BIOCONTROL OF ANTAGONISTIC BACTERIA FLUORESCENT *Pseudomonas* STRAINS AGAINST *Xanthomonas oryzae* pv. *Oryzae* CAUSING BACTERIAL LEAF BLIGHT OF RICE IN THE GREEN HOUSE CONDITIONS

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ABSTRACT

Flourescent Pseudomonads are indigenous inhabitants of the rice rhizosphere and effective biocontrol agents against bacterial leaf blight of rice (BLB), caused by Xanthomonas oryzae pv. oryzae (Xoo). This study aimed to evaluate the potential involvement of selected fluorescent Pseudomonas strains in controlling BLB disease under greenhouse conditions. The experiment was designed in a completely randomized design (CRD) with 5 treatments and 4 replications, in which antagonistic bacteria strains were applied foliarly to the rice plant at 2 days before inoculation and 2 days after inoculation of Xoo. The results indicated that three strains (Ps.HG-11, Ps.HG-82, and Ps.HG-44) had good disease control ability for bacterial leaf blight. In which the Ps.HG-11 strain has the highest control ability, this is desmontrated by the lowest percentage of lesion length (PLL) and effectively reduces the PLL by 57.8% at 14 days after pathogen inoculation.

Keywords: Fluorescent Pseudomonas, Biocontrol, *Xanthomonas oryzae* pv. *oryzae*.

INTRODUCTION

Xanthomonas oryzae pv. *oryzae* (*Xoo*) is widely prevalent and causes bacterial leaf blight (BLB) in rice grown in different areas of the Mekong Delta of Vietnam. There is a need to use environmentally safe approaches to overcome the loss of grain yield in rice due to this disease. Biocontrol is the potential use of antagonistic bacteria as biocontrol agents that can suppress pathogens by producing bioactive secondary metabolites, including antibiotics, siderophores, and volatile compounds. Natural products are a consistent source of antimicrobial metabolites and are largely produced by soil-dwelling microorganisms (Yang et al. 2023). The fluorescent *Pseudomonas* group represents: 1) phytopathogenic cytochrome c oxidase-positive species, such as *P. cichorii*, *P. marginalis*, and *P. tolaasii*; 2) non-phytopathogenic, non-necrogenic strains, such as *P. fluorescens*, *P. putida*, *P. chlororaphis*, *P. aureofaciens*, and *P. aeruginosa* type species; and 3) phytopathogenic necrogenic fluorescent *Pseudomonas spp* without cytochrome c oxidase: *P. syringae* and *P. viridiflava* (Botelho and Mendonça-Hagler 2006). Fluorescent *Pseudomonas* strains produce many antimicrobial natural products and growth-promoting substances, which make them well adapted to environmental stress and suitable as biocontrol agents of plant pathogens (Gade and Koche 2022). On rice plants, some bacterial strains belonging to the genus *Pseudomonas* which can antagonize *Xanthomonas oryzae* pv. *oryzae*, which reduces the diseased leaf area by 60%, but this research did not specify which species Pseudomonas belongs to (Yasmin et al. 2016). According to Shivalingaiah and Umesha (2013), *Pseudomonas fluorescens* possesses

antibacterial activity against the *Xanthomonas oryzae* pv. *oryzae*, the bacterial leaf blight pathogen of rice. Inheriting the above research works with the aim of selecting fluorescent *Pseudomonas* strains that are capable of reducing important diseases in rice plants, which are: rice leaf blight caused by *Xanthomonas oryzae* pv. *oryzae (Xoo.)* in the greenhouse conditions.

Therefore, the study "Biocontrol of antagonistic bacteria by fluorescent *Pseudomonas* strains against *Xanthomonas oryzae* pv. *oryzae (Xoo.)* causing bacterial leaf blight of rice in the greenhouse conditions" was carried out with the objective: To find out the fluorescent *Pseudomonas* strains which exhibited high biocontrol effects against the bacterial pathogen *X. oryzae* pv. *oryzae* in the greenhouse conditions.

