Efficient utilization of agricultural by-products using lactic acid-producing fungi

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Summary

The project has been conducted for basic research on fungi that quickly ferment agricultural by-products, such as potato pulp, and the use of the fermented products as animal feeds and ingredients for functional foods. We found that *Rhizopus oryzae* strains, the lactic acid-producing fungi, are classified into two groups producing predominantly lactic acid and fumaric acid, and we elucidated these differences genetically. As for the application, a large-scale method to ensile potato pulp using *Amylomyces rouxii*, which is closely related with *R. oryzae*, was developed. The resultant potato pulp silage can be used as animal feeds with a high degree of acceptance by cows and sheep. Potato pulp fermented by lactic acid-producing fungi showed cholesterol-lowering effects in rats, and it has potential for applications in cereal-based foods as a fiber supplement, an ingredient that is often lacking in the typical human diet of the present times.

Keywords: potato pulp, lactic acid, Rhizopus oryzae, Amylomyces rouxii

Introduction

Potatoes are a principal rotation crop in Hokkaido, the northernmost island of Japan. Each year, the starch industry uses about one million tons of harvested potatoes, and, simultaneously, pulp is produced, corresponding to 10% of the raw material. Potato pulp, which contains starch, cellulose, hemicelluloses, and pectin, is produced in large amounts at the end of the potato season; if left untreated, spoilage is a concern. Therefore, the pulp is usually composted and used regionally as an organic fertilizer in spite of its relatively high nutrient value. In other countries, potato pulp is used as cattle feed despite the high cost of drying it. Sometimes it is used for the microbial production of enzymes. I have previously isolated amylolytic lactic acid bacteria to ferment starch in food by-products without saccharification by enzymes. However, the selected strain, *Lactobacillus amylovorus* JCM 10628, which has shown a high productivity of lactic acid from raw starch in a liquid medium, failed to reduce the pH of potato pulp by acid synthesis. Potato pulp may lack a necessary carbon source and other minor nutritional components that are required for the vigorous growth of the lactic acid bacterium. Starch in potato pulp is not susceptible to amylases without damage to the cell walls even when the enzyme has the capacity for high activity for raw starch. I then turned our attention to the fungal genus *Rhizopus* that produces lactic acid and planed the project for basic research on fungi that quickly ferment agricultural by-products, such as potato pulp, and the use of the fermented products as animal feeds and ingredients for functional foods. National Agricultural Research Center for Hokkaido Region organized the research consortium including Hokkaido University, Obihiro University of Agricultural and Veterinary Medicine, Fuji Women's University, Hokkaido Food Processing

Research Center, Snow Seed Co. and Shihoro Agricultural Cooperative. The present paper reports the results of this project, which was by Special Coordination Funds for Promoting Science and Technology (Leading Research Utilizing Potential of Regional Science and Technology) of the Ministry of Education, Culture, Sports, Science and Technology of the Japanese Government from October, 2001 to March, 2003. Details of the results and evaluation are shown at the following sites:
http://www.chousei-seika.com/search/info/inforesult.aspx?sendno=1
http://www.mext.go.jp/b_menu/houdou/16/12/04121501.htm

Microbiological study of lactic acid producing fungi

Thirty-seven strains of *Rhizopus oryzae* were grown on the sterilized potato pulp to compare their productivity of lactic acid with the reference strain NRRL 395 (Oda et al. 2002). The highest amount of L(+)-lactic acid (10 mg/g fresh matter) was observed in the pulp fermented for six days by *Rhizopus oryzae* NBRC 4707. Strains NBRC 4707 and NRRL 395 developed comparable activities of xylanase, cellulase, α-amylase, and glucoamylase, while the polygalacturonase activity of strain NBRC 4707 was twice that of strain NRRL 395. The addition of commercial pectinase enhanced the formation of metabolite, suggesting that the degradation of pectic substances determines the fermentation of the potato pulp by *R. oryzae* (Saito et al. 2003, 2004b). From these findings, strain NBRC 4707 was used for ensiling experiments of potato pulp in the autumn of 2002.

When forty-eight strains of *R. oryzae* were grown in a liquid medium, lactic acid dominated organic acids in the culture filtrate of 27 strains, and both fumaric and malic acids were principally produced by the other 21 strains. These strains, which differed in pattern of organic acid accumulation, were separated into two groups (Oda et al. 2003). The strains producing predominantly lactic acid were shown to possess two genes, *ldhA* and *ldhB*, encoding NAD-dependent lactate dehydrogenases (Saito et al. 2004a). Variation in nucleotide sequence was identified for each gene from different strains, and similar phylogenetic trees were obtained based on the nucleotide sequences of both genes. The other strains producing predominantly fumaric and malic acids contained a single ORF of *ldhB* (Saito et al. 2004a). Compared to the strains producing predominantly lactic acid, a lower degree of sequence divergence was found in *ldhB*, resulting in a separate cluster in the phylogenetic tree. The high similarity (>90%) spanning the ORF and adjacent regions demonstrates that *ldhA* and *ldhB* are derived from the same ancestor gene. The strains producing predominantly fumaric and malic acids lack functional *ldhA*, which plays a role in lactic acid synthesis and may form a lineage separated from the strains producing predominantly lactic acid in the genus *Rhizopus*.

