INVESTIGATION OF THE ANTAGONISTIC CAPABILITY OF *BACILLUS* BACTERIA AGAINST *RHIZOCTONIA SOLANI* KUHN AND THEIR EFFECTIVENESS IN CONTROLLING OF RICE SHEATH BLIGHT DISEASE IN THE SCREEN HOUSE CONDITION

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ABSTRACT

Investigation of the antagonistic capability of Bacillus strains against Rhizoctonia solani Kuhn in vivo by green house experiments for rice sheath blight control was conducted. The results indicated that three bacterial antagonist strains LM2.15et, LM3.16et, PH5.8et belong to the Bacillus group were potential biocontrol agents of the rice sheath blight disease equally to Bacillus amyloliquefaciens and Carbenda Supper 50SC fungicide. At 14 days after infection, the treatment with Bacillus strains LM2.15et, LM3.16et, and PH5.8et by spraying one day after inoculation with pathogen retained disease suppression of 45,67%, 43,81% và 47,59%, respectively, and there is not significantly different from B. amyloliquefaciens. Treatment of antagonists by spraying one day after inoculation with pathogen had higher disease suppression (45.25%) than those with one day before pathogen inoculation treatment (27.37%) or seed-coating treatment (26.91%).

Keywords*: bacterial antagonists, biocontrol, R. solani, rice sheath blight disease*

INTRODUCTION

Sheath blight caused by *Rhizoctonia solani* Kuhn is considered as the second most important disease after rice blast in reducing yield and quality (Park *et al.,* 2008). This fungus thrives in the tropics of Vietnam, especially in the Mekong Delta, where the climate conditions are hot and humid. Furthermore, the favorable conditions for development and harmfulness of fungi as problems need to be addressed. The applications of management measures based on biological control are not only a logical choice but also a imperative requirement. According to Lai Van E (2003), the applications of biological control with improvement in farming techniques are highly effective.

Among the agents of biological control, the bacteria of the genus *Bacillus* are considered as safe biological agents and have high potential in biological control (Silo-Suh *et al.,* 1994), which are great significance in maintaining the biological balance and natural ecosystems (Alabouvette & Cordier, 2011). Thus, rice sheath blight control based on beneficial bacteria is a potential tool that can replace and reduce the reliance on chemical pesticides. This is considered as key measure in controlling rice sheath blight.

MATERIALS AND METHODS

The study was carried out in the greenhouse conditions of Plant Protection Department, College of Agriculture and Applied Biology, Can Tho University.

Preparation of *Bacillus* strains

Three prospecting and outstanding *Bacillus* strains were isolated from laboratory experiments and *B. amyloliquefaciens* and Carbenda Supper 50 SC (used for positive control) were used to test the ability of inhibiting the growth *R. solani in vivo*. The samples of the isolated bacteria are quoted: location, field order, the order from colonies of

different fields, the endogenous or exogenous bacteria (e) with heat-treated or not (t). The *Bacillus* strains were grown in King's B liquid media for 48 hours.

Investigation of the antagonistic capability of *Bacillus* strains isolated against *Rhizoctonia solani* Kuhn *in vivo*

The experiment was followed a completely randomized design with 2 factors and four replications.

+ Factor A (6 levels): disease controlling agents

- Three strains of *Bacillus* with good potential selected from previous experiments; one fungicide (Carbenda Supper 50SC) with strong inhibition againts *R. solani in vitro;* one strain of *B. amyloliquefaciens* from the laboratory of Plant Protection department; one untreated treatment as negative control.

+ Factor B (level 3): processing time for disease control agents

- Seed-coated with *Bacillus* strains for 12 hours before sowing

- Two days before pathogen inoculation treatment (38 days after sowing)

- Two days after pathogen inoculation treatment (42 days after sowing)

Supper Carbenda 50SC was treated by spraying at a dosage recommended by manufacturers.

RESULTS AND DISCUSSION

The effectiveness of controlling rice sheath blight disease of *Bacillus* strains was assessed via disease index (%) (DI) and the effectiveness of disease reducing (%) in comparison with positive controls *B. amyloliquefaciens* and Carbenda Supper 50SC.

