HARNESSING *Streptomyces* spp. AS RICE PLANT GROWTH PROMOTER AND BIOCONTROL AGENT AGAINST BACTERIAL PANICLE BLIGHT

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree Doctor of Philosophy

January 2024

FBSB 2024 3

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Bacterial panicle blight (BPB) disease caused by *Burkholderia glumae* is an alarming disease of rice. Controlling BPB using chemical control and BPB-resistant rice cultivars is expensive and inconsistent due to chemical resistance strains' development. Using plant growth-promoting bacteria to enhance rice plant growth and suppress pathogens is considered safe, environmentally friendly, long-lasting and inexpensive. This study aimed to investigate the roles of *Streptomyces* spp. as rice plant growth promoter and biocontrol agent against *B. glumae*. A total of 50 *Streptomyces* isolates were obtained from rice fields, and 22% showed antagonistic activity against *B. glumae*. The best three plant growth-promoting *Streptomyces* (PGPS) isolates, *S. corchorusii* TBR11, *S. corchorusii* JAS2 and *S. misionensis* TBS5 with plant growth-promoting (PGP) abilities and biocontrol activity against *B. glumae* were identified and characterised. The identity of PGPS isolates was confirmed based on 16S rRNA and phylogenetic tree analyses and multilocus sequence analysis (MLSA) by amplifying *gyrB*, *rpoB* and *trpB* using the polymerase chain reaction (PCR). Through API® ZYM analysis, the PGPS isolates showed enzymatic activities crucial in plant growth promotion. The PGPS isolates showed the highest (80%) PGP traits, including fixing nitrogen, solubilising phosphate and producing siderophore and indole-3-acetic acid. For antagonistic activity, TBS5 (26.35 ± 0.14 mm) exhibited the most antagonistic activity against *B. glumae* ($p \leq 0.05$). The supernatant of PGPS isolates reduced the survival viability of *B. glumae* more than 50.7%. Scanning electron microscopy (SEM) indicated that substantial morphological changes occurred in the cell membranes of *B. glumae* after the PGPS treatment. Rice seeds treated with PGPS isolates improved vigour index by more than 100% compared to the control plants. Under the greenhouse study, TBR11 (149%) and TBS5 (141%)-treated plants showed a higher rice yield than the control (100%). The BPB disease severity was reduced by up to 27% and 39% for TBR11 and TBS5-treated plants, respectively. The talc-kaolin-*Streptomyces* bioformulations (BioSTBR11, BioSJAS2 and BioSTBS5 containing TBR11, JAS2 and TBS5, respectively) were successfully prepared and showed no phytotoxic effect on rice plants. Bioformulations were more stable when stored under refrigeration than at room temperature, with more than 50% spore viability at 180 days after storage. Treatment with BioSTBR11 and BioSTBS5 reduced BPB disease severity by up to 7% and 10%, and increased rice yield by up to 301% and 170% relative to control (100%), respectively. Transcript levels of defense-related genes (*Gns1*, *OsPR1a*, *Oswrky45* and *OsJAMYB*) were up-regulated by the treatment with BioSTBR11 and BioSTBS5 at 7 days after infection than the control. Results suggest that the PGPS isolates, especially *S. corchorusii* TBR11 and *S. misionensis* TBS5 are promising candidates for enhancing rice yield and suppressing BPB disease. Overall, this

study offers biocontrol solution for suppressing BPB disease by utilising PGPS, which can be incorporated as one of the BPB disease management strategies.

Keywords: Biocontrol, *Burkholderia glumae*, Plant growth-promoting *Streptomyces*, Rice sustainability

SDG: GOAL 2: Zero Hunger, GOAL 12: Responsible Consumption and Production, GOAL 15: Life on Land

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGGUNAAN *Streptomyces* spp. SEBAGAI PENGGALAK TUMBESARAN PADI DAN AGEN BIOKAWALAN TERHADAP HAWAR BULIR BAKTERIA

