Walailak J Sci & Tech 2008; 5(1): 29-38.

Efficacy of Antifungal Metabolites of *Bacillus* spp. for Controlling Tomato Damping-off Caused by *Pythium aphanidermatum*

Warin INTANA[1], Prakong YENJIT[1], Taksin SUWANNO[1], Supalak SATTASAKULCHAI[1], Manoon SUWANNO[1] and Chiradej CHAMSWARNG[2]

[1]School of Agricultural Technology, Walailak University, Nakhon Si Thammarat 80161, Thailand
[2]Department of Plant Pathology, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Nakhon Pathom 73140, Thailand

(E-mail: iwarin@wu.ac.th)

ABSTRACT

A total of 4 strains of bacteria were isolated from the leaf surface of the rambutan using a tissue transplanting technique. They were characterized, by a dual culture test, for their efficacy to inhibit mycelial growth of *Pythium aphanidermatum*, a causal agent of the damping-off on tomato. All 4 strains significantly inhibited mycelial growth of *P. aphanidermatum* on potato dextrose agar (PDA) at room temperature (27 °C). B-NST-02 and B-NST-03 gave values of inhibition of 62.0 % and 57.5 %, respectively. All strains were identified as *Bacillus* spp. Antifungal metabolites extracted from all 4 strains were tested at 1,000 mg/l. Tomato seedlings treated in the laboratory with metabolites from B-NST-03 and B-NST-02 showed germination of 85.5 % and 82.0 %, respectively. Under glasshouse conditions, seedling treated with metabolites from B-NST-03 and B-NST-02 provided seed germination rates were 92.5 % and 92.0 %, respectively, while the controls treated with either sterile water or 2 % methanol had only 28.0 % and 26.5 % seed germination rates, respectively. In *P. aphanidermatum* viability test, mycelia of *P. aphanidermatum* treated with antifungal metabolites from 4 strains of *Bacillus* spp. showed no visible growth, while the control with sterile water or 2 % methanol, mycelia of *P. aphanidermatum* rapidly grew and covered the whole surface of the PDA in the Petri dish within 5 days.

Keywords: Antifungal metabolite, tomato damping-off, *Bacillus* spp., biological control

INTRODUCTION

Damping-off disease caused by *Pythium aphanidermatum* is a major disease of several plants including tomato. This fungus causes severe damage to the tomato in both pre and post emergence stages. This disease can be controlled by some fungicides such as metalaxyl. However, side effects from fungicide usages were noted such as acquisition of resistance of this pathogen and potential oncogenic risks of the fungicide. The application of biological control agents (BCAs) and their antifungal metabolites to control this disease could be an interesting alternative to fungicides. *Trichoderma* spp. and *Bacillus* spp. [1,2] have been successfully used for disease control in a variety of important plant pathogens such as *P. aphanidermatum*, *Cercospora* sp., *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* and *Rhizoctonia solani* [3-7]. The mechanisms underlying these bacterial antagonisms for plant pathogens involve antibiosis, competition for nutrients or space, enhancement of root and plant development, induction of plant resistance, solubilization and sequestration of inorganic nutrients and/or inactivation of the pathogen's enzymes [8]. Antibiosis, in particular, is the most important mechanism for control of a plant disease. The aims of this investigation were to: 1) Evaluate the *in vitro* inhibition effects of *Bacillus* spp. on *P. aphanidermatum* mycelial growth, 2) Evaluate the effect of antifungal metabolites from *Bacillus* spp. under laboratory and glasshouse conditions, and 3) Identify the genus of promising strains of *Bacillus* spp.

MATERIALS AND METHODS

Microorganisms

Bacteria were isolated from rambutan leaf surfaces in various orchards in Nakhon Si Thammarat using a tissue transplanting technique modified from Agrios [9]. The plant materials were cut to small pieces (0.5×0.5 cm^2) and washed with sterile water. Then, the plant samples were dried on sterile soft paper before transferring to a Petri dish containing nutrient glucose agar (NGA). The Petri dish was then sealed with plastic wrap and incubated at room temperature (27 ± 2 °C) for 3 days. The growing colonies were subcultured on nutrient agar (NA) for single colony isolation and each single colony was resubcultured in NA slant. The slant was kept at 10 °C and used as a stock culture. This stock culture was subcultured every 3 months.

Isolation of a pathogen from tomato plants with damping-off symptoms was performed on potato dextrose agar (PDA) by the tissue transplanting technique as described above. The most pathogenic isolates were selected for further study.

