Original paper

Hydrolysis of Pork Myofibrillar Proteins during Fermentation Using Starter Cultures of Lactobacillus bulgaricus and Streptococcus Thermophilus

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We examined the effects of Lactobacillus delbrueckii subsp. bulgaricus (Lb. bulgaricus) and Streptococcus thermophilus (St. thermophilus) starter cultures on the proteolytic processes occurring in fermented pork. The hydrolysis of myofibrillar or myosin B proteins was evaluated using SDS-PAGE. Significant hydrolysis was observed in myofibrillar proteins from the fermented pork with lactic acid bacteria (LAB) cultures and the incubated pork with glucono-delta-lactone (GDL), in contrast to the control sample incubated without LAB or GDL. Although the specific proteolytic activities of each strain towards myofibrils were unclear, their hydrolysis of myosin B was clearly demonstrated. We concluded that myofibrillar protein hydrolysis occurring during the fermentation of pork is due to the combined action of meat endogenous proteases and proteases from Lb. bulgaricus and St. thermophilus.

Keywords: Lactobacillus bulgaricus, Streptococcus thermophilus, fermentation, proteolysis, myofibril, myosin B

Introduction

Lactic acid bacteria (LAB) are widely used throughout the world in fermented products such as yogurt and cheese. LAB produce many metabolites such as organic acids (e.g., lactic acid or acetic acid), ethanol, aromatic compounds, bacteriocins, expopolysaccharides (EPS), and several enzymes (Axelsson et al., 2004; Leroy and Vuyst, 2004; Leroy et al., 2006). LAB prolong the shelf life and enhance microbial safety, improve texture, and contribute to the pleasant sensory profile of the end products. Because of such benefits, LAB are often used as starter cultures in fermented foods.

In recent years, consumers have become concerned about the relationship between food and health. Consequently, the market for foods with health-promoting effects, so-called “functional foods”, has seen remarkable growth during the last decade. The use of functional starter cultures in the dairy industry has recently become popular. Several probiotic LAB strains in fermented milk products have health-promoting properties, such as the modulation of intestinal flora, anti-carcinogenic activity, alleviation of symptoms of cedar pollinosis, and immune enhancement (Työppönen et al., 2003; Leroy and Vuyst, 2004; Salminen et al., 2004; Guo, 2009).

In the meat industry, LAB are commonly used as starter cultures in dry-fermented sausages. In particular, Lactobacillus (Lb.) sakei, Lb. curvatus, Lb. plantarum, Lb. fermentum, Lb. brevis, Lb. casei, Lb. pentosus, Pediococcus (Ped.) acidilactici, and Ped. pentoseaeus are widely used as meat starter cultures in the processing of fermented sausages (Työppönen et al., 2003; Feiner, 2006). Several researchers have proposed the use of probiotic meat starter cultures for dry-fermented sausage manufacture (Hugas and Monfort, 1997; Arihara et al., 1998; Incze, 1998; Papamanoli et al., 2003; Pennacchia et al., 2004; Ammor and Mayo, 2007). While numerous LAB are used in the manufacture of various fermented dairy products, the application of LAB in meat products is rare, except for dry-fermented sausages.

Recently, we developed a dry-fermented meat product,
fermented pork jerky, using *Lb. bulgaricus* and *St. thermophilus* starter cultures which are often used in the manufacture of yogurt (Ohashi and Negishi, 2011; 2012). The pork jerky is processed using LAB fermentation for 6 h at 43°C, followed by seasoning and then drying at 20°C. Fermented pork jerky processed using a combination of strains had a tender texture compared to common commercial jerky (Ohashi and Negishi, 2012), possibly due to hydrolysis of myofibrillar proteins during fermentation (Ohashi and Negishi, 2012).

Numerous reports indicate that sarcoplasmic and myofibrillar proteins degrade during the fermentation or ripening of dry-fermented sausages (Fadda et al., 1999a, b; Kenneally et al., 1999; Sanz et al., 1999a, b; Hughes et al., 2002; Fadda et al., 2010a, b; Sriphochanart and Skolpap, 2010). *Lactobacillus* spp., e.g., *Lb. plantarum* (Fada et al., 1999a, b; 2010a, b), *Lb. sakei* (Sanz et al., 1999b; 2002), *Lb. casei* (Sanz et al., 1999a), and *Lb. curvatus* (Fada et al., 1999b), have been used as LAB starter cultures for fermented sausages. The proteolytic effects of *Lb. bulgaricus* or *St. thermophilus* starter cultures in fermented meat have not been reported. Further, it is unclear whether the effects of proteolysis during ripening of the fermented sausages are due to the activity of the starter cultures or to the action of endogenous meat enzymes (Casaburi et al., 2007).

