EVALUATION OF ACTINOMYCETES STRAINS AGAINST *Fusarium moniliforme* SHELDON CAUSING BAKANAE DISEASE OF RICE *IN VITRO* CONDITIONS

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ABSTRACT

Biological control is one of the useful methods of plant disease management because of its economic efficiency, minimization of chemical resistance and environmentally friendly approach. Using actinomycetes for managing Bakanae disease in rice has been mentioned in many previous studies. In this study, the antagonistic ability of actinomycetes against the Fusarium moniliforme fungus causing Bakanae disease in rice was conducted in the laboratory of the Plant Protection Department, CuuLong Delta Rice Research Institute. One (FU-CT12) out of twenty pathogenicity strains of F. moniliforme with the highest rating in disease incidence, disease severity, and plant death from Can Tho City was used as the fungal pathogen in vitro antagonistic evaluation experiment. Based on a preliminary assessment of the antagonistic ability of 120 actinomycetes strains against F. moniliforme, 10 antagonist effect strains were screened for antifungal activity against the FU-CT12 strain under in vitro conditions. The results showed that four actinomycetes strains, AC-CT03, AC-CT09, AC-CT13, and AC-CT78, had strong antagonistic activity against FU-CT12. Three strains, AC-CT03, AC-CT09, and AC-CT13, showed the high inhibitory effect on fungal spore germination of the FU-CT12 strain.

Keywords: Bakanae disease, *Fusarium moniliforme*, actinomycetes, antagonist.

INTRODUCTION

Bakanae disease of rice is caused by *Fusarium moniliforme* (*Gibberella fujikuroi*), which is one of the most common diseases and is distributed throughout rice-growing regions of the world (Ou 1985). In Vietnam, the disease has appeared in many regions and caused serious harm to rice in some provinces of the Red River Delta, such as Hai Duong, Hung Yen, Thai Binh, Nam Dinh (Vu and Le 1998). In the Mekong Delta, Bakanae disease thrived and spread throughout the provinces during the Winter-Spring Season 2005-2006 with an average infection rate of 10-20%, some places up to 40-45%, especially in intensive rice areas with three seasons (Pham 2006).

Methods to prevent rice disease are mainly the use of chemicals, however, the abuse of chemicals in disease management easily leads to chemical resistance of pathogens, and also causes environmental pollution and chemicals residues in rice products that will affect the health of consumers. Therefore, biological control could be a great potential to manage rice disease effectively and minimize environmental pollution. The study "Evaluation of actinomycetes strains against *Fusarium moniliforme* fungus causing Bakanae disease of rice *in vitro* conditions" was carried out to select the best inhibiting strain of actinomycetes for the growth of *F. moniliforme* fungus *in vitro* conditions. Further, this strain would be used to

control Bakanae disease in rice as a biological method.

MATERIALS AND METHODS

Materials

The experiment was conducted at the laboratory of the Plant Protection Department, Cuu Long Delta Rice Research Institute (CLRRI) by using Jasmine 85 as a susceptible variety to Bakanae disease. Sources of fungal pathogens including 20 strains of *F. moniliforme* were isolated from rice plants infected with Bakanae disease, have been collected in Can Tho city. The antagonistic actinomycetes strains were provided by the Department of Plant Protection, CLRRI.

Methods

Disease sampling, isolation, and pathogenicity validation of *F. moniliforme* strains

Rice plants infected Bakanae disease in the fields at 70 days after sowing were collected according to the symptoms that were described by Ou (1985) in the 4 districts of O Mon, Thoi Lai, Thot Not, and Vinh Thanh in Can Tho City. The strains of *F. moniliforme* were isolated by using the published method (Kazempour and Elahinia 2007) with modifications. Isolated *F. moniliforme* strains were determined to be pathogenic in rice plants at the germination stage by placing seeds on rolled paper (ISTA 1993). The experiment was carried out in a completely randomized design with four replications, in which each strain of *F. moniliforme* represented a treatment. The control test was treated with distilled water. The rate of disease incidence and plant death were scored at 7 days after inoculation on rice seeds.