Taxonomy for genus of bacterial strains

Morphological characteristics of all bacteria grown on NA at room temperature for 2 days were observed. Gram test of bacteria was perform by using 3 % KOH [10],

while the test for endospore formation was conducted by staining with 5 % malachite green and safranin O [10].

In vitro evaluation of the inhibition effects of *Bacillus* spp. on *Pythium aphanidermatum* mycelial growth

Three-day-old cultures of *P. aphanidermatum* on PDA and 2-day-old bacterial antagonists on NA were used in this experiment. A mycelial plug of *P. aphanidermatum* was cut from the colony margin by a 0.8 cm diameter cork borer and placed onto the center of a Petri dish containing PDA. After transferring the pathogen, each bacterial isolate was added to the dish as 4 spots in a cross design, each spot was 3 cm apart from the center of the pathogen's plug. The dish was sealed with plastic wrap and incubated at room temperature for 3 days. Percent inhibition of mycelial growth of *P. aphanidermatum* and clear zone caused by bacterial isolates were recorded daily. The design used for this experiment was a complete randomized design (CRD) with 4 replications [4]. A Petri dish without any bacteria antagonist was also used as a control.

Extraction of antifungal metabolites

Two-day-old cultures of bacteria on NA were flooded with sterile water and cells of bacteria were scraped with a sterilized glass rod. The cell suspension was measured with a spectrophotometer and adjusted to 0.2 optical density (wavelength 600 nm, Spectronic 1001, Bausch and Lomb) with sterile water in order to obtain 1.0×10^8 cell/ml suspension. Three ml of cell suspension of each strain was inoculated into 1 l of nutrient broth (NB) and incubated at 27 ± 2 °C on a rotary shaker (160 rpm). Four days after incubation, the bacterial culture was extracted with ethyl acetate (EtOAc) and EtOAc was later removed at 40 °C using a rotary evaporator. The dry weight of antifungal metabolites as crude extracts were recorded before being mixed with 25 ml, 2 % methanol, and adjusted to a concentration of 1,000 mg/l. Then the stock solution was kept at 10 °C [11].

Viability of *Pythium aphanidermatum*

The pathogen was subcultured on PDA covered with a dialysis membrane for 2 days. Then, the membrane covered with mycelia was cut (0.5×0.5 cm^2), placed into a sterile Petri dish and flooded with 10 ml of crude extracts of *Bacillus* spp. (1,000 mg/l) [11]. After 24 h of incubation, the membrane was rinsed with sterile water 3 times and placed onto sterile soft paper. Then, the membrane was put into a Petri dish containing PDA supplemented with 1 mg/l streptomycin sulfate and incubated at room temperature. The mycelial growth of *P. aphanidermatum* was evaluated 5 days later. The design used for this experiment was a complete randomized design (CRD) with 5 replications, 10 membranes per a replicate [11]. Treatment without any chemical and treatment with metalaxyl were used as control 1 and 2, respectively.

Evaluation of the effects of antifungal metabolites from *Bacillus* spp. under laboratory conditions

Sterile filter paper discs (Whatman No. 1), 1.3 cm in diameter, were placed onto PDA Petri dishes inoculated with *P. aphanidermatum*. These dishes were sealed with plastic wrap before incubation at 27 ± 2 °C until discs were well colonized with *P. aphanidermatum*. The discs were removed and placed on sterile moistened filter paper in Petri dishes, 10 discs per Petri dish, before being covered with a paper disc imbibed with 1,000 mg/l crude extracts of *Bacillus* spp. Disinfected tomato (*Lycopersicon esculentum*) seeds (dipped in 2 % sodium hypochlorite for 30 sec and rinsed with sterile water for 5 min, 3 times) were placed on filter paper discs, one seed per disc. Percent of seedling germination was recorded 14 days after treatment. Three controls used comprised of surface disinfected tomato seeds placed on sterile filter paper discs. Sterile filter paper discs colonized with *P. aphanidermatum* were covered with sterile discs dipped in sterile water and 2 % methanol for 10 min, respectively. Paper disc impregnated with 1,000 mg/l of metalaxyl, a systemic fungicide was also used as a control. The design used for this experiment was a complete randomized design (CRD) with 5 replications, 10 seeds per a replicate [11].

