

New Probiotic Effect of Bifidobacteria for Human Health

Yoshimi Benno

RIKEN BioResource Center, Japan

More than 500 species of human gut microbiota is excreted with the feces. The whole analysis of gut microbiota is gradually coming into focus. Recently, an association has been found between gut microbiota and many diseases beyond those of the gastrointestinal system, including today's three leading causes of death - cancer, heart disease, and cerebrovascular disease - as well as allergies and dementia. Our group is trying to capture the whole analysis of gut microbiota, which constantly changes with age and lifestyle-related factors such as diet, and apply it to preventive medicine.

Various molecular-biological approaches using the 16S rRNA gene sequence have been used for the analysis of human colonic microbiota. Terminal- restriction fragment length polymorphism (T-RFLP) analysis is suitable for a rapid comparison of complex bacterial communities. Terminal-restriction fragment (T-RF) length can be calculated from a known sequence, thus one can predict bacterial species on the basis of their T-RF length by this analysis. Finally, we can build a phylogenetic assignment database for T-RFLP analysis of human colonic microbiota (PAD-HCM), and demonstrate the effectiveness of PAD-HCM compared with the results of 16S rRNA gene clone library analysis. PAD-HCM was completed to include 342 sequence data obtained using four restriction enzymes. Approximately 80% of the total clones detected by 16S rRNA gene clone library analysis were the same bacterial species or phylotypes as those assigned from T-RF using PAD-HCM. Moreover, large T-RFs consisted of common species or phylotypes detected by both analytical methods. All pseudo-T-RFs identified by mung bean nuclease digestion could not be assigned to a bacterial species or phylotype, and this finding shows that pseudo-T-RFs can also be predicted using PAD-HCM. We conclude that PAD-HCM built in this study enables the prediction of T-RFs at the species level including difficult-to-culture bacteria, and that it is very useful for the T-RFLP analysis of human colonic microbiota.

Probiotics have been defined in several ways, depending on our understanding of the mechanisms of action of their effects on health and well-being of humans. Now, the definition was as follows: Probiotics are live microbial; food ingredients that have a beneficial effect on human health. The health effects of probiotics seem to be induced by improvement of intestinal environment, which depends on

metabolites produced by probiotics or improved intestinal microbiota composition. *Bifidobacterium animalis* subsp. *lactis* Bb-12 is a probiotic strain with ideal properties including acid-tolerance and adhesion to human intestinal mucosa. The improvement of intestinal environment by intake of the Bb-12 yogurt was examined using polyamine, haptoglobin and mutagenicity as indexes, which directly reflect the health condition of the host. The concentration of spermine in feces increased significantly by 3-fold ($P < 0.05$) at week 2 of the Bb-12 yogurt intake compared with before administration, and that of putrescine and spermidine also tended to increase with the yogurt intake. Fecal mutagenicity was measured using fecal extract and fecal precipitate. Both preparations showed similar significant decrease ($P < 0.05$) by the Bb-12 yogurt intake, as well as a negative correlation with polyamine content. These results indicate that antimutagenicity due to the Bb-12 yogurt intake was not based on binding of the mutagen to the bacterial cell wall.

In conclusion, the outline of new probiotic functions is now shown by the development of culture-independent analysis of human gut microbiota.