



UNIVERSITI PUTRA MALAYSIA

PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF *Pantoea stewartii* SUBSPECIES *stewartii* CAUSING BRONZING DISEASE OF JACKFRUIT (*Artocarpus heterophyllus*) IN MARAN REGION

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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

Department of Plant Protection
Faculty of Agriculture
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This project report entitle Phenotypic and Molecular Characterization of *Pantoea Stewartii* Subspecies *Stewartii* Causing Bronzing Disease of Jackfruit (*Artocarpus Heterophyllus*) in Maran Region is prepared by Muzainah Binti Mohd Zakiyuddin and submitted to the Faculty of Agriculture in fulfilment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science.

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LIST OF ABBREVIATIONS

%	percent
°C	degree celcius
Bp	base pair
CABI	Commonwealth Agricultural Bureaux International
DNA	deoxyribonucleic acid
DOA	Department of Agriculture
EDTA	ethylene-diamine-tetraacetic acid
FAO	Food and Agriculture Organization
g	gram
h	hour
kb	kilobase pair
L	liter
M	molar
Mb	megabase pair
Min	minutes
ml	milliliter
mm	milimeter
mM	milimolar
ng	nanogram
nm	nanometer
PCR	polymerase chain reaction
rpm	rotation per minute

sec	seconds
TAE	tris-acetic EDTA
Taq	<i>Thermus aquaticus</i>
TM	melting temperature
U	unit
UV	ultra-violet
V	voltan/volt
x g	gravity force
μg	microgram
μg/ml	microgram per milliliter
μl	microliter
μM	micromolar
μm	micrometer



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Abstract

Jackfruit (*Artocarpus heterophyllus*) is one of the most widely grown fruit crop in Malaysia. Under the Third National Agricultural Policy, it has been recognized as having the potential to meet domestic demand, especially for export and as a replacement for imports. Fruit bronzing disease caused by the bacterium *Pantoea stewartii* subspecies *stewartii* (*P. stewartii* subsp. *stewartii*) is frequently found in jackfruit, which eventually leads to production loss. The disease is characterized by yellowish- orange to reddish discoloration of the affected pulps and rags in the fruit. Once infected, the fruit quality is compromised and could discourage the consumers and processors. The objectives of this study are; 1) to isolate and identify *P. stewartii* subsp. *stewartii* in local jackfruit varieties using phenotypic characterization and 2) to determine the genetic relationship of *P. stewartii* subsp. *stewartii* strains isolated via molecular identification and phylogenetic analyses. In January 2017, symptoms of infected jackfruit with bronzing were observed in Ladang Taman Kekal Pengeluaran Makanan (TKPM) Serengkam, Maran Pahang region. To identify the pathogen, 8 disease fruits resembled bronzing symptom were collected from the field by randomly to the disease, excised and cultured onto nutrient agar (NA) medium and incubated overnight at 35⁰C. Two representative bacteria colonies were obtained

and selected for further characterization. Bacteria colonies were yellow-pigment, round, smooth and translucent. All strains were Gram-negative rod-shaped, positive for Kovac oxidase and catalase tests and negative for potassium hydroxide (KOH) reaction. These are characteristics of *Pantoea* spp. For the pathogenicity test, 10 ml of 10⁸ CFU/ml bacterial suspension of four selected strains were injected into healthy detached jackfruits of J33 (Tekam Yellow) variety. Fruits that were infiltrated with sterile distilled water served for negative control. Inoculations with all strains were performed in two replications, as well as the uninoculated control detached fruits. The inoculated fruits produced bronzing symptoms as observed on naturally diseased samples. Besides that, control fruits remained asymptomatic. Strain culture were re-isolated and showed the morphological and biochemical characteristics as previously described. The PCR amplification of 16S rDNA 8F and 1492R primer set produced a 1400 bp amplicon in each strain. To our knowledge, this is the first report of bronzing disease caused by *P. stewartii* subsp. *stewartii* in jackfruit in Malaysia.

