

**CHARACTERIZATION OF NOVEL THERMOPHILIC
MICROORGANISMS FROM HOT SPRINGS**



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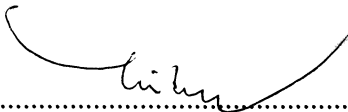
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ABSTRACT

Introduction: Thermophilic bacteria can be isolated at high temperatures (60°C to 80°C) in hot springs of neutral or alkaline pH, as well as in hot water or in natural waters subjected to thermal population. This is in contrast to mesophiles which normally survive at 37°C.

Objective: To isolate and characterize thermophilic bacteria from the hot springs in Malaysia and to identify useful products from these bacteria.

Materials and Methods: Water samples from six hot springs (Selayang, Setapak, Klah, Labis, Gadek, and Pedas) were collected using the LaMotte bottom sampling dredge and transported in cold boxes back to the laboratory at UiTM. Portions of these water samples were mixed with CTYE medium and another portion without CTYE medium and were incubated at 60°C for 1 to 2 days. After 24 h incubation, turbid samples were plated and incubated at the same temperature using 3% agar in CTYE medium. Pure colonies were chosen from each culture plate for identification by (i) conventional biochemical methods and (ii) molecular characterization. DNA were extracted from positive cultures and amplified for 16S rDNA gene by polymerase chain 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. Reference strain *Thermus aquaticus* (ATCC® 25104™) was included as control.

Results: Most of the organisms from the hot springs can be maintained in the laboratory using CTYE medium at 60°C. The water temperature ranged from 48°C to 95°C with pH 6.8 to 7.9. By conventional tests and microscopic examination, five new strains of bacteria (Klah, Klah 2, Labis, Gadek, and Pedas) were isolated and confirmed to be closely related to *Geobacillus* spp. These bacteria were Gram-positives, motile, oxidase and catalase positive. In these same locations, another 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) were also found. Out of the four Gram-negatives, two strains (Gadek 1 and Klah 1) were found to be closely related to *Thermus* strains. All the Gram-positives were related to thermophilic *Bacillus* spp. Based on 16S rDNA study, five new strains of bacteria (Klah, Klah 2, Labis, Gadek, and Pedas) were confirmed to be closely related to *Geobacillus* spp. (Gram-positive bacilli, motile, oxidase and catalase positive). By skim milk test, five strains (Gadek, Gadek2, Labis, Klah3, and Klah2) were suspected to be protease producers out of eight thermophiles (Gadek, Gadek2, Labis, Pedas, Klah3, Klah2, Sly, and Sly1) that