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Enzymatic Screening and Genotypic Characterization of Thermophilic Bacteria from the Hot Springs of Sarawak, Malaysia

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Abstract

Owing to their eccentric thermostable ability, thermophiles are among the most utilized extremophiles in various industries, such as manufacturing, and clinical research. Researchers believe that many unknown thermophiles are yet to be discovered. This study aimed to genotypically characterize the diversity of thermophiles and screen them for the potential production of enzymes in the recreational hot springs located at Northwest Coast of Borneo. Water samples were collected at 45 °C–50 °C from Annah Rais and Panchor hot springs during the sampling period from January 2018 to January 2019. Three samples (water and sediment) were collected twice in a 3-week interval from each pool of the sampling sites. Each water sample was diluted up to 10^{-3} and plated on thick nutrient agar at 55 °C for 24 h. Customized nutrient agar plus Bacto-agar plates were used for the optimum growth analysis of the isolates at 40 °C–90 °C for 24 h. The thermophiles were isolated, characterized biochemically, and amplified molecularly using DNA fingerprinting and 16S rRNA gene sequencing. Lipase, protease, gelatinase, amylase, catalase, and nitrate reductase enzymatic production was examined. Twenty-one thermophilic isolates were successfully characterized into seven clusters of *Amnoxybacillus* spp. and *Geobacillus* spp. by studying their phylogenetic dendrograms. Isolates AR10 and AR15 could produce most of the tested enzymes. All the isolates showed negative results in gelatinase and lipase production. PC14 was the only isolate that did not produce any of the enzymatic reactions in this experiment. The results showed that most of the thermophiles isolated from the two Borneo hot springs can synthesize enzymes and have potential to be thermostable. In conclusion, the search for the thermophilic producers of novel enzymes in Borneo is successful; further research must focus on their applications.

Keywords: Borneo, enzyme, genotypic, recreational hot springs, thermophiles

Introduction

The unique enzymatic potential of thermophilic and hyperthermophilic microorganisms was discovered two decades ago, prompting explorations on their potential biotechnological, industrial, and clinical applications. Thermophiles are the extremophiles that optimally grow in hot environments with temperatures ranging from 45 °C to 122 °C [1]. Thermophiles are grouped in three major clusters, namely, moderate thermophiles (survives at temperatures from 55 °C–65 °C), extreme thermophiles (survives at temperatures from 65 °C–80 °C), and hyperthermophiles (survives at temperatures higher than 80 °C) [2]. They exhibit normal function and metabolism in tremendously hot environments. Most thermophiles are classified as members of the *Bacillaceae* family, including *Thermobacillus*, *Coprobacillus*, *Anoxybacillus*, *Sulfobacillus*, *Halobacillus*, *Salibacillus*, *Marinibacillus*, *Virgibacillus*, *Amphibacillus*, *Alicyclobacillus*, *Gracilibacillus*, *Geobacillus*, *Jeotgalibacillus*, *Breviballicus*, *Paenibacillus*, *Aneurinibacillus*, and *Ureibacillus*, as identified using 16S rRNA gene sequences [3–5]. Thermophiles are found in habitats such as hot springs, deep-sea cores, petroleum reservoirs, deep-sea hydrothermal vents [6], and man-made facilities such as compost piles, slag heaps, and water heaters [7].