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Evaluation of Actinomycetes Culture Media for Control of Bakanae Disease of Rice Seeds

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Abstract Six strains of actinomycetes, OMA60-45, OMA60-46, CSA60-25, SEA120-12, SEA120-25 and SEA120-31, isolated from natural soil samples from Suthep-Pui National Park, Chiang Mai, Thailand, were evaluated for their antifungal activities against *Fusarium moniliforme* isolate Pk105St_62 causing bakanae disease of rice. There were no significant differences observed among the actinomycete strains in inhibitory effects on the colony growth of *F. moniliforme* (61.3 – 68.0%) when screened using the dual culture method. Then, cultures of the pathogen were grown on two actinomycete culture media, a non-filtered culture medium (NF) and filtered culture medium (F), by incubation with shaking in an enzyme production medium (EPM) for 3, 5 and 7 d. The non-filtered culture medium inhibited colony growth of *F. moniliforme* by 30.8 to 59.0%, while the filtered type caused a 22.5 to 51.4% inhibition. The 5- and 7-d-old culture media inhibited conidial germination of *F. moniliforme* by 22.8 – 99.6% and 28.3 – 98.6%, respectively. Moreover, abnormal morphological changes occurred in conidia treated with culture media of OMA60-45 and OMA60-46. Furthermore, at 21 d after treatment, the percentage of rice seed infected by *F. moniliforme* was significantly reduced by treatment with the NF media of both strains (43.50 and 62.50%, respectively) compared to 100% infection in the control, with no effects on rice germination. This reduction was equivalent to treatment with a commercial fungicide (captan; Orthocide® 50) (38.75%). In addition, seeds treated with the NF of OMA60-45 and OMA60-46resulted in 75.0 and 70.0% germination of rice seeds, respectively, in pathogen infested soil, which was equivalent to treatment with captan (70.0%), while the germination of non-treated seeds was only 5.0%.

Keyword: *Fusarium moniliforme*, rice, bakanae, actinomycetes, non-filtered and filtered culture medium

Introduction

Rice (*Orysa sativa* L.) is one of the most important staple foods for the world's rapidly increasing population (Sasaki, 2005). For Thailand, Thai jasmine rice (cv. Kao Dok Mali 105, KDML 105) is the long grain rice which is well known for its fragrance and taste all around the world, and is one of the

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main export products of the central and north eastern region of Thailand. Therefore, rice production in Thailand, especially Thai jasmine rice, represents a significant portion of the Thai economy. However, one of major diseases contributing to loss in the production of rice is bakanae disease caused by the fungal pathogen, *Fusarium moniliforme*. The typical symptoms of Bakanae are slender, chlorotic and elongated primary leaves that seem to be induced by the production of gibberellin by the pathogen (Ou, 1987; Amoah *et al*., 1995). However, not all infected seedlings show these symptoms, as crown rot is also seen, resulting in stunted rice plants (Ou, 1987; Amoah *et al*., 1995). Crop losses caused by Bakanae may reach 40% (Ou, 1987). The pathogen can be both seed-borne and soilborne. Generally, the seed-borne inoculum provides initial foci for secondary infection. Under favorable environmental conditions, infected plants in different foci have the capacity to produce numerous conidia that subsequently infect proximal healthy plants (Ou, 1985).

Biological control has been widely studied as an alternative method of controlling plant diseases, since the increasing use of fungicides has caused development of pathogen resistance, problems with environmental pollution and human and animal health risks (Compant *et al*., 2005). Actinomycetes are responsible for the production of about half of the discovered bioactive secondary metabolites, notably antibiotics (Berdy, 2005) comprising three quarters of all known products, and *Streptomyces* spp. are especially prolific antibiotic producers (Lechevalier, 1989; Mansour *et al*. 1996; Saadoun and Gharaibeh, 2003). They also have an important capacity for the degradation of polymers such as lignin, chitin, cellulose and starch (Crawford *et al*., 1988; Nolan *et al*., 1988) which improves their biocontrol potential against plant pathogens (Getha *et al*., 2005; Minuto *et al*., 2006). Therefore, the objective of present study was to investigate the potential of soilborne actinomycetes as biocontrol agents *in vitro* and *in vivo* for controlling bakanae disease of rice.

