Biological Control of Rhizoctonia Damping Off of Chinese Cabbage using a Bioformulation Containing *Streptomyces* sp. MOST-1

Sirirat Siripornvisal[1] and Teerayuth Suddee[1]

Abstract

In the present study, a talc based biological control product containing an antagonistic Streptomyces strain MOST-1 was formulated and tested for its stability and efficacy against soil borne diseases of *Brassica chinensis*. Stability of the formulation was tested over a 9 month period, at 4 °C and at room temperature (25-35 °C). It was found that the formulation showed high stability since it retained 100 % survivability of the antagonist after 6 months storage and up to 80 % after 9 months. The formulation was then experimentally applied to control Rhizoctonia damping off diseases of *Brassica chinensis* under environmental controlled chamber condition. The results revealed that the formulation effectively control the diseases since it provided up to 100 % suppression when it was applied at the rate of 10 g per 1 kg of potting material. In addition, it was found that application of the formulation led to the promotion of plant growth. These results indicated appropriateness talc as a bulk carrier for bioformulation of Streptomyces sp. MOST-1 for biocontrol use. The potential of the bioformulation as an effective product for biocontrol of Rhizoctonia damping off diseases of Chinese cabbage was also confirmed in this study. However, in order to declare it commercial suitable, larger scale or field application should be further evaluated

Keywords : antagonistic Streptomyces, bioformulation, biocontrol, *Brassica chinensis*

[1] Faculty of Science and Technology, Phranakhon Si Ayutthaya Rajabhat University, Phranakhon Si Ayutthaya, Thailand

Introduction

Rhizoctonia solani is an important soil-borne fungal pathogen of many horticultural plants including cabbage plants (brassica plants) (Shiraishi et al., 2003; Chung et al., 2005). Damping-off of seedlings is the most common disease caused by *R. solani* (Moussa, 2002). Application of chemical fungicides is the most preferable and effective method to control *R. solani* (Grissbuhler et al., 1982; Minuto et al.,2006). However, throughout the past decade, concerns on the side effects of chemical fungicides have been rising dramatically. Today, it is widely recognized that the intensive use of chemical fungicides has not only created problems of fungicide resistance and increased contamination of environment, but may also have adverse high toxicity on microbial communities and a degradative effect on the ozone layer. In addition, chemical controls are not completely effective, and *Rhizoctonia* disease remains a persistent problem (Gees and Coffe, 1989; Huang et al., 2011). Therefore, it is necessary to search other environmentally safe products as alternatives to chemical fungicides for management of damping off disease caused by *R. solani*.

Biological control, by means of using antagonistic microorganisms in fighting plant pathogens, is now widely recognized as a safer and more sustainable alternative to the chemical based strategy. During the past decades, various groups of microorganisms including bacteria, yeasts and filamentous fungi have been screened for antagonistic activities against certain plant pathogens and numerous effective antagonists have been reported (Siripornvisal, 2010). Actinomycetes have long been recognized as sources for several antimicrobial metabolites (Goodfellow and Simpson, 1987; Goudjal et al., 2014) and some of them (mainly *Streptomyces* spp.) have been reported as potential biocontrol agents (BCA) against fungal pathogens of plants (Goudjal et al., 2014; Toumatia et al., 2015; Sabaratnam and Traquir, 2002). *Streptomyces* sp. MOST-1, an antagonistic actinomycete previously isolated from the rhizosphere of field-grown tomato (*Lycopersicon esculentum* Mill.), has been tested to suppress damping-off of tomato transplants caused by *Rhizoctonia solani* under controlled environmental conditions. In these experiments, damping-off was controlled by mixing the talc based formulation containing vegetative propagules of *Streptomyces* sp. MOST-1 with potting media prior sowing.

Materials and Methods

Strains and Inoculum Preparation

Streptomyces sp. MOST-1, was previously isolated from the rhizosphere of field-grown tomato (*Lycopersicon esculentum* Mill.), and has been tested in our laboratory to aggressively suppress *Rhizoctonia solani* under controlled environmental conditions.

R. solani was isolated from tomato fields in Nakhon Pathom province, Thailand. The fungus was maintained on PDA slant at 4°C. In order to prepare fresh inoculum for infestation of potting media, agar plugs (0.6 cm in diameter) containing mycelial mats of *R. solani* were picked from 3-day-old cultures grown on PDA plate. Glass bottles containing granulated maize-sand medium were steam sterilized at 140 kPa for 45 min, and then inoculated with mycelial discs (1 cm

diameter). The bottles were incubated for 15 days at 28 ºC.

Preparation of *Streptomyces* sp. MOST-1 Formulation

Streptomyces sp. MOST-1 was formulated as a wettable powder by mixing propagules with talcum powder and Kaolin carrier. A hundred grams of Kaolin was mixed thoroughly with 100 ml suspension (5×10^9 cfu/ml) of the *Streptomycete* propagules and 900 g of talcum powder in a homogenizer and sieved through a 0.2-mm nylon screen to obtain particles of a uniform size. The powder formulation was weighed and stored at 4°C and 24°C in the dark.

