Enterotoxigenicity of *Staphylococcus* Strains Isolated from Spanish Dry-Cured Hams

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The ability of 135 *Staphylococcus* strains isolated from Spanish dry-cured hams to produce enterotoxins in culture was investigated by the reversed passive latex agglutination method. A high percentage of enterotoxigenic *Staphylococcus aureus* strains (85.9%) was recorded, and 54.3% of these produced enterotoxin A. One of the two *Staphylococcus epidermidis* strains produced enterotoxin C. The reversed passive latex agglutination method yielded satisfactory results.

Dry-cured hams are frequently implicated in outbreaks of staphylococcal poisoning (6, 11). *Staphylococcus aureus* has been the secondmost frequently reported microorganism causing outbreaks of food poisoning and food-borne toxic infections in Spain in recent years (2). During this same period, meat products have been the secondmost frequently reported food involved in such outbreaks (1). Humans are the most important source of staphylococci: food handlers may contaminate raw materials, equipment, and finished products via cuts in the hands and throat infections (13).

S. aureus has been isolated from hams by researchers (11, 18). The presence of this bacterium may pose a threat to health if the strains present are enterotoxigenic and conditions are favorable for growth (10).

Data on the enterotoxigenicity of *S. aureus* strains isolated from human beings and food in general (17) indicate that more than 50% of strains are enterotoxigenic.

Although on the whole coagulase-negative *Staphylococcus* species do not produce enterotoxins, some researchers (19, 25) have described enterotoxigenic strains of coagulase-negative staphylococci that have included organisms responsible for outbreaks of food poisoning (5). Consequently, the enterotoxigenicity of such strains when present in large numbers in food, as often occurs in dry-cured hams, requires further study.

No studies of the enterotoxigenicity of staphylococci isolated from dry-cured hams during the production process or from the different types of spoilage affecting Spanish dry-cured hams have been published. Therefore, in view of the large number of cases in which ham is implicated in outbreaks of staphylococcal food poisoning, the present article reports the results of a study of the enterotoxigenic capacity of *Staphylococcus* strains isolated from dry-cured hams in an attempt to determine the types of produced enterotoxins, the incidence of such strains in dry-cured hams, and the possible risk of food poisoning attached to the consumption of contaminated hams.

Recent advances in immunological techniques have resulted in fast and sensitive methods for detecting staphylococcal enterotoxins, such as the reversed passive latex

agglutination (RPLA) method. This method uses latex particles sensitized with purified anti-staphylococcal enterotoxin immunoglobins that agglutinate in the presence of homologous enterotoxins. Although the RPLA assay is easy to perform and requires no special equipment, it occasionally yields nonspecific agglutination (4). Wieneke (26) reported that *S. aureus* produced greater amounts of enterotoxin in brain heart infusion (BHI) broth than in foodstuffs and that the RPLA method was sufficiently sensitive to detect all enterotoxin-producing strains, hence it was selected for use in the present study.

Samples. A total of 233 samples were analyzed. The numbers of samples from each category were as follows: (i) raw, 46; (ii) cured, 168 (slow, 90; fast, 78); (iii) spoiled, 19 (slow, 14; fast, 5).

The stages of ham dry-curing are: salting, postsalting, and drying. In the fast dry-curing process, the salting and postsalting are done at 4°C for 11 and 25 days, respectively, and the drying is done in two stages (45 days at 17°C and 15 days at 30°C). In the slow dry-curing process, the salting and postsalting are done at 4°C for 7 to 9 and 45 days, respectively, and the drying is done in four stages (30 days at 14°C, 25 to 30 days at 25°C, 30 days at 32°C, and 10 to 15 days at 35°C), and finally, the hams are stored for 60 days at room temperature.

The fast dry-curing process took between 5 and 6 months, with 4.6% salt, 5.5 ppm nitrates, 1.21 ppm nitrites, and 0.919 water activity in the final product. Slow dry-curing took from 9 to 12 months, with 7.3% salt, 46.9 ppm nitrates, 1.6 ppm nitrites, and 0.8844 water activity in the final product.

Samples taken from the hams that spoiled during the fast dry-curing were from four greenish hams, which had green areas inside the muscle but no off odors, and one "bone-tainted" ham, which had both an unpleasant odor and a gas pocket inside the muscle. For hams spoiled during the slow dry-curing process, there were 10 "bone sour" hams which gave off a putrefied smell from the area around the hip bone or the area around the joint, one "bone-tainted" ham, two vacuum-packed boned hams, and one "slimy" ham, which had a pinkish-orangish slimy surface.

Samples were collected aseptically from the surface and/or from inside of the muscle near the hip bone during the different stages of both dry-curing processes. Samples from the spoiled hams were taken directly from the affected areas,

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TABLE 1. Enterotoxigenic *Staphylococcus* strains isolated from fast and slow dry-curing ham processes

Dry-curing process	Ham sample	No. of strains	No. of nonspecific strains (%)	No. of enterotoxigenic strains (%)	% of enterotoxigenic strains producing enterotoxin:								
					A	B	C	A and B	A and C	B and C	A, B, and C	A, B, and D	A, B, C, and D
Fast	Normal	26	2 (7.7)	24 (92.3)	62.5			20.8			4.2		12.5
	Spoiled	2	1 (50)										
Slow	Normal	89		79 (88.8)	58.2	3.8	2.5	14	2.5	1.3	3.8	2.5	11.4
	Spoiled	18	2 (11.1)	13 (72.2)	15.4		76.9		7.7				
Total		135	5 (3.7)	116 (85.9)	54.3	2.6	10.3	13.8	2.6	0.9	3.4	1.7	10.3

which were predominantly areas with a well-developed vascular system near the joints.

