Journal of Applied Microbiology 1998, **85**, 905–912

Evaluation of proteolytic activity of micro-organisms isolated from dry cured ham

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6587/02/98: received 9 February 1998, revised 17 June 1998 and accepted 22 June 1998

M. RODRÍGUEZ, F. NÚÑEZ, J.J. CÓRDOBA, M.E. BERMÚDEZ AND M.A. ASENSIO. 1998. In order to determine the possible contribution of micro-organisms to the ripening of meat products, 48 cocci, 18 moulds and 20 yeasts isolated from dry-cured Iberian ham were evaluated for proteolytic activity. Two specific methods were used: the ability to hydrolyse myosin in broth and, for those strains showing high activities, hydrolysis on both myofibrillar and sarcoplasmic proteins on pork slices. Moulds and cocci showed the highest proteolytic activity for myosin in broth. Both myofibrillar and sarcoplasmic proteins were recovered at lower rates from inoculated than from sterile incubated pork. The deepest changes in myofibrillar and sarcoplasmic proteins were originated by one strain each of *Penicillium chrysogenum* and *Staphylococcus xylosus*, respectively. Only small changes were observed in the concentrations of free amino acids from inoculated pork slices, except for the samples with *P. chrysogenum*, where there were increases in all free amino acids. Thus, *P. chrysogenum* makes a significant contribution to proteolysis during the ripening of dry-cured meat products.

INTRODUCTION

Dry-cured ham is obtained from uncooked hams after 8–24 months of ripening and is characterized by a typical flavour. During this time, an uncontrolled microbial population grows on the surface. Moulds, yeasts and Gram-positive, catalase-positive cocci have been reported as the dominant organisms on the surface of different types of dry-cured ham for most of the ripening time (Giolitti *et al.* 1971; Langlois and Kemp 1974; Francisco *et al.* 1981; Huerta *et al.* 1988; Rodríguez *et al.* 1994; Núñez *et al.* 1996a,b). Most of the Gram-positive bacteria have been characterized as *Staphylococcus* spp., *Micrococcus* spp. and unidentified cocci with a 42·3–51·5% guanine + cytosine content (Baldini *et al.* 1977; Rodríguez *et al.* 1994, 1996). *Penicillium commune*, *P. chrysogenum*, *P. expansum*, *P. aurantiogriseum*, *Eurotium repens*, *E. herbariorum*, *Debaryomyces hansenii* and *D. marama* have been described as the main fungi on dry-cured ham (Comi and Cantoni 1983; Monte *et al.* 1986; Huerta *et al* 1987a; Núñez *et al.* 1996a,b). Micro-organisms may contribute to the proteolysis which takes place on hams, as has been reported for other dry-cured meat products (Sajber *et al.* 1971). However, toxigenic strains of both cocci and moulds have been found on the surface of dry-cured ham (Núñez *et al.* 1996b; Rodríguez *et al.* 1996).

Given the decisive role attributed to proteolysis in the genesis of flavour in meat products (Ventanas *et al.* 1992; Aristoy and Toldrá 1995), any measure which aims to prevent microbial growth on dry-cured ham may lead to a loss of quality. However, the contribution of micro-organisms to proteolysis in dry-cured ham remains unclear. Proteolysis in dry-cured meat products has been attributed mainly to endogenous enzymes (Toldrá *et al.* 1992a). On the other hand, the higher increase of amino acids such as Val, Met, Ile, Leu, His, Phe and Tyr at the surface than at depth in dry-cured ham (Córdoba *et al.* 1994b) could be related to the proteolytic micro-organisms present on the surface.

The regulation of the microbial population growing on ham requires the determination of the proteolytic activity of the micro-organisms concerned. Suitable microbial populations could then be used as starter cultures to prevent the growth of toxigenic strains without affecting the quality of the final product and perhaps shortening the ripening time.

In order to study the proteolytic activity of micro-organisms, an adequate substrate should be used. Casein and gelatine are amongst the substrates which have been used to test the proteolytic activity of micro-organisms isolated from dry-cured ham (Huerta *et al.* 1987b, 1988; Cornejo and Car-

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rascosa 1991). However, the proteases of micro-organisms isolated from meat products may not be active on these substrates. As the protein most hydrolysed in dry-cured ham is myosin (Córdoba *et al.* 1994a), this may be a good choice for the evaluation of organisms for dry-cured meat products. Also, a direct test of isolates for myofibrillar and sarcoplasmic protein hydrolysis on meat samples might be very useful for showing the microbial contribution to dry-cured ham ripening.

