Utilization of *Lactobacillus gasseri* and *Bifidobacterium bifidum* for meat fermentation

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BACKGROUND

Intestinal lactic acid bacteria, such as *Lactobacillus acidophilus* group and *Bifidobacterium* sp., beneficially affect humans by improving the properties of the indigenous microflora (Havenaar, 1992). These bacteria have been utilized for making fermented dairy products (Arihara et al., 1994). In a past decade, probiotic bacteria, such as intestinal lactic acid bacteria, have been paid more attention for the industrial use. However, to date, these bacteria have not been utilized in the meat industry.

Recently, we demonstrated that several strains of intestinal (probiotic) lactic acid bacteria were applicable to meat fermentation (Arihara et al., 1996, 1997). Of six type strains of *Lactobacillus acidophilus* group species tested, *Lactobacillus gasseri* (predominant *Lactobacillus* species in human intestinal tracts) JCM1131^T exhibited greatest fermentation performance in model sausages. Also, of 25 strains of *Bifidobacterium* tested, *Bifidobacterium bifidum* K202 was selected for meat fermentation.

OBJECTIVES

In this study, *Lactobacillus gasseri* JCM1131^T and *Bifidobacterium bifidum* K202, originated from human intestinal tracts, were applied to meat fermentation.

MATERIALS & METHODS

BACTERIAL STRAINS AND CULTURE CONDITIONS

Lactobacillus gasseri JCM1131^T was obtained from Japan Collection of Microorganisms (Wako, Japan). *Bifidobacterium* bifidum K202, which was isolated from microflora of human intestinal tracts, was from our laboratory collection. All cultures were maintained as frozen stocks kept at -55°C in MRS broth plus 10% glycerin, and prior to use, they were passed at least twice at 37°C in MRS broth. Viable cells of lactic acid bacteria were counted by plating on MRS agar.

PREPARATION OF MODEL SAUSAGE

Fresh pork trim (ham) was ground, mixed with glucose, NaCl, $NaNO_2$, sodium ascorbate, and starter lactic acid bacteria (10^6-10^8 cfu/g meat). The batter (50g each) was stuffed into a high density polyethylene pouches and incubated at 30/37°C for 12-48 h.

GENERATION OF MUTANTS

To obtain the mutants of *Lactobacillus* and *Bifidobacterium* strains which are resistant to both NaCl and $NaNO_2$, cells were plated on the MRS agar containing 3.3% NaCl and 200 ppm $NaNO_2$, and were exposed under UV light for 10 seconds. After two days incubation, colonies generated on the surface of agar plates were picked and tested for their properties.

RESULTS & DISCUSSION

Although both *Lactobacillus gasseri* JCM1131^T (Figure 1) and *Bifidobacterium bifidum* K202 grew well at 30/37°C in model sausages, respectively (Table 1), a single culture of *Bifidobacterium bifidum* gave undesirable flavor due to its acetic acid production. Since *Bifidobacterium* is most significant bacteria contributing to the human health in the intestinal tracts, utilization of this bacterium is desirable for producing a healthy meat product. Next experiments were conducted to use the mixed culture of

Lactobacillus gasseri and *Bifidobacterium bifidum* to improve the quality of products. We have established the conditions for fermenting meats by using the mixture of *Lactobacillus gasseri* 1131^T (10^7 cfu/g meat) and *Bifidobacterium bifidum* K202 (10^8 cfu/g meat). For depressing the overgrowth of *Lactobacillus gasseri* JCM1131^T during fermentation and storage, it was desirable that fermentation was carried out at 30°C in meat containing 0.5% glucose.

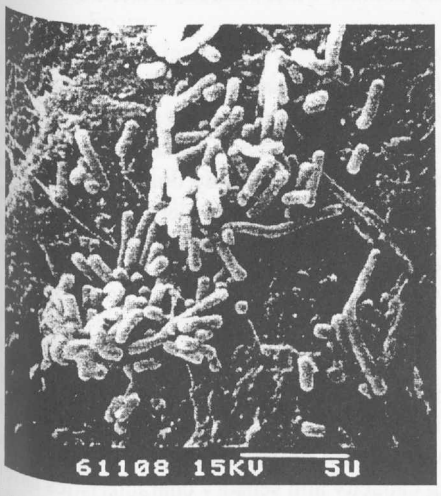

FIGURE 1. SEM of the inside dissected surface of a Model Sausage Fermented with *L. gasseri* JCM1131T.

TABLE 1. Changes in Viable Cells of *L. gasseri* JCM1131^T and *B. bifidum* K202 in Model Sausages during Fermentation at 37°C and Storage at 4°C.

Species/Strains	Log_{10} cfu/g sausage					
	Fermentation (h)			Storage (d)		
	0	12	24	1	4	7
L. gasseri JCM1131^T	6.0	6.7	8.9	8.9	8.7	8.3
B. Bifidum K202	6.0	6.3	7.2	7.4	7.8	8.2

Sausages were fermented with 10^6cfu of each strain/g meat.

Since both two strains were relatively sensitive to NaCl and $NaNO_2$, further efforts were directed to obtain the mutants of these strains showing resistance to these components. Several mutants (e. g. 1131-M8) of *Lactobacillus gasseri* JCM1131^T, which resisted to both components, were obtained by UV irradiation (Table 2). Protein extracted from the cell surface of these mutants were slightly different from those of the original strain. Similar experiments for obtaining the mutant of *Bifidobacterium bifidum* K202 are now in progress in our laboratory.

TABLE 2. Resistance of *L. gasseri* Strains to NaCl and $NaNO_2$ in Model Sausages.

Strain	pH of Sausages after 24h Fermentation	
	Without NaCl, $NaNO_2$	With 3.3% NaCl, 200 ppm $NaNO_2$
JCM1131^T	4.3	5.8
1131-M8	4.4	4.6

CONCLUSIONS

Intestinal lactic acid bacteria strains, *Lactobacillus gasseri* JCM1131^T and *Bifidobacterium bifidum* K202, were applicable to meat fermentation. Especially, a combination of these two strains has great possibility for producing a new type healthy meat product. Also, the mutants of *Lactobacillus gasseri* JCM1131^T improved its utility as a meat starter culture.

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