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RESEARCH ARTICLE OPEN ACCESS

Comparison of the Antagonistic Potential of the Entomopathogenic Bacterium *Serratia nematodiphila* GCSR38 with other Effective Microorganisms for the Control of Rice Bacterial Leaf Blight

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Abstract

The efficacy of antagonistic microorganisms and secondary metabolites of entomopathogenic bacteria was evaluated *in vitro* and *in vivo* against *Xanthomonas oryzae* pv. *oryzae (Xoo),* a causal agent of rice bacterial leaf blight. The effect of the culture filtrates of the microorganisms was tested against *Xoo,* using the agar well diffusion assay. *Trichoderma* sp. UB05/3 was the most effective against *Xoo.* However, the results were not statistically different from those of the entomopathogenic bacterium *Serratia nematodiphila* GCSR38 with a 22.97 mm and 19.15 mm zone of inhibition, respectively. The effect of the secondary metabolite crude extracts of antagonistic microorganisms (2,000 µg/ml) on *Xoo* inhibition was tested by the paper disc diffusion method. Only *S. nematodiphila* GCSR38 was able to control *Xoo,* with an inhibition zone of 17.60 mm. Minimum inhibitory concentration (MIC) was determined using a 96-well microtiter plate. The MIC of secondary metabolites crude extracts of *S. nematodiphila* GCSR38 was 1,000 µg/ml and the minimum bactericidal concentration (MBC) was 1,000 µg/ml. The efficacy of the secondary metabolite crude extract of *S. nematodiphila* GCSR38 against *Xoo* was tested using the detached leaf technique; the secondary metabolite crude extracts controlled the disease, with 24.45% and 15.56% disease severity when used before and after inoculation with *Xoo,* respectively. Inoculation with *Xoo* alone resulted in a disease severity of 44.45%. The secondary metabolite crude extracts of *S. nematodiphila* GCSR38 can reduce disease severity of bacterial leaf blight in rice by 28.89%, whereas zinc thiazole causes a disease severity of 22.22% and *Xoo* alone causes a disease severity of 66.67%.

Keywords: Entomopathogenic bacteria, Minimum Inhibitory Concentration (MIC), secondary metabolite

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INTRODUCTION

Rice (*Oryza sativa* L.) is a major economic crop in Thailand, and it is the staple food for more than half of the world's population. In many places worldwide, including the Asia-Pacific region, North America, South America, and Africa, rice is used as the main source of energy. The disadvantage of Thai rice is its high production cost and low yield per hectare, which affect the efficiency of its export in the ASEAN trade market. One major factor affecting the productivity of Thai rice is the bacterium *Xanthomonas oryzae* pv. *oryzae (Xoo),* which causes bacterial leaf blight (BLB).[1-4] It occurs in both rainfed and irrigated fields in all regions of Thailand and has a high epidemic potential in many parts of the world, causing severe crop losses of up to 50–60%.[5,6] Various disease control strategies have been used to reduce productivity losses and prevent rice disease spread. However, chemical-based strategies were unsuccessful because of differential sensitivity of the rice pathogenic species to the chemicals. In addition, the use of antibiotics and chemicals in the control of BLB is limited because of their toxic residues.[7] Currently, BLB in rice is controlled by various chemicals, along with the use of disease-resistant rice cultivars such as Suphan Buri 60, Suphan Buri 90, Suphan Buri 1, Suphan Buri 2 and Kor Khor 23. However, it is still not possible to control the disease effectively because of genetic variations in the pathogens, unstable resistant rice varieties, sensitivity of pathogen to different antibiotics or other environmental factors, and chemical resistant pathogens, which limit the potential of management strategies. The increasing desire to reduce pesticide use has led to the development of integrated pest management methods that make the best use of natural resources. Therefore, host plant-resistant varieties are the first important strategy for disease control.

Biological controls are on the list of alternative management strategies with high potential. Use of biological controls is a cost-effective approach, which is less toxic to non-target species and environment friendly.[6,8-11] To reduce the loss of productivity due to disease, it is necessary to use environmentally friendly methods. *Trichoderma* spp. is a powerful antagonistic fungus that controls a wide range of plant diseases; it not only acts as a biological regulator, but also stimulates plant defense mechanisms against pathogens and promotes plant growth, resulting in higher crop yields. Biological control mechanisms include mycoparasitism, antibiosis, competition, and stimulation of plant defense mechanisms against pathogens.[12] Several studies[13-15] have reported the efficacy of *Trichoderma* in controlling *Xoo*, the causal agent of BLB in rice, *in vitro,* under greenhouse conditions, and in the field. For example, a comparison of the antagonism of *Trichoderma* against *Xoo* showed that *T. harzianum* showed the fastest growth with 62.3% mycelial growth after 72 h of incubation. Several species of *Trichoderma* completely inhibit the growth of *Xoo*.[13] Volatile compounds produced by *T. harzianum* resulted in the lowest average *Xoo* growth radius, followed by *T. hamatum*. Non-volatile compounds of *T. harzianum* at a concentration of 6.25% inhibited *Xoo* growth by 51.1%. Results showed that *T. harzianum* was the most effective isolate for reducing disease severity, with 48.26% and 59.22% reduction in disease severity, respectively. *T. harzianum* isolated from the phylloplane of rice can reduce disease severity and lead to higher yields compared to isolates from other sources.[15]

