UNIVERSITI PUTRA MALAYSIA

ENHANCEMENT OF SPORE PRODUCTION OF ANTAGONISTIC Penicillium oxalicum T3.3 VIA SOLID STATE FERMENTATION

AIN NAJIHAH AZMI

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ENHANCEMENT OF SPORE PRODUCTION OF ANTAGONISTIC *Penicillium oxalicum* T3.3 VIA SOLID STATE FERMENTATION

By:

AIN NAJIHAH AZMI

Thesis submitted to the Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, in fulfilment of the requirement for the Degree of Bachelor of Science (Hons) Biotechnology

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FACULTY OF BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES
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LETTER OF PERMISSION

It is thereby to state that I, AIN NAJIHAH AZMI (Matric No: 163161) have done a final year project entitled "**Enhancement of spore production of antagonistic *Penicillium oxalicum* T3.3 via solid state fermentation**" under supervision of Assoc. Prof. Dr. Umi Kalsom Md. Shah from the Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

I hereby give permission to my supervisor to write and prepare manuscript from the results of this research to be published in any form, if I do not do so in six (6) months from the date above, in condition that my name is also added as one of the article's authors. The arrangement of the name depends on the supervisor herself.

Yours sincerely,

…….
(AIN NAJIHAH AZMI)

FACULTY OF BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES
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APPROVAL SHEET

This thesis entitled "**Enhancement of spore production of antagonistic *Penicillium oxalicum* T3.3 via solid state fermentation**" is submitted by AIN NAJIHAH AZMI (Matric No: 163161) in fulfilment of the requirement for the Degree of Bachelor of Science (Hons) Biotechnology in Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

Approved by,

…….. Date:

(Assoc. Prof. Dr. Umi Kalsom Md. Shah)
Project Supervisor
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ABSTRACT

Abstract of thesis presented to the Faculty of Biotechnology and Biomolecular Sciences in fulfilment of the requirement for the Degree of Bachelor of Science (Hons.) Biotechnology

ENHANCEMENT OF SPORE PRODUCTION OF ANTAGONISTIC *Penicillium oxalicum* T3.3 VIA SOLID STATE FERMENTATION

By:

AIN NAJIHAH AZMI

JUNE 2015

Supervisor : Assoc. Prof. Dr. Umi Kalsom Md. Shah

Faculty : Faculty of Biotechnology and Biomolecular Sciences

Anthracnose disease is a disease in which cause extensive pre- and postharvest damage to chilli crops caused by pathogen, *Colletotrichum gloeosporioides*. Recently, rather than using chemical control, spores produced from *Penicillium oxalicum* T3.3 raised high attention as biocontrol agent against *C. gloeosporioides*. Hence, this study was conducted in order to prove the antagonistic activity of *P. oxalicum* T3.3 toward *C. gloeosporioides* and to study the effect of cultural conditions on the production of *P. oxalicum* T3.3 spore in solid state fermentation. In this study, antagonistic activity of *P. oxalicum* T3.3 against *C. gloeosporioides* was tested in vitro by dual culture assay. *P. oxalicum* T3.3 was found to exhibit strong antagonistic activity against *C. gloeosporioides* with percent growth inhibition (PGI) of 55.86%. Then, solid state fermentation of *P. oxalicum* T3.3 was carried out in 250 mL flask containing 12 g of peat/vermiculite/soya bean meal (1:1:1, wt/wt/wt), sterilized and then inoculated with 4 discs of agar plugs (5mm in diameter) from 7 days old *P. oxalicum* culture and incubated at 28°C for 12 days. *P. oxalicum* T3.3 produced the highest spore concentration on 8th day of incubation time with 2.19×10^{9} spores ml^{-1} recorded. After that, effect of culture conditions on *P. oxalicum* T3.3 spore production was determined. The highest production of *P. oxalicum* T3.3 spore was obtained when mycelia was used as inoculum type, 4 discs (5 mm) agar plug as inoculum size, media (peat/vermiculite/soya bean meal) at proportion of 1:1:1 (wt/wt/wt), soya bean meal as nitrogen source, temperature at 28°C, and pH 6

as initial medium pH. From the findings, it can be concluded that *P. oxalicum* T3.3 was able to possess antagonistic activity against *C. gloeosporioides* and spore production can be significantly affected by culture condition factors in solid state fermentation.

Keywords: Biocontrol, *Penicillium oxalicum, Colletotrichum gloeosporioides,* spore, dual culture.