In the manufacture of fermented pork jerky, we paid attention to the hydrolysis of myofibrillar proteins during fermentation as one of the major factors determining meat tenderness. Thus, the hydrolytic effects of *Lb. bulgaricus* or *St. thermophilus* isolated from the LAB starter cultures, and the combination of both strains, on myofibrillar proteins were studied using SDS-PAGE. Our objective was to examine the effects of *Lb. bulgaricus* or *St. thermophilus* on the proteolytic processes occurring in fermented pork to determine if proteolysis is due to the action of endogenous meat enzymes or the activity of proteolytic LAB enzymes, or both.

**Materials and Methods**

**Pork** Frozen pork round was purchased from a commercial processor (Aichi, Japan) and thawed for 3 days at 2–3°C prior to use. Pork semimembranosus muscle was removed from the round and cleaned of visible fat and connective tissue, then sliced into 3.5 mm pieces using a meat slicer (AC-300C, Takazi Co. Ltd., Tokyo, Japan). The sliced pork was immediately vacuum-packed using plastic bags (nylon (ON) 15 µm/polyethylene (PE) 60 µm) and stored until use at −30°C after quick freezing at −35°C.

**Preparation of pork myofibrils** Myofibrils were prepared by a modification of the procedure of Olson and Parrish (1977), as previously described (Negishi and Yoshikawa, 1993). The sliced semimembranosus pork (−30°C) was thawed and ground (diameter, 3 mm). The ground muscle (4 g) was homogenized (15,000 rpm) under ice-cold water with a Waring Blender for 1 min at 4°C in 10 vol (v/w) isolation medium (100 mM KCl, 20 mM KH₂PO₄ (pH 7.0), 5 mM EDTA, 1 mM MgCl₂ and 1 mM NaNO₃), then again homogenized for 1 min after pausing for 30 s. The homogenization steps were repeated twice. The homogenate was centrifuged at 1,000 × g for 15 min and the supernatant was decanted. The sediment was re-suspended in 10 vol (v/w) of isolation medium containing 0.2% Triton X-100 (w/v) (Kitamura et al., 2010), centrifuged again at 1,000 × g for 15 min, and the supernatant was decanted. This procedure was repeated twice. The sediment was again re-suspended in 2.5 vol (v/w) of isolation medium and passed through a stainless steel strainer (18 mesh) to remove connective tissue and debris. An additional 2.5 vol (v/w) of isolation medium was used to wash the myofibrils on the strainer, the myofibrils were centrifuged, and finally, the sedimented myofibrils were suspended in 5 vol (v/w) of the original isolation medium.

**Preparation of pork myosin B** Myosin B from pork was prepared by using a modification of the procedure of Briske and Fukazawa (1971). The ground m. semimembranosus described above was blended in 3 vol (v/w) of 4°C Weber-Eddsall solution (0.06 M KCl, 0.04 M NaHCO₃ and 0.01 M Na₂CO₃) and incubated at 4°C for 24 h. Then, the suspension was mixed after adding 2 vol (v/v) of Weber-Eddsall solution and centrifuged at 20,000 × g for 60 min at 4°C to provide the supernatant. The supernatant was mixed with 3 vol (v/v) distilled water for 5 min and centrifuged at 80,000 × g for 10 min at 4°C. The precipitate was re-suspended with 3 vol (v/w) of 1 M KCl and the sediment was removed by centrifugation at 20,000 × g for 60 min at 4°C. The resultant supernatant was again blended with 3 vol (v/v) distilled water for 5 min and centrifuged at 80,000 × g for 10 min at 4°C. The resuspension in distilled water and centrifugation procedure was repeated twice. The resultant myosin B precipitate was re-suspended in the same volume (v/w) of 1 M KCl and dialyzed for 6 h at 4°C against 0.6 M KCl. Following dialysis, the supernatant containing myosin B was obtained by centrifugation (20,000 × g for 60 min at 4°C) and used to evaluate the hydrolytic action of LAB.

