



**UNIVERSITI PUTRA MALAYSIA**

***CHARACTERIZATION AND PATHOGENICITY OF *Bacillus Pumilus*  
ISOLATED FROM TRUNK BULGES OF RRIM 3001 SUPERCLONE  
RUBBER TREES IN PENINSULAR MALAYSIA***

**SAFWAN BIN MAZLAN**

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By

**SAFWAN BIN MAZLAN**

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

**February 2020**

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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 AND PATHOGENICITY OF *Bacillus Pumilus*  
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**February 2020**

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*Hevea brasiliensis* (rubber tree) is well known as the tree of life for its by-products such as wood and latex. Nonetheless, the growth and performance of rubber trees are reduced substantially by bacterial species that causes trunk bulges. Trunk bulges are one of the most important diseases in rubber plantations especially in RRIM 3001 superclone. This study provides information that the causal pathogen responsible for the development of trunk bulges in rubber tree is *Bacillus pumilus* (*B. pumilus*) through isolation, identification, characterization and pathogenicity test. During October 2017 to December 2017, samples collection of infected rubber trees with trunk bulges disease was performed in four different locations with reported outbreaks. At the very first screening, all isolates were confirmed as *Bacillus* species by producing creamy white, filamentous and circular features on nutrient agar, and yellow pigment, circular, raised and entire form on *Bacillus* differentiation agar. These isolates appeared as Gram-positive rods and were positive for the catalase test. All isolates showed negative reaction for potassium hydroxide (KOH) and oxidase tests, positive for motility but negative for sulfide and indole tests. In carbohydrates fermentation, all isolates were able to utilize glucose, inositol, mannitol, sucrose, xylose and trehalose but unable to utilize lactose, maltose and arabinose. Further identification of isolates using 16S rRNA, Bsub specific gene and gyrase B gene sequence analyses disclosed that all 35 strains were clustered to the published *B. pumilus* from other regions worldwide. Meanwhile, phylogenetic analyses of 16S rRNA, Bsub specific gene and gyrase B gene further showed that all 35 strains were grouped with 99% bootstrap value to the published *B. pumilus*. By comparing phylogenetic analysis constructed from all genes tested, it showed that phylogenetic of gyrase B gene is significantly higher genetic variation compared to the 16s rRNA and Bsub specific gene. For pathogenicity study, 10 from 35 strains were chosen for this assay based on the differences in nucleotides showed in the different clades in the phylogenetic tree of Bsub specific gene. All 10 strains were virulence towards the RRIM 3001 superclone rubber tree exhibiting trunk bulges symptoms after seven days of

inoculation with different degrees of virulence; weakly virulent, moderately virulent and highly virulent. Strain KD15.5 was the most virulent, while strains KD3.1 and SD12.9 were weakly virulent. Statistical results revealed relationships between their severity and geographical origins of isolates, with the most and the lowest severity were isolates from Kedah and Selangor. This study represents the first evidence on the introduction of *B. pumilus* associated with trunk bulges disease of rubber tree in Peninsular Malaysia. These findings provide constructive documentation on the existence of *B. pumilus* causing trunk bulges disease of rubber tree in Peninsular Malaysia since rubber tree has been recognized as the important commodity crop with a high economic value in this country.

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**PERINCIAN DAN KEPATOGENAN *Bacillus Pumilus* DIPENCILKAN  
DARIPADA BENJOLAN BATANG PADA KLON POKOK GETAH RRIM 3001  
DI SEMENANJUNG MALAYSIA**

