UTILIZATION OF PROBIOTIC LACTIC ACID BACTERIA FOR MEAT PRODUCTS

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BACKGROUND

Probiotics have been defined as a culture of live microorganisms that, applied to humans or animals, beneficially affect the host by improving the properties of the indigenous microflora[2]. Probiotic lactic acid bacteria from human intestinal tracts, such as *Lactobacillus acidophilus* grouping and *Bifidobacterium* spp., have been widely utilized for healthy fermented dairy products (e.g., acidophilus milk)[1]. However, these bacteria have not been applied to meat products, although several non-intestinal *Lactobacillus* species/strains have been utilized for fermented meats[3]. Thus, meat products with probiotic lactic acid bacteria would have great possibility as unique healthy products in the market.

OBJECTIVES

In this study, we screened lactic acid bacteria (*Lactobacillus*, *Bifidobacterium*, *Enterococcus*) from the intestinal tracts for the meat starter cultures. Efforts were also directed to characterize the selected strains and apply them to meat fermentation for developing novel healthy meat products.

MATERIALS & METHODS

BACTERIAL STRAINS

Type strains of *Lactobacillus acidophilus* grouping (*L. acidophilus* JCM1132^T, *L. crispatus* JCM1185^T, *L. amylovorus* JCM1126^T, *L. gallinarum* JCM2011^T, *L. gasseri* JCM1131^T, *L. johnsonii* JCM2011^T) used in this study were obtained from Japan Collection of Microorganisms (Wako, Japan). Cultures of intestinal *Lactobacillus* (200 strains) and *Bifidobacterium* (25 strains) used for the screening were obtained from our collection. Twenty-four *Enterococcus* strains were isolated from human faeces on MRS (Difco Laboratories, Detroit MI) agar plates containing 3.3% NaCl and 200ppm $NaNO_2$ under anaerobic condition at 37°C. 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 screw-capped test tubes containing MRS broth.

Four enterotoxin-producing *Staphylococcus aureus* strains, R090101 (enterotoxin A), R090104 (enterotoxin B), R090105 (enterotoxin C) and R090106 (enterotoxin D), were obtained from our culture collection.

PREPARATION OF MODEL SAUSAGES

Fresh pork trim (ham) was ground, mixed with glucose, NaCl, $NaNO_2$, sodium ascorbate, and starter lactic acid bacteria (10^6-10^7cfu/g meat) and/or *Staphylococcus aureus* strains (10^6cfu/g meat). The batter (50g each) was stuffed into a high density polyethylene pouches and incubated for 12-48 h.

MICROBIOLOGICAL ANALYSES

Viable counts of lactic acid bacteria and *S. aureus* were determined by plating on MRS and Vogel-Johnson agar (Eiken, Tokyo, Japan), respectively.

Enterotoxin production by *S. aureus* was detected by reversed passive latex agglutination method. This assay was carried out by a commercial kit, SET-RPLA-SEIKEN (Denka Seiken Co., Tokyo, Japan).

ACID AND BILE TOLERANCE OF LACTIC ACID BACTERIA

Low pH and bile-tolerance were tested to estimate the resistance of lactic acid bacteria under

conditions simulating those of the stomach and intestines of men. The survival of lactic acid bacteria strains was studied by the addition of the cell suspension into a series of sterile distilled water at pH 1, 2, 3. The incubation mixture was maintained at 37°C, and the viable organisms were enumerated at 0, 0.5, 1.0, 3.0, and 5.0 h. Also, the cell suspension were plated on MRS agar plates containing various concentrations (0 to 2,000ppm) of bile. The bile tolerance was estimated by the cell growth on the plates.

RESULTS & DISCUSSION

Of 6 type strains of *Lactobacillus acidophilus* grouping tested, *L. gasseri* JCM1131^T demonstrated the highest growth rate in model sausages. Also, this strain inhibited the growth of *Staphylococcus aureus* and accompanying enterotoxin production in meat during fermentation. From these results, some intestinal lactic acid bacteria would be applicable to meat fermentation. Although *L. gasseri* JCM1131^T ferments meat well, this strain was relatively sensitive to $NaNO_2$. Since it is regulated to use 200ppm $NaNO_2$ and 3.3% NaCl, and process under 20°C for non-heat treated meat products in Japan, further screening of probiotic *Lactobacillus* strains for meat fermentation were carried out.

Of 200 strains of intestinal lactobacilli tested, 2 strains (*Lactobacillus rhamnosus* FERM P-15120 and *L. casei* ssp. *alactosus* FERM P-15121) cleared these regulations. These 2 strains were sufficiently resistant to gastric acid and bile, thus they are capable of surviving against hurdles of the low pH environment of the stomach and the presence of bile acids in the intestines. These strains also gave products with satisfactory sensory properties.

For their therapeutic activity, *Bifidobacterium* spp. are widely used for dairy products especially in Japan. Of 25 strains of *Bifidobacterium* strains tested, several strains (e.g., *B. bifidum*) showed the growth in meat and dropped its pH. However, they were all sensitive to 200ppm $NaNO_2$, and gave undesirable flavor due to their acetic acid production. Utilizing a mixed culture of microorganisms (for example, with *Lactobacillus* spp.), is one possible way for applying *Bifidobacteium* spp. to meat fermentation.

Enterococcus spp. are another group of probiotic lactic acid bacteria. Twenty-four newly isolated strains from human faeces in this study were identified as *Enterococcus faecium* (23 strains) and *E. faecalis* (1 strain), respectively. Since these strains demonstrated the rapid growth in meat and were resistant to gastric acid and bile, further studies for characterizing some of these strains as starter cultures are now in progress. Although enterococci are often isolated from cheese, and have been mentioned for use as starter cultures[4], recent studies have established the pathogenic potential of these organisms. However, since the enterococci identified in this study were non-hemolytic, they are presumably non-pathogenic strains.

CONCLUSIONS

Although to date, probiotic lactic acid bacteria have not been utilized for meat products, in this study it was demonstrated that the selected strains are applicable to the meat fermentation. Thus, the probiotic bacteria have great potential for developing unique healthy meat products.

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