Material and methods

Pathogen

Fusarium moniliforme isolate Pk105St_62 (sensitive to the fungicide carbendazim), present in the culture collection of the Laboratory of the Department of Entomology and Plant Pathology of Chiang Mai University, Thailand, isolated from naturally infected rice stems, was used for experimental inoculations. Cultures were grown on potato dextrose agar (PDA) at room temperature (RT) for 7 d before use.

Antagonists

This study used six actinomycete strains; OMA60-45, OMA60-46, CSA60-25, SEA120-12, SEA120-25 and SEA120-31, that were previously isolated from natural soil samples from Suthep-Pui National Park, Chiang Mai, Thailand. Strains were grown on glucose yeast malt agar (GYM, Shirling and Gottlieb, 1966) at RT for 10 d.

Antifungal tests on Petri dishes

Antagonism tests of the six actinomycetes strains against *F. moniliforme* isolate Pk105St_62 were carried out on Petri dishes containing GYM using the dual culture method (Fokkema, 1978). The isolates of the antagonistic strains were streaked (4 cm) at 2 cm apart from one side of a Petri dish for 4 d earlier than pathogen inoculation, preassuming the slow growth of these actinomycetes in culture and their secondary metabolite production when incubated at RT. A 5-mm mycelial disc of a 7-d-old pathogen culture was placed on the other side of the dish. Paired cultures incubated at RT for 14 days. Dishes inoculated only with test pathogens served as controls. The experiment was done using a Completely Randomized Design (CRD) with three replications (plates). The growth of the pathogen in both the test and control experiments was recorded. Data were collected as percent inhibition of colony growth (PICG) (modified from Soytong, 1989; Lokesha and Benagi, 2007).

Preparation of culture media

Six actinomycete strains were prepared as non-filtered culture medium (NF) and filtered culture medium (F) by using a modification of the method of Saengnak *et al*. (2014). The cultures were separately grown on an enzyme production medium (EPM) by incubation with shaking for 3, 5 and 7 days, and centrifuged for 20 min at 6,000 rpm (4°C) and the supernatants were collected as the NF. The supernatant was then filtrated through membrane filter pore size 0.2-µm (Minisart®) to get the F (Chareunrat, 1999).

Antifungal activities of the culture media

Efficacy on mycelial growth

Inhibition of pathogen growth by the test compounds was carried out on PDA according to the agar well diffusion method (modified from Perez *et al*., 1990). The PDA consisted of two layers, only the upper layer was inoculated.

Twenty μl of each filtrate were pipetted into 5-mm-diameter wells. Agar plugs from 7-day-old PDA cultures of *F. moniliforme* strains were transferred to the center of the plates, and incubated at RT for 10 d. EPM without actinomycetes and a commercial biofungicide (*Bacillus subtilis* AP-01; Laminar®) at the manufacturer's recommended concentration served as the negative and positive controls, respectively. Data were collected as percent inhibition of radial growth (PIRG) modified from Soytong (1989) and Lokesha and Benagi (2007). Three replications were used arranged in a Two-Factor Factorial Design arranged in a CRD. Factor A represented different treatments: A1 = NF of OMA60-45, A2 = F of OMA60-45, A3 = NF of OMA60-46, A4 = F of OMA60-46, A5 = NF of CSA60-25, A6 = F of CSA60-25, A7 = NF of SEA120-25, A8 = F of SEA120-25, A9 = NF of SEA120-12, A10 = F of SEA120-12, A11 = NF of SEA120-31, A12 = F of SEA120-31, A13 = *Bs* AP-01 (Laminar®) and A14 = EPM. Factor B represented incubation period of the culture medium: B1 = 3 d, B2 = 5 d and B3 = 7 d.

