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Composition and toxigenic potential of the mould population on dry-cured Iberian ham

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Abstract

The fungal population on dry-cured Iberian ham can be essential to the development of the product's unique characteristics, but health hazards due to mycotoxins may be significant. We examined the natural fungal population of Iberian hams during ripening at three different locations. Chloroform extracts from 59 selected isolates were tested for toxicity to brine shrimp larvae and VERO cells, for mutagenicity in the Ames test and for antimicrobial activity against *Staphylococcus aureus*. The diversity of moulds increased during ripening. *Penicillium commune*, *Penicillium chrysogenum*, *Penicillium aurantiogriseum*, *Penicillium expansum* and *Penicillium echinulatum* dominated most of the ripening time; however, the *Eurotium* species, particularly *E. herbariorum* and *E. repens*, increased in the final product. Using the above tests, most moulds were toxigenic. The toxigenic potential of the fungal population increased as the processing progressed. To minimize health hazards from uncontrolled fungal populations, we identified non toxigenic strains of *Penicillium chrysogenum* that could be used as starters in dry-cured hams.

Keywords: Mould; Mycotoxins; Raw ham; Toxicity

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1. Introduction

Iberian ham is an uncooked, dry-cured meat product obtained after 16–32 months of ripening under natural environmental conditions. These characteristics, together with the low a_w reached, favour the development of an uncontrolled fungal population. Moulds are considered beneficial in the ripening of dry-cured meat products due to their positive effects on flavour and appearance (Lücke, 1986). However, mycotoxin production by moulds isolated from meat products is well established (Bullerman et al., 1969a,b; Sutic et al., 1972; Rojas et al., 1991). Therefore, the prevention of all undesirable mould growth has been suggested (Leistner, 1984), but treatments to prevent the growth of undesirable moulds would also prevent the growth of beneficial ones. The use of nontoxigenic strains as starters could help to minimize health hazards associated with Iberian ham, without affecting the quality and character of the product.

The fungi from various fully ripened dry-cured hams have been studied in some detail (Leistner and Ayres, 1968; Sutic et al., 1972; Dragoni et al., 1980a,b; Monte et al., 1986; Huerta et al., 1987a; Rojas et al., 1991). Most isolates were *Penicillium* and *Aspergillus* species. However, little technical information exists about the changes in the fungal populations during the many months of ripening of Iberian dry-cured hams. To select starter cultures, the evolution of the fungal population during ripening as well as the population on the final product need to be known.

Our objective was to assess the toxigenicity of the moulds naturally present during ripening of Iberian ham, so that appropriate actions to minimize health hazards to consumers could be identified.

2. Material and methods

2.1. Sampling

Forty two hams were processed as previously described (Núñez et al., 1996). Samples (10 g, 25 cm^2, 1–4 mm depth) were taken from the gracilis muscle of each of three Iberian hams at three ham factories, in different towns, at the following six stages: (a) fresh; (b) after salting for 8 days and post-salting for 63–70 days in cold rooms at 0–4°C. Then, after ripening under natural, uncontrolled conditions for (c) 3–5 months, in drying rooms with temperature records as high as 25–30°C; and (d) 4, (e) 8, and (f) 15–17 months in a cellar with temperatures ranging from 6–12°C in winter to 20–23°C in summer. Isolations were made from decimal dilutions plated on dichloran rose bengal chloramphenicol agar (Oxoid, Unipath, Basingstoke, UK) and dichloran–18% glycerol agar (Oxoid) as described previously (Núñez et al., 1996). In addition, samples from dilution 1:10 were plated on Aspergillus Flavus and Parasiticus agar (Pitt et al., 1983). All plates were incubated at 25°C for 5 days.

2.2. Identification of isolates

About 20% of the colonies were randomly selected (Ordóñez, 1979) from these plates, and subcultured on nutrient agar (Difco, Detroit, Michigan). Mould colonies were identified according to Pitt and Hocking (1985), Pitt (1986) and Samson and van Reenen-Hoekstra (1988). To confirm the identification of isolates in *Penicillium* subgenus *Penicillium*, selected isolates were grown in neutral creatine sucrose agar (Pitt, 1993).

