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Production of Enterotoxin A by Supposedly Nonenterotoxigenic *Staphylococcus aureus* Strains

ESPERANZA GOMEZ-LUCÍA, JOAQUÍN GOYACHE, JOSÉ A. ORDEN, JOSÉ L. BLANCO, JOSÉ A. RUIZ-SANTA-QUITERIA, LUCAS DOMÍNGUEZ, AND GUILLERMO SUÁREZ*

U. D. Microbiología e Inmunología, Departamento de Patología Animal I (Sanidad Animal), Facultad de Veterinaria, Universidad Complutense, 28040 Madrid, Spain

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The production of staphylococcal enterotoxins A (SEA) and B (SEB) was studied by inoculating six well-defined staphylococcal collection strains into cow's, goat's, or sheep's milk (individually or as a 50% mixture of cow's + goat's or cow's + sheep's), into brain heart infusion, and into a medium generally used to enhance the synthesis of enterotoxins (3+3 medium). Four of the strains used are considered to be SEB producers, another is considered an SEA producer, and the remaining strain is nonenterotoxigenic but produces large quantities of staphylococcal protein A. Staphylococcal protein A masked the results in most cases. Only one strain secreted exclusively SEB, while the other three SEB producers synthesized SEA in different amounts. We conclude that enterotoxin production depends on the natural substrate and may differ from the results obtained when the strain is grown on cellophane over agar to determine its toxigenicity.

Staphylococcal enterotoxins are exoproteins which, when ingested, induce in humans a gastroenteric syndrome. Several types of staphylococcal enterotoxins have been identified on a serological basis and are named A through E. It seems that 5% of the food-borne staphylococcal intoxications are produced by unidentified enterotoxins. It is generally assumed that the enterotoxin types produced by any one strain are constant. However, in a previous study (13) we described the production in Manchego-type cheese of staphylococcal enterotoxin A (SEA) and nonproduction of enterotoxin B (SEB) by *Staphylococcus aureus* S6, which is reputedly a producer of SEB that synthesizes such low amounts of SEA that it was used in early studies as a source for SEB purification (3). Ours is not an isolated description, as previously Lee et al. (19) also found SEA instead of SEB in pasta dough inoculated with *S. aureus S6*. These results should alert public health authorities to the problem posed by ascribing enterotoxigenicity solely on the basis of experimental production, particularly using those techniques such as cellophane-over-agar (14) which are generally used to determine which enterotoxins are produced by staphylococcal strains.

The differences between SEA and SEB are various: SEA is considered as a primary metabolite and, thus, secreted in the exponential phase of growth. SEA detection requires the presence of fewer staphylococci than for detection of SEB, a secondary metabolite synthesized at the late exponential and early stationary phase (1, 21). This, however, is an opinion not sustained by others (3). SEA synthesis is comparatively unaffected by environmental conditions such as pH (22, 26), aeration and salt (6, 8, 20, 21), water activity (22), temperature (22, 26), and additives (21). As a consequence, SEA is associated with about 80% of the confirmed cases of staphylococcal food poisoning (7, 28).

The genetic mechanisms for the synthesis of all staphylococcal enterotoxins are still obscure. However, it has been reported that SEA is encoded by a variable genetic element (the *entA* element) and that this probably accounts for the well-known interstrain variation in ability to produce the toxin (4). Later results (5) suggest that the *entA* gene is carried by a polymorphic family of genes, some of which may be defective. Likewise, transformation and mutation analyses have suggested that the *entB* gene is structurally unstable, mobile, and carried by a very large transposable element, a bacteriophage or a site-specific transposon (24).

In the present work several well-known *S. aureus* collection strains, reported to produce exclusively SEB or, in one case, mainly SEB and small amounts of SEA, were studied with regard to enterotoxin production in different liquid media. The liquid media included milk from three domestic animal species, either alone or in combination, as the initial assumption was that the composition of milk might affect enterotoxin synthesis. The aim of the study was to determine whether these reported SEB-producing strains synthesized nonreported enterotoxins (SEA) in the media tested.

