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Detection of the antibacterial efficacy of *Paenibacillus polymyxa* against *Xanthomonas oryzae* pv. *oryzae* in rice seedlings, and the antimicrobial related genes

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Abstract

Paenibacillus polymyxa bacterium has been known as a biocontrol agent (BCA) for various plant diseases, and it promotes the growth of several treated plants. On the other hand, *Xanthomonas oryzae* pv. *oryzae* (Xoo) is an important bacterial pathogen that causes bacterial leaf blight (BLB) to rice plants, and cause high yield loss during the growing season. In this study, *P. polymyxa* SX3 inhibited the *in vitro* growth of Xoo by 80 % and affected the biofilm formation. Also, the cell membrane of Xoo lost its permeability after treating it with culture filtrate of *P. polymyxa* at its MIC (40 %). Transmission electron microscopic (TEM) observations showed an influence of the culture filtrate of *P. polymyxa* on the cell morphology of Xoo. Applying *P. polymyxa* decreased the severity of bacterial leaf blight on rice seedlings to 4.9 %, compared to 42.5 % of the control. Moreover, this strain also improved the growth parameters of the rice seedlings significantly by increasing the shoot length, root length, fresh and dry weight by 166.7 %, 168.2 %, 100 %, and 255.6 %; respectively, compared to the positive control (Xoo only). Three antimicrobial related genes; *pmxB*, *pmxD* and *Fusdel* were detected in *P. polymyxa* SX3 using nine specific gene markers. The aims of the study were to investigate the impact of *P. polymyxa* SX3 on *in vitro* growth of Xoo, *in vivo* growth of the rice seedlings in the greenhouse, and to detect the presence of related antimicrobial genes.

Keywords: *Paenibacillus polymyxa*, *Xanthomonas oryzae* pv. *oryazae*, Biocontrol, Antimicrobial genes, Rice

1. Introduction

Various pathogens threat rice plants during the growing season. *Xanthomonas oryzae* pv. *oryzae* (Xoo) the causal agent of bacterial leaf blight (BLB) is considered as one of the most serious diseases that attack rice plants. A study conducted by Yasmin *et al*., (2017) revealed that BLB is deployed worldwide especially in the United States, Latin America, Australia, and in many Asian countries during the heavy rains of the monsoon season. Sharma *et al*., (2017) added that it causes high yield loss that may reach up to 50 %. A recent research work of Thanh *et al*., (2018) highlighted that in rice-producing countries

such as Asia, millions of hectares of rice paddies have been seriously combating every year due to the infestation by Xoo. Numerous management strategies have been tested to control BLB including; chemicals, breeding for resistant cultivars, and biological control. MacManus *et al*., (2002) documented that due to the hazardous impact of chemicals on the environment, mammals and beneficial microbes' communities, and due to its toxic residues, application of chemicals on rice plants has several limitations. Also George *et al*., (1997) previously revealed that the extensive use of antibiotics may develop bacterial strains with mutations that make them highly resistant to these antibiotics. According to Shanti *et al*., (2010), although breeding for resistance is the safest strategy for controlling plant diseases; however, it costs money and time. In addition, some single gene-based resistance i.e. Xa4, could be defeated by evolution of Xoo sub-populations. Therefore, the use of biological control strategy is the more suitable way to control BLB, where most of the antagonistic rhizospheric bacteria not only suppress the pathogen, but also can indirectly increase the plant resistance by improving the growth of the rice plant. Nowadays, many studies have approved the successful application of different bacterial groups in controlling plant pathogens, and in promoting growth of the host plants (Marra *et al*., 2012).

Paenibacillus polymyxa formerly known as *Bacillus polymyxa* is a Gram-positive bacterium, and has been studied as an interesting BCA for numerous plant diseases (Lal and Tabacchioni, 2009; Zhou *et al*., 2016; Shi *et al*., 2017). *P. polymyxa* is a potential source of bioactive metabolites, and acts as a plant growth promoting rhizobacteria (Kim *et al*., 2016). In addition, it forms spores with unique properties to resist the harsh environmental conditions including; extreme pH, temperature, high pressure, UV irradiation, aridity, and chemical infiltration (Huo *et al*., 2012).

As a potent bioagent, *P. polymyxa* produces diverse antibacterial agents such as the polymyxins antibiotics; which are synthesized by the non-ribosomal peptide synthetase (NRPS) mechanism (He *et al*., 2007). Also, it produces lipopeptides which are active against a wide range of bacteria, fungi, and oomycetes (Martin *et al*., 2003; Ongena and Jacques, 2008).

On the other hand, several studies of Chithrashree *et al*., (2011); El-shakh *et al*., (2015); Yasmin *et al*., (2017); Abdallah *et al*., (2019) reported that the plant growth promoting Pseudomonads such as *P. polymyxa* and *Bacillus* spp. act as effective bioagents against Xoo. However, the way how *P. polymyxa* suppresses Xoo growth and promote rice plant growth is still not well studied. The objectives of this study were to investigate the *in vitro* and *in vivo* antibacterial potency of *P. polymyxa* SX3 against pathogenic *Xanthomonas oryzae* pv. *oryzae*, and to detect some of the antibacterial related genes in the tested *P. polymyxa* SX3, by using nine specific gene markers. These are in addition to studying the impact of *P. polymyxa* SX3 on the growth parameters of rice seedlings infested by Xoo in the greenhouse.