The aim of this work was to evaluate the proteolytic activity of moulds, yeasts and Gram-positive, catalase-positive cocci isolated from dry-cured ham, using two specific methods, both to study the contribution of micro-organisms to proteolysis in dry-cured meat products and to select strains for their use as starter cultures.

MATERIALS AND METHODS

Microbial cultures

Forty-eight Gram-positive, catalase-positive cocci, 18 moulds and 20 yeasts isolated from dry-cured Iberian ham at different stages of processing were used. The isolates of cocci had previously been characterized as *Staphylococcus xylosus* (29 strains), *Staph. equorum* (five strains), *Staph. saprophyticus* (five strains), *Staph. cohnii* (three strains), *Micrococcus kristinae* (four strains) and two strains of an unidentified organism with a 42·3–51·5% G + C content (Rodríguez *et al.* 1994, 1996). The isolates of moulds and yeasts had previously been characterized as *Eurotium herbariorum* (two strains), *Penicillium commune* (four strains), *P. chrysogenum* (two strains), *P. echinulatum* (two strains) and one strain each of *P. aurantiogriseum*, *P. brevicompactum*, *P. jensenii*, *P. restrictum*, *P. rugulosum*, *P. viridicatum* and *Paecilomyces variotii* (Núñez *et al.* 1996b) and *Debaryomyces hansenii* (13 strains), *Candida zeylanoides* (two strains) and one strain each of *Candida blankii*, *C. intermedia*, *Pichia carsoni*, *Rhodotorula rubra* and a non-identified yeast (Núñez *et al.* 1996a).

Proteolytic activity on myosin in broth

Organisms were grown in nutrient broth diluted 1 : 50 in water, containing 50 mg ml^{-1} NaCl, 0·71 mg ml^{-1} myosin (Sigma) and 50 $\mu g\ ml^{-1}$ cycloheximide (Sigma) for cocci, and 200 $\mu g\ ml^{-1}$ chloramphenicol (Sigma) for yeasts and moulds. Due to different growth rates of the micro-organisms tested, samples inoculated with cocci were incubated at 30 °C for 7 d, those with moulds at 25 °C for 15 d and yeast at 25 °C for 10 d. The proteins were denatured by boiling 0·1 ml of culture media for 5 min in 0·01 mol l^{-1} phosphate buffer, pH 7·1, with 1·5% SDS and 1% 2-mercaptoethanol. Proteins were then electrophoresed on 5% SDS-PAGE (Weber and Osborn 1969), loading the slots of the electrophoresis gel with 10 μl of samples. Density of the myosin band was measured in an Investigator 2-D Electrophoresis System (Millipore, MA, USA).

Proteolytic activity on meat slices

Pork sirloins were removed from carcasses 24 h after slaughter. The exterior of the muscles was sterilized by searing as described by Dainty and Hibbard (1980). The burnt tissues were removed down to a depth of about 5 mm using sterile instruments in a laminar flow cabinet Bio Flow II (Telstar, Madrid, Spain). Sterile tissues were cut into 1 cm thick slices, and samples of about 50 g were placed in pre-sterilized 250 ml conical flasks and 2·5 g sterile NaCl added. The slices were inoculated with 0·1 ml of cultures, containing approximately 10^6 cfu ml^{-1} of the selected strain, and incubated at 25 °C for 30 d. Sterile slices were also incubated as controls. All samples were incubated in triplicate.

After incubation, total viable counts were determined on Plate Count Agar (Oxoid) at 30 °C, 48 h. Sarcoplasmic and myofibrillar proteins were extracted from 2 g of sample, respectively, with 0·03 mol l^{-1}, pH 7·1, sodium phosphate buffer and 1·1 mol l^{-1} IK + 0·1 mol l^{-1} sodium phosphate pH 7·4, buffer. Both extracts were electrophoresed on SDS-PAGE (Córdoba *et al.* 1994a). The density of the bands obtained was measured as described above for myosin.

