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Outline of Research Result

Analysis of Microbial Purine Metabolism and Its Application for Hyperuricemia Prevention

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Research aims

Hyperuricemia is a disease, which results from the over-production and/or underexcretion of uric acid and is greatly influenced by a high dietary intake of purine. It is usually an asymptomatic condition, which is hypothesized to play a role in cardiovascular disease and hypertension. Some hyperuricemic individuals develop gout, a common disease with a worldwide distribution and are mainly caused by deposition of monosodium urate crystals in joints and other tissues as a result of extracellular urate supersaturation. Although most uric acid is derived from the metabolism of endogenous purine, eating foods rich in purines contributes to the total pool of uric acid. Consumption of meat, seafood and alcoholic beverages in moderation and attention to food portion size is important. Low purine diet, moderation in the consumption of not only beer but also other forms of alcohol is essential. But traditional low-purine diets, once a mainstay of hyperuricemia management, are difficult for patients to adhere to because it is not easy to keep the nutritional balance of their diets.

We attempted to design a new prophylaxis for hyperuricemia using probiotic effects of microorganisms. The term probiotic refers to live microorganisms that survive passage through the gastrointestinal tract and have beneficial effects on the host. We assumed that probiotic bacteria administered orally could help degrading purine and purine metabolites derived from food in human intestine, which lead to lowering the elevated serum uric acid level. In this report, we first described about the screening of microorganisms which catalyze inosine and guanosine degradation and evaluated their effects on serum uric acid level reduction. Furthermore, through the analysis of purine metabolism, mechanism of the probiotic effects of the potential strains was elucidated.

Methods

Lactic acid bacteria (267 strains in total), including *Bifidobacterium*, *Lactobacillus*, *Enterococcus*, *Leuconostoc*, and *Pediococcus* species, were cultivated anaerobically in MRS medium and their washed cells were prepared. The washed cells were added to the reaction mixture containing 1.25 mM inosine, 1.25 mM guanosine, and 0.1 M potassium

phosphate buffer (pH 7.0) and incubated anaerobically 2 h at 37°C. After the reaction, the concentration of inosine and guanosine was monitored and the strains showed higher reduction were selected.

The effects of selected strains on the serum uric acid level were investigated using the rats pretreated with the uricase inhibitor potassium oxonate as animal models for hyperuricemia. The non-treated group was fed the diets containing 0.25% oxonate. The control group was fed the diets containing 0.25% oxonate and 1% RNA as purine source. The test group was fed the diets containing 0.25% oxonate and 1% RNA together with washed cells of lactic acid bacteria (1.0×10^9 CFU). After 2, 5, and 8 days of feeding, the serum was collected and their uric acid level was evaluated.

As to the strains showing serum uric acid level reduction, their purine metabolism was analyzed. These strains were cultivated in MRS medium under anaerobic conditions at 28°C for 48 h. 2 ml of the reaction mixture comprising 100 mM potassium phosphate buffer (pH 7.0) and 1.25 mM AMP, 1.25 mM IMP, 1.25 mM GMP, 1.25 mM adenosine, 1.25 mM inosine, 1.25 mM guanosine, 1.25 mM adenine, 1.25 mM hypoxanthine, 1.25 mM guanine, 1.25 mM xanthine, or 1.25 mM uric acid were added into the test tube containing the wet cells from 3 ml culture broth and incubated anaerobically at 37°C for 2 h. After the incubation, the purine contents in the supernatants were analyzed.

Results

Microorganisms were screened for their ability to degrade purine. As the result of screening in *Bifidobacterium*, *Lactobacillus*, *Enterococcus*, *Leuconostoc* and *Pediococcus* species, 13 strains of bacteria, such as *L. mali*, *L. vaccinos-tercus*, *L. brevis*, *L. fermentum*, *L. homohiochii*, and *L. pentosus*, were selected as good catalysts for degradation of inosine and guanosine. Among these 13 strains, 11 strains were isolated from fermented food and good for probiotic purposes. As to these 11 strains, the effects on the serum uric acid level were investigated using the rats pretreated with the uricase inhibitor, potassium oxonate, as animal models for hyperuricemia. During the testing term, no significant difference in weight and dietary intake was observed with each group. The serum uric acid level of con-

trol group was increased until 5 days and reached maximum. On the other hand, the test group with lactic acid bacteria intake showed repressing effects on serum uric acid level elevation. The ability to lower elevated level of serum uric acid was found in *L. fermentum* ONRIC b0185, *L. fermentum* ONRIC b0195, and *L. pentosus* ONRIC b0223 (Fig. 1).

