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ISOLATION OF ALKALIPHILIC BACTERIA PRODUCING HIGHLY ALKALINE THERMOSTABLE LIPASE

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PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk “Isolation of Alkaliphilic Bacteria Producing Highly Alkaline Thermostable Lipase” telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh Azian Ezeila binti Mad Zain (162413) sebagai syarat untuk kursus BMY 4999 projek.

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ABSTRACT

Lipase known as triacyglycerol hydrolases (EC3.1.1.3) is use to catalyze the hydrolysis of triaclyglycerol to glycerol and fatty acid. Naturally found in nature and basically derived from various sources such as bacteria, archaea, animals, plants and fungi. Lipase is industrially important and commercially used in industrial such as detergent, food, leather and bioremediation. The aim of this thesis is to screen and isolate bacteria producing highly alkaline and thermostable lipase from hot springs in Malaysia. The identification was done by biochemical tests and 16S rDNA gene sequencing. Follow by characterization of crude lipase on different temperature and pH. Forty eight isolates were selected during first screening on tributyrin agar plate that showed clear halo zones. All forty eight isolates were tested for gram staining for morphology identification. Total of sixteen isolates were chosen from the gram staining and test for second screening on rhodamine B agar plate for the confirmation as lipase producing bacteria. Then, all sixteen isolates were test for lipase production and five isolates were further choosen based on higher lipase activity. All five isolates were gram positive rod. Biochemical test results revealed that all five isolates (T4, T3, B1,G and 20) belong to genus *Bacillus*. Identification of 16S rDNA showed all isolates were *Bacillus licheniformis* with different strain. Lipases from two isolates identified as *Bacillus licheniformis* G and *Bacillus licheniformis* 20 were characterized. Lipase isolated from *B. licheniformis* strain G exhibited an optimum temperature of 65 °C and optimum pH at 8.0. while, *B. licheniformis* strain 20 produced extracellular lipase with optimum temperature and pH of 50 °C and pH 8.0 respectively.

ABSTRAK

Lipase dikenali sebagai hidrolase triasilglycerol (EC 3.1.1.3) adalah digunakn untuk mengurai triasilglycerol kepada gliserol dan asid lemak. Boleh dijumpai di alam semulajadi dan biasanya berasal dari pelbagai sumber seperti bakteria, archaea, haiwan, tumbuhan dan kulat. Enzim lipase digunakan dalam pelbagai industri seperti bahan pencuci, makanan, kulit dan bioremediasi. Tujuan tesis ini adalah untuk pengasingan dan pemeriksaan bakteria yang meghasilkan lipase yang sangat beralkali dan tahan panas dari kolam air panas di Malaysia. Identifikasi dilakukan dengan ujian biokimia dan 16S rDNA. Kemudian, diikuti dengan pencirian bakteria lipase pada suhu dan pH yang berbeza. Empat puluh lapan bacteria telah dipilih dari persam[elan pertama pada agar tributirin yang menunjukan zon halo yang jelas. Semua empat puluh lapan bakteria diuji dengan perwarnaan gram untuk morfologi. Daripada empat puluh lapan bacteria, enam belas backteria telah dipilih dan dilakukan ujian pengesanan kedua pada agar rodamine B untuk pengesahan bakteria menghasilkan lipase. Kemudian, semua enam belas bakteria dijalankan ujian pengeluaran lipase dan lima bakteria telah dipilh berdasarkan aktiviti lipase yang lebih tinggi. Kesemua lima bakteria adalah gram rod positif. Keputusan ujian biokimia mendedahkan bahawa kesemua lima bakteria (T4, T3, B1, G dan 20) tergolong dalam genus *Bacillus*. Pengenalpastian 16S rDNA menunjukkan semua bakteria adalah *Bacillus licheniformis* dengan strain yang berbeza. Lipases dari dua isolat dikenal pasti sebagai *Bacillus licheniformis* G dan *Bacillus licheniformis* 20 telah dicirikan. Lipase diasingkan daripada *B. licheniformis* strain G menunjukkan suhu optimum pada 65 ° C dan pH optimum pada 8.0. Manakala, lipase yang dihasilkan daripada *B. licheniformis* strain 20 mempunyai suhu optimum dan pH pada 50 ° C dan pH 8.0.

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CHAPTER 1

INTRODUCTION

Lipases (EC 3. 1. 1. 3) known as triaclyglycerol acylhydrolases, which can catalyze the hydrolysis of triaclyglycerol to glycerol and fatty acid. These enzymes are normally found in nature and basically derived from various sources such as bacteria, archae, animals, plants and fungi. Among them, microbial lipases gain more attention due to great ability of catalytic activities, easy to be genetically modified and best growth on inexpensive media. Besides that, microbial lipases are more stable compared to enzyme derived from plants and animals and safe for research application and industrial purpose (Verma and Sharma, 2014). Many microorganisms are known as potential producers of extracellular lipases including bacteria, fungi and yeast. Among bacterial lipases being exploited, those from *Bacillus* exhibit interesting properties that make them potential candidates for biotechnological applications. The most common bacterial lipases are *Bacillus subtilis, Bacillus licheniformis, Bacillus pumilus, Bacillus coagulans, and Bacillus alcalophilus*. In addition, *Pseudomonas* sp., *Pseudomonas aeruginosa, Burkholderia multivorans, Burkholderia cepacia,* and *Staphylococcus caseolyticus* are also reported as bacterial lipase producers. Meanwhile, lipase from *Bacillus thermoleovorans* is one of the bacteria lipases which could stand at high pH and most active at pH 9.0-10.0. But, only certain type of bacteria can grow at high temperature such as *Bacillus, Chromobacterium, Pseudomonas, and Staphylococcus* (Bora et al., 2013). *Bacillus* sp. is very useful in industry because it can stand at harsh condition and able to be active at alkaline environment (Bora and Bora, 2012; Gundala et al., 2013). Alkaline

and thermostable lipases are widely used in industry because of their ability to retain activities in harsh conditions. Thermostable alkaline lipases are used in many areas of commercial interest like detergent, leather, pharmaceutical and bioremediation industry (Zuridah et al, 2011). In detergent industry for the enzyme based detergent, alkaline lipase are highly demand because it possess higher cleaning ability compared to chemical based detergents. Lipase also can be used for environmental management areas especially in bioremediation processes where they are needed to raise wastes of lipid processing factories. Other than that, lipases are important in leather processing because it needs necessity to be carried out at alkaline pH for removal of subcutaneous fat and dehairing (Ray, 2012; Bora et al., 2013). The aim of this thesis is to screen and isolate bacteria producing highly alkaline and thermostable lipase from hot springs in Malaysia.

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