Omonrice 9: 79-86 (2001)

Current status and future prospects in biological control of rice sheath blight in Mekong Delta

Lai Van E[1], Nguyen Thi Phong Lan[1], Pham Van Du[1], and T.W. Mew[2]

ABSTRACT

This research paper addresses the beneficial bacteria covering the aspects of biological control and plant growth promotion in terms of sheath blight (ShB) disease control. Six strains of beneficial bacteria (NF1, NF3, NF 52, NF 49, CT 6-37, and W 23) were selected from 300 strains isolated from seeds and other components of the rice ecosystem in Mekong Delta. These strains were tested for suppression of sheath blight disease, caused by Rhizoctonia solani Kuhn and their ability to promote seeds germination, seedling development. They significantly reduced sheath blight development and spread both under greenhouse and field conditions. To enable the introduction of a number of bacterial cells high enough to allow the expression of their beneficial activities under field conditions, the strains should be mixed with chemical fungicide at a low rate. These results strongly suggest that the combination of antagonistic bacteria with chemical fungicide is needed for more efficient control effects, especially under flooded paddy soil conditions. An obvious understanding of the specificity and mode of action of antagonists as well as pathogen can lead to improved biocontrol. Sclerotia floating on the water surface are considered as a main source of primary inoculum. However, mycelium on the diseased rice straws may act as a primary inoculum. The diseased weeds such as water hyacinth are not considered as a initial inoculum but it may cause the water supply to contaminate. We have found that bacterial suspension should not be applied before maximum tillering stage because of the ShB pathogen rarely works at that time under flooded paddy soil condition.

Key words: Sheath blight, *Rhizoctonia solani*, biocontrol, BCA, antagonistic bacteria

INTRODUCTION

Disease management in rice has been largely depended on host plant resistance, chemical control which may not be consistent in term of sustainable agricultural production. Biological control has been recently considered as a promising option. Sheath blight (ShB) caused by *Rhizoctonia solani* Kuhn is one of the most important rice diseases in the tropical areas. Due to the lack of high level of rice varietal resistance to sheath blight, it has become necessary to find alternative means to manage the disease. The initial bilogical control researches on sheath blight have been implemented in Mekong delta to meet the demand since early 1980's. In 1996, much attentions have been paid to beneficial bacteria so far. It included (1) screening antagonists; (2) evaluating effectiveness of beneficial bacteria targeted different diseases under greenhouse and field conditions. Some of them have been demonstrated in a large scale area of rice production. Biological control bacteria are expected to colonize and persist on plants and to inhibit the ability of plant pathogenic agents to cause damage and spread disease. For further studies to know how to improve the effectiveness of biological control, more efforts will be implemented to answer: (1) Do BCAs (biological control agents) live on the rice? (2) What is the relationship between microorganisms (plant pathogens and nonpathogen) and host plants?

This study aims at (1) determining the initial source of ShB inoculum in irrigated rice, (2) testing the effects of antagonistic bacterial originated from rice at seed germination stage, (3) understanding *R. solani* sclerotial viability. In field trials, chemical fungicide and biocontrol combination treatment should be

[1] CuuLong Delta Rice Rearch Institute,Omon, Cantho, Vietnam
[2] Entomology and Plant Pathology Division, IRRI, Los Banos, Philippines

considered to deal with the efficiency of antagonistic bacterial colonization of the phylloplane or rhizoplane in the presence of these chemicals.

MATERIALS AND METHODS

Antagonistic bacterial strains

Six bacterial strains that were used as biocontrol agents for sheath blight of rice are listed in Table 1. These strains were selected by bioassays from 300 strains of antagonistic bacteria which were isolated from the rice (rice seeds, diseased or healthy plant tissues, weeds). The strains were grown on NA medium containing 5 g peptone, 3 g beef extract, 5g NaCl, 15 g Bacto agar per liter for 24 hours at room temperature. The concentration of bacterial suspension was adjusted to log 8 cfu/ml.

Pathogen

Rhizoctonia solani belonging to anastomosis group AG1-IA that was isolated from infected rice fields in Mekong Delta, was used in the study as a source of pathogen inoculum. For *in vitro* tests, sclerotia that were harvested from PDA medium were used as a primary source of inoculum, whereas mycelia in the rice hull rice grains medium were used for greenhouse and field tests.

