# Use of Promising Bacterial Strains for Controlling Anthracnose on Leaf and Fruit of Mango Caused by *Colletotrichum gloeosporioides*

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## ABSTRACT

A total 146 isolates of bacteria were taken from leaf surface, fruit skin, and blossom of mango (var. Nam Dorkmai). They were tested for the inhibition of mycelial growth of *Colletotrichum gloeosporioides*, a causal agent of anthracnose, on potato dextrose agar (PDA). Seventy-four bacterial isolates inhibited the growth of fungal mycelia by 24.51-49.10%. The 40 highly effective isolates out of 74 isolates were further tested for the potential to reduce the development of anthracnose lesion on detached leaves of mango marcotages at 24 h after inoculation of pathogen. Results indicated that 12 isolates provided high efficacy for inhibiting disease by 51.39-86.11%. Application of these bacteria on mango fruits at 24 h prior to the inoculation of the pathogen revealed that isolates B46 and B12 suppressed disease by 50.36 and 44.13% respectively while *Trichoderma harzianum* CB-Pin-01 provided 37.30% of the inhibition. For controlling post-harvest disease, an isolate B12 or B12 integrated with hot water treatment (55$^{o}$C) provided 91.33 and 88.00% of disease severity reduction respectively when applied at 24 h before inoculation of pathogen. Isolates B12 and B44 were identified as *Bacillus subtilis* while B46 and K112 were *B. licheniformis* and *B. cereus* respectively. The mechanism of these isolates for controlling *C. gloeosporioides* was the reduction of spore germination and the inhibition of germ-tube elongation.

**Key words:** Antagonist - *Colletotrichum gloeosporioides* - Biocontrol - Mango disease - Disease suppression

## INTRODUCTION

Anthracnose disease caused by *Colletotrichum gloeosporioides* (*Glomerella cingulata* Spauld & Schrenk) is one of the most common and serious diseases of mango (*Mangifera indica* L.) in the tropics (1). The disease occurs at any stage of fruit growth and the pathogen causes the disease on a wide range of hosts such as apple, pear, guava and mango (2). The most visible evidence of disease occurs on post-harvest mango fruit by latent infection (3) which usually results in commercial losses (4).

*C. gloeosporioides* is sensitive to temperature and humidity; germination and growth increase with the increase in temperature and humidity. Severe symptom occurs at 90% RH and 25-30$^{o}$C (5,6). Fallen infected leaves and blossoms under the tree are an important source of inoculum for further infection. The spores can be dispersed by wind and rain (7). The hyphal masses directly infect the leaves, blossom and fruit of mango, causing anthracnose symptom on leaves and latent infection on fruit skin.

Disease control methods include the prophylactic use of fungicides such as benomyl, prochloraz, mancozeb, carbendazim, iprodione and thiabendazol (8,9,10,11). Dipping of mango fruit with hot water at 50$^{o}$C for 30 min or 55$^{o}$C for 10 min (12) or with hot water at 52$^{o}$C supplemented with 0.1% of benomyl for 1-3 min can reduce antracnose, injury, and the remnants of chemical from hot water and chemical control (13). For biocontrol, antagonistic microorganisms are isolated and used to control anthracnose disease such as *Bacillus subtillis*, *B. cereus*, *B. licheniformis* and *Pseudomonas fluorescent* (14,15,16,17,18).

This investigation was aimed at screening antagonistic bacteria for effective suppression of anthracnose disease on detached leaf of mango and using selected antagonistic bacteria for the control of pre- and post-harvest antracnose on mango fruit.

