UNIVERSITI TEKNOLOGI MARA

PRODUCTION OF TANNASE BY LOCALLY ISOLATED *ASPERGILLUS NIGER* THROUGH SOLID-STATE FERMENTATION USING SELECTED AGRI-INDUSTRIAL BY-PRODUCTS

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MSc

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Tannase or tannin acyl hydrolase (EC 3.1.1.20) is an important industrial enzyme widely used in beverages, food, feed, leather, pharmaceutical, chemical and cosmetics industries. Tannase is an inducible extracellular enzyme that hydrolyses ester bonds of hydrolysable tannin such as tannic acid and gallotannin into gallic acid and glucose. Tannase is used in the manufacturing of gallic acid, an intermediate for the synthesis of trimethoprim and propyl gallate, which are used in pharmaceutical and food industries, respectively. In beverage industry, tannase helps to reduce the astringent taste and acts as a clarifying agent in the fruit juice, coffee, coffee-flavoured soft drink and instant tea. Despite the huge industrial application of tannase, its usage is still limited due to high production cost of tannase. Industrial production of tannase is generally through submerged fermentation by *Bacillus* sp. using tannic acid as carbon source and inducer, which is costly. Therefore, this study aimed to screen for best tannase-producing fungal strain and select agri-industrial by-products as potential substrate and subsequently, improve the fermentation parameters for maximum tannase production under solid-state fermentation using the one-factor-at-time (OFAT) approach. A total of 51 tannase-producing fungal strains, isolated from various types of agri-industrial by-products (ABP) and 20 fungal strains from the CFFC MARDI were used for the screening of the potential tannase producer. Fungal strain labelled PN1 isolated from the banana peel was selected as the best tannase-producing fungal strain based on its ability to produce the largest diameter of hydrolytic zone (60.7 ± 0.60 mm) on tannic acid plate agar and highest tannase activity of (6.86 ± 0.02 U/mL) and was identified as *Aspergillus niger*. Among the 3 selected agri-industrial by-products (rice bran, brewer's rice and spent coffee ground) screened, rice bran was found as the best substrate for tannase production with highest tannase activity of 149.40 U/gds followed by brewer's rice with 133.54 U/gds and spent coffee ground with 116.10 U/gds. Improvement of fermentation parameters using OFAT has revealed that 1x10^8 spores/mL, initial moisture level of 49.36%, initial pH at 6.50 and supplementation of 4% of spent coffee ground at 30 ºC of incubation time were found could enhance tannase production by 2.48 folds with maximum activity of 182.41 U/gds compared to the unoptimised conditions (73.45 U/gds). Therefore, solid-state fermentation of rice bran by *Aspergillus niger* under optimum parameters was a great alternative for enhanced tannase production.

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"*One child, one teacher, one book, and one pen, can change the world*." Malala Yousafzai

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