**FENOTIP DAN PENCIRIAN MOLECULAR KE ATAS *Pantoea stewartii*
SUBSPECIES *stewartii* YANG MENYEBABKAN PENYAKIT
PEMERANGAN DALAM NANGKA (*Artocarpus heterophyllus*) DI DAERAH
MARAN**

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Abstrak

Nangka (*Artocarpus heterophyllus*) adalah salah satu tanaman buah-buahan yang popular dan paling banyak ditanam di Malaysia. Di bawah Dasar Pertanian Negara Ketiga, nangka telah diiktiraf sebagai tanaman yang mempunyai potensi untuk memenuhi permintaan dalam negeri, untuk eksport dan sebagai pengganti import. Walaubagaimanapun, satu penyakit yang dikenali pemerangan ataupun karat buah telah ditemui di dalam nangka. Penyakit ini disebabkan oleh *Pantoea stewartii* (*P. stewartii*), dimana ia mempunyai ciri-ciri seperti berwarna kuning-oren kemerahan yang telah menyerang pada bahagian isi dan jerami didalam buah nangka yang menyebabkan nangka mengalami kerugian yang besar. Apabila buah nangka dijangkiti, ia boleh menjejaskan kualiti buah nangka dan penawaran terhadap buah nangka oleh pengguna dan pemproses. Objektif kajian ini ialah; 1) untuk mengasingkan dan mengenalpasti *P. stewartii* subsp. *stewartii* pada varieti buah nangka tempatan dengan menggunakan ciri-ciri fenotip dan 2) untuk mengenalpasti hubungan genetik strain *P. stewartii* subsp. *stewartii* strains melalui pengenalan

molekul dan analisis filogenetik. Pada Januari 2017, gejala pemerangan dalam nangka telah diperhatikan di Ladang Taman Kekal Pengeluaran Makanan (TKPM) Serenggam, Maran Pahang. Untuk mengenalpasti patogen, sebanyak 8 biji nangka yang mempunyai penyakit yang menyerupai gejala pemerangan diambil secara rawak, dipotong dan dibiakkan ke media nutrient agar (NA) dan dieram semalaman pada 35⁰C. Sebanyak dua koloni bakteria diperolehi dan dipilih untuk pencirian lanjutan. Koloni bakteria berwarna kuning-berpigmen, berbentuk bulat, licin dan jernih. Kesemua strain adalah Gram-negatif dengan berbentuk rod pendek, positif untuk Kovac xidase dan catalase, dan negatif untuk tindak balas kalium hidroksida (KOH). Berikut adalah ciri *Pantoea* spp. untuk ujian kepatogenan, 10 ml of 10⁸ CFU/ml empat jenis yang telah dipilih disuntik ke dalam nangka sihat jenis J33 (Tekam Yellow). Manakala, buah yang digunakan sebagai kawalan negatif telah disuntik dengan air suling yang telah di steril. Inokulasi terhadap semua strain telah dijalankan dalam dua replikasi, serta satu inokulasi buah kawalan. Buah yang telah disuntik menghasilkan gejala pemerangan sebagaimana yang berlaku ke atas sampel yang berpenyakit secara semula jadi. Kultur strain telah diasingkan semula dan menunjukkan ciri morfologi dan biokimia seperti yang telah dinyatakan sebelum ini. PCR amplikasi 16S rDNA 8F dan 1492R menghasilkan 1400bp amplicon dalam setiap strain. Ini merupakan laporan yang pertama, penyakit pemerangan disebabkan oleh *P. stewartii* subsp. *stewartii* pada nangka di Malaysia.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Jackfruit (*Artocarpus heterophyllus*) is one of the popular fruits which belong to the family of Moraceae (Haq, 2006). It's originated from southwest India and spreads in ancient times throughout Southeast Asia and then to tropical Africa (Love and Paull, 2011). Jackfruit was introduced to Malaysia from Indian in 1877 (MOA, 1995). In Malaysia, the planted areas for jackfruit have increased from the year 2009. In 2013, the production of jackfruit increase to 33,907.4 tonnes with the planted areas is 4,902.9 hectare (DOA, 2015).