Stability of *Streptomyces* sp. MOST-1 Formulation during Storage

Viable propagules of *Streptomyces* sp. MOST1 was obtained by growing the bacterium in YMG broth supplemented with 0.1% (w/v) casamino acids.

The shelf life stability of *Streptomyces* sp. MOST-1 formulation stored under cold (4°C) and room temperature (25-35 °C) were determined at 1-month intervals over a 9-month period by dilution plate count on YMGA. Approximately 1g of the formulation was suspended and serially diluted in 10 mM phosphate buffer. Aliquot 0.1 ml of each dilution was spread separately on an YMGA plate. The analysis was done in three replicates. The dilution plates were incubated for 3–5 days at 25°C. The number of visible colonies was counted and the number of cfu/g formulation was calculated.

To evaluate the life time efficacy of the formulation during storage, a timely *in vitro* efficacy testing was done at 1-month intervals over a 9-month period. The efficacy was measured as the percentage of *Streptomyces* colonies capable of reducing the in *vitro* mycelial growth of *R. solani* by 5 mm at least. Approximately 1g of the formulation was serial diluted and plated onto YMGA. After incubation at 25°C until *Streptomyces* colonies appeared. Mycelial plugs (6 mm in diameter) cut from the margin of 5-day-old colonies of *R. solani* were placed 2 cm away from widely separated *Streptomyces* colonies on the dilution plates containing no more than six colonies per plate. Twenty replicate plates were used. Controls were the dilution plates of heat-sterilized formulations. Assay plates were incubated at 25°C until the fungal colonies had overgrown samples of heat-killed formulation on the control plates. At each storage time, the number of *Streptomyces* colonies that inhibited the mycelial growth of *R. solani* was counted and expressed as the percentage of the viable count or cfu.

Suppression of *Rhizoctonia* Damping-off by *Streptomyces* sp. MOST-1 Formulation

Suppression of *Rhizoctonia* damping-off in Chinese cabbage plug transplants by the formulations of *Streptomyces* sp. MOST-1 was investigated under growth chamber conditions. For the experiment, the formulation was applied to peat based potting substrate (previously infested with *R. solani*) at the rate of 0, 2.5, 5, 10, 20 g per 1 kg of potting media. Seedlings were raised on peat-based potting substrate, in plastic trays. In each experiment, treatments and controls with 3 replicates (20 seedlings /replicate) were randomly placed in a growth chamber (set at 80% relative humidity, 25°C and the photoperiod 14 h day and 10 h night) and grown for 14 days. Seedlings were watered

daily. The number of seedlings showing damping-off was recorded and the percentage damping-off was calculated.

Results and Discussion

Stability of the formulation was tested over a 9 month period, at 4 °C and at room temperature (25-35 °C). It was found that the formulation showed high stability since it retained 100 % survivability of the antagonist after 6 months storage and up to 80 % after 9 months (Fig. 1).

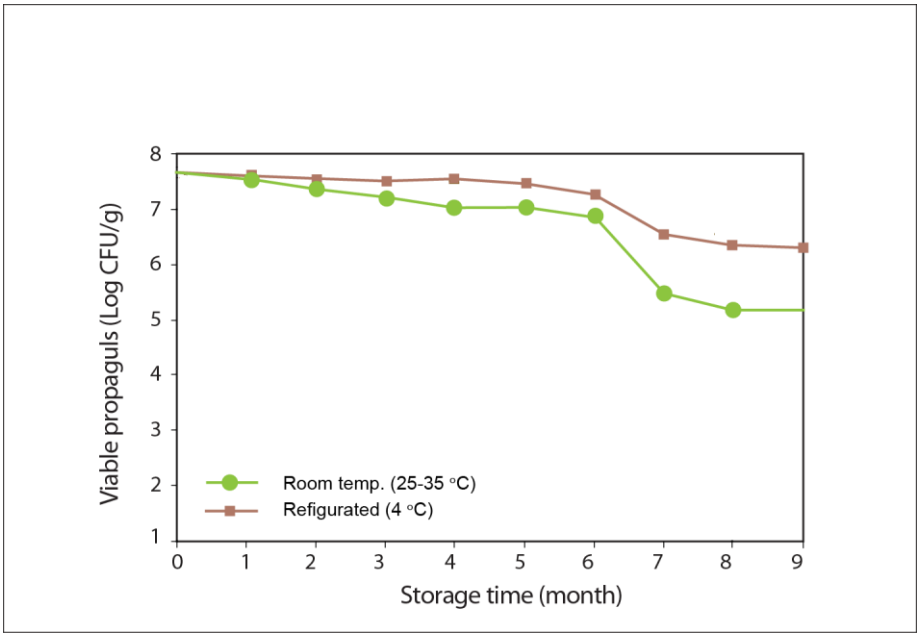

Fig. 1 Shelf life stability of the formulation stored at 4 °C and at room temperature (25-35 °C) over a 9 month period.