2. Material and methods

2.1. Bacterial strains and culture conditions

Xanthomonas oryzae pv. *oryzae* strain GZ 0005 and *P. polymyxa* strain SX3 used in this study were obtained from the Institute of Biotechnology, College of Agriculture and Biotechnology, Zhejiang University, China. Once received the tested bacteria were cultured on Nutrient agar media at 30°C for 24 h. Briefly, Xoo and SX3 cells were isolated in pure cultures by streaking on nutrient agar (NA) plates. Loopfuls of the bacterial cells from freshly grown SX3 colonies were suspended in 5 ml of nutrient broth (NB), and then the inoculated tubes were incubated overnight at 30°C. Population of the SX3 was estimated by reading the optical density at 600 nm (OD_{600}) using lambda 35 UV/vis spectrophotometer (PerkinElmer), according to Abdallah *et al*., (2019).

2.2. *In vitro* antibacterial potential of *P. polymyxa* against *Xanthomonas oryzae*

The antibacterial activity of *P. polymyxa* SX3 against Xoo GZ 0005 was detected using the agar diffusion technique of Monteiro *et al*., (2005), with minor modifications. An aliquot of 200 µl of Xoo (10^8 cells/ ml) obtained from 24h old cultures on nutrient broth (NB) media was added to the surface of solidified NA plate, and then spread using a sterile glass spreader. After that, three spots of 5 µl suspensions of (10^8 cells/ ml) of *P. polymyxa* SX3 strain were placed individually at equidistance on the surface of the seeded agar plate. Distilled water was added as a control. Five replicate plates were incubated at 28ºC for 48 h. The antibacterial potential was estimated by measuring the diameter of inhibition zone using a calibrated ruler. The assay was repeated twice.

2.3. Determination of the minimum inhibitory concentration (MIC) of the antagonist's culture filtrate

The *P. polymyxa* strain SX3 was cultured on NB at 37°C on a rotary shaker at 160 rpm for 48 h. The broth culture was centrifuged at 15000 rpm for 15 min. at 4ºC, and then sterilized using 0.22-l millipore filter, according to Yoshida *et al*., (2001). A loopful of the sterilized culture filtrate streaked on NA plate and then incubated to confirm the no cells were left.

The MIC is defined as the lowest concentration of the antagonistic filtrate that completely inhibited the bacterial pathogen growth. In reference to Nguyen *et al*., (2016), the bacterial growth was estimated by determining the optical density at 600 nm (OD_{600}). Different concentrations of the culture filtrate were used mainly; 30, 40, 50, 60 and 70 %. An aliquot of 10 µl of the Xoo cultural suspension and 50 µl of *P. polymyxa* SX3 filtrate were added individually into each well of 96-well microtiter plates. The total volume was adjusted to 200 µl in each well depending on the amount of the filtrates concentration. The plate was incubated at 28°C for 24 h without shaking. Wells filled with Xoo alone was considered as negative controls. After incubation, the OD_{600} of the culture in each well was measured using a Microplate Photometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Three replicate microtiter plates were used for each treatment, and the assay was repeated twice.

2.4. Inhibition of *Xanthomonas oryzae* biofilm formation

The ability of *P. polymyxa* to inhibit the biofilm formation by *X. oryzae* pv. *oryzae* was examined using 96-well microtiter culture plates (Nijland *et al*., 2010). About 5 µl of Xoo culture broth (OD_{600} = 0.8; 10^8 cells/ ml) was added to 95 µl of NB in 96-well plates, and then the plate was kept at 30°C for 24 h. An aliquot of 100 µl of 40% culture filtrate of *P. polymyxa* SX3 was added to each well. NA broth only was used in six wells as negative controls. Consequently, the plate was further incubated h at 30°C for 24. After incubation, the non-attached pathogen cells were removed by discarding the culture medium, rinsing the plate gently four times with sterile dist. water, and then the plate was dried at room temperature for 1 h. The attached biofilm material was stained with 1% crystal violet (CV) and then incubated for 25-30 min. at room temperature. After incubation, the stained solution was solubilized by adding 150 µl Acetic acid (33%), to solubilize the crystal violet bound to the biofilm. Finally, the microplate OD was measured at 570 nm using a spectrophotometer (PerkinElmer, Norwalk, CT, USA). Three replicate plates were used for each treatment, and the assay was repeated twice.

2.5. Integrity of the bacterial pathogen cell membrane

The cell membrane integrity of Xoo cells challenged with *P. polymyxa* was examined as described by Wang *et al*., (2012). To harvest the bacterial cells, Xoo broth culture was centrifuged at 5,000 rpm for 5 min., washed twice using sterile dist. water, and then re-suspended in sterile 0.9% saline. The final cell suspension was adjusted to 0.6 at OD_{630} and 0.8 at OD_{420} to measure the OD_{260} and OD_{280}, respectively. Measurement of OD_{260} represents the release of DNA, while that of OD_{280} refers to the

release of proteins from the bacterial cells. Culture filtrate of the antagonistic bacterium was added according to its MIC (40 % concentration), to the pathogen bacterial suspension at the ratio of 1:3. The release of DNA and proteins after 0.5, 1, 2 and 4h of incubation periods were recorded using a lambda 35 UV/vis spectrophotometer (PerkinElmer, Norwalk, CT, USA). The assay was repeated twice and five replicates were used in each treatment.

2.6. Transmission electron microscope (TEM)

Xanthomonas strain GZ0005 was prepared for observation by TEM through adding culture filtrate of SX3 (MIC, 40%) to the cell suspension of Xoo (10^8 cells/ ml). NA broth was used as a control. After 4 h of incubation, Xoo suspension was centrifuged and then the cells were washed and transferred to another centrifuge tubes containing 2.5% glutaraldehyde, finally incubated overnight at 4°C. The samples were dehydrated using a graded series of ethanol solutions (70, 80, 90 and 100 %, v/v), post-fixed with 1 % (w/v) Osmium tetroxide (OsO_4) solution for 1 h at room temperature, and then washed three times. After that, the samples were embedded in agar using a micropipette. Thin sections of the tested specimens were cut on an Ultra cut Ultramicrotome (Super Nova; Reichert-Jung Optische Werke, Wien, Austria), and then double-stained using saturated uranyl acetate and lead citrate. Grids containing the tested samples were examined with a JEM-1230 transmission electron microscope (Hitachi, Tokyo, Japan) at an operating voltage of 75 kV, as described by Ahmed *et al*., (2016).

