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Gram-Positive, Catalase-Positive Cocci from Dry Cured Iberian Ham and Their Enterotoxigenic Potential

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Iberian ham is an uncooked, cured meat product ripened under natural uncontrolled conditions for 18 to 24 months. Gram-positive, catalase-positive cocci are the main microbial population in Iberian ham for most of the ripening time. Since some of these organisms are able to produce enterotoxins, adequate characterization and toxicological study are needed. For this, 1,327 gram-positive, catalase-positive cocci, isolated from Iberian hams at different stages and locations, were characterized by physiological and biochemical tests. Selected isolates were further characterized by guanine-cytosine (G+C) content and restriction enzyme analysis of genes coding for 16S rRNA. The toxigenic potential of these organisms was tested with specific DNA gene probes for staphylococcal enterotoxins A, B, C, and D and confirmed by semiquantitative sandwich enzyme immunoassay. The majority of the isolates were identified as *Staphylococcus* spp. and *Micrococcus* spp. Non-identified gram-positive, catalase-positive cocci which were moderately halophilic and showed a 42 to 52% G+C content were detected. A great variety of staphylococcal strains were found within the different species at any sampling time. Two strains of *Staphylococcus xylosus*, one *Staphylococcus cohnii* strain, and four of the non-identified organisms with 42 to 52% G+C contents hybridized with some of the DNA probes for C and D staphylococcal enterotoxin genes. *S. xylosus* hybridizing with C-enterotoxin probe reacted with both C and D enterotoxins in the immunological test. In addition, enterotoxin D was confirmed in the nonidentified strains. Some toxigenic organisms were isolated from the final product, posing a health hazard for the consumer.

Iberian dry cured ham, the most valuable meat product of Spain, is produced from uncooked hams of the Iberian breed of pigs by a traditional method that takes at least 18 to 24 months of ripening. Gram-positive, catalase-positive cocci have been identified as the predominant organisms, for most of the ripening time, in different types of dry cured ham (6, 10, 15, 24). In Iberian ham, these organisms reach 10^8 CFU/g at the surface and 10^4 CFU/g in deep tissues at the end of the post-salting period (24).

These organisms may contribute to development and stabilization of color, inhibition of rancidity, and improvement of the flavor of cured ham by reductases, catalases, and lipases (17). However, toxigenic strains of *Staphylococcus aureus* have been isolated from dry cured ham (18). In addition, the ability to produce staphylococcal enterotoxins has been reported for coagulase-negative staphylococci (1, 2, 12, 18, 27). Close to 95% of 457 gram-positive, catalase-positive cocci isolated from Iberian ham consisted of a great diversity of coagulase-negative *Staphylococcus* spp. (24). Furthermore, *Staphylococcus* spp. and *Micrococcus* spp. with characteristics common to several species have been found in Iberian ham (24). Thus, adequate characterization and toxigenic evaluation of the gram-positive, catalase-positive cocci from dry cured ham are necessary to prevent health hazards caused by enterotoxigenic staphylococci.

In the present work, gram-positive, catalase-positive cocci from dry cured Iberian ham were characterized and their toxigenic potential was investigated.

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MATERIALS AND METHODS

Processing of the hams. The study involved 42 hams from Iberian pigs processed in large-scale batches in the traditional way, at three different production plants (I through III), as described previously (22, 24). The hams were rubbed with sea salt containing 1% potassium nitrate and trace amounts of nitrites. The first part of the processing was done at 0 to 5°C, including 8 days of dry salting in piles of salt with alternate beds of hams and sea salt, used repeatedly for different batches, and 63 to 70 days of postsalting storage. The remaining processing took place under natural uncontrolled conditions, including 3 to 5 months during spring and summer in drying rooms with temperature records as high as 25 to 30°C and 15 to 17 months in a cellar with temperatures ranging from 6 to 12°C in winter to 20 to 23°C in summer.