Evaluation of the inhibition of actinomycetes on the mycelial growth of *F. moniliforme*

The inhibition of actinomycetes on the mycelial growth of *F. moniliforme* was identified by the published method (Kazempour and Elahinia 2007) with modifications. The experiment was carried out in a completely randomized design with four replications. The treatment cultured with the test pathogen *F. moniliforme* served as the control. The colony radius of *F. moniliforme* mycelium was recorded at 3, 5, 7, and 9 days after treatment. The inhibitory effect of actinomycetes on the growth of *F. moniliforme* mycelium was calculated by the formula of Montealegro et al. (2003).

$$\text{Inhibitory effect (\%)} = \frac{G1 - G2}{G1} \times 100$$

G1: Colony radius on control treatment

G2: Colony radius on treatments

Evaluation of the inhibition of actinomycetes culture solution on spore germination of *F. moniliforme*

The experiment was carried out a completely randomized design for 12 treatments (10 strains of actinomycetes, Jivon 6WP chemical, and distilled water), with 4 replications. The potato dextrose agar was used for *F. moniliforme* (FU-CT12) culture, and their spores were obtained according to the published method of Ruangwong and Liang (2012) with modifications. The actinomycetes culture solution was prepared by the method of Alam et al. (2012) with modifications. Dilution of actinomycetes in liquid culture was made into different concentrations of 10%, 20%, and 40% according to Alam et al. (2012), while Jivon 6WP was diluted at three concentrations of 10%, 20%, and 40%. Inhibition of actinomycetes on germination rates of spores of *F. moniliforme* was evaluated by Saleh et al. (2007) and germination inhibitory effect (Bindu and Padma 2009).

▪ Germination rate (Saleh et al. 2007)

$$\text{Germination rate (\%)} = \frac{\text{Number of germination spores}}{\text{Total number of observed spores}} \times 100$$

▪ Germination inhibitory effect (Bindu and Padma 2009)

$$\text{Germination inhibitory effect (\%)} = \frac{A - B}{A} \times 100$$

A: Germination rate of the control treatment

B: Germination rate of the treatments

Statistical analysis

The basic statistics of data were analyzed by SAS statistical software.

RESULTS AND DISCUSSION

Pathogenicity test of *F. moniliforme* strains in laboratory conditions

A total of 20 strains of *F. moniliforme* were obtained from Bakanae - diseased rice plants collected from four districts Thoi Lai, O Mon, Thot Not, and Vinh Thanh of Can Tho city. Results in **Table 1** showed that *F. moniliforme* strains with the highest disease incidence rates were FU-CT20, FU-CT12, FU-CT13, FU-CT06, and FU-CT03. The highest disease severities were associated with strains FU-CT12 and FU-CT20. The highest plant death rates were observed in strains FU-CT12, FU-CT15, and FU-CT20. Thus, the FU-CT12 strain harboring the highest disease incidence and severity as well as plant death rate was selected as the fungal pathogen for further *in vitro* experiments.

Table 1. Disease incidences-severities and plant death rates at 7 days after inoculation of *F. moniliforme* fungus on rice seeds, Jasmine 85 variety, CLRRI.

No.	Treatments	Disease incidence – severities and plant death rates (%)		
		Disease incidences	Disease severities	Plant death rates
1	FU-CT01	78.75^{c}	47.19^{f-i}	22.50^{c}
2	FU-CT02	77.50^{c}	46.41^{ghi}	25.00^{c}
3	FU-CT03	90.00^{ab}	56.09^{cd}	39.38^{b}
4	FU-CT04	70.00^{d}	42.81^{ij}	22.50^{c}
5	FU-CT05	76.25^{cd}	45.47^{hij}	26.25^{c}
6	FU-CT06	90.63^{ab}	57.97^{c}	40.38^{b}
7	FU-CT07	76.88^{cd}	51.56^{def}	22.50^{c}
8	FU-CT08	72.50^{cd}	41.56^{j}	23.75^{c}
9	FU-CT09	76.25^{cd}	45.94^{g-j}	23.75^{c}
10	FU-CT10	88.13^{b}	56.09^{cd}	39.38^{b}
11	FU-CT11	76.88^{cd}	45.78^{g-j}	25.63^{c}
12	FU-CT12	95.63^{a}	72.03^{a}	51.88^{a}
13	FU-CT13	91.88^{ab}	56.09^{cd}	28.13^{c}
14	FU-CT14	77.50^{c}	49.53^{fgh}	23.13^{c}
15	FU-CT15	88.13^{ab}	60.16^{c}	42.75^{b}
16	FU-CT16	87.50^{b}	58.28^{c}	39.38^{b}
17	FU-CT17	87.50^{b}	55.47^{cde}	40.00^{b}
18	FU-CT18	77.50^{c}	50.31^{fg}	27.50^{c}
19	FU-CT19	89.38^{ab}	57.34^{c}	36.88^{b}
20	FU-CT20	96.25^{a}	65.16^{b}	41.36^{b}
	F	**	**	**
	CV (%)	2.8	3.6	10.8

