

## Chapter 6

### General conclusion

Probiotic effects are known for some lactic acid bacteria (LAB) and bifidobacteria strains, which are living microorganisms that confer a health benefit to the host when administered in adequate amounts. The effects of probiotics on the regulation of the intestine and the immunomodulatory activity of the host are reported as a function of probiotics. Also, many studies have isolated original probiotic strain and investigated their probiotics potential and health benefit to the host. Mongolia is one of the world's traditional dairy countries. Mongolian dairy products include many unique products used several types of milk from domestic animals including cows, sheep, goats, yaks and horses. These products are interesting as bioresources of LAB and are expected to yield candidate LAB strains as probiotics.

I collected 66 samples classified into five kinds of traditional Mongolian dairy products, from which 543 LAB strains were isolated and identified. In the identified LABs, the species that frequently described not only from the dairy products but also from the environment such as the silage were observed. Furthermore, all LAB isolates were screened for tolerance to low pH and bile acid, gas production from glucose, and adherence to Caco-2 cells. *In vitro*, it was found 10 strains possessed probiotic properties, and were identified as *Lactobacillus* (*L.*) *plantarum* or *L. paracasei* subspecies, based on 16S ribosomal DNA and carbohydrate fermentation pattern. These strains were differentiated from each other individually.

Of the 10 probiotic LAB, *L. paracasei paracasei* 06TCa19 strain was deemed to be the most promising of the candidate strains for use in developing dairy products, especially yogurt. To determine if the 06TCa19 strain reaches and survives in the intestine and to

evaluate the effects of the 06TCa19 strain on the human bowel and intestinal environment, I was conducted a randomized, double-blind, crossover study with 46 young, healthy women who consumed either the 06TCa19 strain or control fermented milk. The 06TCa19 strain was observed from *Lactobacilli* colonies from cultures of feces of subjects who consumed the 06TCa19 fermented milk, suggesting that this strain reached and survived in the human intestine. After consumption of either fermented milk, the subjects' defecation frequencies were significantly increased. However, subjects who consumed the 06TCa19 fermented milk also exhibited significantly improved defecation characteristics, particularly regarding fecal shape and color, compared to the control group, indicating that the 06TCa19 strain was superior as a probiotic that regulated the human intestine. It is presumed that the mechanism of action is through an increased level of L-lactic acid produced by the 06TCa19 strain and/or an increased number of *Lactobacillus (L.)* and *Bifidobacteria* in the intestine after the ingestion of 06TCa19 fermented milk.

In another study, to evaluate the immunomodulatory activity of the 10 probiotic strains from traditional Mongolian dairy products, the effects of the strains were assessed on T-helper (Th) 1 cytokine production in a mouse macrophage cell line and spleen cells and mouse natural killer (NK) activity after administering the strains to mice. The results of co-culture with J774.1 cells and mouse spleen cells showed that 4 of the 10 strains promoted Th1 cytokine production. Furthermore, the administration of the *L. plantarum* 06CC2 strain, one of the strains that enhanced Th1 cytokine production, resulted in the highest level of mouse NK cell activity of the 10 strains. Therefore, the 06CC2 strain was expected to have superior immunomodulatory activity for the host and an effect on symptoms such as type I allergic diseases and infection by bacteria, parasites and viruses.

Also, the immunomodulatory activity of the 10 probiotics strains from Mongolian dairy products was assessed on IFV infection in IFV-infected mice. The immunomodulatory

activity of probiotics is expected to affect IFV infection because cytokine production locally and systemically and NK cell activation are important in promoting the host-immune defense and alleviating symptoms, especially in early stage IFV infection. Oral administration of boiled strain 06CC2, one of the 10 LABs, was significantly effective against symptoms of infected mice and for reducing viral yields in the lungs after infection without toxicity. Also, it was found that 06CC2 alleviated influenza symptoms in mice in correlation with the augmentation of NK cell activity associated with enhancement of IFN- $\alpha$  and Th1 cytokine production through intestinal immunity and the reduction of TNF- $\alpha$  in early stage infection. Therefore, the 06CC2 strain exhibited immunomodulatory activity in IFV-infected mice, resulting in the alleviation of IFV infection.

Finally, the diversity of LAB isolated from traditional Mongolian dairy products was investigated and the probiotic potential of the isolated strains was estimated *in vitro*. The results showed that 10 strains possessed probiotic properties. In those strains, a *L. paracasei paracasei* 06TCa19 strain was found to reach and survive in the intestine and regulate the human bowel and intestinal environment favorably. Moreover, *L. plantarum* 06CC2 strain had superior immunomodulatory activity not only in healthy mice but also in IFV-infected mice, resulting in the alleviation of IFV infection. I anticipate the development of functional dairy products such as yogurt using these strains. Many other probiotic functions have been demonstrated such as the prevention of allergy and cancer. The LAB isolated in this study required investigations on their efficacy. Further studies on probiotics are needed to verify their novel effects and investigate their mechanisms.