Sampling. Samples of approximately 10 g were taken aseptically from the surface and the depth of fresh ham and at the end of postsalting, drying, and cellar ripening periods. Additional samples were taken at 4 and 8 months of cellar ripening in location I. Surface samples were obtained from 25-cm^2, 1- to 4-mm-deep *Gracilis* muscle. Samples from the interior were obtained from the excised *Biceps femoris* muscle after it was dipped in absolute ethyl alcohol and flamed for a few seconds. Three hams were tested at every sampling, and two additional ones were sampled at the end of cellar ripening. Samples were also collected from the salt in the piles on which the fresh hams were laid.

The samples were homogenized in peptone water (1%, wt/vol) with a Stomacher Lab Blender 400 (Seward Medical UAC House, London, United Kingdom) for 5 min. Serial dilutions were made, and 0.1-ml fractions were plated on mannitol salt agar (MSA) (Oxoid, Unipath Ltd., Basingstoke, United Kingdom) and Baird-Parker agar (Oxoid, Unipath Ltd.). MSA plates were incubated at 30°C and Baird-Parker agar plates were incubated at 37°C, both for 48 h.

Identification. Approximately 20% of the colonies on plates from both culture media were subcultured on nutrient broth (Oxoid, Unipath Ltd.) for identification. Colonies from MSA were first recovered in nutrient broth containing 7.5% (wt/vol) NaCl and then subcultured in nutrient broth with no NaCl added. Isolates unable to grow in the latter broth were always cultured in media containing 7.5% (wt/vol) NaCl. For initial characterization, 1,327 isolates from hams and another 28 isolates from salt were tested as described previously (24) for Gram stain, catalase activity, glucose utilization, sensitivity to lysostaphin, growth in nutrient agar supplemented with 7.5 and 10% (wt/vol) NaCl, sensitivity to lysozyme and furazolidone, anaerobic growth in thioglycolate medium, and acid production from glycerol in the presence of erythromycin. For further characterization, the G+C contents of representative strains from the various types according to the previous tests were determined. For this, selected organisms were cultured in nutrient broth at 30°C for up to 48 h. Then, 100 IU of penicillin per ml was added to facilitate cell lysis (26). After additional incubation for 8 h,

TABLE 1. Characterization to genus level of the gram-positive, catalase-positive cocci isolated from Iberian ham

No. of strains	Sensitivity to lysostaphin	Moderate halophile	Sensitivity to novobiocin	G+C content (%)	Genus
1,114	+	−	−	34.5–38.2	*Staphylococcus*
182	+	−	+	31.7–39	*Staphylococcus*
18	−	−	ND[a]	70.1–74.5	*Micrococcus*
13	−	+	+	42.3–51.5	NI[b]

[a] ND, not determined.
[b] NI, not identified.

DNA was extracted (16). DNA was deproteinized to reach a ratio of optical density at 260 nm/optical density at 280 nm of >2 (19). The G+C content was determined from the midpoint value of thermal denaturation (20) by using a spectrophotometer (Perkin-Elmer, model Lambda).

Organisms that could not be identified by the tests described above were characterized by their ability to grow at different salt concentrations and by restriction enzyme analysis of the genes coding for 16S rRNA (16S rDNA). Growth in nutrient broth with final NaCl concentrations of 0, 1, 3, 5, 10, 15, 20, 25, and 30% (wt/vol) for 4 days at 30°C was estimated. Turbidity of cultures was measured at 620 nm. For restriction enzyme analysis of 16S rDNA, reference strains *Planococcus citreus* CCM 316, *Salinicoccus roseus* CCM 3516, and *Marinococcus halophilus* CCM 2706 and one strain of *Staphylococcus xylosus* isolated in this study were included. The 16S rDNAs from the various organisms were amplified by PCR using two universal primers, 5′ GAGAGTTTGATCCTGGC TCAGGA (nucleotides 8 to 30) and 5′ AAGGAGGTGATCCAGCCGCA (nucleotides 1540 to 1521), derived from highly conserved regions of the 16S rRNA (11). Reactions were performed with 50-μl volumes containing 5 μl of 10× buffer (50 mM KCl, 10 mM Tris-HCl, 15 mM $MgCl_2$), 5 μl of deoxyribonucleotides (1.25 mM each), 1 μl of each primer (20 mM), and template DNA at a final concentration of 1 ng/μl. The mixture was denatured at 94°C for 3 min before the addition of 1 U of *Taq* polymerase (Pharmacia Biotech, Uppsala, Sweden). PCR amplification was performed by using a thermal cycler from Biometra (Maidstone, United Kingdom) under the following conditions: 29 cycles of 92°C for 1 min, 57°C for 1 min, and 72°C for 1.5 min, with a final extension at 72°C for 5 min. Approximately 2 μg of PCR product was subjected to digestion with 16 U each of *Sau*3AI, *Rsa*I, *Taq*I, *Cla*I, *Xba*I, and *Spe*I (Pharmacia Biotech) according to the conditions recommended by the manufacturer. The restriction products were separated by electrophoresis in 1.5% agarose gels using 0.5× Tris-borate-EDTA buffer. The gels were stained with ethidium bromide and photographed upon transillumination by UV light.