The data changed arcsin√x for statistical analysis. **: statistically significant in probability 1%.

Inhibition of actinomycetes on mycelial growth of *F. moniliforme* (FU-CT12 strain) under *in vitro* conditions

From a preliminary evaluation of 120 actinomycetes strains provided by the Department of Plant Protection, Cuu Long Delta Rice Research Institute for the ability to antagonize *F. moniliforme*, 10 strains of actinomycetes with high antagonistic activity were selected as constitutive treatments in inhibition assessment experiment of actinomycetes on mycelial growth of *F. moniliforme* (FU-CT12) *in vitro* conditions. Results indicated that, among 10 actinomycetes tested strains, 4 strains AC-CT09, AC-CT03, AC-CT13, and AC-CT78 showed the highest inhibition on mycelial growth of *F. moniliforme* (FU-CT12) (**Table 2**).

Table 2. Inhibitory effect of actinomycetes strains on mycelial growth of *F. moniliforme* (FU-CT12) at different days after treatment, CLRRI.

No.	Treatments	Inhibitory effect on mycelial growth at different DAT (%)			
		3DAT	5DAT	7DAT	9DAT
1	AC-CT03	44.51^{b}	47.65^{b}	54.91^{b}	58.35^{b}
2	AC-CT06	24.97^{fg}	29.05^{fg}	31.64^{ef}	32.42^{ef}
3	AC-CT09	45.46^{b}	48.40^{b}	55.70^{b}	60.14^{b}
4	AC-CT13	42.39^{bc}	45.13^{b}	53.13^{b}	57.72^{b}
5	AC-CT26	33.41^{de}	34.13^{de}	36.63^{d}	37.12^{d}
6	AC-CT72	21.67^{g}	26.66^{g}	29.95^{f}	30.29^{f}
7	AC-CT78	37.34^{cd}	40.18^{c}	46.37^{c}	50.18^{c}
8	AC-CT14	33.69^{de}	37.45^{cd}	45.01^{c}	46.49^{c}
9	AC-CT35	$32,50^{de}$	32.48^{ef}	$34,38^{de}$	35.48^{de}
10	AC-CT60	28.70^{ef}	29.98^{efg}	33.34^{def}	34.22^{de}
11	Jivon 6WP	76.80^{a}	77.95^{a}	79.03^{a}	79.90^{a}
	F	**	**	**	**
	CV (%)	6.4	4.6	3.9	3.5

DAT: Days after treatment; Data changed arcsin √x for statistical analysis. **: statistically significant in probability 1%.