## MATERIALS AND METHODS

### Isolation of Bacteria

Bacteria were isolated from leaf surface, fruit skin, and blossom of mango from 18 orchards in Nakhon Pathom using tissue transplanting technique (placing) modified from Agrios (19) and washing leaf technique (washing) (20). For the tissue transplanting technique, the plant materials were cut into small pieces (0.5×0.5 cm) and then washed with sterilized water. The plant samples were dried on sterilized tissue paper before being transferred to a Petri dish containing King's medium B (KB) (21), nutrient glucose agar (NGA) (21), Thornton's standardized agar (TSA) (22), and casein agar plus glucose (CGA) (23). The Petri dish was sealed with plastic wrap and incubated at room temperature (27$\pm$2$^{o}$C) for 3 days. The growing colony was subcultured on nutrient agar (NA) (23) for a single colony isolation before each single colony was subcultured in NA slant. The slant was kept at 10$^{o}$C in refrigerator and used as a stock culture. This stock was subcultured every 3 months. For washing leaf, a gram of small plant material was mixed with 5 ml of sterilized water in a small flask which was shaken on a shaker at 140 rpm for 30 min. Then 0.1 ml of suspension sample was dropped and spread in a Petri dish containing various media as described above.

Isolation of the pathogen from Nam Dorkmai mango fruit with anthracnose symptom was performed by tissue transplanting technique as described above using potato dextrose agar (PDA) (24).

### Efficacy of Inhibition of Mycelial Growth on Agar

Five-day-old culture of *C. gloeosporioides* (virulent isolate) on PDA and 2-day-old bacterial antagonists on NA were used in this experiment. A mycelial plug of *C. gloeosporioides* was cut from colony margin by a 0.8 cm in diameter cork borer and

placed onto the center of a Petri dish containing PDA. Two days after pathogen transfer, a tested bacterial isolate was transferred to the dish at four spots in a cross manner, each spot was 3 cm away from the center of the pathogen's plug. The dish was sealed with a plastic wrap and incubated at room temperature for 3 days. Percent inhibition of mycelial growth of *C. gloeosporioides* and the clear zone caused by bacterial isolates were recorded daily (16).

**Efficacy of Control of Antracnose on Detached Mango Leaves**

A pathogenicity test of *C. gloeosporioides* isolate on detached mango leaves was conducted. Spore suspension was prepared from 7-day-old culture of *C. gloeosporioides* growing on PDA. Concentration of spores in a suspension was counted and adjusted with haemacytometer to $1\times10^5$ spores per ml. This suspension was sprayed on young detached leaves of mango before the inoculated leaves were incubated in a moist plastic box. The box was placed at room temperature ($27\pm2^oC$) for 4 days and disease severity was daily recorded for comparison with a control treatment of mango leaves sprayed with sterilized water. There were 4 plastic boxes (5 leaves per box) for each treatment.

For disease control efficacy test, a suspension of cells of antagonistic bacteria ($1\times10^8$ cfu) was prepared from 2-day-old culture growing on NGA. A Petri dish was flooded with sterilized water and cells of bacteria were scraped with sterilized glass rod. The cell suspension was measured with spectrophotometer and adjusted to 0.2 OD (optical density) at 600 nm wavelength with sterilized water in order to obtain $1.0\times10^8$ cfu. The cell suspension of each isolate was sprayed on a young Nam Dorkmai mango leaf before the leaf was incubated in a moist plastic box for 24 h. All leaves were then sprayed with spore suspension of *C. gloeosporioides* before further incubated in a plastic box at room temperature for 4 days. Percent of disease control was recorded and compared with a control treatment (detached mango leaf sprayed with sterilized water).

**Efficacy of Antagonistic Bacteria in a Glasshouse**

Nam Dorkmai mango marcotages grown in a glasshouse and a spore suspension of *C. gloeosporioides* ($1\times10^5$ spores per ml) and bacterial antagonists ($1\times10^8$ cfu) were prepared as previously described. Two experiments were conducted separately. For the first experiment, cell suspensions of antagonistic bacteria were sprayed on young leaves of mango marcotage and the protected leaves were covered with a plastic bag for 24 h before they were inoculated with the spore suspension of *C. gloeosporioides* and again covered with a plastic bag for another 24 h. Percent of disease severity was recorded every 3 days for comparison with the chemical and control treatments (sterilized water). For another experiment, all procedures were the same as previously described in the first experiment except for the spraying at 24 h of the cell suspensions of antagonistic bacteria after the inoculation of *C. gloeosporioides.* There were five replications in each treatment and the experiment was designed as randomized complete block design (RCBD).