For species identification, the following tests were used (24): production of pigment; colony diameter; coagulase activity; production of acid from D-xylose, sucrose, D-cellobiose, L-arabinose, D-mannose, raffinose, maltose, and D-turanose; oxidase, urease, β-galactosidase, phosphatase, and DNase activities; production of acetoin; growth in nutrient agar at 10 and 45°C; nitrate reduction; motility; clumping factor; growth in Simmons citrate agar; and hydrolysis of gelatin and casein.

Detection of genes encoding A, B, C, and D staphylococcal enterotoxins. Strains of the main types of detected organisms and two reference strains of *S. aureus* (FRI 1173 and CCUG 9128) were investigated by slot blot hybridization for genes encoding A, B, C, and D staphylococcal enterotoxins. Four specific DNA probes (Sa, Sb, Sc, and Sd) for the genes *sea*, *seb*, *sec*, and *sed* were used (12).

For slot blot hybridization, 10 μl of DNA was denatured by boiling for 5 min in 1× SSC (0.15 mol of NaCl per liter plus 0.015 mol of trisodium citrate per liter) and transferred to Hybond N^+ nylon membranes (Amersham International, Buckinghamshire, United Kingdom) with Millipore slot blot equipment. DNA was fixed to the membrane by placing the membrane on Whatman 3MM filter paper soaked in 0.4 N NaOH for 20 min.

The specific hybridization probes (Sa, Sb, Sc, and Sd) were labeled with fluorescein-dUTP, and hybridization was detected with an enhanced chemiluminescence kit (Amersham International). Each probe was subsequently hybridized at 50°C for 2 h and washed in 1× SSC–0.1% (wt/vol) sodium dodecyl sulfate at 42°C for 30 min. Following the addition of the detection reagents, the blot was exposed to X-ray film for 2 min.

Staphylococcal enterotoxin analysis. Organisms reacting with any of the DNA probes were grown in brain heart infusion (Oxoid, Unipath Ltd.) at 37°C for 18 h. After centrifugation at 10,000 × *g* for 20 min, the supernatants were tested for A, B, C, and D staphylococcal enterotoxins by a semiquantitative sandwich enzyme immunoassay (SET-EIA) (Riedel-de Haen AG, Seelze, Germany).

RESULTS

The majority of the 1,327 strains of gram-positive, catalase-positive cocci isolated from Iberian ham showed sensitivity to lysostaphin (Table 1). Most of these were resistant to novobiocin; only 182 strains were novobiocin sensitive. The G+C contents of all lysostaphin-sensitive isolates ranged from 31.7 to 39%. Therefore, all of these strains were classified as *Staphylococcus* spp.