To elucidate the mechanism to lower the elevated serum uric acid levels, purine metabolism, i.e., the degradation of IMP, AMP and GMP and their metabolites, was evaluated for *L. fermentum* ONRIC b0185, *L. fermentum* ONRIC b0195, and *L. pentosus* ONRIC b0223. *L. fermentum* ONRIC b0185 converted adenosine to adenine, inosine to

hypoxanthine, guanosine to guanine, and guanine to xanthine. This strain strongly degraded inosine to hypoxanthine, and guanosine to guanine (Fig. 2). *L. fermentum* ONRIC b0195 converted adenosine to adenine, inosine to hypoxanthine, guanosine to guanine and xanthine. In a reaction with guanine, guanine was not degraded to xanthine in 2 h. On the other hand, this strain converted guanine to xanthine in a reaction with guanosine (Fig. 2). *L. pentosus* ONRIC b0223 converted adenosine to adenine, inosine to hypoxanthine, and guanosine to guanine. This strain strongly degraded inosine to hypoxanthine (Fig. 2). All of these strains well converted nucleosides to purine bases, indicating that they have high nucleosidase activity.

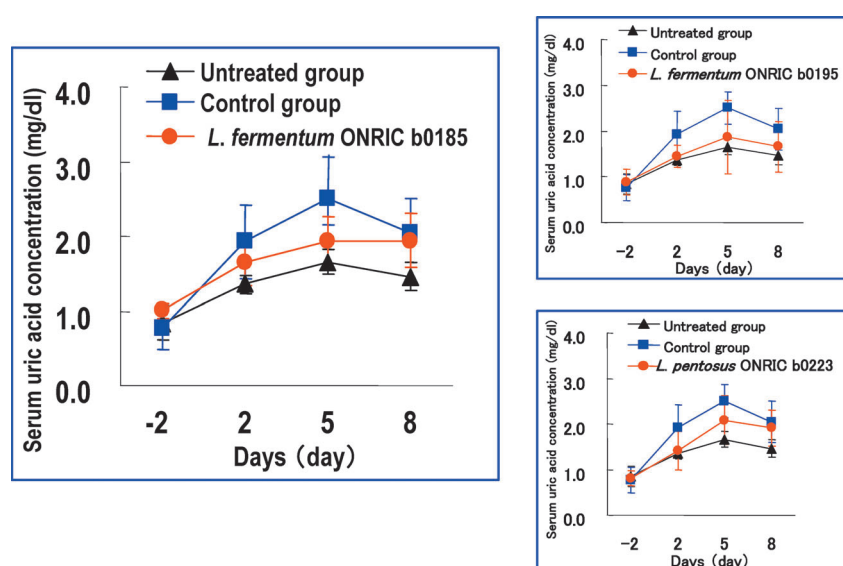


Fig. 1. Results of hyperuricemia model rat experiments. Black triangle; serum uric acid level of non-treated group with oxonate containing diets, blue square; serum uric acid level of control group with oxonate and RNA containing diets, red circle; serum uric acid level of test group with oxonate, RNA, and lactic acid bacteria containing diets.