The most commonly used biological control agents are *Bacillus* spp.; they control pathogens by mechanisms, including antibiosis, competition, parasitism, and induction of systemic resistance, with antibiosis being the most important mechanism.[16] El-Shakh et al.[17] investigated the efficacy of endophytic bacteria in controlling the causal agent of BLB in rice (*Xoo*). Endophytic bacteria and rhizospheric *Bacillus* were tested for their antagonistic activity against *Xoo* in BLB. *In vitro*, all tested bacteria were found to have a high potential for antagonism against *Xoo*. Based on the results of 16S rRNA gene sequencing, *Bacillus* isolates can be classified as follows: *Bacillus amyloliquefaciens, Bacillus methylotrophicus*, and *Bacillus subtilis. B. amyloliquefaciens* FZB42 and *B. amyloliquefaciens* FZB42 produce the antimicrobial compounds difficidin and bacilysin, which inhibit *Xanthomonas* strains. Chen et al.[18] demonstrated the ability of *B. amyloliquefaciens*

FZB42 to target pathogenic microorganisms in rice, using the antibiotics difficidin and bacilysin. Interestingly, *B. amyloliquefaciens* FZB42 promoted plant growth and inhibited plant pathogens. The genome of FZB42 has been sequenced, and it contains a large gene cluster that produces several secondary metabolites with antimicrobial activity. The antibacterial activity is mainly due to the non-ribosomal synthesis of polyketides.[19] *Bacillus* D13 also has antagonistic activity against *Xoo*. The volatile compounds of strain D13 decreased the colony size and motility of *Xoo* cultured on each plate.[20]

Serratia spp. belong to the Enterobacteriaceae family. *Serratia* is a gram-negative bacterium that is rod-shaped, and some species produce red pigments. Some strains of *Serratia* are harmful to pests, when consumed in large amounts. However, sometimes these species can be virulent even when insects are exposed to small amounts. They can kill insect larvae within 48–72 h, with larvae showing symptoms similar to those of a viral infection.[21] In particular, red pigment-producing species have a greater effect on insects than non-pigmented species.[22] *Serratia* sp. EML-SE1 has insecticidal potential that can kill diamondback moths.[23] Control of several insect genera, such as *Anomala, Costelytra,* and *Phyllophaga,* can be achieved by entomopathogenic strains of *Serratia entomophila.*[24] It was also found that control of grass moths (*Costelytra zealandica*) was possible with *S. entomophila* and *S. proteamaculans.*[24-26] This confirms that these two species have insecticidal properties.[27] Long-term pest management guidelines are required for the importation of exotic microorganisms and their subsequent release to destroy pests. Some insect pathogens are also used in this classical microbial management approach, which is related to antibiotic and siderophore production that can inhibit plant pathogens. The objective of this study was to compare the efficacy of secondary metabolites of the entomopathogenic bacterium *S. nematodiphila* GCSR38 with that of antagonistic microorganisms in controlling *Xoo*, the causal agent of BLB in rice, to provide a guide for future management strategies.

MATERIALS AND METHODS

Microorganisms

The causative agent of BLB in rice *Xoo* was obtained from the Plant Protection Research and Development Office of the Ministry of Agriculture and Cooperatives, Thailand. Antagonistic microorganisms, including nine antagonistic bacterial isolates (*Bacillus* sp.), five antagonistic fungal isolates (*Trichoderma* sp.), and one entomopathogenic bacterial isolate (*Serratia nematodiphila* GCSR38) were donated by the Microbiology Laboratory, Department of Biology, Faculty of Science, Mahasarakham University. All isolates were stored in 20% glycerol at -20°C until use.

To confirm the species of GCSR38, the classification was repeated as follows: GCSR38 was grown on nutrient agar (NA) plates for 24 h at 37°C, and colony morphology was observed, such as form, margin, and elevation. They were then Gram stained for microscopic observation. The 16S rRNA gene was classified by sequencing. Genomic DNA of the GCSR38 strain was extracted using the CTAB method.[28] Universal bacteria primers 27F 5'-AGAGTTTGATC MTGGCTC AG-3' and 1492R 5'-TACGGYTAC CTTGTTACGACT T-3' were used for 16S rRNA gene amplification. Amplifications were performed in a Gradient DNA Thermal Cycler programmed for the following parameter: 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 52°C for 30 s, 72°C for 45 s, and a final incubation at 72°C for 5 min. PCR products were commercially sequenced by Macrogen Inc. (Seoul, South Korea). The 16S rRNA gene sequences of bacterium present in the NCBI database were used for alignment and comparison. Sequence data were obtained using BLAST for phylogenetic tree generation of the GCSR38 strain. Closely related sequences were downloaded and aligned using CLUSTAL X.[29] Maximum parsimony (MP), maximum likelihood (ML), and neighbor-joining (NJ) methods were used for sequence analysis.[30]