**Preparation of LAB starter cultures and isolation of LAB strains** *Lb. bulgaricus* and *St. thermophilus* (DVS YC-380) LAB starter cultures were obtained from Christian Hansen (Horsholm, Denmark). The starter was added at 1.5 units/L medium composed of 10% whey powder (Meiji Co., Ltd., Tokyo, Japan), 0.5% D-glucose (Kanto Chemical Co., Inc., Tokyo, Japan) and 0.5% yeast extract (Oxoid, Cambridge, UK) and incubated at 43°C until the pH decreased to 4.6. *Lb. bulgaricus* and *St. thermophilus* strains were isolated from the LAB starter cultures using Lactobacilli MRS Broth (Difco, Detroit, MI, USA) and M17 Broth (Difco) medium, respectively, containing 1.5% (w/v) agar (Wako, Tokyo, Japan). *Lb. bulgaricus*, *St. thermophilus*, and a combination of both strains after fermentation were used in proteolytic assays of pork, myofibrils, and myosin B.

**Hydrolytic action of LAB starter cultures on whole muscles** First, the sliced pieces of pork were immersed in an equal volume (w/w) of LAB starter culture in plastic bags (polyethylene 30 µm)
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and fermented by incubating 2, 4, and 6 h at 43°C. The myofibrils were prepared from the fermented pork according to the procedure described in above. The hydrolysis of the myofibrillar proteins was monitored by SDS-PAGE analysis and the meat pH was determined. Second, the effects of endogenous meat proteases on the hydrolysis of pork during incubation were evaluated as follows. The sliced pieces of pork were incubated for 2, 4 h and 6 h at 43°C in an equal volume (v/w) of 1.0, 2.0 or 3.0% glucono-delta-lactone (GDL) solution in order to exclude the effect of LAB cells. The hydrolysis of myofibrillar proteins during incubation was monitored by SDS-PAGE analysis and the pH was determined. As control samples, pork slices sterilized with 1 mM chloramphenicol (Sigma-Aldrich Co., St. Louis, MO, USA) and 1 mM cycloheximide (Nacalai Tesque, Inc., Kyoto, Japan) were incubated in plastic bags and processed as above.

Hydrolytic action of LAB on myofibrils or myosin B  Lb. bulgaricus, St. thermophilus, or a combination of both strains, were harvested by centrifugation (3,000 × g for 10 min at 4°C) and washed twice in 0.085% (w/v) NaCl. Suspensions of GYP medium and 4.0 mg/mL of myofibrils or 2.0 mg/mL of myosin B were prepared. GYP medium is composed of 1.0 g glucose, 1.0 g yeast extract, 0.5 g peptone, 0.2 g meat extract, 0.2 g sodium acetate, 1 mg MgSO4·7H2O, 1 mg MnSO4·H2O, FeSO4·7H2O, 1 mg NaCl, and 50 mg Tween80 in 100 mL distilled water (pH6.8). The harvested LAB cells were added to GYP medium at 10⁶ cfu/mL and incubated for 6 and 24 h at 43°C. Additionally, GYP medium containing myofibrils or myosin B was incubated for 6 and 24 h in 2.0% GDL to exclude the effect of LAB cells. Control samples were prepared adding 1 mM chloramphenicol and 1 mM cycloheximide to the GYP medium. LAB cells were counted using Plate Count Agar with BCP (BCP, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan). The hydrolysis of myofibrillar or myosin B proteins during incubation was monitored using SDS-PAGE and the pH values of the suspensions were measured.

pH At each sampling point, the pH of the samples was measured using a pH meter (SevenEasy; Mettler Toledo, Greifensee, Switzerland). The pH values of the liquid samples (i.e., the LAB starter cultures, myofibril, or myosin B suspensions) were directly measured. In the case of solid samples (i.e., sliced pork), 5 g samples were blended with 45 mL distilled water using a homogenizer for 2 min at 15,000 rpm, then the pH of the suspension was measured.