Most lysostaphin-resistant isolates had G+C contents of 70 to 74% and were classified as *Micrococcus* spp. The remaining lysostaphin-resistant isolates could not be associated with any of the genera described in the group of gram-positive, catalase-positive cocci (9) because they had G+C contents of 42.3 to 51.5% and were able to grow under both aerobic and anaerobic conditions. They showed optimum growth in nutrient broth with 3 to 5% NaCl (Fig. 1). Growth was fairly good with 1, 10, and 15% NaCl and poor with 0 and 20% NaCl, and no growth was observed with 25 and 30% NaCl after 4 days of incubation. In addition, one of these isolates was subjected to restriction enzyme analysis of 16S rDNA. Patterns with *Taq*I, *Sau*3AI, *Rsa*I, and *Xba*I were different from those of *S. xylosus*, *S. roseus*, *M. halophilus*, and *P. citreus* in at least 36, 19, 29, and 27 bands, respectively (Fig. 2 and 3). Enzymes *Spe*I and *Cla*I did not cut the PCR products from any of the tested organisms.

All of the *Staphylococcus* spp. isolated from the hams were coagulase negative. Most of them (83.9%) were resistant to novobiocin. Of these, *S. xylosus* was the main species (66.8%), followed by *Staphylococcus saprophyticus* (7.6%), *Staphylococcus equorum* (6.3%), and *Staphylococcus cohnii* (2.4%) (Table 2). All of the novobiocin-sensitive staphylococci isolated, *Staphylococcus epidermidis*, *Staphylococcus warnerii*, and *Staphylococcus homini*, were recovered from the *Biceps femoris* muscle, and counts did not reach $>10^4$ CFU/g. The remaining 18 lysostaphin-sensitive isolates did not show the characteristics of any of the species described as staphylococci (9). Among the 18 *Micrococcus* isolates, there were 16 *Micrococcus kristinae* isolates, 1 *Micrococcus halobius* isolate, and 1 isolate with characteristics close to those of *Micrococcus varians* but which produced acid from maltose, without yellow pigmentation, and did not hydrolyze gelatin.

While staphylococci were found at all stages of processing, except in the fresh ham, and at all three processing plants studied, micrococci were isolated only in two samplings and from only two locations. The unidentified organisms with 42.3 to 51.5% G+C contents were the only isolates present in the

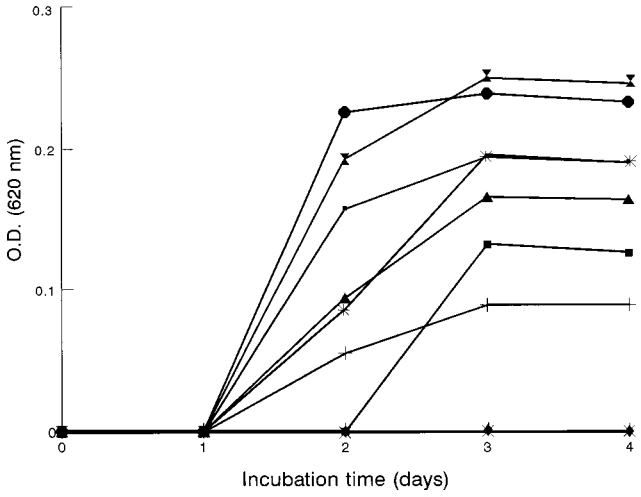

FIG. 1. Growth curves of unidentified organisms in nutrient broth containing 0% (■), 1% (∗), 3% (⧗), 5% (●), 10% (▪), 15% (▲), 20% (+), 25% (◆), and 30% (×) NaCl. O.D., optical density.

