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Contributed Paper

Potential of Selected Mangrove *Streptomyces* as Plant Growth Promoter and Rice Bakanae Disease Control Agent

Wasu Pathom-aree [a], Satidaporn Kreawsa [a], Manita Kamjam [a], Shinji Tokuyama [b], Sada Yoosathaporn*[a] and Saisamorn Lumyong [a]

[a] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

[b] Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, Shizuoka 422-8529, Japan

*Author for correspondence; e-mail: shadhay@gmail.com

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Abstract

Rice is global staple food consumed worldwide. Thai jasmine rice (*Oryza sativa* KDML 105) is Thailand most important economic crop. However, the prevalence of Bakanae diseases from fungal pathogen *Fusarium fujikuroi* is the major problem for rice cultivation in Thailand. The disease affects both quantity and quality of rice resulting in the decreasing of rice yield. Large volume of harmful chemical substances is being used to control the outburst of Bakanae disease. In this study, two selected mangrove *Streptomyces* were tested for their plant growth promoting activities and growth inhibiting activity on *F. fujikuroi*. *In vitro* experiments indicated that, isolate S2-SC16 is the most effective to inhibit the germination of *F. fujikuroi* CMU-F02 conidia. *In vivo* experiment also demonstrated an ability of *Streptomyces* isolate S2-SC16 to control *F. fujikuroi* CMU-F02 infection in rice seeds as the seed infected percentage is reduced from 23% to 6%. Furthermore, in pot experiment, isolate S2-SC16 not only shows an ability to restrict the infection of *F. fujikuroi* CMU-F02, but also shows an ability to promote the growth of rice seedlings by increasing numbers of growth parameters including root length, shoot height, leaf width and weight. Phylogenetic analysis based on 16S rRNA gene sequence indicated that isolate S2-SC16 is closely related to *Streptomyces iranensis* HM 35^{T} (99.55% similarity). From the results of our study, there is the possibility of using mangrove actinomycetes as plant growth promoting and alternative environmental friendly control agents for Bakanae disease in Thai jasmine rice KDML 105. *S. iranensis* isolate S2-SC16 is a candidate for the development as plant supplement and biocontrol agent.

Keywords: plant growth promoting activity, mangrove actinomycetes, *Streptomyces*, Thai jasmine rice KDML105, Bakanae disease, *Fusarium fujikuroi*

1. Introduction

Wide varieties of rice are consumed by more than half of the world population as staple food. One of the most famous varieties is Thai jasmine rice (*O. sativa* KDML 105). This

long grain variety of rice is well known for its jasmine-like color, pandan-like fragrance and its soft and sticky texture. Thai jasmine rice is widely consumed by Thai people and exported worldwide. However, the major constraint in Thai jasmine rice cultivation is Bakanae disease caused by *F. fujikuroi* [1,2,3], which can be transmitted through seeds, soil or air under high humidity condition [4]. The incidence of Bakanae disease has steadily increased in many rice growing countries in Asia and throughout the world [5]. In Thailand, the disease has been reported in rice cultivating area in the northern, western and north eastern parts of the country [6]. Both quantity and quality of rice can be affected by the disease, resulting in drastic yield loss up to 50% [4,7]. Although disease transmission can be controlled by chemical fungicides (prochloraz, mancozeb, carbendazim and myclobutamil), but repetitive application may lead to fungicide resistance in *F. fujikuroi* strains [8]. Chemical fungicides known as an expensive solution for disease control, in fact possess an adverse effect to human health and environment [9,10, 11].

Actinomycetes are large group of Gram positive bacteria with high guanine-cytosine content, capable of producing plant growth promoting substances such as siderophores [12,13,14] and phytohormone [14,15,16]. Several reports indicated the potential of actinomycetes as phosphate-solubilizing microorganisms converting insoluble phosphate in soil to various available forms for plant uptake [17,18,19]. Moreover, as a prolific producer of antibiotics and enzymes, potential of actinomycetes are well-described as biocontrol agents for phytopathogenic fungi [20,21,22] and pathogenic organisms. Actinomycetes are widely distributed in various habitats including marine related environments. Marine and mangrove sediments are known to be a rich source of novel actinomycetes with extraordinary potential to produce useful bioactive compounds including nonribosomal peptide synthetase (NRPS), type I and II polyketide synthases (PKS I and II [23]), antitumor [24,25], anti H1N1 virus [26], antifungal [27,28], antibacterial [29,30] and plant growth promoters such as indole-3-acetic acid (IAA) and siderophore [31]. Some marine and mangrove actinomycetes were reported to exhibit antifungal activity against plant pathogenic fungi such as *Alternaria solani, A. alternata, Colletotrichum gloeosporioides, F. oxysporum, F. solani, Rhizoctonia solani* and *Botrytis cinerea* [32,33,34]. Therefore, this study was conducted to evaluate the potential of selected mangrove actinomycetes as plant growth promoter and biocontrol agent for rice Bakanae disease in Thai jasmine rice (*O. sativa* KDML 105).

2. MATERIALS AND METHODS

2.1 Isolation and Characterization of Pathogen

2.1.1 Morphological and molecular identification

Naturally infected rice leaves (showing Bakanae disease symptoms: yellowish green leaves and abnormal elongation of the stem) were cut into pieces (0.5 x 0.5 cm), surface sterilized with 10% sodium hypochlorite for 5 minutes and rinsed with sterile distilled water (3 times). Sterilized leaf fragments were placed on potato dextrose agar (PDA) media and incubated at 25 °C for 3-4 days. Fungi grew from leaves with morphological characteristics of Bakanae fungi [35] was isolated to pure culture, maintained on PDA plate for further study and stored in 40% glycerol at -20 °C for long term storage.

