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CHARACTERIZATION OF Pantoea SPECIES THAT CAUSES LEAF BLIGHT DISEASE OF RICE IN PENINSULAR MALAYSIA

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CHARACTERIZATION OF *Pantoea* SPECIES THAT CAUSES LEAF BLIGHT DISEASE OF RICE IN PENINSULAR MALAYSIA

By

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CHARACTERIZATION OF *Pantoea* SPECIES THAT CAUSES LEAF BLIGHT DISEASE OF RICE IN PENINSULAR MALAYSIA

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Bacterial leaf blight (BLB) disease is a major constraint in rice cultivation as it reduces yield and quality of grains, hence affecting the economic development of rice production in Malaysia. The disease symptomatic phase exhibited as yellowing color and water soaking from the leaf tip, which later turned into brown stripes. This study was conducted to isolate and characterize *Pantoea* species (*Pantoea* spp.) associated with leaf blight disease of rice in Peninsular Malaysia via phenotypic and molecular approaches. Diseased plants exhibiting blight symptoms were collected from rice fields in Kedah and Selangor. Thirty isolates were characterized via phenotypic, pathogenicity test and molecular approaches. Based on the phenotypic characteristics, all isolated bacteria were Gram-negative facultative anaerobes, motile, positive catalase and KOH, indole positive, incapable of producing hydrosulfuric acid and positive for utilization of carbohyrdates. Colonies were round, smooth with irregular edges, and produced yellow pigment on nutrient agar and King's B agar medium. Pathogenicity test revealed that *Pantoea* species isolates resembled blight symptoms as originally found in the rice fields, and were later confirmed as *Pantoea ananatis* and *Pantoea stewartii* subspecies *indologenes* using *gyrB* and *galE* genes amplification respectively. Statistical analysis revealed that the MR220 CL1 and MR269 rice varieties were highly susceptible to *Pantoea* spp., except for the PA3 isolate which was susceptible only to MR269 rice variety. Based on AUDPC result, MR220 CL1 rice variety was more susceptible towards *Pantoea* spp. compared to MR269 rice variety. All strains were identified based on the *gyrB* sequence of *Pantoea* spp. genome analyses. Fragment of the *gyrB* gene sequence was amplified with

primers PANsp_gyrB-F and PANsp_gyrB-R and BLASTn analysis showed 95 to 99% identical to reference strains in the GenBank database. A phylogenetic analysis constructed using MEGA 7 software of the *gyrB* gene sequence displaying two clusters comprising 22 strains into *P. ananatis* (cluster I), and eight strains into *P. stewartii* (cluster II) in GenBank database with 96% and 99% bootstrap value respectively. The amplification of the *galE* gene revealed that all *P. stewartii* strains were confirmed as *P. stewartii* subspecies *indologenes* with 99% identical to reference strain in the Genbank database. These findings may provide insights into genetic studies and significant documentation of *Pantoea* spp. associated with leaf blight disease of rice in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Sarjana Sains