Effect on conidial germination

Fusarium moniliforme was prepared as conidial suspensions, and adjusted to 1×10^5 conidia/ml using a haemacytometer. An equal volume (100 μl) of treated conidial suspensions from each culture medium was mixed and spread onto a papery GYM plate, then cut into 1×1 cm sections and placed on a microscope slide. Conidial suspensions mixed with equal volumes of EPM served as the negative control. The slide cultures were incubated at RT and checked for conidial germination at 12 h. In this context germination was defined as a germ tube that had developed to longer than the cell width. Three replications were used arranged in a Two-Factor Factorial Design arranged in a CRD. Factor A represented different treatments: A1 = NF of OMA60-45, A2 = F of OMA60-45, A3 = NF of OMA60-46, A4 = F of OMA60-46, A5 = NF of CSA60-25, A6 = F of CSA60-25, A7 = NF of SEA120-25, A8 = F of SEA120-25, A9 = NF of SEA120-12, A10 = F of SEA120-12, A11 = NF of SEA120-31, A12 = F of SEA120-31 and A13 = distilled water. Factor B represented incubation period of the culture medium: B1 = 3 d, B2 = 5 d and B3 = 7 d. To estimate the percent germination, a total of 300 conidia were examined from each treatment (100 per replicate).

Effects of culture media on bakanae disease of rice seeds

Preparation of rice seeds

Rice seeds (*Oryza sativa* L.) cv. Thai Jasmine rice (Khao Dawk Mali 105, KDML 105) were surface sterilized with 10% Chlorox (1% sodium hypochlorite, NaOCl) for 5 min followed by thorough rinsing with sterilized water. Then, sterilized seeds were air dried by blotting on sterile filter paper.

Preparation of pathogen inoculum

F. moniliforme Pk105St_62, 7-d-old culture grown in Petri dishes with PDA at RT, was flooded with 10 ml of sterile distilled water (Singleton *et al.*, 1992). Mycelia were dislodged by scraping the surface of the agar culture with a sterile loop needle. The mycelial suspension was then filtered through sterile cheese cloth. The concentration of conidia in the suspension was determined with a haemocytometer and adjusted to 1×10^4 conidia/ml.

Surface-sterilized rice seeds were inoculated by immersion in the pathogen suspension for 15 min and air dried. Then, inoculated-seeds were immersed in 5-d-old culture media (seed^{NF} or seed^{F}) at a rate of 100 seeds per 5 ml for 60 min and then air dried. Sterilized water (seed^{sw}), and commercial fungicide (seed^{cap}), (captan; Orthocide® 50) at the manufacturer's recommended concentration served as the negative and positive controls, respectively. Germination data was assessed by the blotter method (ISTA, 1999), then collected as percent of germinated seed and infected seed at 7, 14 and 21 d after treatment. Four replications were used arranged in a Two-Factor Factorial Design and in a CRD (100 seeds per replicate).

Effects of culture media as biofungicides under greenhouse conditions

Preparation of pathogen inoculum

Sorghum seeds served as the inoculating media after boiling, sterilization and packing in polythene film (100 g/bag). *F. moniliforme* Pk105St_62 colonies on PDA were cut from a peripheral part of a colony with a 5-mm-diameter cork borer, then transferred to sterilized sorghum seeds in a bag (10 discs/bag) and mixed well. The inoculated bags were incubated at RT for 14 d in the dark before testing.

Pathogen inoculation

Fusarium-inoculum was mixed with a soil-compost mixture (3:1) from Mea Hia Agricultural Research, Demonstrative and Training Center, Chiang

Mai University, Thailand, in a proportion of 100 g inoculum/kg of soil and incubated in 6-in-diameter pots for 10 d.