Efficacy of culture filtrates of effective microorganisms in controlling *Xoo*

The agar well diffusion method was used to test the activity of the culture filtrate. First, antagonistic fungal culture filtrate was prepared by transferring three mycelia discs (8 mm diameter) of

each isolate into 100 ml of potato dextrose broth (PDB), which was then incubated at 28±2°C under static conditions for 7 d. The culture filtrate was collected by separating the fungal mycelia through whatman no. 1 filter paper. The supernatant was centrifuged at 8,000 × g for 15 min to remove suspended particles, and filtered through a 0.22 um sterile membrane filter. Bacterial cultures maintained on NGA slants were aseptically inoculated into 10 ml of nutrient glucose broth (NGB) and then incubated in a shaking incubator at 150 rpm and 28±2°C for 48–72 h. Bacterial cultures were centrifuged at 10,000 × g for 15 min, and the supernatant was collected and filtered through a 0.22 µm sterile membrane filter. *Xoo* cell suspension (10^8 colony forming unit (CFU)/ml) was swabbed on NA plats, and an 8-mm diameter hole was drilled with a sterile cork borer at four points in a cross pattern. Next, 20 µl of culture filtrate was added to the well. The agar plates were then incubated at 28±2°C, and after 24–48 h a clear zone was observed and measured. There were five replicates for each isolate, and 100 ppm rifampicin[31] was used as a control.

Secondary metabolite extraction

For preparing secondary metabolites, antagonistic bacteria were cultured in NGB medium (500 ml) and incubated under shaking at 150 rpm and 28±2°C for 48–72 h. Antagonistic fungi were cultured in PDB medium (500 ml) under static conditions for 7 d. To remove the bacterial cell pellet or fungal mycelia and spores, the culture flask was centrifuged at 10,000 × g at 4°C for 15 min. The supernatant (350 ml of supernatant was extracted with 350 ml ethyl acetate at a 1:1 ratio) and 5 g of NaCl were added for the first extraction. This extraction step was repeated three times. The supernatant in the upper phase of the separatory funnel was collected and pooled, and the solvents were evaporated in a rotary evaporator at 40°C.

The effect of the antagonistic microorganism secondary metabolite crude extract (AMSMCE) on inhibition of *Xoo*

To determine the antibacterial effect of AMSMCE against *Xoo*, the paper disc diffusion method was used. The extract was dissolved in 1% DMSO to a final concentration of 2,000 µg/ml. A sterile filter paper disc (6 mm) containing 10 µl of extract was placed on the surface of NGA plates already inoculated with *Xoo* and incubated at 28±2°C for 24–48 h. The inhibitory effect was determined by measuring the inhibition zone on a millimeter scale.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Determination of MIC was done using the method by Clinical and Laboratory Standards Institute.[32] First, serial two-fold dilutions (1.95–1,000 µg/ml) of the AMSMCE were added to 96-well microtiter plates and 100 µl of *Xoo* (10^8 CFU/ml) was added to each well. Then, 100 µl of Mueller Hinton broth (MHB) was added to well 1 to serve as the negative control, and 100 µl of 100 ppm rifampicin was added to well 12 to act a positive control, and the plate was incubated at 28±2°C for 24 h. The OD600 was then measured using a Asys UVM 340 Microplate Reader (Biochrom)

MBC was assessed by placing samples from wells with concentrations above the MIC and MIC values and below the MIC values on an NA Petri plate, and the plate was incubated at 28±2°C for 24 h. The MBC value was assessed when no colony growth was observed on the NA plates.

The efficacy of AMSMCE in controlling rice BLB

Detached leaf technique

Healthy leaves of Jasmin Rice 105 were selected for the detached leaf method. Rice leaves were washed with tap water for 5 min, sprayed with 70% ethanol, and air-dried. Wounds were inflicted on the leaves using a sterilized inoculation needle (5 wounds/point). A completely randomized block design experiment was planned, consisting of four treatments as follows: (i) 10 µl of AMSMCE was added 24 h before the addition of 10 µl of *Xoo* cell suspension (10^8 CFU/ml), (ii) 10 µl of *Xoo* cell suspension (10^8 CFU/ml) was added

Table 1. Disease severity scale for bacterial leaf blight disease.[32]

Disease Index	Score Lesion area (%)
0	0
1	0-10
3	11-30
5	31-50
7	51-75
9	76-100

Table 2. Efficacy of effective microorganism culture filtrate to control *Xanthomonas oryzae* pv. oryzae by agar well diffusion method

Isolates		Inhibition zone[1/] (mm)
Antagonistic fungi	*Trichoderma* sp. SK24	19.75+0.71 bc
	Trichoderma sp. UB07 special	20.75+0.98 bc
	Trichoderma sp. UB04/3	20.01+1.55 bc
	Trichoderma sp. UB05/3	22.97+1.58 ab
	Trichoderma sp. WU01	20.13+0.33 bc
Antagonistic bacteria	*Bacillus* sp. BWPD-KK23	12.32+0.39 bcd
	Bacillus sp. BWTM-MSU2	17.90+1.87 bc
	Bacillus sp. BWWM-MSU16	19.15+1.00 bc
	Bacillus sp. BWTM-MSU6	8.49+0.43 cd
	Bacillus sp. BWWM-MSU6	8.28+0.46 cd
	Bacillus pumilus FDKF5	12.27+1.35 bcd
	Bacillus sp. GCMR18	12.52+1.00 bcd
	Bacillus sp. GCMR44	9.07+0.79 cd
	Bacillus sp. IKM1	10.59+0.33 bcd
	Serratia nematodiphila GCSR38	18.25+1.25 bc
Negative control	dH_2O	0.00+0.00 d
Positive control	Rifampicin	32.87+1.89 a
	CV (%)	6.61

[1/] Different superscripts within the same column indicate a significant difference ($P < 0.05$).