Fungal isolates were identified based on internal transcribed spacer gene (ITS) sequences. Genomic DNA was extracted from fungal cultured on PDA for 3-4 days, using FavoPrep ™ Tissue Genomic DNA Extraction Mini Sample Kit (Taiwan). DNA quality was checked by electrophoresis in 0.8% agarose gel. The ITS sequence was amplified using PCR primers,

ITS1(5'-TCCGTAGGTGAACCTGCGG-3') and ITS4(5'-TCCTCCGCTTATTGATATGC- 3'). PCR mixture was prepared containing 5x My*Taq* reaction buffer (10 µL), 10 µM of primers ITS1 and ITS4 (1µL each), DNA template (1 µL), My*Taq* HS DNA polymerase (1 µL) and final volume was brought up to 25 µL with PCR grade water. The conditions of PCR amplification were as follow: initial denaturation at 95 °C for 10 min; 30 cycles of denaturation (95 °C for 60 s), annealing (55 °C for 60 s) and extension (72 °C for 90 s); and final extension at 72 °C for 10 min [36]. The amplified products were fractioned on 1.0% agarose gel electrophoresis and purified using Nucleic Acid Extraction Kit: GF-1 AmbiClean Kit (Vivantis). The purified products were outsourced and sequenced by 1stBase, Malaysia (Ward Medic Ltd. Partnership, Thailand). The sequences obtained were aligned in BioEdit and compared with the sequences in the NCBI database.

2.1.2 Pathogenicity test

Pathogenicity of isolated fungi was tested and compared with reference culture of *F. moniliforme* identified and obtained from Division of Plant Pathology, Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Thailand. All fungi were grown on PDA plate and incubated at RT, 5-7 days prior to use. The test was carried out by leaf and seed inoculation methods [37].

For leaf inoculation, healthy detached leaves of 3 weeks old *O. sativa* KDML 105 were surface sterilized and wounded using sterile needle. The leaves were inoculated with mycelial disk of 7 days old fungal isolates grown on PDA plate, 3 replicas per treatment. Leaves inoculated with reference culture were used as positive control, uninoculated leaves were used as negative control. After 5 days of incubation in sterilized moisture box at 25°C, Bakanae symptoms were observed and recorded.

For seed inoculation, conidia suspension was obtained by cultivating isolated pathogenic fungal on PDA plate at 25°C for 7 days. Fungal conidia and aerial mycelia were scraped with sterile glass slide and suspended in sterile distilled water. Then, the suspension was filtered through 2 layers of sterile sheet cloth and adjusted the final concentration to 10^6 – 10^7conidia/ml by haemacytometer. Seeds of *O. sativa* KDML 105 were surface disinfected by immersing in 10% (v/v) sodium hypochrorite for 5 min then rinsed with sterile distilled water 3 times. Three replicas of surface disinfected seeds were submerged for 24 h in fungal conidia suspensions and air dried by blotting on sterile filter paper for 12 h (100 seed/replicas). Surface disinfected *O. sativa* KDML 105 seeds soaked in sterile distilled water were used as control. The seeds were then grown in a sterile moisture chamber at 25°C for 10 days. Percentage of germination and numbers of infected seeds were recorded.

2.2 Taxonomic Characterization and *In vitro* Screening of Plant Growth Promoting Activities of Mangrove Actinomycetes

The collection of mangrove actinomycetes was obtained from Microbiological Resource and Technology Research Laboratory, Microbiology section, Department of Biology, Faculty of Science, Chiang Mai University, Thailand. Among 92 strains of actinomycetes isolated from mangrove sediments, strains S1-SC3 and S2-SC16 were selected for this study based on their records of producing high level of siderophore, a microbial metabolite which is known to help chelating iron and protecting plant against phytopathogens.

Morphological characteristics including color of substrate mycelium and aerial spore mass were observed on International *Streptomyces* Project No. 3 (ISP3) plates, melanin production was observed on International *Streptomyces*

Project No. 6 (ISP 6) after 7-14 days of incubation at 25°C [38]. The strains were classified into color groups based on ISCC-NBS Color-name Charts [39]. Diaminopimelic acid in cell wall hydrolysates were determined using thin layer chromatography (TLC) on cellulose method [40].

Selected plant growth promoting and enzyme production traits were observed as follows. Qualitative and quantitative analysis of siderophores were carried out using iron-perchlorate assay [41] and Arnow's method [42]. IAA production was assessed by standard assay [43,44]. Phosphate solubilization was determined by phosphatase assay according to the method of Fiske and Subbarow [45]. Production of cellulase, chitinase and protease were screened by clear zone observation on solid media: carboxymethyl cellulose agar [46], chitin agar [47] and ISP2 medium with supplemented with 1% skimmed milk [48], for each respective observation.