For free amino acids analysis, 10 g of sample were deproteinized with 5% sulphosalicylic acid ; free amino acids were derivatized with phenyl isothiocyanate and detected on a liquid chromatograph equipped with a u.v. detector, according to Córdoba *et al.* (1994b).

Statistical analysis

Statistical analysis of data was carried out by one-way analysis of variance, and means were separated by Tukey's honest significant difference test using a StatGraphics software package from Statistical Graphics Corp. (Rockville, MD, USA).

RESULTS

Most of the cocci and moulds tested hydrolysed myosin after incubation in 1 : 50 nutrient broth (Table 1). Of the 66 isolates tested in these two groups, only two strains of *Staph. xylosus*, and one each of *Staph. equorum* and *P. variotii*, were unable to reduce the myosin band by 50%, while 57 of the 66 reduced the myosin band by over 80%. On the other hand, yeasts showed a much lower proteolytic activity, as six of the 13 *D. hansenii* assayed did not hydrolyse more than 50% of myosin and just two reduced the myosin band by over 80%.

Strains showing a high ability to hydrolyse myosin, including one each of the most commonly found organisms during the ham ripening process, were tested for myofibrillar and

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Table 1 Effect of the micro-organism tested on myosin (0·71 mg ml^{-1}) in nutrient broth diluted 1 : 50 (data are given as percentage of protein in relation to uninoculated diluted nutrient broth)

Cocci	%	Cocci	%	Moulds	%	Yeasts	%
Staphylococcus xylosus D_1	91	*Staph. xylosus* $2AA_5$	56	*Eurotium herbariorum* 131	80	*Debaryomyces hansenii* 122	38
Staph. xylosus 3DA	100	*Staph. xylosus* $9AA_{13}$	80	*E. herbariorum* 261	100	*D. hansenii* 123	58
Staph. xylosus 5EA	100	*Staph. xylosus* D_2	100	*Penicillium aurantiogriseum* 321	100	*D. hansenii* 131	74
Staph. xylosus $9BA_2$	100	*Staph. xylosus* $5AA_2$	100	*P. brevicompactum* 221	100	*D. hansenii* 133	40
Staph. xylosus 1BA	100	*Staph. xylosus* $2AA_4$	100	*P. commune* 131	82	*D. hansenii* 161	100
Staph. xylosus $11AA_2$	100	*Staph. equorum* 2AB	90	*P. commune* 222	100	*D. hansenii* 262	28
Staph. xylosus $9AA_3$	84	*Staph. equorum* $1AB_2$	100	*P. commune* 332	100	*D. hansenii* 321	61
Staph. xylosus $8BA_2$	40	*Staph. equorum* $8AB_1$	95	*P. commune* 351	100	*D. hansenii* 342	54
Staph. xylosus $8CA_1$	82	*Staph. equorum* $1AB_3$	100	*P. chrysogenum* 221	100	*D. hansenii* 344	21
Staph. xylosus $9AA_6$	93	*Staph. equorum* $8AB_2$	0	*P. chrysogenum* 222	100	*D. hansenii* 345	100
Staph. xylosus $8BA_3$	100	*Staph. saprophyticus* 2AC	59	*P. chrysogenum* 223	100	*D. hansenii* 355	43
Staph. xylosus $9AA_7$	82	*Staph. saprophyticus* 1EC	87	*P. echinulatum* 321	86	*D. hansenii* 361	39
Staph. xylosus $2AA_1$	100	*Staph. saprophyticus* $2DC_1$	100	*P. echinulatum* 351	60	*D. hansenii* 364	67
Staph. xylosus $9AA_8$	100	*Staph. saprophyticus* 9DC	100	*P. jensenii* 261	100	*Candida zeylanoides* 161	86
Staph. xylosus $2AA_2$	100	*Staph. saprophyticus* 18DC	100	*P. restrictum* 341	100	*C. zeylanoides* 221	53
Staph. xylosus 11BA	84	*Staph. cohnii* 2FD	62	*P. rugulosum* 351	100	*C. blankii* 121	59
Staph. xylosus 20AA	100	*Staph. cohnii* 8BD	100	*P. viridicatum* 221	100	*C. intermedia* 321	51
Staph. xylosus 21AA	100	*Staph. cohnii* 8CD	100	*Paecilomyces variotii* 351	28	*Pichia carsoni* 221	32
Staph. xylosus 18AA	100	*Micrococcus kristinae* $M1_1$	100			*Rhodotorula rubra* 321	83
Staph. xylosus $9AA_{10}$	76	*M. kristinae* $M1_2$	100			Non-identified	88
Staph. xylosus $5AA_1$	100	*M. kristinae* $M1_3$	100				
Staph. xylosus $9AA_{11}$	100	*M. kristinae* M4	100				
Staph. xylosus $8CA_2$	41	Non-identified $M1A_2$	100				
Staph. xylosus $9AA_{12}$	80	Non-identified $M1A_5$	100				