Oleh

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*Hevea brasiliensis* (pokok getah) terkenal sebagai pohon hayat untuk produk sampingan seperti kayu dan susu getah. Walau bagaimanapun, pertumbuhan dan prestasi pokok getah berkurangan dengan ketara oleh spesies bakteria yang menyebabkan benjolan batang. Benjolan batang adalah salah satu penyakit yang paling penting di ladang-ladang getah terutamanya pada klon RRIM 3001. Kajian ini menyediakan maklumat tentang punca patogen yang menyebabkan pembentukan benjolan pada batang pokok getah adalah *Bacillus pumilus* (*B. pumilus*) melalui kaedah pengasingan, pengenalan, pencirian dan ujian kepatogenan. Pada Oktober 2017 hingga Disember 2017, pengambilan sampel pokok getah yang dijangkiti dengan penyakit benjolan batang telah dilakukan di empat lokasi yang berlainan yang dilaporkan terjejas. Pada pemeriksaan pertama, semua pencilan bakteria telah disahkan sebagai spesies *Bacillus* dengan menghasilkan morfologi putih berkrim, berfilamen dan bercirikan membulat diatas agar nutrien, dan pigmen kuning, bulat, timbul dan bentuk keseluruhan pada agar pembezaan *Bacillus*. Kesemua pencilan ini menunjukkan bentuk rod, Gram-positif dan positif untuk ujian catalase. Ia juga menunjukkan reaksi negatif untuk kalium hidroksida (KOH) dan ujian oksidase, positif ujian motiliti tetapi negatif untuk ujian sulfida dan indol. Untuk ujian fermentasi karbohidrat, semua pencilan bakteria dapat menggunakan glukosa, inositol, manitol, sukrosa, xilosa dan trehalosa tetapi tidak dapat menggunakan laktosa, maltosa dan arabinosa. Pengenalan lanjutan terhadap pencilan dengan menggunakan analisa jujukan 16S rRNA, gen khusus Bsub dan gen gyrase B menunjukkan semua 35 strain dikelompokkan dengan strain *B. pumilus* yang diterbitkan dari kawasan lain. Sementara itu, analisa filogenetik 16S rRNA, gn khusus Bsub dan gen gyrase B menunjukkan bahawa semua strain telah dikelompokkan dengan 99 % nilai butstrap dengan *B. pumilus* yang diterbitkan. Dengan membandingkan analisa filogenetik daripada semua jujukan gen, ia menunjukkan bahawa filogenetik gen gyrase B adalah lebih tinggi variasi gen berbanding 16S rRNA dan gen khusus Bsub. Untuk kajian kepatogenan, 10 daripada 35 pencilan bakteria telah dipilih untuk cerakin ini

berdasarkan perbezaan pada nukleotida yang ditunjukkan berdasarkan perbezaan klad pada pokok filogenetik gen khusus Bsub. Semua 10 pencilan adalah virulen terhadap pokok klon getah RRIM 3001 dengan menghasilkan simptom benjolan batang selepas tujuh hari inokulasi dengan pelbagai darjah kevirulenan; kurang virulen, sederhana virulen dan paling virulen. Pencilan KD15.5 adalah yang paling virulen, manakala pencilan KD3.1 dan SD12.9 adalah kurang virulen. Analisis statistik menunjukkan hubungan antara tahap virulen dan geografi pencilan bakteria iaitu, yang paling virulen dan paling rendah virulen adalah isolat dari Kedah dan Selangor. Kajian ini merupakan bukti pertama mengenai pengenalan *B. pumilus* yang menyebabkan penyakit benjolan batang pokok getah di Semenanjung Malaysia. Penemuan ini memberikan dokumentasi konstruktif mengenai *B. pumilus* yang menyebabkan penyakit benjolan batang pada pokok getah di Semenanjung Malaysia, kerana pokok getah telah dikenal pasti sebagai tanaman komoditi yang paling penting dengan nilai ekonomi yang tinggi di negara ini.

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## LIST OF ABBREVIATIONS

%	Percent
µL	Microlitre
BDA	<i>Bacillus</i> differentiation agar
bp	Base pair
cfu	Colony forming unit
DAI	Day after inoculation
DNA	Deoxyribonucleic acid
EDTA	Ethylene-diamine-tetraacetic acid
g	Gram
H	Hour
L	Litre
Min	Minute
mL	Millilitre
MRB	Malaysian Rubber Board
NA	Nutrient agar
°C	Degree Celcius
PDA	Potato dextrose agar
rDNA	Ribosomal DNA
RNA	Ribonucleic acid

## CHAPTER 1

### INTRODUCTION

Malaysian Rubber Board (MRB) has produced many rubber tree clones to cater the high demand for planting materials. The development of rubber trees as an attractive forest plantation program provides for the rubberwood industry, particularly for furniture, medium-density fibreboard and molding materials. The development of rubber clones is for dual purposes which are latex and timber also known as Latex Timber Clones (LTC) (Mayati and Izilawati, 2017). RRIM 3001 superclone is one of the latex timber clones produced by the Malaysian Rubber Board. This superclone has a fast-growing clone with high latex production and has a straight bole with minimum branches at the crown part.

After few years planting of this clone, the formation of bulges appeared on the trunk. The appearance of bulging on the tapping panel caused difficulties during the tapping process for harvesting the latex. Tapping process on the uneven bark surface causing damaging of cambium cells and results in the presence of bulging cells on the RRIM 3001 rubber tree affects the quality of wood (Zarawi, 2018). The presence of trunk bulges is higher at the bottom part of the trunk and become consistently low in the upper part.