PENCIRIAN KE ATAS *Pantoea* SPESIES YANG MENYEBABKAN PENYAKIT HAWAR DAUN PADA PADI DI SEMENANJUNG MALAYSIA

Oleh

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Penyakit bakteria hawar daun (BLB) adalah kekangan utama dalam penanaman padi kerana ia merugikan kualiti pengeluaran beras, dengan itu menjejaskan pembangunan ekonomi pengeluaran padi di Malaysia. Penyakit ini biasanya diketahui disebabkan oleh *Xanthomonas oryzae* pv. *oryzae*. Penyakit ini biasanya dipamerkan sebagai warna kuning dan terdapat lecuh basah yang terdapat dari bahagian atas bilah daun dan kemudian bertukar menjadi jalur coklat. Kajian ini dijalankan untuk memencilkan dan mencirikan spesies *Pantoea* yang dikaitkan dengan penyakit daun padi di Semenanjung Malaysia melalui kaedah fenotip dan pendekatan molekular. Aktiviti persampelan telah dijalankan di kawasan sawah yang terletak di Kedah dan Selangor yang menunjukan simptom penyakit hawar daun yang teruk. Tiga puluh pencilan dicirikan melalui fenotip termasuk ujian kepatogenan dan pendekatan molekular. Koloni bakteria dipencilkan pada agar nutrien dan disubkulturkan pada medium agar King's B dan dieram selama 48 jam pada suhu 28°C. Berdasarkan ciri-ciri fenotip, semua bakteria yang diasingkan adalah anaerobes Gram-negatif fakultatif, motil, positif ujian katalase dan KOH, positif indol, tidak berupaya untuk menghasilkan asid hidrosulfurik, dan positif dalam ujian penggunaan karbohidrat. Koloni berbentuk bulat, licin dengan corak tepi yang tidak teratur dan menghasilkan pigmen kuning pada medium agar nutrien dan agar King's B. Ujian kepatogenan mendedahkan bahawa *Pantoea* spp. adalah virulen dan menunjukkan simptom hawar daun yang sama seperti yang dijumpai di sawah padi dan disahkan sebagai *Pantoea ananatis* dan *Pantoea stewartii* subspesies *indologenes* menggunakan teknik amplifikasi gen *gyrB* dan *galE*. Analisis statistik mendedahkan varieti MR220 CL1 dan MR269 yang sangat rentan kepada *Pantoea* spp. kecuali pencilan PA3 yang hanya menyebabkan kerentanan pada varieti MR269. Kesemua pencilan telah dikenal pasti berdasarkan jujukan *gyrB* daripada *Pantoea* spp. genom analisis. Cebisan jujukan *gyrB* gen telah diamplifikasikan dengan menggunakan primers PANsp_gyrB-F dan PANsp_gyrB-R dan BLASTn analisis menunjukkan 96% ke 99% persamaan

dengan strain rujukan di dalam pangkalan data GenBank. Satu analisis filogenetik dibina menggunakan perisian MEGA 7 berdasarkan jujukan *gyrB*, mendedahkan dua kluster yang terdiri daripada 22 strain ke dalam kluster *P. ananatis* (Kluster I) dan lapan strain ke dalam kluster *P. stewartii* (kluster II) dengan 96 ke 99% nilai bootstrap masing-masing. Jujukan *galE* gen mendedahkan bahawa kesemua *P. stewartii* strain adalah disahkan sebagai *P. stewartii* subspesies *indologenes* dengan 99% persamaan dengan strain rujukan di dalam pangkalan GenBank. Penemuan ini boleh memberi maklumat kepada kajian genetik dan dokumentasi penting *Pantoea* spp. yang dikaitkan dengan penyakit hawar daun pada tanaman padi di Malaysia.

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%	percent
°C	degree celcius
AUDPC	Area Under the Disease Progress Curve
BLB	Bacterial leaf blight
CFU	Colony forming unit
bp	base pair
DBT	The Department of Biotechnology
DNA	deoxyribonucleic acid
DOA	Department of Agriculture
DAI	Day after inoculation
DS	Disease severity
EDTA	ethylene-diamine-tetraacetic acid
FAO	Food and Agriculture Organization
g	gram
h	hour
IRRI	International Rice Research Institute
kb	kilobase pair
L	Litre
M	Molar
Mb	Megabase pair
min	minutes
ml	millilitre
mm	millimetre
mM	millimolar
MoEF&CC	Ministry of Environment, Forest and Climate Change
ng	nanogram
nm	nanometer
PCR	Polymerase Chain Reaction
rpm	rotation per minute
sec	seconds
TAE	Tris-acetic EDTA
Taq	*Thermas aquaticus*
TM	melting temperature
U	unit
USDA	United States Department of Agriculture
UV	ultra-violte
V	voltan/volt
µg	microgram
µg/ml	microgram per mililiter
µM	micromolar
µm	micrometer