24 h before the addition 10 µl of AMSMCE, (iii) 10 µL of *Xoo* cell suspension (10^8 CFU/ml) was added, and (iv) 10 µL dH_2O was added. All plants were incubated in a moist chamber for 7 d. For disease rating, a modified scale was used.[33] (Table 1).

Disease severity was calculated with the followed formula:

$SI = [\sum(Ni \times Vi) / (V \times N)] \times 100$

Ni = number of leaves in each disease scale

Vi = disease scale in each leaf

V = maximum disease scale

N = number of leaves

Pot experiments

Jasmin rice 105 was used as the test crop. The experiments in the pots were designed as a completely randomized experiment with four replicates, using three 21-d-old seedlings in each pot. The clipping method with sterilized scissors

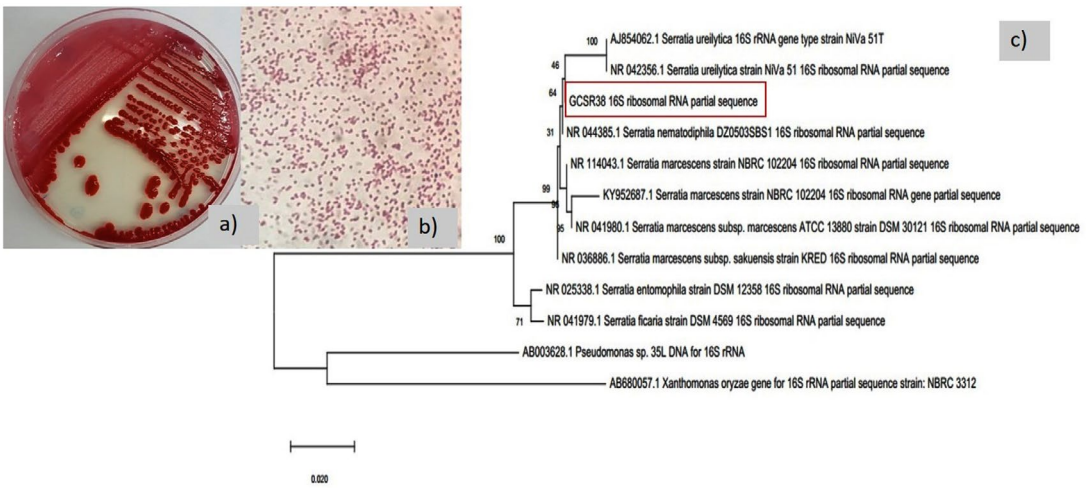

Fig. 1. Characteristic of GCSR3 a) colony morphology b) gram stain c) phylogenetic tree base on 16S rRNA sequence.

Table 3. Effect of antagonistic microorganism secondary metabolites crude extracts (AMSMCE) against *Xanthomonas oryzae* pv. *oryzae* by using agar well diffusion technique

Isolates		Inhibition zone[1/] (mm)
Antagonistic fungi	*Trichoderma* sp. SK24	0.00 + 0.00 c
	Trichoderma sp. UB07 special	0.00 + 0.00 c
	Trichoderma sp. UB04/3	0.00 + 0.00 c
	Trichoderma sp. UB05/3	0.00 + 0.00 c
	Trichoderma sp. WU01	0.00 + 0.00 c
Antagonistic bacteria	*Bacillus* sp. BWTM-MSU2	0.00 + 0.00 c
	Bacillus sp. BWWM-MSU16	0.00 + 0.00 c
	Serratia nematodiphila GCSR38	17.60 + 1.06 b
Negative control	dH_2O	0.00 + 0.00 c
Positive control	Rifampicin	32.70 + 0.52 a
	CV (%)	8.97

1/ Different superscripts within the same column indicate a significant difference *(P* < 0.05).

Table 4. Effect of *S. nematodiphila* GCSR38 secondary metabolites crude extract to control rice bacterial leaf blight disease by using detached leaf technique, 7 days after inoculations

Treatment	Disease Severity (%)[1/]
1. Inoculate GCSR38 extract 24 h before *Xoo*	24.45 + 3.85 b
2. Inoculates *Xoo* 24 h before GCSR38 extract	15.56+ 10.18 b
3. *X. oryzae* pv. *oryzae* (*Xoo*)	44.45 + 3.85 a
4. dH_2O	0.00 + 0.00 c
CV (%)	27.35

[1/] Different superscripts within the same column indicate a significant difference (P <0.05).

was used along with *Xoo* spraying.[34] The four treatments were as follows: (i) *Xoo* treatment 24 h before spraying with AMSMCE, (ii) *Xoo* treatment only, as a negative control, (iii) zinc thiazole treatment as a positive control, and (iv) dH_2O treatment. The inoculated plants were incubated in a humid chamber for 7 d. Disease severity was scored according to the formula above.

RESULTS

Identification of *Serratia nematodiphila* GCSR38

The morphology of *S. nematodiphila* GCSR38 colony on NA plates was as follows: red and circular, with entire margins, umbonate elevation, smooth, gram-negative, short rod, and not endospore-forming. The 16S rRNA sequence showed that GCSR38 had 99.93% similarity with *Serratia nematodiphila* DZ0503SBS1 (Fig. 1).