2.3. Mycotoxin extracts

Selected isolates were grown on malt extract agar (MEA) (Raper and Thom, 1949) and Czapek Dox agar (CDA) (Pitt, 1973) at 25°C for 21 days. Mycotoxins were extracted with chloroform as described by El-Banna et al. (1987). Residues obtained after evaporating the extract to dryness were redissolved with 0.1 ml chloroform per plate extracted.

2.4. Brine shrimp test

The brine shrimp test was performed as described by El-Banna et al. (1987) with 10, 5 and 2 μl of extract per ml of brine shrimp medium (Harwig and Scott, 1971). Dead larvae were counted just before incubation, and after 24 and 48 h at 30°C in a shaker at 80 rpm. Toxicity of extracts was rated as follows: nontoxic (NT) when mortality of larvae showed no statistically significant difference ($P < 0.05$) with spontaneous mortality in controls; toxic (T) if the mortality was $\leq 89\%$ and very toxic (VT) if the mortality was $\geq 90\%$.

2.5. MTT and NR tests

Cytotoxicity was tested using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) and neutral red (NR) tests as described by Reubel et al. (1987) and Borenfreund et al. (1988) with VERO cells (ICN Biomedicals, Thame, UK). Cells were grown in Dulbecco's modified eagle medium (DMEM; Gibco BRL, Gaithersburg, Maryland), supplemented with 10% fetal calf serum (Gibco BRL), 1% penicillin–streptomycin solution (Gibco BRL) and 1% L-glutamine 200 mM (Gibco BRL). Individual wells of microtiter plates were inoculated with 200 μl DMEM containing c.a. 40 000 cells and incubated 24 h in a 5% CO_2 atmosphere at 37°C. To obtain 12.5, 3.13, 0.78 and 0.26 μl of extract per ml of DMEM, appropriate volumes of extract were evaporated to dryness, dissolved in 25 μl dimethylsulfoxide (DMSO) and mixed with 1200 μl of DMEM. To remove non solubilized material, every mix was vigorously shaken and then centrifuged (7200 $\times$ g, 5 min). Thereafter, 200 μl of the supernatant was added to the wells and incubated 24 h at 37°C, 5% CO_2. Every dilution of extract was assayed in triplicate in both tests. Viable cells were quantified after a 3 h incubation with each dye. Extracts were rated as very toxic (VT) when the reduction in the average ab-

sorbance as compared to controls was $\geqslant 90\%$ or toxic (T) if the reduction was $\leqslant 89\%$.

2.6. Ames test

Mutagenicity was assayed by the Ames test (Ames et al., 1975; Maron and Ames, 1984) after 20 min preincubation at 37°C with *Salmonella choleraesuis* TA100 (Spanish type culture collection 881). Tests were run both in the presence and absence of S9 mix obtained from a liver homogenate (Garner et al., 1972) from Wistar male rats induced with phenobarbital and β-naphtoflavone (Ong et al., 1980). Adequate volumes for 50, 5 and 0.5 μl of extract per *Salmonella* plate were evaporated to dryness and dissolved in 50 μl DMSO per plate. All tests were run in duplicate. Extracts were considered mutagenic when the number of revertants was significantly higher ($P < 0.05$) than that in the controls.

2.7. Inhibition of Staphylococcus aureus

Sterile paper discs (5 mm diameter) were saturated with 20 μl of extract, and the solvent evaporated. The discs were placed on P agar (Kloos et al., 1974) plates inoculated with *Staphylococcus aureus* (Spanish type culture collection 976). The area of inhibition zones was determined after incubating first at 4°C for 4 h and then at 37°C for 24 h. Extracts were rated as active (A) if an inhibition zone was visible around the disc, and very active (VA) when the inhibition zone was $\geqslant 100$ mm^2.