MATERIALS AND METHODS

***S. aureus* strains.** Three *S. aureus* strains, reputedly SEB producers (FRI-350, FRI-379, and FRI-1173), one strain considered to produce large quantities of SEB and low amounts of SEA (S6), one SEA-producing strain (FRI-100), and a strain (Cowan-1) which does not secrete enterotoxins but produces high amounts of staphylococcal protein A (SpA) were used in this study. All strains were kindly provided by M. S. Bergdoll (Food Research Institute, Madison, Wis.), previously adsorbed to porcelain beads. For reconstitution, the organisms were allowed to grow in brain heart infusion (BHI) broth. They were then surface streaked on BHI agar, transferred to a medium composed of tryptone (10 g), powdered skim milk (20 g), glycerol (80 ml), and distilled water (320 ml), and frozen until immediately before use. The organisms were grown in BHI broth (Micro-Adsa, Barcelona, Spain) for 18 h at 37°C in a gyratory shaker (100 rpm) and used as inoculum.

Substrates. Seven different substrates were used: milk from goat (G), sheep (S), and cow (C), either alone or combined as a 50:50 mixture of sheep's and cow's milk (SC) or goat's and cow's milk (GC); BHI broth; and 3+3 (3% N-Z Amino NAK [Sheffield Chemical Co.] plus 3% protein hydrolysate powder). S and G were obtained from healthy

* Corresponding author.

animals milked immediately before the experiment. Whole C had been commercially UHT treated. All these substrates were distributed in 100-ml amounts in 120-ml sterile plastic containers which were treated at 70°C for 30 min, three times, with 24 h between treatments. Some heat-treated substrates were left uninoculated, while the others were inoculated with one of the strains described above at a level of 5.2 to 5.6 $\log_{10}$ CFU/ml, as inferred from a curve of A_{540} plotted previously.

Incubation conditions. Inoculated substrates were incubated at 37°C for a maximum of 7 days. Samples were withdrawn after different incubation periods to determine total plate counts, staphylococcal counts, and enterotoxins (SEA and SEB). All samples were done in duplicate. An uninoculated sample of each substrate was run with the inoculated ones.

Bacterial counts. Samples were diluted in 0.1% peptone water (Difco) plus 0.1% Tween 20 (Merck) and surface plated. Two media were used throughout the experiment, Baird Parker medium (BP) (Micro-Adsa) and a plate count medium (PCM) consisting of 8 g of neopeptone, 3 g of yeast extract, 1 g of D-glucose, and 17 g of agar per 1,000 ml of distilled water.

Enterotoxin detection. SEA and SEB were detected by the enzyme-linked immunosorbent assay (ELISA) method described by Freed et al. (11) which in our hands has a detection limit of 0.625 ng of SEA per ml and 1 ng of SEB per ml. Negative samples were further extracted by a procedure similar to that described by Hirooka et al. (15) to be assayed by ELISA. Purified SEA and SEB (20 ng/ml) were added to uninoculated samples and extracted along with the others. The recovery rate of the extraction procedure ranged between 45 and 60%. To remove SpA, samples or extracts were diluted 1:1 in normal rabbit serum with inactivated complement (NRS). The serum-sample mixture was incubated at 4°C for 1 h. Parallel assays with NRS were run with the untreated samples.

Enterotoxin production on solid media. A 500-μl sample of the 18-h BHI broth inoculated with the staphylococcal strains was poured on the surface of cellophane membranes (Spectrapor membrane tubing; Spectrum Medical Industries, Inc., Los Angeles, Calif.) on BHI agar, as described by Hallander (14).

RESULTS AND DISCUSSION

Staphylococcal counts. Staphylococcal counts (arithmetic mean of two identical assays) were similar in the two plating media used (BP and PCM). The values given in Table 1 are the arithmetic mean of the counts on BP and PCM obtained in two identical assays. Growth was good in all substrates, though the poorest growth was obtained in substrates containing G and the highest (about 1.5 $\log_{10}$ higher) was obtained in BHI, generally coinciding with the highest SEB concentrations. Growth maximum was reached usually between days 1 and 3 and declined thereafter.

Enterotoxins. Enterotoxins A and B were detectable by ELISA (Fig. 1A) after the first 24 h in most substrates. Both SEA and SEB concentrations were roughly similar in each sample (Fig. 1B, day 1). We performed several tests to ascertain the validity of our results. The specificity of our ELISA systems was checked by introducing SEA in between the anti-SEB coating and conjugate and vice versa, but only the specific enterotoxins were detected. As SpA has the ability to bind the Fc portion of immunoglobulins, mainly immunoglobulin G, its presence can readily interfere with the ELISA by binding to both coating and the enzyme-conjugated antitoxin. It is produced by most strains of *S. aureus* (12, 16, 18, 25, 27), but the amount released in culture may vary from strain to strain. Berdal et al. (2) determined that the presence of SpA in concentrations greater than 500 ng/ml could affect the detection of staphylococcal enterotoxins. Several methods have been proposed for the neutralization of SpA (2, 10, 17, 23), though the addition of NRS (10) seems the most satisfactory.

