PROTEOLYTIC ACTIVITY OF STAPHYLOCOCCI AND LACTOBACILLI STRAINS ISOLATED FROM DRY-FERMENTED SAUSAGES

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OBJECTIVE

To determine the proteolytic activity of several micrococci and lactobacilli strains isolated from dry-fermented sausages against sacoplasmic proteins and their possible different contribution to the proteolytic events during the curing process.

METHODS

Two staphylococci (S1 and S2) and two lactobacilli (L1 and L2) strains were originally isolated from the natural flora of dry-fermented sausages. For enzymatic assays the staphylococci and lactobacilli strains were grown in Brain Hearth infusion (Oxoid) and MRS broth (Merk), respectively, at 30 °C, overnight. Cells were harvested by centrifugation (6000 g, 10 mins., 4°C) and the supernatant constituted the extracellular fraction (E). Then, cells were washed and resuspended in 50 mM phosphate buffer pH 7.0. The cells disruption was performed by vigorous agitation with glass beads (0.1 mm, Sigma). After a second centrifugation (15000 g, 20 mins 4°C) the cytosolic fraction (C) was recovered in the supernatant and the pellet resuspended in the same buffer constituted the particulate fraction (P), containing the membrane and the cell-wall fragments.

Sarcoplasmic proteins were extracted from pork meat. 5 g were diluted 1:10 with 50 mM phosphate buffer pH 7.0 and homogenized in a Stomacher 400 blender (London, UK) for 3 mins and, then, centrifuged at 10 000 g for 20 mins at 4 °C. The obtained supernatant constituted the sarcoplasmic extract which was sterilized by filtration through a 0.45 µm membrane filter. The sterility of the filtered protein extract was confirmed by checking the absence of growth in Plate Count agar (Merk) after 48 h incubation at 37 °C.

The enzymatic activity was determined by incubating 0.5 ml each enzymatic fraction (extracellular, cytoplasmic and particulate) and 0.5 ml sarcoplasmic extract in eppendorf tubes at 37 °C. Samples were taken after several incubation times (0, 20, 45, 70 and 125 hrs.). Simultaneously, a control without the addition of any enzymatic fraction was incubated in the same conditions to detect the activity due to muscle enzymes. After each incubation time, 50 µl samples were mixed (1:1) with sample buffer A , boiled for 4 mins and applied into the gel. SDS-Polyacrylamide gel electrophoresis was carried out using 10 % polyacrylamide gels. The protein bands were visualized by Coomassie brilliant blue (R-250) stain. The following reference proteins were run simultaeously: myosin (200 KDa), β-galactosidase (116.2), phosphorylase B (97.4 KDa), serum albumin (66.2 KDa), ovalbumin (45 KDa), carbonic anhydrase (31 KDa), trypsin inhibitor (21.5 KDa), lysozyme (14.4 KDa) and aprotinin (6.5 KDa).

RESULTS AND DISCUSSION

The degree of proteolysis caused by the different enzymatic fractions was estimated by comparison of their protein profiles, obtained from the samples incubated for 125 hrs, with that obtained for the initial control (sarcoplasmic extract without incubation, B1) and the final control (sarcoplasmic extract after 125 hrs incubation, B2).

The extracellular fractions of both staphylococci strains did not show relevant proteolytic activity against sarcoplasmic proteins, since their corresponding banding patterns only showed a slightly intensity reduction in the high (160, 110, 100, 84, 76-71 KDa) and low (33-28 KDa) molecular mass bands (see lanes S1E, S2E) when compared to the initial control (lane B1). However, the strain S2 seemed to have higher activity than S1 as can be deduced from the lower intensity detected in the 100 KDa band and the additional formation of a 93 KDa band and other low molecular bands (12-10 KDa) as a result of its proteolytic activity. Despite the fact that the hydrolysis of the high molecular band proteins could be attributed to muscle enzymes, the additional bands observed in the S2E lane, quoted previously, were not present in the controls (see lanes, B). On the other hand, the banding patterns corresponding to the activity from lactobacilli extracellular fractions revealed a strong activity of the assayed strains against muscle proteins being the L2 the most active one (see lanes L1E and L2E). In both cases, the 160 and 110 KDa bands disappeared and the 100 KDa band was clearly weakened, especially in the case of L2. Besides, some protein bands in the range 84-71KDa and the 56 KDa band were also hydrolyzed. The intensity of the strongest bands situated in the range 45-30 KDa was reduced and some of them were completely hydrolyzed (40,33-31 KDa). The changes in these intermediate molecular mass bands were notably stronger than those caused by endogenous enzymes (see lane B2). The hydrolytic activity also reduced the intensity of low molecular mass proteins (23 KDa) and the front was highlighted by the lower molecular mass protein fragments generated. Thus, lactobacilli strains were considered to be capable of taking part in the proteolysis of sarcoplasmic proteins.