Oleh

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Penyakit hawar bulir bakteria (BPB) yang disebabkan oleh *Burkholderia glumae* adalah penyakit padi yang membimbangkan. Pengawalan BPB menggunakan kawalan kimia dan kultivar padi berdaya tahan BPB adalah berkos tinggi dan tidak konsisten disebabkan oleh perkembangan strain rintang kimia. Penggunaan bakteria penggalak tumbesaran tumbuhan bagi meningkatkan tumbesaran padi dan menyekat patogen adalah selamat, mesra alam, tahan lama dan murah. Kajian ini dijalankan untuk mengkaji fungsi *Streptomyces* spp. sebagai penggalak tumbesaran padi dan agen biokawalan *B. glumae*. Sejumlah 50 isolat *Streptomyces* telah diperolehi daripada sawah dan 22% menunjukkan aktiviti antagonis terhadap *B. glumae*. Tiga isolat *Streptomyces* penggalak tumbesaran tumbuhan (PGPS) terbaik, *S. corchorusii* TBR11, *S. corchorusii* JAS2 dan *S. misionensis* TBS5 dengan kebolehan penggalak tumbesaran tumbuhan (PGP) dan aktiviti biokawalan terhadap *B. glumae* telah dikenal pasti dan dicirikan. Pengenalpastian PGPS adalah disahkan melalui analisis 16S rRNA dan pokok filogenetik serta analisis jujukan berbilang lokus (MLSA)

melalui amplifikasi *gyrB*, *rpoB* dan *trpB* menggunakan tindak balas berantai polimerase (PCR). Melalui analisis API® ZYM, isolat PGPS didapati mempunyai aktiviti enzim yang berperanan penting dalam pengalakan tumbesaran tumbuhan. Isolat PGPS menunjukkan ciri-ciri PGP tertinggi (80%), termasuk mampu menetapkan nitrogen dan melarutkan fosfat, serta menghasilkan siderophore dan asid indole-3-asetik. Bagi aktiviti antagonis, TBS5 (26.35 ± 0.14 mm) menunjukkan aktiviti antagonis yang tertinggi terhadap *B. glumae* ($p \leq 0.05$). Supernatan isolat PGPS didapati mampu mengurangkan daya hidup *B. glumae* melebihi 50.7%. Imej *Scanning Electron Microscopy* (SEM) telah menunjukkan bahawa perubahan morfologi berlaku pada membran sel *B. glumae* selepas rawatan PGPS. Benih padi yang dirawat dengan isolat PGPS telah menunjukkan peningkatan indeks kecergasan melebihi 100% berbanding benih kawalan. Bagi kajian rumah hijau, pokok yang menerima rawatan TBR11 (149%) dan TBS5 (141%) menunjukkan peningkatan ketara pada hasil padi berbanding kawalan (100%). Keterukan penyakit BPB telah menurun sehingga 27% dan 39% bagi pokok yang menerima rawatan TBR11 dan TBS5. Bioformulasi talc-kaolin-*Streptomyces* (BioSTBR11, BioSJAS2 dan BioSTBS5 yang mengandungi TBR11, JAS2 dan TBS5) tidak menunjukkan kesan fitotoksik pada pokok padi. Bioformulasi adalah stabil apabila disimpan di bawah keadaan sejuk berbanding suhu bilik dengan lebih daripada 50% kebolehhidupan spora pada 180 hari selepas penyimpanan. Rawatan mengunakan BioSTBR11 dan BioSTBS5 telah mengurangkan keterukan penyakit BPB sehingga 7% dan 10%, serta meningkatkan hasil padi sehingga 301% dan 170% berbanding kawalan (100%). Tahap transkrip gen-gen berkaitan pertahanan (*Gns1*, *OsPR1a*, *Oswrky45* dan *OsJAMYB*) adalah tinggi pada pokok yang

menerima rawatan BioSTBR11 dan BioSTBS5 selepas 7 hari jangkitan berbanding kawalan. Keputusan menunjukkan bahawa isolat PGPS, terutamanya *S. corchorusii* TBR11 dan *S. misionensis* TBS5 adalah calon yang menjanjikan untuk meningkatkan hasil padi dan menyekat penyakit BPB. Keseluruhannya, kajian ini menawarkan peyelesaian biokawalan untuk menyekat penyakit BPB menggunakan PGPS, dimana ia boleh dimanfaatkan sebagai salah satu strategi pengurusan penyakit BPB.

Kata Kunci: Biokawalan, *Burkholderia glumae*, *Streptomyces* penggalak tumbesaran tumbuhan, Kelestarian padi

SDG: MATLAMAT 2: Kelaparan Sifar, MATLAMAT 12: Penggunaan dan Pengeluaran Bertanggungjawab, MATLAMAT 15: Kehidupan di Darat

ACKNOWLEDGEMENTS

In the Name of Allah, The Most Gracious, The Most Merciful and
Prophet Mohammad S.A.W

Alhamdulillah, because of His grace, I was able to finish this project. Therefore, I would like to express my deepest gratitude to my supervisor, Assoc. Prof. Dr. Mohd Termizi Yusof for his guidance, attention, patience and inspiration. I would also like to thank my co-supervisors, Dr. Erneeza Mohd Hata, Prof. Dr. Mohd Razi Ismail, Assoc. Prof. Dr. Noor Baity Saidi and Assoc. Prof. Dr. Nur Ain Izzati Mohd Zainudin for their guidance.