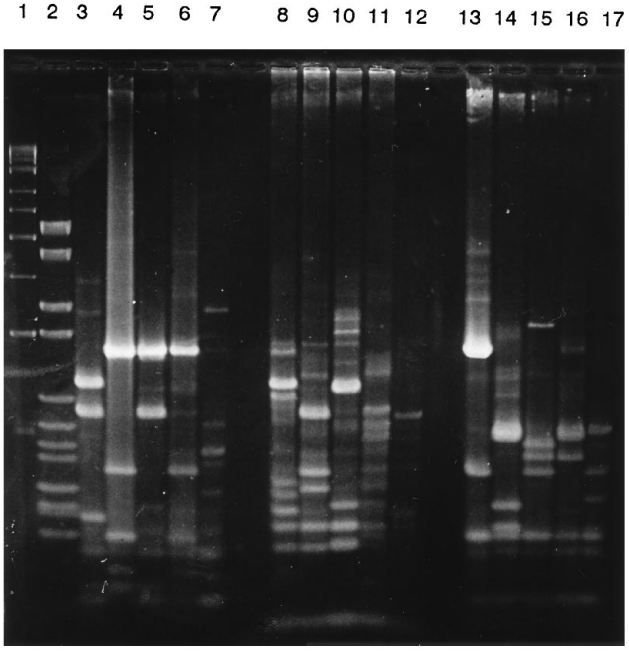

FIG. 2. Agarose gel electrophoresis of restriction endonuclease digests of 16S rDNAs amplified by PCR from an unidentified organism and reference strains. PCR products were digested with *Taq*I (lanes 3 to 7), *Sau*3AI (lanes 8 to 12), and *Rsa*I (lanes 13 to 17). Lane 1, DNA marker with fragment sizes from 10 to 0.5 kb; lane 2, DNA marker with fragment sizes from 2.2 to 0.15 kb; lanes 3, 8, and 13, unidentified organisms; lanes 4, 9, and 14, *S. xylosus*; lanes 5, 10, and 15, *P. citreus*; lanes 6, 11, and 16, *S. roseus*; and lanes 7, 12, and 17, *M. halophilus.*

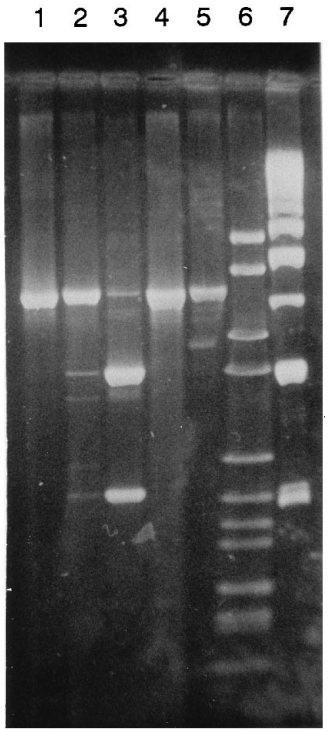

FIG. 3. Agarose gel electrophoresis of restriction endonuclease digests of 16S rDNAs amplified by PCR from an unidentified organism and reference strains. PCR products were digested with *Xba*I. Lane 1, *S. roseus*; lane 2, *M. halophilus*; lane 3, *S. xylosus*; lane 4, *P. citreus*; lane 5, unidentified organism; lane 6, DNA marker with fragment sizes from 2.2 to 0.15 kb; lane 7, DNA marker with fragment sizes from 10 to 0.5 kb.

samples from one factory at the end of processing, and counts reached 10^6 CFU/g.

Additional physiological and biochemical tests revealed a great diversity of strains within each species of novobiocin-resistant organisms (data not shown). Only sensitivity to furazolidone and lysozyme were used to classify novobiocin-resistant organisms into a moderate number of types (Table 2). Four types of novobiocin-resistant species, i.e., *S. xylosus* A, *S. xylosus* B, *S. equorum* B, and *S. saprophyticus* A, as well as the novobiocin-sensitive staphylococci, were found in all samplings and also in all three factories (Table 3).

The 28 gram-positive, catalase-positive cocci isolated from the salt were novobiocin-resistant staphylococci. The main types of isolates recovered from the salt (i.e., *S. xylosus* A and B) were also recovered from the hams in all stages of ripening and also from all three processing plants (Table 3).

The enterotoxigenic potential of representative strains from the main groups, including isolates from different stages and locations of processing, was evaluated (Table 3). Slot blot hybridization of DNA with probes Sa and Sb was negative for all of the selected isolates. On the other hand, one *S. xylosus* A isolate from the postsalting stage and three of the unidentified isolates hybridized with probe Sc (Fig. 4). Also, another *S. xylosus* A isolate, one *S. cohnii* B isolate, and the four unidentified organisms hybridized with probe Sd (Fig. 5). Strains hybridizing with any of the specific DNA gene probes were tested for enterotoxin production by the immunological assay (SET-EIA). Production of C enterotoxin was confirmed for the strain of *S. xylosus* A hybridizing with the Sc probe. However, this strain reacted positively in the immunological test for D enterotoxin, although no hybridization with the Sd probe was observed. The *S. xylosus* A and *S. cohnii* B strains which hybridized weakly with probe Sd were not confirmed for enterotoxin production by the immunological test. Finally, for three of the unidentified isolates, production of D toxin was confirmed (Table 4).