Actinomycetes were identified based on conventional analysis of 16S rRNA gene sequences. Genomic DNA was extracted from actinomycetes cultured on Hickey Treasner's agar [49] for 7 days using FavoPrep ™ Tissue Genomic DNA Extraction Mini Sample Kit (Taiwan). DNA template quality was verified by agarose gel (0.8%) electrophoresis. The 16S rRNA gene was amplified using primers 27F (5' -AGAGTTTG ATCCTGGCTCAG-3') and 1525R (5' -AAGGAGGTGWTCCARCC- 3'; R= A/G, W= A/T). PCR mixture was prepared containing 5x My*Taq* reaction buffer (10 µL), 10 µM of primers 27F and 1492R (1 µL each), DNA template (1 µL), My*Taq* HS DNA polymerase (1 µL) and final volume was brought up to 25 µL with PCR grade water. PCR was conducted using thermal protocol consisting of initial denaturation at 94°C for 4 minutes, 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and elongation at 72°C for 30 s, follow by final extension at 72°C for 10 minutes. PCR amplified products were fractionated in 1.0% agarose gel in 1 x TAE and PCR products were purified using Nucleic Acid Extraction Kit: GF-1 AmbiClean Kit (Vivantis). The purified products were outsourced and sequenced by 1stBase, Malaysia (Ward Medic Ltd. Partnership, Thailand). The sequences obtained were aligned in BioEdit and compared with the sequences in EzBioCloud database [50]. Phylogenetic analysis was performed with MEGA software (Molecular Evolutionary Genetics Analysis, version 7.0). Phylogenetic tree was constructed by neighbor-joining method using with 1000 bootstrap replicates.

2.3 *In vitro* Antagonistic Activity of Strain S1-SC3 and S2-SC16 Against Rice Bakanae Pathogen

2.3.1 Mycelial growth inhibition

Preliminary screening for fungicidal activity was conducted using dual culture method [51]. Mycelial disk (6 mm diameter) of Bakanae fungi was centrally placed upside down on PDA plate and actinomycete disk was placed upside down in straight 3 cm away from center. PDA plate inoculated with mycelial disk of Bakanae fungi (6 mm diameter) without actinomycete was used as control plates. All plates were incubated at 25°C for 5 days. Percentage of inhibition rate growth (PIRG) was calculated using the following equation:

$$\text{PIRG} = [(R1-R2)/ R1] \times 100$$

Where; R1 = radial growth of pathogen in control

R2 = radial growth of pathogen in treatment

Plant growth promoting actinomycetes with PIRG value more than 80% were selected for further study.

2.3.2 Conidial germination inhibition

Mycelial disk of selected actinomycetes were inoculated in 50 mL of enzyme production

medium (EPM) consisting of 3.0 g/L glucose, 1.0 g/L peptone, 0.3 g/L urea, 1.4 g/L $(NH_4)_2SO_4$, 0.3 g/L $MgSO_4.7H_2O$, 0.3 g/L $CaCl_2.6H_2O$, 20 g/L colloidal chitin and 1.0 mL/L trace salt solution containing 5 g/L $FeSO_4.7H_2O$, 1.6 g/L $MnSO_4.7H_2O$ and 1.4 g/L $ZnSO_4.7H_2O$. After 7 days of reciprocal incubation at 25°C, half of total volume of EPM filtered through 0.22 μm filter (minisart®). Actinomycetes liquid culture and filtrated culture were used for conidial germination inhibition test. Conidia suspension of pathogenic fungus was prepared as previously mentioned and inoculated on PDA plate. Conidia germinating inhibition test was done by an agar cup plate method [52]. Sterile distilled water and 50 μg/mL Nystatin was used as negative and positive control, respectively. The plates were incubated at 25°C for 7 days then the inhibition zone was observed and measured.

2.3.3 Fungicidal activity of plant growth promoting actinomycetes on rice seeds and greenhouse experiment

Conidia suspension of pathogenic fungus CMU-F02 was prepared as previously described. Plant growth promoting actinomycetes isolate S2-SC16 was cultivating in enzyme production medium as described before and adjusted the final concentration to 10^8 cfu/ml Seeds of *O. sativa* KDML 105 were divided into 4 different treatments. Treatment 1, seeds were surface disinfected as described before, this group was used to control the efficiency of surface disinfection method. Treatment 2, seed were surface disinfected and inoculated with pathogenic fungal conidia suspension, this group represented the crops infected with Bakanae fungus. Treatment 3, Seeds were surface disinfected and inoculated with pathogenic fungal conidia suspension and 1.5 mg/ml of mancozeb, this group represented the infected crops treated with commercial fungicide. Treatment 4, Seeds were surface disinfected and inoculated with pathogenic fungal and plant growth promoting actinomycete, this group was use to study fungicidal and plant growth promoting activities of actinomycete on rice seeds. The inoculations were done by seed immersion, each immersion took 24 h and between each immersion, seeds were allowed to air dry under sterile condition.

One hundred seeds per each treatment were sowed by moist blotter method. After 10 days, germination percentage, number of infected seeds and number of abnormal seedlings showed elongated, thinner stem with slightly yellow leaves were recorded. Forty uniform growth seedlings from each treatment were randomly selected for greenhouse experiment. Selected seedlings were transplanted in pot with 3 kg sterilized soil and 10 seedlings were transplanted per pot (4 replicates) and all plants were well-watered throughout the experiment. After 21 days, root length, shoot height and width, number of nodes and leaves, color of leaves and fresh weight of seedlings were recorded.

2.4 Data Analysis

Data analysis was carried out using SPSS (version 16.0). One-way analysis of variance (ANOVA) and Turkeys multiple range tests (TMRT) were used to compare mean and determine statistical differences between each treatment ($p = 0.05$).