Evaluation of the effects of antifungal metabolites from *Bacillus* spp. under glasshouse conditions

Pots containing sterilized Mai Long Mai Loo (trade name) [11] potting mix autoclaved at 121 °C for 1 h in 2 consecutive days were used. The disc colonized with *P. aphanidermatum* was transferred into potting mix (10 pairs per pot) at 0.5 cm depth from surface. The disinfected tomato seeds immersed overnight in sterile water were immersed in crude extracts of *Bacillus* spp. for 10 min before placing them over the *Pythium* inoculated discs approximately 0.3 cm from the surface (1 seed per pair of discs). The number of germinated tomato seeds was recorded after 14 days. Control 1 used tomato seeds dipped in sterile water for 10 min before placing above the colonized discs. Tomato seeds dipped in 2 % methanol before placing above colonized discs were used as control 2. The design used for this experiment was a complete randomized design (CRD) with 5 replications, 10 seeds per a replicate [11].

Statistical analysis

All data were analyzed by ANOVA using the GLM procedure of SAS [12] system for Windows and were considered significant when $P \leq 0.05$.

RESULTS

Taxonomy for genus of bacterial strains

Seventeen strains of bacteria were isolated from the rambutan leaf surface. Six strains were gram positive, while 11 strains were gram negative. Six gram positive strains were further investigated for morphological characteristics. Four strains (B-NST-

01, B-NST-02, B-NST-03 and B-NST-04) were identified as *Bacillus* spp. based on varied colony morphology, white colony color, rough colony surface and endospore formation.

In vitro evaluation of the inhibition effects of *Bacillus* spp. on *Pythium aphanidermatum* mycelial growth

Clear zones characterized the inhibition of *P. aphanidermatum* mycelial growth. All strains of bacterial antagonists inhibited mycelial growth of *P. aphanidermatum* on PDA at room temperature by 48.0 - 62.0 % as compared to a control without a bacterial antagonist. Especially, strains B-NST-02 and B-NST-03 which provided 62.0 and 57.5 % of inhibition, respectively (**Table 1**).

Table 1 Mycelial growth inhibition of *Pythium aphanidermatum* by *Bacillus* spp.

Genus	Strains	Mycelial growth inhibition (%) [1/]
Bacillus sp.	B-NST-01	48.0 b[2/]
Bacillus sp.	B-NST-02	62.0 a
Bacillus sp.	B-NST-03	57.5 ab
Bacillus sp.	B-NST-04	56.5 ab

[1/] Percent inhibition of mycelial growth of *Pythium aphanidermatum* on PDA as compared to the control 3 days after inoculation, at room temperature.

[2/] Values followed by the same letter in each column are not significantly different from each other according to Duncan's Multiple Range Test ($p = 0.05$).

Extraction of antifungal metabolites

Antifungal metabolites were successfully extracted from all tested strains of *Bacillus* spp. using ethyl acetate as a solvent. Dried extracts were white-yellow in color and their weights obtained were 6.76, 6.51, 6.33 and 6.03 g/l of the extract for the strains B-NST-01, B-NST-04, B-NST-03 and B-NST-02, respectively.

Viability of *Pythium aphanidermatum*

Mycelia of *P. aphanidermatum* treated with sterile water or methanol (2 %), covered the whole agar surface in the Petri dish within 5 days. No visible growth of mycelia of *P. aphanidermatum* was noted if treated with a suspension of *Bacillus* spp. crude extracts (1,000 mg/l) or metalaxyl.

Evaluation of the effects of antifungal metabolites from *Bacillus* spp. under laboratory conditions

Percent of seed germination in the control 1 (sterile water) and control 2 (2 % methanol) were 17.5 % and 16.0 %, respectively, whereas seeds treated with

1,000 mg/l metalaxyl gave 85.0 % seed germination. The same high germination were obtained after treated with 1,000 mg/l crude extracts of *Bacillus* spp. Especially treatment by strains B-NST-03 and B-NST-02 which resulted in, compared to the control (water), germination rates of 85.5 % and 82.0 %, respectively (**Table 2**).

Table 2 Seed germination of tomato 14 days after placing seeds over paired discs of filter paper precolonized with *Pythium aphanidermatum* and treated with 1,000 mg/l crude extracts from 4 strains of *Bacillus* spp. in laboratory and glasshouse tests.

Treatments	Seed germination (%)	
	Laboratory	Glasshouse
Sterile water (control 1)	17.5 c[1/]	28.0 c[1/]
Methanol (control 2)	16.0 c	26.5 c
B-NST-01	72.5 b	80.0 b
B-NST-02	82.0 ab	92.0 a
B-NST-03	85.5 a	92.5 a
B-NST-04	78.5 b	85.5 ab
Metalaxyl (1,000 mg/l)	85.0 a	88.5 ab

[1/] Values followed by the same letter in each column are not significantly different from each other according to Duncan's Multiple Range Test ($p = 0.05$).

