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Research Note

Proteolytic Activity of Lactic Acid Bacteria Strains and Fungal Biota for Potential Use as Starter Cultures in Dry-Cured Ham

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ABSTRACT

During the processing of dry-cured meat products, sarcoplasmic and myofibrillar proteins undergo proteolysis, which has a marked effect on product flavor. Microbial proteolytic activity is due to the action of mostly lactic acid bacteria (LAB) and to a lesser extent micrococci. The proteolytic capacity of molds in various meat products is of interest to meat processors in the Mediterranean area. Eleven LAB and mold strains from different commercial origins were tested for proteolytic activity against pork myosin, with a view to possible use of these strains as starter cultures for Iberian dry-cured ham. Proteolytic activity was tested by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The LAB strains with the highest proteolytic activity were *Lactobacillus plantarum* (L115), *Pediococcus pentosaceus* (Saga P TM), and *Lactobacillus acidophilus* (FARGO 606 TM). The best fungal candidate was *Penicillium nalgiovense* LEM 50I followed by *Penicillium digitatum, Debaryomyces hansenii,* and *Penicillium chrysogenum*.

Dry-cured ham currently is a key feature of Spanish cuisine and is among the traditional Spanish foods enjoying international renown. Both production and exports have been increasing over recent years, and the need for standardization has prompted growing interest in all factors affecting ham quality, particularly ham sensory properties. During drying and ripening, the meat undergoes a number of changes, which result in the entirely new sensory features for which Iberian ham is greatly prized. Microorganisms play a key role in ripening *(13),* a process that requires the presence of molds (especially native molds) that determine the sensory characteristics specific to each producing area. Dry-cured ham quality is affected by a number of factors, including pH, moisture content, water activity, redox potential, NaCl content, lipolysis, and proteolysis *(8)*. By monitoring these physical and chemical parameters, ham producers may succeed in obtaining an almost ideal sensory profile. Bellati et al. *(1)* reported that ripening quality in Parma prosciutto can be monitored by measuring moisture content, NaCl concentration, and proteolytic index.

During the processing of dry-cured meat products, sarcoplasmic and myofibrillar proteins undergo a process of proteolysis that has a marked effect on product flavor. The changes taking place during proteolysis involve the action of both endogenous and microbial enzymes. Molly et al. *(20)* reported that proteolysis in cured and fermented meat products is predominantly caused by endogenous muscle proteases, certainly for the initial endoproteolytic changes in myosin and actin. These changes have been described in detail by Sentandreu et al. *(32)* and more recently by Flores et al. *(12)* and Mora et al. *(21, 22)*. Relatively little is known about the specific contribution of microorganisms to proteolysis in dry-cured ham *(18)*. However, the addition of proteolytic starter cultures with an influence on the amino acid profile may have a direct impact on the sensory attributes of dry-cured ham *(31)*.

Microbial proteolytic activity comes primarily from lactic acid bacteria (LAB) and to a lesser extent from micrococci. The proteolytic capacity of molds for various meat products in the Mediterranean area has become an area of interest. Certain LAB species, mainly belonging to the genera *Lactobacillus, Pediococcus,* and *Leuconostoc,* have proteolytic activity. Fungi, especially molds, also contain enzymes capable of hydrolyzing muscle proteins, thus contributing to proteolysis and the release of amino acids mainly after the endoproteolytic activity has started. Amino acids play a crucial role in determining food flavor *(23, 34),* an effect clearly enhanced by the activity of proteolytic microorganisms.

Proteolytic activity has been reported in general terms in meat products *(26),* but less research has specifically addressed proteolysis in dry-cured ham, which has a much lower surface/volume ratio *(17)*. The role of molds in dry-cured ham is important; in addition to their proteolytic capacity, molds also regulate carbohydrate metabolism, thus contributing to flavor formation and preventing the ham surface from drying out *(33)*. Various *Penicillium* species contain enzymes with proteolytic activity, which are able to produce peptides and amino acids *(4, 7)*. Amino acids both

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contribute to food flavor and act as precursors of volatile compounds *(16)*. Certain strains of *Penicillium* and *Mucor* isolated from cold meats had proteolytic activity against meat proteins both in vitro and in processed cold meats *(35, 36)*. Binzel *(5)* reported that *Eurotium* and *Penicillium* were able to hydrolyze myoglobin. Strains belonging to these genera that have been isolated in Iberian ham also have considerable activity when exposed to myosin *(28)*. Many of these strains also have aminopeptidase activity, especially against methionine substrates; the presence of these fungi in ham may therefore favor characteristic changes in nitrogen components. Analysis of myosin behavior provides a useful indicator of proteolytic activity in pork *(18)*. An extracellular protease obtained from *Penicillium chrysogenum* isolated from dry-cured ham is of interest for flavor generation in dry-cured meat products *(3)* because it hydrolyzes proteins and shortens the processing time.