2.8. Statistical analysis

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

3. Results

Twenty three species were identified from the 519 mould isolates that we recovered (Table 1). More than 98% of the isolates were either *Penicillium*, *Aspergillus* or *Eurotium* species. For most of the ripening time, the mould population was dominated by *Penicillium* spp. *P. commune*, *P. chrysogenum* and *P. expansum* were the species more frequently isolated. *P. aurantiogriseum* also was found at different samplings, but only in hams coming from location I. *Eurotium herbariorum* and *E. repens* were found in hams from all three producers, but were isolated more frequently than *Penicillium* species only at the last sampling time (15–17 months in cellar). The remaining species were only occasionally isolated, and never from plates of dilutions higher than 1:10 000. No growth was observed in the Aspergillus Flavus and Parasiticus agar at any time, indicating the absence of aflatoxin producing fungi.

Table 1
Moulds isolated from Iberian ham

Mould species	F	PS			D			4MC	8MC	16MC		
		I	II	III	I	II	III	I	I	I	II	III
	10^{-3} [a]	10^{-7}	10^{-4}	10^{-3}	10^{-5}	10^{-3}	10^{-6}	10^{-4}	10^{-5}	10^{-4}	10^{-6}	10^{-6}
Penicillium aurantiogriseum	—	4	—	—	—	—	—	2	15	3	—	—
Penicillium brevicompactum	—	—		1	—	—	—	—	—	—	—	—
Penicillium commune	—	6	2	9	11	2	10	33	14	27	9	19
Penicillium chrysogenum	—	—	—	15	4	1	23	2	3	—	—	—
Penicillium echinulatum	—	1	—	—	—	—	—	—	2	8	1	—
Penicillium expansum	—	—	5	—	3	1	42	7	—	2	—	2
Penicillium jensenii	—	—	—	—	—	—	1	—	—	—	—	—
Penicillium oxalicum	—	—	—	—	—	—	—	—	—	—	1	—
Penicillium restrictum	—	—	—	—	—	—	—	1	—	—	—	—
Penicillium rugulosum	—	—	—	—	—	—	—	—	1	—	—	—
Penicillium viridicatum	—	—	—	5	—	—	—	—	—	—	—	—
Paecilomyces variotii	—	—	—	—	—	—	—	—	1	—	—	—
Aspergillus niger	—	—	—	—	—	—	—	—	—	1	—	1
Aspergillus sydowii	—	—	—	—	—	—	—	—	—	—	5	2
Aspergillus versicolor	12	—	—	—	—	—	—	—	—	—	—	—
Eurotium herbariorum	—	—	—	—	—	4	—	1	—	9	33	13
Eurotium repens	—	—	—	—	—	1	—	—	—	24	32	74
Eurotium rubrum	—	—	—	—	—	—	—	—	—	1	—	1
Alternaria tenuis	—	—	—	—	—	—	—	—	1	—	—	—
Aurobasidium pullulans	—	—	—	—	—	—	—	—	—	—	—	1
Cladosporium herbarum	—	—	—	—	—	—	—	—	—	1	—	—
Curvularia lunata	—	—	—	—	—	1	—	1	—	—	—	—
Syncephalastrum racemosum	—	—	—	—	—	—	—	—	—	—	—	1
Total	12	11	7	30	18	10	76	47	37	76	81	114

F: fresh stage; PS: end of post-salting; D: end of drying; 4MC: 4 months in cellar; 8MC: 8 months in cellar; 16MC: final product.
I, II, III: Locations of ripening.
[a] Higher dilution of isolation.

For the toxicological testing, fifty nine moulds were selected, including isolates from the main species detected, the three locations, and all samplings.

