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Characterization of *Micrococcaceae* isolated from salt used for Spanish dry-cured ham

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M.R. CORDERO AND J.M. ZUMALACÁRREGUI. 2000. The microbial flora of salt used in the production of Spanish dry-cured ham was studied. The results indicated that *Micrococcaceae* constituted the predominant flora. Identification of the 369 isolates belonging to the family *Micrococcaceae* revealed that 60% belonged to genus *Staphylococcus,* 25% to *Micrococcus*, 6% to *Kocuria*, 5·4% to *Dermacoccus* and 0·5% to *Stomatococcus*. The species most often isolated was *Staph. xylosus* (28·9%), followed by *M. lylae* (21·4%), *Staph. equorum* (18·55%) and *D. nishinomiyaensis* (5·4%). The results indicate that the salt during salting process of dry-cured hams offers an ecosystem suitable for the survival of the staphylococci and micrococci.

INTRODUCTION

In Spain about 34 million units of dry-cured hams are produced annually. The hams are obtained from white and Iberian breeds (5% of the total). The method of preparation consists basically of four stages: preparation of pieces, salting, postsalting and ripening.

The Spanish hams are salted with marine salt alone or in combination with nitrate and/or nitrite. The pieces are rubbed with salt mixture before being stored in salt at 3 °C for 8–10 d. During the salting stage a microbial reservoir is created from microorganisms present in the salt, on the surface of hams and in the environment (Cornejo *et al.* 1992). Some of these microorganisms could develop several actions in the brine formed (reduction of nitrates to nitrites, proteolysis and lipolysis) or penetrate the ham contributing to the sensorial properties.

Different authors have studied the microbial flora of brines used in the curing of several types of meat products, such as bresaola (Cantoni 1964), bacon (Gardner and Patton 1978), cooked ham (Papa and Grazia 1991) and raw ham (Schillinger and Lücke 1989). In the majority of cases the most abundant microbial group is the *Micrococcaceae*. Nevertheless, few studies have been carried out on the microbial flora of salt during salting process of dry-cured ham.

The objectives of this research were: (i) The quantification of several microbial groups on salt during the salting stage of the two types of hams produced in Spain; (ii) The characterization of *Micrococcaceae* isolated from the salt.

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MATERIALS AND METHODS

Samples

A total of 29 samples of salt (5 of marine salt, 8 of salt from manufactured Iberian hams and 16 of salt from white hams) were analysed. The samples of salt were obtained from different meat processing plants in Spain. The counts of the different microbial groups were determined in samples obtained at half the lifespan of salt (4–6 months).

Microbiological analysis

Fifteen gram samples were homogenized in a Stomacher (Lab-Blender 400) with 135 ml of tryptone water (Oxoid). A serial decimal dilutions were made using the same medium and plated onto growth media in duplicate. For the lactic acid bacteria 15 g of sample were suspended in 135 ml of quarter strength Ringer Solution (Oxoid) and homogenized. Serial dilutions were then prepared from this initial dilution using as diluent Man Rogosa Sharpe Broth (Oxoid).

Aerobic mesophilic bacteria were enumerated on PC agar (Oxoid), incubated at 30 °C for 3 d; lactic acid bacteria on MRS agar (Oxoid) (37 °C, 72 h in anaerobic jar); *Micrococcaceae* on MS agar (Oxoid) (30 °C, 48 h); yeasts and moulds on ME agar (Oxoid) (25 °C, 3–5 d).

391 colonies of Gram-positive, presumptive *Micrococcaceae*, growing on MSA (18 of marine salt, 169 of salt from elaborated Iberian ham and 204 of salt from white

ham) were randomly isolated. The colonies were characterized using the following tests: Gram stain, cell morphology and catalase reaction. Catalase and Gram-positive cocci were further subjected to the oxidation/fermentation test in Hugh/Leifson's OF medium and to the anaerobic growth in semisolid thyoglycolate medium (Evans and Kloos 1972). For coagulase activity the isolated pure cultures were tested using the tube test with rabbit plasma (Difco) as described by APHA (1976). The cultures were incubated at 37 °C and examined after 6 h and 24 h for a coagulum.

The ID 32-STAPH system (BioMérieux) was used to identify the selected strains, using API Lab. I.D. 32 software for ease of interpretation. Identification was obtained by using the ATB Plus computer program.

RESULTS AND DISCUSSION

The mean logarithms obtained for the counts made on three types of salt samples are shown in Table 1. The *Micrococcacea*e group was present in all samples analysed; 21 samples contained yeasts; 15 contained molds; and 8 contained lactic acid bacteria. *Micrococcaceae*, the predominant group in samples of salt from white and Iberian ham elaboration, were about 3·5 $\log_{10}$ cfu g^{-1}. These values were lower than those reported in brines (Schillinger and Lücke 1989; Papa and Grazia 1991), but were similar to those cited on the surface of Spanish dry-cured ham after the salting stage (Silla *et al.* 1989; Hernández and Huerta 1993). In the marine salt samples *Micrococcaceae* accounted for only 16% of the total count. On the contrary, in the salt samples from white and iberian hams *Micrococcaceae* count was close to the total. These differences may be because marine salt was analysed before application to hams and contains other microbial groups. However, the other two types of salt were sampled during salting stage of hams. Probably, a selection of *Micrococcaceae* takes place during this salting stage.