The disease index at 14 days after inoculation (14 DAI)

The data in Table 1 showed that DI (%) among the treatments shown significant difference at 1% level. However, the DI (%) of *Bacillus* strains LM3.16et, LM2.15et and PH5.8et are higher and significantly different in comparison with the positive control Carbenda Supper 50 SC but not different from *B. amyloliquefaciens.*

Table 1. The DI (%) at 14 DAI

Treatment agents (A)	DI (%) of treatment measures (B)			
	One day before inoculation	***One day after inoculation***	***Seed-coating***	***Average (A)***
LM3.16et	27,22 ^{bc}	22,22 ^{c}	25,00 ^{bc}	24,82 B
LM2.15et	35,56 ^{ab}	23,33 ^{c}	30,00 ^{abc}	29,63 B
PH5.8et	28,83 ^{bc}	21,67 ^{c}	27,22 ^{bc}	25,74 B
B. amylolyquefaciens	28,89 ^{bc}	23,89 ^{bc}	28,89 ^{bc}	27,22 B
Carbenda supper	29,44 ^{bc}	3,33 ^{d}	0,00 ^{d}	21,57 C
Control	41,11 ^{a}	41,11 ^{a}	41,11 ^{a}	41,11 A
Average (B)	31,76 A	22,59 B	30,69 A	
F CV (%)	F(A) ** ; F (B) ** ; F(A x B) ** 15,72%			

*Note: - The average in the same column followed by the same letters or the difference was not statistically significant in Duncan test. ** Significant difference at 1%, * differences at the 5% significance level, ns: not significantly different. The data were transferred to root arcsin (X + 1/4n) / 100 if X = 0%*

Among the treatments, DI (%) of the treatments when treated at one day after inoculation (22.59%) was less than those with one day before inoculation and seed-coating at statistically and significantly different at 1% level.. Over 14 DAI, all prospective *Bacillus* strains are capable of reducing the severity of rice sheath blight.

The effectiveness in disease reducing (%) at 14 DAI

At 14 DAI, the effectiveness in disease reducing among the treatments differ markedly at 1% significance level. In treatments with *Bacillus* strains LM3.16et, LM2.15et and PH5.8et, the effectiveness in disease reduction (%) were not statistically significant compared with the positive control Carbenda Supper 50SC and *B. amyloliquefaciens*. Treatment of antagonists by spraying one day after inoculation with pathogen had higher disease suppression (45.25%) than those treated at one day before pathogen inoculation (27.37%) or seed-coating treatment (26.91%).

Table 2. The effectiveness of disease reducing (%) at 14 DAI

Treatment agents (A)	**The effectiveness of disease reducing (%) of treatment measures (B)**			
	One day before inoculation	**One day after inoculation**	**Seed-coating**	**Average (A)**
LM3.16et	31,44 ^{bc}	45,67 ^{b}	39,39 ^{bc}	38,83 A
LM2.15et	25,92 ^{bc}	43,81 ^{bc}	27,55 ^{bc}	32,43 A
PH5.8et	21,31 ^{c}	47,59 ^{b}	42,18 ^{bc}	37,03 A
B. amylolyquefaciens	32,22 ^{bc}	42,65 ^{bc}	29,88 ^{bc}	34,92 A
Carbenda supper	29,33 ^{bc}	91,80 ^{a}	22,48 ^{c}	45,38 A
Control	0,00 ^{d}	0,00 ^{d}	0,00 ^{d}	0,00 B
Average (B)	27,37 B	45,25 A	26,91 B	
F CV (%)	F(A) ** ; F (B) ** ; F(A x B) ** 30,11%			

*Note: - The average in the same column followed by the same letters or the difference was not statistically significant in Duncan test. ** Significant difference at 1%, * differences at the 5% significance level, ns: not significantly different. The data were transferred to root arcsin (X + 1/4n) / 100 if X = 0%*

Table 2 shows that the spray at one day after inoculation is more effective and stable than the other two measures. This is effective prevention, and treatment of bacterial antagonists is capable of inhibiting pathogens through one or more mechanisms of antibiotics, competition for nutrients and places, more enzymes breaking down fungal cell wall or inducing resistance in crops (Van Loon, 1998). At 14 DAI, *Bacillus* strains still maintain effective because they create endospores when conditions are unfavorable as low humidity and rainfall, they will rely on endospores to survive. PGPR have the potential to reduce the disease as biological control agents, as they have the ability to stimulate other profitable symbiosis, however, bacteria need time to multiply to stimulate some disease resistance (Tran Thi Thuy Ai, 2011).

