学 位 論 文 の 要 旨

論文題目　**Beneficial effects of *Aspergillus*-derived protease preparations on colonic luminal environment**（大腸内環境に及ぼす麹菌由来プロテアーゼ剤の有益な効果）

広島大学大学院生物圏科学研究科
生物機能開発科学　専攻
学生番号　D156196
氏　　名　楊　永寿 Yang Yongshou

Aspergillus is a fungal genus contains more than 200 species found in various ecological niches. Some species belong to this fungus are invasive pathogens which can cause serious diseases in human and animals. However, several *Aspergillus* species have been widely used in traditional fermentation foods and pharmaceuticals due to their enzymes-producing abilities. Particularly, *Aspergillus oryzae* (*A. oryzae*) is widely used for the large-scale production of traditional food products, including soy sauce, sake, miso, rice vinegars, and huangjiu. Due to the vital roles of *A. oryzae* in Japanese food culture, *A. oryzae* has been proposed as the national microorganism in Japan. The catalytic abilities of *A. oryzae*-secreted enzymes may help to increase biological and functional characters of various foodstuffs. In order to know whether dietary of these enzymes exert potentially beneficial effects on health in mammals, here, I investigated the effects of *Aspergillus*-derived protease preparations on the colonic luminal environment in rats.

1. Effects of protease preparations derived from *Aspergillus* on the colonic luminal environment in rats

The preliminary study was conducted to investigate the effects of the consumption of several food-processing proteases derived from *Aspergillus* at the dose of 0.2% in the diet on colonic luminal microflora. Among them, two enzyme preparations, which are Amano protease (Protease A ‘Amano SD’, neutral proteases from *Aspergillus oryzae*) and Orientase (Orientase AY, acid proteases from *Aspergillus niger*), could exert some beneficial effects on colonic environment. Amano protease supplementation markedly elevated the levels of cecal *Bifidobacterium* and *Lactobacillus*, the well-

known health-promoting bacteria. Furthermore, the concentrations of cecal n-butyrate, propionate, and lactate, which are potential mediators playing important roles in gut health, were increased by the Amano protease and Orientase supplementation. Total short-chain fatty acids (SCFAs) concentrations were significantly higher in the Amano protease and Orientase groups compared to the control group. In addition, dietary Amano protease elevated fecal immunoglobulin A (IgA) and mucins, which are responsible for intestinal immune and barrier functions. Taken together, these results suggest that the *Aspergillus*-derived protease preparations may beneficially modify the compositions of cecal microflora, SCFAs, IgA, and mucins in rats fed a high-fat diet.

2. Effect of consumption of an acid protease derived from *Aspergillus oryzae* on gut microflora in rats

The Amano protease preparation contains several digestive enzymes, including acid protease, alkaline protease, amylase, etc. I hypothesized that the digestive enzymes in the protease preparation are responsible for the bifidogenic effect. Then, I tested this hypothesis by investigating whether cecal *Bifidobacterium* levels are elevated by the addition of the purified acid protease (AcP) at the dose equivalent to the level found in the 0.1% Amano protease containing diet, which is considered as the effective dose for generating a bifidogenic effect. The results showed that the cecal and fecal *Bifidobacterium* numbers were not affected by the supplementation with 0.0096% purified AcP at the level equivalent to the AcP amount found in the 0.1% Amano protease diet. However, intriguingly I found that the diet containing 4-fold higher AcP content (0.0384% purified AcP diet) than that found in the 0.1% Amano protease diet significantly elevated the cecal *Bifidobacterium* and *Lactobacillus* numbers and the fecal *Bifidobacterium* numbers in rats. This bifidogenic effect was not observed in the rats fed the inactivated AcP, implying that the enzymatic activity of the protease was a key factor underlying the bifidogenic effect, which was beneficial for the gut luminal environment. As expected from the results of increased cecal numbers of *Bifidobacterium* and *Lactobacillus*, the lactate-producing bacteria, the cecal lactate levels were also elevated by the 0.0384% AcP containing diet.

After this study, I have failed to show any bifidogenic effect of the purified alkaline protease or the purified amylase derived from the Amano protease preparation. At present, it is unclear why AcP exerts a bifidogenic effect, but alkaline protease and

amylase do not. I speculated that AcP is relatively stable in the acidic environment of the stomach and is able to reach to the intestines, where it imparts bifidogenic effects. Further study is in progress to investigate the bifidogenic process. These results are the first to prove that consuming small quantities of active AcP derived from *A. oryzae* exerts a prebiotic-like effect in rats. This finding may provide the insights into novel applications of this protease as a functional food factor, e.g., a dietary supplement for promoting colonic health. The result of this study may challenge the concept of prebiotics, and the *A. oryzae*-derived AcP may be considered as a novel type of prebiotic.

3. Effects of dietary supplemental *Aspergillus* protease preparation on gut-protective amino acids in the cecum of rats

Amino acids are confirmed as one of the main building blocks of protein synthesis and also play critical roles in other functions, such as cell signaling, gene expression, intracellular protein turnover, reproduction, oxidative stress, and immunity regulation. Accumulating evidence indicates that various kinds of amino acids and their metabolites play crucial roles in the intestinal homeostasis. In this study, I hypothesized that some digestive enzymes in the Amano protease preparation may successfully pass through the acidic condition of stomach, and then influence the proteins and amino acids metabolisms in the cecum, which in turn affects gut health. Thus, I investigated changes in profile of free amino acids in the cecal contents of rats fed the 0.1% Amano protease-supplemented diet. As a result, the Amano protease intake markedly increased eleven amino acids, which were valine, cystine, threonine, alanine, glycine, phenylalanine, histidine, proline, taurine, γ-aminobutyric acid (GABA), and ornithine. These amino acids have been suggested to exert positive and protective effects on intestinal health by improving intestinal barrier and integrity, and by suppressing oxidative stress and inflammation. Meanwhile, dietary Amano protease did not affect cecum levels of free leucine, isoleucine, methionine, lysine, serine, aspartic acid, glutamic acid, and tyrosine. Thus, these results suggest the supplemental Amano protease has a beneficial impact on gut health by increasing these eleven gut-protective

amino acids mentioned above. Furthermore, the levels of the amino acids affected by the Amano protease were mostly associated with the relative abundance of cecum *Bifidobacterium* and *Lactobacillus*. Thus, the elevations in cecal *Bifidobacterium* and *Lactobacillus* by the Amano protease intake appear to be linked to the increased amino acids in the cecal contents.

In conclusion, these studies suggest that dietary *A. oryzae*-derived protease preparation exerts the prebiotic-like and beneficial effects on gut health by increasing the levels of *Bifidobacterium* and *Lactobacillus,* various gut-protective amino acids, SCFAs, IgA, and mucins. Over the years, various *Aspergillus* protease preparations have been widely used for food production and as components of digestive enzyme preparations all over the world. My studies may provide an insight into the novel application of *Aspergillus* protease and the novel benefits of fermentation foods prepared with *Aspergillus spp*. This result has a great impact on several academic fields, including food science, nutrition, prebiotics, microflora, pharmacology, gastroenterology, digestive enzymology, and microbial biotechnology. Further studies are necessary to clarify the underlying mechanisms of the beneficial effects of *Aspergillus* protease preparations.