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SUPPRESSION OF *Botrytis cinerea*, CAUSATIVE AGENT OF GRAY MOLD OF TOMATO BY ANTAGONISTIC BACTERIA

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SUPPRESSION OF *Botrytis cinerea*, CAUSATIVE AGENT OF GRAY MOLD OF TOMATO BY ANTAGONISTIC BACTERIA

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A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science

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ENDORSEMENT

This project report entitled "Suppression of *Botrytis Cinerea*, Causative Agent of Gray Mold of Tomato by Antagonistic Bacteria" is prepared by Nurul Huda bt Badarunzaman and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science.

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TABLE OF CONTENT

CHAPTER 3: Materials and methods

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
℃	Celcius
Cm	Centimetre
CRD	Completely Randomized Design
NA	Nutrient Agar
et al	et alia 'and others'
PCR	Polymerase Chain Reaction(PCR).
eDNA	Extracellular DNA
B.cinerea	*Botrytis cinerea*
LSD	Least significant differences
Hr	Hours
µl	Microlitre
µm	Micrometer
mg	Milligram
ml	Millilitre
et al	et alia 'and others'
PIRG	Percentage Inhibition of Radial Growth
PBS	Phosphate Buffered Saline
B. brevis	*Botrytis brevis*
PDA	Potato Dextrose Agar
NB	Nutrient Broth

Abstract

Botrytis cinerea is one of the most important fungal pathogen in tomato (*Lycopersicon esculentum Mill*.) worldwide. This fungal pathogen is also a major pathogen in other vegetables especially during postharvest stage. *Botrytis cinerea*, produces characteristic gray mold symptoms on fruits, leaves and stems within seven days following contamination. Thus, the objective of this study was to isolate and identify potential biocontrol agent (BCA) which have the ability to suppress growth of *B. cinerea in-vitro*. To achieve the objective, BCA was isolated from diseased and healthy tomato fruits. The *botrytis cinerea* was cultured in PDA medium and the bacteria was cultured in NA medium. The screening test was done via dual cultural test, culture filtrate test and spore suppression test. Finally, five potential BCA were identified in this test from bacteria isolate code BC4, BC23, BM4, BM11, and BM13 were found to be the most effective in suppressing growth of *B. cinerea*.

Abstrak

Botrytis cinerea, salah satu daripada patogen kulat yang paling penting dalam tomato (*Lycopersicon esculentum Mill*.) Hal Ini disebabkan kulat adalah patogen utama dalam sayur-sayuran lain di seluruh dunia terutama pada tanaman lepas tuai. *Botrytis cinerea*, menghasilkan ciri gejala acuan kelabu pada buah-buahan, daun dan batang dalam masa 7 hari selepas pencemaran. Oleh itu, objektif kajian ini adalah untuk mengasingkan dan mengenal pasti agen kawalan biologi yang berpotensi (BCA) yang mempunyai keupayaan untuk menyekat pertumbuhan *B. cinerea* dalam *in-vitro*. Untuk mencapai matlamat tersebut, BCA akan diasingkan daripada buah-buahan tomato berpenyakit dan sihat. Pathogen dari fungus *botrytis cinerea* dikultur dalam PDA media manakala bacteria dikultur di dalam NA media. Ujian saringan dilakukan melalui ‘dual culture test’, ‘culture filtrate test’, dan ‘spore suppression test’. Akhirnya, lima berpotensi BCA telah dikenal pasti dalam ujian ini adalah bakteria kod bernombor BC4, BC23, BM4, BM11, dan BM13 menunjukan potensi yang paling berkesan dalam menghalang dan mencegah pertumbuhan *Botrytis cinerea*.

CHAPTER 1

INTRODUCTION

Microorganisms such as bacteria, yeasts and fungi have gained attention recently as they were exhibited biological control ability that can help to reduce or contain certain diseases to be spread in crops. Generally, over the last two decades, the studied has been found that the biocontrol of *botrytis*-incited diseases with filamentous fungi, bacteria, and yeast capable to suppress the disease speared (Blakeman *et al.*, 1993). Biocontrol is the alternative to the conventional methods for the disease control because application of biocontrol control agents (BCAs) are the intensive way and environmental friendly while it also can reduce the mode of action the risk of resistance development. Since the world market for *Botrytis* control products increase by the year, the biocontrol product can help farmer to reduce their cost of pesticides either for conventional system or organic farming.