In addition, I also want to express my sincere thanks to the following individuals for assisting and helping me to get all the facilities that were necessary to complete this project:

- Members of the Faculty of Biotechnology and Biomolecular Sciences, UPM.
- Members of the Faculty of Agriculture, UPM.
- Members of the Sustainable Agronomy and Crop Protection, Institute of Plantation Studies, UPM.
- Members of the Institute of Bioscience, UPM.
- The farmers that contributed soil and rice plant samples for use in this study.

I personally express my tremendous gratitude to the following individuals, who are important to me and kept me focused and going forward even when I ran into difficulties during the project:

- My parents: Ngalimat Kayat, Masseah Tapah, Mohd Ali Ismail and Siti Saerah Sellan.
- My wife: Nur Shidaa Mohd Ali.
- My family members: Junidah Ngalimat, Suliyatiawidanita Ngalimat, Siti Normala Ngalimat, Vizia Raha Ngalimat, Mohd Rudi Ngalimat, Siti Nor Baiyah Ngalimat, Siti Khairunisa Ngalimat, Abdul Jalil Ngalimat, Nur Aidaa Mohd Ali and Mohamed Azman Mohd Ali.

Last but not least, I would also like to thank the Ministry of Higher Education Malaysia (MoHE) for providing financial support through the Long-Term Research Grant Scheme, LRGS (LRGS/1/2019/UPM/01/2/2), Program: Development of climate ready rice for sustaining food security in Malaysia, Project: Sustainable short and medium to long-term strategies for managing bacterial panicle blight (BPB) disease under climate-resilience rice production. Thanks to the Special Graduate Research Allowance Scheme (SGRA) scholarship for providing financial support from this study's 1^{st} and 2^{nd} semesters. Also, thanks to MoHE for providing financial support through the MybrainSC scholarship from the 3^{rd} to 6^{th} semesters of this study.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Date: 11 July 2024

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α	Alpha
β	Beta
°C	Degree celsius
%	Percentage
µL	Microliter
µm	Micrometer
µg/mL	Microgram per microliter
16S rRNA	16 Svedberg ribosomal ribonucleic acid
ACC	1-Aminocyclopropane-1-carboxylate
bp	Base pair
BCA	Biological control agents
BPB	Bacterial panicle blight
CAS	Chrome azurol S
CFU	Colony-forming unit
CRD	Completely randomized design
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
g	Gram
g/L	Gram per litre
HCN	Hydrogen cyanide
IAA	Indole-3-acetic acid
ISR	Induce systemic resistance
JA	Jasmonic acid
kb	Kilobase
M	Molar
MAFF	Ministry of Agriculture, Forestry and Fisheries
mg/L	Miligram per litre
mL	Mililitre
MLSA	Multilocus sequence analysis
mm	Milimeter
mM	Milimolar

NBRC	NITE Biological Resource Center
NCBI	National Center for Biotechnology Information
NF	Nitrogen fixation
ng/µL	Nanogram per microlitre
PCR	Polymerase chain reaction
PGP	Plant growth-promoting
PGPA	Plant growth-promoting agents
PGPS	Plant growth-promoting *Streptomyces*
pv.	Pathovar
RNA	Ribonucleic acid
rpm	Revolutions per minute
SEM	Scanning electron microscopy
sp.	A single unnamed species
spp.	More than one unnamed species
subsp.	Subspecies
TPC	Total plate count
UK	United Kingdom
USA	United States
UPM	Universiti Putra Malaysia
v/v	Volume over volume
w/v	Weight over volume

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

Rice is a staple food in Asia and parts of African countries. Over 90% of the world's rice is produced in the Asian region and consumed as the main food source. Ensuring food security for the general population is challenging with an ever-increasing human population. To counter the demand, rice production must be increased to fulfill this need (Firdaus et al., 2020). However, throughout the growing season, a reduction in rice yields has been detected and is caused by various pathogens, including bacteria. Bacterial rice diseases, including bacterial panicle blight (BPB), have become a main constraint to sustainable rice productivity.