3. RESULTS

3.1 Isolation and Characterization of Pathogen

3.1.1 Morphological and molecular identification

From Bakanae infected rice leaves, 3 fungal strains were successfully isolated and designated as CMU-F01, CMU-F02 and CMU-F03. Preliminary characterization was made according to The *Fusarium* Laboratory Manual [35]. Only isolate CMU-F02 showed similar characteristics to reference culture of *F. monoliforme* on PDA

plate. CMU-F02 formed white mycelia which later became greyish magenta. Microscopic observation revealed isolate CMU-F02 produced oval microconidia with 0-1septa and slender with tapered apical macroconidia with 3-4 septa (Figure 1). PCR fragment of 489 bp from ITS amplification of CMU-F02 was analyzed and compared with NCBI database using BLAST program. The isolate was closely related to *F. fujikuroi* (KT362205.1) with 100 % similarity.

3.1.2 Pathogenicity test of *F. fujikuroi*

Healthy detached leaves of Thai jasmine rice (*O. sativa* KDML 105) inoculated with *F. fujikuroi* CMU-F02 and *F. moniliforme* (reference culture) were both showed similar symptoms of Bakanae disease, the inoculated leaves turned yellowish green to brown and mycelium growth can be observed on the leaf surface. While control leaves without fungal inoculation, no hyphal growth nor symptoms of Bakanae disease were observed (Figure 2). Pathogenicity test on Thai jasmine rice (*O. sativa* KDML 105) seeds showed negative effect on seed germination of fungal treated rice seeds (Table 1). In innoculated seeds, both *F. moniliforme* and *F. fujikuroi* CMU-F02, non-germinated seeds were covered with fungal mycelium and germinated seeds showed abnormal shape and insufficient growth (Figure 3). Germination of rice seeds innoculated with *F. fujikuroi* CMU-F02 was 14.25 % lower than control and percentage of infected seeds was 7.25 % higher than control.

The lowest percentage of seed germination and the highest percentage of infection were observed in seeds inocculated with *F. fujikuroi* CMU-F02. This pathogenicity test has confirmed *F. fujikuroi* CMU-F02 as a virulence strain which was selected for further study.

3.2 Taxonomic Characterization and *In vitro* Screening of Plant Growth Promoting Activities of Mangrove Actinomycetes

Mangrove actinomycete isolates S1-SC3 and S2-SC16 were examined based on selected taxanomic characterization and plant growth promotion traits (Table 2). Colony appearances

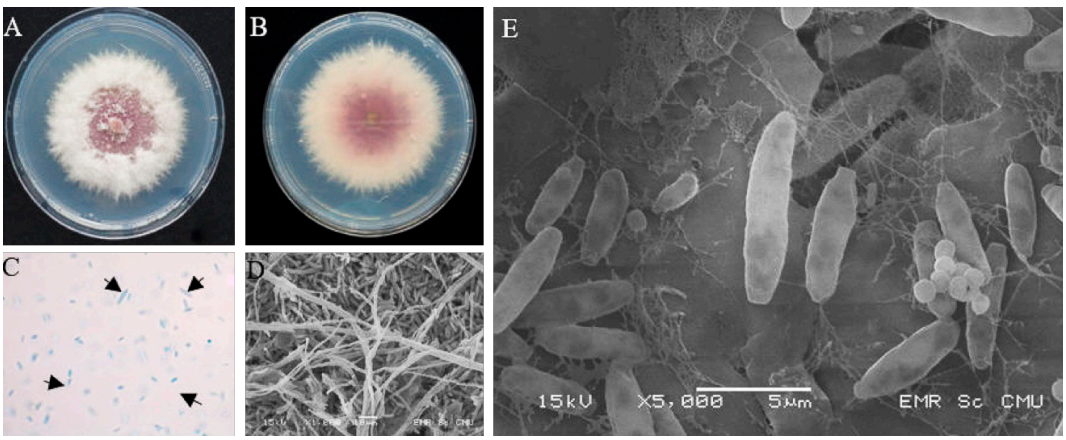

Figure 1. Morphological characteristics and microscopic characteristics of Bakanae fungus isolate CMU-F02. Arial mycelium (A) and substrate mycelium (B) growth on PDA plate after 5 days of incubation at 25°C. CMU-F02 spore under compound microscope at 400X magnification (C), CMU-F02 mycelium under scanning electron microscope (SEM) at 1000X (D) and CMU-F02 conidia under scanning electron microscope at 5000X (E).

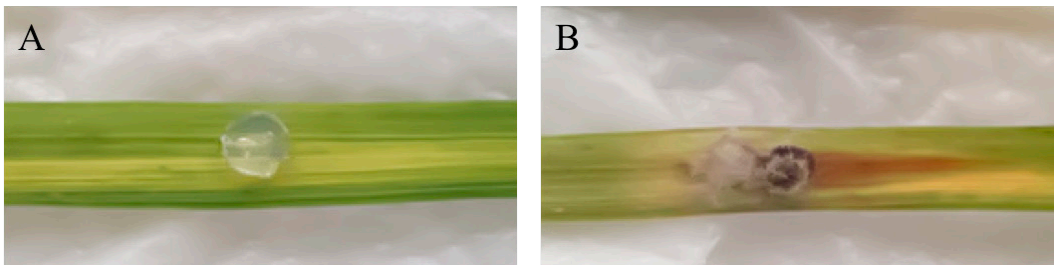

Figure 2. Leaf of Thai jasmine rice (*O. sativa* KDML 105) inoculated with agar disc without fungal pathogen (A) and rice leaf inoculated with *F. fujikuroi* CMU-F02 (B).