3.1. Brine shrimp test

This assay revealed a high incidence of toxigenic moulds in dry-cured Iberian ham. From isolates grown on MEA, 54% of the extracts were toxic to the larvae within 24 h (Table 2), and 76% of the extracts were toxic, most very toxic, by 48 h. Even for *P. brevicompactum*, where no toxicity was detected in any of the tests in 24 h, all three dilutions from both culture media were VT after 48 h. Extracts from CDA gave similar or slightly lower levels of toxicity (data not shown) than those from MEA. The main exceptions were *A. versicolor*, rated VT in all tests when cultured on CDA but NT when grown on MEA, and one isolate each of *P. chrysogenum* and *P. echinulatum* rated T in CDA but NT when grown on MEA. All

Table 2
Toxicity in brine shrimp test of Iberian ham moulds grown on malt extract agar

Mould species	10 μl/ml[a] incubated for:						2 μl/ml incubated for:					
	48 h			24 h			48 h			24 h		
	VT	T	NT	VT	T	NT	VT	T	NT	VT	T	NT
Penicillium aurantiogriseum	4	—	—	3	1	—	1	2	1	1	2	1
Penicillium brevicompactum	1	—	—	—	—	1	1	—	—	—	—	1
Penicillium commune	13	2	—	7	6	2	1	2	12	—	—	15
Penicillium chrysogenum	—	1	5	—	—	6	—	—	6	—	—	6
Penicillium echinulatum	1	2	2	—	2	3	—	—	5	—	—	5
Penicillium expansum	1	1	—	—	—	2	—	—	2	—	—	2
Penicillium jensenii	—	—	1	—	—	1	—	—	1	—	—	1
Penicillium oxalicum	1	—	—	1	—	—	—	—	1	—	—	1
Penicillium restrictum	—	1	—	—	—	1	—	1	—	—	1	—
Penicillium rugulosum	—	1	—	—	—	1	—	1	—	—	—	1
Penicillium viridicatum	—	—	1	—	—	1	—	—	1	—	—	1
Paecilomyces variotii	—	1	—	—	1	—	—	—	1	—	—	1
Aspergillus niger	2	—	—	1	1	—	1	—	1	—	—	2
Aspergillus sydowii	2	—	—	1	—	1	—	2	—	—	—	2
Aspergillus versicolor	—	—	1	—	—	1	—	—	1	—	—	1
Eurotium herbariorum	3	1	—	2	1	1	1	—	3	—	1	3
Eurotium repens	3	1	—	2	—	2	1	1	2	1	—	3
Eurotium rubrum	2	—	—	2	—	—	1	—	1	—	1	1
Aurobasidium pullulans	—	—	1	—	—	1	—	—	1	—	—	1
Cladosporium herbarum	—	—	1	—	—	1	—	—	1	—	—	1
Curvularia lunata	1	—	1	1	—	1	1	—	1	—	1	1
Syncephalastrum racemosum	—	—	1	—	—	1	—	—	1	—	—	1
Total	34	11	14	20	12	27	8	9	42	2	6	51

VT: Number of very toxic isolates; T: Number of toxic isolates; NT: Number of non toxic isolates.
[a] Concentration of chloroform extract in brine shrimp medium.

Aspergillus and *Eurotium* isolates and 82% of the *Penicillium* isolates tested had some toxicity in the brine shrimp test. *Eurotium rubrum* and *P. aurantiogriseum* showed the highest activity, as 2 μl extract/ml from any of the two culture media was toxic following 24 h of incubation.

3.2. Cytotoxicity

Toxicity to VERO cells was similar to that in the brine shrimp test. When grown on MEA, 71.1% of the isolates were cytotoxic in the MTT test, but only 62.7% in the NR test (Table 3). With extracts from CDA, the number of cytotoxic isolates was slightly lower in both tests. In addition, all isolates rated VT on CDA were at least toxic on MEA, while 5 isolates rated VT on MEA showed no cytotoxicity when cultured on CDA. Again, all the *Aspergillus* and *Eurotium* species, but only 77% of the *Penicillium* isolates were toxic to the VERO cells. The highest level of cytotoxicity, detected with 0.78 μl extract/ml DMEM from both media and in both tests, was shown by *P. commune* and *P. oxalicum*.

