UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF *Pseudomonas fuscovaginae* CAUSAL AGENT SHEATH BROWN ROT OF RICE IN PENINSULAR MALAYSIA

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By

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Thesis Submitted to the School of Graduate Studies, University Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Do whatever you can do, nothing can stop you. To be able to go ahead in life, you have to overcome the struggle and strive. Keep trying till you succeed, such determination is all that you need". – Nor Farah Shamira (Beloved wife and friend)

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

CHARACTERIZATION OF *Pseudomonas fuscovaginae* CAUSAL AGENT SHEATH BROWN ROT OF RICE IN PENINSULAR MALAYSIA

By

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NOVEMBER 2014

Chairman: Associate Professor Dr. Kamaruzaman Bin Sijam
Faculty: Agriculture

Rice (*Oryza sativa*) is a staple food and strategic crop for economic development in Malaysia. There are several downfall factors that affect rice productivity, which are pests and diseases. Sheath brown rot (SBR) has been highlighted as one of the most important diseases that affect rice plants by contaminating the rice seeds. It causes rot to the sheath, panicle and leaves of rice plants, thus leading to a decline in rice production. In this study, 25 bacteria isolates were studied and characterized morphologically, including hypersensitivity reaction and pathogenicity test, biochemical test as well as molecular identification. These 25 isolates had the characteristics of *Pseudomonas fuscovaginae* which were fluoresced under UV illumination on King's B Selective (KBS) medium, positive for both hypersensitivity reaction (HR) on *Nicotiana tabacum* cv. *xanthi* and pathogenicity tests on *Oryza sativa*. They also showed positive results for several biochemical reactions such as oxidase, arginine dihydrolase and trehalose utilization tests. Based on these preliminary tests, all isolates were identified as *Pseudomonas fuscovaginae*. The 16S rDNA sequence analysis showed that all the isolates had 97-99% sequence similarities to *P. fuscovaginae* in the GenBank database. This result was also supported by the constructed phylogenetic tree with the *Acidivorax avenae* outgroup. It was noted that all 25 isolates originated from the same node with the *P. fuscovaginae* group. To evaluate the effect of sheath brown rot pathogen on Malaysian rice varieties, disease severity levels of different bacterial isolates on MR219 variety were determined. Most of the bacterial isolates had virulent and intermediate severity level. Virulent isolate, T1 from Selangor, Malaysia had highest percentage of diseased sheath area. As the effects of sheath brown rot pathogen on Malaysian rice varieties were evaluated, most of the varieties recovered from the disease as they matured. Moreover, results also showed that the panicle's number and grain's weight of infected rice plants were lower than control rice plants but not significant. These findings conclude that under greenhouse conditions, the pathogen of sheath brown rot could not affect Malaysian rice varieties. The information on characteristics of *P. fuscovaginae* in Peninsular Malaysia and its pathogenicity on Malaysian rice varieties could hopefully be used in future studies especially on Malaysian *P. fuscovaginae* biology and their control in Malaysia.

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PENCIRIAN *Pseudomonas fuscovaginae* AGEN PENYEBAB PENYAKIT REPUT SELUDANG PADI DI SEMENANJUNG MALAYSIA