Table 1. Pathogenicity of *F. fujikuroi* in Thai Jasmine Rice (*O. sativa* KDML 105) seedlings.

Strain	% germination *	% infection *
Negative Control	97.00 ± 1.15[a]	10.00 ± 1.31[b]
Positive Control *(F. moniliforme)*	84.00 ± 0.76[b]	16.00 ± 1.93[a]
***F. fujikuroi* CMU-F02**	82.75 ± 0.89[b]	17.25 ± 1.16[a]

*Data represent the average of 3 independent experiments. Mean average followed by the same letters were not significantly different by post hoc Duncan's test ($p \leq 0.05$).

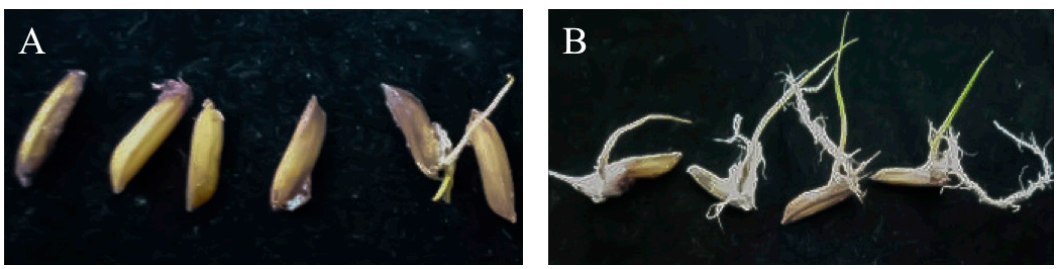

Figure 3. Seeds of Thai jasmine rice (*O. sativa* KDML 105) infected by *F. fujikuroi* CMU-F02. non-germinated seeds covered with fungal mycelium (A) and insufficient germinated seeds (B).

was observed on ISP3 medium where both isolates, S1-SC3 and S2-SC16 formed a flat powdery colony. However, both demonstrated a distinctive substrate and aerial mycelium color on ISP3 media. Isolate S1-SC3 produced white substrate mycelium and dark grey conidia, while isolate S2-SC16 produced yellowish white substrate mycelium with grey conidia. Isolates S1-SC3 and S2-SC16 contained *LL*- diaminopimelic acids in their cell wall hydrolysates.

Both isolates were subjected to 16S rRNA amplification and were successfully produced DNA fragment size of approximately 1400 bp. The sequences were then analyzed and compared with EzBioCloud database using identify program. Based on EzBioCloud database, both isolates belonged to the genus *Streptomyces* which known as dominant genus of actinomycetes. Isolates S1-SC3 and S2-SC16 were closely related to *S. yogyakartensis* (99.38% similarity) and *S. iranensis* (99.55% similarity), respectively. The sequences of isolate S1-SC3 and S2-SC16 were deposited in the DNA Data Bank of Japan (DDBJ) database under accession

Table 2. Some properties of plant growth promoting actinomycete.

Properties	Isolate	
	S1-SC3	**S2-SC16**
Morphological characteristic		
- Color		
- Substrate mycelium	white	white yellow
- Aerial spore mass	dark grey	Grey
Diaminopimelic acid type	*LL*-DAP	*LL*-DAP
Siderophore type	Hydroxamate	Hydroxamate
Siderophore production (μmol/L)	1,260.0 ± 6.6[b]	1,725 ± 25.0[a]
IAA production (μg/mL)	3.08 ± 0.09[b]	7.71 ± 0.19[a]
Phosphate solubilization (mg/L)	11.77 ± 1.96[b]	28.49 ± 8.02[a]
Melanin production	-	-
Protease production	-	-
Chitinase production	-	-
Cellulase production	+	+

*Data represent the average of 3 independent experiments. English alphabets are a statistical comparison between groups using ANOVA and post hoc Duncan's test. Different letters on each row indicate significant differences among treatments ($p \leq 0.05$).

number KP339493 and KM678004. Phylogenetic trees were constructed using *Kitasatospora setae* as an out group. Comparison of isolate S1-SC3 and S2-SC16 of 16S rRNA gene sequence with other corresponding sequence of phylogenetically-related species with valid published name indicated that it formed a branch in the *Streptomyces* 16S rRNA gene tree (Figure 4).

Both isolates produced hydroxamate type siderophores, 1,260 μmol/L for isolate S1-SC3 and 1,725 μmol/L for isolate S2-SC16. Isolates S1-SC3 and S2-SC16 produced 3.80 and 7.71 μg/mL of IAA, respectively. Solubilized phosphate of 11.77 and 28.49 μg/mL were measured from isolates S1-SC3 and S2-SC16, respectively. Both isolates also demonstrated positive result for cellulase production and negative result in melanin, chitinase and protease production.

3.3 *In vitro* Antagonistic Activity of Strain S1-SC3 and S2-SC16 Against Rice Bakanae Pathogen

3.3.1 Mycelial growth inhibition

Based on dual culture test, *F. fujikuroi* CMU-F02 hyphal growth was inhibited by actinomycete isolate S2-SC16 by 82.42% of inhibition percentage (Figure 5), followed by S1-SC3 (81.02%) with respect to the control after 7 days of inoculation.