Table 1 Average of microbial counts ($\log_{10}$ cfu/g) in the three types of salt analysed

Microbial groups	Marine salt	Iberian ham salt	White ham salt
Total aerobic bacteria	2·45	3·77	3·83
Micrococcaceae	1·66	3·63	3·59
Yeasts	2·11 (1)	1·54 (5)	2·96 (15)
Moulds	–	1·72 (7)	1·29 (8)
Lactic acid bacteria	1·84 (1)	0·48 (2)	0·88 (5)

Numbers in parenthesis indicate number of samples in which the microbial groups studied are present.

A total of 369 strains of the 391 isolated from MSA plates were Gram-positive and catalase-positive cocci and assigned to the *Micrococcaceae* family. The other 22 were catalase-negative and therefore excluded.

The strain differentiation of the staphylococci group from 'micrococci' on the basis of the fermentation of glucose and anaerobic growth in thioglycolate medium was not possible. According to the glucose oxidation/fermentation test, only 119 strains would be considered staphylococci, but taking into account the ability to grow anaerobically, a total of 187 could be classified within the genus *Staphylococcus*. With regard to the 'micrococci', 85 strains grew under anaerobic conditions, this property is atypical of this microbial group. Similar results have been obtained by De la Rosa *et al.* (1990) in *Micrococcaceae* isolated from semipreserved meat products. In this study, differentiation of the 'micrococci' group from staphylococci was based on the results obtained with the ID 32-STAPH system.

Table 2 shows the percentages of the different *Micrococcaceae* (*Staphylococcus*, *Micrococcus, Dermacoccus*, *Kocuria* and *Stomatococcus*) isolated from salt. There was a predominance of staphylococci (224 isolates) over other micrococcaceae (143), as has been reported in almost all the works on the characterization of microbial flora in brines (Papa and Grazia 1991) and fermented meats (Montel *et al.* 1992; Torriani *et al.* 1994). These results can be explained by the higher resistance to NaCl and particularly the lower oxygen demands of staphylococci.

Fifteen different *Staphylococcus* species were identified. *Staph. xylosus* (107 isolates) and *Staph. equorum* (67) were the most abundant species. *Staph. xylosus* is the principal species on brines (Papa and Grazia 1991), dry cured ham (Carrascosa and Cornejo 1991; Cornejo and Carrascosa 1991; Rodríguez *et al.* 1994) and dry sausages (Montel *et al.* 1992; Torriani *et al.* 1994; Coppola *et al.* 1997). *Staph. equorum* has rarely been described in meat products. The presence of *Staph. capitis*, *Staph. hominis and Staph. epidermidis*, species susceptible to novobiocin and considered to be host specific for humans, suggests that human isolates are transmitted to the salt. Among *Staphylococcus*, only one species was coagulase positive and identified as *Staph. intermedius*. *Staph. aureus* was not found on the samples tested.

With regard to the other micrococcaceae, five species were isolated. *Micrococcus lylae* (81 isolates) and *Dermacoccus nishinomiyaensis* (20 isolates) were the predominant ones. These two species has also been identified by De la Rosa *et al.* (1990) in semipreserved meat products and *D. nishinomiyaensis* by García (1994) in Spanish dry-cured beef 'cecina'. Although *Kocuria kristinae* has frequently been isolated from cured meat products, it was not found in this study. Two strains of *Micrococcaceae* are characterized as *Stomatococcus mucilaginosus*, a microorganism

Table 2 *Micrococcaceae* species isolated from salt

Species	Marine salt	White ham salt	Iberian ham salt	%
Staphylococcus				
Staph. xylosus	10	51	46	28·99
Staph. equorum	5	20	42	18·15
Staph. saprophyticus	–	7	9	4·33
Staph. kloosii	3	4	–	1·89
Staph. capitis	–	6	–	1·62
Staph. sciuri	–	4	2	1·62
Staph. gallinarum	–	3	2	1·35
Staph. simulans	–	–	3	0·78
Staph. hominis	–	2	–	0·54
Staph. schleiferi	–	2	–	0·54
Staph. caprae	–	1	–	0·27
Staph. chromogenes	–	–	1	0·27
Staph. epidermidis	–	–	1	0·27
Staph. intermedius	–	–	1	0·27
Staph. lentus	–	1	–	0·27
Micrococcus				
M. lylae	–	50	31	21·95
M. luteus	–	15	–	4·06
Kocuria				
K. roseus	–	11	7	4·87
K. varians	–	–	7	1·89
Dermacoccus				
D. nishinomiyaensis	–	12	8	5·42
Stomatococcus				
S. mucilaginosus	–	–	2	0·54

present normally in the mouth and upper respiratory tract of human.

In conclusion, a remarkable variety of Gram-positive, catalase-positive cocci are present in the salt used in elaborated white and Iberian hams. The results indicate that the salting stage, although the *Micrococcaceae* counts are relatively low, offers an ecosystem suitable for the survival of the staphylococci and micrococci.

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