The data we present, as well as those regarding staphylococcal growth and enterotoxin production in yogurt (unpublished data), suggest that SpA may be causing false-positive results in some samples. However, this was not the case in our previous study with Manchego-type cheese inoculated with *S. aureus* S6 (13). In this study we did not observe any SEB, the detection of which would be possible if SpA were present.

Figure 1B shows the concentration of both enterotoxins after the milk was diluted 1:1 with NRS to block possible SpA present. On adding NRS, the amounts of enterotoxin detected by ELISA decreased 50% due to the dilution to which the extracts were subjected. After this treatment only strain FRI-1173 produced only SEB while, surprisingly, the other three SEB producers synthesized SEA and SEB at different amounts depending on the substrate. Thus, FRI-350, considered not to produce SEA, paradoxically synthesized four times more SEA than SEB, while FRI-379 synthesized equal amounts of both and S6 produced 3.5 times more SEB than SEA. In the substrates inoculated with FRI-100, the SEA producer, only this enterotoxin was detected, while no enterotoxin was present in the Cowan-1 substrates after the addition of NRS.

The substrates we used in the experiments influenced the production of enterotoxins. G and the 50% GC mixture seemed to favor the synthesis of SEB, while, overall, very little or no SEA was detected in G or GC inoculated with the four SEB-producing strains. However, these well-known non-SEA-producing collection strains synthesized more SEA than SEB in S, C, or SC.

Strains were suspected of being contaminated with staphylococci producing SEA. To eliminate this possibility, they were grown on cellophane over agar (14) as is generally done to determine the enterotoxigenicity of a strain (Table 2). To evaluate the differences corresponding to a different period of incubation, membranes were harvested after 24 and 48 h (Table 2). By this method and after the addition of NRS, the six strains produced the enterotoxins they had been reported to synthesize, namely, SEA by FRI-100, SEB and SEA by S6, and only SEB by FRI-350, FRI-379, and FRI-1173. Thus, results with membrane over agar did not agree with results of growth on liquid media.

Strains S6, FRI-350, FRI-379, and FRI-1173 used in this experiment are well-known collection strains. They have always been regarded as strains for SEB production. These studies are mainly based on the cellophane-over-agar technique (14) for ascertaining the enterotoxigenicity of the strains. Our results show that the physical state of the substrate, i.e., a membrane or a liquid, and the composition of the substrate on which the strains are grown affect enterotoxin production, and thus, the findings reached may not agree with those obtained with the conventional techniques for determining enterotoxigenicity.

Enterotoxigenicity in staphylococcal outbreaks is routinely examined by plating suspected food in a suitable medium and searching for enterotoxigenic staphylococci. The enterotoxin found to be produced is then analyzed in the

TABLE 1. Growth and enterotoxin production in the different substrates by six *S. aureus* strains[a]