2.7. Effect of *P. polymyxa* on severity of bacterial leaf blight (BLB) and rice growth traits in the greenhouse

The ability of *P. polymyxa* to decrease the severity of BLB and improve the growth of rice seedlings were tested *in vivo* according to the methods recommended by Yasmin *et al*. (2017); Abdallah *et al*., (2019), with minor modifications. About 50 rice seeds (cv. IIyou 023) were added into 9 cm Petri plates, soaked in 10 ml of *P. polymyxa* SX3 suspension (10^8 cells/ ml) for 4 h, and then sown in plastic pots. The assay was performed using completely randomized block design and repeated twice. Four seeds were planted in each 4 inches pot; five replicates pots were used for each treatment. After sowing, the pots were placed in the greenhouse at ~28°C, 80 % relative humidity with a photoperiod of 16 h light and 8 h dark. Two weeks later the rice seedlings were challenged with pathogenic Xoo strain GZ 0005 (10^8 cells/ ml) using the leaf-clip method of Kauffman *et al*., (1973), where the rice seedlings leaf tips were cut off with Xoo suspension moistened scissor. A negative control was used, where rice seedlings were treated with sterilized water only without inoculation with *P. polymyxa* or Xoo, and a positive control where the seedlings were inoculated with Xoo only. One week after inoculation, the percentage of diseased leaf areas (DLA %) was calculated according to the following equation of Abdallah *et al*., (2019);

DLA (%) = Total lesion area of the test sample/Total leaf area of the test sample × 100

In addition, the effect of *P. polymyxa* on improving the growth of rice seedlings was also estimated according to Abdallah *et al*., (2019), by measuring the shoot height, root length, fresh and dry weight of the rice seedlings.

2.8. Detection of the antibacterial related genes and estimation of their expression

The DNA was extracted from the antagonistic P. polymyxa SX3 using TIANamp bacteria DNA Kit (Tiangen Biotech (Beijing) co., Ltd.), according to the manufacturer's instructions. Nine primers reported by Li *et al*., (2007); Kim *et al*., (2015) based on the coding regions were used to detect the presence of nine antimicrobial genes in *P. polymyxa* SX3 (Table. 1). Amplifications were carried out in a Programmable Temperature Cycler (PTC-200, MJ Research, USA). The Polymerase chain reaction (PCR) was carried out in a total volume of 20 µl containing 10 µl PCR buffer, 8 µl H_2O, 0.5 µM of each primer, and 1 µl of genomic

DNA. The cycling conditions for amplification of all the target genes were: 95°C for 4 min., 40 cycles of 94 °C for 1 min., annealing temperature for 1 min., and 70°C for 1 min. A final extension step at 70°C for 5 min. and then kept a 4°C. The annealing temperature was set up to 57°C for fusdel, PmxA, PmxD, bac and pmxB; 61 °C for plpD and pmxE; 66 °C for plpE and 64°C for plpF. The amplification products were analyzed in a 2% agarose gel in 1x Tris- Borate-EDTA (TBE, pH 8.0), run for 25 min. at 120 V, and then viewed. Bands size comparisons were made with a DL 2,000 DNA marker (TaKaRa, Shanghai, China). The Gel images were captured with an imaging UV light system.

2.9. Statistical analysis

Data were subjected to analysis of variance (ANOVA) using SAS, 2003 software (SAS Institute, Cary, NC, USA). General linear model (GLM) procedure was used to check the significant differences among main treatments. Individual comparisons between the mean values were performed using Duncan's method ($p < 0.05$).

Table 1. Oligonucleotide primers used to detect the antimicrobial genes

Gene name	Primer	Primer sequence	Annealing T ºC
pmxA	PmxAF pmxAR	TAACGTTTTCACCCCATTGG GGGAGCTTGGAGCTTTGCTG	57
pmxB	PmxBF pmxBR	TCCACAACTCGAGCTAAGCC ACTTACCGCTCCAGTACTGTTC	57
pmxD	PmxDF pmxDR	CAGGAATTTACCGAGTCTGCC GTCGCATTCGCAAGCAGGAAG	57
pmxE	PmxEF pmxER	GAGCGGCTGAAACGTCAGGAAGCC CTGCTTCGCCTGTATGATTGTC	61
Fusdel	FusdelF FusdelR	AGCTCCATTGCTGCGGGTCG ATCTTCACATACGACTGCCAC	57
plpD-A1	plpDA1F plpD-A1-R	CTAGCCATGGAAAACATTTTGACCCG CACCTCGAGTTCGTACTCCGCTCCG	61
plpE-A3	plpE-A3F plpEA3R	CTAGCCATGGCGGCGGAGCAGACAC CCCAAGCTTCGCGACGTAGTCGGCTC	66
plpF-A1	plpF-A1-F plpF-A1-R	CTAGCTAGC TTGTCCGACTCCGAG GCGGATCC TCACTCCAGTCCGGTCT	64
bacA	bac-F bac-R	CAGCTCATGGGAATGCTTTT CTCGGTCCTGAAGGGACAAG	57

3. Results

3.1. *In vitro* antagonism against *Xanthomonas oryzae* pv. *oryzae*

The antibacterial potency of *P. polymyxa* SX3 was tested using agar diffusion technique as shown in Fig. (1 a, b). *P. polymyxa* SX3 significantly suppressed the in vitro growth of the pathogenic Xoo, compared to the untreated control. It expressed inhibition zone diameters that averaged 2.3 cm, when applied on Xoo NA culture.

