
Serratia marcescens* KMITL2020 as a plant growth stimulant and bacterial antagonist to control brown leaf spot of rice caused by *Drechslera oryzae

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Abstract *Serratia marcescens* KMITL2020 proved to be growth stimulant and antagonistic to *Drechslera oryzae* causing brown leaf spot of rice caused by *Drechslera oryzae*. The brown leaf spot disease was isolated the causal agent which identified as *Drechslera oryzae*. Bi-culture test between *D. oryzae* and *S. marcescens* KMITL2020 inhibited spore production of the tested pathogen 44.12 %. It showed that crude extract from *S. marcescens* KMITL2020 gave effectively against *D. oryzae* which the effective dose (ED₅₀) was 92.57 µg/ml. Result showed crude extract from *S. marcescens* and carbendazim treatments significantly controlled brown leaf spot caused by *D. oryzae* which reduced the disease of 25 % when compared to the non-treated control in 28 days. Plant growth parameters revealed that crude extract from *S. marcescens* gave significantly higher tillers, plant height, plant fresh weight, root fresh weight, plant dried weight, root dried weight than carbendazim treatment when compared to the non-treated control.

Keywords: *Serratia marcescens*, crude extract, *Drechslera oryzae*, biological control

Introduction

Rice (*Oryza sativa* L.) is one of the major food crops in Asia and it is daily diet in other regions of the world. It is one of the most important staple food to serve the increasing world population, especially in Asia. Asian farmers are still account for 87% of the world's total rice production. Rice is mainly exported of Thailand. Rice diseases are damaged and reduced yield by abiotic and biotic factors. The biotic factors are mainly caused by bacteria, virus and fungi. The brown leaf spot of rice caused by *Drechslera oryzae* is one of the

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destructive rice disease in cultivated rice fields in Thailand and one of the important disease in the world. It causes seriously disease and both quantity and quality yield losses. The averaged yield loss was about 5% in lowland ricefields in South and Southeast Asia. It was reported that some areas were severely infected fields caused 45% yield loss (IRRI, 2019). The traditional chemical fungicides have been used for years and some case the pathogens become resistance to those chemical fungicides. However, there are many researcher were reported to use the biocontrol agents to control disease. Biological control of plant pathogens has become widely used to reduce the disease incidence. The use of toxic chemical fungicides confirmed the occurrence of pollution to surrounding environment. Queiroz and Meo (2006) found that *Serratia marcescens* R-35 isolated from citrus rhizosphere inhibited *Phytophthora parasitica* over 50% of the disease occurrence and promoted plant growth. Jaiganesh *et al.* (2007) stated that a new antagonist, *S. marcescens* proved to control of *Pyricularia oryzae* causing rice blast which reported to produce chitinolytic enzymes to degrade the fungal cell walls, plant defence induction. The objectives of reserch project were proved *Serratia marcescens* KMITL2020 to promote plant growth, and control brown leaf spot of rice var. Chainart1.

Materials and methods

Isolation of Drechslera oryzae from rice brown leaf spot disease

The brown leaf spot symptoms were collected from infested field planted to rice var. Chainart 1. The samples were isolated by tissue transplanting method. Leaf symptoms were cut in advanced margin into rectangular small pieces, soaked 10 % sodium hypochlorite, and steriled water, placed on water agar (WA), and incubated at room temperature (28-30°C). observe everyday until hypha appear cut and move onto potato dextrose agar (PDA) to get pure culture.

Antagonistic bacterium

The antagonistic bacterium, *Serratia marcescens* KMITL2020 is obtained from Biological control Research Unit, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand and It was confirmed by morphological and molecular phylogenetic from previous work, and subcultured on nutrient agar (NA) for further experiment.

Pathogenicity test

The spore concentration of pathogen, *D. oryzae* was adjusted to 1×10^5 spores/ml. It was inoculated on wounded leaves of rice var. Chainart 1 of the 21 days of seedlings stage by spraying 15 to 20 ml. for one wounded leaf, then kept in moisten chamber at room temperature. The non-inoculated was served the control. Each treatment was repeated four times. The infected lesion on inocuated wound leaves were observed as lesion size. Disease index was scored on a 0 ± 10 point scales for the scoring of brown leaf spot of rice caused by *Drechslera oryzae* given by IRRI (2019).

