UNIVERSITI TEKNOLOGI MARA

IDENTIFICATION AND CLONING OF LIPASE AND NUCLEOSIDE PERMEASE ENZYME FROM THERMOPHILIC BACTERIA

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ABSTRACT

Lipase enzymes can be isolated from thermophiles in hot springs. This present study involves the identification and retaining the interested genes by cloning. Previous thermophiles (A1, A3, A4, A5, A6, A8, A13 and A14) which were kept in glycerol stocks were subcultured onto Castenholtz Tryptone Yeast (CTYE) agar for further study. Rhodamine B-olive oil agar plate culture was used to screen for lipase enzyme followed by PCR. Isolates A1, A3, A4 and A5 was a circular, pin point, creamy colonies with entire margin on CTYE media, whilst A6, A8, A13 and A14 was irregular, spreading, wavy, and creamy colonies. Four isolates (A1, A3, A4 and A13) showed the presence of orange fluorescence around colonies on rhodamine B olive oil agar plate thus indicating the presence of lipase. Amplification and sequencing of lipase gene showed that A1, A3, and A4 were 98% similar to *Geobacillus* and *Bacillus sp.* compared to other lipase sequences in the GenBank. Interestingly, A14 was 90% similar to nucleoside permease gene from *Anoxybacillus flavithermus* WK1. A14 was further identified by 16S rRNA gene which showed 93% similarities to *Anoxybacillus flavithermus* WK1. On further gene-protein translation study, A14 was found to contain an amino acid sequence highly homologous to the putative domain hits to nucleoside permease protein. Lipase gene from isolate A1 and nucleoside permease gene from isolate A14 were cloned using pBAD TOPO expression kit cloned. Lipase gene of A1 was not successfully cloned; however, the cloning of nucleoside permease gene of A14 was successful and further sequenced and reconfirmed as such. In conclusion, three lipase producing bacteria, *Geobacillus* spp. (A1, A3, A4) and one nucleoside permease producer, *Anoxybacillus* sp. (A14) were identified in this study. Lipase and nucleoside permease enzymes are recommended for further characterization and its suitability for industrial usage.

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INTRODUCTION

1.1 INTRODUCTION

The crucial criteria when deal with bioprocess at high temperature for sustainable operation is the stability of the enzyme or catalyst at this temperature (Rahman, Leow, Salleh and Basri, 2007). The industrial use of enzyme has rapidly increased during the last two decades. Lipases have gained a lot of attention in the area of organic synthesis compared to protease and amylase. Lipases are classified as water insoluble enzymes that catalyzed the hydrolysis of ester bonds in lipid substrate (Savitha et al., 2007). Lipases possessed several advantages such as more environment-friendly over bulk chemical syntheses, able to manufacture a high quality product, ease of recovery and re-use of the lipases in continuous operation (Hamid et al., 2003; Akanbi et al., 2009).

Due to these positive values, many research have divert their attention to seek for alternative sources of lipase, and therefore efforts have been focused on identification of thermophilic bacteria because they are important source of heat stable enzymes (Rahman et al., 2007). Thermophiles are important source of heat stable enzymes (Hamid et al., 2003). Psychrophile, mesophile and thermophile have their own optimum growth temperature and this forms the basis to categorize them into distinct classes by their position in a temperature spectrum (Mohr and Krawiec, 1980). Thermophilic organisms have their optimal growth temperature between 50^0C to 80^0C (Brown, 2005). Enzyme derived from thermophilic organism is known to be thermostable and resistant to the action of organic solvents, thus, these enzymes have found a number of commercial applications (Radaideh, Malkawi, Omari, and Deeb, 2010).