Incubation conditions: 7 d at 30 °C for cocci, 15 d at 25 °C for moulds, and 10 d at 25 °C for yeasts.

sarcoplasmic protein hydrolysis on sterile meat. In addition, one strain each of cocci, moulds and yeasts showing low proteolytic activity against myosin were also assayed on sterile meat, as controls.

Proteolysis in uninoculated sterile pork slices kept at 25 °C for 30 d was quite strong (Table 2). Three myofibrillar and one sarcoplasmic protein could not be detected by PAGE after incubation. In addition, the amounts of another of the remaining sarcoplasmic proteins and two further myofibrillar proteins, were lower than 56% of that in fresh pork.

When pork slices were inoculated with the micro-organisms tested, a further reduction in the area of most proteins was observed (Table 2), even with *Staph. equorum* $8AB_2$, *P. variotii* 351 and *D. hansenii* 344, used as controls for low proteolytic activity.

The decrease in inoculated samples was more pronounced for myofibrillar than for sarcoplasmic proteins. The myofibrillar protein of 67 kDa, reduced to 56% in the uninoculated samples, was not detected at all in any of the inoculated samples. The 37 kDa protein which was relatively unaffected during incubation of sterile pork slices, showed a reduction from about 50% to 100%, depending on the strain inoculated. Nearly all the remaining myofibrillar proteins decreased to less than 50% of the area found in uninoculated samples. The most significant changes were obtained in the samples inoculated with *P. chrysogenum* 222 and *P. commune* 131, where the 220 kDa band (corresponding to myosin) was not detected after incubation.

In the sarcoplasmic fraction, the changes were less significant and more strain-dependent. *Penicillium* spp. were less active than any other group, while *Staph. xylosus* originated the most intense changes. However, most sarcoplasmic proteins found in uninoculated samples after 30 d incubation were still detected in all inoculated samples.

For a few proteins, the amount after incubation was higher in samples inoculated with certain micro-organisms than in the uninoculated ones. The most outstanding examples were found for the 92 kDa myofibrillar protein with cocci and yeast, and the 56 kDa sarcoplasmic protein with the three moulds (Table 2).

The initial concentration observed for most amino acids was typically 10–100 μmol 10^{-2} g^{-1}, with higher levels only

Table 2 Effect of the selected micro-organisms on sarcoplasmic and myofibrillar proteins of sterile pork slices (data are given as percentage of protein in relation to uninoculated samples before incubation)