BPB disease was first reported in the 1950s in Japan. It is a severe seed-borne bacterial disease of rice. BPB disease is primarily caused by *Burkholderia glumae*, a Gram-negative, rod-shaped and polar flagellated bacterium (Goto & Ohata, 1956; Tanii et al., 1976). BPB disease has been recorded in rice production areas in several Asian (Mondal et al., 2015; Weny et al., 2019), South and Central American (Mulaw et al., 2018; Quesada-González & García-Santamaría, 2014; Riera-Ruiz et al., 2014) and African countries (Zhou, 2014). The reduction of rice yield caused by BPB disease has reached up to 75% worldwide due to the inhibition of seed germination, floret sterility, grain rot, grain weight reduction and grain abortion (Zhou, 2019).

In Malaysia, the BPB-like symptomatic plants with the symptoms of seedling rot, panicle blighting and leaf-sheath browning were first reported in 2016 at Sungai Manik and Kerian, Perak. The disease symptoms were also detected in 2017 at Sungai Ache, Penang. In 2018, the symptoms were also found in Kampung Banir, Kelantan. Until now, BPB disease was reported to have affected rice granaries in various states in Peninsular Malaysia by up to 50% of the disease incidence (Ramachandran et al., 2021a). In 2021, the causal agent of BPB disease in Malaysia was confirmed caused by *B. glumae* based on phenotypic characterisation, molecular identification and Koch's postulates (Ramachandran et al., 2021a).

So far, the main strategies to control BPB disease worldwide include chemical control by applying oxolinic acid (Zhou-qi et al., 2016) and utilising BPB-resistant rice cultivars (Zhou et al., 2011). However, these strategies are expensive and inefficient due to chemical resistance development within the pathogenic strains (Hikichi et al., 2001; Karki et al., 2012). Interestingly, biocontrol offers a potential alternative strategy for managing BPB disease by using beneficial microbes such as bacteria as biological control agents (BCA) to control pathogens with additional benefits as plant growth-promoting agents (PGPA) to enhance plant growth (Suárez-Moreno et al., 2019).

The implementation of biocontrol strategy to control plant diseases has received great attention because it is potentially safe, environmentally friendly, long-lasting and inexpensive (Aghighi et al., 2004). Many studies have reported the applicability of bacteria to control pathogens in an attempt to replace existing strategies of chemical fertilisers that frequently lead to environmental pollution

(Betancur et al., 2017; Ham & Kim, 2018; Shang et al., 2018). As a management strategy to control plant diseases, bacteria demonstrated as an excellent alternative in controlling pathogens while enhancing rice plant growth under greenhouse and field studies (Jin et al., 2020). Among bacterial groups, *Streptomyces* spp. have been widely examined as BCA and PGPA (Hata et al., 2021; Mohd Hata et al., 2015; Suárez-Moreno et al., 2019).

1.2 Problem statement

The BPB-like symptomatic rice plants were first reported in Malaysia at Sungai Manik and Kerian, Perak, in 2016. In 2017 and 2018, the disease symptoms were also detected at Sungai Ache, Penang, and Kampung Banir, Kelantan, respectively. To date, rice yield losses caused by BPB disease in various states of Peninsular Malaysia have reached up to 50%. It has been confirmed that the causal agent of BPB disease in Malaysia was *B. glumae* (Ramachandran et al., 2021a).

So far, the main strategies to control BPB disease in Malaysia include chemical control by applying oxolinic acid to produce pathogen-free seeds (Ramachandran et al., 2021b). However, these strategies are expensive and inefficient due to chemical resistance development within the pathogenic strains (Hikichi et al., 2001; Karki et al., 2012). Interestingly, biocontrol offers a potential alternative strategy for managing BPB disease by using beneficial microbes such as bacteria as biological control agents (BCA) to control pathogens with additional benefits as plant growth-promoting agents (PGPA) to enhance plant growth (Suárez-Moreno et al., 2019).

