

CHAPTER 5

CONCLUSIONS

In this study, a potential probiotic LAB strain from Thai fermented food origins was selected to be functional starter for soybean milk fermentation and further developed to be nutraceutical agents and other plant starter. The LCC 150 isolated from a pickled vegetable or som pak (mixed leek, cabbage and pork skin) and collected from Sakonnakorn which were isolated from 480 Thai fermented food samples. The selected LCC 150 was the best to perform probiotic properties out of total 928 lactobacilli strains and was identified as *Lactobacillus plantarum* base on biochemical tests and partial nucleotide sequenced of the 16S rDNA determination. The conclusion of selected potential probiotic properties of *L. plantarum* LCC 150 was displayed in Table 5.1.

From 928 isolated lactobacilli strains, the SC 359 and LCC 150 showed the key probiotic properties such as ability of 0.3 % (w/v) bile salts tolerance, survival in pH 2 to 8 conditions, ability of starch, protein and lipid utilization, and strong antagonistic activities against the growth of all seven microbial indicators.

Especially, the SC 359 showed strong activity against *C. albicans*, which should be further studied the efficiency to be antifungal agents in opportunistic patients causing *C. albicans* infection such as HIVs and candidiasis. For the adherence inhibition tests, two strains expressed the ability to obstruct the Caco-2 cell

adherence of three bacterial pathogens (*Escherichia coli*, *Salmonella* spp. and *Shigella* spp.) with the percentages of inhibition ranged 33.50 to 74.39. The adherent inhibition against three pathogens by SC 359 and LCC 150 were related with the strong antimicrobial activities in present study.

For the safety evaluation, the antibiotic resistance and haemolytic activity of lactobacilli strains were evaluated. The SC 359 and LCC 150 were accepted to be safe strains for host which were found susceptibility to nine antibiotic tests and showed non-haemolytic activities. Beside the basically probiotic properties, the useful enzyme activities such as β -glucosidase and bile salt hydrolase (BSH) of lactobacilli were observed. The β -glucosidase enzyme activities were detected both extracellular and bound forms with the significant ($P<0.05$) maximum activity at 18-24 h. Therefore, the cholesterol lowering properties and plant glycosides biotransformation of the SC 359 and LCC 150 were to largely improve with the activities of the BSH and β -glucosidase enzyme were found, respectively.

Results of cholesterol lowering of SC 359 and LCC 150 in *in vitro* tests, the ability to decrease cholesterol from suspension by active or viable cells of lactobacilli were significantly ($P<0.05$) highest than non-active cells (dead and resting cells). In this study, the SC 359 and LCC 150 strain were detected for the cholesterol-lowering activity via BSH enzyme activity and the capability of bacterial cell to remove cholesterol from culture broth. The active cells of these 2 strains exhibited significantly the highest ability ($P<0.05$) to decrease cholesterol from broth and increase in pellet. The strains with BSH activity exhibited cholesterol-lowering property higher than the strains without BSH activity. These

results suggest that the BSH ability supported the mechanism for the *in vitro* lowering of cholesterol of the cells (Parvez et al., 2006; Kim et al., 2008). The high activities of active cells that means the mechanisms were related with cell metabolisms as follows hydrolysis bile salts becomes free bile salts by the BSH enzyme provide increasing the new formation of bile salts, therefore, the mechanisms for removal of cholesterol from media by lactobacilli due to: the decrement of bile salt precursor, assimilation cholesterol from suspension into the cells during growth and binding cholesterol to the cell surface.

The SC 359 and LCC 150 were identified using the 16S rDNA gene and all of them belonged to *L. plantarum*. Based on functional properties of lactobacilli strains from this study, only one isolate was selected to be inoculants for soybean milk fermentation. The LCC 150 was selected to be functional starter culture due to the strain was the highest efficient probiotics. It may provide the potential functional effect on human host or provide the potential starter culture of fermented foods and beverages. All these will lead to select it to be the starter of functional foods from soybean milk fermentation. Furthermore, heat-killed LCC 150 is potent inducers of different cytokines on Caco-2. Production of IL-6 and IL-10 is also induced by heat-killed strain. Induction of proinflammatory cytokines IL-6 and anti-inflammation cytokine IL-10 by heat-killed lactobacilli could indicate that heat-killed lactobacilli can stimulate nonspecific immune responses. Hence, heat-killed LCC 150 might prevent or reduce inflammatory responses. Application of heat-killed LCC 150 will have a beneficial effect as physiologically functional soybean milk product with much stability than using live cell *Lactobacillus*. It is possible

development to enhance the functional properties of soybean milk product during storage over long period.

Total 11 treatments of fermented soymilk which designed by the 2^3 factorial experiments in central composite and 3 days fermentation were investigated. The appropriate levels of soybean milk ingredients, water extraction ratios at 11 per 1 part of soybean (w/w), initial inoculum of LCC 150 starter at 6 log cfu/ml and sucrose added to soybean milk at 10% (w/v) was the significantly ($P < 0.05$) responded to the high-range score of sensory evaluation, maximum enhanced total acidity and maximum increased of viable cell of starter with greater than 9 log cfu/ml. It was the high amount of viable cells relate to the suitable dosage used of probiotic microbes recommended at least 6 log cycles of viable cells.