Proteins (kDa)	Sterile	*Staphylococcus xylosus* $9AA_8$	*Staphylococcus xylosus* 5EA	*Staphylococcus equorum* $8AB_2$	*Staphylococcus equorum* $1AB_2$	*Penicillium chrysogenum* 222	*Penicillium commune* 131	*Penicillium variotii* 351	*Debaryomyces hansenii* 344	*Debaryomyces hansenii* 131
Sarcoplasmic										
91	nd*	1 ± 0·1^{a}	nd	nd	nd	28 ± 28·3^{b}	11 ± 11·2^{ab}	nd	nd	nd
56	27 ± 2·7^{a}	14 ± 3·7^{a}	15 ± 7·7^{a}	49 ± 2·7^{ab}	21 ± 7·7^{a}	80 ± 12·2^{b}	65 ± 0·3^{b}	65 ± 16·7^{b}	28 ± 28·4^{a}	15 ± 3·7^{a}
43	84 ± 0·3^{a}	13 ± 0·4^{b}	9 ± 5·1^{b}	31 ± 0·2^{b}	11 ± 6·7^{b}	62 ± 34·2^{a}	37 ± 12·9^{ab}	78 ± 26·7^{a}	24 ± 6·6^{b}	38 ± 0·0^{ab}
41	72 ± 2·2^{a}	7 ± 2·2^{b}	13 ± 3·7^{bc}	27 ± 1·3^{bc}	17 ± 2·7^{bc}	21 ± 8·6^{bc}	16 ± 3·2^{bc}	33 ± 17·3^{c}	32 ± 6·8^{bc}	8 ± 0·9^{b}
36	77 ± 5·7^{a}	8 ± 0·2^{b}	11 ± 4·7^{b}	56 ± 0·3^{ac}	20 ± 3·4^{bc}	49 ± 24·2^{ac}	20 ± 7·9^{bc}	47 ± 17·8^{bc}	42 ± 23·2^{bc}	64 ± 0·1^{bc}
29	71 ± 3·9^{a}	4 ± 0·2^{b}	10 ± 5·8^{b}	57 ± 1·2^{a}	12 ± 2·4^{b}	46 ± 0·6^{a}	23 ± 8·9^{b}	nd	51 ± 30·3^{a}	nd
Myofibrillar										
220	47 ± 3·3^{a}	17 ± 5·1^{b}	13 ± 5·5^{bc}	6 ± 0·1^{bc}	3 ± 3·0^{c}	nd	nd	nd	17 ± 7·7^{b}	3 ± 3·1^{c}
92	nd	7 ± 7·3	3 ± 3·0	7 ± 7·4	10 ± 9·5	nd	nd	nd	11 ± 3·5	22 ± 1·5
80	nd	4 ± 3·8	nd	nd	nd	nd	nd	nd	nd	nd
70	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
67	56 ± 2·7	nd	nd	nd	nd	nd	nd	nd	nd	nd
43	79 ± 28·6^{a}	23 ± 4·6^{b}	17 ± 3·1^{b}	37 ± 0·1^{c}	18 ± 9·1^{b}	10 ± 9·7^{b}	9 ± 5·1^{b}	25 ± 16·2^{b}	54 ± 8·9^{ac}	21 ± 5·4^{b}
37	98 ± 3·5^{a}	24 ± 5·9^{bc}	13 ± 4·4^{b}	52 ± 0·1^{c}	nd	nd	nd	27 ± 18·7^{bc}	40 ± 6·6^{c}	7 ± 7·3^{b}

Samples were incubated for 30 d at 25 °C.
* Not detected. Values with different letters as superscript are significantly different ($P < 0{\cdot}05$).

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for Gly, Ala and Lys (Table 3). During the incubation of sterile pork slices, the concentration of free amino acids showed only small changes, resulting in no significant difference with those in fresh pork except for Leu and Lys. When the samples were incubated with each of the different micro-organisms, the concentrations of most amino acids did not differ significantly ($P < 0{\cdot}05$) from those obtained in sterile samples. The only change induced by several micro-organisms was a decrease in Asp, Arg and Leu. In addition, increases in the concentration of free amino acids were rarely observed, except when pork was inoculated with *Penicillium* spp. In these samples, the concentrations of some free amino acids after incubation were higher than for the remaining inoculated samples. With *P. chrysogenum* 222 in particular, nearly all the amino acids showed increases, reaching higher concentrations than in all other samples.

DISCUSSION

Most of the organisms tested showed high proteolytic activity for myosin in broth. This contrasts with the weak proteolytic activity against gelatine or powdered milk reported for Gram-positive, catalase-positive cocci (Molina and Toldrá 1992), *Aspergillus* spp. and *Penicillium* spp. (Huerta *et al.* 1987b) isolated from dry-cured ham. The application of myosin, as a specific meat substrate, was more suitable for the detection of proteolytic activity of organisms from meat products than other non-meat substrates, such as gelatin or powdered milk. In addition, growth in a poor medium supplemented with a meat protein could promote proteolytic activity of the micro-organisms.