With regard to yeasts, *Debaryomyces hansenii* strains isolated from Iberian ham have considerable aminopeptidase and proteolytic activity *(24, 28)*. Although activity has mainly been evaluated against specific substrates such as myosin, some strains also are active when inoculated directly into meat *(14, 28)*. Proteolytic activity varies depending on the substrate used *(9)*. Martín et al. *(16)* identified *P. chrysogenum* and *D. hansenii,* the most abundant yeasts in fermented cold meats *(10, 11, 30),* as potential starter cultures on the basis of their proteolytic activity.

The main objective of this work was to test 11 commercial LAB and mold strains for proteolytic activity against pork myosin and to evaluate their possible use as starter cultures for Iberian dry-cured ham.

MATERIALS AND METHODS

The following commercial LAB and mold strains were tested: LP1, *Lactobacillus plantarum* L115 (Rhodia Ibérica, Madrid, Spain); LP2, *L. plantarum* (Raspini SCA, Scalenghe, Italy); PP1, *Pediococcus pentosaceus* (Saga P TM; Lab Amerex, Madrid, Spain); PP2, *P. pentosaceus* (SKW Biosystems, Waukesha, WI); LA1, *Lactobacillus acidophilus* (FARGO 606 TM; Lab Amerex); LA2, *L. acidophilus* (Raspini SCA); PD1, *Penicillium digitatum* (CECT 2954, Burjassot, Spain); PN1, *Penicillium nalgiovense* LEM 50I (Rhodia Ibérica); PN2, *P. nalgiovense* (Chr. Hansen, Hørsholm, Denmark); PC1, *Penicillium chrysogenum* (Schneider TM; Schneider-Soprosal, Bloney-Vevey, Switzerland); and DH1, *D. hansenii* LEM 50I (Rhodia Ibérica). Proteolytic activity was monitored using myosin from pork muscle (myosin M-0273, Sigma, St. Louis, MO) and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) *(2)*. Samples were prepared following the method of Rodríguez et al. *(28)*.

Sample preparation. The myosin solution was prepared by dissolving 50 mg of myosin in a 50% glycerol solution with 0.6 M potassium chloride and potassium phosphate buffer (pH 6.8). The culture medium comprised 1 g of lauryl tryptose broth (31149, Difco, BD, Sparks, MD), 50 ml of distilled water, and 2.5 g of sodium chloride. Later, 25 μg of cycloheximide (Sigma) and 100 μg of chloramphenicol (Sigma) were added to the tubes, which were to be inoculated with LAB and yeasts-molds, respectively. Samples were prepared in a sterile tube, adding 59 μl of the myosin solution and 416 μl of the culture medium. Each tube was then inoculated with the respective microorganism, which had been previously cultured on de Man Rogosa Sharpe agar (LAB) or on potato dextrose agar (fungal strains). Samples were incubated at 30°C for 7 days (LAB) or at 25°C for 15 days (fungal strains). For control purposes, an appropriate molecular weight marker (marker M3788, Sigma) was added to the myosin solution and incubated under the same conditions.

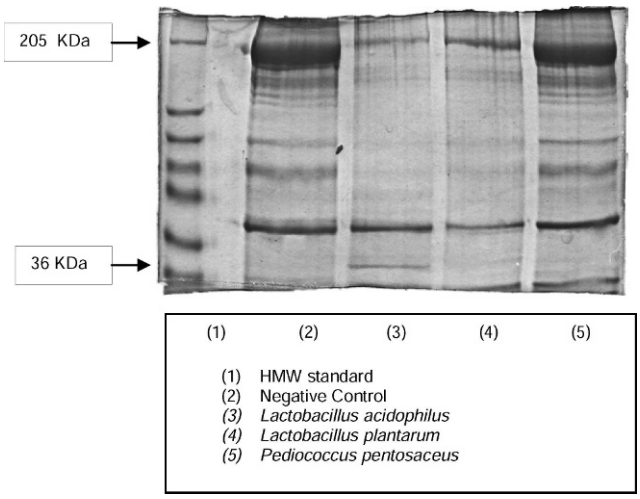

FIGURE 1. *Electrophoretic profile (proteolytic activity when used with pork myosin) of different LAB strains commercialized in Spain.*

