

ISOLATION OF Ceratocystis-INHIBITING BACTERIA IN Acacia mangium

NATASHA DEANNA CHAN BINTI JEFFREY CHAN

FH 2018 21

ISOLATION OF Ceratocystis-INHIBITING BACTERIA IN Acacia mangium



By

NATASHA DEANNA CHAN BINTI JEFFREY CHAN

A Project Report Submitted in Partial Fulfilment of the Requirements for the Degree of Bachelor of Forestry Science in the Faculty of Forestry

Universiti Putra Malaysia

2018

DEDICATION

For my family: Nornida Idris Jeffrey Chan Abdullah Joshua Daniel Chan Adam Chan Nugget and Pudding Chan

To my supervisor, Dr Razak Terhem

Not forgetting my friends, Who gave me encouragement and motivation when I had none, For anchoring me when I returned from my manic, For always reaching out a hand to haul me up when I found myself sinking deeper, For being understanding and patient with me while I was going through my rough patches, Especially, Nur Aisyaa Azman Nur Suraya Anis Binti Zainal Abidin, Intan Nur Farisa Binti Mohd Fauzi, Nur Atigah Binti Saad, Muhammad Hafizuddin Bin Rahmat To my lab partner, Nur Aina Adlina Binti Aznan, thank you for being the friend I never knew I needed, the polar opposite of myself, for being the perfectionist to the rebellious child, for selflessly making my problems her own.

To the new-found friends I made along this path, and to everyone whom I did not have the opportunity to mention, thank you.

ABSTRACT

Acacia mangium is an important plantation species, especially for pulp and paper production, as well as wood composite products. Several pathogen species have been proven to be extremely detrimental to this species in the last 20 years, namely *Ceratocystis* sp., which is fatal in juvenile trees. The aim of this study is to isolate endophyte bacteria in healthy *A. mangium* trees, and to test these isolated endophyte bacteria species against *Ceratocystis* sp. Bacteria exhibiting positive inhibiting abilities would be helpful as a means of future biological control to restore *A. mangium* plantations against it. The roots and leaves of *Acacia mangium* seedlings and trees were sterilized and cultured on 1% Nutrient Agar medium to culture endophyte bacteria. Each bacteria sample obtained was tested against *Ceratocystis* sp. on 1% Potato Dextrose Agar medium. Endophyte bacteria which possessed *Ceratocystis*-inhibiting abilities were successfully isolated, derived mostly from primary root of healthy *Acacia mangium* seedlings. In a total of 50 endophytic bacteria samples, ten endophytic bacteria samples possessing Ceratocystis-inhibiting abilities were successfully isolated.

ABSTRAK

Acacia mangium adalah spesies perladangan penting, terutamanya untuk pengeluaran pulpa dan kertas, serta produk komposit kayu. Beberapa spesies patogen telah membuktikan sangat memudaratkan spesies pokok ini dalam tempoh 20 tahun yang lalu, terutamanya Ceratocystis sp., yang membawa maut kepada pokok-pokok juvana. Tujuan kajian ini adalah untuk mengisolasi bakteria endophyte dalam pokok A. mangium yang sihat, dan untuk menguji spesies bakteria endophyte ini terhadap *Ceratocystis* sp. Bakteria yang mempamerkan kebolehan menghalang yang positif akan membantu sebagai satu cara kawalan biologi masa depan untuk memulihkan ladang A. mangium terhadapnya. Akar dan daun dari pokok matang dan juga anak pokok Acacia mangium telah disterilkan dan dibiakkan pada medium 1% Nutrient Agar untuk membiak bakteria endophyte. Setiap sampel bakteria yang diperolehi, diuji terhadap Ceratocystis sp. pada medium 1% Potato Dextrose Agar. Bakteria endophyte yang memiliki kebolehan menghalang Ceratocystis berjaya diasingkan, dengan kebanyakannya berasal dari akar tunjang A. mangium yang sihat. Dalam jumlah 50 sampel bakteria endophyte, hanya sepuluh sampel berjaya menghalang pertumbuhan Ceratocystis sp.