Sources of initial inoculum for rice sheath blight in irrigated rice in Mekong Delta.

The percentage of infected plants in rice field with different sources of inoculum of *R. solani* such as sclerotia, mycelium in the diseased rice straws, infected weeds, water contaminated with infected plant debris was determined to clarify whether a main source of inoculum for sheath blight infection in rice fields. For the check plots, water supply was filtered to prevent from contamination of sclerotia floating and mycelium in plant debris. Data collected from the field experiment were repeated twice.

Effects of antagonistic bacteria on rice seed germination and seedling development.

Rice seeds were soaked with antagonistic bacterial suspensions for 48 hrs, then placed in petri plates at 25 seeds/plate. The plate contained 2-3 layers of sterilized blotter papers moistened with sterilize distilled water. Plates were incubated at 28^{o}C for 4 days at an alternating cycle of 12hr darkness and 12hr light. The seeds were soaked with sterilize distilled water as control. Seed germination, radicle and hypocotyl measurements were recorded at the fourth day and continuing through the tenth day of incubation.

Rhizoctonia solani sclerotial viability

One hundred of sclerotia of *R. solani* were soaked in bacterial suspensions for 24, 48 and 72 hrs, then placed on PDA medium or directly observed with microscope. Sclerotia were soaked with distilled water as control. The number of germinated sclerotia was recorded after 48 hrs incubating at room temperature.

Suppression of rice sheath blight by using combination of antagonistic bacteria and fungicide

Rice cultivar OM2031 was planted in greenhouse (1x1m^2 / plot) and in rice field (50m^2 / plot) to evaluate the biocontrol agents. Rice plants at maximum tillering stage, were sprayed with 48hr old cell suspension of antagonistic bacteria alone and combined with chemical fungicide (Validacin 5SP) at 1/2, 1/4, 1/6, and 1/8 of the recommended rate, at two days before and after artificial inoculation with the sheath blight pathogen. The percentage of infected hills at different distance form the focus or sheath blight expansion from an initial inoculum source was recorded at 7 days after inoculation and 7-day interval. A control was also observed in three replications.

Table1. Bacterial strains specifications

Code	Origin
CT6-37	Rice seedlings
W 23	Weeds
NF 1	Diseased plant tissues
NF 3	Diseased plant tissues
NF 49	Rice seeds
NF 52	Weeds

The effectiveness index (EI) was calculated on the basic of percentage of infected hill (P) as

$$EI(\%) = \frac{P_{Control} - P_{Treatment}}{P_{Control}}$$

The EI value may range from 0 (no effect) to 100 (total effectiveness).

RESULTS AND DISCUSSION

Sources of initial inoculum for rice Sheath Blight in irrigated rice ecosystem in Mekong Delta

The number of diseased hills in the plots amended with sclerotia obtained the highest values both in Dry and Wet seasons (Fig.1). The disease incidence in the plots which were planted with water hyacinth showed higher than that in the plots which were amended with diseased rice straw in Dry season whereas mycelium in infected rice straw is considered as a main source of primary inoculum in Wet season. Previous studies in different countries indicated that the primary inoculum for rice sheath blight consists mostly of sclerotia floating on the water surface after pudding and mycelium in plant debris may also act as primary inoculum in tropical regions (Takashi et al. 1997). However, the inoculum from the diseased weeds is also very important in disease outbreaks although it is not considered as a primary inoculum but it may cause contamination of water supply.