ABSTRAK

Abstrak tesis diserahkan kepada Fakulti Bioteknologi dan Sains Biomolekul bagi memenuhi keperluan untuk Ijazah Bacelor Sains (Kepujian) dalam Bioteknologi.

PENINGKATAN PENGHASILAN SPORA BERMUSUHAN *Penicillium oxalicum* T3.3 MELALUI FERMENTASI FASA PEPEJAL.

Oleh:

AIN NAJIHAH BINTI AZMI

JUN 2015

Penyelia : Prof. Madya Dr. Umi Kalsom Md. Shah

Fakulti : Fakulti Bioteknologi dan Sains Biomolekul

Penyakit anthracnose adalah sejenis penyakit yang menyebabkan kerosakan pra dan pasca tuaian yang teruk terhadap tanaman cili, disebabkan oleh pathogen *Colletotrichum gloeosporioides*. Baru-baru ini, daripada menggunakan kawalan kimia, spora yang dihasilkan daripada *Penicillium oxalicum* T3.3 telah menarik perhatian yang tinggi sebagai agen kawalan biologi terhadap *C. gloeosporioides*. Oleh itu, satu kajian telah dijalankan untuk membuktikan aktiviti antagonistik di antara *P. oxalicum* T3.3 dan *C. gloeosporioides* dan untuk mengkaji kesan keadaan kultur terhadap pengeluaran spora *P. oxalicum* T3.3 dalam fermentasi fasa pepejal. Dalam kajian ini, aktiviti antagonistik antara *P. oxalicum* T3.3 dan *C. gloeosporioides* diuji secara in vitro menggunakan kultur dual. *P. oxalicum* T3.3 didapati mempamerkan aktiviti antagonistik yang kuat terhadap *C. gloeosporioides* dengan peratus perencatan pertumbuhan (PGI) sebanyak 55.86%. Kemudian, fermentasi fasa pepejal *P. oxalicum* T3.3 telah dijalankan dalam kelalang 250 mL mengandungi 12 g gambut / vermikulit / kacang soya (1: 1: 1, berat / berat / berat), disterilkan dan kemudian disuntik dengan 4 cakera agar (5mm diameter) dari kultur *P. oxalicum* yang sudah ditumbuhkan selama 7 hari dan diinkubasi pada suhu 28^{o}C selama 12 hari. *P. oxalicum* T3.3 menghasilkan spora yang tertinggi pada hari ke-lapan tempoh inkubasi dengan 2.19 × 10^{9} spora ml^{-1} direkodkan. Selepas itu, kesan keadaan kultur terhadap penghasilan spora daripada *P. oxalicum* T3.3 dikaji. Didapati,

penghasilan spora yang tertinggi dihasilkan apabila mycelia digunakan sebagai jenis inokulum, 4 cakera (5 mm) agar sebagai saiz inokulum, nisbah medium (gambut/vermikulit/kacang soya) pada 1:1:1 (berat/berat/berat), kacang soya sebagai sumber nitrogen, suhu 28^{o}C, dan pH 6 sebagai pH awal media. Merujuk kepada hasil kajian, dapat disimpulkan bahawa *P. oxalicum* T3.3 boleh mempunyai aktiviti antagonistik terhadap *C. gloeosporioides* dan pengeluaran spora boleh dipengaruhi dengan ketara oleh faktor-faktor keadaan kultur di dalam fermentasi fasa pepejal.

Kata kunci: Agen kawalan biologi, *Penicillium oxalicum, Colletotrichum gloeosporioides,* spora, kultur dual.

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LIST OF ABBREVIATION

SSF:	Solid state fermentation.
SmF:	Submerged fermentation.
CVMV:	Chilli veinal mottle virus.
CMV:	Cucumber mosaic virus.
Maneb:	Manganese ethylenebisdithiocarbamate.
PDA:	Potato dextrose agar.
PGI:	Percent growth inhibition.
ANOVA:	Analysis of variance.
GIC:	Growth inhibition category.
LSD:	Least- significant difference.