ACKNOWLEDGEMENTS

I would like to take this opportunity to express my utmost gratitude and thanks to my supervisor, Dr Razak Terhem, whom without his guidance, patience and knowledge, I would not have succeeded this far.

I would also like to convey my appreciation to Assoc. Prof. Dr. Rozi for allowing me to utilise her laboratory, also to Dr Lee, Kak Aida, Kak Farah, Kak Imah, and Encik Syahmi from the Faculty of Forestry's Forest Biotechnology Laboratory for supervising and aiding me in carrying out critical steps in this study. I would also like to thank Kak Fatimah from the Faculty of Forestry's Wood Deterioration and Treatment Laboratory, for supervising me during my utilisation of the Wood Deterioration Laboratory.

My sincerest gratitude goes to FRIM's Mycology and Pathology Department, namely Dr Mohd Farid Ahmad, Cik Siti Nuratikah Binti Derman and most importantly Encik Ahmad Syazwan Bin Samsuddin for sacrificing precious time and energy into teaching and sharing their knowledge.

Not forgetting, my parents, siblings, and closest friends for their help throughout this whole study, especially Nur Aisyaa Azman, Nur Suraya Anis, Intan Nur Farisa, Nur Atiqah Saad, Muhammad Hafizuddin Raja Nazrin, Muhamad Lutfi and last but not least, my partner throughout this study, Nur Aina Adlina.

iv

APPROVAL SHEET

I certify that this research project entitled "Isolation of *Ceratocystis*-Inhibiting Bacteria in *Acacia mangium*" by Natasha Deanna Chan Binti Jeffrey Chan has been examined and approved as a partial fulfilment of the requirements for the Degree of Bachelor of Forestry Science in the Faculty of Forestry, Universiti Putra Malaysia.



Dr. Razak Terhem Faculty of Forestry Universiti Putra Malaysia (Supervisor)

Prof. Dr. Mohamed Zakaria Bin Hussin Dean Faculty of Forestry Universiti Putra Malaysia

Date: 8 January 2018

TABLE OF CONTENTS

ABST ABST AKNO APPR LIST LIST	ICATION TRACT TRAK OWLEDGEMENTS ROVAL SHEET OF TABLES OF FIGURES OF ABBREVIATIONS	PAGE i iii iv v vii viii ix
CHAF 1	PTER INTRODUCTION 1.1 Background 1.2 Justification/Problem Statement 1.3 Objective	1 3 5 3
2	LITERATURE REVIEW 2.1 Acacia mangium 2.2 Ceratocystis sp.	4 6
3	 METHODS AND MATERIALS 3.1 Selection of Material 3.1.1 Sources of Fungal Isolates 3.1.2 Selection of <i>A. mangium</i> parts for the study 3.2 Sterilization 3.3 Culturing of Endophyte Bacteria 3.3.1 Culturing of selected <i>A. mangium</i> parts on medium 3.3.2 Isolation of bacteria 3.4 Antagonistic testing 	7 7 7 8 8 8 8
4	 RESULTS AND DISCUSSION 4.1 Summary 4.2 Diversity and Distribution of Endophytic Bacteria in <i>A. mangium</i> 4.3 Antagonistic activity of Isolated Endophytic Bacteria 4.3.1 Antagonistic activity against <i>Ceratocystis</i> sp. 4.3.2 Antagonistic activity against fungal isolate 8.1 	11 12 18 24 27
5 CC	ONCLUSION AND RECOMMENDATIONS	31
REFE	ERENCES	33
APPE	ENDICES	35

LIST OF TABLES

TABLE		
4.1	The distribution of endophytic bacteria derived from healthy <i>A. mangium</i> parts	12
4.2	Observation of selected A. mangium parts cultured on NA	14
4.3	Observation of selected A. mangium roots cultured on PDA	17
4.4	Antagonistic activity of endophytic bacteria derived from <i>A.</i> mangium against fungal isolates	23
4.5	Antagonistic effect of bacterial samples against <i>Ceratocystis</i> sp. (FRIM 1162) in dual culture test	25
4.6	Antagonistic effect of bacterial samples against fungal isolate 8.1 in dual culture test	28