DISCUSSION

The majority (97.6%) of isolates obtained from Iberian ham at the different stages of processing were *Staphylococcus* spp.,

TABLE 2. Frequency and main characteristics of the different types of novobiocin-resistant staphylococci isolated from Iberian ham

No. of strains	Biochemical test result[a]			Sensitivity to:		Species	Type
	Xyl	Suc	Cel	Furazolidone	Lysozyme		
57	+	+	+	+	−	*S. equorum*	A
28	+	+	+	+	+	*S. equorum*	B
320	+	+	−	+	−	*S. xylosus*	A
555	+	+	−	+	+	*S. xylosus*	B
8	+	+	−	−	+	*S. xylosus*	C
85	−	+	−	+	−	*S. saprophyticus*	A
11	−	+	−	+	+	*S. saprophyticus*	B
5	−	+	−	−	−	*S. saprophyticus*	D
19	−	−	−	+	−	*S. cohnii*	A
8	−	−	−	+	+	*S. cohnii*	B
17	+	−	−	+	−	*Staphylococcus* sp.	A
1	+	−	+	+	+	*Staphylococcus* sp.	B

[a] Xyl, acid production from xylose; Suc, acid production from sucrose; Cel, acid production from cellobiose.

TABLE 3. Distribution of the gram-positive, catalase-positive cocci isolated from Iberian ham and salt and sources of the strains selected for enterotoxigenic evaluation

Type	No. of isolates from the indicated source or stage of processing[a]												Total no. of strains
	Salt	PS			D			4MC	8MC	16MC			
		I	II	III	I	II	III	I	I	I	II	III	
S. equorum													
A	2	17	1	23			2	7	5				57
B	2	2		13	1		4	3	1		2		28
S. xylosus													
A	12	85	2	103	4		32	11	21	2		48	320
B	11	52	22	73	147		139	14	66		1	30	555
C			1					6	1				8
S. saprophyticus													
A	1	20		2	4	1	5	26	2	1	11	12	85
B		2	1				3		5				11
C								5					5
S. cohnii													
A		6						5				8	19
B					5						1	2	8
Staphylococcus sp.													
A		11		1	3			1				1	17
B							1						1
Sns[b]			1		50	54	10	5	6	7	13	36	182
Micrococcus spp.								17				1	18
NI[c]											13		13
Total no. of strains	28	195	28	215	214	55	196	100	107	10	41	138	1,327

[a] Underlined numbers indicate strains used for enterotoxigenic evaluation. PS, postsalting; D, drying; 4MC, 4 months of cellar storage; 8MC, 8 months of cellar storage; 16MC, 16 months of cellar storage; I, II, and III, processing plants I to III.
[b] Sns, novobiocin-sensitive staphylococci.
[c] NI, unidentified isolates with 42.3 to 51.5% G+C.

as has been reported previously for other dry cured hams (7, 21). Only organisms characterized by a 42.3 to 51.5% G+C content were present in high numbers at the end of processing at one of the factories. Since these organisms showed optimum growth at 3 to 5% NaCl, they are moderately halophilic (14). According to Gram stain, catalase reaction, G+C content, and growth with moderately high levels of NaCl, these organisms are close to members of the genera *Planococcus*, *Salinicoccus*, and *Marinococcus* or even *Staphylococcus*. However, the un-

