



UNIVERSITI PUTRA MALAYSIA

**IDENTIFICATION AND CHARACTERIZATION OF FUNGAL PATHOGEN
CAUSING CHARCOAL ROT DISEASE OF *Ananas comosus* IN JOHOR
AND NEGERI SEMBILAN**

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FP 2016 44

IDENTIFICATION AND CHARACTERIZATION OF FUNGAL PATHOGEN
CAUSING CHARCOAL ROT DISEASE OF *Ananas comosus* IN JOHOR AND
NEGERI SEMBILAN

BY

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A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in
fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the
degree of Bachelor of Agricultural Science

Faculty of Agriculture

Universiti Putra Malaysia

2015/2016

ACKNOWLEDGEMENT

First of all I want to show my gratitude and appreciation to all those who had gave me the strength and help through completing this final year project. Alhamdulillah, all praises to Allah S.W.T for the strength and His blessing upon on completion of this project report.

I would like to give a special appreciation to my supervisor, Dr Nusaibah Binti Hj. Syd Ali for her valuable guidance, advices, help and support throughout this project. Furthermore, my fullest gratitude to the entire laboratory staffs of Plant Protection Department that had help, co-operated and gave guidance to me especially Mrs. Asmalina Binti Abu Bakar, Mr. Johari Bin Mohd Sarikat and Mr. Mohamed Nazri Bin Abdul Rahman. Not to forget, to Ms. Muniroh binti Saad, my supervisor's post-graduate student for all her guidance and help along this project.

Moreover, my deepest gratitude and appreciation to my family especially my beloved parents, Maarof Bin Omar and Feridah Binti Perdi for their support, encouragement, understanding and endless love for me while in completing this final year project.

Sincerely thanks to all of my friends especially Alawiah Binti Daud, Siti Zubaidah Binti Mohd Zahri and Nurnashirah Binti Latif for their kindness and moral support during my study. Thanks for the encouragement, friendship and memories.

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LIST OF ABBREVIATION

| | |
|-------|---|
| AE | Extraction buffer |
| BLAST | Basic Local Alignment Search Tool |
| Bp | Basepair |
| DNA | Deoxyribonucleic acid |
| DS | Disease severity |
| DSI | Disease severity index |
| ITS | Internal Transcribed Spacer |
| LCB | Lactophenol Cotton Blue |
| MPIB | Malaysian Pineapple Industrial Board |
| MT | Metric tonnes |
| NCBI | National Center for Biotechnology Information |
| PDA | Potato Dextrose Agar |
| PCR | Polymerase Chain Reaction |
| RNA | Ribonucleic acid |
| RCBD | Randomized Complete Block Design |
| UPM | Universiti Putra Malaysia |
| UV | Ultra violet |

ABSTRACT

Ananas comosus or commonly known as pineapple is from the family *Bromeliaceae*. It is a commercial fruit cultivated in dry, well-drained tropical habitats. In Malaysia, Johor and Negeri Sembilan states are the major producers of pineapple. Charcoal rot disease is one of the most significant diseases of pineapple in Malaysia causing decrease in yield and quality of the fruit. In order to take preventive and curative measures against charcoal rot of pineapple, the identity of the causal pathogen is essential. Thus, this study was conducted with objectives of i) To isolate the causal fungal pathogen from the symptomatic *Ananas comosus* tissues and ii) To identify the isolated fungal pathogen via morphological and molecular characteristics. The fungi were isolated from symptomatic *Ananas comosus* tissues and characterized morphologically based on cultural and microscopic characteristics. 11 isolates that met the criteria of the black (charcoal rot) fungi were chosen from 46 total isolates of the total samples for further molecular identification that was carried out by extracting genomic DNA using QIAGEN DNeasy Plant Mini Kit and amplification of internal transcribed spacer (ITS) region of the DNA via ITS1 and ITS4 primer sets and finally sequenced. Later, sequences obtained were analysed using BLAST application on GenBank sequence database to achieve species identity. Finally, pathogenicity test was carried out on susceptible pineapple seedlings to reproduce similar symptoms of charcoal rot and further confirm the causal pathogen. In this study, *Lasiodiplodia theobromae* and *Neoscytalidium dimidiatum* were identified as the causal pathogen of charcoal rot disease in *Ananas comosus* because they produce the same symptoms produced by the previous isolated diseased plants.

ABSTRAK

Ananas comosus atau lebih dikenali sebagai nanas adalah daripada family *Bromeliaceae*.