Bi-culture test

The interaction between antagonistic bacterium, *Serratia marcescens* KMITL2020 and *Drechslera oryzae* causing brown leaf spot of rice was done using Completely Randomized Design (CRD) with 4 replications. The four culture plugs (0.5 cm) of *Serratia marcescens* were placed in equally distance of rectangular in potato dextrose peptone agar (PDPA) petri dish. A culture plug (0.5 cm) of *Drechslera oryzae* was moved the middle of four culture agar plugs of antagonist in the medium. Bi-culture plates were incubated at room temperature and observe for 20 days. The colony diameter was measured and number of spores were counted by haemocytometer. Percent inhibition of colony growth and spore production were calculated by the following formula: Growth inhibition; $GI = R1 - R2 \times 100$, where R1 = colony diameter or conidia number of pathogen in control R2 = colony diameter or conidia number of pathogen in control in dual culture plate. Data were analyzed analysis of variance (ANOVA) and use Duncan Multiple's Range Test (DMRT) at $P = 0.05$ and 0.01 .

Testing crude extract from serratia marcescens KMITL2020 to control Drechslera oryzae

The crude metabolic extract of *S. marcescens* KMITL2020 was done by culturing in the flasks of nutrient broth (NB), and shake at room temperature for 150 rpm by orbital shaker until appeared red color, then centrifuged at 2800 rpm 20 min. The bacterial suspension was filtered out of bacterial cells, and kept the supernatant for extraction with rotary evaporator at 55°C to yield crude extract. The crude extract was tested to control *Drechslera oryzae* (rice brown leaf spot). The experiment was set up in Completely Randomized Design (CRD) with 4 replications. Crude extractv was dissolved in 2 % dimethyl sulfoxide, mixed into PDA, autoclaved for 30 mins at 121°C , 15 lbs.

Treatments were concentration of crude extracts of 0,10,50,100, 500, and 1000 ppm. The agar plug of tested pathogen was transferred into the middle to each concentration, incubated at room temperature and observed until the pathogen in the control plates was full grown. Spore number and colony diameter (cm.) were recorded, then statistically computed analysis of variance (ANOVA), and mean comparison was calculated using Duncan Multiple's Range Test (DMRT) at P = 0.05 and 0.01. The probit analysis program was computed the effective dose (ED₅₀).

The efficacy test of crude extract from *Serratia marcescens* KMITL2020 to control rice brown leaf spot

Randomized completely block design (RCBD) was performed with four repeated experiments. Treatments were set up as follows: T1= inoculated control, T2= crude extract from *S. marcescens* KMITL2020 at the concentration of 1,000 ppm, and T3= carbendazim fungicide (1 g/1L). The seedlings of rice var Chainart were planted in pots containing sterilized soil (loamy soil: organic compost as 10:1 ration w/w) in each experimental unit. The tested pathogen, *D. oryzae* was cultured on PDA for 15 days, then made the spore suspension of 1×10^5 spores/ml, and inoculated to all tested plants by spraying onto the wounded lesion on leaves. The inoculated plants were sprayed either crude extract of *S. marcescens* or carbendazim fungicide. All tested plants were maintained in greenhouse for one month. Data were collected as plant height (cm), fresh and dried weight of plants (g). Disease index was scored as 1= no symptom, 2= 1-25 %, 3= 26-50%, 4= 52-75 %, and 5= 76- 100 %.

Results

Isolation of *Drechslera oryzae* from rice brown leaf spot disease

The brown leaf spot disease was isolated the causal agent which identified as *Drechslera oryzae*. The pathogen was proved to be pathogenic isolate according to *D. oryzae* belongs to Pleosporaceae, Kingdom Fungi. The fungus grew on PDA for 19 days with brown black in color of colony. It produces conidia on conidiophore either in the top or side of conidiophore, conidia 3-9 septa (Figure 1). With this, brown leaf spot of rice is reported to infect rice either in seedlings or mature.

Antagonistic bacterium

The antagonistic bacterium, *S. marcescens* KMITL2020 was morphological reconfirmed identification. It showed a smooth shine red colony, rod shape, and found to gram negative (Figure 2).