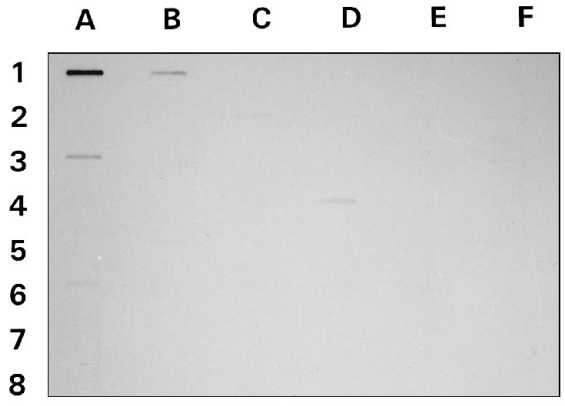

FIG. 4. Slot blot hybridization with the Sc probe. A1, control fluorescein-labeled DNA; B1, *S. aureus* producing C enterotoxin; C1, *S. xylosus* A; D1, *S. xylosus* A; E1, *S. xylosus* B; F1, *S. xylosus* C; A2, *Micrococcus* sp.; B2, *S. equorum* B; C2, NI2; D2, *S. saprophyticus* D; E2, *S. xylosus* B; F2, *S. xylosus* B; A3, NI3; B3, novobiocin-sensitive staphylococcus; C3, *S. xylosus* A; D3, *S. xylosus* C; E3, *S. equorum* A; F3, *S. saprophyticus* A; A4, *S. equorum* A; B4, *S. xylosus* B; C4, *S. cohnii* B; D4, NI1; E4, *S. xylosus* A; F4, *S. xylosus* C; A5, *S. xylosus* B; B5, *Micrococcus* sp.; C5, *S. xylosus* B; D5, *S. saprophyticus* D; E5, *Micrococcus* sp.; F5, *S. xylosus* B; A6, *S. xylosus* A; B6, *S. xylosus* A; C6, *S. xylosus* A; D6, *S. xylosus* A; E6, *S. xylosus* B; F6, *S. xylosus* B; A7, NI4; B7, *S. xylosus* A; C7, *S. xylosus* B; D7, *S. xylosus* A; E7, *S. xylosus* B; F7, *S. xylosus* A; A8, *S. xylosus* A; B8, *S. xylosus* B; C8, *S. xylosus* A; D8, *S. xylosus* B; and E8, *S. xylosus* B.

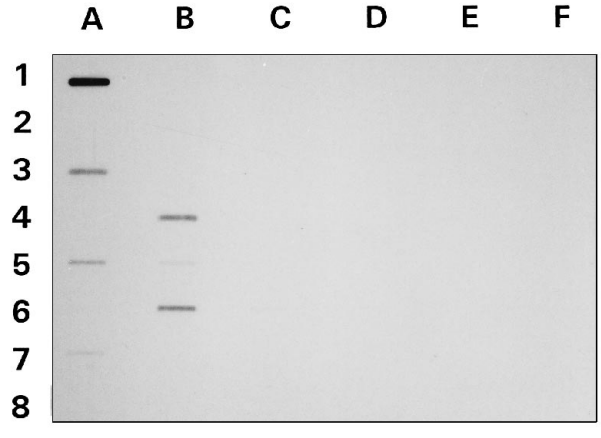

FIG. 5. Slot blot hybridization with the Sd probe. A1, control fluorescein-labeled DNA; B1, *S. equorum* A; C1, *S. saprophyticus* A; D1, *S. xylosus* A; E1, *S. equorum* B; F1, *Micrococcus* sp.; A2, *S. xylosus* B; B2, *S. xylosus* C; C2, *S. xylosus* B; D2, novobiocin-sensitive staphylococcus; E2, *S. xylosus* B; F2, *S. xylosus* A; A3, NI2; B3, *S. xylosus* B; C3, *Micrococcus* sp.; D3, *S. xylosus* A; E3, *S. xylosus* B; F3, *S. equorum* A; A4, *S. xylosus* C; B4, NI1; C4, *S. xylosus* A; D4, *S. saprophyticus* D; E4, *S. xylosus* A; F4, *S. xylosus* B; A5, NI3; B5, *S. xylosus* A; C5, *S. xylosus* B; D5, *S. saprophyticus* D; E5, *S. xylosus* B; F5, *S. xylosus* A; A6, *S. xylosus* A; B6, NI4; C6, *S. xylosus* A; D6, *S. xylosus* B; E6, *S. saprophyticus* A; F6, *S. xylosus* A; A7, *S. cohnii* B; B7 *S. xylosus* B; C7, *S. xylosus* A; D7, *S. xylosus* B; E7, *S. xylosus* A; F7, *S. xylosus* B; A8, *S. xylosus* C; B8, *S. xylosus* B; C8, *Micrococcus* sp.; and D8, *S. xylosus* B.

