ISOLATION AND IDENTIFICATION OF AMYLASE PRODUCING *Bacillus* sp. FROM LOCAL HOUSE WASTE CONTAMINATED SOIL

By

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AUTHOR'S DECLARATION

I hereby declare that this thesis is my original work and has not been submitted previously or currently for any other degree at UiTM or any institutions.

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ABSTRACT

Amylase is an enzyme that breaks the starch molecules into dextrins and smaller glucose units. It is one of the most important enzymes comprising about 30 % of the world's enzyme production. Amylase can be obtained from different sources such as plants, animals, and microorganisms however, *Bacillus* species (*B.subtilis*, *B.licheniformis* and *B.amyloliquefaciens*) is most commonly use in the industrial production of amylase. Recently, the search for novel amylases is growing worldwide since the application of these enzymes has spread in many industrial sectors. Considering the importance of amylase in industry, this study was conducted to isolate and identify amylase producing *Bacillus* sp. from local house waste contaminated soil. The soil samples were collected from two different locations, Kampung Bukit Kuching Tengah and Felda Bukit Cherakah in Selangor, Malaysia. Two sites which were site A and B were selected from Felda Bukit Cherakah while Site C from Kampung Bukit Kuching Tengah. The isolation of amylase producing *Bacillus* sp. began with heat treatment method whereby only aerobic endospore forming bacteria (AEFB) were isolated. Starch hydrolysis test was used to screen for potent amylase producer. The isolates were then subjected to Polymerase Chain Reaction (PCR) for amplification of 16S rDNA gene before further proceed with sequencing and BLAST analysis for identification. Based on the results, a total of 12 bacterial isolates were obtained from the soil, among them only eight isolates named as A1, A3, B1, B2, B3, C1, C4 and C6 were amylase producing *Bacillus* sp. The BLAST result showed that A1 was found to be *B.cereus JKR62* with 100% homology whereby the probable identity of A3 was *B.amyloliquefaciens LEM97*and *B.subtilis H-70* with 99% homology. Furthermore, B1 showed 99% homology with three different *Bacillus* strains which was *B.cereus BVC77, B.thuringiensis serovar morrisoni* and *B.anthrasis* isolate 1111TES13M4 whereas B2 and B3 showed 99% similarities with *B.amyloliquefaciens AR-2* and *B.amyloliquefaciens ARC225* respectively. Moreover, all the isolates from site C (C1, C4, and C6) were identified as *B.subtilis b+, B.subtilis IARI-V-7* and *B.subtilis DL47* respectively. In conclusion, this study proved that amylase producing *Bacillus* sp. can be isolated from local house waste contaminated soil whereby these isolates can be further used for production of amylase to support the industrial need.

Keyword: Amylase, *Bacillus* sp, waste contaminated soil

CHAPTER 1

INTRODUCTION

1.1 Background of study

Amylase is an enzyme that breaks the starch molecules into dextrins and smaller glucose units (Sahnoun, Bejar, Sayari, Triki, Kriaa, & Kammoun, 2012). It is one of the most important enzymes comprising about 30 % of the world's enzyme production (Prasad, 2014) . Amylase is used in baking, brewing, textile, detergent, paper and distilling industries (Ashwini, Kumar, Karthik, & Bhaskara, 2011). In addition, amylase from a fungal source is the first enzyme commercially produced for the treatment of digestive disorders (John Ravindar & Elangovan, 2013). Amylase can be obtained from different sources such as plants, animals, and microorganisms (fungi and bacteria). Production of amylase by microorganism is more advantageous than other sources since they fulfilled the need of industry and can be easily manipulated to obtain enzymes with favorable characteristic. Although amylase is produced by many other microbes, *Bacillus* species (*B.subtilis*, *B.licheniformis* and *B.amyloliquefaciens*) is most commonly use in the industrial production of amylase (Gaur, Jain, & Bajpai, 2012). They have the ability to produce amylase and other enzymes in large quantities which make them among the most important industrial enzyme producer. This is proven by the facts that about 60 % of commercially available enzymes is produced by different species of *Bacillus* (Bakri, Ammouneh, El-khouri & Thonart, 2012).

Recently, the search for novel amylases is growing worldwide since the application of these enzymes has spread in many industrial sectors (Devi, Dowarah, Unni, Wann, & Samanta, 2012). To accomplish this purpose, isolation of amylase producing microorganism from the soil has been the most favorable way since soil harbor numerous numbers of microorganisms. According to Ministry of Housing and Local Government Kuala Lumpur (MHLG) as cited in Ting, De Cruz, & Chan (2008), amylase producing bacteria is mainly isolated from soil that is contaminated with food, organic or paper wastes. This is supported by the study by Ting *et al.*, (2008)