CHAPTER I

INTRODUCTION

Foods fermented with lactic acid bacteria (LAB) play and important role in human diet and in the production of foods throughout the world. Lactic acid produced by LAB is applied in food, pharmaceutical, cosmetic and industrial. The LAB also has long been used as probiotics for beneficial effects and well being on humans and animals health. Recently, neutraceuticals, a wide range of foods and food components with claimed medical or health benefit, can be produced by metabolic engineering of LAB (Hugenholtz *et al.*, 2002). Realizing their various abilities and products, LAB are potential source of enzymes and natural products for use in many applications so its have been subjected to considerable research and commercial development over the past decade. More recently, genome sequences of the LAB are now undergoing (Klaenhammer *et al.*, 2002). Comparative and functional genomic analysis has resulted in important scientific breakthroughs and has led to new applications (Makarova *et al.*, 2006).

It was reported that the LAB, especially *Lactobacillus* spp. have glutamic acid decarboxylase (L-glutamate 1-carboxy-lyase, Gad; EC 4.1.1.15) activity and produces γ-aminobutylate (GABA) from glutamic acid (Ueno et al., 1997). GABA has several physiological functions of: (1) facilitating a blood flow in the brain to increase the supply of oxygen so as to accentuate a brain metabolism; (2) accelerate a carbohydrate metabolism; (3) hypertensive and diuretic effects (de Wardener, 2001; Hayakawa et al, 2004). So it has been used for medicine and also added in some functional foods commercially for improvement of brain metabolic function and hypertension. GABA containing fermented food processing by *Lactobacillus brevis* IFO3960 was patented on 2002 (US Patent Pub. No. US2002/0106424 A1). Predicted glutamate decarboxylase genes (*gadB*) were found in released genome sequence analysis of lactobacilli by comparison of the known functional genes of the others microorganism. (The US Department of Energy Joint Genome Institute http://www.jgi.doe.gov/) However, the predicted *gadB* genes have not been proved for their functional properties to date. In order to produce large amount of GABA, GAD gene cloning and further study in molecular level are important and useful for production of functional foods with beneficial effect for health.

The genus *Lactobacillus* and *Enterococcus* are by far the most well known LAB widely isolated from various fermented foods and ecology niches (Tanasupawat et al., 1995; 1998). Interestingly, some species of *Lactobacillus* and *Enterococcus* are able to hydrolyze starch and protein by catalytic activities of amylase and proteinase respectively. Amylase genes from *Lactobacillus amylovorus*, *Lactobacillus plantarum*, and *Lactobacillus manihotivorans* were comparative analyzed (Rodiguez-Sanoja et al., 2005). New endopeptidase genes were identified from genomic sequence of *Lactobacillus helveticus* and their properties in hydrolysis of model bitter peptides were revealed (Sridhar et al., 2005). Both enzymes are important application for production of food-grade lactic acid and in food industries.

The present study was designed to isolate *Lactobacillus* and *Enterococcus* which were able to produce glutamate decarboxylase, amylase and/or proteinase from fermented food and various sources in Thailand. The selected strains were subjected for systematic studies by both classical and molecular methods and the genes of interest were investigated based on comparative analysis and sequence similarity prediction from genome sequence data available on database. Herein report, the newly identified glutamate decarboxylase gene (*gadB*) of the novel high GABA producing isolate *Lactobacillus senmaizukensis* sp. nov. L13^T was successfully cloned and expressed.

The main objectives of this investigation were as follows

1. To isolate and select *Lactobacillus* and *Enterococcus* strains which were able to produce glutamate decarboxylase, amylase and/or proteinase.
2. To identify the selected strains of *Lactobacillus* and *Enterococcus.*
3. To comparatively analyze of the genes involving in production of glutamate decarboxylase, amylase and/or proteinase from the selected strains.
4. To clone and express of glutamate decarboxylase gene from high GABA producing strain in order to produce large amount of GABA.