CHAPTER 4

CONCLUSION

The study on inhibitory activity of *Bacillus subtilis* BCC 6327 metabolites against growth of aflatoxigenic fungi isolated from bird chili powder can be concluded as following:

1. *Aspergillus flavus* was mostly found aflatoxigenic fungi that were isolated from the contaminated bird chili powder sample. Analysis of 18s rRNA gene sequence showed that it was closely related to *A. flavus* ATTC 11489, *A. flavus* ATTC 9643, and *A. flavus* ATTC 11497. The amount of aflatoxin B_1 was found to be 27.6 µg/L in the culture medium of the isolated *A. flavus*.

2. On potato dextrose agar medium, isolated aflatoxigenic fungus (*A. flavus*) was yellow-green mycelia colonies forming sandy beige after 4 days of incubation. The morphological characterization by microscope (40X) showed the conidia which originate from a basal cell located on the hyphae and terminate in a vesicle like gloves vesicles.

3. The amount of aflatoxin B_1 in 3 bird chili powder samples by MicroELISA assay was shown to be 7.6, 4.4, 31.0 µg/Kg from sample 1, 2 and 3 respectively. The amount of aflatoxin B_1 in each sample was correlated with the frequency of occurrence of *A. flavus* that isolated from these bird chili powder.

4. The antagonistic activity of antagonist *B. subtilis* against aflatoxigenic *A. flavus* was tested by dual culture both in broth medium and on agar plate,

exhibiting inhibitory effect on *A. flavus* growth. *B. subtilis* inhibitory properties are considered the most efficient to antagonistic activity.

5. The extracellular metabolites in cell free supernatant of *B. subtilis* showed a strong inhibitory effect on the hyphal grew of *A. flavus*, inducing the decrease of fungal development. The morphology of *A. flavus* that growth on cell free supernatant slide culture, had no conidiospore of *A. flavus* and fungal mycelia appeared less dense.

6. On plate screening hydrolytic enzymes, *B. subtilis* BCC 6327 produced protease, chitinase and cellulase whereas amylase and lipase were not obviously produced. But, the cellulase activity assay showed negligible activity.

7. Protease production of *B. subtilis* started in the early stationary phase (24 h) of growth and gradually increased during 24 to 32 h of growth. The maximum protease specific activity (1.449 U/mg protein) was achieved at 32 h of growth and rapidly decreased after 32 h of growth at stationary phase.

8. Chitinase production of *B. subtilis* closely associated with the growth patterns. Chitinase production began in the logarithm phase of growth (24 h) and rapidly increased at 24 h up to 36 h of growth in the logarithm phase and stationary phase. The maximum specific chitinase activity of 0.1218 U/mg protein was achieved at 36 h of incubation. After 36 h, enzyme production rapidly decline that was associated with the decreased cell number of growth during death phase.

9. Nutrient broth culture media of *B. subtilis* showed antifungal activity. Moreover, the stationary phase of all cell free culture media possessed a significantly higher antifungal potential than the exponential culture filtrate. The increased antifungal activity by the stationary culture filtrate of *B. subtilis* was possibly a

consequence of the production of extracellular secondary antifungal compound. In this way, the antifungal potential of *B. subtilis* culture filtrate related to the increased production of hydrolytic enzymes, particularly protease and chitinase.

10. Production of extracellular β-1, 3-glucanase, chitinase and protease increased significantly when *B. subtilis* were grown on media supplemented with dried mycelia of *A. flavus*. The hydrolytic enzymes produced by *B. subtilis* play an important role in destruction main structural cell wall components of *A. flavus* (Chitin, β -1, 3-glucan and protein).

11. More sugar released (total reducing sugars, glucose, and N-acetylglucosamine) from *B. subtilis* grown in media supplemented with dried mycelia suggested that this material can act as an inducer of lytic enzymes synthesis. *B. subtilis* had the potential to produce cell wall degrading enzymes when chitin or *A. flavus* cell wall material was present in the growth medium.

12. The *B. subtilis* cell free supernatant was decreased the growth of *A. flavus* when supplemented onto dried bird chili powder.