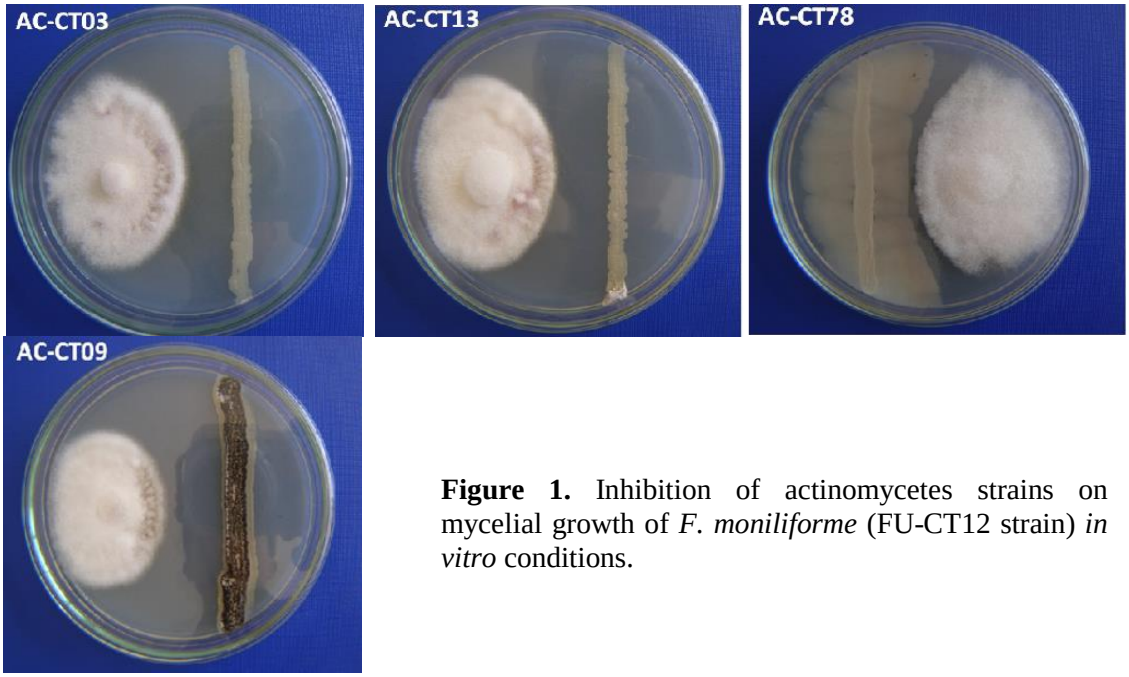

Figure 1. Inhibition of actinomycetes strains on mycelial growth of *F. moniliforme* (FU-CT12 strain) *in vitro* conditions.

Inhibition of actinomycetes culture solution on spore germination of FU- CT12 under *in vitro* conditions

Evaluation of inhibition of actinomycetes culture solution on spore germination of *F. moniliforme* (strain FU-CT12 strain) showed that the liquid culture (PDA liquid culture medium) of actinomycetes was able to inhibit the germination of *F. moniliforme* fungal spores (FU-CT12 strain) *in vitro* conditions.

Results in **Table 3** indicated that among the actinomycetes tested treatments, 3 treatments related to the lowest spore germination rates of FU-CT12 at 3 concentrations of 10%, 20%, and 40% were found on strains AC-CT03, AC-CT09, and AC-CT13. Whereas treatment with the highest spore germination rate at 2 concentrations (20% and 40%) was observed for strain AC-CT26.

Inhibitory effect of actinomycetes culture solutions on germination of fungal spores of FU-CT12 were shown in **Table 4,** which also found that 3 out of 10 actinomycetes tested treatments with the highest germination inhibitory effect at concentrations 10%, 20%, and 40% were strains AC-CT09, AC-CT03, and AC-CT13. Treatment had the lowest inhibitory effect on spore germination at concentrations of 10%, 20%, and 40% belonged to strains AC-CT26.

From the results of inhibition of actinomycetes solutions for spore germination of FU-CT12 *in vitro* conditions confirmed that, among 10 tested strains of actinomycetes, 3 strains had the highest inhibition on spore germination and the lowest germination inhibitory effect of *F. moniliforme* (FU-CT12 strain) at all experimental concentrations.

Table 3. Spore germination rates (%) of FU-CT12 after inoculation in actinomycetes solution at three concentrations, CLRRI.