**Inhibition of Spore Germination and Germ-tube Elongation**

Antagonistic bacteria and *C. gloeosporioides* were prepared as cell and spore suspensions as described above. For inhibition of spore germination test, the cell suspension of bacteria ($1\times10^8$ cfu) was mixed with spore suspension of *C. gloeosporioides* ($1\times10^5$ spores per ml) at ratio 1:1 by volume in a test tube and kept for 12 h at room temperature before the sample was stained with lactophenol cotton blue. The mixed sample was observed and recorded under compound light microscope (25).

In germ-tube elongation test, spore suspension of *C. gloeosporioides* was incubated at room temperature for 12 h for most of spores germinated. Then the spore suspension was mixed with cell suspension of antagonistic bacteria and incubated at room temperature for 12 h. The mixed sample was stained with lactophenol cotton blue before the length of germ-tube was measured under light microscope and compared with a control treatment.

**Taxonomy for Species of Promising Isolates**

Promising strains of bacterial antagonists were tested for producing endospore by straining with malachite green. After that they were identified as species by using Biolog Microlog System, Release 4.2.

**Post-harvest Control of Anthracnose on Mango Fruits**

Nam Dorkmai mango fruits in post-harvest stage were used in this experiment. The fruit was disinfected with 70% alcohol before getting pierced with a sterilized needle to open three wounds on each fruit. Two experiments were separately performed. In the first experiment, 0.1 ml spore suspension of *C. gloeosporioides* ($1\times10^5$ spores per ml) was inoculated at the wounded areas on the mango fruits which were then incubated at room temperature for 24 h prior to the spray of cell suspension of bacterial antagonist ($1\times10^8$ cfu, OD=0.2). For the second experiment, mango fruits were sprayed with the bacterial antagonist and kept for 24 h at room temperature before they were inoculated with *C. gloeosporioides*. The fruits from two experiments were incubated at room temperature ($27\pm2^oC$) before disease severity was observed and recorded.

**Statistical Analysis**

All data were analyzed by ANOVA using the GLM procedure of the SAS system for Windows. The model used was $Y_{ij} = \mu + treatment_i + e_{ij}$, where $Y_{ij}$ = measured value such as percentage of disease control, number of spore germination, $\mu$ = general mean, $treatment_i$ = various strains of bacterial antagonist, and $e_{ij}$ = residual error. Duncan's multiple range test (DMRT) was used to evaluate the different response values between treatment groups.

## RESULTS

### Isolation of Bacteria

A total of 146 isolates of bacteria were isolated from mango plants (leaves surface, fruit skin and blossom). Ninety-seven, 35, and 14 isolates were isolated from leaf surface, fruit skin, and blossom of mango respectively. Of the 97 isolates from leaf surface, 60 and 37 isolates were recovered by using tissue transplanting and washing leaf techniques respectively. Out of the 35 isolates from fruit skin, 24 and 11 isolates were isolated by using tissue transplanting and washing leave techniques respectively. Of the 14 isolates from blossom, 10 and 4 isolates were obtained by means of tissue transplanting and washing leaf techniques respectively **(Table 1)**.