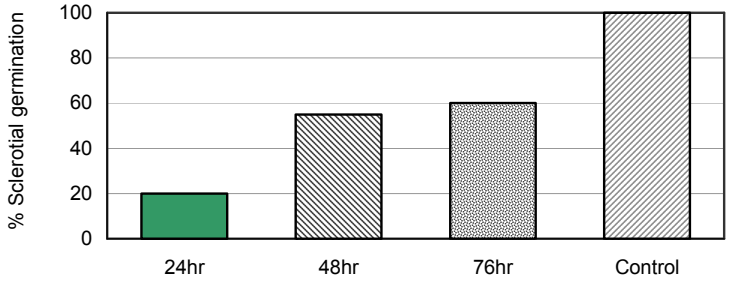


Fig. 1. Percentage of diseased hill in plot with different initial sources of *R. solani* inoculum in the field conditions. (AUDPC : area under disease progress curves of the sheath blight incidence over time)

Effects of some promising antagonistic bacterial isolates on *R. solani* sclerotial viability and rice seed germination

In preliminary tests, antagonistic bacteria did not affect rice seed germination but they influenced seedling development. Some of them enhanced seedling development and other not effective at all or deleterious, especially radical length. Among four bacteria strains used, CT6-37 that was isolated from

rice seeds, indicated more effectiveness on seedling development than those of that were isolated from weed or other parts of rice (Fig 2 & Table 1). Indirect effect of bacteria on sclerotial germination would be a significant effect on disease incidence under field condition. All of tested strains inhibited mycelial growth and affected sclerotial viability *in vitro* test. The number of sclerotial germination was the lowest when sclerotia were soaked in bacterial suspensions for 24 hrs, then placed on cultural medium whereas higher value of viability of sclerotia was recognized at 48 and 76 hrs after soaking (Fig. 3). There was a negative correlation found between the population of antagonistic bacteria and incubation period. Antagonistic bacteria obtained from the long period of incubation suspension was not able to prevent from sclerotial germination.

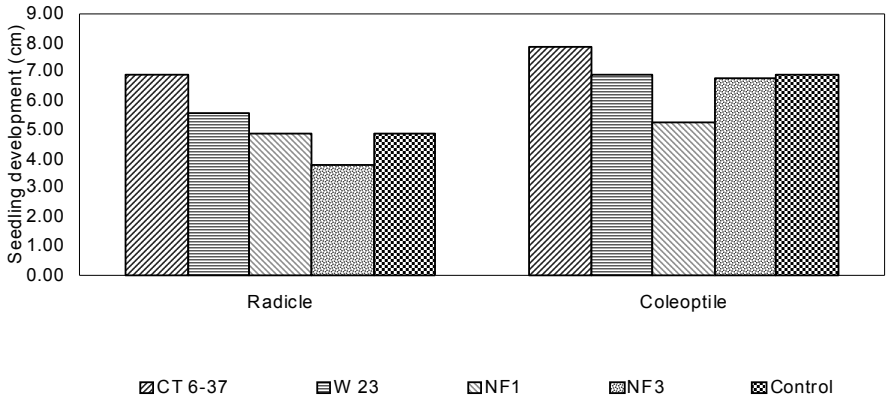


Fig. 2. Effect of antagonistic bacteria originated from seeds and other components of the rice ecosystem in Mekong Delta on seedling development.

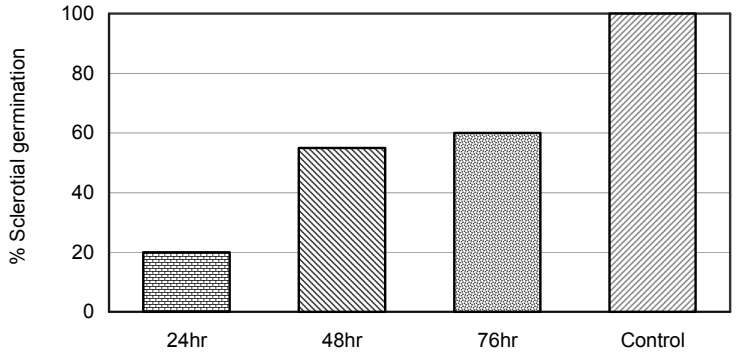


Fig. 3. Effect of antagonistic bacteria incubation periods on viability of R. solani sclerotia.

Suppression of rice sheath blight by using combinations of antagonistic bacteria and chemical fungicide.

