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

ANTAGONISTIC ACTIVITIES OF EPIPHYTIC BACTERIA ON BLACK POD DISEASE OF COCOA

By

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A study was conducted to determine the antagonistic activity of epiphytic bacteria isolated from cocoa pod surface on *Phytophthora palmivora* (Butler) Butler, the causal agent of black pod disease of cocoa. Out of 233 isolates of epiphytic bacteria obtained from non symptomatic and diseased cocoa pods, only 8 were found to have antagonistic activities against the fungal pathogen when screened by the dual-culture method. Three isolates, (LKM/B/1, LKM/B/35, LKM/B/76c) were identified as *Pseudomonas putida* Biotype A, 2 isolates (LKM/B/5 and LKM/B/62b) were identified as *Pseudomonas aeruginosa* and one isolate each was identified as *Pseudomonas spinosa* (LKM/B/2), *Burkholderia glidioli* (LKM/B/4) and *Burkholderia* sp (LKM/B/6). Identification of the epiphytic bacteria was done using

the BIOLOG® Identification System. Percentage inhibition of the radial growth (PIRG) of *P. palmivora* by the epiphytic bacteria ranged from 66.0% to 82.1%. All isolates of epiphytic bacteria showed optimum antagonistic activities at 30^0 C. pH has no influence on the antagonistic activities of the epiphytic bacteria.

Detached pod studies showed that pods treated with the epiphytic bacteria retarded the growth of black pod lesion up to 12 days after inoculation.

Isolate *Burkholderia glidioli* (LKM/B/4), *Pseudomonas aeruginosa* (LKM/B/5) and *Pseudomonas putida* Biotype A (LKM/B/76c) produced volatile substances that affect the growth of *P. palmivora*, while isolates LKM/B/1 and LKM/B/35 of *Pseudomonas putida* Biotype A and isolate LKM/B62b of *Pseudomonas aeruginosa* did not show the production of volatile substances. Isolate LKM/B/76c also produced diffusible metabolites that could significantly inhibit the growth of *P. palmivora* compared to other epiphytic bacteria especially isolate LKM/B/35 which showed good antagonistic through dual culture method.

Microscopic observations of *P. palmivora* at the periphery of the inhibition zone indicated that all isolates of epiphytic bacteria inhibited *P. palmivora* by the process of cell wall degradation and growth retardation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Pertanian

AKTIVITI ANTAGONISTIK OLEH BAKTERIA EPIFIT KE ATAS PENYAKIT BUAH HITAM KOKO

Oleh

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Kajian ini di lakukan untuk mengenalpasti aktiviti antagonistik oleh bakteria epifit yang di pencilkan daripada permukaan buah koko ke atas kulat *Phytophthora palmivora* (Butler) Butler, agen penyebab penyakit buah hitam. Daripada 233 pencilan bakteria epifit yang di dapati, hanya 8 pencilan sahaja yang menunjukkan aktiviti antagonistik terhadap kulat penyebab penyakit melalui kaedah penyaringan 'dual-culture'. Tiga daripada pencilan iaitu LKM/B/1, LKM/B/35 dan LKM/B/76c telah dikenalpasti sebagai *Pseudomonas putida* Biotype A, dua pencilan iaitu LKM/B/5 dan LKM/B/62b di kenalpasti sebagai *Pseudomonas aeruginosa* dan setiap satu pencilan masing-masing di kenalpasti sebagai *Pseudomonas spinosa* (LKM/B/2), , *Burkholderia glidioli* (LKM/B/4) dan *Burkholderia* spp (LKM/B/6). Pengenalpastian

bakteria epifit dilakukan menggunakan kaedah pengenalpastian 'BIOLOG®' . Peratusan perencatan pada jejari pertumbuhan di kawasan perencatan adalah antara 66.0% hingga 82.1%. Kajian pada aktiviti antagonistik ke atas beberapa kesan suhu terhadap kesemua 8 isolat bakteria epifit menunjukkan terdapat interaksi antagonistik yang jelas di kawasan perencatan pada suhu 30^0C. Kajian pada kesan pH menunjukkan bakteria epifit tersebut tidak mempengaruhi aktiviti antagonistik.

