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Staphylococcal Enterotoxin A: a Chromosomal Gene Product†

WILLIAM M. SHAFER AND JOHN J. IANDOLO*
Division of Biology, Microbiology Group, Kansas State University, Manhattan, Kansas 66506

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Physical and genetic analysis of the plasmid make-up of *Staphylococcus aureus* strains S6 and FRI-100 has shown that staphylococcal enterotoxin A is a chromosomal gene product.

Staphylococcus aureus produces at least five extracellular immunologically distinct enterotoxins that are responsible for the clinical manifestations of staphylococcal food poisoning. Of these, enterotoxin A (SEA) is the type most frequently found associated with such incidents. Surprisingly, however, little is known about the regulation of SEA synthesis, particularly with regard to its genetic management. Before information can be obtained, a more precise estimation of the gene locus of SEA must be made. At present, SEA is assumed to be a chromosomal rather than a plasmid gene product because of the phenotypic stability it exhibits (1), even after mutagenesis for high-level production (2). There is reason, however, to examine this question experimentally, especially because of the variability observed in the locus of other enterotoxin genes. Enterotoxin B (SEB) appears to be plasmid borne in strain DU-4916 (6) but chromosomal in strains S6 and 277 (5). In this study we present evidence to suggest that SEA production in strain S6 (a producer of SEA and SEB) and in strain FRI-100 (produces only SEA) is determined by a chromosomally associated gene.

S. aureus strains S6 and FRI-100 were propagated in 3% NAK-PHP broth (3, 5) for routine passage of the cultures and for toxin determinations. Cultures were incubated at 37°C for 36 h with high aeration (4) to optimize SEA production. Test solutions were prepared by centrifugation of the media at 10,000 × *g* for 15 min. The cleared supernatant fluid was removed and, when necessary, concentrated 10× by pervaporation. The presence of SEA was then analyzed by Ouchterlony double diffusion (5). Monospecific antisera to SEA and reference toxin were provided by R. Bennett (Food and Drug Administration, Washington, D.C.).

Labeling of plasmid DNA, clear-lysate production, centrifugal analysis, transduction, and plasmid curing have been described by us (5).

Examination of the extrachromosomal DNA profile by centrifugation of labeled cleared lysates in cesium chloride-ethidium bromide equilibrium density gradients of strain S6 revealed a satellite peak of radioactivity (fraction no. 30 in Fig. 1A) indicative of covalently closed circular DNA. Strain FRI-100 exhibited only a single peak (fraction no. 36) sedimenting in the density region corresponding to chromosomal DNA and plasmid DNA existing as a relaxed molecule.

To resolve the individual plasmid species, including those that may exist as relaxed molecules, we centrifuged concentrated, cleared lysates directly in 5 to 20% neutral sucrose gradients. Results from the neutral sucrose gradients showed that only strain S6 contained plasmid DNA (Fig. 1B). A single plasmid species was resolved from S6 lysates and from the position of sedimentation had an apparent molecular weight of 17.5×10^6 (37S). As expected, no monodisperse peaks of radioactivity were resolved from strain FRI-100 when cleared lysates were centrifuged directly in neutral sucrose gradients. These ultracentrifugal data and the phenotypic stability of SEA when strain FRI-100 was subjected to repeated nitrosoguanidine mutagenesis (2) strongly suggest that SEA is a chromosomal gene product in this strain.

We have examined the 37S plasmid in strain S6 to substantiate a possible chromosomal locus for SEA in this strain. We have shown (5) that this plasmid codes for cadmium resistance (Cad^r) and therefore used that criterion in the selection of transductants and cured cells. Introduction of Cad^r into the plasmid-negative, SEA^- strain 8325 via phage 29-mediated transduction was carried out (transduction frequency, 6×10^{-7}). Subsequent neutral sucrose gradient analysis of the strain 8325 Cad^r transductants demonstrated that the 37S plasmid was present (profile identical to S6 in Fig. 1B), but toxin (SEA) analysis of concentrated culture supernatants of these clones was negative. To confirm that the SEA gene was not a component of this plasmid in strain S6, it was eliminated by growth in 3% NAK-PHP broth containing 9×10^{-6} M ethid-