SDS-PAGE SDS-PAGE was performed according to the method of Laemmlli (1970) using a 5 – 20% gradient gel (ATTO Corp., Tokyo, Japan), or a 5% stacking gel combined with a 12.5% separation gel (Negishi et al., 1996). Samples were dissolved in a solution of 8 M urea, 2.5% SDS, 1 mM DTT and 10 mM Tris-HCl (pH 8.0) and boiled for 2 min. Gels were stained with Coomassie Brilliant Blue R-250. For SDS-PAGE of myosin B, the gels were stained with a Silver Stain Kit (AE-1360 EzStain Silver; ATTO Co., Tokyo, Japan) to provide more sensitive protein visualization. Silver staining was performed according to the manufacturer’s instructions. Wide Range SDS-PAGE Protein Standards (TEFCO, Tokyo, Japan) were used as molecular weight markers. The protein concentrations of the myofibril and myosin B suspensions were determined using the biuret procedure, with bovine serum albumin as the standard.

Results and Discussion

Proteolysis of the myofibrillar proteins from LAB-fermented pork SDS-PAGE patterns of the myofibrillar proteins from pork during incubation for 6 h at 43°C are shown in Fig. 1. Significant hydrolysis was observed in the myofibrillar proteins of the pork fermented with LAB cultures, in contrast to the control sample without LAB cultures. The concentration of the bands with molecular weights above 227 kDa (range A), and the myosin heavy chain (MHC) band with a molecular weight of 200 kDa, decreased during incubation. Slight decreases in intensity of the bands at ~100 kDa (possibly α-actinin) and at ~37 kDa (possibly troponin T) were observed during incubation for 4 h or 6 h of the LAB-fermented pork. Further, several new bands at ~50 kDa, ~30 kDa and ~60 – 90 kDa (range B) appeared during incubation. In commercial preparations, the stuffing of dry-fermented sausages includes starter cultures, then the sausages are fermented for 24 – 48 h at 18 – 24°C. During fermentation, the pH falls to 5.0 or lower (Ordóñez and de la Hoz, 2007). Compared to manufactured dry-fermented sausages, the fermented pork we tested was incubated at a much higher temperature (43°C) after addition of the LAB starter cultures, like a yogurt-fermentation. As a result, the pH rapidly dropped to about 4.0, and proteolysis of myofibrils in the fermented pork occurred. The degradation of myofibrillar protein may be similar to the proteolysis of myofibrillar proteins previously reported in dry-fermented sausages (Kenneally et al., 1999; Hierro et al., 1999; Sanz et al., 1999a, b; Fadda et al., 1999b, 2010a; Hughes et al., 2002; Dura et al., 2004; Casaburi et al., 2007, 2008; Sripochanart and Skolpap, 2010). Spaziani et al. (2009) demonstrated that most of the proteolytic activity observed in myofibrillar proteins in traditional Italian low-acid sausages was due to endogenous enzymes, although a contribution of bacterial proteases to the degradation was observed. Fadda et al. (2010a) reported that meat protein degradation occurred during sausage fermentation that may be the result of synergistic action of meat endogenous proteases, LAB proteolytic activity, and acid-induced changes arising from bacterial fermentation metabolism. The degradation of myofibrillar proteins we observed suggested that the activation of acidic muscle proteases, i.e., cathepsins (Saunders, 1994; Zhao et al., 2005; Kemp et al., 2010) and/or proteases (Fada et al., 1999a, b; 2010a, b; Sanz et al., 1999a, b; 2002; Toledano et al., 2011) by the LAB contributed to the hydrolysis of the myofibrillar proteins in the fermented pork with LAB cultures. However, we could not conclude whether the effect of proteolysis of the myofibrils in LAB-fermented pork is due to the activity of...
the LAB cultures or to the action of meat endogenous enzymes, as reported for dry-fermented sausages (Casaburi et al., 2007).

**pH changes in pork treated with GDL** GDL was used as an acidulant to acidify the meat in order to exclude the effect of LAB activity. GDL is recognized as a “Generally Recognized as Safe” food additive and is hydrolyzed to gluconic acid in aqueous solution. Fig. 2 shows the pH changes in pork incubated with LAB cultures or with 1.0%, 2.0% and 3.0% GDL for 6 h at 43°C. The pH values of the samples during incubation are shown under the lane number. Lane 1: molecular weight markers; Lane 2–5: control, 0, 2, 4 h and 6 h, respectively; Lane 6–9: LAB cultures, 0, 2, 4 h and 6 h, respectively. Range A: bands with molecular weights above 227 kDa, range B: bands with molecular weights of 60–90 kDa, range C: bands with molecular weights of 14.3–35.7 kDa. MHC: myosin heavy chain, TnT: troponin-T.

