CHAPTER V

CONCLUSION

A bacterium, TU05, was isolated from soil in Pathumwan, Bangkok, Thailand. TU05 was identified and classified as *Microbacterium* sp.. *Microbacterium* sp. TU05 showed the ability to produced chitinase when grown in the presence of colloidal chitin and flake chitin for 2 days and 10 days, respectively. These results suggested that both CC and FC can induce chitinase production of *Microbacterium* sp. TU05, but it can use colloidal chitin easier than flake chitin and grow faster. Then we observed high enzymatic activity in less incubation time.

When crude enzyme was characterized, the optimum pH, the enzyme was observed chitinase activity in broad pH between 4 to 8 with the highest chitinase activity at pH 5.0, 50.12 mU/ml and pH 7.0, 42.16 mU/ml. For optimum temperature, chitinase activity was observed in range 30 to 50 °C with the optimum temperature of crude chitinase at 40 °C, 52.451 mU/ml, similar to chitinase from *Aeromonas hydrophilia* H-2330. From substrate specificity of crude enzyme, we conclude that chitinase from *Microbacterium* sp. TU05 can hydrolyze amorphous chitin better than crystalline chitin. In the group of amorphous chitin, chitinase from *Microbacterium* sp. TU05 prefers substrate that contained high non-reducing end, suggesting that crude enzyme from *Microbacterium* sp. TU05 contained high exochitinase activity. There may be some chitosanase activity from crude enzyme of *Microbacterium* sp. TU05. Chitinolytic activity of crude enzyme was showed at least 2 bands with molecular weight 65 and 30 kDa on 10% SDS-PAGE, suggest that has at least two species of chitinolytic enzyme. When hydrolysis products were analyzed by HPLC, a mixture of products, monomer (*N*-acetylglucosamine) and dimer (*N*, *N*'-diacetylglucosamine) were obtained, suggesting that crude enzyme contained chitinase and chitobiase activity.

Partially purified chitinase peak1 was found one activity band at 65 kDa, while 2 activity bands at 55 kDa and 30 kDa was found in partially purified chitinase peak2. The partially purified chitinase peak1 showed the highest activity at pH 5.0 and 40 °C. From substrate specificity of partially purified chitinase peak1 suggested that the partially purified chitinase peak1 was mainly exochitinase and has no chitosanase activity.

We did not find any positive clones on selective media from shotgun cloning. This might be caused by many sites of *Pst*I inside chitinase gene of *Microbacterium sp.* TU 05 or chitinase gene was not trancribed, translated, and processed to active chitinase in the *E. coli* JM109 or chitinase was produced inside cell but can not export into the medium. We use primers, designed from conserved amino acid sequence of *Bacillus* sp. family 18 chitinase gene, to amplified partial chitinase gene. The PCR product, 636 bp, was found and showed highest similarity to chitinase from *Arthrobacter* sp. (71%), which differs from *Microbacterium* sp. in the family level. *Arthrobacter* sp. is classified in family Micrococcaceae, but *Microbacterium* sp. is classified in family Microbacteriaceae. We conclude that *Microbacterium sp.* TU05 produced extracellular family 18 chitinase.

For the future work, we will use the partially chitinase gene as a probe for detect chitinase gene from *Microbacterium* sp. TU05 DNA library.