To achieve better disease suppression under field conditions, the combination treatments between antagonistic bacteria and chemical fungicides are needed. Results from the experimental microplots in greenhouse and rice field indicated that sheath blight incidence in rice plants treated with the mixture of bacteria and Validacin was significantly lower than that of the checks which were treated by antagonistic bacteria alone or by water. They significantly reduced sheath blight development and spread both in greenhouse and rice field. Two strains NF49 and NF52 were particularly active. A mixture of two strains in a ratio of 1:1 gave significantly better control of the disease than each of the strains used individually. However,

under high disease pressure condition, antagonistic formulation amended with Validacin at 25% of recommended rate has been showed the increased efficacy of the biological control of *R. solani* under natural conditions (Fig. 4). Similar results were observed for other strains, such as NF3, CT6-37, W23, under greenhouse conditions.

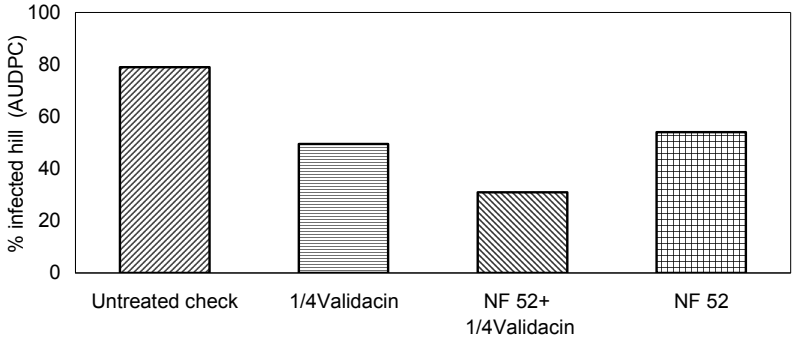
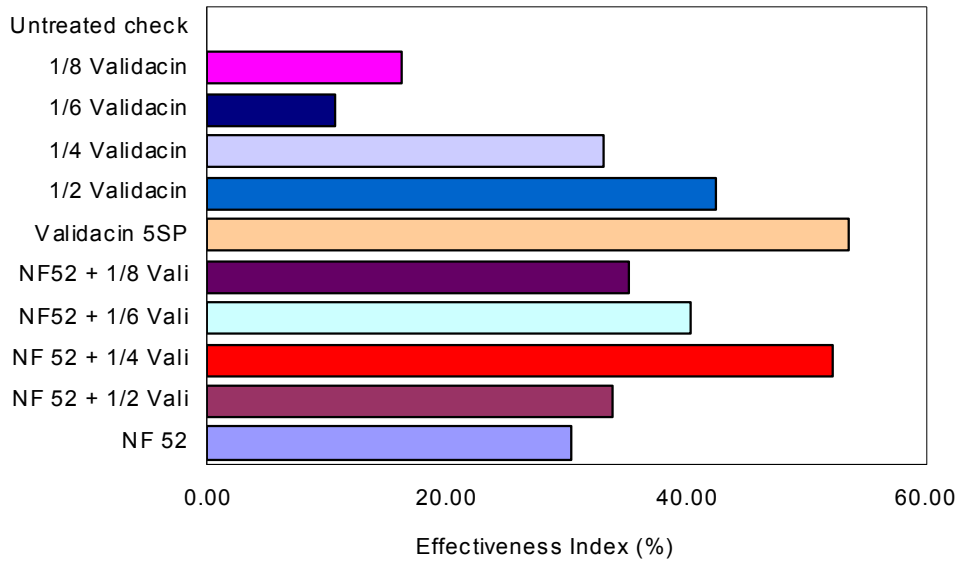


Fig. 4. Greenhouse test on comparison of control effect against sheath blight between antagonistic bacteria alone and combination with chemical fungicide (audpc: area under disease progress curves of percentage of diseased hill in plots over time).

Combination of effective organisms and manipulation of microbial communities rather than using a single strain was suggested for more consistent and effective control (Mew et al.1994). This study also demonstrates that combination of antagonistic bacteria and chemical fungicides significantly increased the suppression of rice sheath blight expansion. Fungicide addition to bacterial suspension at low rates significantly increases suppressive effect of bacterial formulation. The fungicide addition may possible directly prevent rice from sheath blight infection and increase the activity of antagonistic bacteria for preventing secondary infection by colonization on host plant surface or induce resistance. The percentage of infected hills was low at very near from the focus (inoculated hill), supplementation of bacterial suspensions with 25% of recommended rate of Validacin 5SP further enhanced the suppressive effects, the EI was greater than 50%, particularly in preventing disease expansion from the focus under flooded paddy soil conditions (Fig. 5), it could be explained by the effect of interaction between antagonist and fungicide.