Kajian pada buah koko yang dirawat dengan bakteria epifit menunjukan kesemua 8 pencilan bakteria epifit masing-masing merencatkan pertumbuhan lesion penyakit buah hitam berbanding dengan buah koko yang tidak dirawat dengan bakteria epifit selepas 12 hari inokulasi dilakukan. *Burkholderia glidioli* pencilan LKM/B/4, *Pseudomonas aeruginosa* pencilan LKM/B/5 dan *Pseudomonas putida* Biotype A pencilan LKM/B/76c mengeluarkan kesan bahan peruwapan yang memberi kesan terhadap pertumbuhan kulat *P. palmivora* manakala *Pseudomonas putida* Biotype A pencilan LKM/B/1 dan LKM/B/35 dan *Pseudomonas aeruginosa* pencilan LKM/B/62b tidak menunjukkan kesan peruwapan yang ketara. Pencilan LKM/B/76c juga mengeluarkan bahan metabolit peresap yang boleh merencat pertumbuhan kulat tersebut dengan bererti

berbanding dengan pencilan bakteria epifit yang lain terutama pencilan LKM/B/35 yang mana telah menunjukkan kesan antagonistk yang baik melalui kaedah penyaringan ‘dual-culture’.

Pemerhatian melalui mikroskop terhadap misilium kulat *P. palmivora* pada kawasan pertumbuhan yang aktif di zon perencatan menunjukkan kesemua 8 pencilan bakteria epifit merencat pertumbuhan kulat *P. palmivora* melalui proses penguraian dinding sel .

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LIST OF ABBREVIATIONS

CRD	Completely Randomised Design
HSD	Honestly Significant Difference
MARDI	Malaysian Agriculture Research and Development Institute
SAS	Statistical Analysis System

CHAPTER 2

LITERATURE REVIEW

2.1 Black pod disease management

For many years, black pod caused by *Phytophthora palmivora* (Butler) Butler is the most serious disease of cocoa (*Theobroma cacao* L.) in cocoa producing countries such as Malaysia, Indonesia, Papua New Guinea, Trinidad and Brazil. In some other countries in West Africa such as Ghana, Cote d'Ivoire, Cameroon, Nigeria and Togo the disease is also caused by *Phytophthora megakarya* (Akrofi and Opoku, 2000). This disease is a major limiting factor to successful cocoa production. The occurrence of *P. megakarya* in Ghana has considerably changed the black pod disease situation as pod losses in affected areas ranging from 60 % to 100 % (Akrofi and Opoku, 2000). However, infection of the disease differs from one region to another regions and depends on the management practices. The pathogen affects leaves, shoots, stems, roots, flower cushions, cherelles and pods. Sources of initial inoculum are mycelia, sporangia, zoospores and chlamydospores present in soil, pod piles, mummified pods previously infected, flower cushion, bark of cocoa and shade trees, and bark cankers (Opeke and Lorenz, 1974; Maddison and Griffin,1981). The pathogen survives as chlamydospores when conditions are adverse.

Conducive conditions for disease development are warm temperature of 15^{o}C to 30^{o}C, high relative humidity of 80% to 100% and high rainfall (Tarjot,1974;

Wood, 1985) . Gregory (1974) and Mackenzie *et al.* (1983) reported that black pod disease was more prevalent in areas of high rainfall with temperatures between 22-26^{o}C. The management of this disease relies heavily on chemical control which can be costly and labour intensive at times.

Pod infection is considered the most important element related to direct economic losses. Normally, cocoa pods can be infected by the pathogen at any stage of pod development. However most significant economic losses are incured due to the loss of the immature pods. Currently, there are several methods used in controlling cocoa black pod.

2.1.1 Cultural control

Cultural control is the most effective and economical method for management of the disease. Reducing the quantity of initial inoculum by removing the infected pods is essential before they begin to sporulate. Cultural control which include regular pruning, sanitation practices and frequent removal of infected pods can be effective in reducing the spread of the pathogen, but sometimes it becomes very labor intensive. Pruning the canopy during the low cropping season to increase the sunlight penetration is recommended to reduce the disease incidence. Pruning is also recommended to maintain the height of cocoa trees to facilitate the removal of infected pods. The differences in shade levels on farm have direct barrier on the incidence of black pod disease.

Cook (1978) pointed out that no single control measure has proven completely adequate for control, but a combination of one or more practices can reduce losses to a minimum.

2.1.2 Chemical Control

For over the years, cocoa pod have been sprayed with fungicides to minimize losses from black pod disease, but not always economically . These control procedures have been reviewed (Gregory, 1969 ; Thorold,1975 ; Cook,1978). At present, black pod disease is controlled using fungicides particularly during the wet season and during the main peak crop period when the disease incidence is high. Spraying fungicide reduce the population of the pathogen or to protect the pod from infection by the pathogen. It can be very effective but it is not cost effective and may have hazardous effects on health, environmental safety and may give detrimental effects on non-target organisms.