**Proteolysis of myofibrillar proteins in GDL-treated pork** SDS-PAGE patterns of the myofibrillar proteins following incubation of pork with 2.0% or 3.0% GDL for 6 h at 43°C are shown in Fig. 3. The SDS-PAGE degradation patterns were similar for pork treated with 2.0% or 3.0% GDL. The concentration of bands with molecular weights above 227 kDa (range A) decreased during incubation. The concentration of the band corresponding to MHC (∼200 kDa) slightly decreased during incubation. Further, the concentration of bands with molecular weights between 14.3 kDa–35.7 kDa (range C) decreased by 6 h. Therefore, we confirmed that the hydrolysis of pork myofibrils occurs at acidic pH, as shown in the LAB cultures or to the action of meat endogenous enzymes, as reported for dry-fermented sausages (Casaburi et al., 2007).

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h incubation, the incubation was continued for 24 h (Table 1). Figure 4 shows the SDS-PAGE patterns of myofibrillar proteins after incubation for 6 h and 24 h with Lb. bulgaricus, St. thermophilus, and the combination of both strains, respectively. No degradation of myofibrillar proteins in the control (lacking the LAB strains) was observed after incubation for 6 h or 24 h. Fermentation with the LAB strains showed no significant changes after incubation for 6 h, similar to the control. However, after incubation for 24 h, a decrease in the concentration of bands with molecular weights of ~116 – 200 kDa and ~43 kDa, and an increase in the bands with masses of 45 – 116 kDa, were observed. As shown in Table 1, the pH of the medium containing myofibrils with Lb. bulgaricus, St. thermophilus, and the combination of both strains, was 5.86, 5.99, and 5.58 after incubation for 6 h, respectively, whereas after 24 h, the pH decreased to 4.05, 4.09, and 4.05, respectively. As shown in Table 1 and Fig. 4, the pH of the myofibrils incubated with 2% GDL decreased to 4.47 and 4.41 during 6 h and 24 h incubation, respectively. Furthermore, the SDS-PAGE profiles indicated similar patterns of proteolysis after incubation for 24 h with the LAB strains, as described above. The hydrolysis of myofibrillar proteins with the addition of 2% GDL may be due to acid-induced changes. It has been reported that several meat-derived Lactobacillus spp. such as Lb. plantarum (Fada et al., 1999a, b; 2010a, b; Toledano et al., 2011), Lb. sakei (Sanz et al., 1999a, b; 2002), Lb. casei (Sanz et al., 1999a), and Lb. curvatus (Fada et al., 1999a) show proteolytic activity for sarcoplasmic and myofibrillar proteins (Fada et al., 1999a, b; 2010a, b; Sanz et al., 1999a, b; 2002). However, we could not conclude if the effect of proteolysis

**Table 1. pH changes in pork muscle, myofibrils or myosin B during incubation**

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pork muscles</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.56</td>
<td>5.55</td>
<td>5.57</td>
<td>5.56</td>
<td>-</td>
</tr>
<tr>
<td>2.0% GDL</td>
<td>5.30</td>
<td>4.45</td>
<td>4.26</td>
<td>4.20</td>
<td>-</td>
</tr>
<tr>
<td>3.0% GDL</td>
<td>5.23</td>
<td>4.10</td>
<td>4.07</td>
<td>3.93</td>
<td>-</td>
</tr>
<tr>
<td><strong>Myofibrils</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.56</td>
<td>-</td>
<td>-</td>
<td>6.48</td>
<td>6.39</td>
</tr>
<tr>
<td>Lb. bulgaricus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.86</td>
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<tr>
<td>St. thermophilus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.99</td>
<td>4.09</td>
</tr>
<tr>
<td>Lb. bulgaricus + St. thermophilus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.58</td>
<td>4.05</td>
</tr>
<tr>
<td>2.0% GDL</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.47</td>
<td>4.41</td>
</tr>
<tr>
<td><strong>Myosin B</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.50</td>
<td>-</td>
<td>-</td>
<td>6.40</td>
<td>6.30</td>
</tr>
<tr>
<td>Lb. bulgaricus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.77</td>
<td>3.72</td>
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<td>St. thermophilus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.82</td>
<td>3.71</td>
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<tr>
<td>Lb. bulgaricus + St. thermophilus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.61</td>
<td>3.69</td>
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<tr>
<td>2.0% GDL</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.37</td>
<td>4.30</td>
</tr>
</tbody>
</table>

1Sliced pieces of pork muscle were incubated for 0 – 6 h at 43°C in 2.0 or 3.0% GDL solution. 2GYP medium containing myofibrils or myosin B were incubated with Lb. bulgaricus, St. thermophilus, and Lb. bulgaricus + St. thermodilus for 6 h or 24 h at 43°C. Dashes show data not determined.

