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Production of Staphylococcal Enterotoxin A on Cellophane-Over-Agar

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Up to approximately 50 μg of staphylococcal enterotoxin A per plate was produced by cellophane-over-agar cultures. Enterotoxin was determined by a latex agglutination technique.

Increased yields of staphylococcal enterotoxins have been observed and reported in the literature from cultures of *Staphylococcus aureus* grown on cellophane-over-agar media (1, 2). Results of these studies were reported in terms of microslide gel-diffusion titers. This communication provides information acquired by recovery and detection of enterotoxin A from cellophane cultures of *S. aureus* with a latex agglutination procedure.

Cellophane discs, 9 cm in diameter, were cut from dialyzing tubing [3¼ inches (approx. 8.3 cm) wide, S.S.D.C., Union Carbide], soaked in several changes of distilled water, sterilized in an autoclave (121 C, 15 min), cooled, and aseptically applied to the surface of Trypticase soy (TS) agar (1.5%; BBL, BioQuest) in plastic petri dishes (100 by 15 mm; Falcon, BioQuest). An 18-hr culture of *S. aureus* strain 100 was washed three times in phosphate buffer (*p*H 7.4) and resuspended in buffer, and 0.1 ml of the suspension, or an appropriate dilution thereof, was applied to the cellophane disc and evenly distributed over the surface with a sterile bent glass rod. Plates were incubated upright at 37 C for appropriate periods of time. Staphylococci and enterotoxin were harvested from the cellophane with two applications of 1.0 ml of sterile 0.85% saline solution. One milliliter of the pooled washes was used to determine numbers of staphylococci (pour plates of TS agar and 2 days of incubation at 37 C). The remaining sample was centrifuged (4,000 rev/min, 10 C, 5 min), and the supernatant fluid was recovered with a Pasteur pipette. A 1:10 dilution of the supernatant fluid was made in borate-saline buffer (*p*H 8.2) containing 0.07% bovine serum albumin.

The latex agglutination procedure of Salomon and Tew (3) as currently applied in the laboratory of M. S. Bergdoll (The Food Research Institute, University of Wisconsin) was followed. Proper controls were included in each determination to detect false-positive reactions.

Enterotoxin was not detected on the plates immediately after application of cells to the cellophane. In one experiment, a detectable quantity of enterotoxin (0.1 μg per plate) was recovered from the cellophane culture after only 4 hr. After 8 and 24 hr of incubation, yields of 2 and 20 μg of enterotoxin per plate, respectively, were observed. Table 1 lists enterotoxin values obtained after 24 hr from plates treated with different-sized inocula of staphylococci. Yields achieved with the largest inoculum ranged from 26 to 51 μg per plate. Although there is considerable variation inherent in the latex agglutination procedure, the data suggest that lower yields of toxin seem to be associated with smaller initial inocula of organisms.

Yields of enterotoxin encountered in this

TABLE 1. *Production of enterotoxin A by different-sized inocula of Staphylococcus aureus on cellophane-over-Trypticase soy agar (BBL) after 24 hr of incubation at 37 C*

No. of staphylococci per plate[a]		Amt of enterotoxin per plate (μg)	
Inoculum	After 24 hr	Trial 1	Trial 2
7×10^6	4×10^{10}	51	26[b]
9×10^5	4×10^{10}	13	13
9×10^4	1×10^{10}	3	3

[a] Similar results obtained in each trial.

[b] Results obtained on a succeeding day were 32 and 51 μg when plates contained initial numbers of 7×10^6 organisms.

study are probably equivalent to those reported by Jarvis and Lawrence (2), if one assumes that (i) the lowest level of enterotoxin A which they detected with the microslide technique was 1 μg/ml, (ii) the lowest titer of 10 corresponds to the minimum toxin detectable, and (iii) time and temperature of incubation (which are not stated by the authors) were similar to those used in this investigation. If these assumptions are correct, then the quantities of toxin recovered by these authors from cellophane-over-Brain Heart Infusion and -casein hydrolysate agar were 20 and 10 μg per plate, respectively.

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