Rice seeds of KDML 105 were immersed in the NF (seed^{NF}), captan (seed^{cap}) or sterilized water (seed^{sw}) (100 seeds/5 ml) for 60 min and air dried. Various methods of treatment included: (T_1); seed^{sw} sown in sterilized soil, (T_2); seed^{sw} sown in *Fusarium*-infested soil for 5 d, (T_3); seed^{sw} sown in *Fusarium*-infested soil for 10 d, (T_4); seed^{cap} sown in *Fusarium*-infested soil for 5 d, (T_5); seed^{cap} sown in *Fusarium*-infested soil for 10 d, (T_6); seed^{NF} of OMA60-45 sown in *Fusarium*-infested soil for 5 d, (T_7); seed^{NF} of OMA60-45 sown in *Fusarium* infested soil for 10 d, (T_8); seed^{NF} of OMA60-46 sown in *Fusarium*-infested soil for 5 d and (T_9); seed^{NF} of OMA60-46 sown in *Fusarium*-infested soil for 10 d. T_1 and T_2-T_3 served as negative and positive controls, respectively. T_4-T_5 served as evaluation of the commercial fungicide. Pots containing five seeds of each treatment were transferred to a greenhouse. Four replications were used arranged in a Randomized Completely Block Design (RCBD) (5 seeds per replicate). Data were collected at 14 d after inoculation as percent seedling survival.

Statistical analyses

Treatment means were statistically compared using the Least Significant Difference (LSD) at $P \leq 0.05$.

Results and discussion

Antifungal tests in Petri dishes

The six actinomycetes were not significantly different in inhibition of the colony growth of *F. moniliforme* Pk105St_62, ranging from 61.3 to 68.0% (Figure 1), and were categorized as having high antagonistic activity (Soytong, 1989).

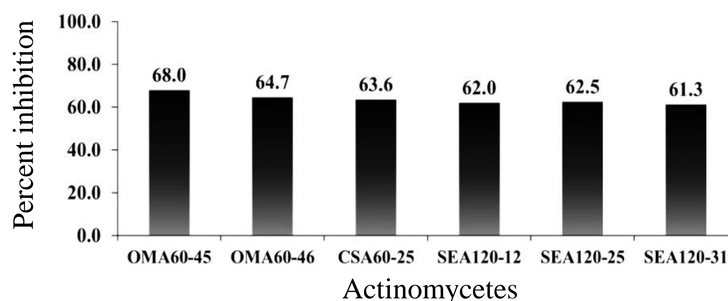

Figure 1. Percentage inhibition of the colony growth of *Fusarium moniliforme* Pk105St_62 caused by the six actinomycete strains on glucose yeast malt agar (GYM) after 14 d. Radial growth means the colony size of *F. moniliforme*

Antifungal activities of the culture media

Efficacy on mycelial growth

The NF culture media of the actinomycetes inhibited colony growth of *F. moniliforme* Pk105St_62 from 30.8 to 59.0%, while the F type produced a 22.5 to 51.4% inhibition. However, the commercial biofungicide, *B. subtilis* AP-01 (Laminar®) and the enzyme production medium inhibited the colony growth by only 19.3 to 39.3% and 3.0 to 10.6%, respectively. Moreover, our results showed that the incubation periods of actinomycetes culture media did not significantly affect the colony growth of *F. moniliforme* Pk105St_62. Among the six actinomycete strains, OMA60-45 and OMA60-46 produced the significantly highest inhibition of colony growth, followed by SEA120-31, SEA120-12, SEA120-28 and CSA60-25. More importantly, the NF produced higher average inhibitory effects than the F (Table 1).

Effect on conidial germination

The NF actinomycetes culture media inhibited conidial germination of *F. moniliforme* Pk105St_62 ranging from 22.8 to 99.6%, while F type produced a 23.7 to 99.1% inhibition. Inhibition of conidial germination in the negative control was only 8.30 to 16.2%. Furthermore, the actinomycetes culture media incubated for 3 d produced the significantly lowest inhibition of conidial germination of *F. moniliforme* Pk105St_62, 26.3 to 47.3%, comparable to incubation periods of 5 and 7 days which had inhibitions ranging from 22.8 to 99.6% and 28.3 to 98.6%, respectively. Among the six actinomycetes strains, OMA60-45 and OMA60-46 were significantly highest in inhibition of conidial germination, followed by SEA120-31, SEA120-12, SEA120-28 and CSA60-25 which were not significantly different. In addition, NF media produced average inhibitory effects equivalent to the F media (Table 2).