There are numerous diseases of jackfruit and one of them is fruit bronzing. Fruit bronzing disease can give impact to production of jackfruit because this disease attacks the fruit area such as pulps and rags. The symptoms can be seen when the fruits are opened. When this disease attacks the jackfruit, the quality of fruit reduced as well as the production (Gapasin *et al.*, 2014).

1.2 Statement of the Problem

Fruit bronzing disease is a current major problem that leads to loss of production. Once infected, the quality of fruit is compromised and could discourage the consumers and processors. Jackfruit was recognized as having the potential to meet domestic demand under the Third National Agriculture Policy by Malaysia government, circumstances occurrence of this disease problem have been the most important and major constraint to the production of jackfruit since it could discourage to consumers and processors (DOA, 2013;Gapasin *et al.*, 2014). A locally named jackfruit bronzing disease cause by *Pantoea sterwatii* subspecies *sterwatii* is yet unreported and unstudied (Gapasin *et al.*, 2014).

1.3 Significant of the Study

Artocarpus heterophyllus or commonly known as jackfruit is one of the importance economic- driven fruit crops in Malaysia especially for local and export markets .Views from the point, none of the disease have been well documented in Malaysia. The result of this study will be an important documentation of bronzing disease of jackfruit in Maran Pahang. Considering this matter, our study would be a major platform on generating details documentation of bronzing disease and its causal pathogen *Pantoea stewartii* in jackfruit crop in Malaysia, by using combination of phenotypic characterization and molecular phylogenomics approaches.

1.4 Objective of the Study

Our study was carried out with the following objectives:

1. To isolate and identify *P. stewartii* subsp. *stewartii* in local jackfruit varieties using phenotypic characterization.
2. To determine the genetic relationship of *P. stewartii* subsp. *stewartii* strains isolated via molecular identification and phylogenetic analyses.

The output from this research perhaps may improve and increase efficiency in the development of accurate molecular diagnostic test for detection and identification of *P. stewartii* subsp. *stewartii*, thus bettering the jackfruit industry in Malaysia.

REFERENCES

- Ahmed, N.N., Islam, M., Hossain, M.A., Meah, M.B. and Hossain, M.M. (2013).
Determination of Races and Biovars of *Ralstonia solanacearum* Causing
Bacterial Wilt Disease of Potato. *Journal of Agricultural Science*, 5: 86-93.
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. (1990). Basic
local alignment search tool. *Journal of Molecular Biology*, 215: 403-410.
- Azad, H. R., Holmes, G. J. and Cooksey, D. A. (2000). A new leaf blotch disease of
sudangrass caused by *Pantoea ananas* and *Pantoea stewartii*. *Plant Disease*,
84(9): 973-979.
- Baliga, M. S., Shivashankara, A. R., Haniadka, R., Dsouza, J. and Bhat, H. P. (2011).
Phytochemistry, nutritional and pharmacological properties of *Artocarpus*
heterophyllus Lam (jackfruit): A review. *Food Research International*, 44(7):
1800-1811.
- Brady, C., Cleenwerck, I., Venter, S., Vancanneyt, M., Swings, J. and Coutinho, T.
(2008). Phylogeny and identification of *Pantoea* species associated with
plants, humans and the natural environment based on multilocus sequence
analysis MLSA. *Systematic and Applied Microbiology*, 31(6): 447-460.

Burbank, L. (2014). Global Regulation of Virulence Determinants During Plant Colonization in the Bacterial Phytopathogen, *Pantoea stewartii* subsp. *stewartii*.

CABI. (2013). Towards improved market access for ASEAN agricultural commodities: final technical report, November 2009 – May 2013, CAB International Southeast Asia (CABI-SEA), Selangor, MY.

Chaudhry, Z. and Rashid, H. (2011). Isolation and Characterization of *Ralstonia solanacearum* from Infected Tomato Plants of Soan Skesar Valley of Punjab. *Pakistan Journal of Botany*, 43: 2979-2985.