The strains showing high proteolytic activity against myosin were assayed for hydrolysis of myofibrillar and sarcoplasmic proteins in pork slices. The reduction in the bands of myofibrillar and sarcoplasmic proteins observed in uninoculated samples can be attributed to protein hydrolysis by endogenous enzymes, as expected during ripening of different meat products (Córdoba *et al.* 1994a; García *et al.* 1997; Monin *et al.* 1997). In addition, this reduction could also be related to the partial insolubilization of proteins described in meat products (Córdoba *et al.* 1994a; García *et al.* 1997; Monin *et al.* 1997).

The higher reduction in myofibrillar than sarcoplasmic proteins observed in both inoculated and sterile ripened pork has also been reported during ripening of meat products (Toldrá *et al.* 1992b; García *et al.* 1997). Thus, myofibrillar proteins seem to be more easily hydrolysed by enzymes of both microbial and endogenous origin than sarcoplasmic proteins.

The differences found in both groups of proteins for inoculated and uninoculated pork slices revealed proteolytic activity from micro-organisms. Given that the temperature and salt concentration used for pork slices are similar to those found during the ripening of most hams (Córdoba *et al.* 1994a), proteolysis on hams may be due not only to endogenous, but also to microbial, enzymes. Therefore, starter cultures could be used during the ripening of dry-cured ham to control proteolysis caused by microbial enzymes. In addition, the use of safe starter cultures could minimize hazards resulting from the growth of toxigenic strains.

It should be possible to benefit from the complementary ability of some of the cocci and moulds tested to hydrolyse sarcoplasmic and myofibrillar proteins by using combined starter cultures. In addition, these two microbial groups should not interfere with each other, as their growth takes place at different times during dry-cured ham processing (Rodríguez *et al.* 1994; Núñez *et al.* 1996b).

As the micro-organisms selected by the test with myosin in diluted nutrient broth showed higher proteolytic activity on pork slices than those used as controls, this test seems to be appropriate for the screening of proteolytic activity in organisms isolated from meat products. However, the evaluation of changes in sarcoplasmic and myofibrillar proteins in sterile pork provides detailed information on the microbial effect on inoculated samples.

In meat samples inoculated with most of the organisms tested, free amino acids did not increase as expected, despite the fact that extensive hydrolysis of sarcoplasmic and myofibrillar proteins took place. This could be due to limited proteolysis yielding peptides rather than extensive protein hydrolysis producing free amino acids. In addition, the amounts of some free amino acids may decrease as they are transformed to new products, which contribute to flavour, such as aldehydes, acids, ketones and amines (Gottschalk 1985; Tschabrun *et al.* 1990; Hinrichsen and Andersen 1994).

The strain showing the highest proteolytic activity, according to the extension of hydrolysis on myofibrillar proteins, and increase of most free amino acids, was *P. chrysogenum* 222. Free amino acids in pork slices inoculated with this strain reached similar levels in 30 d to those found in dry-cured ham after approximately 6 months of ripening (Córdoba *et al.* 1994b). As free amino acids play a major role in taste formation (Berdagué *et al.* 1993) and odour development (Hinrichsen and Andersen 1994), proteolysis yielding free amino acids should be regarded as one of the most positive characteristics in dry-cured meat products. Given that *P. chrysogenum* 222 showed no toxicity in bioassays (Núñez *et al.* 1996b) and the highest proteolytic activity, it is the most appropriate micro-organism for use in starter cultures. The strains of *Staph. xylosus* tested, both in their ability to hydrolyse muscle proteins and their lack of enterotoxin production (Rodríguez *et al.* 1996), could also be considered for starter cultures.

From these results it can be concluded that myosin hydrolysis is a suitable test for preliminary screening of

Table 3 Effect of the selected micro-organisms on free amino acids of sterile pork slices (data are given as concentration in μmol 10^{-2} g^{-1})

