

HIGH CELL DENSITY CULTIVATION OF *Hendersonia* sp. FOR THE
APPLICATION OF BIOLOGICAL CONTROL OF OIL PALM DISEASE

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to my mom and dad for bringing me into this wonderful world

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ABSTRAK

Malaysia merupakan salah satu pengeluar dan pengeksport produk kelapa sawit yang terbesar di dunia. Pada 2011, Malaysia telah mengesport sebanyak 24.27 juta tan produk kelapa sawit yang membawa kepada nilai eksport sebanyak RM 80.4 billion. Walau bagaimanapun, pokok kelapa sawit mudah terdedah kepada penyakit, terutamanya Basal Stem Rot yang berpunca dari *Ganoderma*. Penyakit ini telah menyebabkan kerugian yang besar kepada ekonomi. *Hendersonia* sp. adalah strain kulat yang telah menunjukkan kesan efektif dalam menangani jangkitan *Ganoderma*. Kajian ini dijalankan bagi membina suatu platform pengkulturan *Hendersonia* yang komprehensif untuk pengeluaran skala industri. Bagi kajian screening medium agar, medium yang terbaik terdiri daripada (g/L): Ekstrak Malt, 20; Glukosa, 20; Pepton, 1; Ekstrak Yis, 5; Serbuk Agar, 20. Bagi kajian screening shake flask pula, medium yang terdiri dari (g/L): Ekstrak Malta, 20; Glukosa, 20; Pepton, 1; Ekstrak Yis, 5, memberi CDW yang tertinggi iaitu 7.45 ± 0.6 g/L. Ini diikuti dengan medium yang terdiri dari of (g/L): Sukrosa, 40; KH_2PO_4 , 1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0; KCl, 0.5; FeSO_4 , 0.01; Ekstrak Yis, 2, yang memberi CDW sebanyak 6.15 ± 0.14 g/L. Optimasi medium telah dibuatkan dengan menggunakan Metodologi Response Surface. Formulasi medium yang telah dioptimasi terdiri dari (g/L): Sukrosa, 60; K_2HPO_4 , 0.5; Ekstrak Yis, 3; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1; KCl, 0.5; FeSO_4 , 0.01. Bagi pengkulturan bioreaktor 150-L, dengan menggunakan pH tanpa kawalan, CDW sebanyak 13.55 g/L telah diperolehi. Selain itu, kesan kelajuan agitasi kepada jisim sel telah dikaji di dalam bioreaktor 150-L. CDW yang tertinggi telah diperolehi dengan menggunakan kelajuan agitasi 250 rpm pada 70 jam. Kesimpulannya, kajian ini telah mencadangkan kaedah yang kos efektif dalam menghasilkan *Hendersonia* dalam skala industri.

ABSTRACT

Malaysia is one the world's biggest producers and exporters of oil palm products. In 2011, Malaysia has exported 24.27 million tonnes of oil palm products, which accounted for RM 80.4 billion in total export revenue. However, the oil palm is susceptible to plant diseases, especially Basal Stem Rot caused by *Ganoderma*. This disease has caused tremendous losses to the economy. *Hendersonia* sp. is a novel fungus strain that has shown effective results in controlling *Ganoderma* infection. The aim of this study was to establish a comprehensive *Hendersonia* cultivation platform for industrial-scale production. In an agar medium screening study, the best medium was composed of (g/L): Malt Extract, 20; Glucose, 20; Peptone, 1; Yeast Extract, 5; Agar powder, 20. Based from the shake flask media screening study, the medium composed of (g/L): Malt extract, 20; Glucose, 20; Peptone, 1; Yeast extract, 5, gave the highest CDW of 7.45 ± 0.6 g/L. This is followed by the medium composed of (g/L): Sucrose, 40; KH_2PO_4 , 1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0; KCl, 0.5; FeSO_4 , 0.01; Yeast Extract, 2, which resulted with CDW of 6.15 ± 0.14 g/L. The optimization of the medium was applied by using Response Surface Methodology. The new optimized medium formulation was composed of (g/L): Sucrose, 60; K_2HPO_4 , 0.5; Yeast Extract, 3; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1; KCl, 0.5; FeSO_4 , 0.01. For the 150-L bioreactor cultivation, by utilizing un-controlled pH throughout the cultivation, 13.55 g/L of CDW was obtained. In addition, the effects of agitation speed on the cell mass during the 150-L bioreactor cultivation were studied. The highest CDW (15.6 g/L) was obtained with agitation speed of 250 rpm at 70 h. In conclusion, the present study has proposed a reliable and cost-effective approach in mass producing *Hendersonia* in industrial scale.

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LIST OF ABBREVIATIONS

CDW	-	Cell dry weight
DO	-	Dissolved oxygen
FeSO ₄	-	Ferrous sulfate
KCl	-	Potassium chloride
KH ₂ PO ₄	-	Di-potassium phosphate
MgSO ₄ ·7H ₂ O	-	Magnesium sulfate heptahydrate

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CHAPTER I

INTRODUCTION

1.1 Research background

Being one of the world's largest producers and exporters of oil palm products, Malaysia exported 24.27 million tonnes of oil palm products in 2011. These oil palm products, which include palm oil, palm kernel oil, palm kernel cake, oleochemicals, biodiesel and finished products, account for RM 80.4 billion in total export revenue (Malaysian Palm Oil Board, 2012).

However, like most crops, oil palm is susceptible to diseases. One of the major diseases of oil palm is known as basal stem rot (BSR), which is caused by pathogenic fungal species especially *Ganoderma* (Pilotti, 2005). This disease poses a serious threat to the cultivation and the production of oil palm, which leads to severe economic losses.

Over recent years, many attempts have been done to control basal stem rot in oil palm. The usage of chemical-based fungicide as control showed only mediocre results due to the characteristics of *Ganoderma* which is soil borne (Susanto *et al.*, 2005). Thus,

researchers are currently focusing in studying the biological control of the disease using endophytic microorganisms including *Pseudomonas*, *Trichoderma*, and *Hendersonia*.

One of the major advantages of using endophytic microorganisms as control is they can be easily introduced to the roots without causing harm to the host plants. Moreover, these endophytic microorganisms can suppress the growth of pathogens by limiting their nutrient and space.

1.2 Problem statement

Hendersonia has been researched for its biological control properties, especially in controlling fungal diseases. Resulting from this, there is a growing need of these fungi in oil palm cultivations across Malaysia and other oil palm producing countries. Thus, in order to cater for these demands, there must be some means to produce this strain in large scale. In this study, we aim to design an efficient high cell density cultivation of *Hendersonia* sp. As of the time of writing this thesis, there has been no previous study on both shake flask and bioreactor cultivation of *Hendersonia* sp.

1.3 Research objectives

The main objectives of this research are:

- (i) To establish a comprehensive *Hendersonia* sp. cultivation platform;
- (ii) To select the optimum medium for the cultivation of *Hendersonia* sp.

1.4 Research scope

In order to achieve the objectives of this study, the scope of research were applied:

- (i) Medium screening for agar cultivation;
- (ii) Medium screening for shake flask cultivation;
- (iii) Medium optimization for shake flask cultivation;
- (iv) Effect of pH and agitation speed on cell growth in 150-L bioreactor.

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