Moreover, conidia treated with culture media of OMA60-45 and OMA60-46 appeared abnormal. They showed branched hyphae, formed segments and could not develop into mycelium, while the mycelium of untreated conidia was elongated (Figure 2). Therefore, these two effective strains were selected to test in next series of experiments.

Table 1. Inhibition of colony growth of *Fusarium moniliforme* Pk105St_62 by actinomycete culture media (NF and F) after incubation for 3, 5 and 7 d

Treatments		**Percent inhibition of colony growth**[x]					
		3 d		5 d		7 d	
OMA60-45	NF	49.0	Aab[y]	53.4	Aab	47.6	Aab
	F	41.4	Aab	51.4	Aab	48.7	Aab
OMA60-46	NF	39.4	Bab	58.4	Aa	51.7	ABa
	F	36.5	Ab	48.7	Aab	47.2	ABab
SEA120-25	NF	37.5	Ab	41.5	Ab	34.5	Abc
	F	37.5	Ab	20.8	Bc	34.5	Abc
SEA120-12	NF	42.2	Aab	39.8	Ab	33.5	Abc
	F	39.5	Aab	40.9	Ab	29.8	Abc
CSA60-25	NF	40.5	Aab	42.6	Ab	30.8	Abc
	F	36.5	Ab	23.5	Bc	22.5	BCbc
SEA120-31	NF	50.0	Aa	35.6	Bbc	44.6	ABab
	F	44.2	Aab	38.1	Ab	34.2	Abc
***Bs* AP-01 (Laminar®)**		19.3	Bc	36.9	Ab	39.3	Ab
EPM		10.6	Ac	6.6	Ad	3.0	Ad
A (treatment(			***	$LSD_{0.05}$ = 7.06			
B (incubation period of NF and F)			ns				
A*B			***	$LSD_{0.05}$ = 12.22			
CV (%)			20.20				

NF = non-filtered culture medium, F = filtered culture medium, *Bs* = *Bacillus subtilis*

[x]The agar well method was used. Radial growth was the average of three replicates.

[y]Values of each column (a, b, c) and row (A, B) followed by different letter indicate that they are significant different by $LSD_{(P<0.05)}$.

ns: non significant *** significantly different at $P<0.001$

Table 2. Efficacy of actinomycetes culture media (NF and F); incubated for 3, 5 and 7 d, on inhibiting the conidial germination of *Fusarium moniliforme* Pk105St_62 on potato dextrose agar inspected 12 h after treatment

Treatments		**Percent inhibition of conidial germination**[x]					
		3 d[3]		5 d		7 d	
OMA60-45	NF	28.6	Cb[y]	99.1	Aa	73.9	Bb
	F	36.9	Bab	99.1	Aa	94.8	Aa
OMA60-46	NF	29.0	Bb	99.6	Aa	97.9	Aa
	F	36.9	Bab	99.1	Aa	98.6	Aa
SEA120-25	NF	33.7	Ab	37.1	Ad	28.3	Ae
	F	47.3	Aa	43.5	Acd	38.9	Ade
SEA120-12	NF	29.4	Bb	33.6	Bde	55.8	Ac
	F	23.7	Bb	31.8	Bde	59.8	Ac
CSA60-25	NF	26.4	Ab	22.8	Ae	35.1	Ade
	F	29.7	Bb	55.9	Ac	30.7	Be
SEA120-31	NF	26.3	Bb	24.8	Be	71.0	Ab
	F	38.6	Bab	67.3	Ab	44.1	Bd
distilled water		8.3	Ac	9.2	Af	16.2	Af
A (treatment(			***	$LSD_{0.05}$ = 6.39			
B (incubation period of NF and F)			***	$LSD_{0.05}$ = 3.07			
A*B			***	$LSD_{0.05}$ = 11.06			
CV (%)			14.26				

NF = non-filtered culture medium, F = filtered culture medium

[x]The silde culture technique was used. Conidial germination was averaged of three replicates (100 conidia/replicate).

