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

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A dissertation submitted in partial fulfillment of the requirements for the award of the degree of Master of Engineering (*Bioprocess*)

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JANUARY 2013

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

ACKNOWLEDGEMENT

Praise be upon Allah, the Almighty Who is Kind and Merciful. For it is by His grace and blessing that gave me the endurance and perseverance to complete this project, and ultimately, this dissertation.

I would like to express my sincere gratitude to my supervisor, Prof. Dr. Hesham Ali El Enshasy, for giving me the opportunity to revive my long lost passion for research. There won't be anything in the world that can compensate his endless support and advice.

I also would like to acknowledge the help and support from everyone who was involved in this project, directly or indirectly, especially the staff members from the Insitute of Bioproduct Development (IBD). Once again, I wish to thank the research officers, Ms. Roslinda and Ms. Zalina, as well as Mr. Solleh and Mr. Yahya for sharing their knowledge and assistance.

My deepest gratitude to all my fellow friends from the lab for their kindness and enormous help throughout the project. Working in the lab would never be fun and enjoyable without them around.

Last but not least, I would like to thank my parents and family for their love and prayer. The success of this project would not be achieved if it were not for their continuous support.

ABSTRAK

Malaysia merupakan salah satu pengeluar dan pengeksport produk kelapa sawit yang terbesar di dunia. Pada 2011, Malaysia telah mengeskport 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; $MgSO_4.7H_2O$, 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; $MgSO_4.7H_2O$, 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; $MgSO_4.7H_2O$, 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; $MgSO_4.7H_2O$, 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.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CDW	-	Cell dry weight
DO	-	Dissolved oxygen
$FeSO_4$	-	Ferrous sulfate
KCl	-	Potassium chloride
KH_2PO_4	-	Di-potassium phosphate
$MgSO_4.7H_2O$	-	Magnesium sulfate heptahydrate

LIST OF APPENDICES

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.

REFERENCES

Abouseoud, M., Maachi, R., and Amrane, A. Biosurfactant production from olive oil by *Pseudomonas fluorescens.* In Mendez-vilas, A. (Ed.) *Communicating current research and educational topics and trends in applied microbiology*. Spain: Formatex. 340-347, 2007

Azevedo, J.L., Maccheroni, J.W., Pereira O.J., and Araujo, L.W. (2000). Endophytic microorganisms: a review on insect control and recent advance on tropical plants. *Journal of Biotechnology*. 3, 40-65.

Bang, S.G., and Choi, C. Y. (1995). DO-stat fed batch production of cis, cis-muconic acid from benzoic acid by *Pseudomonas putida* BM014. *Journal of Fermentation and Bioengineering*. 79, 381-383.

Bingzhang, H. and Yujie, C. (1994). Study on biological characteristics of *Hendersonia acicola. Journal of Northeast Forestry University*. 5, 4.

Butin, H, and Lonsdale, D. (1995). *Tree Diseases and Disorders*. New York; Oxford University Press.

Caldwell, D. (2004). *Basal stem rot / Ganoderma butt rot of palms.* Florida: University of Florida.

Chincholker, S.B. and Mukerji, K.G. (2007). *Biological Control of Plant Diseases*. New York; Haworth Press.

Dikin, A., Sijam, K., Mior-Ahmad, Z.A., and Abu-Seman, I. (2003). Biological control of seedborne pathogen of oil palm, *Schizopyllum commune* Fr. with antagonistic bacteria. *International Journal of Agriculture and Biology*. 4, 507-512.

Doelle, H.W. and Mcgregor, A.N. (1983). The effect of high ethanol and carbon dioxide concentrations on the ultrastructure of *Zymomonas mobilis*. *Applied Microbiology and Biotechnology*. 17, 44-48.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., and Smith, F. (1956). Colorimetric method for determination of sugars and related substance. *Analytical Chemistry*. 28, 350-356.

Ellis, M.B. (1997). *Microfungi on Land Plants: An Identification Handbook.* United Kingdom: The Richmond Publishing Co. Ltd.

Gao, L., Sun, M.H., Liu, X.Z., and Che, Y.S. (2007). Effects of carbon concentration and carbon to nitrogen ratio on the growth and sporulation of several biocontrol fungi. *Mycological Research*. 3, 87-92.