Currently, available fungicides that are widely used to control black pod are copper based fungicides, metalaxyl and fosetyl-aluminium. Metalaxyl and fosetyl-aluminium are systemic fungicides. They are effective to reduce and suppress the black pod disease and recommended when the infestation of disease is very high (Tey and Lee,1994; Tey and Bong,1990). Systemic fungicides are

effective to reduce and suppress the black pod disease and recommended when the infestation of disease is very high (Tey and Lee,1994; Tey and Bong,1990). Systemic fungicides are chemicals that can penetrate plant surface and translocate within the plant. Since systemic fungicides are active inside the plant, they could not be removed by rain or irrigation. Spraying with a copper fungicide is the standard control measure but never completely effective. Copper based fungicide is a contact fungicide. These fungicides can only prevent the disease from spreading but do not kill the pathogen, once it has entered the plant tissues. These chemical also remains on the plant surface for a short duration. The combination with appropriate cultural practices can reduce the level of disease infection.

2.1.3 Use of resistant planting materials

The use of resistant varieties of cocoa is the best way to control the disease . Resistant varieties are not only environment friendly, but also require little additional disease control inputs from farmers. Many promising cocoa varieties with various degrees of resistance to black pod have been reported (Bong *et al*, 1998; Ahmad Kamil and Yahya 2000), but none has been found to be fully resistant to the disease. A few clones such as PBC 123, BR 25 are resistant to the black pod disease(Ahmad Kamil and Yahya, 2000). Some of the clones used are susceptible to moderately tolerant to black pod although most of the clones are fairly

resistant to vascular streak dieback. Estates sector are virtually planted with monoclonal system, composed almost entirely of clone PBC 123. Under rehabilitation cocoa programme conducted by Malaysian Cocoa Board, most of the farmers are recommended to conduct the practice of budding the old cocoa plant with clone PBC 123 and BR 25, which is the best resistant clone to the disease at the moment.

2.1.4 Biological control

Potential biological control agent for the control of the fungal pathogen have been reported. *Pseudomonas flourescence*, isolated from the cocoa pod has been reported to be antagonistic to *P. palmivora* and was more effective than copper oxide and chlorathalonil (Galindo, 1992). Biological control agents isolated from healthy cocoa pods and infected pod surface (resident antagonists) can interfere with the growth of the pathogen. Epiphytic microorganisms, especially bacteria, are capable of inhibiting the growth of *P. palmivora* (Attafuah, 1965; Tarjot,1974; Frais and Garcia, 1985; Galindo, 1992). These evidance showed that biological control using microorganisms are highly promising for used to control black pod disease. Biological control also offers an environmental friendly approach to the management of cocoa disease and can be incorporated with cultural and physical control and limited chemical usage for effective integrated disease management system. Biological control avoids other problems of

chemical controls such as the development of pathogen resistance to the chemical and it is non-polluting and free of outbreak of secondary pests and diseases.

2.2 Prospects of biological control of plant pathogens

Currently, there is a great deal of interest in developing biological control technologies for disease management. Biological control of diseases on aerial plant parts is an important component of an intergrated approach to disease management. Additional research is needed to provide an understanding of the habitat in the phylloplane. Blakeman and Fokkema (1982), reported that there is strong evidence that natural biological control provides protection against many diseases in the field. Resident microorganisms multiply on healthy surfaces without affecting the plant. Resident microorganisms are specific to particular plants. The main microorganisms present on the plant surfaces are bacteria, fungi and yeasts (Blakeman and Fokkema, 1982 ; Cook and Baker, 1983). Sources of nutrients for epiphytic microorganisms and foliar pathogens are pollen, flower parts and other debris that fall on aerial parts (Cook and Baker, 1983). Lopez (1980), found that sugar, amino acids and phenols have been found in leachates from the pod wall of cocoa.

The environment on the aerial parts of the plant is characterized by wide and rapid fluctuations of moisture and temperature. Humidity on the phylloplane is one of the most important factors affecting growth and survival of epiphytic microorganisms like bacteria. To support growth of this epiphytic microorganisms, the surface of aerial plant parts must have a ‘film’ of water or a relative ambient humidity above 95 %. These conditions are met during rainfall or when water condenses at night and are frequently found in cocoa plantations (Alvim, 1977 ; Dickinson and Preece, 1976).

Microorganisms are exposed to sunlight and ultraviolet radiation. The main micro-climatic components of the plant environment are radiation, wind speed, temperature, humidity and carbon dioxide concentration. These factors are controlled by prevalent macro climatic conditions, crop density and by the shape, size and roughness of the surface of the plant tissue (Baker and Cook, 1974 ; Cook and Baker, 1983). In crops with a high canopy like cocoa, most of the radiation is absorbed by the leaves in the upper strata (Alvim,1977).