3.2. The MIC of the antagonist's culture filtrate

All concentrations of *P. polymyxa* SX3 culture filtrate reduced the number of Xoo cells, as clear in Fig. (2). However, the higher concentrations of 40, 50,

60, and 70 % inhibited the *in vitro* growth of Xoo significantly, compared to the 30% culture filtrate. The observed MIC is 40 %, as it inhibited the growth of Xoo by approximately 80 %, and there is no significant difference between 40% and the higher concentrations of *P. polymyxa* SX3 culture filtrate.

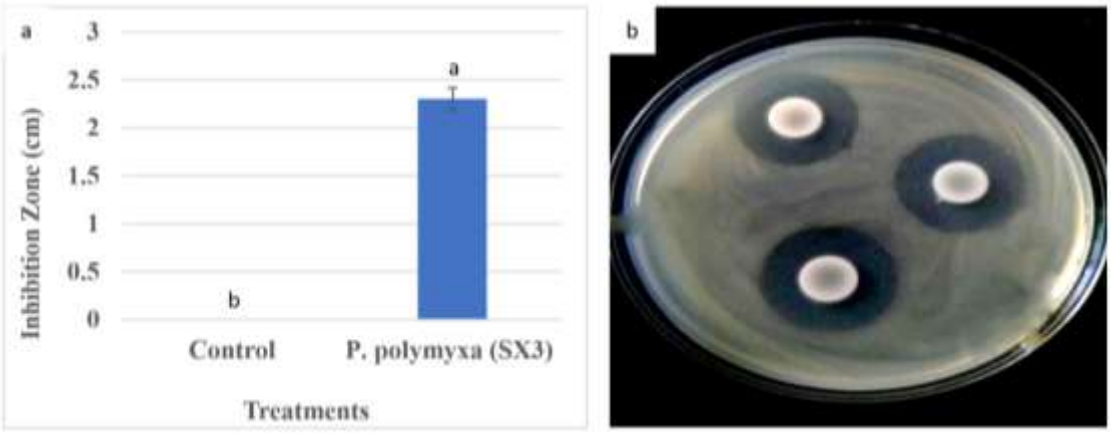

Fig. 1. (a) Chart representing the diameter of inhibition zones, vertical bars represent standard errors of the means (n=3). Bars followed by the same letter (s) are not significantly different ($p \leq 0.05$). (b) *In vitro* antibacterial potential of *P. polymyxa* (SX3) against Xoo on nutrient agar (NA) plate

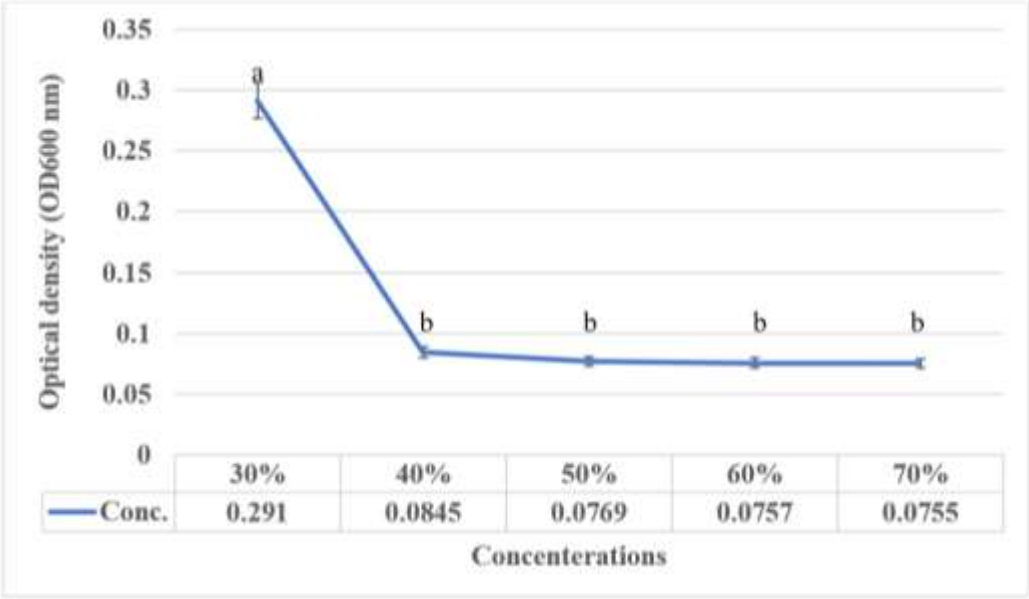

Fig. 2. Minimum inhibitory concentration (MIC) of *P. polymyxa* SX3 culture filtrate

3.3. Inhibitory effect of antagonistic *P. polymyxa* on biofilm formation by *X. oryzae*

P. polymyxa SX3 significantly inhibited the biofilm formation by the treated Xoo cells after 24 h of incubation at 30°C, compared to the untreated control. *P. polymyxa* SX3 was able to reduce the OD_{570} values by 77.42%, compared to the control (Fig. 3).

3.4. Integrity of the pathogenic *X. oryzae* cell membrane

The integrity of Xoo cell membrane challenged with *P. polymyxa* SX3 culture filtrate was tested during various incubation periods using absorbance at OD_{260} and OD_{280} (Table 2). Results demonstrated that the incubation period didn't show a significant effect on the values of OD_{260} and OD_{280}. In general, the release of DNA and proteins from *X. oryzae* pv. *oryzae* strain GZ0005 cells treated with the antagonist's culture filtrate was significantly increased compared to the control, regardless of the incubation period. However, the culture filtrate showed an increase of OD_{260} values compared to OD_{280}. Moreover, the highest OD_{260} value of 0.8736 was recorded after 0.5 h of treating the *X. oryzae* cells with *P. polymyxa* culture filtrate, while the highest value of 0.7606 at OD_{280} was observed after 2 and 4 h of incubation.