3.3. Ames test

Results from the mutagenicity tests were less consistent than those from the toxicity tests. Of the nineteen isolates showing mutagenic activity with 50 μl of extract per plate (Table 4), only one isolate each of *P. aurantiogriseum* and *P. commune* produced active extracts when cultured on both media, and another *P. commune* was mutagenic with and without S9 mix. No activity was found using 0.5 μl or 5 μl of extract per plate from either MEA or CDA, except for one isolate each of *Curvularia lunata* and *E. repens*. In addition, the number of revertants was always under 52, rather low to be considered significant when compared to the numbers reached in similar conditions with mycotoxins (Wehner et al., 1978). Therefore, no tested isolate revealed a strong mutagenic potential.

3.4. Antimicrobial activity against Staphylococcus aureus

Chloroform extracts from 43 mould isolates were able to inhibit *S. aureus* (Table 5). A higher percentage of active isolates was found in the *Aspergillus* and *Eurotium* than in *Penicillium* species. However, the highest levels of inhibition from both media used were recorded with extracts obtained from *P. oxalicum*, *P. rugulosum* and *Paecilomyces variotii*. In addition, extracts with larger inhibition zones (> 100 mm^2) were obtained from strains isolated at the last two samplings.

4. Discussion

In contrast to the observations with both micrococci and yeasts from Iberian ham (Rodríguez et al., 1994; Núñez et al., 1996), the diversity of the mould population increased throughout the ripening process. *P. commune* was the only

Table 3
Cytotoxicity in MTT and NR tests of Iberian ham moulds grown on malt extract agar

Mold species	MTT test						NR test					
	12.5 μl/ml[a]			3.13 μl/ml			12.5 μl/ml			3.13 μl/ml		
	VT	T	NT	VT	T	NT	VT	T	NT	VT	T	NT
Penicillium aurantiogriseum	2	2	—	—	1	3	—	4	—	—	1	3
Penicillium brevicompactum	—	—	1	—	—	1	—	—	1	—	—	1
Penicillium commune	8	1	6	1	6	8	3	5	7	—	6	9
Penicillium chrysogenum	2	1	3	—	1	5	1	1	4	—	2	4
Penicillium echinulatum	4	1	—	—	1	4	1	4	—	—	1	4
Penicillium expansum	—	2	—	—	1	1	—	2	—	—	—	2
Penicillium jensenii	—	—	1	—	—	1	—	—	1	—	—	1
Penicillium oxalicum	1	—	—	1	—	—	—	1	—	—	1	—
Penicillium restrictum		—	1	—	—	1	—	—	1	—	—	1
Penicillium rugulosum	—	—	1	—	—	1	—	1	—	—	—	1
Penicillium viridicatum	—	—	1	—	—	1	—	—	1	—	—	1
Paecilomyces variotii	—	—	1	—	—	1	—	—	1	—	—	1
Aspergillus niger	2	—	—	—	1	1	—	2	—	—	2	—
Aspergillus sydowii	2	—	—	1	—	1	1	1	—	—	1	1
Aspergillus versicolor	1	—	—	—	1	—	1	—	—	—	—	1
Eurotium herbariorum	2	1	1	—	—	4	1	1	2	—	1	3
Eurotium repens	3	1	—	2	1	1	3	1	—	2	1	1
Eurotium rubrum	2	—	—	1	1	—	1	1	—	1	1	—
Aurobasidium pullulans	—	1	—	—	—	1	—	—	1	—	—	1
Cladosporium herbarum	—	—	1	—	—	1	—	—	1	—	—	1
Curvularia lunata	1	1	—	—	1	1	—	1	1	—	—	2
Syncephalastrum racemosum	—	1	—	—	1	—	—	—	1	—	—	1
Total	30	12	17	6	16	37	12	25	22	3	17	39

VT: Number of very toxic isolates; T: Number of toxic isolates; NT: Number of non toxic isolates.
[a]Concentration of chloroform extract in Dulbecco's modified eagle medium.