Detection of proteolytic activity on myosin. After incubation, 0.1 of each cultured sample was mixed with 10 μl of a 0.01 M phosphate buffer (pH 7.1) containing 1.5% (wt/vol) SDS and 1% (wt/vol) β-mercaptoethanol. Samples were boiled for 5 min to denature proteins. Myosin hydrolysis was monitored by 12.5% (wt/vol) SDS-PAGE *(2)*. Myosin (220 kDa) and myoglobin (14 kDa) (both from Sigma) were used as standards. Electrophoresis was carried out at 30 mA and 10 to 15°C. Proteins were visualized by staining with Coomassie brilliant blue R-250.

RESULTS AND DISCUSSION

Figures 1 and 2 show the proteolytic activity on myosin of the following LAB strains marketed in Spain and their equivalents marketed in Italy: *L. acidophilus, L. plantarum,* and *P. pentosaceus*. Analysis of electrophoresis profiles indicated that *L. plantarum* (L115), *P. pentosaceus* (SAGA P TM), and *L. acidophilus* (FARGO 606 TM) had higher levels of proteolytic activity than did *L. acidophilus* (Raspini SCA), *P. pentosaceus* (SKW Biosystems), and *L. plantarum* (Raspini SCA). Thus, the former three strains were considered the best candidates for use as starter cultures.

Of the selected LAB strains (Fig. 1), *L. acidophilus* had the highest level of proteolytic activity on myosin; of the nonselected strains (Fig. 2), the highest activity was recorded for *L. plantarum*. Similar findings were reported by Molina and Toldrá *(19),* who identified *P. pentosaceus* and *Staphylococcus xylosus* as the microorganisms most commonly isolated from dry-cured ham; both species had marked in vitro proteolytic activity. Other *Staphylococcus* species have been reported to have high levels of activity on dry-cured ham *(28)*. A number of studies on different foods also have highlighted the high proteolytic activity of LAB. Pescuma et al. *(27),* in a study of the proteolytic activity of

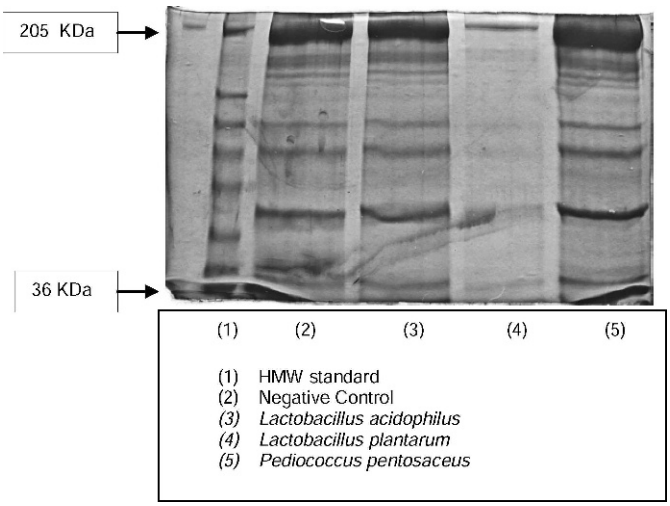

FIGURE 2. *Electrophoretic profile (proteolytic activity when used with pork myosin) of different LAB strains commercialized in Italy.*

various LAB species in whey-based beverages, found intense activity for *Lactobacillus delbrueckii* subsp. *bulgaricus* CRL 454, *L. acidophilus* CRL 636, and *Streptococcus salivarius* subsp. *thermophilus* CRL 804 when used with lactose, alpha-lactalbumin, and beta-lactoglobin. Benito et al. *(3)* noted that *Pediococcus acidilacti* is a major source of proteolytic activity in meat products, including dry-cured chorizo and salchichon sausages.