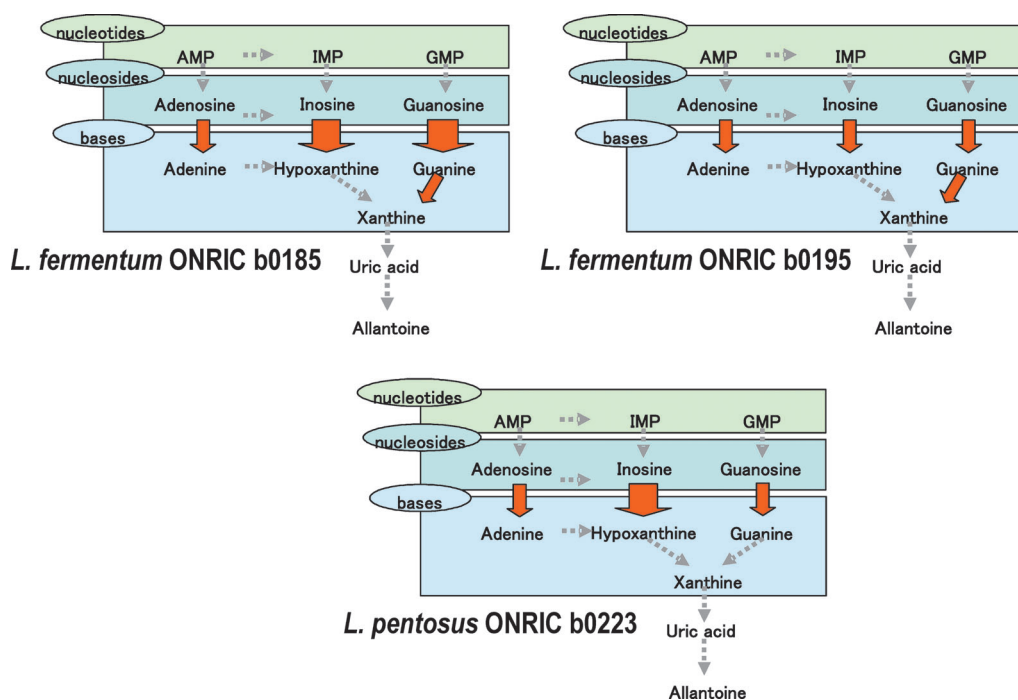


Fig. 2. Purine degradation pathway of lactic acid bacteria. Dot-arrow; no or trace activity, solid arrow; significant activity. The boldness of the arrow represents the level of activity.

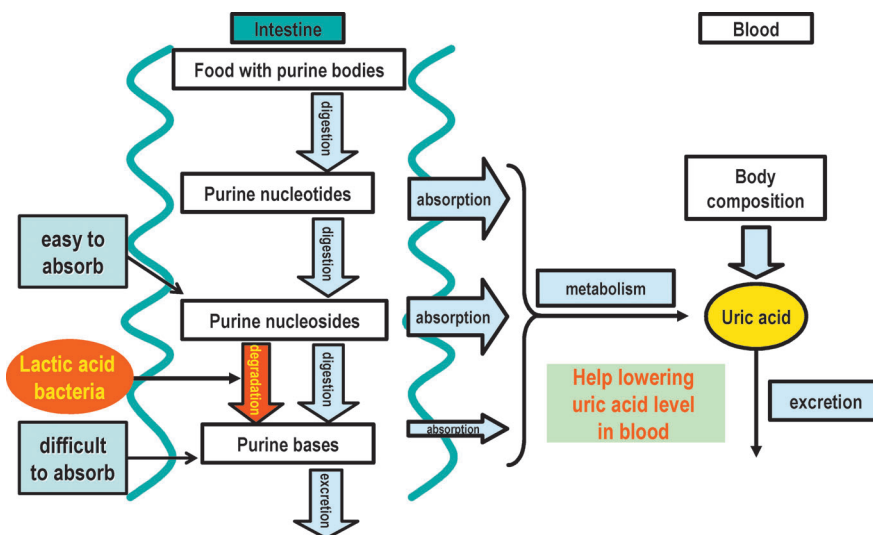


Fig. 3. Proposed mechanism of lowering serum uric acid level by lactic acid bacteria.

Conclusion

The ability to lower elevated level of serum uric acid was found in *L. fermentum* ONRIC b0185, *L. fermentum* ONRIC b0195, and *L. pentosus* ONRIC b0223. These strains showed high nucleosidase activity converting nucleoside to corresponding nucleobase.

The previous research about nucleoside transport and metabolism in isolated rat jejunum reported that luminal inosine and hypoxanthine increased the serosal uric acid concentration significantly and that the nucleoside gave a significantly greater rate of serosal uric acid appearance than the base. The research also reported that luminal guanosine and guanine increased the serosal uric acid concentration significantly and that the serosal uric acid appeared signifi-

cantly faster from the nucleoside than it did from the base. Taking these facts into account, the result of the analysis of purine metabolism of these three strains shows that these strains converted purine nucleosides to purine bases, less easy form in intestine to be absorbed into serosal side. This conversion enabled these strains to lower the elevated serum uric acid level in animals. This result suggested that this lowering effect might be beneficial in the treatment of patients with hyperuricemia using probiotics.

References

1. J. Ogawa, C.-L. Soong, S. Kishino, Q.-S. Li, N. Horinouchi, S. Shimizu "Screening and industrial application of unique microbial reactions involved in nucleic acid and lipid metabolisms." *Biosci. Biotechnol. Biochem.*, **70**, 574–582 (2006)