Coplin, D. L., Majerczak, D. R., Zhang, Y., Kim, W. S., Jock, S. and Geider, K. (2002). Identification of *Pantoea stewartii* subsp. *stewartii* by PCR and strain differentiation by PFGE. *Plant Disease*, 86 (3): 304-311.

Correa, V. R. (2010). Functional genomics of *Pantoea stewartii* subsp. *stewartii* and partial genome sequence of the maize stolbur *Phytoplasma solani*, two insecttransmitted bacterial pathogens of maize (Doctoral dissertation, The Ohio State University).

Correa, V. R., Majerczak, D. R., Ammar, E. D., Merighi, M., Pratt, R. C., Hogenhout, S.

A., ... and Redinbaugh, M. G. (2012). The bacterium *Pantoea stewartii* uses two

different type III secretion systems to colonize its plant host and insect vector.

Applied and Environmental Microbiology, 78 (17): 6327-6336.

Corner, E.J.H. (1938) Notes on the systematics and distribution of

Malayan phanerogams II. The jack and the chempedak. *Gardener. Bull.*

10:56-81.

Department of Agriculture Malaysia. (2013). *Fruit Crop Statistics 2013*.

Department of Agriculture Malaysia. (2015). *Fruit Crop Statistics 2015*.

Drummond, A.J. and Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by

sampling trees. *BMC Evolutionary Biology*, 7: 214.

EPPO/CABI. (2004). *Data Sheets on Quarantine Pests Ralstonia solanacearum*.

Prepared by CABI and EPPO for the EU under Contract 90/399003.

EPPO/CABI. (2006) EPPO Diagnostic Standard PM 7/77 (1). Documentation and

reporting on a diagnosis. *Bulletin OEPP/EPPO Bulletin* **36**, 459–460.

Elevitch C.R., & Manner H.I. (2006). Species profiles for Pacific Island agroforestry.

Retrieved from <http://www.traditionaltree.org>

Fahy, P.C. and Hayward, A.C. (1983). Media and methods for isolation and diagnostic tests.

Farmers' Organization Authority. (2011). Differences between Mantin (J32), Tekam Yellow (J33) and Mastura (J35).

Gapasin, R. M., Garcia, R. P., Advincula, C. T., De la Cruz, C. S. and Borines, L. M. (2014). Fruit Bronzing: a New Disease Affecting Jackfruit Caused by *Pantoea stewartii* (Smith) Mergaert et al. *Annals of Tropical Research*, 36(1): 17-31.

Gerry McMahon, Senior Technical Officer, DPIFM Darwin (2003). Fact Sheet FF7 Jackfruit:1-2.

Gouy, M., Guindon, S. and Gascuel, O. (2010). SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution*, 27: 221-224.

Gram, C. (1884). The differential staining of Schizomycetes in tissue sections and in dried preparations. *Fortschritte der Medizin*, 2: 185-9.

Haq, N. (2006). *Jacfruit: Artocarpus heterophyllus*. Southampton Centre for Underutilised Crops, University of Southampton, Southampton: UK.

He, L.Y., Sequeira, L. and Kelman, A. (1983). Characteristics of strains of *Pseudomonas solanacearum* from China. *Plant Disease*, 67: 1357-1361.

Halebian, S et al. 1981. Rapid Method That Aids in Distinguishing Gram-Positive from Gram-Negative Anaerobic Bacteria. *J Clin Microbiol.* 13(3):444-448.

<http://www.moa.gov.my/web/guest/buah-buahan> (Date of accessed: 10th November 2015).

Isenberg, H.D. (1992). *Clinical microbiology procedures handbook*. American Society Microbiology, 1992.

Ismail, N. and Kaur, B. (2013). Consumer Preference for Jackfruit Varieties in Malaysia. *Journal of Agribusiness Marketing*, 6: 37-51.

Katoh, K., Misawa, K., Kuma, K.I. and Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, 30: 3059-3066.

Kovacs, N. (1956). Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature*, 178: 703.