The LCC 150 and its fermented soybean milk were detected for the high efficacy to reduce cholesterol concentration in animal within cholesterol supplemented group. Furthermore, fermented soybean milk with LCC 150 was observed for the significantly ($P < 0.05$) highest activity to reduce serum cholesterol, triglyceride and LDL cholesterol. *L. plantarum* LCC 150 and its soybean milk product were observed for slightly effect on HDL cholesterol in both normal and high cholesterol group. The level of serum cholesterol, triglyceride, HDL and LDL cholesterol of normal rats within treated group compare to untreated group were reported with percentage of lowering ranged 1.10-2.21, 6.49-10.16, 2.35-10.63 and 2.98-4.08, respectively. In case of cholesterol supplemented rats, serum cholesterol, triglyceride and LDL cholesterol within treated group compare to untreated group

were reported with percentage of lowering ranged 18.11-28.29, 21.39-27.63 and 22.64-26.56, respectively. Only HDL cholesterol was detected enhancing within cholesterol supplemented group with the percentage of increment as 8.38-21.96. The decrease in LDL-cholesterol combined with an increase in HDL-cholesterol within treated rats might provide strong against heart disease in agreement with Ooi and Liong (2010).

The fermented soybean milk product containing LCC 150 was detected for greater activity of cholesterol lowering than only LCC 150 strain administration. It might be due to the synergistic effects of both LCC 150 strain activity and bioactive compound in soybean milk such as dietary isoflavones on cholesterol lowering in rats. It was in agreement with the results in the present study that the *L. plantarum* LCC 150 showed the cholesterol lowering properties in *in vitro* investigation. Furthermore, the isoflavone contents were detected in fermented soybean milk, particularly isoflavone aglycones were increased after fermentation.

The fermented soybean milk products were considered using the 9-point hedonic scale by the 20 tasters. The evaluated scores of the smell and overall products were more acceptant sensory. The fermented soybean milk containing LCC 150 did not exhibit beany and rancid smell. The products with 3-7 d of fermentation should be store at 2-8°C to maintain the viable cells of functional starter at least 6 log cfu/ml at maximum for 1 month.

The *L. plantarum* LCC 150 is the most fulfilling selected strain of functional properties as the previously described. Hence, this selected strain could

be promoted as functional starter culture for producing fermented plant-derived foods and beverages. Its fermented soybean milk products show the cholesterol lowering property and have enhanced the bioactive contents such as isoflavone aglycones. The soybean aglycones are reported that their phytoestrogenic activity which can be the hormone replacement therapy (HRT) in post menopausal women. Therefore, the LCC 150 could be claimed as probiotic starter to produce functional soybean milk fermentation. Furthermore, its products with bioactive isoflavones enhancing could provide beneficial effects on human differ from fermented cow's milk.

Table 5.1 The probiotic properties of a selected *L. plantarum* LCC 150 strain

Determination	LCC 150 characteristics
1. Origin source	Pickle leek and cabbage containing pork skin collected from northeastern Thailand
2. Bile salt tolerance	0.15 and 0.30% (w/v)
3. pH tolerance for 2 h of incubation with survival rate greater than 50%	2, 3, 4, 5 and 8
4. Nutrient utilization	Utilize starch, protein and lipid
5. Antimicrobial activity against <i>E. coli</i> ATCC 25922, <i>Staphylococcus aureus</i> ATCC 25923, <i>Bacillus cereus</i> , <i>Pseudomonas aeruginosa</i> ATCC 27853, <i>Salmonella typhi</i> , <i>Shigella sonnei</i> and yeast <i>Candida albicans</i> ATCC 90028	Against all of microbial indicators in 3 parts (normal supernatant, supernatant pH 7.0 and supernatant pH 7.0 containing catalase enzyme)
6. susceptibility to ampicillin, chloramphenicol, erythromycin, gentamicin, kanamycin, rifampicin, streptomycin and tetracycline.	susceptible to the lower concentration breakpoints of all antibiotics
7. Hemolytic activity	No
8. Bile salt hydrolase (BSH) activity	Yes

Table 5.1 (Continued)

Determination	LCC 150 characteristics
9. <i>In vitro</i> cholesterol lowering property	Exhibit maximum activity to lowering cholesterol were found in active cell
10. Beta-glucosidase enzyme activity	Detected both extracellular and cell-bound or cell-associated form
11. <i>In vitro</i> adherence inhibition against <i>E. coli</i> , <i>S. sonnei</i> and <i>S. typhi</i>	Detected in three ways inhibition; elimination, competition and displacement The inhibition was strong in the way elimination
12. Ability to be starter for soybean milk fermentation	Yes
13. Cholesterol lowering property in rat	Yes, the selected probiotic strain LCC 150 reduced rat serum cholesterol, triglyceride and LDL cholesterol within cholesterol supplemented group. Furthermore, the fermented soybean milk product containing this strain LCC 150 was detected for greater activity of cholesterol lowering than only LCC 150 strain administration

Suggestion

1. According to the results obtained in this research, the LCC 150 and its fermented soybean milk showed the cholesterol lowering property in *vitro* and in *vivo*. Further studies should be carried out to investigate the activity in human.
2. In further study, mechanisms and activity in human clinical trials, pharmaceutical dosage form, safety and stability of this potential probiotic strain and its products should be studied to assure as probiotic and its product used in human host.
3. Although the SC 359 was not selected to produce fermented soybean milk, the strain showed strong activity against the growth of *C. albicans*. It should be further studied the efficiency to be antifungal agents in opportunistic patients causing *C. albicans* infection.
4. The result obtained from this research shown that the fermented soybean milk products showed the cholesterol lowering property, it should be further investigated the certain dose of bioactive compounds and also nutritional values that provide to requirement of consumers.
5. The model for plant fermentation with containing the selected probiotic lactobacilli strain as starter culture was set up from only soybean. The model should be further verified by the data obtained from another plant, medicinal plant and food origins fermentation.
6. The efficient LCC 150 strain should be further develop as bio-agent or pharmaceutical products which could be claimed the probiotic, cholesterol lowering property and biotransformed activity with enhancing the phytoestrogen (isoflavone aglycones).