TABLE 4. Enterotoxigenic evaluation of strains hybridizing with any of the DNA probes

Strain	Hybridization with probe:				Enterotoxin production[a]			
	Sa	Sb	Sc	Sd	A	B	C	D
S. xylosus A	−	−	+[wb]	−	−	−	+	+
	−	−	−	+[w]	−	−	−	−
S. cohnii B	−	−	−	+[w]	−	−	−	−
NI1	−	−	+[w]	+	−	−	−	−
NI2	−	−	+[w]	+	−	−	−	+
NI3	−	−	+	+	−	−	−	+
NI4	−	−	−	+	−	−	−	+

[a] Determined by SET-EIA.
[b] +[w], weak positive.

identified organisms were able to grow under anaerobic conditions and showed a pattern different from those of reference strains in the restriction enzyme analysis of 16S rDNA. For this reason, they could be included in a new taxon.

S. xylosus was the species most frequently isolated from most samples, as has also been reported for other types of dry cured ham (3, 4, 21). The additional physiological and biochemical tests revealed a great diversity of strains within each species. Many of the species of the genus *Staphylococcus* are composed of a high percentage of strains carrying plasmids, and at least some of them may exchange genetic information (13). Such exchange could contribute to the great diversity of strains in Iberian ham. However, the salt seems to be an essential source of these organisms, because the main types isolated from hams in all stages of ripening were also found in the salt but not in fresh ham.

One *S. xylosus* A strain hybridized with probe Sc, showing production of C and D enterotoxins. This might represent an artifact of immunological assays due to cross-reacting staphylococcal antigens, or a variant in the staphylococcal enterotoxin genes that do not hybridize with the specific probe, as has been hypothesized for *S. aureus* (12). Several strains hybridized with the DNA probes but did not produce toxins (Table 4). Defects in toxin expression have been shown to be due to point mutations which convert the toxin genes to silent genes (23). These genes could be reactivated by a single mutational event and could be expressed under appropriate conditions (8). Therefore, not only enterotoxin production, but also detection of these enterotoxin genes must be considered for all gram-positive, catalase-positive cocci used in dry cured meat products.

Organisms with all of the biochemical and physiological characteristics of the enterotoxin-producing strains of *S. xylosus* were detected only from green hams at the postsalting and drying stages. Therefore, the actual risk for these organisms can be considered negligible. However, lack of control of the microbial population during processing could facilitate their presence in high numbers even in the final product.

Among the organisms with G+C contents of 42.3 to 51.5%, all four isolates tested hybridized with DNA probes, but D-enterotoxin production was confirmed for only three of them. Because these organisms were isolated from the final product at 10^6 CFU/g, they pose a potential hazard for the consumer. The concern is increased by the fact that organisms with similar physiological and biochemical characteristics and G+C contents have been isolated from dry cured sausages (25) and cheese (5). In addition to precise characterization of these organisms, the incidence in foods and conditions for enterotoxin production should be known in order to assess the actual risk for consumers. In addition, measures to prevent the occurrence of toxigenic organisms are necessary. Given that gram-positive, catalase-positive cocci contribute to raw-ham ripening (17), the use of safe, technologically tested strains as starter cultures should be considered for this purpose. Strains of types recovered from all samplings and also from all three locations that do not hybridize with any of the probes tested could be evaluated for use as starter cultures.

In conclusion, there is a great diversity in the gram-positive, catalase-positive cocci found in Iberian ham, including toxigenic strains from both coagulase-negative staphylococci and unidentified, moderately halophilic organisms that could constitute a new taxon. Appropriate steps to minimize the health hazard due to these toxigenic organisms in dry cured ham should be taken.

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