Strain	Sub-strate	Day 1					Day 2					Day 3	Day 4	Day 5	Day 7		
		Growth (log)	Enterotoxin (ng/ml of substrate)				Growth (log)	Enterotoxin (ng/ml of substrate)				Growth (log)	Growth (log)	Growth (log)	Growth (log)	Enterotoxin (ng/ml of substrate)	
			SEA	SEA+	SEB	SEB+		SEA	SEA+	SEB	SEB+					SEA+	SEB+
FRI-100	G	8.4	10.1	3.7	2.9	0	8.6	1.8	0	0	0						
	C	9.2	21.7	10.6	15.9	0	8.9	19.5	9.7	10.5	0						
	GC	8.8	21.4	12.3	11.3	1.1	8.5	20.9	7.3	15.9	0						
	BHI	9.0	34.4	11.5	22.3	0	9.0	39.6	12.6	22.2	0						
	3+3	8.8	28.8	19.3	22.5	0	8.8	42.8	13.9	34.1	0						
S6	G	6.6	0	0	0	0	7.6	2.0	0	0	0	7.5		6.9	6.0	10.0	10.0
	S	6.6	0	0	0	0	8.0	0	0	0	0						
	C	8.6	22.4	1.3	14.4	2.1	8.6	10.9	0	9.2	2.8		8.2	6.6	6.0	6.0	24.0
	GC	8.8	12.9	0	12.1	1.1	8.1	1.9	0	2.7	0	7.5		6.3	5.8	12.0	28.0
	SC	8.9	17.6	2.5	17.4	2.1	9.2	20.0	0	17.3	0						
	BHI	9.5	20.6	2.3	33.6	15.0	8.8	28.4	0	30.6	12.1						
	3+3	8.7	32.0	8.4	39.7	12.5	8.5	40.9	10.0	47.0	13.3						
FRI-350	G	7.1	0	0	0	0	0	1.2	0	0	0		8.3	8.0	7.2	20.0	6.0
	S	8.7	22.0	8.5	12.0	0	8.3	18.4	8.0	7.0	1.4						
	C	8.8	18.6	5.8	12.8	0	8.2	17.6	7.5	12.3	1.6	8.0		8.0	7.9	0	0
	GC	7.2	0	0	0	0	5.6	3.5	0	0	0	7.9		7.3	7.2	20.0	5.0
	SC	8.3	18.0	9.9	9.0	0	9.2	21.7	8.8	5.6	1.8						
	BHI	9.0	49.0	9.3	33.0	2.7	9.0	50.7	11.7	29.9	5.3						
	3+3	8.7	25.0	8.5	20.0	2.9	8.6	28.1	11.1	16.0	3.5						
FRI-379	G	8.4	0	0	0	0	8.3	0	0	0	0	8.4		8.4	8.9	18.0	6.0
	S	8.8	16.5	0	12.1	0	9.4	10.6	0	9.7	2.6						
	C	8.6	18.9	4.2	10.4	1.2	9.2	8.4	2.9	3.0	0		7.1	5.0	4.3	0	0
	GC	8.4	4.7	0	1.9	1.4	8.6	0	0	0	0	8.2		7.4	7.3	14.0	5.0
	SC	8.6	12.9	2.9	11.6	1.5	9.0	6.8	0	4.0	1.1						
	BHI	8.8	18.8	5.8	21.6	8.1	9.1	20.1	8.0	23.0	9.6						
	3+3	8.8	27.7	5.5	33.3	11.2	8.8	23.4	10.2	60.0	12.3						
FRI-1173	G	9.1	0	0	9.0	7.1	8.3	0	0	2.0	4.0	7.9		6.9	7.2	0	0
	S	9.4	1.0	0	6.9	1.4	7.9	0	0	0.9	0						
	C	8.9	6.0	0	14.1	5.6	8.8	2.9	0	7.1	2.9	8.0		4.0	0	0	20.0
	GC											6.4		6.2	5.5	0	20.0
	SC	8.7	0	0	2.0	2.0	8.7	0	0	1.8	2.0						
	BHI	9.4	11.3	0	25.9	15.1	9.4	7.0	0	21.6	13.7						
	3+3	8.5	14.7	0	23.9	15.5	8.7	10.0	0	22.0	16.0						
Cowan-1	G	8.7	0	0	1.8	1.0	7.9	0	0	0	0						
	S	6.0	2.0	0	0	0	5.3	0	0	0	0						
	C	8.6	17.1	0	22.8	0	8.7	8.9	0	11.8	0						
	GC	7.5	0.9	0	0	0	7.2	0	0	0	0						
	SC	7.4	3.2	0	1.3	0	6.4	0	0	0	0						
	BHI	9.0	26.4	0	29.6	1.5	8.9	15.9	0	21.6	0						
	3+3	8.8	22.6	0	26.2	1.6	9.1	13.5	0	27.3	0						
Avg Total		8.5	14.3	3.4	13.7	2.9	8.2	12.8	3.0	12.2	2.7	7.8	7.9	6.8	6.1	8.3	10.3
By strain[b]																	
FRI-100		8.8	23.3	11.5	15.0	0.2	8.8	24.9	8.7	16.5	0						
S6		8.2	15.1	2.1	16.7	4.7	8.4	14.9	1.4	15.3	4.0	7.5	8.2	6.6	5.9	9.3	20.7
FRI-350		8.3	18.9	6.0	12.4	0.8	7.0	20.2	6.7	10.1	1.9	8.0	8.3	7.8	7.4	13.3	3.7
FRI-379		8.6	14.2	2.6	13.0	3.4	8.9	9.9	3.0	14.2	3.7	8.3	7.1	6.9	6.8	10.7	3.7
FRI-1173		9.0	5.5	0	13.6	7.8	8.6	3.3	0	9.2	6.4	7.2		5.7	4.2	0	13.3
Cowan-1		8.0	10.3	0	11.7	0.6	7.6	5.5	0	8.7	0						
By substrate[c]																	
G		8.1	2.0	0.6	2.3	1.4	6.8	0.8	0	0.3	0.7	7.9	8.3	7.6	7.3	12.0	5.5
S		7.9	8.4	1.7	6.2	0.3	7.8	5.8	1.6	3.5	0.8						
C		8.8	17.5	3.7	15.1	1.5	8.7	11.4	3.4	9.0	1.2	8.0	7.6	5.9	4.6	1.5	11.0
GC		8.1	8.0	2.5	5.1	0.7	7.6	5.3	1.5	3.7	0	7.5		6.8	6.4	11.5	14.5
SC		8.4	10.3	3.1	8.3	1.1	8.5	9.7	1.8	5.7	1.0						
BHI		9.1	26.8	4.8	27.7	7.1	9.0	27.0	5.4	24.8	6.8						
3+3		8.7	25.1	7.0	27.6	7.3	8.8	26.5	7.5	34.4	7.5						