No.	Treatments	Germination rate of FU-CT12 fungal spores (%)		
		10%	20%	40%
1	AC-CT03	77.00^{fg}	60.00^{gh}	36.00^{f}
2	AC-CT06	97.00^{ab}	87.00^{bc}	71.50^{bc}
3	AC-CT09	71.00^{g}	56.50^{h}	33.00^{f}
4	AC-CT13	77.50^{efg}	63.00^{fgh}	40.50^{f}
5	AC-CT14	92.00^{bc}	83.00^{bcd}	64.00^{cd}
6	AC-CT26	97.00^{ab}	88.50^{b}	78.50^{b}
7	AC-CT35	82,000^{def}	70.50^{efg}	51.00^{e}
8	AC-CT60	86.00^{cde}	74.50^{def}	57.50^{de}
9	AC-CT72	89.00^{cd}	78.00^{cde}	61.00^{d}
10	AC-CT78	90.50^{c}	81.00^{b-e}	61.50^{d}
11	Jivon 6WP	32.00^{h}	23.50^{i}	15.00^{g}
12	Controls	99.00^{a}	98.50^{a}	98.00^{a}
	F	**	**	**
	CV (%)	6.4	8.6	7.4

Data changed arcsin√x for statistical analysis; **: statistically significant in probability 1%.

Table 4. Inhibiting effect of actinomycetes culture solution on spore germination of FU-CT12 (%) at three concentrations, CLRRI.

No.	Treatments	Inhibiting effect on spore germination of CF-CT12 (%)		
		10%	20%	40%
1	AC-CT03	22.27^{bc}	39.12^{bc}	63.22^{b}
2	AC-CT06	2.02^{f}	11.68^{gh}	26.99^{ef}
3	AC-CT09	28.31^{b}	42.66^{b}	66.26^{b}
4	AC-CT13	21.76^{bc}	36.07^{bcd}	58.63^{b}
5	AC-CT14	7.08^{e}	15.73^{fgh}	34.65^{de}
6	AC-CT26	2.02^{f}	10.16^{h}	19.87^{f}
7	AC-CT35	17.20^{cd}	28.45^{cde}	47.93^{c}
8	AC-CT60	13.15^{de}	24.39^{def}	41.29^{cd}
9	AC-CT72	10.12^{de}	20.83^{efg}	37.72^{d}
10	AC-CT78	8.59^{e}	17.78^{e-h}	37.23^{d}
11	Jivon 6WP	67.74^{a}	76.19^{a}	84.72^{a}
	F	**	**	**
	CV (%)	19.1	16.2	8.8

Data changed arcsin√x for statistical analysis. **: statistically significant in probability 1%.