3.3.2 Conidial germination inhibition

The germination inhibition of *F. fujikuroi* CMU-F02 conidia by mangrove actinomycetes was observed. Only non-filtrate culture media of mangrove actinomycete isolate S2-SC16 showed inhibitory activity against *F. fujikuroi* CMU-F02 with a clear zone of 1.28 cm (Figure 6). A slightly bigger clear zone was

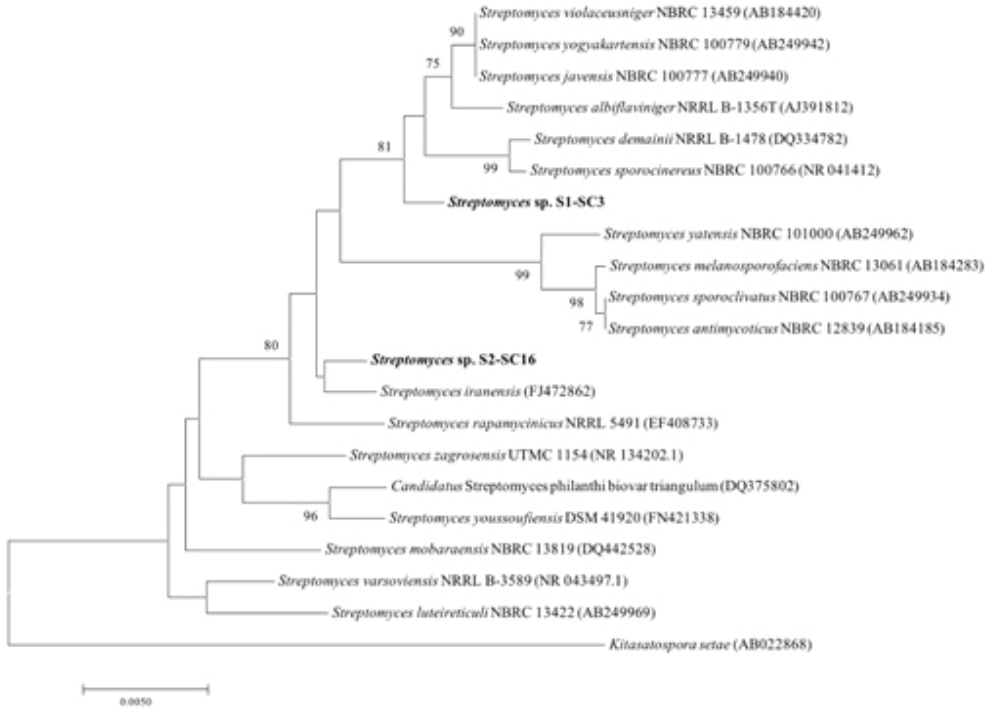

Figure 4. Phylogenetic tree of isolate S1-SC3 and S2-SC16 based on neighbor-joining method, showing its relationship with closely related reference strains from the database.

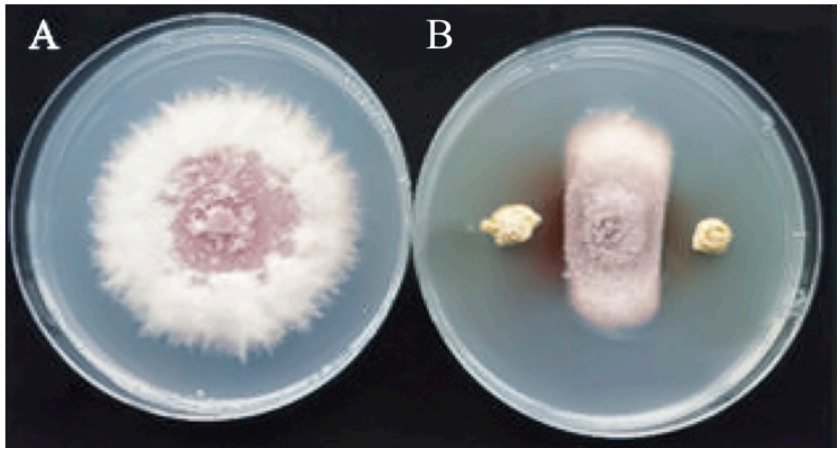

Figure 5. Normal mycelial growth of *F. fujikuroi* CMU-F02 (A) and *F. fujikuroi* CMU-F02 Mycelial growth inhibition by mangrove actinomycete isolate S2-SC16 (B).

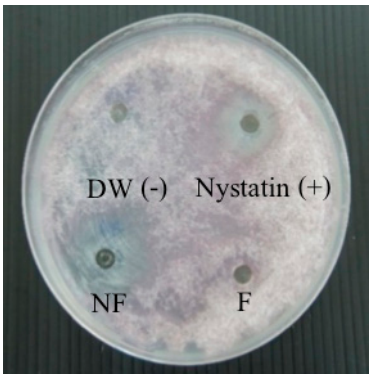

Figure 6. Conidial germination inhibition of *F. fujikuroi* CMU-F02 by nystatin and non-filtrated culture of mangrove actinomycete isolates S2-SC16 (NF).

obtained from a positive control of 50 μg/mL nystatin (1.45 cm). Based on the test result, isolate S2-SC16 was proceeded to further study.