MATERIALS AND METHODS

Materials

Xoo. bacteria *(Xoo.*HG-08 Strain*)* was isolated from diseased leaves of bacteria leaf blight that was collected in Vi Thanh district, Hau Giang province. Three strains of fluorescent *Pseudomonas* (Ps.HG-11, Ps.HG-44, and Ps.HG-82) with high antagonistic ability against *Xoo.* bacteria were selected from *in vitro* antagonistic screening experiment that was conducted at Department of Plant Protection, Cuu Long Delta Rice Research Institute. The reference chemical was Anti-Xo 200WP. The media for culturing of pathogen *Xoo.* bacteria and biocontrol agents fluorescent *Pseudomonas* bacteria.

Methods

***Xoo.* pathogen inoculation:** Rice seeds were sterilized with 1% sodium hypocloride, sown in the pots in greenhouse conditions and plants were raised by maintaining the normal agronomical practices with a fertilizer formula applied 100N-40P_2O_5-30K_2O (Pham Sy Tan 2005). The 40 day-old-plants were inoculated with a virulent strain of *X. oryzae* pv. *oryzae* (*Xoo*.HG-08 strain). The bacterial pathogen strain was inoculated into NB medium and incubated on a shaker with 200 rpm at 28^oC for 18h until the logarithmic growth period, collected, and then diluted with sterile 0.9% NaCl to a density of 10^8 cfu/ml. The leaf-cutting method was used for inoculation of *Xoo*. by which rice leaves were clipped using scissors dipped in *Xoo* culture suspension (Kaufman et al. 1973).

Antagonistic bacteria application: The antagonistic fluorescent *Pseudomonas* strains were applied to rice leaves infected with *Xoo.* bacteria with a density of 10^8 cfu/ml at two days pre-inoculation and two days post-inoculation of the bacterial pathogen. The experiment was arranged in a completely randomized design (CRD) with 5 treatments and 4 replications, in which each biocontrol treatment consisted of a fluorescent *Pseudomonas* strain, a bactericide of Anti-Xo 200WP was used as the referent chemical treatment, and water application was served as the control treatment.

Data collection: Observation of the disease occurrent on experimental treatments was conducted at 6, 10, and 14 days after the inoculation. Measure the lesion length and the corresponding leaf length from which to calculate the ratio of lesion length (%) according to the formula by Gnanamanickam et al. (1999).

$$\text{Ratio of lesion length (\%)} = \frac{\text{Lesion length}}{\text{Leaf length}} \times 100$$

Statistical analysis: Data from the experiment was proccessed by Excel 2010 and subjected to analysis of variance (ANOVA) by SPSS version 2.0 sofware.

Table 1. The application concentrations and times of spraying in the experiment.

No.	Treatments	Concentrations	Times of spraying (day)
1	Ps.HG-11	10^8 cfu/ml	2DBI + 2DAI
2	Ps.HG-82	10^8 cfu/ml	2DBI + 2DAI
3	Ps.HG-44	10^8 cfu/ml	2DBI + 2DAI
4	Anti-Xo 200WP	3.75 gram/1 L water	2DBI + 2DAI
5	Control/ inoculation	--	*Xoo*. and water

(DBI) Days before inoculation; (DAI) Days after inoculation; L: liter, (--) not application of antagonistic bacteria and bactericide.

RESULTS AND DISCUSSION

The ratio of lesion length on treatments applied antagonistic bacteria strains

The evaluation results of the biocontrol ability of fluorescent *Pseudomonas* strains against the pathogen bacteria *Xoo*.HG-08 that caused bacterial leaf blight disease under greenhouse conditions indicated that all application treatments with antagonistic bacteria strains resulted in a significant decrease in ratio of lesion length when compared to the untreated control. At 6 DAI, the ratio of lesion lengths from three treatments of fluorescent *Pseudomonas* strains (Ps.HG-11, Ps.HG-82, and Ps.HG-44) were lower than the untreated control and were statistically significant different. In which, strain Ps.HG-11 had the lowest ratio of lesion length and strain Ps.HG-44 had the highest ratio of lesion length. There were a statistically significant different among fluorescent *Pseudomonas* strains. At 10 DAI and 14 DAI, the biocontrol activity of antagonistic bacteria strains remained at a high level through the assessment time points. Treatment Ps.HG-11 had a ratio of lesion length lower than the rest of biocontrol treatments and chemical control treatment. A statistically significant difference among biocontrol treatments (Ps.HG-11, Ps.HG-82 & Ps.HG-44) as well as chemical treatment (Anti-Xo 200WP) were found.