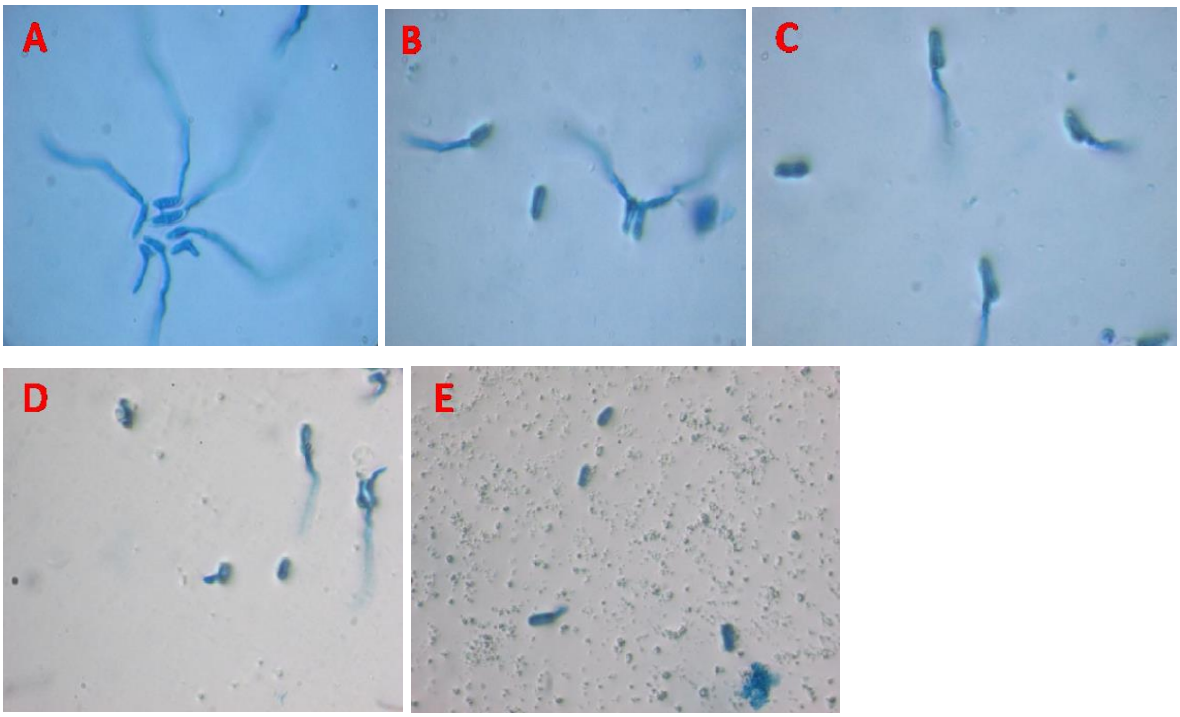

Figure 2. Spore germination of the FU-CT12 strain after treatment with actinomycetes (*AC-CT03* strain) solution at three concentrations, magnification x400. (**A**: Control; **B**: 10%; **C**: 20%; **D**: 40%; **E**: Concentration of Jivon 6WP 10% compared to the recommended concentration).

CONCLUSIONS

The FU-CT12 strain of *F. moniliforme* exhibited the highest pathogenicity on the seedling of the Jasmine 85 rice variety, which was selected as a fungal pathogen for *in vitro* antagonistic evaluation of actinomyces strains. Four actinomycetes strains, AC-CT03, AC-CT09, AC-CT13, and AC-CT78 were strongly antagonized by *F. moniliforme* (FU-CT12 strain) and caused Bakanae disease. Three actinomycetes strains, AC-CT03, AC-CT09, and AC-CT13 showed the highest inhibition on spore germination of *F. moniliforme* (FU-CT12 strain).

COMPETING INTERESTS

The authors declare they have no conflict of interest, financial or otherwise.

AUTHOR'S INFORMATION AND CONTRIBUTIONS

Tran Phuoc Loc conducted the experiment and provided data for the manuscript. Tran Thi Nam Ly wrote the draft version of the manuscript in English. Nguyen Duc Cuong edited and approved the final draft.

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ĐÁNH GIÁ KHẢ NĂNG ĐỐI KHÁNG CỦA XẠ KHUẨN ĐỐI VỚI NẤM *FUSARIUM MONILIFORME* SHELDON GÂY BỆNH LÚA VON TRONG ĐIỀU KIỆN PHÒNG THÍ NGHIỆM

Biện pháp phòng trừ sinh học là một trong những biện pháp đem lại hiệu quả kinh tế, giảm thiểu tính kháng thuốc hóa học và không ảnh hưởng đến môi trường. Sử dụng xạ khuẩn để phòng trừ là một trong những biện pháp đã được nhiều nghiên cứu đề cập đến. Nghiên cứu này đã tiến hành đánh giá khả năng đối kháng của xạ khuẩn đối với nấm F. moniliforme gây bệnh lúa von trong phòng thí nghiệm tại bộ môn Bảo vệ thực vật, Viện lúa Đồng bằng sông Cửu Long. Chủng nấm F. monilifome FU-CT12 có tỷ lệ bệnh, chỉ số bệnh và tỷ lệ cây chết cao nhất được phân lập tại thành phố Cần Thơ đã được sử dụng như tác nhân để đánh giá khả năng đối kháng trong phòng thí nghiệm. Qua kết quả đánh giá sơ khởi về khả năng đối kháng của 120 chủng xạ khuẩn đối với nấm F. moniliforme đã chọn ra 10 chủng xạ khuẩn có hiệu quả đối kháng với dòng FU-CT12 trong điều kiện phòng thí nghiệm. Kết quả ghi nhận cho thấy bốn chủng xạ khuẩn AC-CT03, AC-CT09, AC-CT13 và AC-CT78 có khả năng đối kháng cao đối với chủng nấm FU-CT12. Ba chủng AC-CT03, AC-CT09 và AC-CT13 cho hiệu quả ức chế cao nhất đối với sự mọc mầm của bào tử nấm thuộc chủng FU-CT12.

Từ khóa: *Bệnh lúa von, Fusarium moniliforme, xạ khuẩn, đối kháng*.