CHAPTER 4

DISCUSSION

1. Enumeration of viable bacteria in the formulations

Five granule formulations prepared for spray application contained viable bacteria about 10^7 CFU/g (Table 6). The loss in number of viable bacteria after granule production was not detected. The result agree with another study, bacterial suspension (storage at 2-8°C for 2 days) containing viable bacteria about 10^{10} CFU/ml was used as an active ingredient in two granule formulations (30 ml in 100 g of each formulation). The formulations contained viable bacteria about 10^9 CFU/g.

All finished tablet formulations prepared for spray application contained viable bacteria about 10^6 CFU/g (Table 7). The result agrees with a previous study by Phiharn and Markbumrung (2002) who reported that the number of viable bacteria in enteric coated *Lactobacillus acidophilus* tablets decreased from 9.26×10^9 CFU/g to 1.20×10^7 CFU/g after compression. In another study, Wongwiwat and Nhumard (2003) also reported that the number of viable bacteria in *B. megaterium* fast released tablets greatly declined after compression. The bacteria were mixed with either skim milk or lactose and dried by lyophilization. The skim milk powder contained the viable bacteria 4.6×10^8 CFU/g and the lactose powder contained the viable bacteria 1.9×10^8 CFU/g. After compression, the skim milk tablets contained the viable bacteria 2.54×10^7 CFU/g and the lactose tablets contained the viable bacteria 4.71×10^6 CFU/g. The reduction of viable bacteria in these finished tablet formulations may be the result from the compression force and the heat occurred during the compression of granules to form tablets, so that these effects on the number of viable bacteria in the tablet formulations should be investigated.

2. Evaluation of physical properties of *B. megaterium* formulations

2.1 Evaluation of physical properties of bacterial granules for spray application (GS)

The deposition site of the particle size 3 - 5 μm was lower airway (Thompson, 1992). The granules which have particle size more than 1 mm are considered safe from getting into the lower airway of human by inhalation (Table 8). These products can be measured easily or weighed out, in contrast to dusts (Paau, 1998). All formulations dissolved well at room temperature except formulation GS-MC (Table 9). This might be because of methylcellulose, one of the ingredients in the formulation GS-MC, which has better solubility in cold water (2-8°C) (Wade and Weller, 1994). The pH value of all formulations was in the range of 4.97 – 6.97 (Table 10). This pH would not affect the growth of the plants that can grow at pH 4-9 (Brady and Weil, 1996) and bacteria as *Bacillus* spp. can grow at neutral pH (Boer and Diderichsen, 1991).

Formulation GS-Alg and GS-SCMC were selected for testing under greenhouse conditions because the disintegration time to prepare 1% w/w solution of granules in water was much less than those of other formulations.

2.2 Evaluation of physical properties of bacterial tablets for spray application (TS)

All tablets met the requirement for weight variation of USP27 (Table 12). The advantage of less friable tablets was the less damage that might be occurred during production, transportation and storage. However, the tablets with high hardness value could affect the disintegration of those tablets when applied to aquatic environment.

In practical term, the disintegration time is quite long (Table 13). To solve this problem, it is possible to shorten the time required to dissolve the tablets by immerging the tablets in the water. The preliminary experiment with tablet formulation TS-15 revealed that the tablets would be totally dissolved by immerging these tablets in the water for 30 min and stirring for 10 min afterward. The neutral pH of the tablet solution presented in Table 14 had no effect on plant growth and bacteria survival (Boer and Diderichsen, 1991). The degree of viscosity is varied dependent upon the proportion of SCMC 1500 in the tablets. Formulation TS-5, TS-10 and TS-15 were composed of SCMC 1500 5% w/w, 10% w/w and 15% w/w, respectively (Table 15).

Formulation TS-15 was selected for testing the efficacy under greenhouse conditions because the viscosity of tablet solution in water (5% w/w and 10% w/w solution) was quite high compared to other formulations. The high viscosity solution in the formulation was suitable for spray application because it can adhere on plant surface. The survival of bacteria in all tablet formulations after production was quite high in which approximately 10^6 CFU/g of viable bacteria were counted.

2.3 Evaluation of physical properties of bacterial granules for broadcast application (GB)

The density of granules was less than 1 g/ml. Thus, the granules could float on the water surface. HVO was an excellent floating material in granules because of their hydrophobicity and low density (Wade and Weller, 1994). In previous study, the granule formulations composed of 49% w/w HVO showed good floatability (Kanjanamaneesathian *et al*., 1998).

In this study, the formulation GB-19, GB-29 and GB-39 composed of HVO 19% w/w, 29% w/w and 39% w/w, respectively. Viability of bacteria in all formulations remained high at approximately 10^8 CFU/g after production and the

density of formulations were less than 1 g/ml. Formulation GB-19 was selected for testing the efficacy under greenhouse conditions because it composed of HVO only 19% w/w. HVO is a mixture of triglycerides of fatty acids (Wade and Weller, 1994). A large amount of HVO in the formulation might have a capacity to trap the bacteria in the formulation.