Oleh

MOHD ZAFRI BIN AB WAHAB

NOVEMBER 2014

Pengerusi: Profesor Madya Dr. Kamaruzaman Bin Sijam
Fakulti: Pertanian

Padi (*Oryza sativa*) adalah makanan ruji dan tanaman strategik untuk pembangunan ekonomi di Malaysia. Terdapat beberapa faktor yang memberi kesan kepada kejatuhan produktiviti beras, iaitu perosak dan penyakit. Reput seludang padi merupakan salah satu penyakit penting yang memberi kesan kepada tanaman padi. Selain mencemarkan benih padi, penyakit ini menyebabkan reput pada seludang, tangkai dan daun pokok padi, seterusnya menyebabkan penurunan dalam pengeluaran beras. Dalam kajian ini, sebanyak 25 pencilan bakteria telah dikaji dan diciri secara morfologi, termasuk tindak balas hipersensitiviti dan ujian kepatogenan, ujian biokimia serta kenal pasti secara molekular. Keseluruhannya, 25 pencilan ini mempunyai ciri-ciri *Pseudomonas fuscovaginae* yang bercahaya bawah pencahayaan UV di atas media 'King's B Selective' (KBS), positif untuk kedua-dua tindak balas hipersensitiviti (HR) pada *Nicotiana tabacu*m cv. *xanthi* dan ujian kepatogenan pada *Oryza sativa*. Mereka juga menunjukkan hasil yang positif untuk beberapa tindak balas biokimia seperti oxidase, dihydrolase arginina dan ujian penggunaan trehalosa. Berdasarkan ujian awal, semua pencilan telah dikenal pasti sebagai *P. fuscovaginae*. Analisis jujukan 16S rDNA menunjukkan bahawa semua pencilan mempunyai persamaan jujukan 97-99% dengan *Pseudomonas fuscovaginae* dalam pangkalan data GenBank. Keputusan ini juga disokong oleh pohon filogeni yang dijana dengan *Acidivorax avenae* sebagai 'outgroup'. Berdasarkan pemerhatian, semua 25 pencilan berasal dari nod yang sama dengan kumpulan *P. fuscovaginae*. Untuk menilai kesan patogen reput seludang pada varieti padi Malaysia, tahap kepatogenan penyakit oleh pencilan bakteria yang berbeza pada varieti MR219 telah ditentukan. Kebanyakan pencilan bakteria mempunyai kepatogenan ditahap virulen dan virulen intermidiat. Pencilan yang virulen, T1 dari Selangor, Malaysia mempunyai peratusan tertinggi dalam kawasan seludang berpenyakit. Apabila kesan patogen reput seludang pada varieti padi Malaysia telah dinilai, kebanyakan varieti pulih daripada penyakit ini setelah mereka matang. Selain itu, keputusan juga menunjukkan bilangan 'panicle' dan berat bijirin tanaman padi dijangkiti adalah lebih rendah daripada tanaman padi kawalan tetapi tidak ketara. Kesimpulannya, di dalam keadaan rumah hijau, patogen reput perang seludang tidak dapat menjejaskan varieti padi Malaysia dengan ketara. Maklumat ciri-ciri *P. fuscovaginae* di Semenanjung Malaysia dan kepatogenannya pada varieti padi Malaysia diharap dapat digunakan di masa hadapan terutamanya dalam kajian tentang biologi *P. fuscovaginae* di Malaysia dan kawalannya.

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LIST OF ABBREVIATIONS

SBR	Sheath brown rot
KMB	King's B Medium
KBS	King's B selective medium
NaCLO	Sodium hypoclorite
v/v	volume over volume
mL	mili Liter
μL	micro Liter
cfu	colony forming unit
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
rDNA	Ribosomal DNA
^{o}C	degree Celsius
SDW	Sterile distill water
DAI	Day after inoculation
Psi	unit of pressure (pounds *per square inch)*

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CHAPTER 1

INTRODUCTION

Rice (*Oriza sativa*) is the major food crop of nearly half of the world's populationwhich provides carbohydrates and important nutrition for human.In recent years, most countries in the world are facing a major problem regarding the issue of food crisis especially rice production. Overall, rice-cropped area and rough rice production of the world in 2012 were 158.40 million hectares and 467.60 million tons, respectively (IRRI, 2014). However, the available resources probably cannot support the increasing world population which results in scarcity of food. Many researches of rice have been carried out to improve the rice production (FAO, 2003).

Unfortunately, for many rice varieties, the potential of producing high yield has been affected by many pest and disease problem. Sheath brown rot (SBR) of rice caused by *Pseudomonas fuscovaginae* is one of the devastating bacterial diseases of rice where the first outbreak of this disease was first discovered by Tanii *et al.*, (1976) in Japan, now has been disseminated throughout the world (CABI, 2012). The effects of SBR on rice plant were sheath lesions, grain sterility and grain discolouration (Rott, 1987). As cited in Azmi *et al.*, (2009), Cahyaniati & Mortensen, (1997) reported yield losses as high 72.2% occurred in Indonesia, while in Madagascar, severe infection lead to 100% yield loss (Rott, 1987).

In Malaysia, this disease had been well distributed in Peninsular Malaysia, where the first incidence was reported by Marzukhi *et al.*, (1991) in Seberang Perak, Malaysia with yield loss 46%. Later, Saad *et al.*, (2003) said sheath brown rot is a potentially devastating bacterial disease of rice in Malaysia. Up to date, Azmi *et al.*,(2009) discovered that more than half of SBR disease incidences were found to occur in major granary in Peninsular Malaysia and severe infection of SBR was also recorded in east region of Peninsular Malaysia.

Since the establishments of SBR in Malaysia, the characteristics of the causal agent have not yet been described or documented in any report. The characteristics of the pathogen are important for accurate identification in basic research on its biology and control. Actual measurements of SBR effects on commercialized and farmer's regularly used Malaysian rice varieties also have not been reported.

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This study was encouraged by the report of *P. fuscovaginae* in Malaysia by Azmi *et al.*, (2009), Saad *et al.*, (2003) and Marzukhi *et al.*, (1991) which is based on the fact that the characteristics and pathogenicity of the Malaysian *P. fuscovaginae* had not previously been investigated. Therefore, this study was undertaken with following objectives:

1. To characterize the causal agent of sheath brown rot of rice in Peninsular Malaysia.

2. To evaluate the effect on Malaysian rice varieties after being inoculated with sheath brown rot pathogen.

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