Newhook and wood (1951) discovered the common species of microorganism with antagonistic *Fusarium* spp. and *Penicillium claviforme* that originated from the same crop that can prevent primary progression of *B. cinerea*. They also investigated about saprophytic activity on dead lettuce tissue was found and it responsible to be control under the natural condition. The controlled of gray mold on glasshouse tomatoes by spraying method with the spore suspension of *Cladosporium herbarum* and *Penicillium* sp. on floral debris attached to the fruit. Besides that, the effective control of *B. cinerea* also can use *C. herbarum* as idea to control strawberry gray mold by protecting the flower under the fields condition (Bhatt and Vaughan,1962).

In addition, the research on chrysanthemum and beetroot leaves reported that there was the antagonistic effect of bacteria on *B. cinerea* (Blakeman and Brodie, 1976). Subsequently, *Trichoderma* spp. has been commonly used for control *B. cinerea* to the postharvest fruits such as grape, bean blossom, strawberry, and greenhouse crop (Eled *et al*., 1995; O'Neill *et a*l.,1996). The effectiveness in controlling *B. cinerea* using bacteria such as *Bacillus* and *Pseudomonas* has been proven to suppress the *Botrytis* conidiation (Redmond *et al.*,1987; Edward and Seddon,1992; Elad *et al.*,1994b)

Biocontrol agents has their strategy and ability to control the pathogens. For example *Botrytis* has behavior during the pre-penetration phase it can develop on healthy plant surface and the relative important of necrotic tissue within the crop as an inoculums source determine the ability plant to protect themselves with unfavourable condition. *Botrytis* was the strongly susceptible to competition condition because its dependence on an exogenous supply nutrients for germination, growth and infection on host. Sometime, *botrytis* conidia or germ-tube were sensitive to the effect of antibiotics and lytic enzymes that produced by microorganisms on plant surface. So, the antibiotic activity on plant that will inhibit germination to disease suppression may be based on reduction of pathogen saprophytic ability, spore dissemination or induced resistance in the host plant (Elad and Freeman,2002).

Microorganism such as bacteria was capable change the wettability of plant surface. Edward and Seddon(1992) discover the plant surface on Chinese cabbages applied with *bacillus brevis* can cause the water drops to spread and dry. Thus, decreasing the wetness and limit the period was suitable for *B. cinerea* germination or infection. It was common same with the effects when the formulation applied to plant surface for providing a secondary benefit from biocontrol research.

Antibiosis in biocontrol mechanism has been reported for many microorganisms. In natural ecosystem sometime hard to predict the importance inhibitory compound that was produced *in vitro* by antagonists. However, the ultimate prove for the role of these compounds in biocontrol was the loss of activity in non-producing mutants of the antagonist but in many case the mutant still operate as biocontrol activity because of the other mode of action. *B. cinerea* has been controlled by antibiotic producer of certain *bacillus* species in various hosts for example *B. brevis* secretes the antibiotic gramicidin S that was known to be effective against *B. cinerea* (Edward and seddon,1992).

In this study, *B. cinerea* pathogen are being tested *in vitro* by using non-identical bacteria that produce in tomato. Antagonistic bacteria were evaluated for their effectiveness in postharvest control of gray mould on fresh-market tomatoes. According to M. Mari *et.al,* (1996), Gray mould was reduced in fresh-market tomatoes treated with antagonists and artificially inoculated with *Botrytis cinerea* and stored at 20°C for at least 7 days. One strain, 5PVB (*Raciltus amyfoliquefaciens*) was particularly effective.

There was a lot of research carried out to investigate the biological control in *Botrytis cinerea* using bacteria. The reduction in inoculums production of *B. cinerea* especially in pathogen's ability will be suppressed and it also may built the positive effect over the other disease cycle in photosystem. Peng and Sutton (1991) found the several microorganisms have been reported to suppress sporalation of *B. cinerea* on strawberry. The same phenomenon was later exploited in various crops around the world especially postharvest crop. Due to its self-protective structure to defend itself ,pathogen will resistance to the fungicides if we still continue using same chemical control. Thus, in this study, the bacterial as a biocontrol agent is used to test the inhibition the growth of *Botrytis cinerea* in tomato by antagonist bacteria. The antagonist bacteria are normally isolate from endophytic bacteria within tissue of healthy fruits (Pratella, Mari, Guizzardi and Folchi, 1993). The objective of this study was to isolate and identify BCA agents against *B. cinerea* and assess its antagonistic activity.

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