[y]Values of each column (a, b, c) and row (A, B) followed by different letter indicate that they are significant different by $LSD_{(P<0.05)}$.

*** significantly different at P<0.001

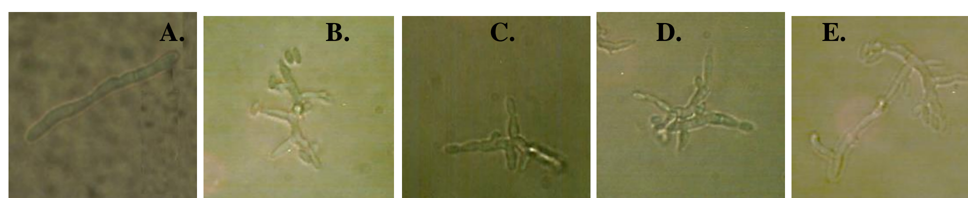

Figure 2. Conidial abnormalities of *Fusarium oxysporum* Pk105St_62 causing rice Bakanae disease after treatment with culture media on potato dextrose agar for 12 h; A. negative control, B. treated NF of OMA60-45, C. treated F of OMA60-45, D. treated NF of OMA60-46 and E. treated F of OMA60-46 (40X)

Effects of culture media on bakanae disease of rice seeds

At 7 d after treatment, rice seeds of all treatments were not significantly different in seed infection; seeds infected by the pathogen ranged from 0.75 – 2.00%. The treatments clearly differed at 14 d after incubation. Seed^{NF} of OMA60-45, OMA60-46 and seed^{F} of OMA60-45 were infected with pathogen at levels of 25.50, 44.50 and 51.00%, respectively, which were not significantly different the seed^{cap} treatment (38.75%). In contrast, the highest infected seed was found in seed^{F} of OMA60-46 (80.50%), which did not significantly differ from the pathogen inoculated seed (65.50%). Finally, seed^{NF} of OMA60-45 had the significantly lowest infection (43.50%) and was equivalent to seed^{cap} (38.75%) at 21 d after incubation, followed by seed^{NF} of OMA60-46 and seed^{F} of OMA60-45. Conversely, seed^{F} and inoculated seeds were completely (100%) infected by the pathogen, while *F. moniliforme* was isolated at a low rate (3.00% in non-treated seeds (Table 3).

Percent seed germination of rice immersed in various treatments were successively collected at 7, 14 and 21 d after treatment, which found an over 90% germination. No significant differences in seed germination were observed among treatments during the test period (Table 4).

Table 3. Effects of actinomycetes culture media (NF and F); incubated for 5 d, on reduction *Fusarium moniliforme* Pk105St_62 infected on rice seeds inspected 7, 14 and 21 d after treatment

Treatments		**Percent seed infection**^{x}				
		7 d	14 d		21 d	
non treated control		1.00	1.75	E^{y}	3.00	e
inoculated control		1.00	65.50	ab	100.00	a
captan (Orthocide® 50)		0.75	38.75	cd	38.75	d
OMA60-45	NF	0.75	25.50	d	43.50	cd
	F	2.00	51.00	bc	76.75	b
OMA60-46	NF	1.25	44.50	cd	62.50	bc
	F	1.25	80.50	a	100.00	a
F-test		ns	***		***	
LSD$_{0.05}$		-	20.16		19.28	
CV (%)		109.69	31.2		21.62	

NF = non-filtered culture medium, F = filtered culture medium

^{x}The blotter method was used. Percent seed infection was averaged of four replicates (100 seeds/replicate).