Pathogenicity test

Drechslera oryzae isolated from brown leaf spot of rice var. Chainart 1 that was proved to be virulence for disease infection to rice var Chainart 1 causing brown leaf spot. The inoculated *D. oryzae* showed high disease index of level 5. The non-inoculated control revealed no infection.

Bi-culture test

Bi-culture test between *D. oryzae* and *S. marcescens* KMITL2020 control brown leaf spot of rice showed that inhibiting the growth of colony by measuring the diameter of *D. oryzae* (5.00 cm), compared with control (9.00 cm.), colony inhibition of 44.44%. The spore production in control plate and bi-cultuer plate found 6.47×10^5 spores/ml and 3.63×10^5 spores/ml., respectively and showed spore inhibition of 44.12% (Figure 3).



Figure 1. Morphological characteristics of *Drechslera oryzae*

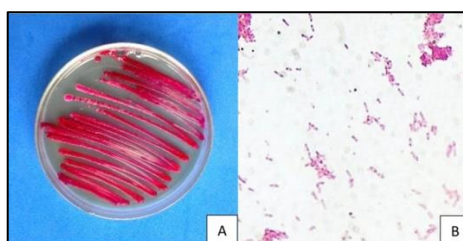


Figure 2. Morphological characteristics of *Serratia marcescens* KMITL2020
A= colony, B= cells of *Serratia marcescens* KMITL2020 after gram strain

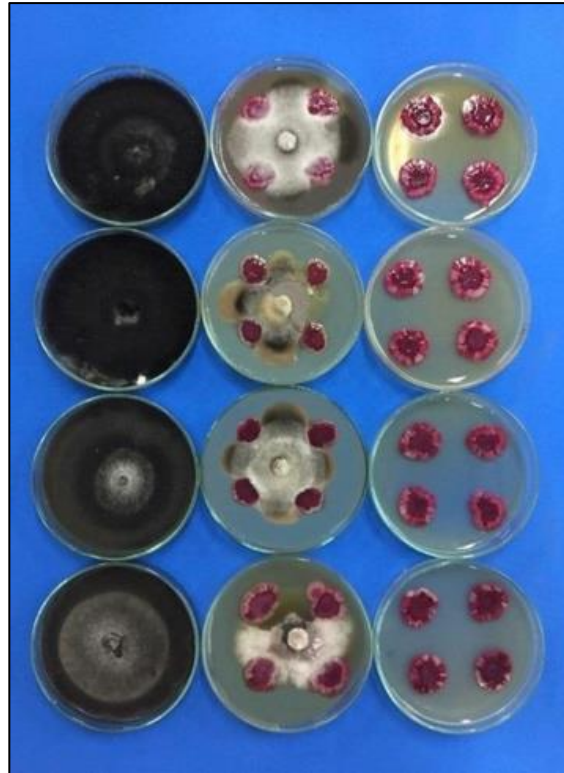


Figure 3. Bi-culture test between *Drechslera oryzae* and *Serratia marcescens* KMITL2020, left = *Drechslera oryzae*, middle = Bi-culture and right = *Serratia marcescens* KMITL2020

Testing crude extract from Serratia marcescens KMITL2020 to control Drechslera oryzae

It showed that crude extract from *S. marcescens* KMITL2020 (Figure 4) at concentration 1,000 ppm significantly inhibited colony and spore production of 26 % and 94 % respectively which the colony diameter of 3.70 cm., and spore production was 0.16×10^5 spores/ml. when compared to the control (colony diameter of 5 cm., and spore production of 2.98×10^5 spores/ml). Crude extract from *S. marcescens* KMITL2020 significantly inhibited spores of *D. oryzae* at concentration 10, 50, 100, 500, 1000 ppm which were 11.9, 16.41, 58.4, 91.58, and 94. 58 ppm., respectively. It was shown that crude extract from *Serratia marcescens* KMITL2020 gave effectively against *D. oryzae* which the effective dose (ED_{50}) was 92.57 $\mu\text{g/ml}$ (Table 1, Figure 5).

