CHAPTER V

CONCLUSION

1. Thermotolerant bacterial strain BC1 was identified by morphological and biochemical properties followed by Bergey's manual of determinative bacteriology. It's morphological and biochemical properties suggested that strain BC1 was closely similar to *Brevibacillus brevis*. In contrast, 16S rRNA whole gene was amplified by using chromosomal DNA and single fresh colony as sources of DNA templates. Partial sequence of the gene was analyzed and compared with available 16S rRNA sequence of *Bacillus* species by using the EMBL-GenBank-DDBL database. The highest homology was found between 16S rRNA gene of thermotolerant bacterial strain BC1 and that of *Bacillus badius*. In addition, phylogenetic tree showed that strain BC1 gave the closest evolutionary distance values with *Bacillus badius* while *Brevibacillus brevis* was phylogenetically distinct from it. The obtained results indicated that strain BC1 was *Bacillus badius*. This conclusion is supported by *Tm* determination. The melting temperature of chromosomal DNA from strain BC1 was 50 ^{o}C in the same range with that from *Bacillus badius*. In addition, strain BC1 could grow in the medium containing NaCl up to 5% similar to that of *Bacillus badius*. This property is usually used to distinguish *Bacillus badius* from *Brevibacillus brevis*. Thus, the results confirm that thermotolerant bacterial strain BC1 belongs to *Bacillus badius*.

2. Eight peptide fragments of phenylalanine dehydrogenase obtained by the lysyl endopeptidase digestion were sequenced with as the sequence at N-terminus peptide to be as follow TSIIKDFTLFEKMSEHEQVVFANDPATGLR and internal peptide sequences: GMTYKXAASDVDFGGGK AVIIGDPQKDKSPELFRAFGQFVDSLGGRFYTGTDM GTNMEDFIHAMK, ATNK, DDLGGVTYAIQGLGKVGYKVAEGLLEEGAHLFVT, AIAGSANNQLLTEDHGRHLADK, ERVLAK, and WDIRN. This obtained amino acid sequences were then used for designing degenerated primers for phenylalanine dehydrogenase gene sequencing and cloning.

3. Full length phenylalanine dehydrogenase gene from *Bacillus badius* BC1 was sequenced by cassette-ligation mediated PCR and successfully cloned into *E. coli* JM109.

This gene has a large single open reading frame of 1140 nucleotides, which is capable of encoding a polypeptide of 380 amino acids and has GC content about 40%.

4. The percentage of identical nucleotide sequences of phenylalanine dehydrogenase from *Bacillus badius* BC1 compared with that of *Bacillus badius*, *Thermoactinomyces intermedius*, *Sporosarcina ureae* and *Bacillus sphaericus* was 96, 85, 83 and 81%, respectively.

5. The *E. coli* clones that showed phenylalanine dehydrogenase activity had total activity in the range of 0 to 360 units/100 ml culture. The highest total activity produced by the *E. coli* clone was about 60 times higher than that of *Bacillus badius* BC1.

6. The optimum time for induction phenylalanine dehydrogenase gene by IPTG was 120 minutes.

7. Stability of phenylalanine dehydrogenase gene from recombinant clones that showed high phenylalanine dehydrogenase activity was studied by (1) daily subculturing for 15 days. Each clone showed varied expression level of phenylalanine dehydrogenase gene. Five from six recombinant clones were still stable without host cell deletion process after subculturing for 15 times while inserted gene fragment of the other one was removed. (2) Retransformation into host cell *E. coli* JM109 by electroporation. Four retransformed plasmids, which represented each type of plasmid pattern, showed that allof them gave the same pattern with their original recombinant plasmids and phenylalanine dehydrogenase activities of all retransformants were still constantly remained.