^{y}Values of each column (a, b, c) and row (A, B) followed by different letter indicate that they are significantly different by LSD$_{(P<0.05)}$.

ns: non significantly *** significantly different at P<0.001

Table 4. Effects of actinomycete culture media (NF and F); incubated for 5 d, on rice seed germination after inoculation with *Fusarium moniliforme* Pk105St_62, inspected 7, 14 and 21 d after treatment

Treatments		Percent seed germination[x]		
		7 d	14 d	21 d
non treated control		97.00	98.00	98.00
inoculated control		91.00	92.50	92.50
captan (Orthocide® 50)		92.00	92.00	94.00
OMA60-45	NF	95.00	95.00	96.00
	F	95.00	95.50	95.50
OMA60-46	NF	93.50	95.00	96.00
	F	93.50	95.00	96.00
F-test		ns	ns	ns
$LSD_{0.05}$		-	-	-
CV (%)		3.46	3.27	3.22

NF = non-filtered culture medium, F = filtered culture medium
[x]The blotter method was used. Percent seed germination was averaged of four replicates (100 seeds/replicate).
ns: non significant

Effects of culture media as biofungicides under greenhouse conditions

At 10 d after inoculation with the pathogen in soil, $seed^{NF}$ of OMA60-45 and OMA60-46 before sowing had equivalent germinations to $seed^{cap}$, 75, 70 and 70%, respectively. In contrast, non-treated seed sown in infested and sterilized soil had germinations of 5 and 95%, respectively (Table 5; Figure 3).

Table 5. Effects of non-filtrated actinomycete culture media (NF); incubated for 5 d, on rice seeds germination after inoculation with *Fusarium moniliforme* Pk105St_62 in soil under greenhouse conditions, inspected 14 d after treatment

Treatments	Percent seed germination[x]
non treated control	95.0 a[y]
inoculated control	5.0 c
captan (Orthocide® 50)	70.0 b
OMA60-45	75.0 ab
OMA60-46	70.0 b
F-test	***
$LSD_{0.05}$	21.97
CV (%)	22.63

[x]Percent seed germination was the average of four replicates (5 seeds/replicate).
[y]Values of each column (a, b, c) followed by different letter indicate that they are significantly different by $LSD_{(P<0.05)}$.
*** significantly different at $P<0.001$

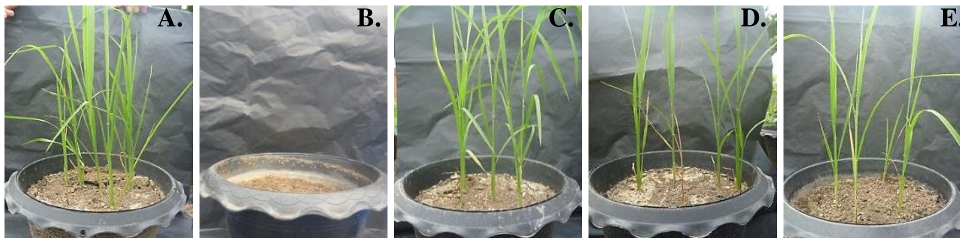

Figure 3. Effects of non-filtered actinomycete culture media (NF); incubated for 5 d, on rice seeds germination in soil infested with *Fusarium moniliforme* Pk105St_62 under greenhouse conditions, inspected 14 d after treatment; A. non treated control, B. inoculated control, C. rice seeds treated with captan (Orthocide® 50), D. rice seeds treated with the NF of OMA60-45 and E. rice seeds treated with the NF of OMA60-46

Discussion

Selected actinomycetes, especially in the genus *Streptomyces*, have been used in many studies for the direct biocontrol of various plant diseases (Yuan and Crawford, 1995; Abd-Allah, 2001; Neeno-Eckwall *et al*., 2001; Getha and Vikineswary, 2002; Shekhar *et al*., 2006). The present investigation evaluated the potential of six actinomycetes; OMA60-45, OMA60-46, CSA60-25, SEA120-12, SEA120-25 and SEA120-31, to control *Fusarium moniliforme* Pk105St_62 causing bakanae disease of rice. These six actinomycetes were isolated from natural soil samples from Suthep-Pui National Park, Chiang Mai, Thailand; which was the same source of actinomycete isolates NSP1, NSP2, NSP3, NSP4, NSP5 and NSP6 (Suwan *et al*., 2012a; Suwan *et al*., 2012b; Saengnak *et al*., 2013; Saengnak *et al*., 2014), and based on their morphology (data not shown), thus they most probably belong to the genus *Streptomyces*. Their bioactive component is chitinase (Totree *et al*., 2011). This enzyme has ability to inhibit fungal pathogens, and this is one of several properties associated with actinomycetes that might explain their ability to act as biocontrol agents.