The efficacy test of crude extract from Serratia marcescens to control rice brown leaf spot

Result showed crude extract from *S. marcescens* and carbendazim treatments significantly controlled brown leaf spot caused by *D. oryzae* which reduced the disease of 25 % when compared to the non-treated control in 28 days. Disease index (DI) in crude extract from *S. marcescens* and carbendazim treatments were 4.5 which significantly higher DI than the non-treated control (DI=6.0) as seen in Table 2. Plant growth parameters revealed that crude extract from *S. marcescens* gave significantly higher tillers (15.50), plant height (65.75 cm), plant fresh weight (25.32 g), root fresh weight (6.21g), plant dried weight (4.27 g), root dried weight (0.77 g) than carbendazim treatment (10.50, 52.75 cm, 19.46 g, 3.02 g, 2.84 g and 0.43 g, respectively) when compared to the non-treated control (11.75, 56.25 cm, 23.45 g, 3.61g, 4.04 g and 0.46 g, respectively) (Table 3 and Figure 6).



Figure 4. Crude extracts from *Serratia marcescens*

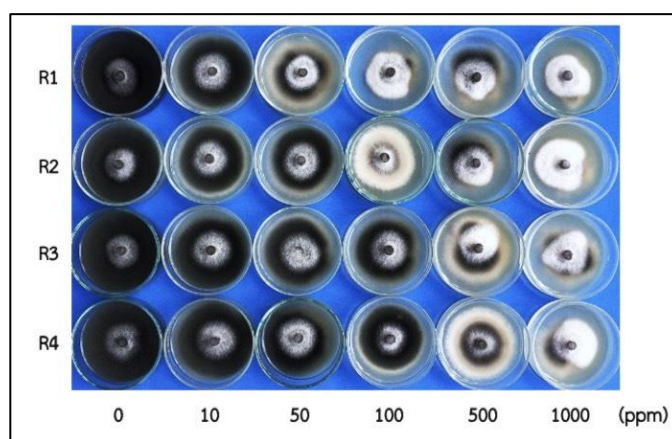


Figure 5. Crude extract test of *Serratia marcescens* KMITL2020

Table 1. Crude extract of *Serratia marcescens* KMITL2020 against *Drechslera oryzae*

Concentrations ($\mu\text{g/ml}$)	<i>Drechslera oryzae</i>				
	Colony diameter(cm)	% inhibition of colony	Spores production	% inhibition of spores	ED ₅₀ ($\mu\text{g/ml}$)
0 (control)	5.00 ^a	0.00 ^b	2.98 ^a	0.00 ^c	92
10	4.62 ^a	7.50 ^b	2.62 ^a	11.90 ^c	
50	4.52 ^a	19.50 ^a	2.48 ^a	16.41 ^c	
100	3.85 ^b	23.00 ^a	1.21 ^b	58.30 ^b	
500	3.87 ^b	22.50 ^a	0.24 ^c	91.58 ^a	
1000	3.70 ^b	26.00 ^a	0.16 ^c	94.58 ^a	
CV%	6.38	38.87	29.04	36.40	

¹ Means of four replications, means comparison in each column were significantly differed by DMRT at P =0.01.

Table 2. efficacy of crude extract from *Serratia marcescens* KMITL2020 to control rice brown leaf spot in 28 days

Treatments	Disease Index			
	Day 7	Day 14	Day 28	% Disease reduction
T1 control	4.50	3.70 ^{b1}	6.00 ^a	-
T2 crude extract	4.50	3.75 ^b	4.50 ^b	25
T3 carbendazim	4.50	4.50 ^a	4.50 ^b	25
CV%	-	35.86	16.33	-

¹ Means of four replications, means comparison in each column were significantly differed by DMRT at P =0.0

Table 3. Plant growth parameters from treatment of crude extract from *S. marcescens* compared to chemical fungicide in rice var. Chainart1 at 28 days

Treatments	tillers	Plant height (cm)	Roots height (cm)	Plant fresh weight (g)	Roots fresh weight (g)	Plant dried weight (g)	Roots dried weight (g)
T1 Control	11.75 ^{b1}	56.25 ^{ab}	31.00 ^a	23.45 ^a	3.61 ^b	4.04 ^a	0.46 ^b
T2 Crude extract	15.50 ^a	65.75 ^a	25.50 ^b	25.32 ^a	6.21 ^a	4.27 ^a	0.77 ^a
T3 Carbendazim	10.50 ^b	52.75 ^b	25.50 ^b	19.46 ^b	3.02 ^b	2.84 ^b	0.43 ^b
CV%	16.70	10.04	18.41	24.70	47.66	16.31	41.31

¹ Means of four replications, means comparison in each column were significantly differed by DMRT at P =0.01.