Evaluation of the effects of antifungal metabolites from *Bacillus* spp. under glasshouse conditions

Significantly higher seed germination than in control 1 (sterile water) and control 2 (2 % methanol) were obtained following treatments with 1,000 mg/l crude extracts of *Bacillus* spp. and metalaxyl. Especially, strains B-NST-03 and B-NST-02 that showed germination of 92.5 % and 92.0 %, respectively, while control 1 (sterile water) and control 2 (2 % methanol) had 28.0 % and 26.5 % seedlings germination rates, respectively, whereas seeds treated with 1,000 mg/l metalaxyl gave 88.5 % seed germination (**Table 2**).

DISCUSSION

Four bacterial strains were isolated from the leaf surface of rambutan indicating that some bacteria could grow and survive on the leaf surface of rambutan. This is probably due to the fact that plant leaf usually produces some nutrients suitable for the growth of bacteria [13].

Since a tissue transplanting technique was used for bacterial isolation, only bacteria which had significantly colonized the rambutan leaf surface were isolated. In

this research, there was a strong intention to prove that phylloplane associated bacteria from the rambutan leaf surface can be potential antagonists against some soilborn pathogens, especially *P. aphanidermatum* [4,11].

All strains of bacterial antagonists inhibited mycelial growth of *P. aphanidermatum.* This suggests that bacteria on the rambutan leaf surface were potent antagonists of *P. aphanidermatum*. Similar evidence was reported by Koomen and Jeffries [14], who showed that most strains of *Bacillus* spp. have potential as bacterial antagonists to many plant pathogens including *P. aphanidermatum*.

Yoshida and co-workers [15] reported that *Bacillus* spp. produces a clear zone (maybe this clear zone contains some antibiotics) to inhibit pathogens on an agar test and that bacterial antagonists which were able to produce antibiotics, provided better efficacy to inhibit mycelial growth of plant pathogens than bacterial antagonists without antibiotic production. This indicated the important role of bacterial metabolites in the inhibition of mycelial growth of plant pathogens [15-20]. Our results showed that all strains of bacterial antagonists could produce clear zones on agar and confirmed the results of previous studies. Moreover several researchers reported that *Bacillus* spp. produce a large number of antifungal metabolites such as bacitracin, gramicidin S, polymyxin, tyrotricidin, bacilysin, chlotetaine, iturin A, mycobacillin, bacilomycin, mycosubtilin, fungistatin and subsporin [7,21,22] which are able to control plant diseases.

Successful use of antifungal metabolites extracted from *Bacillus* spp. to control cucumber damping-off caused by *P. aphanidermatum* has been reported [5]. Our study demonstrated that control of *P. aphanidermatum* could also be achieved in tomato plantations. Both in the laboratory and glasshouse conditions, crude extracts (1,000 mg/L) produced by all *Bacillus* spp. strains gave high and satisfactory control of damping-off disease resulting in an increased percent germination rate of tomato seeds.

In this research, we obtained interesting and promising results for successful control of tomato damping-off caused by *P. aphanidermatum* using biological natural products from *Bacillus* spp. The efficacy of antifungal metabolites obtained from all strains of *Bacillus* spp. was not statistically and significantly different to those in which metalaxyl was used. However, use of *Bacillus* in field control of *P. aphanidermatum* with an equal biological controlling effect but without any oncogenic risk associated with metalaxyl will be in the long term safer for the environment and animal populations. Therefore, it is important to research further into the mass production and application techniques of the antifungal metabolites for the control of tomato and other plant diseases in the field. In particular, isolation, purification and structural characterization of the secondary metabolites from the bacterial crude extracts as well as assessment of their control effects of fungi are warranted.

ACKNOWLEDGEMENTS

The authors thank the Scientific and Technology Equipment Center and the Tropical Fruits Research Unit (Walailak University) for the use of their facilities. We would like to thank Prof. Dr. Serge Belloncik for proofreading on this manuscript. This research was supported by the grant No. 02/2550 from the Institute of Research and Development (Walailak University).

REFFERENCES

[1] H Wolfhechel and DF Jensen. Use of *Trichoderma harzianum* and *Gliocladium virens* for the biological control of post-emergence damping-off and root rot of cucumber caused by *Pythium ultimum*. *J. Phytopathol.* 1992; **136**, 221-30.