[a] Enterotoxin is expressed as the average of two identical experiments. +, After diluting 1:1 in NRS.
[b] Average over all media.
[c] Average over all strains.

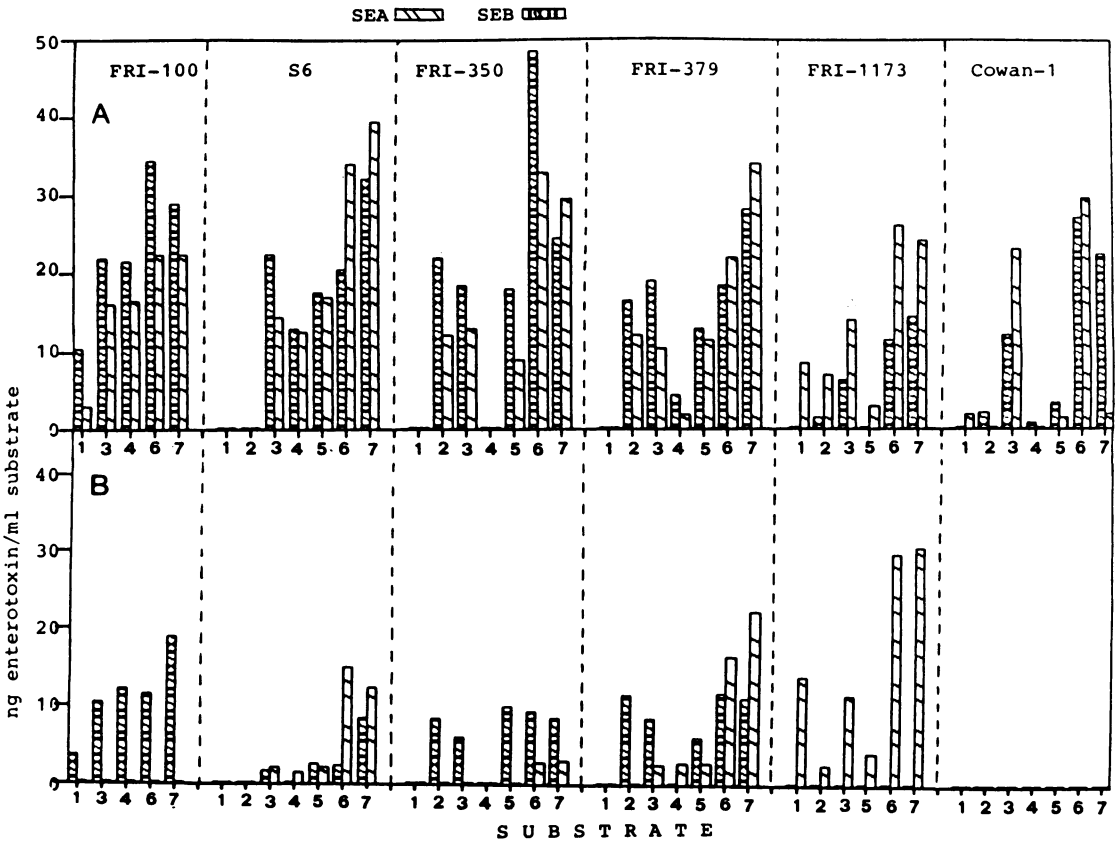

FIG. 1. Enterotoxin concentration detected in day 1 (average of two experiments). (A) Before the addition of NRS; (B) after the addition of NRS. The substrates used were: 1, G; 2, S; 3, C; 4, GC; 5, SC; 6, BHI; 7, 3+3.