Table 4
Mutagenicity of moulds isolated from Iberian ham in the Ames test, with and without S9 mix for metabolic activation

Mould species	Number of isolates used	Number of mutagenic isolates			
		With S9 mix		Without S9 mix	
		MEA	CDA	MEA	CDA
Penicillium aurantiogriseum	4	1	—	2	1
Penicillium brevicompactum	1	—	—	—	—
Penicillium commune	15	2	3	2	1
Penicillium chrysogenum	6	—	—	—	1
Penicillium echinulatum	5	1	—	—	—
Penicillium expansum	2	—	—	—	—
Penicillium jensenii	1	—	—	—	—
Penicillium oxalicum	1	—	—	—	—
Penicillium restrictum	1	—	—	—	—
Penicillium rugulosum	1	—	—	—	—
Penicillium viridicatum	1	—	—	—	—
Paecilomyces variotii	1	—	—	—	—
Aspergillus niger	2	—	—	1	—
Aspergillus sydowii	2	—	—	—	1
Aspergillus versicolor	1	—	—	—	—
Eurotium herbariorum	4	—	1	1	—
Eurotium repens	4	1	—	—	1
Eurotium rubrum	2	—	1	—	—
Aurobasidium pullulans	1	—	—	—	—
Cladosporium herbarum	1	—	—	—	—
Curvularia lunata	2	1	—	—	—
Syncephalastrum racemosum	1	—	—	—	—
Total	59	6	5	6	5

MEA: Extracts from malt extract agar; CDA: Extracts from Czapek Dox agar.

species isolated in all samplings after salting. Only when surface a_w values reached 0.88–0.79 (Rodríguez et al., 1994; Núñez et al., 1996) did *Aspergillus* and *Eurotium* species become dominant. Thus, the hazard posed by the development of *Aspergillus* spp. could be reduced by altering the end value of a_w reached in the hams. However, the moulds that dominate the final product, including the main *Eurotium* spp. also were detected at the earlier processing stages.

All of the moulds that we found have also been found in other types of country-cured hams (Leistner and Ayres, 1968; Sutic et al., 1972; Dragoni et al., 1980a,b; Monte et al., 1986; Huerta et al., 1987a; Rojas et al., 1991). The succession of *Penicillium* spp. by *Aspergillus* spp. and *Eurotium* spp. has been reported in hams with low a_w or in those ripened under low relative humidities (Leistner and Ayres, 1968; Spotti et al., 1989; Hernández and Huerta, 1993). However, only *Aspergillus* was recovered from hams with shorter ripening times (Huerta et al., 1987a; Rojas et al., 1991), while *Eurotium* was more frequent during the ripening of Iberian ham

(Table 1) and in the final product (Monte et al., 1986). The *Eurotium*/*Aspergillus* ratio in the final product seems to increase with the ripening time.

The results of the toxicity tests were homogeneous, with most isolates being positive in more than one test. The incidence of toxic moulds and the level of toxicity obtained was similar to that reported by others for moulds isolated from meat products (El-Banna and Leistner, 1987; El-Banna et al., 1987; Fink-Gremmels et al., 1988; Fink-Gremmels and Leistner, 1990). In the brine shrimp test, 13 isolates required more than 24 h of incubation time to express toxicity (Table 2). Of these 13 isolates, only *Penicillium brevicompactum* showed no toxicity in the other tests. The toxicity of this fungi in the brine shrimp test can be explained by fungal fatty acids toxic for the shrimp larvae (Curtis et al., 1974). The remaining isolates were positive in the other tests. Thus, the 48 h incubation improved the detection of toxic moulds with the brine shrimp test. Six isolates, including one *P. chrysogenum* and the only *P. jensenii*, were cytotoxic but not toxic to the shrimp larvae. These strains might synthesize mycotoxins to which the larvae are relatively

Table 5
Inhibition of *Staphylococcus aureus* by extracts of moulds grown on CDA and MEA

