CHAPTER V

CONCLUSION

Bacillus circulans PP8, isolated from soil, showed the ability to produce chitinase and chitosanase when grew on colloidal chitin and powdered chitin minimum medium. *Bacillus circulans* PP8 produced chitinase at 12 hours (ChiA) and 24 hours (ChiB) and then slowly dropped and vanished after 48 hours where as chitosanase activity was detected at 60 hours when cultured on colloidal chitin minimum medium. On powdered chitin minimum medium, chitinase activities were detected at the same time as on colloidal chitin minimum medium, but chitosanase activities was detected earlier, at 24 hours.

Crude chitinases were characterized. ChiA has optimum pH and temperature at pH 7 and 50°C respectively. ChiB has optimum pH and temperature at pH 6 and 30°C. Crude chitosanase was characterized. The optimum pH and temperature are pH 7 and 60°C.

SDS-PAGE and activities staining of crude enzymes show at least three types of chitinases. The estimate molecular weights of the major chitinase species were 200, 55, and 42 kDa.

*Pst*I digested DNA fragment between 2 to 9 kb was cloned into *E. coli* DH5αF' using pBluescript/SK$^-$ as a vector. Two transformants from 1,800 colonies showed

positive result, clear zone around the colony. Recombinant plasmid was extracted and retransformed into other hosts, which all showed clear zones demonstrating that the DNA fragments contain genes that can hydrolyze chitin.

After cut with *Pst*I for analyzing the insert sizes, clone 847 and 1691 presented approximately 6 and 7 kb insert fragments, respectively.

SDS-PAGE and activities staining of crude enzyme of two recombinant clones show at least one type of chitinase. The molecular weight of chitinase from clone 847 is approximately 55 kDa, which is the same as chitinase in *Bacillus circulans* PP8. However the activity from clone 1691 was very low and was not analyzed for molecular weight.

Chitinase activities of two recombinant clones were also detected by using colorimetric method. Both of two clones had activities with colloidal and powdered chitin. Clone 847 had chitinase activity with colloidal chitin as same as with powdered chitin, while clone 1691 had chitinase activity with powdered chitin higher than colloidal chitin.

Further studies

Three experiments should be done. Firstly, two recombinant encoding chitinase genes should be sequenced and mapped to determine the position of chitinase genes and

study further for overproducing chitinase. Secondly, we should clone and characterize chitosanase because it has high activity and very stable. Thirdly, chitin deacetylase detection is necessary to ensure that *Bacillus circulants* PP8 produce this enzyme and use the pathway of enzyme production as predicted above. Moreover, chitin deacetylase from this bateria should be cloned and characterized.