



UNIVERSITI PUTRA MALAYSIA

**ISOLATION OF ALKALIPHILIC MICROBES PRODUCING HIGHLY
ALKALINE THERMOSTABLE PROTEASE**

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PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk “Isolation of Alkaliphilic Microbes Producing Highly Alkaline Thermostable Protease” telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh Choo Kin Yan (163668) sebagai syarat untuk kursus BMY 4999 projek.

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ABSTRACT

For many decades, alkaliphilic bacteria that can produce highly alkaline thermostable protease had been the focus of the intense research since they were able to perform well under high temperature and pH during many industrial processes. Proteolytic bacteria that were isolated from Selayang and Kuala Kubu Bharu hot springs in Malaysia were screened using skim milk agar plates. Isolates which showed better proteolytic activity were chosen for further identification.

Bacillus licheniformis strain 1 and *Bacillus licheniformis* strain H were identified by using gram staining, biochemical tests and 16S rDNA sequence analysis. Protease produced by *Bacillus licheniformis* strain 1 reached optimum proteolytic activity at 65°C, pH 9. Its stability showed broad temperature ranges between 40 to 65°C, whereas it was stable from pH 4 to 6 for pH ranges. On the other hand, the maximum proteolytic activity showed by *Bacillus licheniformis* strain H was at 65°C, pH 9. Protease produced by this strain also had the potential to be stable over wide ranges of temperature and pH, that were between 40 to 60°C and from pH 4 to 11.

ABSTRAK

Untuk beberapa dekad, bakteria alkaliphilic yang boleh menghasilkan protease sangat alkali tahan panas telah menjadi tumpuan penyelidikan yang sengit kerana mereka dapat stabil dan dapat menunjukkan prestasi yang baik pada suhu yang tinggi dan pH dalam proses industri banyak. Bakteria proteolitik yang diasingkan daripada Selayang dan Kuala Kubu Bharu mata air panas di Malaysia telah disaring menggunakan susu skim plat agar. Isolat yang menunjukkan aktiviti proteolitik yang lebih baik telah dipilih untuk pengenalpastian selanjutnya.

Bacillus licheniformis strain 1 dan *Bacillus licheniformis* strain H telah dikenal pasti oleh 16S rDNA analisis turutan dan sistem BLAST. *Bacillus licheniformis* strain 1 telah mencapai aktiviti proteolitik optimum pada 65°C, pH 9. Kestabilannya menunjukkan julat suhu yang luas antara 40 hingga 65°C, sedangkan ia stabil dari pH 4 hingga 6 untuk julat pH. Manakala aktiviti proteolitik maksimum ditunjukkan oleh *Bacillus licheniformis* strain H adalah pada 65°C, pH 9. Strain ini juga mempunyai potensi untuk menjadi lebih stabil julat luas suhu dan pH, yang adalah di antara 40 hingga 60°C dan dari pH 4 hingga 11.

CHAPTER 1

INTRODUCTION

The most concentrated and widespread occurrence of organisms can be generally observed in moderate environments. It has been known that there are extreme environments on earth which thought to prevent any existence of life. And normally in these habitats, environmental factors such as temperature, salinity concentrations and pH are either extremely high or low. Organisms that specifically adapted to these habitats are known as extremophiles and can be divided into alkaliphiles, thermophiles and acidophiles. (Ulukanli, 2002). There are no precise definitions of what characterize alkaliphilic microbes. Alkaliphilic microbes or alkaliphiles are terms used to describe organisms that are able to survive or grow well in pH values above 9, normally around 10 to 12. However, they cannot survive at near neutral pH of 6.5. (Horikoshi, 1991). Recently, microbial enzymes have become the focus of research by bioprocess engineers and manufacturing biochemists for their environmentally- friendly applications in industrial process. Amongst the industrial enzymes, (Bajaj and Jamwal, 2013). According to Kasana et al. (2011), highly alkaline thermostable protease is one of the essential groups of enzymes that can be produced by alkaliphilic microorganisms. Paliwal et al. (1994) proposed that alkaline thermostable protease can be applied in many fields such as food industry, leather processing, waste treatment and others in order to replace chemical catalysts. Protease with high activity and stability in alkaline range are very useful in bioengineering and biotechnological applications. (Pathak and Deshmukh, 2012). Highly alkaline protease is preferred to be used compared to conventional catalysts because it has high catalytic activity and exhibits high degree of substrate specificity. In addition, it is also economically viable and can be produced in large

amounts. Highly alkaline protease can be characterized according to effects of pH, temperature, surfactants. Hence, the objectives to carry out this project are to screen and isolate alkaliphile proteolytic bacteria producing highly alkaline thermostable protease, to identify the producers and to characterize the highly alkaline thermostable protease.



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