food extract. This procedure is followed in most laboratories which do not have sensitive analytical techniques and thus may detect enterotoxins only by immunodiffusion, a method which demands concentration to very small volumes and in which it is not possible to check the extract for several toxins. However, data presented here prove that immunodiffusion is not a good method for determining enterotoxigenicity in food as the different components in it may inhibit or enhance synthesis of enterotoxin.

For this experiment we chose two reference media, BHI and 3+3. BHI broth is a very rich and complex medium which allows staphylococcal growth and renders high enterotoxin production. The medium 3+3 is recommended for the purification of enterotoxins (9) as it stimulates production. In these two media we registered the highest levels of production of SEA and SEB.

TABLE 2. Enterotoxin harvested by cellophane-over-agar procedure with and without NRS

Strain	Day	Enterotoxin yield (ng/ml of substrate)			
		SEA		SEB	
		−NRS	+NRS	−NRS	+NRS
FRI-100	1	44.8	27.8	33.6	0
	2	44.1	28	27.8	0
S6	1	29.4	6.8	29.6	15.5
	2	25.8	7.5	27.8	15.2
FRI-350	1	4.6	0	24.7	17.1
	2	9.8	0	29.4	16.4
FRI-379	1	6.8	0	20.9	16.7
	2	5.5	0	26.8	17.6
FRI-1173	1	3.3	0	23.5	19.7
	2	7.1	0	23.1	21.7
Cowan	1	16.2	0	23	0
	2	22.7	0	30	0

For a given strain, using laboratory media, more SEB may be produced than SEA; however, in foods, these same strains may produce more SEA. By extension of this observation, it seems possible that a strain could produce only SEA when grown in foods and no detectable toxin at all when laboratory media are used. This information is important to food industry and regulatory agencies.

In brief, enterotoxin synthesis may differ in the natural and in the laboratory conditions used to ascertain enterotoxigenicity, and results should only be considered positive after the addition of NRS. These two facts should be taken into account when determining the enterotoxin-producing ability to field strains isolated from outbreaks of food-borne illness.

LITERATURE CITED

1. **Bennett, R. W., and W. T. Amos.** 1982. *Staphylococcus aureus* growth and toxin production in nitrogen-packed sandwiches. J. Food Prot. **45**:157–161.
2. **Berdal, B. P., O. Olsvik, and T. Omland.** 1981. A sandwich ELISA method for detection of *Staphylococcus aureus* enterotoxins. Acta Pathol. Microbiol. Scand. **89**:411–415.
3. **Bergdoll, M. S., J. K. Czop, and S. S. Gould.** 1974. Enterotoxin synthesis by the staphylococci. Ann. N. Y. Acad. Sci. **236**: 307–316.
4. **Betley, M. J., S. Lofdahl, B. N. Kreiswirth, M. S. Bergdoll, and P. Novick.** 1984. Staphylococcal enterotoxin A gene is associ-