Table 2. The ratio of lesion length (%) on the treatments applied antagonistic bacteria strains and bactericide chemical at different times of observation.

No.	Treatments	The ratio of lesion length (%)		
		6DAI	10DAI	14DAI
1	Ps.HG-11	2.7^{e}	6.8^{c}	12.0^{e}
2	Ps.HG-82	3.0^{d}	8.0^{bc}	15.0^{c}
3	Ps.HG-44	4.5^{b}	9.7^{b}	17.0^{b}
4	Anti-Xo 200WP	3.2^{c}	9.6^{b}	13.0^{d}
5	Control	10.0^{a}	18.9^{a}	28.5^{a}
	F	**	**	**
	CV (%)	2.0	6.5	1.6

Means followed by a common letter are not significantly different at 1% level by DMRT. ** statistically significant in probability 1%; DAI: days after the inoculation.

Reduction efficiency in the ratio of lesion length on treatments applied antagonistic bacteria and chemical bactericide

Suppression of BLB was measured in terms of a reduction in the ratio of bacterial blight lesion length on treated leaves compared to untreated control. The result from **Table 3** confirmed that reduction efficiency in the ratio of lesion length of antagonistic bacterial application treatments was highest at 6 DAI and gradually decreased until 14 DAI. All strains of fluorescent *Pseudomonas* were effective in reducing in the ratio of lesion length of BLB disease, which was higher than the control treated which applied the chemical bactericide Anti-Xo 200WP. At 6DAI, the effectiveness of reduction of the ratio of lesion length (RLL) was highest on strains Ps.HG-11 (73.0%) and Ps.HG-82 (70.0%). The lowest control effectiveness was observed on strain Ps.HG-44 (55.0%). There was a statistically significant reduction in RLL between strain Ps.HG-44 and the rest antagonistic strains (Ps.HG-11 and Ps.HG-82). At 10 DAI, the reduction of RLL of experimental treatments was gradually decreased compared to 6 DAI. There were no statistically significant differences among antagonistic bacteria strains as well as chemical control treatment. At 14 DAI, treatment Ps.HG-11 had the highest efficiency in reduction of RLL among the biocontrol treatments (57.8%), whereas treatment Ps.HG-44 had the lowest control effectiveness among these treatments. A comparison between antagonistic bacteria applied treatments and chemical control treatment confirmed that treatment Ps.HG-11 exhibited higher efficiency in reduction of RLL compared to chemical control treatment, whereas the efficiency in reduction of RLL of treatment Ps.HG-82 was at par with chemical control treatment.

Table 3. Reduction efficiency in ratio of lesion length on treatments applied antagonistic bacteria strains and chemical bactericide.

No.	Treaments	Reduction efficiency in ratio of lesion length (%)		
		6DAI	10DAI	14DAI
1	Ps.HG-11	73.0^{a}	63.2^{a}	57.8^{a}
2	Ps.HG-82	70.0^{a}	57.5^{a}	47.2^{b}
3	Ps.HG-44	55.0^{b}	47.5^{a}	40.2^{c}
4	Anti-Xo 200WP	68.0^{a}	49.6^{a}	50.3^{b}
	F	**	ns	**
	CV (%)	2.1	9.6	3.5

Means followed by a common letter are not significantly different at 1% level by DMRT. ** statistically significant in probability 1%; DAI: days after the inoculation. ns: not significantly different.

Assessment results on the biocontrol ability of prospective fluorescent *Pseudomonas* strains against bacterial leaf blight disease on Jasmine 85 variety under greenhouse conditions (**Tables 2, 3 and Figure 1**) indicated that prospective antagonistic bacteria strains all showed good biocontrol of *Xoo.* bacteria (*Xoo.*HG-08 strain) caused bacterial leaf blight disease, in which the antagonistic strain *Ps*.HG-11 exhibited the highest biocontrol efficiency to bacterial leaf blight disease, with the lowest lesion length rate and the highest reduction efficiency on the ratio of lesion length was obtained among the fluorescent *Pseudomonas* strains evaluated (**Figure 1**).