Ia merupakan tanaman komersial yang ditanam di kawasan tropika yang kering dan bersaliran baik. Di Malaysia, negeri Johor dan Negeri Sembilan merupakan pengeluar nanas yang utama. Namun begitu, penyakit reput arang yang merupakan salah satu penyakit nanas yang signifikan di Malaysia yang sering mengakibatkan penurunan hasil dan kualiti pada nanas. Bagi mengambil langkah-langkah pencegahan dan penyembuhan, identity pathogen kulat penyebab penyakit reput arang adalah sangat penting. Oleh itu, kajian ini dilaksanakan dengan objektif-objektif berikut i) Untuk memencilkan patogen kulat penyebab daripada tisu-tisu pokok *Ananas comosus* yang mempamerkan simptom-simptom penyakit reput arang dan ii) Untuk mengidentifikasi pathogen kulat yang dipencilkan berdasarkan pencirian secara morfologi dan molikul. Kulat dipencilkan daripada sampel-sampel tisu yang mempamerkan simptom-simptom penyakit reput arang nanas dan seterusnya dikaji secara morfologi berdasarkan ciri-ciri kultur dan mikroskopik. 11 isolat yang menunjukkan ciri-ciri kulat hitam reput arang dipilih daripada keseluruhan 46 isolat yang ada untuk pencirian secara molikul dijalankan dengan mengekstrak DNA genomik menggunakan Kit Mini Tumbuhan DNeasy QIAGEN dan DNA tersebut diamplifikasi menggunakan set pencetus ITS1 dan ITS4 dan akhirnya produk PCR dihantar untuk proses penjujukan DNA. Jujukan nukleotida setiap isolat kulat yang diperolehi dianalisis menggunakan aplikasi BLAST pada pangkalan data jujukan di GenBank untuk mengenalpasti identiti spesies. Akhirnya, ujian kepatogen dijalankan keatas anak pokok nanas yang tidak rentan bagi menghasilkan semula simptom-simptom

penyakit reput arang serta mengesahkan identiti patogen kulat penyebab. Dalam kajian ini, kulat *Lasiodiplodia theobromae* dan *Neoscytalidium dimidiatum* telah dikenalpasti sebagai patogen penyebab penyakit reput arang pada pokok *Ananas comosus* kerana mereka telah menghasilkan simptom-simptom yang sama sebagaimana pokok nanas yang dijangkiti penyakit reput arang yang dipencilkan.



CHAPTER 1

INTRODUCTION

Ananas comosus is the scientific name of pineapple. It is a perennial crop that has successor plant from the initial plant. Pineapple is a very important industrial fruit to certain countries due to its high export value and demand. Hossain *et al.* (2015) reported that, request for pineapple in the world market is increasing daily. Ripen pineapple fruit is consumed fresh or either as juice. Nevertheless, there are numerous food items produced from pineapple such as jam, jelly and pickles. It is also a well-known source of essential minerals and vitamins with some medicinal values when consumed. Pineapple contains vitamin C, fiber, significant amount of calcium, and potassium. Other than that, pineapples were also used as animal feed and as an ornamental value plant (Joy, 2010). These plants' leaves were also used as a source of fiber and in clothing industry in many countries. Pineapple fruit differ in the quality depending on the growing environment and variety (Hossain *et al.*, 2015).

Malaysia as one of the pineapple producer in Asia exports fresh pineapples to its biggest importer namely China followed by Singapore and Middle East countries which are now Malaysia's main export markets to cope with the demand which has been increasing yearly (MPIB, 2014). In relation to that, Malaysia plans to increase the existing 15,000ha of areas planted with pineapples to 24,000ha by the year 2020 (MPIB, 2014). These show that pineapple is an important commercial fruit crop in Asia.

Diseases in pineapple plant which includes pre and postharvest diseases cause a drop in production scale. This fruit is very prone to diseases. As a way of preventing the disease in pineapples, more research must be done thoroughly to identify the symptoms and causal agents of these diseases in order to avoid more losses in pineapple production. One of the common disease of pineapple in Malaysia is the charcoal rots disease that infects the plant during fruit maturing stage which is the most important stage in pineapple production.

To date, no research has been conducted to study charcoal rot disease in *Ananas comosus* particularly in Peninsular Malaysia. It is very crucial to know the identity of the causal pathogen for curative and preventive measures of charcoal rot disease. Charcoal rot of *Ananas comosus* is caused by a black fungus with the presence of sclerotia on the infected tissues of the plant especially on or near the plant's stem. Charcoal rot disease in pineapple results in low quality fruits as it will become much more worst in postharvest handling if precautionary steps is not taken during pre-harvest stage.

Thus the objectives of this present study were as follows:

1. To isolate causal pathogen from the symptomatic *Ananas comosus*
2. To characterize and identify the isolated causal pathogen

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