3.3.3 Fungicidal activity of plant growth promoting actinomycetes on rice seeds and greenhouse experiment

The results of seed experiment are shown in Table 3, the inhibitory activity of isolate S2-SC16 against *F. fujikuroi* CMU-F02 on seeds of Thai jasmine rice KDML105 are showed. After 10 days, germination percentage of rice seeds treated with actinomycete isolate S2-SC16 (treatment 4) is the highest among all treatments (94%). The lowest percentage of infection was also observed in this treatment (6%), which was lower than treatment 3 (9%) where the seeds were treated with fungicide (1.5 mg/mL Mancozeb).

After 21 days of cultivation in greenhouse, some physiological growth parameters of rice seedlings treated with different treatments were measured and showed in Table 4. The greatest value of root length, shoot height, shoot width, leaf width and fresh weight were observed in rice treated with isolate S2-SC16. Stem elongation is one of the obvious symptom of Bakanae disease as observed in treatment 3. Shoot height of infected seedlings are greater than observed in treatment 2 where seedlings were not infected with pathogenic fungus. Since the results from seeds experiment showed significantly low percent infection of seeds treated with mangrove actinimycetes, then the maximum shoot height observed in treatment 5, where seedlings were treated with mangrove actinomycete isolate S2-SC16 in green house experiment is unlikely to be the result of Bakanae disease.

4. DISCUSSION

Fungal isolate CMU-F02 displays Bakanae fungus traits as described by [35]. Colony of

Table 3. Effect of isolate S2-SC16 on rice seeds treated with Bakanae fungus.

Treatment*	% germination**	% infection**
Treatment 1	92 ± 0.57 ^{b}	8 ± 0.57 ^{b}
Treatment 2	77 ± 1.52 ^{c}	23 ± 1.53 ^{a}
Treatment 3	91 ± 1.52 ^{b}	9 ± 1.15 ^{b}
Treatment 4	94 ± 2.00 ^{a}	6 ± 1.17 ^{b}

**Data represent the average of 3 independent experiments. English alphabets are a statistical comparison between groups using ANOVA and post hoc Duncan's test. Different letters on each column indicate significant differences among treatments ($p \leq 0.05$).

Table 4. Growth parameters measured from greenhouse cultivated rice treated with different treatments (description in Table 1).

Treatment	Root length* (cm)	Shoot height* (cm)	Leaf width* (mm)	Fresh weight* (g)
Treatment 1	20.41 ± 0.07 ^{bc}	13.73 ± 0.06 ^{d}	3.85 ± 0.12 ^{b}	1.15 ± 0.09 ^{b}
Treatment 2	22.72 ± 0.08 ^{a}	15.05 ± 0.07 ^{c}	3.55 ± 0.16 ^{bc}	1.14 ± 0.03 ^{b}
Treatment 3	16.78 ± 0.15 ^{c}	15.56 ± 0.16 ^{bc}	3.48 ± 0.08 ^{c}	0.91 ± 0.06 ^{c}
Treatment 4	23.55 ± 0.08 ^{a}	18.1 ± 0.13 ^{a}	4.86 ± 0.18 ^{a}	1.81 ± 0.07 ^{a}

*Data represent the average of 4 independent experiments. English alphabets are a statistical comparison between groups using ANOVA and post hoc Duncan's test. Different letters on each column indicate significant differences among treatments ($p \leq 0.05$).

CMU-F02 was covered in cottony white aerial mycelium with greyish violet to magenta substrate mycelium. Microscopic observation revealed 2 types of conidia, an oval colorless, single microconidia and macroconidia, which were colorless and slender with tapered apical with 3-4 septum. From pathogenicity test, isolate CMU-F02 caused infection in Thai jasmine rice *(O. sativa* KDML 105) seeds, resulted in the reduction of seed germination as compared to control and seeds infected with *F. moniliforme*. Isolate CMU-F02 also induced weak, abnormal, and insufficient growth of rice seedlings such as elongated, leaves stunted and chlorosis similar to those reports in previous studies [4,53,54]. In addition, ITS gene analysis confirmed a close relationship between isolate CMU-F02 and *F. fujikuroi* with 100 % similarity. Therefore, isolate CMU-F02 was identified as *F. fujikuroi*.

Diaminopimelic acids (DAP) determination was conducted to classify actinomycetes as streptomycete which contained *LL*-DAP and non-streptomycete which contained *meso*-DAP in their cell walls. Mangrove actinomycete isolates S1-SC3 and S2-SC16 contained *LL*-type of diaminopimelic acids in their cell walls which indicated that these 2 isolates belonged to the genus *Streptomyces* [55]. This chemotaxonomic based assignment was in agreement with 16S rRNA gene sequence analysis. Isolate S1-SC3 showed close phylogenetic relationship with *S. yogyakartensis* NBRC 100779^{T} with 99.38% similarity and isolate S2-SC16 is closely related to *S. iranensis* HM 35^{T} with 99.55 % similarity. Therefore, isolate S2-SC16 was identified as *S. iranensis*. For isolate S1-SC3, its position on 16S rRNA gene phylogentic tree suggested that it may represent a novel taxon though sharing high 16S rRNA gene similarity with the closest neighbors. A more refine polyphasic taxonomic approach is needed to clarify its exact position within the *Streptomyces* genus. Hence, it was identified as *Streptomyces* sp. isolate S1-SC3.