The sequences of ITS (Internal Transcribed Spacer) region of *R. oryzae* strains were determined and revealed to conserve mutations correspond to lactic acid production. We have devised these mutations for a novel method to identify the strains producing predominantly lactic acid, by designing specific PCR primers on them (Abe et al. 2003). Touch down PCR using these primers amplified the ITS region of these strains specifically. By this method, one fungal strain was isolated from Indonesian fermented foods. However, the isolate was quite different from that of *R. oryzae* in morphology, such as poor sporangia formation and abundant chlamydospore formation. These characters indicated us that the isolated strain is similar to *Amylomyces rouxii* that plays a crucial role in the making of Asian fermented foods (Abe et al. 2004). Then, we tested fermentation of potato pulp by the seven strains of *A. rouxii* and finally selected strain CBS 438.76 derived from *look pang* (Figure 1), the fermentation starter in Thailand. Although lactic acid production by *A. rouxii* CBS 438.76 was less *R. oryzae* NBRC 4707, its growth acidified the pulp in seven days to less than pH 4.0, the level found in conventional silage fermented by lactic acid bacteria. *A. rouxii* was preferable to *R. oryzae* for recycling potato pulp and other agricultural by-products into food materials

because this fungus was being consumed long before written history, which attests to its safety for humans. In this project, *A. rouxi*i CBS 438.76 was used for ensiling experiments of potato pulp after 2003 instead of *R. oryzae* NRRL 4707.

Figure 1. Appearance of look-pang

Ensiling of agricultural by-products using lactic acid-producing fungi

Research included in this subtheme had seasonal restriction. The experiments required number tons of fresh potato pulp generated from starch manufacturing plants between September and November. In 2002, the first year of this project, the starter culture grown on wheat bran was found to be effective to stabilize the quality of silage. In the next year, thirty tons were ensiled with and without the addition of *R. oryzae*. When these two silages were supplied side by side, the cattle significantly preferred the silage fermented by *R. oryzae*. In the final year, potato pulp produced from a starch manufacturing plant was immediately mixed with the starter of *A. rouxii* and packed in bags each at 500 kg (Figure 2). After fermentation for three weeks, feeding experiments were carried out for beef cattle and lactating cow in usual farms.

Figure 2. Silage fermentation of potato pulp in bags

As for beef cattle, types of feed were total mixed ration as a reference and that in which 20% was replaced to potato pulp fermented with and without addition of *A. rouxii*. Blood

composition and body weight were determined every week for six week during experiments. Potato pulp fermented with *A. rouxii* elevated average weight gain per day probably because of its higher acceptability.

Three types of feed were offered to the same group of lactating Holstein cow with different periods for one month to measure production and composition of milk. In the period with fermented potato pulp, milk production tended to be improved although milk fat content decreased slightly. Fermented potato pulp may also be effective for lactating cow by considering constitution of feed.

Application of agricultural by-products fermented by lactic acid-producing fungi as food materials

The nutritional and physiological functions of fermented potato pulp were evaluated in rats (seven week old). Experimental diet containing 10% of potato pulp was fed for three weeks to determine body weight, feed consumption, serum composition, weight of internal organs and expression of enzymes related metabolism. Fermented potato pulp lowered increase of serum cholesterol concentration more than raw potato pulp. The effects of fermented pulp on gastric and liver disorders induced by free radical generation were also examined. The diet supplemented with 0.5% acetoaminophene was fed as described above. Activity of alkaline phosphatase as a marker of liver disease was reduced by fermented potato pulp with the increase of glutathione concentration. Fermented potato pulp may improve internal metabolism of cholesterol and restore liver disorders.

Fermented potato pulp is inappropriate to consumed directly as foods irrespective of its desirable effects for health. We tested the potential for applications in cereal-based foods as a fiber supplement, an ingredient that is often lacking in the typical human diet of the present times (Yamauchi et al. 2004). The use of raw potato pulp significantly diminished the baking quality of wheat flour by degrading physical properties of the dough to release the gas retention. The bread-making quality of dough with fermented potato pulp was much better than that of dough with raw potato pulp. In particular, the bread quality such as specific loaf volume, aspect and crumb grain, except for staling and color, were good. Increase of gas retention and gassing power were the primary reasons for its improvement. They seemed to be related to the decomposition of starch and fiber in potato pulp. Similar results were obtained when residues from the processing of soybean, adzuki bean, sweet potato or sugar beet were fermented by lactic acid producing fungi.

Conclusion and future prospects

In this project, we have characterized lactic acid producing fungi, *Rhizopus oryzae* and *Amylomyces rouxii*, as the basic aspect and perfected making process of silage from potato pulp using lactic acid producing fungi as the application aspect. Fermented potato pulp was found to be beneficial for living organisms and used as a supplement for cereal-based products. Promised technology derived from these results has been developed. Numbers of other results are as follows: 11, applications of a patent; 18, original papers; 5, communications; 11, international meetings, 49, domestic meeting.

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