCTION

1.1 Background of study

Microorganisms are found in almost most habitats on earth. Extremophiles are microorganisms that can live in an extreme environment. Extremophiles can be categories in different parameters such as extreme in geochemical parameters (pH, salinity or oxygen species), physical parameters (temperature, pressure and radiation, or biological extremes (high concentration in nutrient or parasite). (Khalil, 2011, Pinzón-Martínez, 2010, Yoko, 2011). Microorganisms can be categories into four main groups according to their living temperature which are psychrophiles (cold-living microbes), mesophiles (moderate-temperature-loving microbes, thermophiles (heat-loving microbes) and hyperthermophiles (Madigan, 1999).

Thermophilic microorganism survives in high temperature (between 45 and 80 ℃) are only found in prokaryotes, especially Archaea domain (Adiguzel, 2009).

Generally, thermophiles are separated into three main categories based on their basic growth temperature: thermophiles (35-70 ℃), extreme thermophiles (55-85 ℃) and hyperthermophiles (75-113 ℃).

Thermophiles can be found in hot springs, deep sea hydrothermal vents or any heated locations (Adiguzel, 2009, Sudip, 2010). Discovery and research on thermophiles and their enzymes have received attention due to their enzyme ability to resist denaturation and remain active at high temperatures, chemical reagents and extreme pHs than that to mesophiles (Synowiecki, 2010, Pinzón-Martínez, 2010). Thermostable microbes and their enzyme have high potential in industrial application in several industrial sectors such as laundry, pharmaceutical, food, petroleum, biomedical and agricultural (Synowiecki, 2010, Ramesh, 2011).

Hot springs are heaven to thermostable microbes and their enzymes (Sudip, 2010). Malaysia consisted of more than 40 naturally geothermal sites. The temperatures for these hot springs vary in the range of 27-103 ℃. Approximately 25 hot springs in Peninsular Malaysia exhibit annual temperature of higher than 45 ℃. Examples are Kg Ganun Gadek, Alor Gajah (59 ℃), Kg. Air Panas, Bemban (Malacca) (59 ℃), Gua Musang (Johor) (76 ℃), Dusun Tua (Selangor) (75 ℃), Hulu Slim River (Perak) (103 ℃) and Pedas, (Negeri Sembilan) (60 ℃).

Apart from that, Kg Ganun Gadek, Alor Gajah and Kg Air Panas， Bemban which both are located at Malacca state are chosen as study sites for isolation and identification potential of thermophilic microorganism for this study as they are in the suitable temperature range for thermophile.

1.2 Statement of study

Isolation of novel thermophilic have being focus extensive among the scientific world globally because of their biotechnological importance as they possess unique enzymes with thermal activity and stability at high temperatures. Thermozymes from these thermophilic microorganisms are used in most of industrial applications such as biocatalysis, biotransformation and biodegradation due to their extreme stability (Burgess *et al.*, 2010, Fields, 2001, Haki and Rakshit, 2003). Therefore, this research is conducted to obtain other possible type of microorganisms present in Malacca hot spring for biotechnology applications.

1.3 Objective

The objectives of this project are:

I. To isolate and screen for thermophilic microorganisms from Malacca hot spring
II. To characterise the morphological and physiological properties of the isolated thermophilic microorganisms
III. To identify the strains of thermophilic microorganisms using PCR amplication of 16S rRNA gene fragments

1.4 Scope of Research

i. Isolation and cultivation of potential thermophilic microorganisms in the samples at different growth conditions such as varying the temperature, pH and composition of agar medium.
ii. Biochemical analysis for selected strain
iii. Genome isolation, amplication of 16S rRNA gene, sequencing and identification of bacteria identity by using the Blastn program

1.5 Research Significance

This research is useful to the local researches as a thermophile culture collection. The strains can later be used to produce beneficial thermozymes in biotechnology applications.

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