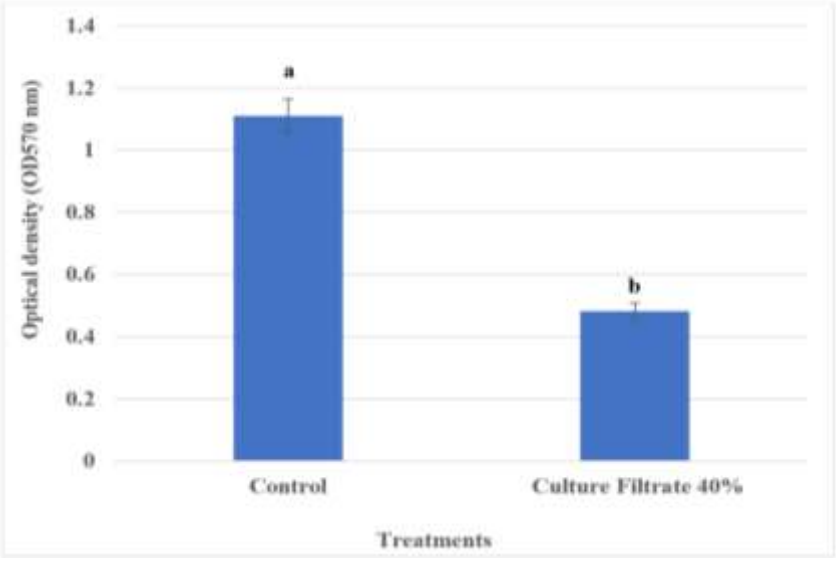

Fig. 3. Inhibitory effect on biofilm formation of *X. oryzae* pv. *oryzae* by using culture filtrate of *P. polymyxa* SX3 at 40 % concentration. Vertical bars represent standard errors of the means (n=3), while bars followed by the same letter (s) are not significantly different ($p \leq 0.05$)

Table 2. Release of cell materials (DNA and proteins) from *X. oryzae* pv. *oryzae* strain GZ0005 treated with 40 % culture filtrate of *P. polymyxa* SX3, recorded at absorbance's of OD_{260} and OD_{280}

Time/hour	OD_{260}		OD_{280}	
	GZ0005	GZ0005+Sx3	GZ0005	GZ0005+Sx3
0	0.0413^{a}	0.8396^{a}	0.0240^{ab}	0.7273^{b}
0.5	0.0046^{a}	0.8736^{ab}	0.0070^{b}	0.7280^{b}
1	0.0316^{a}	0.7740^{b}	0.0160^{ab}	0.6996^{a}
2	0.0276^{a}	0.8263^{ab}	0.0323^{a}	0.7606^{c}
4	$.0906^{b}$	0.8510^{a}	0.0143^{ab}	0.7606^{c}
MSD	0.0288^{**}	0.0200^{*}	0.0072^{*}	$0.0210^{NS(P<0.2721)}$

Values within a row with different superscript letters differ significantly at p <0.05; NS=not significant. *Demonstrates significance of releasing materials from the cells through the time

3.5. Transmission electron microscope (TEM)

The micrographs of TEM showed normal and visible cell membranes of the non-treated control Xoo cells. However, disruption of cell wall and change in cell morphology were observed in Xoo cells treated with 40% culture filtrate of *P. polymyxa* SX3 (Fig. 4). In some of the treated Xoo cells, the membranes were severely destroyed, which may cause loss of the membrane permeability and flow of the intracellular substances outwards.

3.6. Effect of antagonistic *P. polymyxa* on severity of BLB and rice seedlings growth traits

There were no symptoms of BLB disease on the rice seedlings treated with *P. polymyxa* alone, compared to the positive control which showed yellowing of the leaves starting from the tip. Also, co-inoculation of rice seedlings with *P. polymyxa* and Xoo significantly reduced the percentage of diseased leaf area (DLA) to 4.9 %, compared to 42.5 % for the positive control (Fig. 5).

On the other hand, application of *P. polymyxa* SX3 alone significantly improved the rice seedlings growth compared to the other treatments. *P. polymyxa* SX3 caused a 25.3%, 47.5% increase in the height of shoot and length of root; respectively, compared to the controls (Fig. 6, 7). On the other hand, this strain increased shoot height by 142% and root length by 91.3%. In addition, treatment with *P. polymyxa* SX3 increased the fresh and dry weight of the rice seedlings by 66.6 % and 100 %; respectively, compared to the controls. On the contrary, inoculation of rice seedlings with Xoo alone impeded their growth, causing 48, 41.7% reduction in the shoot length and the root length; respectively, compared to the control. Moreover, this pathogenic strain significantly reduced the fresh and dry weight by 50 % and 105 %; respectively. However, the fresh and dry weights of the rice seedlings co-inoculated with Xoo and SX3 1:1 are increased by 66.6, 23.5 %; respectively, compared to the positive control.

3.7. Detection of the antimicrobial genes

DNA from *P. polymyxa* was screened for the presence of nine antimicrobial genes mainly: *pmxA*, *pmxB*, *pmxD*, *pmxE*, *Fusdel*, *plpD-A1*, *plpE-A3*, *plpF-A1*, and *bacA*, using nine specific primers. Only three genes *pmxB*, *pmxD*, and *Fusdel* are clearly detected in the DNA of *P. polymyxa* SX3 (Fig. 8). The use of specific markers successfully resulted in the detection of a single band of size 300 pb, which refers to the presence of *pmxB* gene. The bands between 750 and 1000 pb confirmed the detection of *Fusdel*, and *pmxD* genes. However, there are no clear bands that insure the presence of *pmxA*, *pmxE*, *plpD-A1*, *plpE-A3*, *plpF-A1*, and *bac* genes.