Mould species	A			NA
	MEA and CDA	MEA	CDA	MEA and CDA
Penicillium aurantiogriseum	2	1	1	—
Penicillium brevicompactum	—	—	—	1
Penicillium commune	6	4	2	3
Penicillium chrysogenum	1	1	1	3
Penicillium echinulatum	3	—	1	1
Penicillium expansum	1	—	—	1
Penicillium jensenii	—	1	—	—
Penicillium oxalicum	1	—	—	—
Penicillium restrictum	—	—	—	1
Penicillium rugulosum	1	—	—	—
Penicillium viridicatum	—	—	—	1
Paecilomyces variotii	1	—	—	—
Aspergillus niger	1	—	—	1
Aspergillus sydowii	1	—	1	—
Aspergillus versicolor	1	—	—	—
Eurotium herbariorum	1	2	—	1
Eurotium repens	2	—	2	—
Eurotium rubrum	2	—	—	—
Aurobasidium pullulans	1	—	—	—
Cladosporium herbarum	—	—	—	1
Curvularia lunata	—	1	—	1
Syncephalastrum racemosum	—	—	—	1
Total	25	10	8	16

A: Number of active isolates; NA: Number of non active isolates.
MEA: Extracts from malt extract agar; CDA: Extracts from Czapek Dox agar.

insensitive (Harwig and Scott, 1971), such as penicillic acid in the former (Davis and Diener, 1987) or griseofulvin in the latter (Frisvad, 1987). Thus, detection of toxic moulds from dry-cured ham should not rely only on the brine shrimp test. Even though differences in cytotoxicity were found with the MTT and NR tests (Table 3), all but two isolates positive in the latter were also toxic in the former. At the same time, toxicity of extracts from MEA was the same or higher than the corresponding extracts from CDA, except for *A. versicolor*. Thus, the highest efficiency screen for toxigenic moulds was the MTT test using extracts from cultures grown on MEA.

The Ames test was of little value as a screen since all isolates that were positive in this test had been rated VT in some of the previous assays. Experimentally, this test often gave inconsistent results, and, when detected, mutagenic activity was usually low. At the same time, the mutagenic potential of 31% of the selected isolates indicates the potential for problems as a result of an uncontrolled fungal population on this kind of dry-cured meat product.

Similar rates of antimicrobial activity against *S. aureus* have been described for moulds from meat products (Leistner and Ayres, 1968; Huerta et al., 1987b). Most isolates showing activity against *S. aureus* were also toxic either to *A. salina* or to the VERO cells. *Paecilomyces variotii*, one of the most active isolates against *S. aureus*, showed no toxicity in previous tests. This activity, even though not related to toxicity, is not desirable in cured meats as it could inhibit nitrate reducing Gram positive, catalase positive cocci. Consequently, the *S. aureus* assay allows the detection of unwanted microbial activities in dry-cured hams.

Some of the more toxic extracts were obtained from fungi detected only at the end of the curing process. Others, like *P. aurantiogriseum*, are associated with the place of processing. However, *P. commune* was detected at every sampling time after salting and at every processing location. The fact that cured ham is not an appropriate substrate for the production of some mycotoxins at high levels (Bullerman et al., 1969b; Rojas et al., 1991) does not eliminate health hazards, as *Penicillium* spp. and *Aspergillus* spp. have a proven ability to produce mycotoxins in appreciable amounts on country cured ham (Escher et al., 1973; Wu et al., 1974). In addition, little antimycotoxigenic effect of *Lactobacillus* species (Gourama and Bullerman, 1995) can be expected, as such bacteria are in low numbers for the last months of ripening (Baldini et al., 1977; Huerta et al., 1988; Silla et al., 1989). Only 5 strains (i.e. three *P. chrysogenum* and one each of *Cladosporium herbarum* and *P. viridicatum*) showed no toxicity in any of the assays we used. These fungi might be used as starter cultures, in combination with hygienic measures to reduce fungal contamination, to minimize the hazards resulting from the growth of toxigenic moulds on Iberian ham. As *P. chrysogenum* was commonly recovered, we suggest that nontoxigenic strains of this species be selected for further evaluation.

In conclusion, the natural fungal population of Iberian ham increases in diversity with ripening time and include numerous toxigenic moulds. Thus, it is essential to control the fungal population growing on dry-cured ham. Total exclusion of mould from Iberian ham, in addition to being difficult to accomplish, could affect the quality and character of the product. The use of non toxic strains as starter cultures

may be the most effective method to minimize public health risks due to fungal growth on this product.

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