The efficacy of the effective microorganism culture filtrate in controlling *Xoo*

The efficiency of antagonistic bacteria and antagonistic fungi culture filtrate against *Xoo* was tested using the agar well method. *Trichoderma* sp. UB05/3 showed the best inhibition of pathogen growth and was not statistically different from 100 ppm rifampicin with inhibition zone diameters equal to 22.97+1.58 mm and 32.87+1.89 mm, respectively (Table 2). All isolates of the antagonistic microbial culture filters showed *Xoo* inhibition compared to the inhibition observed in case of dH2O. The inhibition by other seven isolates was not statistically different from that of *Trichoderma* sp. UB05/3, *Trichoderma* sp. SK24, UB07 special, UB04/3, WU01, *Bacillus* sp. The efficacy of BWTM-MSU2, BWWM-MSU16, and *S. nematodiphila* GCSR38 was tested in the next step.

The effect of AMSMCE on the inhibition of *Xoo*

AMSMCE was tested for *Xoo* inhibition using the paper disc diffusion method. The results showed that only the crude extract of secondary metabolites from *S. nematodiphila* GCSR38 could inhibit the growth of *Xoo* with a 17.60+1.06 mm inhibition zone, while rifampicin (positive control) showed a 32.70+0.52 mm inhibition zone (Table 3).

Determination of MIC and MBC

The MIC of *S. nematodiphila* GCSR38 secondary metabolite crude extract against *Xoo* was 1,000 µg/ml. The MBC screening indicated that *S. nematodiphila* GCSR38 secondary metabolite crude extracts tested against *Xoo* at 1,000 ug/ml showed no colony-forming ability, indicating that the MBC value for this extract was 1,000 µg/ml (Fig. 2).

Table 5. Effect of *S. nematodiphila* GCSR38 secondary metabolites crude extract to control rice bacterial leaf blight disease in potted rice plants under greenhouse conditions, 7 days after inoculations

Treatment	Disease Severity (%)[1/]
1. Inoculates *Xoo* 24 h before GCSR38 extract	28.89 + 3.85 b
2. *X. oryzae* pv. oryzae	66.67 + 0.00 a
3. Zinc thiazol	22.22 + 3.85 c
4. dH_2O	0.00 + 0.00 d
CV (%)	9.24

1/ Different superscripts within the same column indicate a significant difference (P <0.05).

The efficacy of GCSR38 secondary metabolite crude extracts against *Xoo*

Detached leaf technique

Seven days after inoculation, the results showed that *S. nematodiphila* GCSR38 secondary metabolite crude extracts could reduce disease severity with a severity index less than that of the only *Xoo* treatment. Using the extracts 24 h before and after inoculation with *Xoo*, we found that the disease severity index was not significantly different and was 24.45% and 15.56%, respectively (Table 4).

Pot experiments

Inoculation of *Xoo* 24 h before spraying *S. nematodiphila* GCSR38 secondary metabolite crude extracts reduced the incidence of rice BLB by 28.89%; spraying with zinc thiazol yielded significantly different results compared to those by other treatments, with 22.22% disease severity (Table 5).

DISCUSSION

Morphological and molecular characterization of strain GCSR38 indicated that it was *S. nematodiphila*. This result is similar to that by Zhang et al.,[35] who reported the isolation of the bacterium *Serratia* DZ0503SBS1(T) from the intestine of a nematode (*Heterorhabditidoides chongmingensis*); it produced red pigment, was gram-negative, mobile, fluorescent, odd-shaped, and had a unilateral flagellum. A comparative analysis of 16S rRNA gene sequences showed that DZ0503SBS1(T) is a novel species named *S. nematodiphila* sp. nov. The culture filtrates

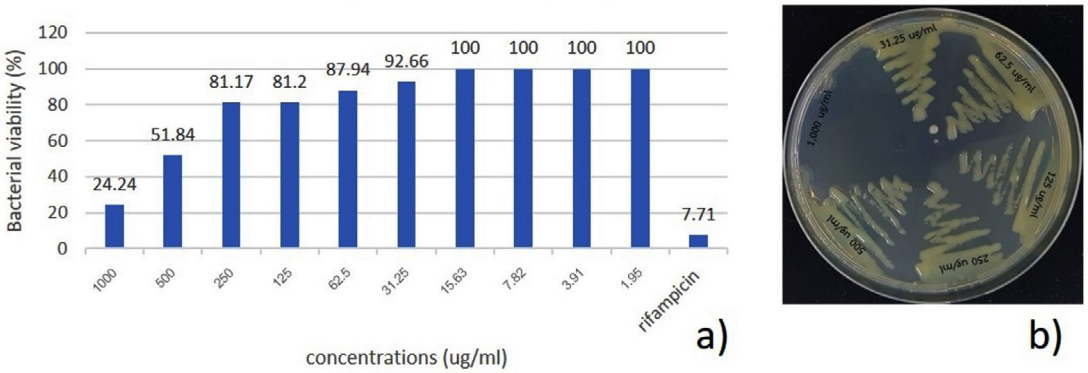

Fig. 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of secondary metabolites crude extracts against *X. oryzae* pv. *oryzae* a) MIC b) MBC.