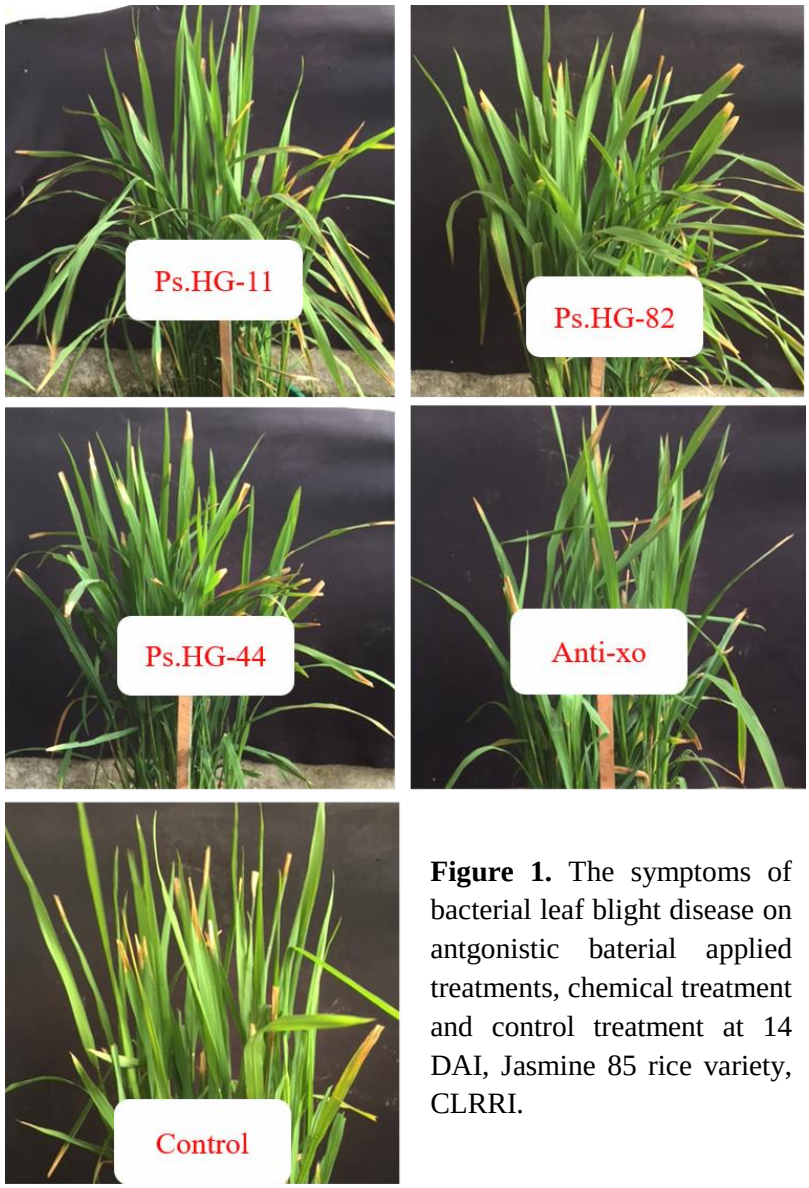

Figure 1. The symptoms of bacterial leaf blight disease on antgonistic baterial applied treatments, chemical treatment and control treatment at 14 DAI, Jasmine 85 rice variety, CLRRI.

CONCLUSIONS

All antagonistic strains of fluorescent *Pseudomonas* showed good control activity against *Xoo*. (*Xoo*.HG-08 strain) caused bacterial leaf blight on Jasmine 85 variety under greenhouse conditions, in which strain Ps.HG-11 exhibited the best effectiveness in controlling BLB disease as shown by the lowest percentage of lesion length and the highest effective reduction of the ratio of lesion length. Thus, this strain was considered as a promising biocontrol agent for BLB disease under field conditions. It is necessary to continue to identify, at the specie level, promising fluorescent *Pseudomonas* strains before evaluating their control efficacy against bacterial leaf blight disease under field conditions.