Figures 3 and 4 show the proteolytic activity of the fungal species *P. nalgiovense* LEM50I, *P. nalgiovense* PN-2, *P. digitatum, P. chrysogenum,* and *D. hansenii*. Analysis of electrophoresis profiles (Fig. 3) indicated that *P. chrysogenum* had higher proteolytic activity on pork myosin than did *P. digitatum* and *D. hansenii*. Rojas *(29)* reported that *Penicillium* is the genus most commonly isolated from dry-cured ham (43.35%), followed by *Aspergillus* (36.99%); the most common species are *P. chrysogenum* and *Aspergillus glaucus*. No significant differences were detected between *P. nalgiovense* LEM 50I and *P. nalgiovense* PN-2 (Fig. 4), which both displayed proteolytic activity similar to that of *P. chrysogenum*. *P. nalgiovense*

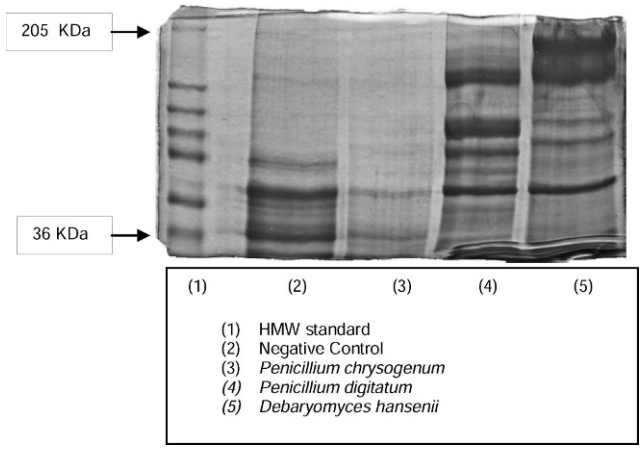

FIGURE 3. *Electrophoretic profile (proteolytic activity when used with pork myosin) of different fungal strains.*

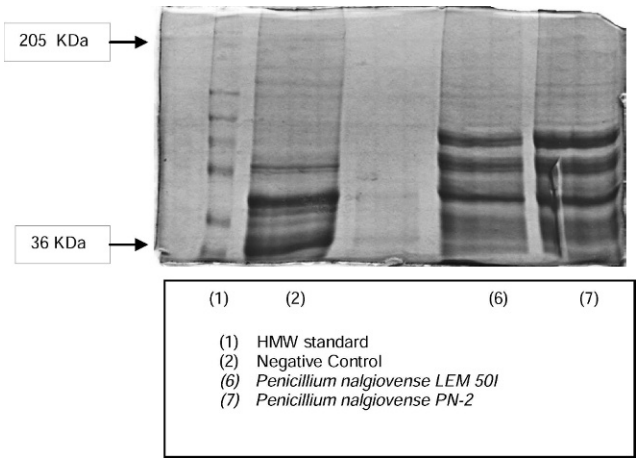

FIGURE 4. *Electrophoretic profile (proteolytic activity when used with pork myosin) of different fungal strains.*

LEM 50I was the only fungal species of potential interest as a starter culture, in combination with *P. digitatum, D. hansenii,* and *P. chrysogenum*.

The results obtained here with molds match those reported by Rodríguez et al. *(28)* and Martín et al. *(16),* who found that *P. chrysogenum* had the highest proteolytic activity on dry-cured ham. Ockerman et al. *(26)* tested five mold strains on Spanish meat products and concluded that *P. chrysogenum* had the highest proteolytic activity. Martín et al. *(15, 17)* noted that the activity of *P. chrysogenum* and *D. hansenii* makes them suitable candidates as starter cultures, for improving the sensory and health-related attributes of meats. Ockerman et al. *(25),* studying five *Penicillium* strains, found that *Penicillium aurantiogriseum* and *Penicillium camemberti* produced the highest quality flavor values in Spanish ham. Bolumar *(6)* reported that *D. hansenii* hydrolyzed pork muscle sarcoplasmic proteins, thus generating large amounts of free amino acids and peptides that contributed considerably to the flavor of meat products.

In conclusion, the proteolytic activity of microorganisms in dry-cured ham can be enhanced by using specific starter cultures. The results of this work and that of other authors indicate that *L. acidophilus* (an LAB) and *P. chrysogenum* (a fungus) were the best candidates of the tested microorganisms for potential use as starter cultures in dry-cured ham.

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