Figure 1. Expression rice sheath blight in 14 DAI when treating with disease control agents

3.3. Characteristics of *Bacillus* colonies

Table 3. Characteristic colonies of prospective *Bacillus* strains

Ser. No.	The *Bacillus strains*	Characteristics of *Bacillus* colonies
1	LM3.16et	Milky white, wrinkled, protruding edges
2	LM2.15et	Yellowish white, flat edge, flat center
3	PH5.8et	Milky white, shinning edge, smooth center

Table 4. Three characteristics of prospective rhizosphere bacteria have been tested in green house conditions when observed at 100X lens

Isolates	The cells size μm	Gram	Endospores
LM3.16et	1,21 x 2,12	+	x
LM2.15et	1,23 x 2,17	+	x
PH5.8et	1,21 x 2,10	+	x

According to Bien Van Minh *et al.* (2006), a number of bacteria at the end of the growing stage, the nutrients in the environment and toxicity depleted through excessive exchange, or due to a sudden change in the growth conditions likely causing sporulation inside cells (endospores). The ability in creating endospores in the 3 strains indicated that there may be one factor helping the survival of this strain in the drought conditions. The maintaining density ability of the rhizosphere bacterial strains after a long period of time can also be explained by the ability in creating endospores (Monteiro *et al*., 2005).

