UNIVERSITI TEKNOLOGI MARA

ISOLATION AND CHARACTERIZATION OF THERMOPHILIC BACTERIA FROM THE HOT SPRINGS IN MALAYSIA

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Candidate's Declaration

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ABSTRACT

Thermophiles can be defined as organisms capable of growing at high temperatures (60°C to 80°C) as compared to the mesophiles (37°C). These organisms can be isolated in hot springs and most studies aim for their thermostable enzymes. Water samples from six hot springs (Selayang, Setapak, Klah, Labis, Gadek, and Pedas) were collected using the LaMotte bottom sampling dredge. Samples were mixed with CTYE medium and another portion without CTYE medium. These samples were transported in cold boxes and in the laboratory were incubated at 60°C for 1-2 days. Reference strain *Thermus aquaticus* (ATCC® 25104™) was included as control. After 24 h incubation, turbid samples (indicating bacterial growth) were plated and incubated in the same condition using CTYE supplemented with 3% agar. Pure colonies were chosen from each culture plate for identification by (i) conventional biochemical methods and (ii) molecular characterization. Another portion of the water samples were processed directly for DNA. Extracted DNA were used to amplify 16S rDNA gene by PCR method. The amplicons were sent for sequencing and results obtained were aligned with representative 16S rDNA sequences of related taxa using CLUSTAL W 1.83 software. Based on 16S rDNA study, five new strains of bacteria (Klah, Klah 2, Labis, Gadek, and Pedas) were isolated and confirmed to be closely related to *Geobacillus* spp. These strains were earlier identified by conventional tests and found to be Gram-positive bacilli, motile, oxidase and catalase positive. More isolates were identified from the same locations: four Gram negative bacilli (Sly, Sly 2, Klah 1, and Gadek 1) and three Gram-positive spore-forming bacilli (Sly 1, Klah 3, Gadek 2). Out of the four Gram-negatives, two strains (Gadek 1 and Klah 1) were found to be closely related to *Thermus* strains. Strains Klah 1, Klah 2, Klah 3, Labis, Gadek, Gadek 2 and Pedas were found to be protease enzyme producers. All the Gram-positives were related to thermophilic *Bacillus* spp. Most of the organisms from the hot springs can be maintained in the laboratory using CTYE medium at 60^0C. The hot springs water temperature was in the range of 48°C to 95°C and pH 6.8 to 7.9. Morphological examination, conventional biochemical tests and physiological studies were found to be useful in the preliminary identification of new isolates. However, 16S rDNA sequences and phylogenetic analysis has proven to be more discriminative tool for accurate determination of unknown organism. In conclusion, this study therefore provides evidence that the hot springs in Malaysia supports the growth of both *Thermus* and *Bacillus* spp. These bacteria may have numerous potentials as useful enzyme producers for biotechnological applications.

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CHAPTER 1

INTRODUCTION

1.0 Background of the Study

Organisms living in extreme environments such as hot springs, cold arctic water, acidic and alkaline water, saturated salt brines and pressurized abyssal water (Stetter, 1999) are becoming an interest among scientists for their unique adaptations to the extreme environments. Over the past decades, studies of the microbiology of high-temperature hot springs by both molecular-ecological and culture-based approaches have revealed phylogenetic and physiological diversity (Nakagawa & Fukui, 2003).

1.1 Definition of Extremophiles

Extremophiles can be defined as organisms that are capable to grow in a severe environment (Rossi *et al.,* 2003). Specifically, it is a condition where only some organisms are able to grow under that particular condition, whereas others cannot (Brock, 1978). Rossi *et al.* (2003) have reviewed some extreme environments like high or low temperature, acidic and alkaline conditions, high salt concentrations, and high pressure. One of these environmental extreme conditions usually becomes a factor to live for some organisms that can tolerate but will be a lethal factor to other organisms that cannot tolerate these extreme conditions. Brock (1978) has given three ways of how organisms can adapt to an environmental extreme: (i) able to exclude the