of all fungal isolates (*Trichoderma* spp.) could inhibit the rice BLB pathogen, while the culture filtrates of all antagonistic bacterial isolates could inhibit the pathogens, but their efficacy was lesser than that of the antagonistic fungi. When extracting secondary metabolites with ethyl acetate, only the entomopathogenic bacterium *S. nematodiphila* GCSR38 was found to inhibit *Xoo in vitro*. Gangwar and Sinha[14] screened *Trichoderma* spp. and studied their biocontrol abilities against *Xoo* isolates obtained from rice-growing areas of various states. *Trichoderma* spp. isolated from Pantnagar were able to inhibit the growth of *Xoo* isolated from Pantnagar and from other locations by 100%. *Trichoderma* spp. can inhibit the radial growth of *Xoo* with 89.8 % inhibition for both isolates from Kashipur; however, 100% inhibition was obtained using *Trichoderma* spp. isolated from Pantnagar and Haldwani. GC-MS was used for further analysis of antibacterial activity against the rice BLB pathogen. The results revealed that the dual culture of *Trichoderma* spp. showed a larger inhibition zone than the monoculture. This indicates that better stimulation of novel secondary metabolites can be achieved by dual culture of beneficial fungi and is better than monoculture.[36] Prabawati et al.[37] performed isolation, selection, and identification of phyllosphere bacteria against *Xoo*. The plug agar method was used to screen the antagonistic activity of 52 isolates of phyllosphere bacteria. The results showed that the seven isolates could inhibit *Xoo*. The morphology and molecular characteristics of these antagonistic bacteria were used to classify them in the genera *Bacillus* and *Arthrobacter*. Nagendran et al.[38] managed BLB using endophytic bacteria. The results of the *in vitro* study showed a significantly higher inhibition of *Xoo*, including five isolates of *B. subtilis* var. *amyloliquefaciens.* Halim et al.[39] screened and characterized bacterial isolates that had potential antagonistic activity against *Xoo*. Only 17 endophytic bacterial isolates showed positive antagonistic activity, which was indicated by inhibition zones against *Xoo* around bacterial colonies on NA plates. While isolating and identifying soil bacteria antagonistic to *Xoo*, it was found that among 830 isolates, CT-66, CT-78, and CT-88 strains exhibited strong antagonistic effects against the pathogen. These effects are associated with antibiotics. Based on the 16S rRNA gene sequence, morphology, and biochemical properties, strain CT-78 was identified as *S. nematodiphila.*[40] The biochemical synthesis of bacterial secondary metabolites involves multiple enzymatic pathways and structural heterogeneous reactions that give rise to their highly specific mechanisms of action. The genus *Serratia* lives in a wide variety of environments and has a large amount of plaque, which is considered a source of exotic and biologically important structural secondary metabolites with potential activities include antioxidants, antimicrobial and immune-suppressive properties.[41] *S. nematodiphila* RL2 produces a red pigment called prodigiosin. Prodigiosin is an antimicrobial agent that is effective in controlling *Listeria* spp., *Pseudomonas* spp., *Yersinia* spp., and *Shigella* spp.[42]

The MIC of GCSR38 secondary metabolite extracts against *Xoo* was 1,000 µg/ml, and the MBC value for this exact was also 1,000 µg/ml. *S. nematodiphila* GCSR38 secondary metabolite extracts can reduce the disease severity of BLB *in vitro* and *in vivo*. This is similar to the findings of Shi et al.,[43] who reported the role of *Streptomyces roseoverticillatus* 63 (Sr-63) against *Xoo*; disease suppression was accomplished under glasshouse conditions. In addition, carbazomycin B isolated from the fermented broth of strain Sr-63 was found to be active against *Xoo*, with an MIC of 8 µg/ml. Bioactivity analysis showed that the compounds isolated from *Pseudomonas aeruginosa* (LN strain) inhibited three *Xanthomonas* strains under greenhouse conditions. *In vivo* testing under greenhouse conditions for fractions with antibiotic activity and their bioactivity evaluation is essential. Spraying plants with semi-purified fractions can control *Xanthomonas* spp.[44]

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by the author.