The occurrence of trunk bulges of RRIM 3001 superclone was likely due to the reaction to the bacterial attacks and tree defense mechanism for survival. It was presumed that the trunk of the rubber tree was attacked by soil-borne bacteria (Mokhter and Aris, 2018). Previous studies on scots pine proved that *Bacillus pumilus* is the cause of wetwood, fireblight, canker and tumour-like bacteriosis (Kovaleva *et al.*, 2015). This bacterial pathogen is a widespread systemic bacterial disease that commonly proceeds in a chronic form and affects the central core of many forest trees (Kovaleva *et al.*, 2015). It was supported with other report that *B. pumilus* infect other crops such as ginger rhizome rot (Peng *et al.*, 2013), potato rot (Bathily *et al.*, 2010), twig dieback of Asian pear trees (Li *et al.*, 2009), and mango leaf blight (Galal *et al.*, 2006).

The outbreak of trunk bulges has further become crucial in the rubber industry since the disease is amongst the most severe trunk diseases in Malaysia where it causes critical yield losses, widespread rapidly and can rigorously impact the rubber sawn timber. Rubber industries are important to deliver RM230.9 billion of gross national income (GNI) by 2020 (MRB, 2011). Occurrences of trunk bulges have been the most crucial and cause a major reduction in the production of latex and rubberwood (Mokhter and Aris, 2018).



The outcomes from this study may increase the efficiency in the development of suitable molecular diagnostic tests for the detection and characterization of *B. pumilus* associated with trunk bulges. Indeed, the findings obtained will as serve useful information for the suppression of trunk bulges spread and quarantine purposes, hence improving the rubber industry in Malaysia.

This study would be a major platform on generating details information of *B. pumilus* is the causal pathogen in the development of trunk bulges of rubber trees in Malaysia using a combination of phenotypic identification and molecular phylogenetic approaches. Therefore, this study was conducted with the following objectives:

1. To isolate and identify potential pathogen-associated with trunk bulges of RRIM 3001 superclone rubber tree in Peninsular Malaysia by using phenotypic characterization.
2. To characterize *B. pumilus* as a potential causing of trunk bulges of RRIM 3001 superclone rubber tree via molecular and phylogenetic analyses.
3. To identify the *B. pumilus* as a pathogen causing trunk bulges by pathogenicity assessment.

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## **BIODATA OF STUDENT**

Safwan bin Mazlan was born on January 9, 1994, in Baling, Kedah. He completed his formal education with Sijil Pelajaran Malaysia in Sekolah Menengah Kebangsaan Seri Iskandar, Perak. In 2012, he pursued his study in Science Matriculation program at Kolej Matrikulasi Perak. A year after that, he continued his undergraduate in Bachelor of Science with honors (Resource Biotechnology) at Universiti Malaysia Sarawak (UNIMAS) and graduated in November 2016.

He was employed as an agronomist in the plant breeding at Green World Genetics Sdn Bhd from November 2016 until August 2017. He later enrolled as a Master of Science (Plant Pathology) candidate in Department of Plant Protection, Faculty of Agriculture, UPM since September 2017 until present under the supervision of Dr. Dzarifah Zulperi.

## LIST OF PUBLICATIONS

### Journal

Mazlan, S., Zulperi, D., Wahab, A., Jaafar, N. M., Sulaiman, Z., & Rajandas, H. (2019). First Report of *Bacillus pumilus* Causing Trunk Bulges of Rubber Tree (*Hevea brasiliensis*) in Malaysia. *Plant Disease*, 103(5), 1016.

Mazlan, S., Jaafar, N. M., Wahab, N. M., Sulaiman, Z., & Rajandas, H., & Zulperi, D. Molecular characterization and phylogenetic analysis of *Bacillus pumilus* causing trunk bulges of RRIM 3001 superclone rubber tree in Malaysia. *European Journal of Plant Pathology*, accepted.

Mazlan, S., Jaafar, N. M., Wahab, A., Rajandas, H., & Zulperi, D. (2019). Major Diseases of Rubber (*Hevea brasiliensis*) in Malaysia. *Pertanika Journal of Scholarly Research Reviews*, 5(2).

### Poster and Proceeding

Mazlan, S., Zulperi, D., Wahab, A., Jaafar, N. M., Sulaiman, Z., & Rajandas, H. (2019). Molecular phylogeny of *Bacillus pumilus* strains causing trunk bulges disease of rubber tree in Malaysia. International Society for Southeast Asian Agricultural Sciences, Serdang, Malaysia.

### Award

1. UPM Graduate Research Funding for Post-graduate student.

### GenBank

Deposited sequences:

1. 16S rRNA gene (GenBank accession nos. MH401100-MH401102, MK483879- MK748178).
2. Bsub specific gene (GenBank accession nos. MH428001-MH428003, MK483841- MK483878).
3. Gyrase B gene (GenBank accession nos. MK645724-MK645756).