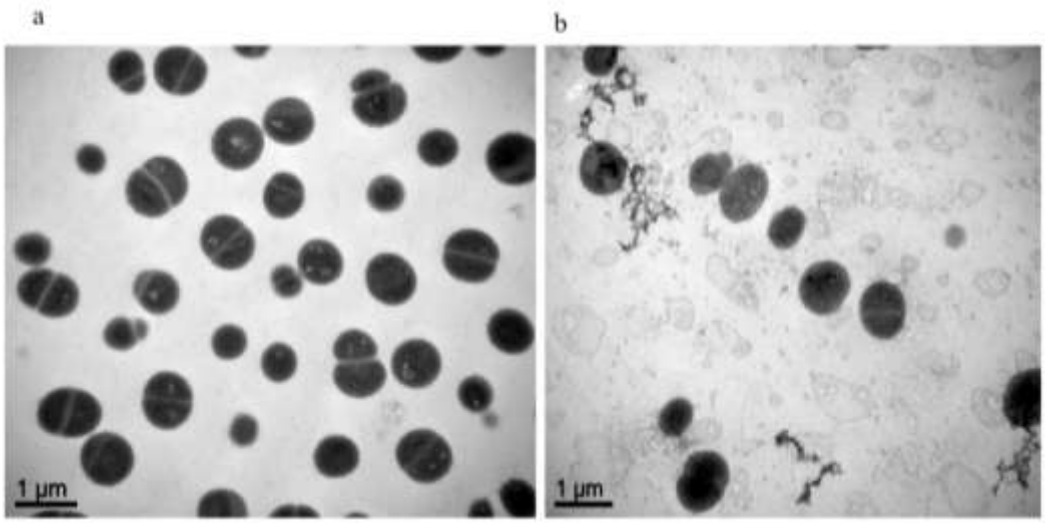

Fig. 4. Transmission electron microscope (TEM) of: (a) non-treated control Xoo cells grown on nutrient broth (NB) medium, (b) Xoo cells treated with culture filtrate of *P. polymyxa* SX3 (40 %)

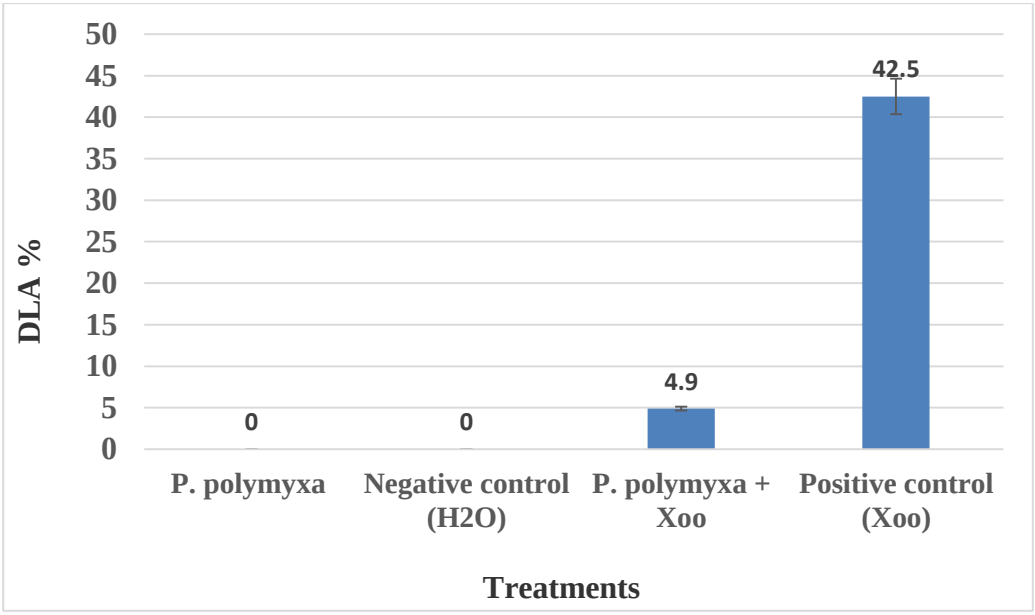

Fig. 5. Effect of *P. polymyxa* on percentage of diseased leaf area (DLA) of rice seedlings infested with *X. oryzae* pv. *oryzae*. Values above the vertical bars represent percentage of DLA (n=5), whereas, values with different superscripts letters (a, b and c) differ significantly at $p < 0.05$.