 \bigcirc

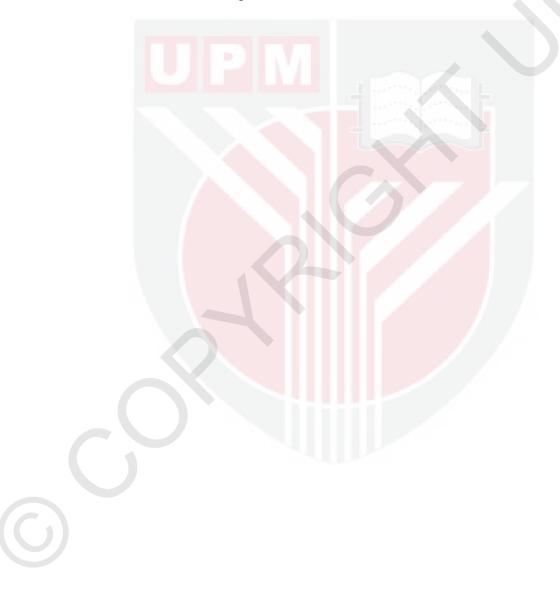
LIST OF FIGURES

FIG	URE	PAGE
3.1	The fungi shown to be inhibited by bacteria in a petri dish of primary tap root of PDA	9
4.1	Endophyte bacteria with antagonistic activity against Ceratocystis sp.	18
4.2	Endophyte bacteria with antagonistic activity against fungal isolate 8.1	19
4.3	Comparison of endophyte bacteria sources and inhibition towards Ceratocystis sp. and fungal isolate 8.1 respectively	20
4.4	Distribution of endophyte bacteria showing antagonism and its overlap between Ceratocystis sp. and fungal isolate 8.1	21

viii

LIST OF ABBREVIATIONS

- NA Nutrient Agar
- PDA Potato Dextrose Agar
- FRIM Forest Research Institute of Malaysia
- PIRG Percentage Inhibition of Radial Growth



CHAPTER 1

INTRODUCTION

1.1 Background

Forest plantations in Malaysia have been going through a hard time for the past 30 years. *Pinus* spp. used to be preferred as pulp and paper plantation species, before intended as general utility timber, but was replaced with species such as Acacia mangium, Araucaria spp., Eucalyptus spp., Gmelina arborea, Maesopsis eminii and Falcatria molucca. Likewise, in Sabah, Pinus spp. was planted during Sabah Softwoods' establishment, but was halted due to slow growth and seed supply problems, and was replaced with A. mangium, F. moluccana, G. arborea and Eucalyptus deglupta. Sabah Forest Industries (SFI) was mostly dependent on hardwood timber from raw natural forest materials, at the same time also planting A. mangium and Eucalyptus spp., A. mangium appeared to be the most promising, based on its stellar performance; superior growth, multiple uses, supposedly lacking of major pest problems and less of a hassle in terms of procuring planting materials. Planted on a 15-year rotation cycle for general utility timber, A. mangium was soon dominating most areas of forest plantations under the programme called the Forest Compensatory Programme in Malaysia. No disease problems were detected during the early and immediate young phase of A. mangium, yet as they aged they turned out to susceptible to quite a few diseases. The most well-known is heart rot, however root rot and phyllode rust came to light to be even more frightening. As of this, A. mangium is no longer a

favoured plantation species in the peninsular, though it is still planted and preferred for pulp and paper production in Sabah and Sarawak.

1.2 Problem Statement

Acacia mangium is a popular plantation species as it is fast-growing, and able to adapt to soils with low fertility. However, the planting of this species is no longer feasible in view of the fast-emerging *Ceratocystis* disease, resulting in wilt canker affecting the stem and root. It is undoubtedly the most serious threat to *Acacia* growers since this species was first introduced in South East Asia 30 years ago. There are three species reportedly affecting *A. mangium*, namely *Ceratocystis adiposa*, *Ceratocystis fimbriata*, and *Ceratocystis manginecans*.