It is evident from inhibition test, that non-filtrated culture of isolate S2-SC16 could control Bakanae causing fungus by inhibiting both hyphal growth and conidia germination of *F. fujikuroi* CMU-F02 *in vitro*. Our results are in agreement with previously reports on inhibitory effects of some *Streptomyces* sp. and mangrove actinomycetes against phytopathogenic fungi. Ningthoujam *et al.* [56] stated the ability of *S. vinaceusdrappus* to suppress the growth of some rice fungal pathogens *in vitro,* including *Curvularia oryzae, Bipolaris oryzae*, *F. oxysporum* and *Pyricularia oryzae.* Punngram *et al.* examined an effective controlling activity of spore

suspension of *S. yogyakartensis* NBRC 100779^{T}isolated from rice field soil against *F. moniliforme* on rice seed with no negative effect on rice growth and development [57]. Sittivate and Nalumpang indicated the application of non-filtrated culture of *Streptomyces* strains isolated from Suthep-Pui National Park, Chiang Mai, Thailand as an efficient biological control agent against rice Bakanae disease cause by *F. moniliforme* both *in vitro* and under greenhouse conditions [58]. Betancur *et al.* [34] also suggested the antagonistic effect of *Streptomyces*, *Micromonospora*, and *Gordonia* recovered from southwest Caribbean Sea against *F. oxysporum* and *C. gloeosporioides.* Antifungal activity of *S. cacaoi* subsp *cacaoi* [M20], Novel mangrove actinomycetes has also been reported by [33]. This novel isolate capable of inhibiting mycelial growth of *F. oxysporum* growth (*in vitro* study) and 10% its culture filtrate was able to support the germination of healthy leguminous plant seeds.

As seed treatment is the most collective way to control rice Bakanae and other soil-borne diseases [59], bio-protective agents produced by selected actinomycetes should not be toxic or have a negative effect on seed germination. *In vitro* and *in vivo* experiments have been conducted in this study to ensure the rice seeds remain as unaffected upon actinomycete treatments. Our results indicated positive growth of Thai jasmine rice (*O. sativa* KDML 105) seedlings when seeds were treated with isolate S2-SC16 Seed infection percentage of seeds treated with *F. fujikuroi* CMU-F02 and S2-SC16 was reduced by 26% comparing to rice seeds treated with *F. fujikuroi* CMU-F02 alone. Bakanae disease occurrence was reduced to 26% by isolate S2-SC16 seed treatment compared to negative control (untreated). The results demonstrated that isolate S2-SC16 is not only able to control rice Bakanae pathogen with no negative effect on seeds but also promotes the germination of Thai jasmine rice (*O. sativa* KDML 105) seeds. This efficacy was likely related to streptomycete ability of producing bioactive compounds such as phytohormones and siderophore where isolate S2-SC16 was also proved to possess these characters. Isolate S2-SC16 capable of producing high amount of siderophore, and according to Hayat et al. [60], a strong iron-chelating compound binds and converts environmental ferric ion to the available forms for plant uptake and hence stimulate plant growth. Siderophore also reported to inhibit the growth of some rice pathogens by scavenging ferric ions from the surrounding environment resulted in reduction of available ferric ions in environment which is required for mycelial growth and conidia germination of rice pathogenic fungi [61, 62, 63].

Moreover, isolate S2-SC16 is able to produce IAA, a phytohormone, which could stimulate rice seeds germination, enhance seedlings growth and root formation. The isolate also produce phosphatase that could solubilize phosphate (existed as insoluble form) into soluble form for plant growth promotion. Although the production level of IAA and phosphatase of strain S2-SC16 are low (7.71 µg/mL and 28.29 µg/mL respectively), comparing to IAA production reported previously (Table 5), this strain could still promote the growth of rice seedling as exemplified by an increaseing plant growth parameters as tested in this study. Our results were in agreement with previous reports on plant growth promoting activity of actinomycetes on wheat [17], mung bean (*Vigna radiata*) [14] and rice [12,14]. However, there were also reports [64,65,66,67] indicated that the overproduction and high concentration of IAA may suppress root formation and elongation and thus negatively affect the rice growth and development.

This isolate was also able to produce cellulase that could degrade cell wall of pathogenic fungus. Similarly, [71] reported the inhibitory

Table 5 Comparison of indole-3-acetic acid (IAA) production, phosphate solubilization and siderophore production of actinomycetes from various related works.

IAA production (µg/mL)	Phosphate released in broth (mg/L)	Siderophore production (µmol/L)	References
144.0	-	-	[68]
79.5	721.30	-	[69]
43.8	-	-	[16]
14.60	-	-	[70]
11.12	224.27	97.50	[14]
7.71	28.49	1,725.00	This study

activity against *Phytophthora cinnamomi*, root rot fungus, by cell wall degradation caused by cellulase producing actinomycetes. Recently, [72] demonstrated the ability of some *Streptomyces* sp. as biocontrol agent of *Alternaria* sp., *F. oxysporum*, *P. oryzae* and *Sclerotium* sp. by producing cellulase, a cell wall degrading enzyme.

5. Conclusion

Our study clearly showed that *S. iranensis* S2-SC16 from mangrove sediment could not only suppress the growth of *F. fujikuroi* CMU-F02 but also improve the growth and development of rice seedlings. This indicated the possibility of using mangrove actinomycetes as plant growth promoter and biocontrol agent for Bakanae disease in Thai jasmine rice (*O. sativa* KDML 105). However, further studies and field experiments are needed, in order to establish a sustainable rice production system using *S. iranensis* S2-SC16.

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