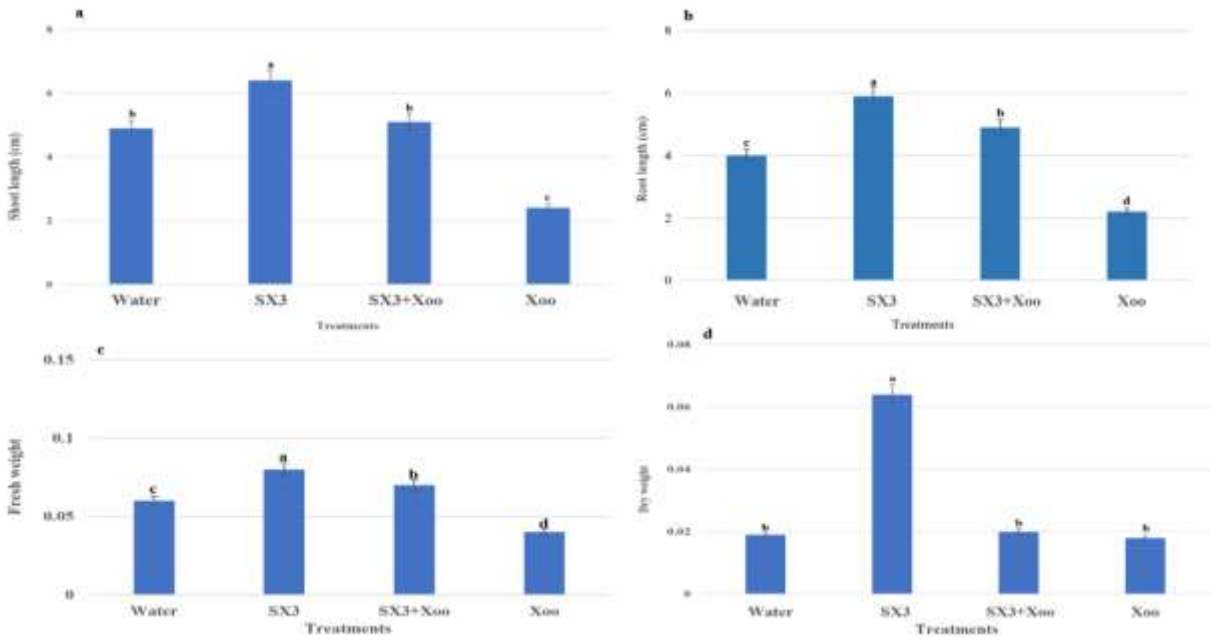

Fig. 6. Effect of *P. polymyxa* on (a) shoot and (b) root length, (c) fresh and (d) dry weight of rice seedlings infested with *X. oryzae* pv. *oryzae*

Fig. 7. Effect of application of *P. polymyxa* SX3 on growth parameters of rice seedlings infested with Xoo compared to the controls; under greenhouse conditions

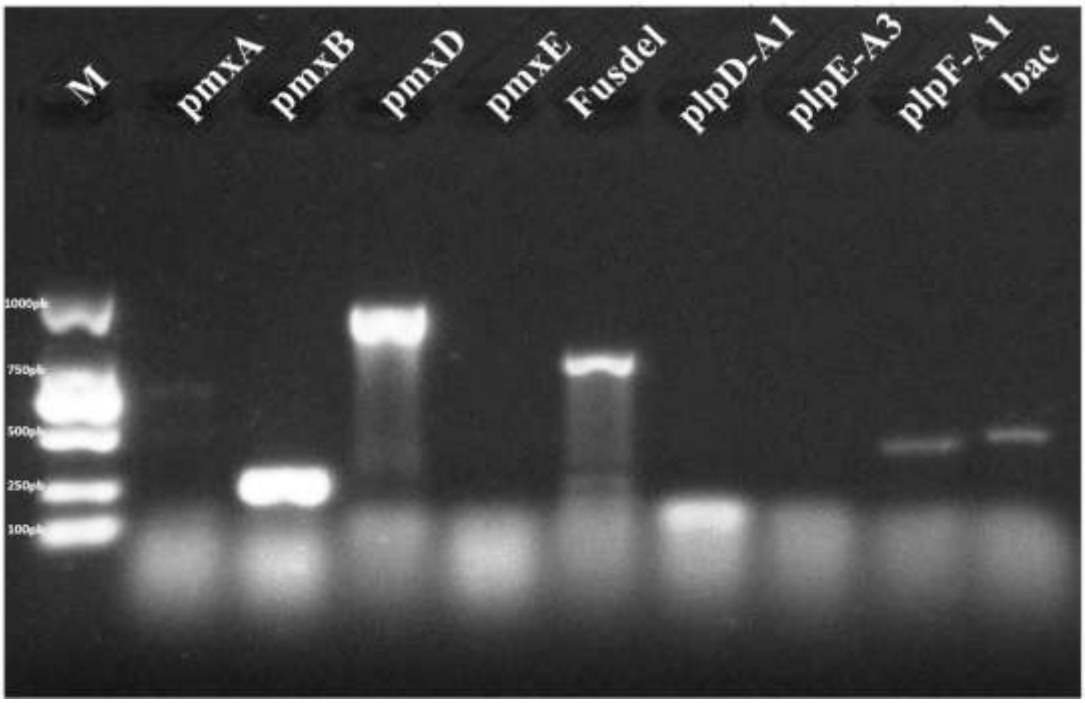

Fig. 8. Electrophoretic amplified pattern of DNA extracted from *P. polymyxa* SX3 using the specific primers of nine genes (1) *pmxA*, (2) *pmxB*, (3) *pmxD*, (4) *pmxE*, (5) *Fusdel*, (6) *plpD-A1*, (7) *plpE-A3*, (8) *plpF-A1*, and (9) *bac*

4. Discussion

Paenibacillus polymyxa strain SX3 was able to inhibit the growth of *X. oryzae* pv. *oryzae* strain GZ 0005 on nutrient agar medium. Also, the culture filtrate of *P. polymyxa* SX3 showed a MIC of 40 % concentration, which decreased the number of Xoo cells with approximately 80 %. The ability of *P. polymyxa* to suppress the plant pathogens was reported previously by several studies (Niu *et al*., 2013; Kim *et al*., 2016; Santiago *et al*., 2016; Shi *et al*., 2017; Yasmin *et al*., 2017; Chávez-Ramírez *et al*., 2020), as it secretes various types of antibacterial compounds against the Gram-positive and Gram-negative bacteria. In addition, *P. polymyxa* was reported by Raza *et al*., (2008) to produce active molecules with antifungal activity such as fusaricidins; a group of cyclic depsipeptides, and the peptide gavaserin. A study conducted by Niu *et al*., (2013) reported the secretion of polymyxin P by a strain of *P. polymyxa* named M-1; which is an active molecule that suppresses the growth of *Erwinia amylovora* and *E. carotovora* bacteria, the causal agents of the blight and soft rot in wheat plants, respectively.