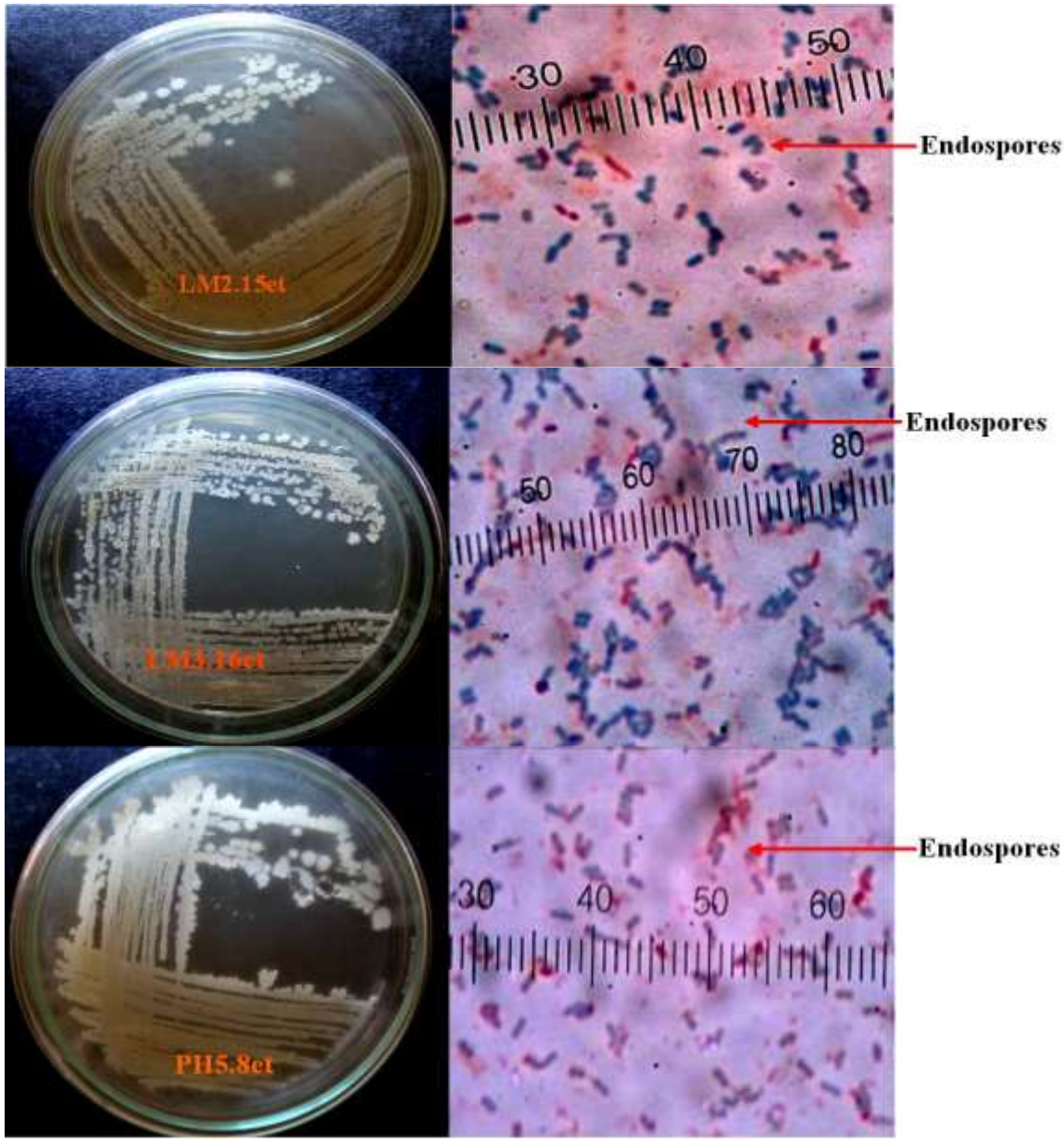

Figure 2. The ability to create endospores of *Bacillus* strains

CONCLUSIONS

Three strains LM2.15et, LM3.16et and PH5.8et have the ability to control rice sheath blight *in vivo* conditions and spraying one day after inoculation with pathogen have disease suppression higher in comparison with one day before pathogen inoculation treatment or seed-coating treatment.

The futher surveying of more effective biological control of rice sheath blight with prospective *Bacillus* strains in field conditions is necessary to incorporated in IPM.

REFERENCES

Alabouvette C., & Cordier C., 2011. Risks of microbial control agens and regulation: are they balance In: *Ehlers R-U (ed) Regulation of biological control agents. Springer, Dordrecht*, pp 157-173.

Bien Van Minh, Pham Van Ty, Kieu Huu Anh, Pham Hong Son, Pham Ngoc Lan, Nguyen Thi Thu Thuy, 2006. Microbiology Lecture, Hue University Publishing.

Lai Van E, 2003. Research using antagonistic microorganisms in biological control of *Fusarium oxysporum* and *Rhizoctonia solani* Kuhn causing deadly diseases on cotton seedlings (*Gossypium hirsutum* L.). Thesis of Master of Science in Plant Protection. College of Agriculture and Applied Biology, Can Tho.

Monteiro L., R. L. R. Mariano and A. M. Souto-Maior, 2005. Antagonism of *Bacillus* spp.

Against *Xanthomonas campestris* pv. *campestris*", Braz Arch Biol Technol 48, pp. 23–29.

Park, D. S., Sayler, R. J., Hong, Y. G., Nam, M. H., and Yang, Y., 2008. A method for inoculation and evaluation of rice sheath blight disease. Plant Dis. 92:25-29.

Silo-suh, L. A.; Lethbridge, B. J.; Raffel, S. J.; He, H.; Clardy, J. and Handelsman, J. 1994. Biological activities of two fungistatic antibiotics produced by *Bacillus cereus* UW85. Applied and environmental microbiology 60: 2023-2030.

Tran Thi Thuy Ai, 2011. Evaluation of efficiency of *Rhizobacteria* biocontrol agents against *Fusarium* wilt on Ginger. Thesis of Master of Science in Plant Protection. College of Agriculture and Applied Biology, Can Tho.

UniversityVan Loon, L.C., P.A.H.M. Bakker and C.M.J. Pieterse, 1998. Systemic resistance induced by rhizosphere bacteria. *Annu Rev Phytopathol*, 36(30), 453–483.

TÓM TẮT

Khảo sát khả năng đối kháng của vi khuẩn *Bacillus* spp. đối với nấm gây bệnh đốm vằn trên lúa (*Rhizoctonia solani* Kuhn) và hiệu quả phòng trị trong điều kiện nhà lưới

Kết quả đánh giá hiệu quả kiểm soát bệnh đốm vằn trong điều kiện nhà lưới, cho thấy cả 3 chủng LM2.15et, LM3.16et, PH5.8et đều có khả năng kiểm soát bệnh tương đương với vi khuẩn Bacillus amyloliquefaciens và cho hiệu quả giảm bệnh tương đương với đối chứng dương (dùng thuốc Carbenda Supper 50SC). Ở thời điểm 14 ngày sau khi lây bệnh, nghiệm thức được xử lý với 3 chủng Bacillus PH2.6t LM2.15et, LM3.16et, PH5.8et có hiệu quả giảm bệnh là 45,67%, 43,81% và 47,59% khi xử lý bằng biện pháp phun sau tương đương với hiệu quả giảm bệnh của vi khuẩn B. amyloliquefaciens. Về biện pháp xử lý, hiệu quả giảm bệnh của xử lý phun sau (45,25%) cao hơn so với xử lý phun 1 ngày trước khi lây bệnh (27,37%) và áo hạt (26,91%)

Từ khóa: *bệnh đốm vằn, phòng trừ sinh học, R. solani, vi khuẩn đối kháng.*