Idris, A.S., Nurrashyeda, R., Maizatul, S.M., Madihah, A.Z., Tarmizi, A.M., Kushairi, A., Wan Azha, W.M., and Tony Peng, S.H. (2012). Biofertiliser *Hendersonia* GanoEF as biological control of *Ganoderma* in oil palm. *Malaysia Palm Oil Berhad Information Series*. ISSN 1511-7871.

Jones, R.P. and Greenfield, P.F. (1982). Effect of carbon dioxide on yeast growth and fermentation. *Enzyme and Microbial Technology*. 4, 210-223.

Khan, S., Misra, A.K., Tripathi, C.K.M., Mishra, B.N., and Bihari, V. (2006). Response surface optimization of effective medium constituents for the production of alkaline protease from a newly isolated strain of *Pseudomonas aeruginosa*. *Indian Journal of Experimental Biology*. 44, 151-156.

Lu, G.Y., Wong, P., He, J.Y., and Li, X.N. (2008). Medium optimization for enzymatic production of L-cysteine by *Pseudomonas* sp. ZJWP-14, using response surface methodology. *Food Technology and Biotechnology.* 46, 395-401.

Malaysia Palm Oil Board. (2011). *Overview of the Malaysian Oil Palm Industry 2010.* Malaysian Palm Oil Board.

Meade, J., Higgins, P., and Ogara, F. (1985). Production and storage of *Rhizobium leguminosarum* cell concentrates as inoculants. *Journal of Applied Microbiology*. 58, 517-524.

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry.* 31 (3): 426–428.

Mitchell, C.P., Williamson, B., and Millar, C.S. (1976). *Hendersonia acicola* on pine needles infected by *Lophodermella sulcigena*. *European Journal of Pathology*. 6, 92-102

Patel, N., Choy, V., Malouf, P., and Thibault, J. (2009). Growth of *Trichoderma reesei* RUT C-30 in stirred tank and reciprocating plate bioreactors. *Process Biochemistry*. 44, 1164-1171.

Pilotti, C. A. (2005). Stem rots of oil palm caused by *Ganoderma boninense*: Pathogen biology and epidemiology. *Mycopathologia*. 159, 129-137.

Preusting, H., Houten, R.Z., Hoefs, A., Langenberghe, E.K.V., Favre-Bulle, O., and Witholt, B. (1992). *Biotechnology and Bioengineering*. 41, 550-556.

Rao, Y.K., Tsay, K., Wu, W., and Tzeng, Y. (2007). Medium optimization of carbon and nitrogen sources for the production of spores from *Bacillus amyloliquefaciens* B128 using response surface methodology. *Process Biochemistry*. 42, 535-541.

Shastry, S., and Prasad, M. (2002). Extracellular protease from *Pseudomonas sp*. (CL 1457) active against *Xanthomonas campestris*. *Process Biochemistry*. 37, 611-621.

Shiloach, J., and Fass, R. (2005). Growing *E. coli* to high cell density - A historical perpsective on method development. *Biotechnology Advances.* 23, 345-357.

Soepena, H. Purba, R.Y., and Pawirosukarto, S. A control strategy for basal stem rot (Ganoderma) on oil palm. In Flood. *et al.* (Ed.) *Ganoderma Disease of Perennial Crops*. United Kingdom: CAB International. 83-88, 2000

Son, H., Kim, K., Kim, H., Kim, Y., and Lee, S. (2000). Enhanced production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by fed-batch cultures of *Pseudomonas sp* EL-2. *Journal of Industrial Microbiology & Biotechnology.* 24, 36-40.

Susanto, A., Sudharto, P. S., and Purba, R.Y. (2005). Enhancing biological control of basal stem rot disease (*Ganoderma boninense*) in oil palm plantations. *Mycopathologia.* 159, 157-157.

Thompson, A. (1931). Stem rot of oil palm in Malaya. *Bulletin of the Department of Agriculture.*

Tripathi, N.K., Sathyaseelan, K., Jana, A.M., and Rao, P.V.L. (2009). High yield production of heterologous protein with *Escherichia coli*. *Defence Science Journal*. 59, 137-146.

Van Loon, L., and Bakker, P. (2004). Signalling in rhizobacteria-plant interactions. In De Kroon, J. and E.J.W. Visser (Eds.) *Ecological Studies, Journal of Root Ecology.* 168, 287-330.

Zaiton, S. Sariah, M. and Zainal-Abidin, M.A. (2008). Effect of endophytic bacteria on growth and suppression of *Ganoderma* infection in oil palm. *International Journal of Agriculture & Biology*. 2, 127-132.