**Table 1.** Isolates, code, method for isolation and source of bacterial isolates from leaf surface, fruit skin, and blossom of mango from Bangkhen and Kamphaeng Saen Districts.

| Isolate | Code[1/] | Method for isolation[2/] | Source |
|---|---|---|---|
| 01-38 | B01-B38 | Tissue | leaf surface |
| 39-52 | B23-B52 | Washing | leaf surface |
| 53-62 | B53-B62 | Placing | fruit skin |
| 63-74 | B63-B74 | Washing | fruit skin |
| 75-80 | B75-B80 | Placing | blossom |
| 81 | B81 | Washing | blossom |
| 82-104 | K82-K104 | Placing | leaf surface |
| 105-126 | K105-K126 | Washing | leaf surface |
| 127-134 | K127-K134 | Placing | fruit skin |
| 135-139 | K135-K139 | Washing | fruit skin |
| 140-143 | K140-K143 | Placing | blossom |
| 144-146 | K144-K146 | Washing | blossom |

[1]Codes of bacterial isolate beginning with B and K were isolated from Bangkhen and Kamphang Saen Districts respectively.
[2]Placing =Tissue transplanting technique; Washing = Washing leaf technique.

### Efficacy for Inhibiting Mycelial Growth on Agar

Seventy-four out of 146 isolates could inhibit a mycelial growth of *C. gloeosporioides* on PDA at room temperature by 24.51-49.10% compared with a control treatment (*C. gloeosporioides* grown in a Petri dish without bacterial antagonist). Especially, isolates K136, K109, B13 and B17 provided 49.10, 48.81, 48.20 and 47.90% of inhibition respectively. There were 55 out of 74 isolates which could inhibit mycelial growth of *C. gloeosporioides* by producing clear zones. Especially, isolates B15, K125 and K142 showed high efficacy for producing clear zones up to 0.5 cm **(Table 2)**.

**Table 2.** Efficacy of bacteria isolated from leaf surface, fruit skin, and blossom of mango for inhibiting mycelial growth of *Colletotrichum gloeosporioides* on various media at 4 days after incubation.

| Isolate | Method for isolation | Medium | Percent inhibition | Clear zone (cm) | Type of action[1] |
|---|---|---|---|---|---|
| B01 | Placing | TSA | 45.85 a-c[2] | 0.2 | A |
| B02 | " | " | 46.10 ab | 0.3 | A |
| B11 | " | PDA | 40.50 cd | 0.2 | A |
| B12 | Washing | CGA | 41.68 cd | - | B |
| B13 | " | " | 48.20 a | 0.4 | A |
| B14 | " | NGA | 40.48 cd | - | B |
| B15 | " | " | 45.25 a-c | 0.5 | A |
| B17 | " | PDA | 47.90 ab | 0.2 | A |
| B19 | " | " | 41.68 cd | 0.2 | A |
| B20 | " | CGA | 41.10 cd | - | B |
| B28 | " | NGA | 35.73 f | - | B |
| B29 | " | " | 44.65 a-c | 0.3 | A |
| B30 | " | YDC | 27.98 g | 0.1 | A |
| B41 | Washing | YMA | 46.41 ab | 0.2 | A |
| B43 | " | YDC | 47.65 ab | 0.4 | A |
| B44 | Placing | NGA | 42.36 b-d | 0.1 | A |
| B45 | " | KB | 47.00 ab | 0.2 | A |
| B46 | " | NGA | 44.41 a-c | 0.1 | A |
| B51 | " | " | 40.50 cb | 0.1 | A |
| B66 | Placing | YDC | 45.21 a-c | 0.2 | A |
| B67 | " | NGA | 45.91 a-c | 0.4 | A |
| B68 | " | CGA | 42.00 a-c | 0.3 | A |
| B69 | " | " | 36.58 ef | - | B |
| B78 | Washing | GYPA | 45.23 a-c | 0.4 | A |
| B79 | Placing | NGA | 24.51 f | - | B |
| B80 | " | " | 44.91 a-c | 0.3 | A |
| K82 | " | CGA | 47.57 ab | 0.1 | A |
| K83 | " | PDA | 39.33 de | - | B |
| K95 | Washing | " | 44.72 a-c | 0.2 | A |
| K102 | " | NGA | 41.15 cd | 0.1 | A |
| K103 | " | YDC | 43.81 b-d | 0.4 | A |
| K105 | Placing | CGA | 47.00 ab | 0.4 | A |
| K106 | " | " | 41.72 cd | - | B |
| K109 | " | " | 48.81 a | 0.3 | A |
| K112 | Washing | " | 43.51 b-d | 0.2 | A |
| K113 | " | GYPA | 38.71 de | - | B |
| K116 | Washing | YMA | 39.30 de | - | B |
| K117 | Placing | TSA | 38.07 de | - | B |
| K122 | " | YDC | 35.70 f | - | B |
| K125 | " | KB | 42.34 b-d | 0.5 | A |
| K126 | " | CGA | 45.81 a-c | 0.3 | A |
| K131 | " | NGA | 42.93 b-d | - | B |
| K132 | Washing | CGA | 43.52 b-d | 0.1 | B |