Application of bacteria as a biological control agent in the field.

To assess the biocontrol suppression of sheath blight by using antagonistic bacteria and their combination with fungicide under field condition for a long time, antagonistic bacteria and fungicide used to control of rice sheath blight must be evaluated for durability effect. At maximum tillering stage, the plants were inoculated with inoculum of *R. solani*. Bacteria were applied at one and two weeks after inoculation. Among six tested applications, both combination methods obtained very good results in sheath blight suppression while bacteria alone applied could not prevent rice plants from sheath blight spreading overtime. When applied together in plant surface, the combination of isolate NF52 and isolate NF49 indicated a better disease suppression as compared to the single strains both in greenhouse and field trials. Among various combinations of bacteria, the formulation containing Validacin at 25% of recommended rate was effective in the suppression of rice sheath blight as compared to both untreated check and bacteria treated alone (Fig 6).

It is necessary to be aware how to improve activity against *Rhizoctonia* when applied at a lower rate and therefore reduce production costs; the ability of a biological agent to exert its effects on pathogens attacking after the chemicals which are no longer providing protection depends to colonization of the phylloplane and rhizoplane with these chemicals' presence.

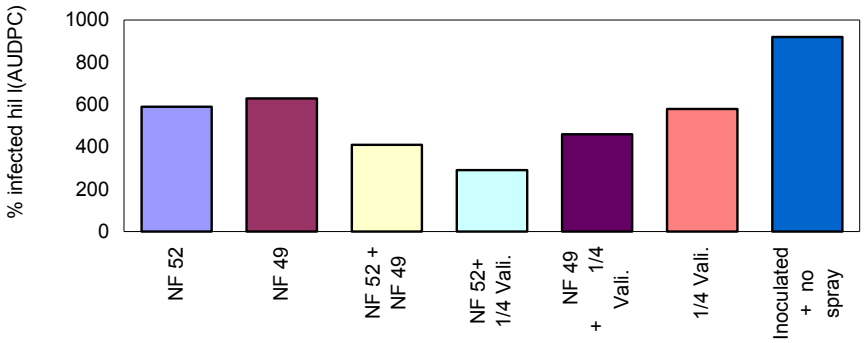


Fig. 6. Effect of different treatments of antagonistic bacteria on the suppression of sheath blight in the field conditions.

CONCLUSION

Under irrigated rice conditions, mycelia in the diseased rice straws are considered as a primary inoculum for rice sheath blight, especially in the Wet season when the plant debris could not be destroyed by burning after harvest. The infected weeds are also very important for causing contamination of water supply.

Antagonistic bacteria isolated from seeds and other components in Mekong Delta rice ecosystem provided significant disease reductions under irrigated rice conditions. This study also indicates the potential benefits using mixture of bacterial strains and chemical fungicide at low rates to suppress diseases under the field conditions.

Several advantages on biological control agents have been reported. However, more efforts to search good antagonistic bacterial strains, to address the optimization of fermentation and protocol for making biological products are still needed

PROSPECTS

In recent years, the progressive developments of biocontrol to plant diseases both in research and application have been recognized in Vietnam. However, several problems have been also created and various obstacles have hindered the development of biocontrol, e.g. lack of stability on its effectiveness. Variability in effectiveness is a commonly encountered problem when biocontrol is employed as a measure of plant disease control. Basic research is fully required before integration between all abio- and/or biofactors which are beneficial to increase effectiveness of biocontrol.

Continuous efforts to search good antagonistic bacterial strains is primarily needed, of which selection of biocontrol agents should focus on finding those which occupy the same ecological niche such as roots, phylloplane and vascular system as the pathogen together with development of new assays for selection. Application of new techniques is also necessary, of which PCR, RFLP and RAPD method wil help not only rapid identification of bacterial speciies but also specific bacterial characteristics importantly involved in either biocontrol or growth promoting mechanisms. These techniques will also be essential for risk assessment of forthcoming genetically modified PGPR after the establishment of guideline as well as public acceptance for the introduction of those into an agronomic situation. In this respects, PGPR and BCAs thus obtained should be recognized and maintained as important genetic (or gene) resource which will be useful for future studies in worldwide.