[2] L Hong, W Duccan, AL Kathryn, B Frank and L Carlo. Biological control of *Botrytis*, *Phytophthora* and *Pythium* by *Bacillus subtilis* Cof1 and CL27 of micropropagated plants in high-humidity fogging glasshouse. *Plant Cell Tiss. Org.* 1998; **52**, 109-12.

[3] GY Yu, JB Sinclair, GL Hartman and BL Bertagnolli. Production of iturin A by *Bacillus amyloliquefaciens* suppressing *Rhizoctonia solani*. *Soil Biol. Biochem.* 2002; **34**, 955-63.

[4] P Yenjit, W Intanoo, C Chamswarng, J Siripanich and W Intana. Use of promising bacterial strains for controlling antracnose on leaf and fruit of mango caused by *Colletotrichum gloeosporioides*. *Walailak J. Sci. & Tech.* 2004; **1**, 56-69.

[5] TC Paulitz, T Zhou and L Rankin. Selection of rhizosphere bacteria for biological control of *Pythium aphanidermatum* on hydroponically grown cucumber. *Biol. Control* 1992; **2**, 226-37.

[6] WGD Fernando, S Nakkeeran, Y Zhang and S Savchuk. Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary by *Pseudomonas* and *Bacillus* species on canola petals. *Crop Prot.* 2006; **26**, 100-7.

[7] DP Collins and BJ Jacobsen. Optimizing a *Bacillus subtilis* isolate for biological control of sugar beet Cercospora leaf spot. *Biol. Control* 2002; **26**, 153-61.

[8] GE Harman. Myths and dogmas of biocontrol changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Plant Dis.* 2000; **84**, 377-93.

[9] GN Agrios. *Plant Pathology*, 5th ed. Academic Press, New York, 2005, p. 703.

[10] E Ryu. On the Gram-differentiation of bacteria by the simplest method. *J. Jpn. Soc. Vet. Sci.* 1938; **17**; 31.

[11] W Intana and C Chamswarng. Control of Chinese-kale damping-off caused by *Pythium aphanidermatum* by antifungal metabolites of *Trichoderma virens*. *Songklanakarin J. Sci. Technol.* 2007; **29**, 919-27.

[12] SAS Institute Inc. SAS Online Doc. Version 8 with PDF files. SAS Institute Inc., USA, 2000.

[13] DR Fravel and HW Spurr. Biocontrol of tobacco brown spot disease by *Bacillus cereus* subsp. *Mycoides* in a controlled environment. *Phytopathology* 1977; **67**, 930-2.

[14] I Koomen and P Jeffries. Effects of antagonistic microorganisms on the post-harvest development of *Colletotrichum gloeosporioides* on mango. *Plant Pathol.* 1993; **42**, 230-7.

[15] S Yoshida, S Hiradate, T Tsukamoto, K Hatakeda and A Shirata. Antimicrobial activity of culture filtrate of *Bacillus amyloliquefacien* RC-2 isolated from mulberry leaves. *Phytopathology* 2001; **91**, 181-7.

[16] CJ Baker, JR Stavely, CA Thomas, M Sasser and JS MacFall. Inhibitory effect of *Bacillus subtilis* on *Uromyces phaseoli* and on development of rust on bean leaves. *Phytopathology* 1983; **73**, 1148-52.

[17] GJ Stessel, C Leben and GW Keitt. Partial purification and properties of the antifungal antibiotic, toximycin. *Phytopathology* 1953; **43**, 23-6.

[18] RC Gueldner, CC Reilly, PL Pusey and CE Costello. Isolation and identification of iturins as antifungal peptides in biological control of peach brown rot with *Bacillus subtilis*. *J. Agric. Food Chem.* 1988; **36**, 366-70.

[19] CD McKeen, CC Reilly and PL Pusey. Production and partial characterization of antifungal substances antagonistic to *Monilinia fructicola* from *Bacillus subtilis*. *Phytopathology* 1986; **76**, 136-9.

[20] GS Asante and AL Neal. Characterization of fungistatic substance produced by a *Bacillus* antagonistic to *Ceratocystis ulmi*. *Phytopathology* 1963; **54**, 819-22.

[21] F Drablos, D Nichoson and M Ronning. EXAFS study of zinc coordination in bacitracin A. *Biochem. Biophys. Acta.* 1999; **1431**, 433-42.