Larkin M.A. Blackshields G., Brown N.P., Chenna R., McGettigan P.A., McWilliam H., Valentin F., Wallace I.M., Wilm A., Lopez R., Thompson 127 J.D., Gibson T.J.

and Higgins D.G. (2007). ClustalW and ClustalX version 2. *Bioinformatics*, 23: 2947-2948.

Little, E. L., & Wadsworth F. H. (1964). Common Trees of Puerto Rico and the Virgin Islands, Agriculture Handbook No. 249. Washington, D.C.: U.S. Department of Agriculture, Forest Service.

Love, K. and Paull, R. E. (2011). Jackfruit. Published by the College of Tropical Agriculture and Human Resources (CTAHR) and issued in furtherance of Cooperative Extension work, Acts of May 8 and June 30, 1914, in cooperation with the U.S. Department of Agriculture.

Milan, A. R., Valavi, S. G., Peter, K. V. and Thottappilly, G. (2011). Jackfruit in Malaysia. *The jackfruit*, 445-462.

Ministry of Agriculture and Agro-based Industry. (2011). National Agro-Food Policy (2011-2020), Ministry of Agriculture and Agro-based Industry, Kuala Lumpur.

Ministry of Agriculture Malaysia (1999). Third National Agricultural Policy (1998-2010) Executive Summary. Ministry of Agriculture, Kuala Lumpur.

Mohammadi, M., Burbank, L. and Roper, M. C. (2012). *Pantoea stewartii* subsp. *stewartii* produces an endoglucanase that is required for full virulence in sweet corn. *Molecular Plant-Microbe Interactions*, 25 (4): 463-470.

Morales-Valenzuela, G., Silva-Rojas, H. V., Ochoa-Martínez, D., Valadez-Moctezuma, E., Alarcon-Zuniga, B., Zelaya-Molina, L. X., ... and Farfán-Gómez, A. (2007). First report of *Pantoea agglomerans* causing leaf blight and vascular wilt in maize and Sorghum in Mexico. *Plant Disease*, 91(10): 1365-1365.

Morton, J.F. (1987) Fruits of Warm Climates. Creative Resources System, Inc., Winterville, North Carolina, USA: 58-64.

Morton, J.F. (1965) The jackfruit (*Artocarpus heterophyllus*). Its culture varieties and utilization. *Proceedings of the Florida State Horticultural Science Society* 78:336-344.

Orio, A. A., Brücher, E., Plazas, M. C., Sayago, P., Guerra, F., De Rossi, R., ... and Guerra, G. D. (2012). First report of Stewart's Wilt of maize in Argentina caused by *Pantoea stewartii*. *Plant Disease*, 96 (12): 1819-1819.

Orwa, C.; Mutua, A.; Kindt, R.; Jamnadass, R.; Anthony, S. (2009). Agroforestry Database: a tree reference and selection guide version 4.0. World Agroforestry Centre, Kenya.

Pantoea stewartii subsp. *stewartii*. (2006). *Bulletin OEPP/EPPO Bulletin* 36: 111–115.

Rambaut, A. and Drummond, A. (2009). FigTree v1.4.
(<http://tree.bio.ed.ac.uk/software/figtree/>).

Ronquist, F. and Huelsenbeck, J.P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19: 1572-1574.

Roper, M. C. (2011). *Pantoea stewartii* subsp. *stewartii*: lessons learned from a xylem-dwelling pathogen of sweet corn. *Molecular Plant Pathology*, 12 (7): 628-637.

Schaad, N.W., Jones, J.B. and Chun, W. (2001). Laboratory Guide for Identification of Plant Pathogenic Bacteria. Minnesota, USA, APS Press.

Sidhu, A. S. (2012). Jackfruit Improvement in the Asia-Pacific Region–A Status Report. Asia-Pacific Association of Agricultural Research Institutions, Bangkok, Thailand. APAARI.

Sneath P.H.A., Sokal R.R. (1973) Numerical Taxonomy, W.H. & Freeman, San Francisco.

Suslow, T.V., Schroth, M.N. and Isaka, M. (1982). Application of a rapid method for Gram differentiation of plant pathogenic and saprophytic bacteria without staining. *Phytopathology*, 72: 917-918.