REFERENCES

Deborah RF, JWJ Connick and JA Lewis. 1998. Formulation of microorganisms to control plant diseases. In Formulation of microbial biopesticides - Beneficial microrganisms, nematodes and seed treatments, ed. H.D. Burges, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp187-233.

Hashiba T and S Mogi. 1976 Development changes in sclerotia of the sheath blight fungus. Phytopathology 65: 159-162.

Hashiba T. 1984. Estimation method of severity and yield loss by rice sheath blight disease. Bull. Hokuriku

Natl. Agric. Exp. Stn. 26: 115-164 (in Japanese with English summary)

Mew TW, AM Rosales, and GV Maningas. 1994. Biological control of Rhizoctonia sheath blight and blast of rice . In *Improving Plant Productivity with Rhizosphere Bacteria*. Ed. M. H. Ryder, PM. Stephens and G.D. Bowen. Pp 9-13, International Workshop on Plant Promoting Rhizobacteria, Adelaide, Australia.

Sung KC, YR Chung. 1997. Enhanced suppression of rice sheath blight using combination of bacteria which produce chitinases or antibiotics. In: Plant Growth Promoting Rhizobacteria : Present Status and Future Prospects. Eds. A. Ogoshi, K. Kobayasshi, Y. Homma, F. Kodama, N. Kondo, and S. Akino. pp. 370- 372. Proceeding of the Fourth International Workshop on PGPR Japan-OECD Joint Workshop.

Takashi K, TW Mew TW, and T Hashiba. 1997. Relationship between incidence of rice sheath blight and Primary inoculum in the Philippines: Mycelia in plant debris and sclerotia. Ann. Phytopathol. Soc. Jpn. 63:324-327.

SUMMARY IN VIETNAMESE

Hiện trạng và triển vọng của phòng trừ sinh học bệnh đốm vằn trên lúa ở đồng bằng sông Cửu Long

Hiện nay, chúng ta chưa có giống lúa kháng bệnh đốm vằn do nấm Rhizoctonia solani Kuhn gây ra. Phòng trừ sinh học được xem như một biện pháp có ích, hạn chế sự ô nhiễm môi trường do dùng hóa chất (thuốc diệt khuẩn). Cở sở phòng trừ sinh học yêu cầu trước hết phải tìm hiểu nguồn lây lan mầm bệnh đốm vằn trong ruộng lúa nước vùng ĐBSCL. Hạch nấm trôi nổi trong nước được xem như là nguôn lây bệnh chủ yếu. Khuẩn ty tồn tại trên rơm rạ đã nhiễm bệnh từ vụ trước cũng được xem xét là nguồn gây bệnh quan trọng, đặc biệt trong mùa mưa. Lục bình bị nhiễm bệnh đốm vằn trôi nổi trong những kênh mương không phải là nguồn lây bệnh trực tiếp, nó là nguyên nhân gián tiếp gây tạp nhiễm nguồn nước tưới trong ruộng lúa.

Sáu dòng vi khuẩn đối kháng CT6-37, NF1, NF3, NF49, NF52, và W23 được phân lập. Chúng gây ảnh hưởng: (1) kích thích sự phát triển cây mạ, (2) ức chế sự phát triển của khuẩn ty Rhizoctonia solani Kuhn, (3) làm giảm sức sống của hạch khuẩn, và (4) ngăn chận sự phát triển của bệnh trong điều kiện ngoài đồng ruộng. Kết hợp một lượng thuốc rất ít (Validacin 5SP%) với việc sử dụng dung dịch vi khuẩn đối kháng trong cả hai thí nghiệm ở nhà lưới và ngoài đồng ruộng đều cho kết qủa kiểm soát bệnh tốt, đặc biệt trong trường hợp áp lực bệnh hại cao trên ruộng lúa.