**Table 2.** (Continue)

| Isolate | Method for isolation | Medium | Percent inhibition | Clear zone (cm) | Type of action[1] |
|---|---|---|---|---|---|
| K133 | " | GYPA | 42.61 b-d | 0.3 | A |
| K136 | " | YDC | 49.10 a | 0.4 | A |
| K138 | Placing | " | 36.31 ef | - | B |
| K139 | " | KB | 42.90 b-d | 0.4 | A |
| K141 | " | PDA | 42.89 b-d | 0.2 | A |
| K142 | " | CGA | 47.58 ab | 0.5 | A |
| K143 | " | KB | 46.43 ab | 0.2 | A |
| K146 | " | YDC | 39.91 df | - | B |
| Control | - | - | 0 | - | - |

[1]Types of action on PDA plate: A = inhibit mycelial growth of *Colletotrichum gloeosporioides* and produce clear zone; B = inhibit mycelial growth of *C. gloeosporioides* but do not produce clear zone; C = no inhibitive effect.
[2]Means in a column followed by the same letter(s) are not significantly different according to DMRT (P=0.05).

**Efficacy for Controlling Antracnose on Mango Leaves**

Twelve out of 40 isolates of antagonistic bacteria could control antracnose disease on mango leaves in laboratory. Diameter of lesion was markedly reduced when compared with a control treatment. Especially, isolates B46, B12, B44, K122 and K132 provided high efficacy for controlling disease with 86.11, 70.83, 65.28, 63.89 and 62.50% of inhibition respectively as compared with a control treatment **(Table 3)**.

**Table 3.** Efficacy of promising antagonistic bacteria for controlling anthracnose disease on detached leaves of mango in laboratory.

| Treatment | Average diameter of lesion (cm) | Disease inhibition (%) |
|---|---|---|
| B02 | 0.75 cd[1] | 58.34 bc[1] |
| B12 | 0.53 b | 70.83 b |
| B44 | 0.63 bc | 65.28 bc |
| B46 | 0.25 a | 86.11 a |
| K83 | 0.85 c-e | 52.78 c-e |
| K103 | 0.70 cd | 61.11 bc |
| K105 | 0.73 cd | 59.72 bc |
| K112 | 0.65 bc | 63.89 bc |
| K122 | 0.88 c-e | 51.39 c-e |
| K132 | 0.68 bc | 62.50 bc |
| K136 | 0.78 cd | 56.95 b-d |
| K139 | 0.80 c-e | 55.56 b-d |
| Control | 1.75 f | 0.00 f |

[1]Means in a column followed by the same letter(s) are not significantly different according to DMRT (P=0.05).

**Efficacy for Controlling Disease under Glasshouse Condition**

All four promising isolates of antagonistic bacteria demonstarted high efficacy for controlling anthracnose disease on young mango leaves when sprayed before or after inoculation of *C. gloeosporioides* as compared with a control treatment. All treatments sprayed with bacterial antagonists at 24 h before pathogen's inoculation provided higher efficacy for controlling anthracnose than treatments with bacterial spray after inoculation of a pathogen for 24 h. A treatment sprayed with isolate B46 provided the highest efficacy for controlling plant disease (50.36% of inhibition) while treatments sprayed with *Trichoderma harzianum* CB-Pin-01 (commercial strain of antagonistic fungus) or benomyl provided 37.30 and 43.85% of inhibition respectively as compared with a control treatment **(Table 4)**.