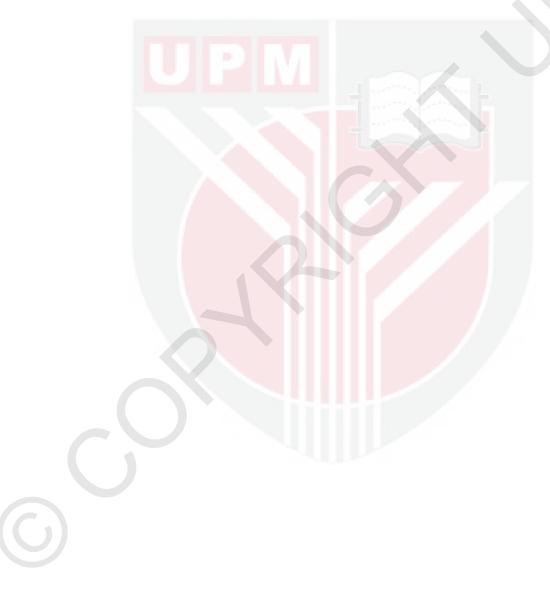
Ceratocystis disease causes wilt and die back in young *A. mangium* plants, most seriously affecting those ranging from six months to two years old. Among symptoms of plants infected with *Ceratocystis* disease are vascular disease, characterized by wilting of foliage, crown dieback, tree death, blackened stem, and patches of foamy exudate on the stem accompanied by a sour fermentation odour. Infected trees are easily recognizable by the presence of distinctive blueblack streaks and lesions under the bark.

1.3 Objective

Thus, in line with the disease affecting the productivity, therefore the objectives of the study were:

1) To isolate endophyte bacteria present in healthy Acacia mangium.

2) To isolate Ceratocystis-inhibiting bacteria in Acacia mangium



REFERENCES

Begum, M. M., Sariah, M., Abidin, Z. M. A., Puteh, A. B., & Rahman, M. A. (2008). Antagonistic potential of selected fungal and bacterial biocontrol agents against Colletotrichum truncatum of soybean seeds. *Pertanika Journal of Tropical Agricultural Science*, *31*, 45-53.

Brigalow, M., & Wattle, S. (1996). Acacia mangium Willd. Development, 19, 21.

Campanile, G., Ruscelli, A., & Luisi, N. (2007). Antagonistic activity of endophytic fungi towards Diplodia corticola assessed by in vitro and in planta tests. *European Journal of Plant Pathology*, *117*(3), 237-246.

Francis, J. K. (2002). Acacia mangium Willd. US Depart. Agr. Forest Service: Tropical Seed Manual, 256-257.

Ghai, S., Sood, S. S., & Jain, R. K. (2007). Antagonistic and antimicrobial activities of some bacterial isolates collected from soil samples. *Indian Journal of Microbiology*, *47*(1), 77-80.

Ginting, R. C. B., Sukarno, N., Widyastuti, U., Darusman, L. K., & KANAYA, S. (2013). Diversity of endophytic fungi from red ginger (Zingiber officinale Rosc.) plant and their inhibitory effect to Fusarium oxysporum plant pathogenic fungi. *HAYATI Journal of Biosciences*, *20*(3), 127-137.

Hadi, S., & Nuhamara, S. T. (1997). Diseases of species and provenances of acacias in West and South Kalimantan, Indonesia. In K. M. Old, S.S. Lee, & J. K. Sharma (Eds.), *Diseases of Tropical Acacias: Proceedings of an International workshop held at Subanjeriji, South Sumatra 28*, 23-47.

Hegde, M., Palanisamy, K., & Yi, J. S. (2013). Acacia mangium Willd.-A Fast Growing Tree for Tropical Plantation. *Journal of Forest and Environmental Science*, *29*(1), 1-14.

Ismail, F. S., Malahubban, M., Sajili, M. H., & Aziz, Z. F. A. (2016). Plant growthpromoting properties of cultivable endophytic root nodule bacterial isolates from Acacia mangium Willd. *Research in Plant Biology*, *6*, 14-18.

