Utilization of spore-forming lactic acid bacteria for meat products

K. Arihara, M. Itoh, and Y. Kondo

Faculty of Animal Science, School of Veterinary Medicine and Animal Sciences,
Kitasato University, Towada-shi 034-8628, Japan

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BACKGROUND

Probiotic lactic acid bacteria, such as *Lactobacillus acidophilus* group and *Bifidobacterium* sp., have been long utilized for fermented dairy products (Arihara et al., 1994). These bacteria beneficially affect humans by improving the properties of the indigenous microflora (Havenaar, 1992). In a past decade, probiotic bacteria (e.g., intestinal lactic acid bacteria) have been paid more attention for the industrial use. We have been studied application of intestinal (probiotic) lactic acid bacteria to meat fermentation (Arihara et al., 1997, 1998; Sameshima et al., 1998). Several intestinal strains of lactic acid bacteria were well applicable to meat fermentation. However, these strains of bacteria are relatively sensitive to heat treatment. Therefore, if we expect therapeutic effects of these bacteria, we can utilize them only for meat products without heat treatment.

It has been reported that spore-forming lactic acid bacteria (e.g., *Bacillus coagulans*) are stable to heat treatment (Yanagida et al., 1987). *B. coagulans* have been utilized for probiotics and foods industrially. However, to date, application of the spore-forming lactic acid bacteria to meat products have not been studied. Since *B. coagulans* have probiotic effects, meat products with these bacteria would have great possibility as unique healthy products .

OBJECTIVES

In this study, efforts were directed to characterize spore-forming lactic acid bacteria (*Bacillus coagulans*) and apply them to meats for the development of new healthy meat products.

MATERIALS & METHODS

Bacillus coagulans strains were recovered from a commercial food additive product LACRIS-S (Sankyo, Tokyo, Japan), that contains *B. coagulans* cells (approx. 5.0 x 10^9 cfu/g). MRS agar plates were used for their isolation at 37°C. Cultures were maintained as frozen stocks kept at -55°C in MRS broth plus 10% glycerin. For some experiments, bacteria were propagated in MRS broth and their washed cells were freeze-dried. Viable cells of *B. coagulans* were counted by plating on MRS agar at 37°C for 2 days.

Heat-tolerance of bacteria was tested in a series of distilled water containing *B. coagulans* cells (10^8 cfu/ml). Solutions were heated at 4-200°C for 0-2 h. After respective periods for heating, the viable cells of solutions were counted on agar plates.

Low pH (acid) and bile-tolerance were tested to estimate the resistance of bacteria under conditions simulating those of the stomach and intestines of men. The survival of bacteria 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 0-5 h. Also, the cell suspensions were plated on MRS agar plates containing various concentrations (0-2,000 ppm) of bile. The bile tolerance was estimate by the cell growth on the plates.

Model sausages were prepared in this study. Fresh pork trim (ham) was ground, mixed with glucose, sodium chloride, sodium nitrite, sodium ascorbate, and *B. coagulans* cells (10^6-10^8 cfu/g meat). The batter (50g each) was stuffed into a high density polyethylene pouches and incubated.

RESULTS & DISCUSSION

Bacterial strains isolated from LACRIS-S were gram-positive, sporulating, catalase-positive rods, and produced a considerable amount of L(+)-lactic acid. Since other phenotypical properties were identical with those of *Bacillus coagulans,* all isolates in this study were identified as *B. coagulans.* These isolates reduced pH of liquid media and model sausages containing glucose. The pH reached to 4.6 after extensive incubation (fermentation).

In distilled water, *B. coagulans* cells survived at 100°C for more than 2 h or at 150°C for 1 h. Also, levels of viable cells of *B. coagulans* in model sausages were well maintained during the boiling procedure (70°C, 30-60 min).

B. coagulans strains exhibited resistance against sodium chloride and sodium nitrite. They grew in distilled water or model sausages containing 5% sodium chloride and 300 ppm sodium nitrite (as evidenced by a pH drop after incubation). During storage of the model sausage with *B. coagulans* (with/without fermentation periods), levels of viable cells were maintained at least for two weeks. This aspect is important for the utilization of probiotic bacteria. Also, such strains must survive in the environment with gastric acid and bile, when viable cells go through the gastrointestinal tract. *B. coagulans* tested in this study had satisfactory resistance characteristics. They survived at pH 1 for 12 h and grew with 2,000 ppm bile acids.

Model sausages extensively fermented with *B. coagulans* slightly deteriorate their sensory properties. Thus, utilization of these bacteria without fermentation periods is one approach to give therapeutic (probiotic) properties to meat products. It is also to be expected that combination of several strains of lactic acid bacteria having flavor developing ability.

CONCLUSIONS

Spore-forming lactic acid bacteria, *Bacillus coagulans,* were highly stable against heat treatment. Also, these organisms resisted sodium chloride, sodium nitrite, gastric acid and bile. The results from this study, along with previous work about the probiotic effects of *B. coagulans*, suggest that spore-forming lactic acid bacteria could be effectively utilized for meat products to develop healthy meat products.

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