REFERENCES

1. Saha S, Garg R, Biswas A, Rai AB. Bacterial Diseases of Rice: An Overview. *J Pure Appl Microbiol.* 2015;9(1): 725-736.
2. Hastuti RD, Lestari Y, Suwanto A, Saraswati R. Endophytic *Streptomyces* spp. as biocontrol agents of rice bacterial leaf blight pathogen (*Xanthomonas oryzae pv. oryzae*). *HAYATI J Biosci*. 2012;19(4): 155-162. doi: 10.4308/hjb.19.4.155
3. Li B, Liu B, Shan C, et al. Antibacterial activity of two chitosan solutions and their effect on rice bacterial leaf blight and leaf streak. *Pest Manag Sci*. 2013;69: 312–320. doi: 10.1002/ps.3399
4. Wu L, Wu H, Chen L, Yu X, Borriss R, Gao X. Difcidin and bacilysin from *Bacillus amyloliquefaciens* FZB42 have antibacterial activity against *Xanthomonas oryzae* rice pathogens. *Sci Rep*. 2015;5:1-9. doi: 10.1038/srep12975.
5. Gnanamanickam SS, Priyadarisini VB, Narayanan NN, Vasudevan P, Kavitha S. An overview of bacterial blight disease of rice and strategies for its management. *Current Sci*. 1999;77(11):1435- 1443.
6. Yasmin S, Zaka A, Imran A, et al. Plant growth promotion and suppression of bacterial leaf blight in rice by inoculated bacteria. *PLOS One*. 2016;11(8):1-19. doi: 10.1371/journal.pone.0160688
7. MacManus PS, Stockwell VO, Sundin GW, Jones AL. Antibiotic use in plant agriculture. *Ann Rev Phytopathol.* 2002;40: 443-465. doi: 10.1146/annurev.phyto. 40.120 301.093927
8. Nelson LM. Plant growth promoting rhizobacteria (PGPR): Prospects for new inoculants. Online. *Crop Management*. 2004. doi:10.1094/CM-2004-0301-05-RV.
9. Chithrashree, Udayashankar AC, Nayaka SC, Reddy MS, Srinivas C. Plant growth promoting rhizobacteria mediate induced systemic resistance in rice against bacterial leaf blight caused by *Xanthomonas oryzae* pv. oryzae. *Biol Control*. 2011;59: 114–122. doi: 10.1016/j.bio control.2011.06.010
10. Bhattacharyya PN, Goswami MP, Bhattacharyya LH. Perspective of beneficial microbes in agriculture under changing climatic scenario: *J Phytol.* 2016;8: 26-41. doi: 10.19071/jp. 2016.v8.3022
11. Liu K, Garret C, Fadamiro H, Kloepper JW. Antagonism of black rot in cabbage by mixture of plant growth-promoting rhizobacteria (PGPR). *Bio Control*. 2016; 61: 605–613. doi: 10.1007/s10526-016-9742-3
12. Naher L, Yusuf U, Ismail A, Hossain K. *Trichoderma* spp.: A biocontrol agent for sustainable management of plant diseases. *Pak J Bot*. 2014;46(4): 1489-1493.
13. Gangwar GP, Sinha AP. Comparative antagonistic potential of *Trichoderma* spp. against *Xanthomonas oryzae pv. oryzae*. *Ann Pl Protec Sci*. 2010; 18(2): 458-463. https://www.researchgate.net/publication/221655149_Comparative_antagonistic_potential_of_*Trichoderma*_spp_against_*Xanthomonas_oryzae*_pv_*oryzae*
14. Gangwar GP, Sinha AP. Comparative antagonistic potential of fungal and bacterial bioagents against isolates of *Xanthomonas oryzae* pv. *oryzae*. *Ann Pl Protec Sci.* 2012;20(1): 154-159. https://www.indianjournals.com/ijor.aspx?target=ijor:apps&volume=20&issue=1&article=035
15. Gangwar GP. Field efficacy of formulation of fungal bioagents against bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. oryzae (Uyeda and Ishiyama) Dowson. *J Appl Nat Sci*. 2013;5(2):423-426.
16. Thomashow LS, Weller DM. Current concepts in the use of introduced bacteria for biological disease control: mechanisms and antifungal metabolites. In: Stacey G, Keen NT (Eds.). *Plant-Microbe Interactions*. New York: Chapman and Hall 1, 1996, 87-235.
17. El-shakh ASA, Kakar KU, Wang X, et al. Controlling bacterial leaf blight of rice and enhancing the plant growth with endophytic and rhizobacterial *Bacillus* strains. *Toxicol Envi Chem.* 2015;97(6):766-785. doi:10.1080/02772248.2015.1066176
18. Chen XH, Koumoutsi A, Scholz R, et al. Genome analysis of *Bacillus amyloliquefaciens* FZB42 reveals its potential for biocontrol of plant pathogens. *J Biotechnol*. 2009;140: 27–37. doi: 10.1016/j.jbiotec.2008.10. 011
19. Chen XH, Vater J, Piel J, et al. Structural and functional characterization of three polyketide synthase gene clusters in *Bacillus amyloliquefaciens* FZB 42. *J Bacterio*. 2006;188: 4024–4036. doi: 10.1128/JB.00052-06
20. Cock LS, Guarnizo AFC, Guerrero CAR. Antimicrobial activity and fermentation kinetics of Weissella confusa against *Xanthomonas albilineans. Acta Agron.* 2013; 52(2):97-104.
21. Lysyk TJ, Kalischuk-Tymensen LD, Selinger LB. Comparison of selected growth media for culturing *Serratia marcescens, Aeromonas sp.*, and *Pseudomonas aeruginosa* as pathogens of adult *Stomoxys calcitrans* (Diptera: Muscidae). *J Med Entomol.* 2002;39: 89–98. doi: 10.1603/0022-2585-39.1.89
22. Grimont PAD, Grimont F, Dulongderosnay HLC, Sneath PHA. Taxonomy of the genus *Serratia*. *J Gen Microbiol.* 1977;98:39–66. doi: 10.1099/00221287-98-1-39.
23. Jeong HU, Mun HY, Oh HK, et al. Evaluation of insecticidal activity of a bacterial strain, *Serratia* sp. EML-SE1 against diamondback moth. *J Microbiol*. 2010; 48(4): 541–545. doi: 10.1007/s12275-010-0221-9
24. Nunez-Valdez ME, Calderon MA, Aranda E, et al. Identification of a putative Mexican strain of *Serratia entomophila* pathogenic against root-damaging larvae of Scarabaeidae (Coleoptera). *Appl Environ Microbiol*, 2008;74: 802–810. doi: 10.1128/AEM. 01074-07
25. Jackson TA, Boucias DG, Thaler JO. Pathobiology of amber disease, caused by *Serratia* spp., in the