**Table 4.** Efficacy of antagonistic bacteria for controlling antracnose disease on mango leaves when sprayed before and after 24 h of pathogen inoculations under glass house condition.

| Treatments | Spray before inoculation of pathogen | Spray after inoculation of pathogen |
|---|---|---|
| | Inhibition (%)[1] | Inhibition (%)[1] |
| B12 | 44.13 a[2] | 36.48 ab[2] |
| B44 | 38.91 ab | 29.49 ab |
| B46 | 50.36 a | 34.56 ab |
| K112 | 18.24 b | 9.54 c |
| Benomyl | 43.85 a | 41.35 a |
| T- CB-Pin-01[3] | 37.30 ab | 23.82 b |

[1]Calculation by a formula (∅ of control treatment-study treatment)/ ∅ of control treatment) × 100 when ∅ represented diameter of disease lesion.
[2]Means in a column followed by the same letter(s) are not significantly different according to DMRT.
[3]A commercial strain of antagonistic fungus *Trichoderma harzianum.*

**Inhibition of Spore Germination and Germ-tube Elongation**

All promising isolates of bacterial antagonists significantly (P=0.05) inhibited a spore germination of *C. gloeosporioides* after incubation of the mix of bacteria and pathogen suspensions at room temperature for 12 h. Especially, isolates B44 and B46 provided the highest efficacy for reducing percent of spore germination by 20.00 and 22.67% respectively while all isolates significantly (P=0.05) inhibited germ-tube elongation of *C. gloeosporioides* with isolate B46 provided the highest efficacy forinhibition. The length of germ-tube of *C. gloeosporioides* when treated with cell suspension of B46 was 24.00 µm while control was 55.50 µm, **(Table 5)**.

**Table 5.** Efficacy of bacterial antagonists for inhibiting spore germination and germ-tube elongation of *Colletotrichum gloeosporioides* within 12 h of incubation.

| Treatments | Spore germination (%)[1] | Length of germ-tube (㎜)[2] |
|---|---|---|
| B12 | 24.00 b[3] | 21.30 b[3] |
| B44 | 20.00 a | 22.13 b |
| B46 | 22.67 b | 24.00 c |
| K112 | 29.67 c | 12.30 a |
| Control | 52.00 d | 55.50 d |

[1]Mean of spore germination calculated from 4 replications (100 spores per replication).
[2]Mmean of germ-tube's length from 4 replications (100 germ-tubes per replication).
[3]Mmeans in a column followed by the same letter(s) are not significantly different according to DMRT (P=0.05).

**Taxonomy for Species of Promising Isolates**

All promising bacterial antagonists showed green pigment when tested with malachite green. Identification of promising bacterial isolates by using Biolog Microlog System, Release 4.2 showed that bacterial antagonists were *Bacillus* spp., isolates B12 and B44 were *B. subtilis*, while B46 and K112 were *B. licheniformis* and *B. cereus* respectively.

**Post-harvest Control of Anthracnose on Mango Fruits**

All promising isolates of bacteria and T-CB-Pin-01 (a commercial strain *of Trichoderma harzianum*) could reduce disease severity on mango fruits. Spray application of bacterial antagonist before inoculation of *C. gloeosporioides* for 24 h revealed that treatments with B12 and B12 combined with hot water treatment (55$^{o}$C for 5 min) provided 91.33 and 88.00% of disease control respectively. The treatment with benomyl combined with hot water provided only 49.33% of disease control.

