CHAPTER V

CONCLUSION

Four rod-shaped of γ-aminobutyric acid (GABA) producing bacteria, two rod-shaped and three cocci in chains of starch hydrolyzing bacteria including two rod-shaped of proteinase producing bacteria were isolated from various sources in Thailand. On the basis of the phenotypic characteristics, *rpoA*, *pheS* and 16S rDNA sequencing, phylogenetic analysis, and DNA-DNA similarity, the GABA producing strains, LSF8-13 was identified as *Lactobacillus brevis*, FS73-1, SEA62-2 and SR11-2 were identified as *L. plantarum*. The rod-shaped starch hydrolyzing strains SB2-3 and U3-1 were identified as *L. plantarum.* The starch hydrolyzing cocci in chains, FP 15-1 and N2-1A were the novel species in *Enterococcus* and N12-9 was *E. gallinarum*. The weekly starch hydrolyzed strain FP15-1 isolated from fermented tea leaves is proposed as *Enterococcus camelliae* sp. nov. The proteinase producing strains, SMC1 and SCR1 were *E. faecalis* and *L. sakei* respectively. A high GABA producing lactobacilli L13 isolated from a traditional Japanese pickle (senmaizuke) was polyphasic studied. This strain is belonged to a new species in *Lactobacillus*

Novel glutamate decarboxylase (GAD) encoding gene (*gadB*) of the novel high GABA producing species *Lactobacillus* sp. L13 was successfully identified, cloned, and expressed. *gadB* genes of other GABA producing isolates LSF8-13, FS73-1, SEA62-2 and SR11-2 were identified. Nucleotide sequence analysis of the expressed L13 *gadB* revealed that the open reading frame (ORF) consisted of 1437 bases and encoded a protein of 479 amino acid residues while the unexpressed LSF8-13 *gadB* consisted of 1410 bp which revealed 470 amino acid residues. The deduced amino acid showed 82.2, 51.5, and 51.3 % identity to the predicted GAD of *L. brevis* ATCC 367, *L. plantarum* WCFS1 and that of the isolated LSF 8-13 *gadB*, respectively. Compared with the known GADs of *Lactococcus lactis* subsp. *lactis* and the well characterized GAD of *Escherichia coli*, the deduced amino acid also showed low identity at 52.0 % and 36.9-37.1%, respectively. The molecular weight of the purified L13 and LSF8-13 GADs were approximately 59 kDa. The recombinant GAD of L13 in *E.coli* JM109 showed GAD activity significantly depended on a present of PLP in enzyme reaction while that of LSF8-13 had no activity. Deduced amino acids and 3D structures were comparatively analyzed. PLP-binding lysine (K) is conserved

among all isolated GADs. By comparison of the 3D models and alignment of deduced amino acids, the residues phenylalanine (F) 120, glycine (G) 121, serine (S) 322 and 323 in the strain L13 seem to be important residues that make difference in 3D structure and may cause the high activity compared the other GADs. Predicted dimer structure model is being analyzed. These residues should be further studied by mutation of the expressed clone to check their abilities to produce GABA. Crystal structure of the purified should be analyzed. Addressed here, the newly identified *gadB* gene and its recombinant clone are very useful for further study and also high impact for industrial applications.