**Fig. 3.** SDS-PAGE patterns of myofibrillar proteins from pork during incubation in 2.0% or 3.0% GDL solution for 6 h at 43°C. Control is the same as in Fig. 2. SDS-PAGE was performed using a 5 – 20% gradient gel. The amount of protein loaded onto the gels was 40 µg in each lane. The pH values of the samples during incubation are shown under the lane number. Lane 1: molecular weight markers; Lane 2 – 5: control, 0, 2, 4 h and 6 h, respectively; Lane 6 – 9: 2.0% GDL, 0, 2, 4 h and 6 h, respectively; Lane 10 – 13: 3.0% GDL, 0, 2, 4 h and 6 h, respectively. Range A: bands with molecular weights above 227 kDa, range B: bands with molecular weights of 60 – 90 kDa, range C: bands with molecular weights of 14.3 – 35.7 kDa, MHC: myosin heavy chain.

**Fig. 4.** SDS-PAGE patterns of pork myofibrils during fermentation using Lb. bulgaricus, St. thermophilus, and the combination of both strains, or 2.0% GDL, for 6 h or 24 h at 43°C. SDS-PAGE was performed using gels comprising a 5% stacking gel and a 12.5% separation gel. The amount of protein loaded onto the gels was 30 µg in each lane. The pH values of the myofibrillar protein samples during incubation are shown under the lane number. Lane 1: molecular weight markers; Lane 2 – 4: control, 0, 6 h and 24 h, respectively; Lane 5, 6: Lb. bulgaricus, 6, 24 h; Lane 7, 8: St. thermophilus, 6, 24 h; Lane 9, 10: Lb. bulgaricus + St. thermophilus, 6, 24 h; Lane 11, 12: 2.0% GDL, 6, 24 h, respectively.
was due to acidification by the LAB and action of the meat endogenous enzymes, or to the activity of proteolytic LAB enzymes. Therefore, the proteolytic activities of both LAB strains toward myosin B in the myofibrils were examined according to the above-mentioned procedures.

**Proteolysis of Lb. bulgaricus or St. thermophilus on pork myosin B** The SDS-PAGE profiles of the proteolysis of myosin B using Lb. bulgaricus, St. thermophilus, and Lb. bulgaricus + St. thermophilus are shown in Fig. 5. Significant proteolysis was observed in LAB-fermented myosin B after incubation for 6 h and at 24 h compared to the control or the 2% GDL-added myosin B. As shown in Table 1, the pH changed to pH 4.37 and 4.30 in the 2% GDL-added myosin B after incubation for 6 h and 24 h at 43°C, respectively, but significant proteolysis of myosin B was not observed. A rapid drop in the pH in LAB-fermented myosin B occurred, corresponding to significant hydrolysis of myosin B proteins. The pH decreased to 4.61 – 4.82 and 3.69 – 3.72 after 6 h and 24 h, respectively. The high metabolism of the LAB strains caused the acidification of the medium, and as a result, the proteolytic activities of the LAB strains increased during incubation. The hydrolysis of myosin B after incubation for 6 and 24 h was thus due to the proteolytic systems of Lb. bulgaricus and St. thermophilus.

**Conclusions**

SDS-PAGE profiles of the myofibrillar proteins from fermented pork during incubation with the LAB stains, Lb. bulgaricus and St. thermophilus, for 6 h at 43°C showed significant hydrolysis. We concluded that the hydrolysis of myofibrillar proteins occurring during the fermentation of pork is due to the combined action of meat endogenous proteases and proteases from Lb. bulgaricus and St. thermophilus. Further research is necessary to determine the effects of acid-induced changes in meat during bacterial fermentation metabolism.

**References**


Hydrolysis of Myofibrils during Fermentation