Amino acid	Fresh pork	Incubated samples									
		Sterile	*Staphylococcus xylosus* 9AA$_8$	*Staphylococcus xylosus* 5EA	*Staphylococcus equorum* 1AB$_2$	*Staphylococcus equorum* 8AB$_2$	*Penicillium chrysogenum* 222	*Penicillium commune* 131	*Penicillium variotii* 351	*Debaryomyces hansenii* 344	*Debaryomyces hansenii* 131
ASP	18·8 ± 18·5^{ab}	82·7 ± 18·88^{bcd}	6·0 ± 2·03^{a}	12·8 ± 7·29^{a}	30·1 ± 6·92^{abc}	60·2 ± 3·84^{a}	291·8 ± 2·86^{e}	121·1 ± 81·97^{d}	82·7 ± 30·83^{cd}	1·5 ± 1·58^{a}	nd*
GLU	1·4 ± 0·2^{a}	18·4 ± 1·36^{a}	5·5 ± 3·2^{a}	10·2 ± 7·35^{a}	41·5 ± 2·59^{a}	3·4 ± 1·23^{a}	399·7 ± 120·95^{b}	9·5 ± 5·38^{a}	29·9 ± 30·1^{a}	1·4 ± 0·34^{a}	3·4 ± 1·02^{a}
SER	14·8 ± 1·71^{a}	42·8 ± 14·76^{ab}	nd	5·7 ± 5·43^{a}	415·1 ± 190·6^{abc}	1207·1 ± 115·1^{d}	760·6 ± 207·16^{cd}	138·0 ± 51·79^{ab}	491·2 ± 190·5^{bc}	nd	337·9 ± 337·9^{abc}
ASN	12·9 ± 9·48^{a}	60·6 ± 9·93^{ab}	3·0 ± 0·15^{a}	13·0 ± 6·14^{a}	61·4 ± 33·96^{ab}	68·9 ± 9·09^{ab}	629·9 ± 180·4^{c}	143·3 ± 36·38^{b}	81·9 ± 12·43^{ab}	4·6 ± 0·99^{a}	18·9 ± 15·77^{a}
GLY	211·5 ± 37·11^{a}	670·3 ± 336·6^{ab}	2358·1 ± 135·4^{cd}	1827·4 ± 499·3^{bcd}	251·4 ± 177·42^{a}	65·2 ± 31·79^{a}	6035·5 ± 892·3^{c}	3153·4 ± 274·8^{d}	1131·8 ± 727·4^{ab}	1423·1 ± 96·3^{abc}	1159·8 ± 282·5^{abc}
GLN	34·2 ± 10·34^{a}	6·8 ± 0·62^{a}	81·5 ± 33·76^{a}	56·2 ± 24·86^{a}	15·7 ± 5·62^{a}	181·5 ± 92·88^{b}	94·5 ± 17·1^{ab}	58·2 ± 14·32^{a}	24·7 ± 13·63^{a}	39·0 ± 1·64^{a}	22·6 ± 10·07^{a}
HIS	87·7 ± 60·11^{ab}	13·8 ± 5·22^{bc}	8·4 ± 4·19^{bc}	23·9 ± 6·39^{c}	10·9 ± 5·55^{bc}	4·5 ± 2·39^{c}	110·3 ± 25·8^{a}	123·8 ± 119·07^{a}	8·4 ± 4·9^{c}	22·4 ± 5·87^{bc}	10·3 ± 9·61^{bc}
ARG	11·5 ± 8·51^{ab}	136·8 ± 114·1^{bc}	5·2 ± 3·05^{a}	8·0 ± 3·68^{a}	5·2 ± 3·79^{a}	8·1 ± 0·57^{a}	168·9 ± 115·63^{c}	100·6 ± 99·14^{abc}	8·7 ± 3·79^{a}	6·3 ± 2·47^{a}	8·6 ± 2·47^{a}
TRH	1·7 ± 1·51^{a}	61·3 ± 11·84^{a}	nd	nd	28·6 ± 28·74^{a}	nd	1481·5 ± 112·44^{c}	528·6 ± 473·87^{b}	5·0 ± 4·71^{a}	nd	0·84 ± 0·76^{a}
ALA	420·9 ± 274·5^{a}	131·9 ± 55·96^{a}	874·2 ± 156·38^{a}	786·7 ± 91·98^{a}	1242·4 ± 130·8^{a}	202·7 ± 16·67^{a}	2984·9 ± 2515·1^{b}	209·9 ± 23·15^{a}	744·7 ± 353·41^{a}	944·9 ± 40·29^{a}	483·3 ± 271·38^{a}
PRO	62·6 ± 30·36^{ab}	97·4 ± 14·27^{ab}	150·5 ± 24·8^{bc}	115·7 ± 13·58^{ab}	102·7 ± 16·79^{ab}	38·3 ± 9·14^{a}	229·7 ± 145·12^{c}	71·3 ± 37·24^{ab}	90·5 ± 34·97^{ab}	126·1 ± 31·93^{abc}	35·7 ± 11·14^{a}