2.4 Evaluation of physical properties of bacterial tablets for broadcast application (TB)

The average weights of all tablets met the standard requirement of USP27 (Table 18). The floating ability of tablets was contributed to the amount of HVO in the formulation (Figure 7). Formulation TB-19, TB-29, TB-39 and TB-49 were composed of HVO 19% w/w, 29% w/w, 39% w/w and 49% w/w, respectively. It was found that the formulation composing of high proportion of HVO such as formulation TB-49 had high floating ability. However, some of the bacteria might be trapped in the formulation due to the lipophilicity of HVO.

Formulation TB-39 was selected for testing the efficacy under greenhouse conditions because the percentage of floating tablets was more than 50% and the percentage of bacterial release was more than 99% at 60 min after the formulation was dispersed. On the other hand, formulation TB-19 and TB-29 sank into water at 20 and 35 min, respectively. Formulation TB-49 remained on the water surface up to 5 h after the formulation was dispersed to water but it released the bacteria about 87% at 60 min after the formulation was applied. Some of the bacteria might be trapped in this formulation. The survival of bacteria in all formulations remained high at approximately 10^8 CFU/g after production.

3. Scanning electron microscope (SEM) observation of the selected formulations and bacterial endospores on surface of rice tissues

From the texture of the leaf sheath (Figure 10), the bacterial endospores might not stick on the leaf sheath very well. The sticker in the formulation might increase the adhesion ability and also the number of bacterial endospores on the leaf sheath surface. In contrast, the surface of the leaf blade (Figure 10) composed of a large number of hairs, so the bacterial endospores might be easily trapped between these hairs on the surface.

4. Testing the efficacy of selected granule and tablet formulations containing *B. megaterium* under greenhouse conditions

Rice sheath blight disease is usually most severe during the tillering stage and the disease spread (both horizontally and vertically) occurs very rapidly when the rice canopy is thick and relative humidity is very high (Reissig *et al*., 1986). In this study, the relative humidity was not high enough to create environment suitable for disease development and thus affect the efficacy testing. From the results (Table 19), the bacterial granules for spray application (GS) were more effective than others. When sprayed, the bacteria in the granules might be deposited on various rice plant tissues (such as leaf sheath and leaf blade), and these bacteria might reduce infection and deter disease spread both horizontally and vertically.

When broadcasted, the HVO in the broadcast formulations would be fast released and it covered the water surface. This incidence might reduce the concentration of oxygen in the water because oxygen exchange between air and water interface had been reduced substantially. On the other hand, HVO in the floating pellet formulation had no such effect on the oxygen exchange between air and water interface because it was not readily released when applied (Wiwattanapatapee *et al*., 2004). This slow-released characteristic of the pellet

formulation made it more attractive to use for application to control sheath blight disease. In another study, Pengnoo *et al.* (2000) showed that *B. megaterium* slow-released granules containing HVO 49% w/w for broadcast application performed as well as freshly prepared bacterial antagonists in suppression sheath blight disease in the greenhouse test.

5. Evaluation of *B. megaterium* adhesion on surface of rice tissues

The adhesion on the plant surface of formulations was better than spore suspension (Table 20 and 21). This might be the result from the ingredient such as a gelling agent or a sticker in the formulations. The number of viable bacteria on the leaf sheath was greater than that of the viable bacteria on the leaf blade (Table 20 and 21).

6. Testing the inhibition of *R. solani* mycelial growth

All formulations showed satisfactory effectiveness in suppressing mycelial growth of *R. solani* using dual culture technique after the formulations were stored for 3 and 6 months (Table 22). The antimicrobial activity might be the result from the antibiotic substance that the bacteria produced. The supernatant from *B. megaterium* cultures contained heat stable antibiotic substances and inhibited mycelial growth of *R. solani* (Pengnoo *et al.*, 2000). *B.subtilis* was also report to produce Iturin A that suppress rice sheath blight disease (Pusey, 1989).

7. Viability of bacteria in the selected formulations at room temperature

In general, the number of viable bacteria in the formulation GS-Alg and GS-SCMC had declined noticeably 1 month after production. But the number of viable bacteria remained more or less the same afterward until 6 months. The initial reduction of the number of viable bacteria may be attributed to the dead of vegetative cells of the bacteria in the formulations. The number of viable bacteria in the other formulations also remained high after the formulations were produced. The

stability of the formulation regarding the number of viable bacteria 6 months after production could be attributed to the survival capacity of endospores of *B. megaterium* in the formulations. Wiwattanapatapee *et al.* (2004) also reported that the number of viable bacteria in the *B. megaterium* pellets remained high after the formulations were produced by using the bacterial endospores.