In the current study these six actinomycetes were tested for their inhibitory capabilities as non-filtered culture media (NF) and filtered culture media (F), by incubation with shaking in an enzyme production medium (EPM) for 3, 5 and 7 d. At 3 d they showed moderately inhibitory effects on the colony growth of *F. moniliforme* Pk105St_62, but 5-d-old and 7-d-old culture media showed higher activities on inhibiting conidial germination and some caused morphological abnormalities in conidia. Similarly Saengnak (2012) reported that 5-d-old culture media of the actinomycete NSP (1-6) were significantly highest in inhibiting conidial germination of *Colletotrichum gleosporioides*

NDM_F012 causing mango anthracnose. Conversely, 3-d-old culture media had no effects on conidial germination, although they gave the significantly highest colony growth inhibition of the pathogen. Furthermore, Saengnak *et al*. (2013) reported that conidia of *F. oxysporum* f. sp. *capsici*, causing chili wilt disease, appeared abnormal after being treated with the culture media of NSP (1-6). Jaipin and Nalumpang (2014) also found abnormalities in conidia of *C. gleosporioides*, causing strawberry anthracnose, after being treated with the culture media of NSP (1-6). In addition, Soares *et al*. (2006) reported ability of *Streptomyces* sp. (AC26) on inhibiting the fungal pathogen *Curvularia eragrostides* and *C. gloeosporioides* causing leaf spot of yam, their abilities were related to concentration of a secondary metabolite associated with *Streptomyces* sp.

Furthermore, actinomycetes culture media have ability to reduce the fungal infection in rice seeds, but no effects on seed germination. Moreover, rice seeds associated with the non-filtrated actinomycetes culture media could germinate in pathogen infested soil under greenhouse conditions. These results indicated that both actinomycetes culture media have potential as biofungicides. Similarly, Mutitu *et al.* (2008) evaluated the antibiotic metabolites from two antagonistic actinomycetes isolates for the control of late blight of tomatoes in the greenhouse. The metabolites were found to give a significant control in the management of late blight and delayed the onset of the disease. Punngram1*et al*. (2012) investigated the antagonistic activities of the actinomycete RF 16-12, isolated from rice field soil, against of three rice fungal pathogens: *Fusarium moniliforme*, *Helminthosporium oryzae* and *Rhizoctonia solani.* They found that a spore suspension of RF 12-16 was effective in controlling *F. moniliforme* on rice seed with no effect on the root and shoot development of rice. The molecular identfication showed that the RF 16-12 shared 99.86% similarity with *Streptomyces yogyakartensis* NBRC 100779^{T}. Wellington (2003) reported that the strain DAUFPE 11470 showed great effectiveness for controlling a number of fungal pathogens of seeds, roots and shoots including *Aspergillus* spp., *Cephalosporium acremonium*, *Curvularia lunata*, *Drechslera maydis* and *Fusarium subglutinans*. Our results indicate that the strains OMA60-45 and OMA60-46 reduce the incidence of seed pathogenic fungi and have potential as biological control agents. However, an efficient method of seed treatment with the biological control agents must be developed before they can become agriculturally practical. Finally, the use of these strains at the field level will provide the basis to expand their use to replace chemical fungicides.

Conclusion

The current study demonstrated the potential of actinomycetes isolated from natural soil samples from Suthep-Pui National Park, Chiang Mai, Thailand, as as biological control agents against bakanae disease of rice caused by *F. monoliforme.* The application of non-filtered culture medium (NF) and filtered culture medium (F) extracts showed good *in vitro* antifungal properties, and were also effective under greenhouse conditions. Hence, using these strains as biofungicides in rice fields may be feasible and practical. The actual biocontrol mechanisms of these actinomycetes remain to be studied.

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