The formulation was then experimentally applied to control *Rhizoctonia* damping off diseases of *Brassica chinensis* under environmental controlled chamber condition. The results revealed that the formulation effectively control the diseases since it provided up to 100 % suppression when it was applied at the rate of 10 g per 1 kg of potting material. In addition, it was found that application of the formulation led to the promotion of plant growth (Table 1). These results indicated appropriateness talc as a bulk carrier for bioformulation of *Streptomyces* sp. MOST-1 for biocontrol use. The potential of the bioformulation as an effective product for biocontrol of *Rhizoctonia* damping off diseases of Chinese cabbage was also confirmed in this study.

Conclusion

These results indicated the potential of the formulation as an effective product for biocontrol of Rhizoctonia damping off diseases of Chinese cabbage and possibly other horticultural plants which affected by *Rhizoctonia solani*. However, in order to declare it commercial suitable, larger scale or field application should be further evaluated.

Table 1 Efficacy and effectiveness of the bioformulation in terms of disease reduction and plant growth promotion determined at 14 days after sowing

Rate of application (g/kg)	Disease Incidence (%)	Dry weight (g)	Fresh weight (g)
0.0 (Control)	88.25	0.59 ±0.051[a]	1.64 ±0.27[a]
2.5	60.00	0.84 ±0.072[b]	1.98 ±0.31[c]
5.0	42.75	0.86 ±0.051[b]	1.77 ±0.15[b]
10.0	N	0.86 ±0.051[b]	1.87 ±0.16[b]
20.0	N	0.82 ±0.042[b]	1.95 ±0.23[c]

** Data of the same column marked with different letters are significantly different (Fisher's LSD, P<0.05)*
N= Not observable

Acknowledgements

This work was supported by the National Research Council of Thailand (NRCT) and Phranakhon Si Ayutthaya Rajabhat University.

Referrences

Chung, W.C., J.W. Huang, and H.C. Huang. 2005. Formulation of a soil biofungicide for control of damping-off of Chinese cabbage (*Brassica chinensis*) caused by Rhizoctonia solani. Biological Control. 32(2), 287-294.

Gees, R. and M.D. Coffe, 1989 Evaluation of a strain of Myrothecium roridum as a potential biocontrol agent against Phytophthora cinnamomi. Phytopathology. 79(10), 1079-1084.

Goodfellow, M. and K.E. Simpson. 1987. Ecology of streptomycetes. Front Appl Microbiol. 2, 97-125.

Goudjal, Y., O. Toumatia, A. Yekkour, N. Sabaou, F. Mathieu, and A. Zitouni. 2014. Biocontrol of *Rhizoctonia solani* damping-off and promotion of tomato plant growth by endophytic actinomycetes isolated from native plants of Algerian Sahara. Microbiol. Res. 169(1), 59–65.

Grissbuhler, H., P. Brenneisen and H.P. Fisher. 1982. Frontiers in crop production: chemical research objectives. Science. 217, 505–510.

Huang, X., N. Zhang, X. Yong, X. Yang, and Q. Shen. 2012. Biocontrol of *Rhizoctonia solani* damping-off disease in cucumber with *Bacillus pumilus* SQR-N43. Microbiol. Res. 167(3), 135–143.

Minuto, A., D. Spadaro, A. Garibaldi, and M.L. Gullino. 2006. Control of soilborne pathogens of tomato using a commercial formulation of *Streptomyces griseoviridis* and solarization. Crop Protection. 25(5), 468–475.

Moussa, T.A.A. 2002. Studies on biological control of sugar beet pathogen *Rhizoctonia solani* Kühn. Online Journal of Biological Science.2(12), 800–804.

Sabaratnam, S. and J.A. Traquair. 2002. Formulation of a *Streptomyces* biocontrol agent for the suppression of *Rhizoctonia* damping-off in tomato transplants. Biol. Control. 23(3), 245-253.

Shiraishi, H., Y. Enamia and S. Okanoa. 2003. *Folsomia hidakana* (Collembola) prevents damping-off disease in cabbage and Chinese cabbage by *Rhizoctonia solani*. Pedobiologia. 47(1), 33–38.

Siripornvisal, S. 2010. Biocontrol Efficacy of *Bacillus subtilis* BCB3-19 against tomato gray mold. KMITL Sci. Tech. J. 10(2) 37-44.

Toumatia, O., A. Yekkour, Y. Goudjal, A. Riba, Y. Coppel, F. Mathieu, N. Sabaou, and A. Zitouni. 2015. Antifungal properties of an actinomycin D-producing strain, *Streptomyces* sp. IA1, isolated from a Saharan soil. J. Basic Microbiol. 55(2), 221–228.