According to Raza *et al*., (2008), the current results of growth inhibition, biofilm formation, disruption and loss of permeability of the cell membrane, and leakage of the intracellular substances from the pathogenic Xoo cell wall on treatment with 40% culture filtrate of *P. polymyxa* SX3 could be attributed to the ability of some strains of *P. polymyxa* to lyse the cells of plant pathogens by producing several hydrolytic enzymes including; β-1,3-glucanases, chitinases, cellulases, xylanases, lipases, amylases, and proteases. Later, Deng et al., (2011) confirmed that *P. polymyxa* strain JSA-9 has the ability to secret a glycoprotein, which has an apparent inhibitory efficacy against a broad spectrum of fungi and bacteria. Several recent studies conducted by Santiago *et al*., (2016); Zhang *et al*., (2018); Chávez-Ramírez *et al*., (2020) confirmed the effect of *P.*

polymyxa in decreasing the severity of many plant diseases. In accordance with the current results, Abdallah *et al*., (2019) reported a significant reduction in the diseased leaf area caused by Xoo as a result of treating the rice seedlings with *P. polymyxa* SX3. As revealed by Li *et al*., (2013), the reduction of disease severity was attributed to the suppression of Xoo growth and inhibition of biofilm formation, as it is essential for the phytopathogenic bacteria to colonize their hosts. In addition, some *P. polymyxa* stains secrets hydroxamate- type siderophores (Phi *et al*., 2010) and amylases, which are effective against plant pathogens (Bardin *et al*., 2015). Several research works conducted by Yegorenkova *et al*., (2013); Timmusk *et al*., (2019), reported that *P. polymyxa* induced deformations of wheat seedling root hairs through the formation of exo-polysaccharides, and it increased the plant resistance to the biotic and abiotic stresses.

Currently, in addition to the reducing the severity of BLB, *P. polymyxa* improved the growth of the rice seedlings. Previous studies of Liu *et al*., (2009); Rafigh *et al*., (2014) reported that *P. polymyxa* promotes the plant growth through the production of numerous biologically active substances i.e. antibiotics, phytohormones, and lytic enzymes, as well as through the production of a wide range of exo-polysaccharides. A recent study of Abdallah *et al*., (2019) indicated that *P. polymyxa* SX3 is able to produce IAA and has the ability to solubilize calcium phosphate. The production of IAA, capability of phosphate solubilization and nitrogen fixation are thought to be the vital mechanisms used by *Paenibacillus* spp. to enhance the plant growth (Carrillo *et al*., 2002; Padda *et al*., 2016; Xin *et al*., 2017).

The current study confirmed the presence of three antimicrobial genes mainly; *pmxB*, *pmxD*, and *Fusdel* in the DNA of *P. polymyxa* SX3, by using the specific gene markers. Detection of *pmxB* and *pmxD* genes is an evidence of the ability of this strain to secret the polymyxin antibiotic. Polymyxin is a cationic lipopeptide antibiotic with five positively charged diaminobutyryl (Dab) residues (Huang and Yousef, 2014). Later, a study conducted by Larrouy-Maumus *et al*., (2016) revealed that the first target of the polymyxin in Gram-negative bacteria is the lipopolysaccharide (LPS) of the outer membrane (OM) that acts as a permeability barrier. This results in lysis of the bacterial cell membrane (Rachoin *et al*., 2010; Zhiliang *et al*., 2015a). The obtained results from TEM confirmed that the morphology of Xoo cells were changed with perturbation of their cell wall on treatment with culture filtrates of SX3. In addition, polymyxin antibiotic damaged the physical integrity of the phospholipid bilayer of the inner membrane (IM) by membrane thinning, through overlapping with the interface of the hydrophilic head groups and fatty acyl chains (Huang and Yousef, 2014). This led to lysis of IM and subsequent cell death, as reported by Zhiliang *et al*., (2015b). Moreover, previous research work of Raza *et al*., (2008) highlighted that polymyxins dissolve the fatty acid moiety in the hydrophobic region of the cell membrane and disrupts its integrity. This causes inhibition of the cellular respiration, leakage of the low molecular weight cellular materials and the nucleic acid. The current presence of the two antimicrobial genes *pmxB* and *pmxD* supported the obtained results, and proved that *P. polymyxa* SX3 can inhibit biofilm formation by Xoo. It also confirmed the current results of TEM, which showed leakage of the intracellular substances as the Xoo lost the permeability of its cell membrane that led to cell death.

Furthermore, another important gene Fusdel has been detected which is responsible for producing fusaricidin. Choi *et al*., (2008); Li and Jensen, (2008); Vater *et al*., (2015) revealed that fusaricidin is a peptide antibiotic consisting of six amino acids, and has been identified as an antifungal agent produced by *P. polymyxa*.

Conclusion

Application of *P. polymyxa* SX3 to rice seedlings inhibited the growth and biofilm formation of the pathogenic Xoo. Moreover, this antagonistic bacterial strain killed the cells of Xoo, decreased the severity of

BLB, and improved the growth of the rice seedlings. Current detection of *pmxB* and *pmxD* genes in *P. polymyxa* SX3 indicated the secretion of polymyxin antibiotic that contributes to the antibacterial potential of this strain.

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Conflict of interest

No conflict of interests exists between the authors of this study.

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Ethical Approval

Non-applicable.

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