COMPETING INTERESTS

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

AUTHOR'S INFORMATION AND CONTRIBUTIONS

Tran Thi Kieu provided data and wrote the draft version of manuscript in English. Tran Thi Nam Ly read the draft version of manuscript in English. Nguyen Duc Cuong edited and approved the final draft.

REFERENCES

Botelho GR, and Mendonça-Hagler LC (2006) Fluorescent *pseudomonads* associated with the rhizosphere of crops - an overview. Braz J Microbiol 37:401-416.

Gade RM, and Koche MD (2022) Enhancing the growth and disease suppression ability of *Pseudomonas fluorescens*. In Singh H. B. and Vaishnav A. (Eds.), Sustainable Agriculture: Advances in Microbe-based Biostimulants, Pages 351-368.

Gnanamanickam SS (2009) Biological control of rice diseases. Springer science and Business media B. V., 108 pages.

Kaufman HE, Reddy APK, Hsien SPY, and Merca SD (1973) An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. Plant Dis Rep 57:537-541.

Tan PS (2005) Research results to improve fertilizer efficiency for high yielding rice in the Mekong Delta. In the book series "20 years of innovation in science and technology of agriculture and rural development". Volume 3, Pages 315-327. National Political Publisher, Hanoi. (In Vietnamese).

Yang R, Shi Q, Huang T, Yan Y, Li S, Fang Y, Li Y, Liu L, Liu L, Wang X, Peng Y, Fan J, Zou L, Lin S, and Chen G (2023) The natural pyrazolotriazine pseudoiodinine from *Pseudomonas mosselii* 923 inhibits plant bacterial and fungal pathogens. Nat Commun 14(1):734.

doi.org/10.1038/s41467-023-36433-z

Shivalingaiah, Umesha S (2013) *Pseudomonas fluorescens* inhibits the *Xanthomonas oryzae* pv. *oryzae*, the bacterial leaf blight pathogen in rice. Can J Plant Prot 1(5):147-153.

Yasmin S, Zaka A, Zahid MA, Imran A, Yousaf S, Rasul G (2016) Plant growth promotion and suppression of bacterial leaf blight in rice by inoculated bacteria. PloS One 11(8):e0160688. doi:10.1371/journal.pone.0160688.

PHÒNG TRỪ SINH HỌC CỦA VI KHUẨN ĐỐI KHÁNG *Pseudomonas* PHÁT HUỲNH QUANG ĐỐI VỚI VI KHUẨN *Xanthomonas oryzae pv. oryzae* GÂY BỆNH BẠC LÁ LÚA TRONG ĐIỀU KIỆN NHÀ LƯỚI

Pseudomonas phát huỳnh quang là vi sinh vật bản địa sống trên vùng rễ lúa đồng thời cũng là tác nhân phòng trừ sinh học hiệu quả đối với bệnh bạc lá lúa do vi khuẩn Xanthomonas oryzae pv. oryzae gây ra. Nghiên cứu này nhằm mục đích đánh giá khả năng phòng trừ của các chủng Pseudomonas phát huỳnh quang đã được chọn đối với bệnh bạc lá lúa trong điều kiện nhà lưới. Thí nghiệm được bố trí theo thể thức hoàn toàn ngẫu nhiên (CRD) với 5 nghiệm thức và 4 lần lặp lại, trong đó chủng vi khuẩn đối kháng được phun trên lá cây lúa vào thời điểm 2 ngày trước khi chủng và 2 ngày sau khi chủng vi khuẩn Xoo. gây bệnh bạc lá lúa. Kết quả cho thấy, 3 chủng (Ps.HG-11, Ps.HG-82 và Ps.HG-44) có khả năng phòng trừ tốt vi khuẩn Xoo.HG-08 gây bệnh bạc lá lúa. Trong đó chủng Ps.HG-11 có khả năng phòng trừ cao nhất, thể hiện qua tỷ lệ chiều dài vết bệnh thấp nhất và hiệu quả giảm tỷ lệ chiều dài vết bệnh là 57,8% tại thời điểm 14 ngày sau chủng bệnh.

Từ khóa: *Fluorescent Pseudomonas, kiểm soát sinh học, Xanthomonas oryzae pv. oryzae*