New Zealand grass grub, *Costelytra zealandica*. *J Invertebr Pathol*, 2001;78(4): 232–243. doi: 10.1006/ jipa.2002.5078

26. Sikorowski PP, Lawrence AM, Inglis GD. Effects of *Serratia marcescens* on rearing of the tobacco budworm (Lepidoptera: Noctuidae). *Am Entomol,* 2001;47: 51–60. doi:10.1093/ ae/47.1.51
27. Patil CD, Patil SV, Salunke BK, Salunkhe RB. Insecticidal potency of bacterial species *Bacillus thuringiensis* SV2 and Serratia nematodiphila SV6 against larvae of mosquito species *Aedes aegypti, Anopheles stephensi*, and *Culex quinquefasciatus*. *Parasitol Res*, 2012;110: 1841–1847. doi: 10.1007/s00436-011-2708-6
28. Ausubel FM, Brent R, Kingdton RE, et al. Current protocols in molecular biology. New York: Greene Publishing Association; Wiley-Interscience, 1992. v.1.
29. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 1997;25: 4876–4882. doi: 10.1093/nar/25.24.4876
30. Mirza BS, Welsh A, Rasul G, Rieder JP, Paschke MW, Hahn D. Variation in *Frankia* populations of the Elaeagnus host infection group in nodules of six host plant species after inoculation with soil. *Microb Eco*. 2009;58: 384–393. doi: 10.1007/s00248-009-9513-0
31. Cheng CM, Tu J, Yang CC, Kuo TT. Rifampicin: an inhibitor of Xp12- specific protein phosphorylation. *FEMS Microb Lettes.* 1996;143: 141-149.
32. Clinical and Laboratory Standards Institute. Method for dilution antimicrobial susceptibility teste for bacterial that grow aerobically. 11th Edition. Clinical and Laboratory Standards M07. 2018. https://clsi.org/ media/1928/m07ed11_sample.pdf
33. Chaudhary RC. Fukuyi. Internationalization of elite germplasm for farmers: Collaborative mechanisms to enhance evaluation of rice genetic resources. In: New Approaches for Improved use of Plant Genetic Resources, Japan.1996:26. https://www.researchgate. net/publication/318494793
34. Kauffman HE, Reddy APK, Hsieh SPY Merca SD. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. *Plant Dis Rep.* 1973; 57: 537-541.
35. Zhang CX, Yang SY, Xu MX, et al. *Serratia nematodiphila* sp. nov., associated symbiotically with the entomopathogenic nematode *Heterorhabditidoides chongmingensis* (Rhabditida: Rhabditidae). *Int J Syst Evol Microbiol,* 2009;59(7):1603-1608. doi: 10.1099/ ijs.0.65718-0
36. Shobha B, Lakshmeesha TR, Ansari MA, et al. Mycosynthesis of ZnO nanoparticles using *Trichoderma* spp. isolated from rhizosphere soils and its synergistic antibacterial effect against *Xanthomonas oryzae* pv. *oryzae*. *J fungi.* 2020;6(3): 181. doi: 10.3390/ jof6030181
37. Prabawati A, Susilowati A, Sugiyato S. Phyllosphere bacteria as a candidate of biocontrol agents against *Xanthomonas oryzae pv. oryzae* (*Xoo*) causes bacterial blight disease. *Pros Sem Nas Masy Biodiv Indon.* 2019; 5(2): 256-262. doi:10.13057/psnmbi/m050219
38. Nagendra K, Karthikeyan G, Raveendran M, Prabakar K, Raguchander T. Management of bacterial leaf blight disease in rice with endophytic bacteria. *World Appl Sci J.* 2014;28(12):2229-2241. doi: 10.5829/idosi. wasj.2013.28.12.2009
39. Halim RA, Hasan NA, Ramachandran K. Screening of endophytic bacteria as biocontrol agents against bacteria leaf blight (*Xanthomonas oryzae*). HAYATI J Biosci. 2020;27(3): 215-220. doi: 10.4308/hjb.27.3.215
40. Khoa ND, Giàu NDN, Tuấn TQ. Effects of *Serratia nematodiphila* CT-78 on rice bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*. *Bio Control*. 2016; 103: 1–10. doi: 10.1016/j.biocontrol.2016.07.010
41. Al-Ghanem MM. Serratia a novel source of secondary metabolites. *Adv Biotech & Micro*. 2018;11(3):1-5. doi: 10.19080/AIBM.2018.11.555814.
42. Gondil VS, Asif M, Bhalla TC. Optimization of physicochemical parameters influencing the production of prodigiosin from *Serratia nematodiphila* RL2 and exploring its antibacterial activity. *3 Biotech*. 2017;7: 338. doi: 10.1007/s13205-017-0979-z
43. Shi T, Guo X, Zhu J, Hul J, He Z, Jiang D. Inhibitory effects of carbazomycin B produced by *Streptomyces roseoverticillatus* 63 against *Xanthomonas oryzae pv. oryzae*. *Front Microbiol.* 2021;12(6):1-13. doi: 10.3389/ fmicb.2021.616937
44. Spago FR, Mauro CSI, Oliveira AG, et al. *Pseudomonas aeruginosa* produces secondary metabolites that have biological activity against plant pathogenic *Xanthomonas* species. *Crop Protec*. 2014;62:46-54. doi: 10.1016/ j.cropro.2014.04.011