ated with a variable genetic element. Proc. Natl. Acad. Sci. USA **81**:5179–5183.
5. **Betley, M. J., and J. J. Mekalanos.** 1985. Staphylococcal enterotoxin A is encoded by phage. Science **229**:185–187.
6. **Carpenter, D. F., and G. J. Silverman.** 1974. Staphylococcal enterotoxin B and nuclease production under controlled dissolved oxygen conditions. Appl. Microbiol. **28**:628–237.
7. **Casman, E. P., R. W. Bennett, A. E. Dorsey, and J. A. Issa.** 1967. Identification of a fourth staphylococcal enterotoxin. enterotoxin D. J. Bacteriol. **94**:1875–1882.
8. **Czop, J. K., and M. S. Bergdoll.** 1970. Synthesis of enterotoxin by L-forms of *Staphylococcus aureus*. Infect. Immun. **1**:1969–1973.
9. **Fey, H., H. Pfister, and C. Muller.** 1984. Simple production of staphylococcal enterotoxins (SET) with chromatofocusing and isoelectrofocusing in flat bed gels. Zentralbl. Vet. Med. B **31**:508–517.
10. **Fey, H., H. Pfister, and O. Ruegg.** 1984. Comparative evaluation of different enzyme-linked immunosorbent assay systems for the detection of staphylococcal enterotoxins A, B, C, and D. J. Clin. Microbiol. **19**:34–38.
11. **Freed, R. C., M. L. Evenson, R. F. Reiser, and M. S. Bergdoll.** 1982. Enzyme-linked immunosorbent assay for detection of staphylococcal enterotoxin in foods. Appl. Environ. Microbiol. **44**:1349–1355.
12. **Goding, J. W.** 1978. Use of staphylococcal protein A as an immunological reagent. J. Immunol. Methods **20**:241–253.
13. **Gomez-Lucia, E., J. L. Blanco, J. Goyache, R. de la Fuente, J. A. Vazquez, E. F. R. Ferri, and G. Suarez.** 1986. Growth and enterotoxin A production by *Staphylococcus aureus* S6 in Manchego type cheese. J. Appl. Bacteriol. **61**:499–503.
14. **Hallander, H. O.** 1965. Production of large quantities of enterotoxin B and other staphylococcal toxins on solid media. Acta Pathol. Microbiol. Scand. **63**:299–305.
15. **Hirooka, E. Y., S. P. C. De Salzberg, and M. S. Bergdoll.** 1987. Production of staphylococcal enterotoxin A and thermonuclease in cream pies. J. Food Prot. **50**:952–955.
16. **Jensen, K.** 1958. A normally occurring staphylococcus antibody in human serum. Acta Pathol. Microbiol. Scand. **44**:421–428.
17. **Koper, J. W., A. M. Hagenaars, and S. Notermans.** 1980. Prevention of cross-reactions in the enzyme linked immunosorbent assay (ELISA) for the detection of *Staphylococcus aureus* enterotoxin type B in culture filtrates and foods. J. Food Safety **2**:35–45.
18. **Langone, J. J.** 1982. Protein A of *Staphylococcus aureus* and related immunoglobulin receptors produced by streptococci and pneumococci. Adv. Immunol. **32**:157–252.
19. **Lee, W. H., C. L. Staples, and J. C. Olson.** 1975. *Staphylococcus aureus* growth and survival in macaroni dough and the persistence of enterotoxins in the dried products. J. Food Sci. **40**:119–120.
20. **Marcy, J. A., A. A. Kraft, D. G. Olson, H. W. Walker, and D. K. Hotchkiss.** 1985. Fate of *Staphylococcus aureus* in reduced sodium fermented sausage. J. Food Sci. **50**:316–320.
21. **Markus, Z. H., and G. J. Silverman.** 1970. Factors affecting the secretion of staphylococcal enterotoxin J. Appl. Microbiol. **20**:492–496.
22. **Notermans, S., and C. J. Heuvelman.** 1983. Combined effect of water activity, pH and sub-optimal temperature on growth and enterotoxin production of *Staphylococcus aureus*. J. Food Sci. **48**:1832–1840.
23. **Notermans, S., P. Timmerman, and J. Nagel.** 1982. Interaction of staphylococcal protein A in enzyme-linked immunosorbent assays for detecting staphylococcal antigens. J. Immunol. Methods **55**:35–41.
24. **Ranelli, D. M., C. L. Jones, M. B. Johns, G. J. Mussey, and S. A. Khan.** 1985. Molecular cloning of staphylococcal enterotoxin B gene in *Escherichia coli* and *Staphylococcus aureus*. Proc. Natl. Acad. Sci. USA **82**:5850–5854.
25. **Rosten, P. M., K. H. Bartlett, and A. W. Chow.** 1987. Detection and quantitation of toxic shock syndrome toxin 1 in vitro and in vivo by noncompetitive enzyme-linked immunosorbent assay. J. Clin. Microbiol. **25**:327–332.
26. **Scheusner, D. L., L. L. Hood, and L. G. Harmon.** 1973. Effect of temperature and pH on growth and enterotoxin production by *Staphylococcus aureus*. J. Milk Food Technol. **36**:249–252.
27. **Vidal, M. A., C. Bernabeu, and F. P. Conde.** 1982. Binding of human immunoglobulin M to *Staphylococcus aureus* bearing protein A. Immunol. Lett. **4**:311–315.
28. **Wieneke, A. A.** 1974. Enterotoxins production by strains of *Staphylococcus aureus* isolated from food and human beings. J. Hyg. **73**:255–262.