CHAPTER 5

CONCLUSION

Biological control with bacterial antagonists offers an alternative strategy for suppressing rice sheath blight disease. The success of biological agents in disease control depends largely on suitable formulations and methods of application. Recently, bacterial antagonists have been formulated in a variety of forms such as granules, liquid, powder and pellets (Kanjanamaneesathian *et al*., 1998; Arunyanart *et al*., 2001; Kusonwiriyawong *et al*., 1999; Wiwattanapatapee *et al*., 2004). In this study, *B. megaterium* granule and tablet forms for either spray or broadcast application were prepared and evaluated for their physical and biological properties. The selected formulations were finally tested for their efficacy under greenhouse conditions.

Like other *Bacillus* spp., *B. megaterium* offer heat and desiccation-resistant spore that can be formulated in to stable and dry products. Therefore, it was essential to determine the optimal culturing time in order to prepare the bacterial suspension containing high number of viable bacteria endospores. The optimal time for *B. megaterium* cultivation in PDB at 37$\pm$1 °C was 72 h.

Five granule formulations (formulation GS-Alg, GS-HPMC, GS-SCMC, GS-MC and GS-HEC) composed of bacterial suspension, lactose and one of these following binders: Alg, HPMC 4000, SCMC 1500, MC 4000 or HEC 4000 were prepared for spray application. After biological and physical properties evaluation, formulation GS-Alg and formulation GS-SCMC were selected for testing under greenhouse conditions because the disintegration time to prepare 1% w/w solution of granules in water was shortest among various formulations (less than 10 min).

Three tablet formulations (formulation TS-5, TS-10 and TS-15) composed of bacterial suspension, lactose, SCMC 1500, sodium starch glycolate, talcum and magnesium stearate were prepared for spray application. The viable bacteria in all formulations remained high at approximately 10^6 CFU/g after production process. Formulation TS-15 which contained the highest proportion of SCMC 1500 (15% w/w) was selected for testing under greenhouse conditions because the viscosity of tablet solution in water was highest among different formulations. The viscosity value of 10% w/w solution of formulation TS-15 in water was 120.47$\pm$3.01 cps.

Three granule formulations (formulation GB-19, GB-29 and GB-39) composed of bacterial suspension, lactose, Alg and HVO were prepared for broadcast application. Formulation GB-19 which contained the lowest proportion of HVO was selected for testing under greenhouse conditions because the density was less than 1 g/ml and this formulation was composed of HVO only 19% w/w which would have less effect on the oxygen exchange at the air and water interface after application compared to other formulations.

Four tablet formulations (formulation TB-19, TB-29, TB-39 and TB-49) composed of lactose, Alg and HVO were prepared for broadcast application. Formulation TB-39 was selected for testing under greenhouse conditions because this formulation was the best in term of floating property. In addition, the bacterial release was quite quick and almost complete within 1 h.

In the efficacy testing of the selected formulations under greenhouse conditions, treating rice plants with formulation GS-SCMC containing *B. megaterium* (granules for spray application) had the lowest % infection (53.3%). When sprayed, the bacteria in the formulation might be deposit on various rice plant tissues (such

as leaf sheath and leaf blade) and the bacteria might reduce infection and deter disease spread both horizontally and vertically.

The bacterial antagonists are living organism. Therefore, the survival of bacteria in formulations is very important. After the formulations were stored at room temperature (26 - 30°C) for 3 and 6 months, the number of viable bacteria in selected formulations remained high (10^6 CFU/g for spray application formulations and 10^8 CFU/g for broadcast application formulations) and the formulations had a capacity to inhibit *R. solani* mycelial growth effectively.

In conclusion, granule formulation containing *B. megaterium* for spray application (GS-Alg and GS-SCMC) were the suitable form for use to suppress sheath blight disease. This form could be easily produced and the cost of the formulation was not expensive. The cost of the formulation GS-Alg and GS-SCMC was 434.0 and 377.4 Baht per kg, respectively. However, the disintegration time to prepare 1% w/w solution of granule formulation for spray application should be improved. Formulations for broadcast application should contain low amount of HVO. The minimal amount of HVO should be enough for giving floatation but not affect the exchange of oxygen at air and water interface. Other substances which had low density property should be considered to substitute HVO. The viability of bacteria in the formulations should be monitored for 2 years because the 2 years duration is a reasonable period for marketing the products. The actual mechanism of *B. megaterium* in control rice sheath blight disease should be also investigated.