CHAPTER 6

Discussion

Metabolic syndromes becoming a global health issue that encountering people who lives in both developed and developing nations. High fat diet consumption and sedentary lifestyle are major contributors in the development of metabolic syndromes (75) (76) (30). Long term high fat diet consumption resulting in altered otherwise normally balanced gut microbiota that regulate the normal physiological condition of animal and human hosts (33) (21) (37) (77). This disturbed gut microbiota composition links to the imbalanced immune response in the gut tissue resulting in more inflammation occurred in both local tissues and blood (increased serum LPS levels or metabolic endotoxemia) (31) (36) (34). Probiotic bacteria has been extensively used as a newly immunomodulator in manipulating the immune response via the alteration in gut microbiota composition despite the controversial outcomes (27) (56) (78). The lactic acid producing bacteria in genus *Lactobacillus* has been used as a probiotic for therapeutic effect in several inflammatory disease such as inflammatory bowel disease and recently used in obesity and metabolic syndromes (27) (24). However, the major challenge of using probiotic *Lactobacillus* is the strain-specific effects, here I investigated whether the novel strain of probiotic *Lactobacillus paracasei* ST11 (HP4) isolated from the Northern Thai fermented-food (Sirilun et al., unpublished data) could improve metabolic parameters, gut microbiota composition, metabolic endotoxemia and gut inflammation in diet-induced obese rat.

First, I confirm the findings reported in previous studies in which demonstrated that long term consumption of high fat diet resulting in metabolic abnormalities in rat including body weight gain, increased visceral fat accumulation, high serum glucose and dyslipidemia. I also show here that high fat diet consumption results in both low-grade systemic (metabolic endotoxemia or increased serum LPS levels) and local (gut) inflammations. Obese rats fed with high fat diet developed a metabolic endotoxemia and significantly increased the expressions of mRNA levels pro-inflammatory cytokines (IL-

1b and IL-6) in the gut tissue (ileum). These results supported the idea that metabolic disturbances in obesity could be, at least in part, derived from the low-grade inflammations occurred in the site where the food initially encounters the host body (gut) then spread to the systemic (blood circulation) site. However, there is a mild alteration of viable gut microbial population investigated by stool culture methods shown in this study. One of the several advantages for using viable bacterial culture method is to detect the normally non-predominant bacterial population such as the facultative anaerobe Enterobacteriaceae from the vast pools of anaerobic gut microbes such as Bacteroidetes and Firmicutes. Although this method is a relatively simply and cheap compared to the more sophisticated methods such as a quantitative real-time polymerase chain reaction (qRT-PCR) or the state-of-the-art next generation sequencing platforms, the results cannot be truly represented the whole gut dysbiosis. Due to the fact that most of gut microbiota population are non-culturable with the conventional methods. By using this relatively easy approach to study gut microbiota, I found that high fat diet significantly enhances growth of Gram negative-LPS containing bacteria Enterobacteriaceae at the 12^{th} week of feeding. The viable counts of *Bifidobacterium* and *Lactobacillus* were not different between the obese and lean rats. Moreover, the blooming of Enterobacteriaceae observed in obese rat's gut content at the 12^{th} week was disappeared at the end of the study by still unknown mechanism.

To characterize the ability of the newly isolated probiotic *Lactobacillus paracasei* ST11 (HP4) in obesity and metabolic syndrome treatment, obese rats were orally gavaged by 10^8 cfu of probiotic *L. paracasei* ST11 (HP4) daily for 12 week-period. The results showed that only serum cholesterol levels of the obese rats were significantly decreased compared to the non-treated obese and lean rats. Even the obese rats' serum triglyceride levels tend to be decreased by *L. paracasei* ST11 (HP4) ($p = 0.07$). Surprisingly, there were no differences in rat body weight, visceral fat weight, serum glucose, triglyceride, LDL and VLDL cholesterol levels between the treated versus non-treated obese rats. However, *L. paracasei* ST11 (HP4) consumption leads to the attenuation of metabolic endotoxemia and gut inflammations by however unknown mechanism yet. It seems likely that the strain-specific effect of probiotic *Lactobacillus* plays a role in modulating of metabolic syndromes and obesity as previously shown by Wang et al. (56). In their study, the authors found that *Lactobacillus paracasei* CNCM I-4270 and *L. rhamnosus* I-3690 attenuate features of the metabolic syndromes in high fat diet-fed mice (decreased body

weight, improved glucose-insulin homeostasis and attenuated hepatic steatosis); however, with the differences in gut microbiota alterations. More studies also illustrated strain-dependent effect for using probiotic in the treatment of metabolic syndromes (28, 79)(80).

In conclusion, this newly isolated probiotic strain *L. paracasei* ST11 (HP4) demonstrated the potential role as the therapeutic approach for metabolic syndromes and obesity (by the attenuation of serum cholesterol levels, metabolic endotoxemia and gut inflammation) shown in this study. Furthermore, the contribution of this new probiotic strain on the gut microbiota population should be further studied using the culture-independent methods such as qRT-PCR or gut microbiota DNA sequencing.