When bacterial antagonists were applied at 24 h after mango fruits were inoculated with *C. gloeosporioides*, the result showed that all bacterial antagonists, T-CB-Pin-01 and benomyl, could reduce the disease severity. Especially, treatments with B46, B12 combine with hot water, and B12 and benomyl combined with hot water provided 75.53, 66.49, 65.43 and 63.30% of disease reduction respectively as compared with a control treatment **(Table 6)**.

**Table 6**. Efficacy of bacterial antagonists, *Trichoderma harzianum* CB-Pin-01 and benomyl, for reducing antracnose disease on mango fruits when sprayed before or after inoculation of *Colletotrichum gloeosporioides* for 24 h.

| Treatment | Percentage of disease reduction (%)[1] | |
|---|---|---|
| | Spray application at 24 hr before inoculation of *C. gloeosporioides* | Spray application at 24 hr after inoculation of *C. gloeosporioides* |
| B12 | 91.33 a[2] | 65.43 a |
| B46 | 76.67 b | 75.53 a |
| T-CB-Pin-01 | 50.00 c | 25.53 b |
| B12 + hot water | 88.00 a | 66.49 a |
| B46 + hot water | 52.67 c | 29.79 b |
| T-CB-Pin-01+ hot water | 46.00 c | 15.43 b |
| benomyl+ hot water | 49.33 c | 63.30 a |

[1]Percentage of disease reduction was calculated by a formula: (∅ of control treatment- ∅ of testing treatment)/ ∅ of control treatment ×100.

[2]Means in a column followed by the same letter(s) are not significantly different according to DMRT (P=0.05).

## DISCUSSION

Ninety-seven out of 146 bacterial isolates were isolated from surface of mango leaves. This result indicated that most bacteria could grow and survive on the surface of mango leaves. This is probably due to the fact that the leaf has a larger surface area than that of the fruit and blossom. It also produced some nutrients suitable for growth of bacteria (25).

For the efficacy test *in vitro*, seventy-four out of 146 bacterial isolates inhibited mycelial growth of *C. gloeosporioides.* This suggested that most bacteria on leaf surface, fruit skin, and blossom of mango were the potent antagonist of *C. gloeosporioides*. Similar evidence was reported by Koomen and Jeffries (16). Our result showed that most of bacterial antagonists could produce antibiotics since 55 out of 74 antagonistic bacteria produced clear zones on an agar plate. A researcher (26) reported that *B. amyloliquefacien* produced a clear zone (antibiotic) to inhibit pathogen on agar test. Our bacterial antagonists, which were able to produce antibiotics, provided greater efficacy for inhibiting mycelial growth of *C. gloeosporioides* than bacterial antagonists without antibiotic productivity (no clear zone). This indicated the important role of bacterial metabolites in the inhibition of mycelial growth of *C. gloeosporioides*. Results from this study were supported by many reports (26,27,28,29,30,31).

In 1997, some researchers (14) successfully controlled antracnose on mango fruits using *B. subtilis*, *P. fluorescens* and *Sporobolomyces* sp. while, Baker et al (27) using *B. licheniformis* isolated from the skin of mango fruit to control post-harvest antracnose disease on mango. Our findings were consistent with these reports since promising isolates of bacterial antagonists provided high efficacy for controlling

antracnose disease both on young leaves and post-harvest mango fruits. Moreover, Korsten (17) demonstrated that cell suspension of *B. licheniformis* could reduce antracnose disease on mango fruits, and its efficacy was comparable to that of chemical treatment.

Spray application of bacterial antagonist on mango fruits before inoculation with *C. gloeosporioides* provided greater efficacy for controlling antracnose disease than spray application after pathogen inoculation suggested that the bacterial antagonist might require a certain period of time to colonize fruit surface and to produce some antibiotics for protecting the fruit against the pathogen.

Our promising isolates of bacterial antagonists inhibited spore germination and germ-tube elongation of *C. gloeosporioides* when compared with a control treatment. This result was supported by the report of McKeen et al (30) which showed that *B. subtilis* produced antibiotic iturin A that effectively inhibited spore germination of *Monilinia fructicola* while Fiddaman and Rossall (32) found that *B. subtilis* produced antibiotics to inhibit germination of *Rhizoctonia solani*.