Portions (10 g each) of sample were suspended in 90 ml of tryptone water and homogenized aseptically. Serial decimal dilutions were then prepared from this initial dilution.

Reference strains. *S. aureus* (NCTC 10702) and *Staphylococcus epidermidis* (ATCC 14990) were kindly supplied by the Spanish Type Culture Collection.

Isolation and identification of *Staphylococcus* isolates. Isolation of *S. aureus* was carried out by one of two methods: (i) plating 0.1-ml samples of the different dilutions onto the surface of Baird Parker agar (22) and incubating at 37°C for 48 h or (ii) enrichment by inoculating 1-ml samples of the different dilutions in tryptone soya broth (22) containing 1% catalase and incubating at 37°C for 48 h, plating on Baird Parker agar, and reincubating at 37°C for 48 h. Typical colonies were selected for the purpose of obtaining pure cultures on plate count agar.

The strains were identified according to the criteria in *Bergey's Manual* (21). The following tests were carried out: Gram stain, motility, aerobic and anaerobic growth, oxidase and catalase production, oxidation-fermentation from glucose (7), pigmentation, colony morphology, tube coagulase, DNase, mannitol fermentation, acetoin production (9), and heat-stable DNase (14). The biochemical assays of the API STAPH system (Biomerieux) were also carried out.

Enterotoxin production and detection. The strains were cultured in BHI broth at 37°C for 18 h with shaking and then centrifuged at 11,400 × *g* at 4°C for 15 min. The supernatant was used to assay enterotoxin production. The staphylococcal enterotoxins A, B, C, and D present in the supernatant were detected by the RPLA method developed by Shingaki et al. (23) using an RPLA diagnostic kit (Oxoid).

The most probable number (MPN) of *S. aureus* during the fast dry-curing process was between 3 and 9/g, and during the slow dry-curing process it was between 9 and 4.8 × 10^2/g. *S. aureus* was not found in the final product of both curing processes. In the hams that spoiled during the fast dry-curing process, only one greenish ham sample had an MPN of 4/g, while the number of *S. aureus* was between <10 to 10^4 CFU/g in the spoiled hams detected during the slow dry-curing process, with the highest numbers corresponding to the bone-tainted hams.

During the study of both processes of industrial ham curing (fast and slow), as well as from the study of spoiled hams, 135 *Staphylococcus* strains were isolated and identified. Of these, 133 were *S. aureus* and 2 were *S. epidermidis*.

The results are summarized in Table 1. The *S. aureus* reference strain produced enterotoxins A and B.

During the different stages of the fast dry-curing ham process, the percentage of enterotoxigenic strains isolated was higher than in the slow dry-curing process. Most of the enterotoxigenic strains produced enterotoxin A in both processes. More types of enterotoxigenic strains were isolated in the slow dry-curing ham process.

In the hams that spoiled during the slow dry-curing ham process, 18 strains were isolated from 3 bone-tainted ham samples. Sixteen strains were *S. aureus*, and two were *S. epidermidis*. Twelve *S. aureus* strains produced enterotoxins, and 10 (76.9%) of these 12 strains produced enterotoxin C. One of the *S. epidermidis* strains produced enterotoxin C.

The RPLA method gave satisfactory results, since only 3.7% of the strains yielded nonspecific agglutination, i.e., passive agglutination with both sensitized and control latex particles. One possible reason for this would appear to be that the protein A produced by many *S. aureus* strains may either remain attached to the cell wall or may be released into the liquid culture medium (8).

A very high percentage (85.9%) of enterotoxigenic *S. aureus* strains was recorded, in agreement with the findings of other workers for strains isolated from foods (17).

One of the two strains of *S. epidermidis* produced enterotoxin C, which agreed with the findings of other researchers, who have reported that coagulase-negative *Staphylococcus* strains mainly produce this enterotoxin (25).

Most (54.3%) of the enterotoxigenic strains produce enterotoxin A; since human strains predominantly produce this enterotoxin, this might be indicative of contamination by workers handling the hams during processing (3). Moreover, enterotoxin A is most often implicated in cases of staphylococcal food poisoning (18).

Some (32.7%) of the enterotoxigenic strains produced more than one toxin. The combination of enterotoxins A and B was the combination most frequently detected by us and by other researchers (20) for *S. aureus* strains isolated from the throats of healthy carriers. Fewer strains of enterotoxigenic *S. aureus* were isolated from samples taken during the fast dry-curing process than from those taken during the slow dry-curing process. This may have been related to conditions relatively more favorable for bacterial survival and growth during this latter process. No *S. aureus* strains were detected in any of the samples taken from fully cured hams prepared by either process, which agreed with the findings reported by other researchers for dry-cured ham (12, 15, 24). This could be explained by the fact that *S. aureus* organisms do not multiply in the hams during the dry-curing process, because the conditions do not allow their development (16). Thus, there is no risk of staphylococcal food poisoning from eating normal dry-cured hams. However, if there were a change in the technological processes and storage conditions (increase of temperature, pH, and

a_w), the enterotoxigenic strains would grow and produce enterotoxins in the ham.

Enterotoxigenic *S. aureus* strains were not isolated from samples of hams spoiled during the fast dry-curing process, but they were isolated from samples taken from bone-sour hams during the slow dry-curing process. Of these, 75% produced enterotoxin C. The source of these enterotoxin C-producing strains is unclear, but some researchers have related the enterotoxin C to strains of animal origin (3, 25).

Although these strains do not attain hazardous levels during normal processing of hams, their presence represents a potential hazard if production technology is altered.

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