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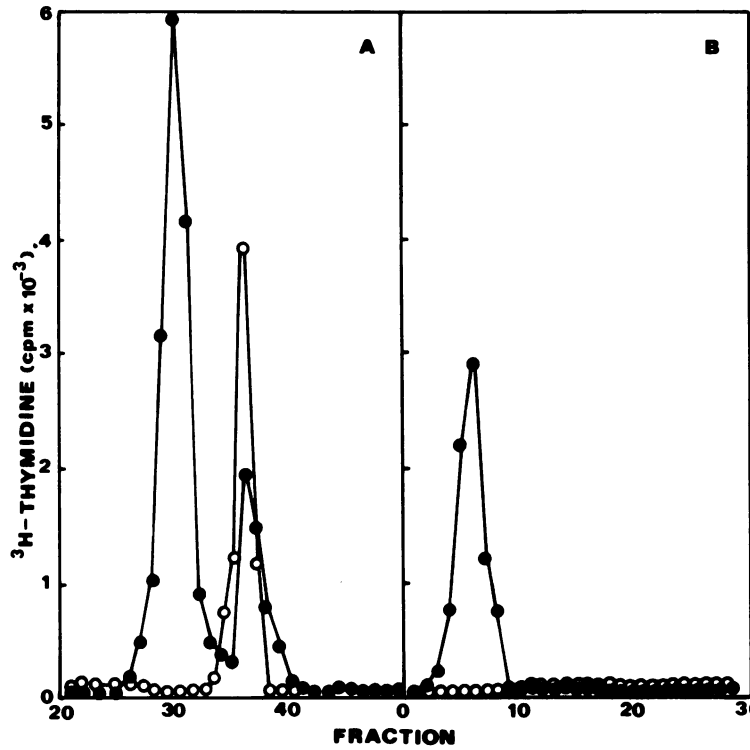

FIG. 1. *(A) Cesium chloride-ethidium bromide equilibrium density centrifugation of clear lysates of S. aureus strains S6 and FRI-100.* (●) *Strain S6;* (○) *strain FRI-100. (B) Neutral sucrose gradient centrifugation of clear lysates of S. aureus strains S6 and FRI-100.* (●) *Strain S6;* (○) *strain FRI-100.*

ium bromide. Cad^r was eliminated at a frequency of 2% (4 out of 200 colonies were Cad^s). Gradient analysis of cured cells confirmed the loss of the 37S plasmid and, most importantly, any other plasmid species of comparable molecular weight. All four S6 (Cad^s) derivatives were grown in broth, and the clarified culture fluid was analyzed for SEA. All of the sensitive derivatives were positive (Table 1).

We conclude that SEA is a chromosomally oriented gene product in at least two typical food isolates of *S. aureus*. In addition, examination of other strains of both single and multiple enterotoxin producers (data not presented) suggest a similar genetic arrangement, and we suspect that the SEA determinant is indeed a chromosomal gene in a more general sense than simply in one or two strains.

One of the interesting problems that these data underscore concerns the regulation of enterotoxin synthesis. Enterotoxins A and B have always been considered to be independently regulated, especially in strains that produce both types (1). SEB is produced in abundant amounts, commonly in excess of 100 to 200 μg/ml, whereas SEA is sparingly produced and seldom exceeds 10 μg/ml. Moreover, transiently high-level SEB producers are easily selected on antibody agar without mutation (1), whereas SEA production is stably fixed and has only been increased by repeated mutagenesis (2). This transient variability in SEB production led to the early conclusion that SEB was a plasmid gene and SEA a chromosomal gene. Our recent finding that SEB is chromosomally located (5) as well as SEA in common food isolates now makes reevaluation of toxin regulation necessary. The most important questions to be answered in this regard concern the relative gene dose of each toxin, the possibility that the SEB gene occupies part of a pseudoplasmid (7), and the longevity of the mRNA of each toxin. We are currently examining aspects of these questions.

TABLE 1. *SEA analysis of test strains*

Strain	Plasmid profile	SEA
FRI-100	None	+
S6	37S	+
S6 (Cad^s)	None	+
8325 (Cad^r)	37S from strain S6	−
8325	None	−

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