[22] GP Trevor, J Daniel, O Sullivan and LM Larry. Identification of bacilysin, chlorotetaine and itirin A produced by *Bacillus* sp. strain CS93 isolated from pozol, a maxican fermented dough. *Appl. Environ. Microbiol.* 2003; **70**, 631-4.

บทคัดย่อ

วาริน อินทนา[1] ประคอง เย็นจิตต์[1] ทักษิณ สุวรรณโน[1] ศุภลักษณ์ เศรษฐสกุลชัย[1] มนูญ สุวรรโณ[1] และ จิระเดช แจ่มสว่าง[2]

ประสิทธิภาพของสารต่อต้านเชื้อราจาก *Bacillus* spp. ในการควบคุมโรคเน่าระดับดินของมะเขือเทศที่เกิดจากเชื้อ *Pythium aphanidermatum*

เชื้อแบคทีเรีย 4 สายพันธุ์ ถูกแยกจากผิวใบของเงาะโดยวิธี tissue transplanting technique เมื่อนำเชื้อแบคทีเรียทุกสายพันธุ์ มาทดสอบประสิทธิภาพการยับยั้งการเจริญของเส้นใยเชื้อรา *Pythium apahanidermatum* สาเหตุโรคเน่าระดับดินของมะเขือเทศในระดับห้องปฏิบัติการ พบว่าทุกสายพันธุ์มีประสิทธิภาพยับยั้งการเจริญของเส้นใยเชื้อรา *P. aphanidermatum* บนอาหาร potato dextrose agar (PDA) ที่อุณหภูมิห้อง (27 ± 2 องศาเซลเซียส) ได้ โดยสายพันธุ์ B-NST-02 และ B-NST-03 มีประสิทธิภาพการยับยั้งสูงที่ 62.0 และ 57.5 เปอร์เซ็นต์ ตามลำดับ เมื่อจำแนกชนิดพบว่าเชื้อแบคทีเรียทุกสายพันธุ์เป็น *Bacillus* spp. ทำการสกัดสารต่อต้านเชื้อราจากทุกสายพันธุ์และทดสอบการควบคุมโรคทั้งในห้องปฏิบัติการและโรงเรือนในระดับห้องปฏิบัติการพบว่าเมล็ดมะเขือเทศที่แช่ในสารสกัดความเข้มข้น 1,000 มิลลิกรัมต่อลิตร จากสายพันธุ์ B-NST-03 และ B-NST-02 มีจำนวนการงอกที่ 85.5 และ 82.0 เปอร์เซ็นต์ ตามลำดับ ส่วนการทดสอบในระดับโรงเรือนพบว่ากรรมวิธีที่แช่เมล็ดมะเขือเทศในสารสกัดความเข้มข้น 1,000 มิลลิกรัมต่อลิตร จากสายพันธุ์ B-NST-03 และ B-NST-02 มีการงอกของเมล็ดที่ 92.5 และ 92.0 เปอร์เซ็นต์ ตามลำดับ ในขณะที่กรรมวิธีควบคุม 1 (น้ำนึ่งฆ่าเชื้อ) และกรรมวิธีควบคุม 2 (เมทานอลความเข้มข้น 2 เปอร์เซ็นต์) มีจำนวนการงอกของเมล็ดที่ 28.0 และ 26.5 เปอร์เซ็นต์ ตามลำดับ ในการตรวจสอบการมีชีวิตของเชื้อรา *P. aphanidermatum* พบว่าเส้นใยเชื้อรา *P. aphanidermatum* ที่แช่ในสารต่อต้านเชื้อราจากเชื้อ *Bacillus* spp. ทั้ง 4 สายพันธุ์ ไม่สามารถเจริญได้ ในขณะที่กรรมวิธีควบคุมที่แช่ในน้ำนึ่งฆ่าเชื้อ หรือ เมทานอลความเข้มข้น 2 เปอร์เซ็นต์ เส้นใยเชื้อรา *P. aphanidermatum* เจริญรวดเร็วและเจริญคลุมทับอาหาร PDA ในจานเลี้ยงเชื้อได้หมดภายใน 5 วัน

[1]สำนักวิชาเทคโนโลยีการเกษตร มหาวิทยาลัยวลัยลักษณ์ อำเภอท่าศาลา จังหวัดนครศรีธรรมราช 80161

[2]ภาควิชาโรคพืช คณะเกษตรกำแพงแสน มหาวิทยาลัยเกษตรศาสตร์ อำเภอกำแพงแสน จังหวัดนครปฐม 73140