CHAPTER 1

INTRODUCTION

Through the centuries, plant diseases have had profound effects on mankind. Based on scientific research on crop protection, it is projected that insects, diseases, weeds and animal pests remove almost half of the foodstuff produced in the world (Ellis & Boehm, 2008). Chilli (*Capsicum* spp) is a crucial economic crop in worldwide especially in tropical and subtropical regions. Chilli is important to the culinary in term of enhancing flavor, aroma, texture, colour as well as contributing to the nutritional value of foods. However, in recent years, chilli production faced a great challenge in which almost up to 50% of the production yield is losses due to the infection with anthracnose disease (Pakdeevaraporn *et al*., 2005). According to Manandhar *et al*. (1995), anthracnose disease affected chilli fruits by causing extensive pre- and postharvest damage, hence led to anthracnose lesions. This disease is one of the major constraints to the chilli production worldwide caused by plant pathogens, *Colletotrichum* spp. Among *Colletotrichum* spp., *Colletotrichum gloeosporioides* have been recognized as one of the pathogens that can cause anthracnose disease in chilli (Than *et al*., 2008).

Due to the emerging concern for this disease, several strategies have been established in order to minimize the incidence and severity of diseased chilli crops. The effective control of anthracnose disease is the use of either one or combination of these approaches: resistant cultivar, cultural control and chemical control (Narayanasamy, 2013). Conventionally, the uses of chemical biocides or pesticides have been long used to control microbial, fungal and insect plant pests. They play a significant part in the stabilization and increment of agricultural yield. Unfortunately, these pesticides may contribute to the atmospheric pollution as well as toxic to mammals and wildlife mainly in developing country (Santamarina et al, 2002).

With growing concern for environmental pollution and toxicity effect of the chemical pesticides issues, biological control approach is receiving an intense research effort in order to lessen the utilization of chemical pesticides in the plant disease treatment

(Johnson, 1994). The term 'biological control' or 'biocontrol' is to indicate the control of "one organism by another organism" (Beirner, 1967). The pathogens can be effectively controlled by other beneficial microorganisms either by competition, parasitism or other modes of action. Moreover, by using microorganism as biocontrol agent, it provides efficient, effective and inexpensive control measures for a range of pests and disease (Zwieten *et al*., 2007). However, until now, methods for biological control for chilli anthracnose disease have not received a lot of attention (Than *et al*., 2008).

In view of this problem, a fungal species, *Penicillium oxalicum* has raised high attention as potential biocontrol agent against anthracnose disease. As reported, *P. oxalicum* Thom is a promising biocontrol agent against vascular wilts caused by *Verticillium* spp. and increase resistance against *Fusarium oxysporum* in tomato plants (De Cal *et al*., 2009). The potential of *P. oxalicum* to produce antagonistic activity can be applied to control or suppress *C. gloeosporioides*, hence decrease the possibility of chilli fruits to be severely infected with anthracnose disease.

In this study, *P. oxalicum* T3.3 was used. The in vitro antagonistic activity interaction between *P. oxalicum* T3.3 and *C. gloeosporioides* can be proved by a dual culture method. The interaction between these two fungi is expected to produce inhibition zone. Application of *P. oxalicum* as biocontrol agent is based on the spore produced from this fungus. Spore of *P. oxalicum* T3.3 can be produced either from solid fermentation or liquid fermentation. For effective control of plant disease, fermentation mode that is able to produce high spore concentration is much desirable. Hence, solid state fermentation (SSF) is more preferable because it produce more spores compared to submerged fermentation (SmF) Larena *et al*., (2002) and allows fungi to produce healthy spores and mycelia Bartlett & Jaronski (1988).

As mention above, SSF is more desirable to produce spore. Yet, to produce spore in masses, a reliable method must be developed. Hence, the effects of six parameters towards production of *P. oxalicum* T3.3 spore will be observed. First, the effect of inoculum type can be determined by inoculating fermentation media with agar plug in which referring to the mycelia, and spore suspensions. Second, the effect of

inoculum size can be obtained by varying the number of agar plugs to be inoculated into the fermentation media. Third, the effect of medium proportion is determined by changing the ratio of the medium. Fourth, the effect of nitrogen source is investigated by observing the outcomes of the spore production in relation to the presence and absence of soya bean meal in the medium. Last but not least, temperature as well as pH of media effects on spore production can be determined by varying the range of incubation temperature and initial pH of the media. The highest amount of spore concentration recorded in each parameter will be considered as the optimum condition for *P. oxalicum* T3.3 to produce spore in SSF.

1.1 Objective of the study

The aims of this study were:

1. To prove the antagonistic activity of *P. oxalicum* T3.3 toward *C. gloeosporioides*

2. To study the effect of cultural conditions; inoculum type, inoculum size, medium proportion, nitrogen source, temperature as well as pH on spore production of *P. oxalicum* T3.3 in solid state fermentation.

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