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Production of High Titers of Enterotoxins for the Routine Testing of Staphylococci

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The cellophane-over-agar technique has been shown to give high titers of enterotoxins A, B, and C for routine testing of staphylococci for enterotoxigenicity.

A variety of methods and media for the production of staphylococcal enterotoxin have been reported. High titers were obtained by using a cellophane sac (5), and low titers were obtained by using semisolid agar (5) and aerated cultures (2). The most common media used are Brain Heart Infusion (BHI) and casein hydrolysates (CH). Hallander (8), using a cellophane-over-agar technique, obtained high yields of enterotoxin B on CH but lower yields on heart infusion agar. This communication demonstrates that this technique may be extended also to the production of high titers of enterotoxins A and C.

The cellophane-over-agar method was compared with shake-flask cultures, by using strains 100, S-6, and 361 for the production of enterotoxins A, B, and C, respectively. Media used were BHI (Difco), *p*H 6.0, and a casein hydrolysate medium (4) modified by adding 2% protein hydrolysate (Trypticase; BBL; 1). For shake-flask cultures, 25 ml of medium was inoculated with 1 ml of overnight culture of staphylococci and incubated on a rotary shaker at 37 C for 24 hr. After centrifugation, the supernatant fractions were tested for enterotoxin. For cellophane cultures, sterile cellophane was placed on agar in a 9-cm petri dish. The surface of the cellophane was inoculated with an overnight culture of staphylococci with a sterile applicator. Cultures were harvested by washing off with 2.5 ml of sterile 0.85% saline and centrifuged, and the supernatant fraction was tested for enterotoxin, by using a microslide gel-diffusion technique (11). The lowest levels of enterotoxins detected were 1.0 to 1.5 μg/ml.

BHI and CH agars were equally suitable for production of all three enterotoxins. The concentration of enterotoxins in preparations by using the cellophane technique was four to eight times greater than in shake-flask supernatant fractions (Table 1). The cellophane preparations have the added advantage of being relatively free from contamination with medium constituents. Cellophane-sac cultures give higher titers (5), but the cellophane-over-agar technique is simpler to use, more economical in medium and incubator space, and therefore particularly suitable for routine screening of staphylococci.

The technique was therefore used to determine the incidence of enterotoxigenicity in staphylococci in New Zealand. Clinical strains of staphylococci were isolated from lesions in hospital patients, bovine strains came from mastitic cows in six herds, and NCTC strains were those used for propagating phages. Ten of 27 clinical strains and 9 of 26 phage-propagating strains were enterotoxigenic, whereas no bovine strains produced enterotoxins A, B, or C (Table 2), supporting similar findings by Casman et al. (6). All strains were phage-typed (3). The enterotoxigenic staphylococci were not restricted to any particular phage groups, in agreement with other investigations (9, 10).

TABLE 1. *Production of enterotoxins A, B, and C in aerated and cellophane-over-agar cultures, by using Brain Heart Infusion (BHI) and casein hydrolysate (CH) media*[a]

Strain	Enterotoxin tested	Aerated cultures		Cellophane-over-agar cultures	
		BHI	CH	BHI agar	CH agar
100	A	20	10	80	40
S-6	B	80	160	400	800
361	C	40	10	80	80

[a] Results are expressed as reciprocals of gel-diffusion titers.

Dornbusch et al. (7) reported a correlation between methicillin resistance and enterotoxin B production. Although 14 of our strains, 7 of which were clinical, produced enterotoxin B, none was

TABLE 2. *Number of strains of enterotoxigenic staphylococci from various sources*

Source of staphylococci	No. tested	Entertoxin produced					
		A	B	C	AB	BC	Negative ABC
Clinical strains.....	27	1	6	2	0	1	17
NCTC strains.....	26	1	3	1	4	0	17
Bovine strains.....	26	0	0	0	0	0	26

methicillin resistant, using sensitivity discs (10 μg, BBL), possibly reflecting the extremely low usage of methicillin in New Zealand.

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