The protein profile corresponding to the proteolytic activity of the particulate fraction from the staphylococci strains were identical showing higher activity than that detected in the extracellular and cytosolic fractions. Thus, in both cases, high molecular bands (100, 110, 84, 76-71 and 62 KDa) were nearly completely hydrolyzed with slightly higher intensity in the case of S1strain (see lanes S1-P, S2-P). The breakdown of these protein bands was more intense than that caused by endogenous enzymes so that it was concluded that bacterial enzymes partly contributed to high molecular weight protein hydrolysis. There were also changes in intermediate molecular mass proteins (45-30 KDa) with the formation of two bands of about 28-25 KDa and a low molecular band of about 10 KDa which were not observed in the control (see lane B2). The protein profile corresponding to the particulate fractions from lactobacilli strains were nearly identical to that corresponding to the activity of muscle enzymes (see lane B2) except for the fact that a double protein band of about 28-25KDa appeared in L2P lane. Therefore, it was deduced that muscle enzymes were mainly responsible for protein hydrolysis in this case.

According to the banding patterns of the cytosolic fractions, low activity was attributed to the staphylococci strains which showed identical protein profiles. The high molecular weight bands were weakened (160-71 KDa) and the 100 KDa and 84 KDa bands completely disappeared. Moreover, the hydrolysis of high molecular mass proteins could be caused by muscle enzymes although their protein profiles were different (see lanes S1-C, S2-C and B2). Additionaly, the changes observed in the range 45-30 KDa were quite similar to that observed in the control (see lane B2) and, consecuently, attributed to endogenous activity. Finally, the proteolytic changes observed in the lanes corresponding to lactobacilli cytosolic fractions were not significative even though higher activity could be assigned to L2 strain (see lanes L1-C and L2-C). High molecular bands were lost or weakened (160- 71 KDa) and similar changes to that observed in the protein profile of the control (see lane B2) were detected in the range 45-28 KDa.

CONCLUSIONS

Lactobacilli strains showed notably higher activity against sarcoplasmic proteins than staphylococci strains. Proteolytic activity slightly differs between strains of the same genus.

Lactobacilli proteases were mainly located in the extracellular fraction and preferently hydrolyzed high and intermediate molecular mass proteins while micrococci proteases remained attached to the cell-envelope (membrane and cell-wall) and mainly hydrolyzed high molecular mass proteins.

Lactobacilli proteases may partly contribute to sarcoplasmic protein breakdown, according to *in vitro* assays and the demostrated accessibility of the enzymes for the substrate.

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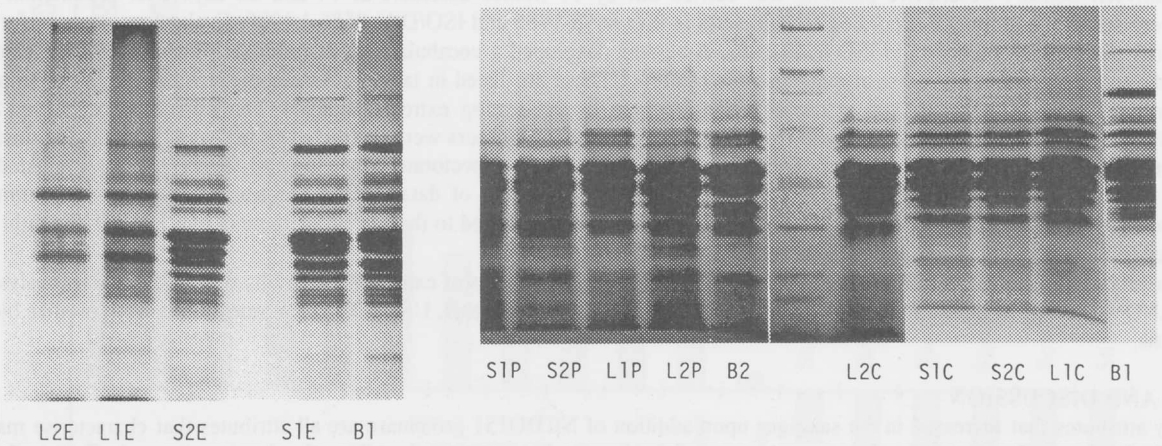

Figure 1.- SDS-polyacrylamide (10%) gel patterns of sarcoplasmic proteins after 125 hours of incubation at 37°C with the extracellular (E), cytoplasmic (C) and particulate (P) enzymatic fractions from two staphylococci (S1 and S2) and two lactobacilli (L1 and L2) strains.