Jousset, A., Becker, J., Chatterjee, S., Karlovsky, P., Scheu, S., & Eisenhauer, N. (2014). Biodiversity and species identity shape the antifungal activity of bacterial communities. *Ecology*, *95*(5), 1184-1190.

Khalid, I., Wahab, R., Sulaiman, O., Mohamed, A., Tabet, T. A., & Alamjuri, R. H. (2010). Enhancing colour appearances of cultivated 15 year-old Acacia hybrid through oil heat treatment process. *International Journal of Biology*, *2*(2), 199-209.

Krisnawati, H., Kallio, M., & Kanninen, M. (2011). Acacia mangium Willd. *Ekologi, Silvikultur dan Produktivitas*.

Lee, S. S. (2005). Diseases and potential threats to *Acacia mangium* plantations in Malaysia. *Mortality*, *30*(20), 10-14.

Lee, S. S. (2017). *Ceratocystis* wilt, A threat to Malaysian forest plantations. *Penyakit Layu: Risiko dan Ancaman Terhadap Perladangan Acacia mangium di Malaysia*, 3-6.

Old, K. M., See, L. S., Sharma, J. K., & Yuan, Z. Q. (2000). A manual of diseases of tropical acacias in Australia, South-East Asia and India. CIFOR, Bogor, Indonesia.

Petatán-Sagahón, I., Anducho-Reyes, M. A., Silva-Rojas, H. V., Arana-Cuenca, A., Tellez-Jurado, A., Cárdenas-Álvarez, I. O., & Mercado-Flores, Y. (2011). Isolation of bacteria with antifungal activity against the phytopathogenic fungi Stenocarpella maydis and Stenocarpella macrospora. *International journal of molecular sciences*, *12*(9), 5522-5537.

Rahman, M. A., Begum, M. F., & Alam, M. F. (2009). Screening of Trichoderma isolates as a biological control agent against Ceratocystis paradoxa causing pineapple disease of sugarcane. *Mycobiology*, *37*(4), 277-285.

Rosenblueth, M., & Martínez-Romero, E. (2006). Bacterial endophytes and their interactions with hosts. *Molecular plant-microbe interactions*, *19*(8), 827-837.

Roux, J., Heath, R. N., Labuschagne, L., Nkuekam, G. K., & Wingfield, M. J. (2007). Occurrence of the wattle wilt pathogen, Ceratocystis albifundus on native South African trees. *Forest Pathology*, *37*(5), 292-302.

Roux, J., & Wingfield, M. J. (2009). Ceratocystis species: emerging pathogens of non-native plantation Eucalyptus and Acacia species. *Southern Forests: A Journal of Forest Science*, 71(2), 115-120.

Tarigan, M., Roux, J., Van Wyk, M., Tjahjono, B., & Wingfield, M. J. (2011). A new wilt and die-back disease of *Acacia mangium* associated with *Ceratocystis manginecans* and *C. acaciivora sp.* nov. in Indonesia. *South African Journal of Botany*, 77(2), 292-304.

Acacia mangium. (n.d). Retrieved from http://www.worldagroforestry.org/treedb/AFTPDFS/Acacia_mangium.PDF

Ceratocystis Wilt of Acacia Mearnsii. (n.d.). Retrieved from <u>http://www.fabinet.up.ac.za/src.fabinet.up.ac.za/tpcp/pamphlets/ceratocystis.pdf</u>

Ceratocystis Wilt of Acacia Mearnsii. (n.d.). Retrieved from http://www.fabinet.up.ac.za/tpcp/pdf/ceratocystis_wattle_wilt.pdf

Metcalf, H. (1912). Gummosis. *Botanical Gazette, 54*(2), 173-174. Retrieved from <u>http://www.jstor.org/stable/2468361</u>

Sabah Softwood Berhad, *Tree Estates*. (n.d.) Retrieved from <u>http://www.softwoods.com.my/ssb_t_estates.cfm</u>

