CHAPTER V

CONCLUSION

Burkholderia cepacia, isolated from soil, showed the ability to produce chitinase when grown in the presence of either colloidal chitin or flake chitin, suggesting its ability to be induced by either substrates. Highest chitinase activity was detected on the second day of cultivation.

Crude chitinase was characterized. The optimum temperature and pH of the crude enzyme is 40°C and 7.0, respectively. To determine chitinase substrate specificity, crude chitinase from *Burkholderia cepacia* was used to digest different substrates. Chitinase activity depended on the structure and biochemical characteristics as well as the solubility of the substrate. We demonstrated that; 1) chitinase from *Burkholderia cepacia* prefers substrate with low crystallinity, 2) substituted group of modified chitin may affect its chitinase activity, 3) no chitosanase activity was detected from cultured medium of *Burkholderia cepacia*.

SDS-PAGE and activity staining of the crude enzyme, showed at least one type of chitinase produced from *Burkholderia cepacia* and secreted into the culture medium. The estimated molecular weight of the major chitinase species was 47.5 kDa. Chitinase from *Burkholderia cepacia* could also be partially purified approximately 27 folds with a 4% yield and specific activity of 120 ($\Delta\%T_{650}$/min/µg protein) in fraction 20 containing highest chitinase activity (see results in Appendix A).

Southern blot analysis was used to determined the DNA fragments, which contains chitinase gene. A chitinase family 18 specific probe , CB1 was used. A 7.0 kb band of *Eco*R I digested chromosomal DNA, 7.8 kb and 10 kb bands of *Bam*H I digested chromosomal DNA, an approximately 23 kb band of *Hin*d III digested chromosomal DNA and 1.8 kb and 1.2 kb bands of *Pst* I digested chromosomal DNA were able to hybridize to the DNA probe.

*Eco*R I digested DNA fragments between 6 to 9 kb and *Bam*H I digested DNA fragments between 6 to 12 kb were cloned into *E.coli* DH5α F' using pBluescript/KS$^+$ and screened for chitinase gene. Eighteen recombinant plasmids gave positive results on dot blot analysis. pKK243B, provided a positive result on Southern blot analysis showing a 7.8 kb band and 1.8 kb band when pKK243B was digested with *Bam*H I and *Pst* I respectively. *E.coli* containing pKK243B shows chitinase activity on phenotypic assay. Low chitinase activity, 6% of relative activity, was also observed from *E.coli* containing pKK1.8PP.

Sequencing of the 1.7 *Pst* I fragment revealed a partial open reading frame of 206 deduced amino acid similar to amino acid sequence of putative sensor proteins and a reading frame of 244 deduced amino acid sequence similar to amino acid sequence of putative 2-component transcriptional regulator. No open reading frame which have similarity to chitinase gene was perceived in pKK1.8PP.

However, both results from Southern blot analysis and phenotypic screening demonstrated that colonies harboring pKK243B contains chitinase gene. Chitinase activity was detected in the medium of bacterial culture containing pKK243B. From these results we reasoned that; 1) chitinase gene is located within the *Bam*H I 7.8 kb fragment, but does not bind to the CB1 probe or 2) this DNA fragment from *Burkholderia cepacia* can induce *E. coli* to produce chitinase like activity, but it does not contain chitinase gene. Recent discovery of a two component sensor-regulator system by Tsujibo *et al.* (1999) in *Streptomyces thermoviolaceus* OPC-520, suggested that our first hypothesis may be correct, since similar gene construct was also found in *Streptomyces thermoviolaceus* OPC-520.

Future studies

Two major experiments should be done. Firstly, the 2.3 kb *Pst* I fragment which is adjacent to the 1.7 kb *Pst* I fragment should be sequenced to determine whether or not there is a family 19 chitinase gene following the putative sensor gene and putative two-component transcriptional regulator gene similar to *Streptomyces thermoviolaceus* OPC-520. Secondly, we should clone and characterize other chitinases

present in *Burkholderia cepacia*, such as family 18 chitinase which hybridize with our probe. Further studies on regulation of chitinase gene in *Burkholderia cepacia* should also be performed to further understand the chitin metabolism in *Burkholderia cepacia*.

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