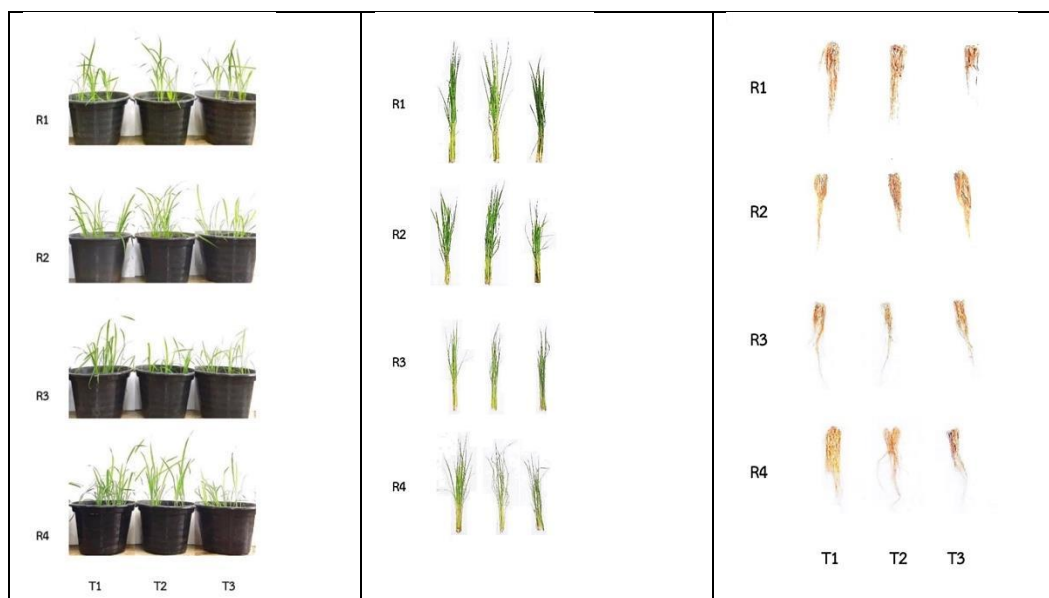


Figure 6. Testing crude extract from *Serratia marcescens* KMITL2020 with comparison to chemical fungicide, R = replications, T1=control, T2=crude extract and T3=carbendazim

Discussion

It found that brown leaf spot disease of rice caused by *Drechslera oryzae*. As reports by Sunder *et al.* (2005) who stated that brown spot of rice caused by *D. oryzae*. Similar reports found with Soyong (2014) noted that brown leaf spot of rice var Pittsanulok 2 caused by *D. oryzae* in Thailand. But Tann and Soyong (2017) found that brown Leaf Spot disease of rice variety IR66 caused by *Curvularia lunata* in Cambodia. Research finding found that *D. oryzae* isolated from brown leaf spot of rice var. Chainart 2. that was virulence infection in rice var Chainart 2. Moreover, Khalili *et al.* (2012) found that brown spot caused by *Bipolaris oryzae* which it is an important rice disease in Southern coast of Caspian Sea, the major rice growing region in Iran.

Serratia marcescens proved to inhibit *Drechslera oryzae* causing brown spot of rice var Chainart 1. However, Purkayastha *et al.* (2018) reported that *S. marcescens* strain ETR17 isolated from tea rhizosphere that showed the effective management of root rot disease in tea. Other research reports found that Soyong (2014) reported *D. oryzae* causing brown leaf spot of rice var Pittsanulok 2 was inhibited mycelial growth and spore production of 83.83 and 71.55 percent, respectively and Yasmin *et al.* (2016) recorded that five

antagonistic bacteria i.e. *Pseudomonas* spp. E227, E233, Rh323, *Serratia* sp. Rh269 and *Bacillus* sp. Rh219 expressed antagonistic activity showing zone of inhibition 1-19 mm, to control bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*. Moreover, Valikuntapu *et al.* (2016) reported that *S. marcescens* (SmChiD) expressed chitinase-D which including chitobiase and transglycosylation activities besides hydrolytic activity. *S. marcescens* (SmChiD) produced a potential enzyme for chitin degradation. It may get involve in control mechanism.