Bacteria from the genera *Bacillus* (Pedraza et al., 2021), *Burkholderia* (Miyagawa, 2000; Miyagawa & Takaya, 2000), *Enterobacter* (Atuesta et al., 2020), *Pantoea* (Kouzai & Akimoto-Tomiyama, 2022) and *Pseudomonas* (Ortega et al., 2020) have been studied as potential BCA against BPB pathogen and PGPA of rice. However, some of the bacterial genera, including *Burkholderia* (Wamishe et al., 2001; Zhou-qi et al., 2016), *Enterobacter* (Cao et al., 2020), *Pantoea* (Azizi et al., 2019; Kini et al., 2017; Lee et al., 2010; Yan et al., 2010) and *Pseudomonas* (Kuwata, 1985; Liu et al., 2012; Tanii et al., 1976; Zeigler & Alvarez, 1990) have been documented as bacterial rice pathogens. Even the bacteria used mostly have been considered nonvirulent; however, the potential of horizontal gene transfer that can lead to antibiotic resistance and virulence developments on closely related species should be considered (Kang et al., 2017). Thus, another alternative of bacterial genera used should be investigated. Interestingly, many studies have documented that *Streptomyces* spp. can be used to control pathogens (Subramaniam et al., 2020) and aid in plant development (Amaresan et al., 2018; Ferrer et al., 2018; Viaene et al., 2016; Vurukonda et al., 2018).

Streptomyces spp. are well known as BCA in agricultural fields. This bacterial genus has been used as BCA as bioinoculants to control bacterial rice pathogens (Aeny et al., 2018; Betancur et al., 2019; El Karkouri et al., 2010; Ilsan et al., 2016; Jaivel et al., 2017; Mohd Hata et al., 2015; Nanjundan et al., 2019; Shang et al., 2018; Suárez-Moreno et al., 2019). Moreover, *Streptomyces* spp. have also been used as PGPA to enhance plant growth (Newitt et al., 2019). *Streptomyces* treatments as bioinoculants were reported to enhance rice plant growth and yield (Awla et al., 2017; Tamreihao et al., 2016); suggesting its potential as the PGPA of rice. However, there is still a lack of comprehensive information on using *Streptomyces* spp. as BCA against *B. glumae* and PGPA of rice (Betancur et al., 2020; Suárez-Moreno et al., 2019), especially in Malaysia. In addition, bioformulations containing *Streptomyces* spp. that are used to enhance rice plant growth and suppress BPB disease are scarcely investigated.

1.3 Significance of the study

In 2021, the standard operation procedure (SOP) for BPB disease management in Malaysia was established by the Malaysian Agricultural Research and Development Institute (MARDI). According to the SOP, the BPB pathogen has been considered a seed-borne pathogen; thus, implementing seed treatments to produce pathogen-free seeds is highly recommended for managing BPB disease (Ramachandran et al., 2021b).

Interestingly, biocontrol strategies by the implementation of beneficial bacteria have been delivered to the rice plants in several ways, including aerial (Shrestha et al., 2016), soil (Chung et al., 2015) and seed (Furuya et al., 1991; Kouzai & Akimoto-Tomiyama, 2022; Ortega et al., 2020; Pedraza et al., 2021) applications. The seed application using beneficial bacteria as bioinoculants enhanced rice plant growth (Mew et al., 2004) and biotic stress resistance (Hata et al., 2021). However, there is a lack of studies using beneficial bacteria as BCA against BPB pathogen with additional benefits to enhance rice plant growth and yield (Betancur et al., 2020; Suárez-Moreno et al., 2019), especially in Malaysia.

This study tried to isolate plant growth-promoting *Streptomyces* (PGPS) from Malaysian rice fields. *Streptomyces* spp. as PGPA of rice and BCA against BPB pathogen, *B. glumae* are highlighted in this study. The bioformulations containing PGPS were also developed and tested for their efficacy in suppressing *B. glumae* infection and enhancing rice plant growth under greenhouse conditions. The effort to use the local strains of PGPS with biocontrol activity against the BPB pathogen in Malaysia is limited and is ongoing. Thus, concerted efforts to investigate the roles of PGPS isolated from rice fields against *B. glumae* and the effect on rice plant growth are necessary and merit scientific attention. The study on the investigation of PGPS from rice fields associated with antagonistic activity against *B. glumae* may provide an alternative for sustainable rice production and BPB disease management.

1.4 Research objectives

The general objective of this study is to investigate the roles of *Streptomyces* spp. isolated from Malaysian rice fields as rice plant growth promoters and biocontrol agents against *B. glumae*. The sub-objectives of this study are:

i. To isolate and characterise PGPS from rice fields associated with antagonistic activity against *B. glumae*.
ii. To evaluate the potential of PGPS to suppress the growth of *B. glumae* and promote rice plant growth under greenhouse conditions.
iii. To investigate the efficacy of *Streptomyces* spp. bioformulations in suppressing the growth of *B. glumae* under greenhouse conditions.

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