There were many reports that *B. subtilis* (14,27,30,32,33), *B. licheniformis* (18), *B. cereus* (15) and *Bacillus* spp. (26,28,29,31) were promising bacterial antagonists for controlling plant diseases. The identification of four promising bacterial antagonists revealed that isolates B12 and B44 were *B. subtilis*, while B46 and K112 were *B. licheniformis* and *B. cereus* respectively. This was consistent with the findings of many previous studies.

## ACKNOWLEDGEMENTS

The authors would like to thank The Graduate School of Kasetsart University for financial support.

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## บทคัดย่อ

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**การใช้แบคทีเรียสายพันธุ์ที่ดีสำหรับควบคุมโรคแอนแทรคโนสบนใบและผลมะม่วงที่เกิดจากเชื้อรา** *Colletotrichum gloeosporioides*

เชื้อแบคทีเรีย 146 ไอโซเลท ถูกแยกจากผิวใบ ผิวผล และช่อดอกของมะม่วง (พันธุ์น้ำดอกไม้) เมื่อนำเชื้อทั้งหมดไปทดสอบประสิทธิภาพบนอาหาร PDA ในการยับยั้งการเจริญของเส้นใยเชื้อรา *Colletotrichum gloeosporioides* ซึ่งเป็นเชื้อสาเหตุโรคแอนแทรคโนส พบว่า 74 ไอโซเลท สามารถยับยั้งการเจริญของเชื้อราสาเหตุโรคได้ในช่วง 24.51-49.10 เปอร์เซ็นต์ นำเชื้อแบคทีเรีย 40 จากทั้งหมด 74 ไอโซเลท ที่มีประสิทธิภาพสูงไปทดสอบความสามารถในการลดขนาดแผลบนใบมะม่วงโดยการฉีดพ่นเชื้อแบคทีเรียหลังจากปลูกเชื้อสาเหตุโรค 24 ชั่วโมง พบว่า 12 ไอโซเลท มีประสิทธิภาพในการควบคุมโรคที่ 51.39-86.11 เปอร์เซ็นต์ เมื่อนำไปประยุกต์ใช้ในการควบคุมโรคบนผลมะม่วงโดยพ่นเชื้อหลังจากปลูกเชื้อสาเหตุโรค 24 ชั่วโมง พบว่าไอโซเลท B46 และ B12 สามารถยับยั้งโรคที่ 50.36 และ 44.13 เปอร์เซ็นต์ ตามลำดับ ขณะที่เชื้อรา *Trichoderma harzianum* CB-Pin-01 สามารถควบคุมโรคที่ 37.30 เปอร์เซ็นต์ สำหรับการควบคุมโรคหลังการเก็บเกี่ยวพบว่ากรรมวิธีที่ฉีดพ่นไอโซเลท B12 อย่างเดียว หรือฉีดพ่น B12 ร่วมกับน้ำร้อน (55 องศาเซลเซียส) เป็นเวลา 24 ชั่วโมง ก่อนปลูกเชื้อสาเหตุโรคพืชพบว่ามีประสิทธิภาพในการลดความรุนแรงของโรคที่ 91.33 และ 88.00 เปอร์เซ็นต์ ตามลำดับ จากการจำแนกเชื้อ พบว่าไอโซเลท B12 และ B44 เป็นเชื้อ *Bacillus subtilis* ขณะที่ไอโซเลท B46 และ K112 เป็นเชื้อ *B. licheniformis* และ *B. cereus* ตามลำดับ ในการศึกษากลไกของเชื้อแบคทีเรียในการควบคุมเชื้อรา *C. gloeosporioides* พบว่า การยับยั้งการงอกของสปอร์และการยับยั้งการเจริญของ germ-tube เป็นกลไกที่สำคัญ

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