Result indicated that *D. oryzae* was inhibited by the crude extract from *S. marcescens* which the ED₅₀ of 92 µg/ml. It was similar to the works of Soyong (2014) found that *D. oryzae* causing brown leaf spot of rice var Pittsanulok 2 was inhibited mycelial growth and spore production by crude extracts of *Chaetomium cochliodes* which ED₅₀ value was 66 µg/ml. Withthis, Wang *et al.* (2013) stated that *S. marcescens* strain JPP1 from peanut hulls in Huai'an city, Jiangsu Province, China showed effectively to inhibit *Aspergillus parasiticus* which it is an aflatoxin production isolate. *S. marcescens* strain JPP1 produced chitinase to degrade cell walls of fungal pathogen. *S. marcescens* JPP1 showed effectively reduced aflatoxin production on peanut seeds.

The crude metabolite from *S. marcescens* KMITL2020 and carbendazim treatments resulted to reduce brown leaf spot caused by *D. oryzae* at 25 %. It was similar result from report of Vareeket and Soyong (2017) that *S. marcescens* actively inhibited *Curvularia lunata* and *D. oryzae* causing brown leaf spot of rice var RD41. Withthis, Purkayastha *et al.* (2018) stated that *S. marcescens* strain gave effective control foliar and root rot disease in tea, and they stated that *S. marcescens* produced hydrolytic enzymes eg chitinase, protease, lipase, cellulase and plant growth promoting metabolites like IAA and siderophore. Moreover, Vareeket *et al.* (2018) stated that nano-particles derived crude metabolite from *Chaetomium brasiliense* gave a good control brown spot of rice caused by *D. oryzae*. *S. marcescens* BTR1.0 was a bacterial antagonists against tomato damping-off *Pythium aphanidermatum* causing damping-off disease in tomato under salt-water irrigation at 50 mM NaCl concentration which suppressed the disease by 68% compared to infected control (Karunasinghe *et al.*, 2019).

It is indicated that crude metabolite of *S. marcescens* KMITL2020 promoted the growth parameters of rice var Chainart 1. Islam and Nandi (1985) noted that *Bacillus megaterium* was not only inhibited brown spot pathogen of rice but also promote plant growth in pot experiment and field trial. Purkayastha *et al.* (2018) also reported that *S. marcescens* strain ETR17 promoted the growth of tea. Further research finding of El Khaldi *et al.* (2015)

found that *S. marcescens* isolated from date palm compost and it expressed antifungal activity against *Rhizoctonia solani* causing stem canker and black scurf diseases of potato. *S. marcescens* was an effective biocontrol agent which reduced the disease incidence over 83.16%. Moreover, Brigida and Itamar (2006) found that *S. marcescens* R-35 from rhizosphere of citrus can be inhibited *Phytophthora parasitica* causing root rot of citrus over 50 % and stimulate the growth of citrus. And Tariq *et al.* (2017) stated *S. marcescens* acts as PGPR could be used as biopesticides and biofertilizers for better plant health and growth improvement. Chakraborty, *et al.* (2010) stated that *S. marcescens* TRS-1 promoted growth in tea seedlings. It was solubilized phosphate which application of *S. marcescens* TRS-1 leading to root and leaf phosphate increased and soil P content decreased, then enhanced soil phosphatase activity. *S. marcescens* TRS-1 also decreased brown root rot of tea caused by *Fomes lamaoensis*. The application of *S. marcescens* resulted to increase phenolics, peroxidase, chitinase, β -1,3-glucanase and phenylalanine ammonia-lyase in tea plants. Further research would be interested to develop as bioproduct to be applied for multipurpose as biofertilizer, biofungicide and bionematicide.

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