TYR	14·9 ± 0·77^{a}	19·3 ± 3·26^{a}	28·2 ± 1·27^{a}	28·2 ± 5·46^{a}	25·9 ± 2·59^{a}	24·8 ± 1·27^{a}	187·7 ± 37·48^{b}	32·6 ± 6·4^{a}	23·2 ± 1·27^{a}	27·6 ± 5·3^{a}	19·3 ± 5·91^{a}
VAL	21·4 ± 1·11^{a}	60·7 ± 9·66^{a}	70·1 ± 7·61^{a}	63·3 ± 15·04^{a}	82·1 ± 23·3^{a}	29·9 ± 6·24^{a}	793·2 ± 295·47^{b}	166·7 ± 58·97^{a}	113·7 ± 22·48^{a}	92·3 ± 2·99^{a}	87·2 ± 22·22^{a}
MET	61·1 ± 3·89^{a}	59·1 ± 12·08^{a}	102·7 ± 30·93^{a}	80·5 ± 11·61^{a}	75·8 ± 21·34^{a}	62·4 ± 11·54^{a}	234·8 ± 211·3^{b}	89·2 ± 16·51^{a}	83·9 ± 7·78^{a}	90·6 ± 18·79^{a}	63·7 ± 14·23^{a}
ILE	81·7 ± 9·01^{ab}	112·9 ± 1·15^{ab}	89·3 ± 5·88^{ab}	95·4 ± 10·15^{ab}	96·2 ± 14·27^{ab}	55·7 ± 17·94^{a}	664·1 ± 118·93^{c}	156·5 ± 30·15^{b}	119·1 ± 17·4^{ab}	140·5 ± 24·81^{ab}	92·4 ± 25·5^{ab}
LEU	9·2 ± 2·29^{a}	382·4 ± 279·39^{b}	18·3 ± 3·36^{a}	31·3 ± 7·33^{a}	65·7 ± 35·27^{a}	24·4 ± 14·04^{a}	1000·8 ± 240·15^{c}	170·9 ± 63·05^{ab}	58·8 ± 11·83^{a}	64·9 ± 0·99^{a}	51·2 ± 10·76^{a}
PHE	10·3 ± 3·09^{a}	38·2 ± 4·12^{ab}	33·9 ± 1·94^{ab}	28·5 ± 4·91^{ab}	35·2 ± 4·97^{ab}	32·7 ± 4·12^{ab}	126·1 ± 42·79^{c}	53·9 ± 13·33^{b}	30·3 ± 1·82^{ab}	25·5 ± 13·1^{ab}	19·4 ± 2·42^{a}
TRP	7·8 ± 3·09	8·8 ± 0·49	13·2 ± 5·0	11·3 ± 2·89	7·8 ± 2·45	9·3 ± 1·27	25·3 ± 7·99	33·8 ± 3·04	42·6 ± 32·79	18·6 ± 9·95	9·3 ± 2·21
LYS	639·4 ± 357·67^{ab}	266·4 ± 0·96^{c}	439·0 ± 150·41^{bc}	381·5 ± 94·73^{bc}	383·4 ± 35·55^{bc}	280·1 ± 32·47^{c}	932·2 ± 13·84^{a}	446·6 ± 102·74^{bc}	316·4 ± 68·56^{c}	510·9 ± 175·96^{bc}	256·2 ± 59·45^{c}

Samples were incubated for 30 d at 25 °C.

* nd, Not detected. Values with different letters as superscript are significantly different ($P < 0·05$).

microbial proteolytic activity. Evaluation of muscle proteins hydrolysis and free amino acids released in sterile pork could then be more appropriate for selecting organisms for use as starter cultures for dry-cured meat products. As *P. chrysogenum* 222 showed a high proteolytic activity in pork slices, a significant contribution of this strain to proteolysis during ripening of dry-cured meat products can be expected. However, confirmation of these results on dry-cured ham is needed.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Spanish Commission Interministerial de Ciencia y Tecnología (ALI95-0237). M. Rodríguez was also the recipient of a grant from the Spanish Ministerio de Educación y Ciencia.

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