CHAPTER 1

INTRODUCTION

Bacterial leaf blight (BLB) disease is typically known caused by *Xanthomonas oryzae pv. oryzae* (*Xoo*). *Xoo* is the oldest bacterial of rice in Asia (Naqvi *et al.,* 2014) and the most severe bacteria in many rice crops in the world (Xu *et al.,* 2010). The yield losses of 10%-20% in moderate conditions and highly losses up to 50% recorded in several Asian and Southeast Asian countries (Mew *et al.,* 1993; Kala *et al.,* 2015). However, BLB also is known to be caused by *Pantoea* species (*Pantoea* spp.) which exhibited similar symptoms as blight disease caused by *Xoo* (Lee *et al.,* 2010; Mondal *et al.,* 2011; Kini *et al.,* 2017). The genus of *Pantoea* has been a major concern as the causal agent of BLB disease of rice in Korea (Lee *et al.,* 2010), India (Mondal *et al.,* 2011), Venezuela (Gonzalez *et al.,* 2015), Benin and Togo (Kini *et al.,* 2017a,b) and Southern Districts of Tamil Nadu (Vinodhini *et al.,* 2017).

Pantoea spp. has been acknowledged as phytopathogenic bacteria associated with plant hosts, including cotton, maize, melon and onion (Walcott *et al.,* 2002; Medrano and Bell, 2007; Kido *et al.,* 2008; Brady *et al.,* 2011). The genus of *Pantoea* also causing devastation to rice crops production worldwide, leading to significant losses in rice productivity and quality. The most well known *Pantoea* spp. are *P. ananatis, P. agglomerans* and *P. stewartii* which causes several diseases on rice such as leaf blight, dieback, grain discoloration, palea browning, seed stalk rot, spot diseases, and stem necrosis (Coutinho *et al.,* 2002; Brady *et al.,* 2008; Morin and Parveen, 2014).

The BLB disease is commonly reported to be caused by *Xoo* in many countries including Malaysia. However, BLB disease outbreaks caused by *Pantoea* spp. Have been reported in many rice producing countries. As rice remains an important agricultural food crop in Malaysia, the constant occurrence of the disease becomes a major constraint in rice production. The disease is nearly affecting all the major rice crops areas in Malaysia and causes critical infection, thus leading to the significant losses in rice productivity and quality of rice (Chukwu *et al.,* 2019). The disease contributes to the yield reduction at the maximum tillering stage as much as 20% to 40% and nearly 50% at early stage (Yasmin *et al.,* 2017). Therefore, a better understanding of BLB pathosystem is critical.

As rice is an important crop and one of the primary sources of food to Malaysia population, scrutinizing records on the current status of BLB caused by *Pantoea* spp. is of significant importance. To date, none of the disease occurrence caused by *Pantoea* spp. has been documented in Malaysia. The findings of this study will be a significant endeavour to the important documentation of BLB disease

caused by *Pantoea* spp. in Malaysia. By taking the disease problem into account, this study would be a major platform on generating details documentation of BLB disease caused by *Pantoea* spp. on rice in Malaysia based on phenotypic characterization and molecular approaches. The findings of this research may contribute to the knowledge in identification of *Pantoea* spp. causing BLB disease on rice and improve the efficiency in the development of the diagnostic tests in the identification of this bacteria. The outputs providing useful information to overcome BLB disease caused by *Pantoea* spp., hence improving the rice production in Malaysia. This study was carried out based on the following objectives:

1. To isolate and characterize *Pantoea* spp. strains using phenotypic characteristics, molecular and phylogenetic analyses.
2. To test the pathogenicity and disease severity of the *Pantoea* isolation.

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LIST OF PUBLICATIONS

Journals

Azizi, M. M. F., Ismail, S. I., Hata, E. M., Zulperi, D., Ina-Salwany, M. Y., & Abdullah, M. A. F. (2019). First Report of Pantoea stewartii subsp. indologenes Causing Leaf Blight on Rice in Malaysia. *Plant Disease*, *103*(6), 1407-1407

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Poster

Azizi, M. M. F., Ismail, S. I., Md Yasin, I. S., Mohd Hata, E., Abdullah, M. A. F., and Zulperi, D. (2018). Phylogeny and identification of Pantoea species causing leaf blight disease of rice in Peninsular Malaysia. *Proceedings of the Joint Symposium of the 8th International Agriculture Congress 2018 and 6th International Symposium for Food & Agriculture UPM, Malaysia*, 269-270.

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