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A Research Note

Production of Staphylococcal Enterotoxins A, D, and E by Sac Culture

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ABSTRACT

The use of the sac culture method for production of relatively large amounts of enterotoxins A (SEA), D (SED), and E (SEE) for use in purification of the enterotoxins was investigated. One-hundred milliliters of medium per sac, made from Union Carbide sausage casing, with a shaking speed of 150 rpm was chosen as the optimum condition for the enterotoxin production. Brain heart infusion (BHI) broth was the medium of choice for production of SEA by strain FRI-722, with over 100 μg/ml being produced. Strain 1151m produced 20 μg/ml of SED in BHI medium which was 10 times that produced in shake flasks. The production of SEE was best in the Biosate + yeast extract medium, with adequate amounts being produced for purification.

Staphylococcal food poisoning is one of the major foodborne diseases in the world, although it does not receive the attention it should because of its relative mildness. Many cases of staphylococcal food poisoning occur in Brazil (do Carmo, personal communication), but in general these are not reported. The causative agent, the staphylococcal enterotoxins (SE), must be present in the food after growth of SE-producing staphylococci to result in the illness. In addition to the characteristic symptoms of vomiting and diarrhea, isolation of SE-producing staphylococci at levels of 10^6 to 10^7/g of food is essential to confirm staphylococcal food poisoning. Determination of SE production is by methods using specific antibodies to the SEs. The SE antibodies are available commercially but are expensive and difficult for developing countries to purchase. Several countries are undertaking the purification of the SEs along with the production of specific antibodies for their own use. A project is being undertaken in Brazil under the direction of one of us (MSB) to purify the SEs and prepare the specific antibodies for use in Brazil. Enterotoxins B (SEB) and C (SEC) are easily purified because they are produced in relatively large amounts (>100 μg/ml); however, production of adequate quantities of enterotoxins A (SEA), D (SED), and E (SEE) for purification is difficult because strains that produce large amounts of these SEs are not available.

To simplify the purification of SEA, SED, and SEE, a study of the sac culture method of Donnelly et al. (*1*) for producing increased amounts of these SEs was undertaken.

MATERIALS AND METHODS

Staphylococcal strains

The following *Staphylococcus aureus* strains were used: FRI-100 (SEA), FRI-722 (SEA), 1151m (SED), and FRI-326 (SEE).

Media for SE production

Six culture media were used: brain heart infusion, 74 g/L, (BHI, Difco Laboratories, Detroit, MI); BHI, 74 g/L, plus 2% yeast extract (BHI+YE); BHI, 74 g/L, plus 0.036% arginine (BHI+Arg), trypticase soy broth, 60 g/L, (TSB, Biobras, Sao Paulo, Brazil); TSB, 60 g/L, plus 2% yeast extract (TSB+YE); and Biosate peptone (Biosate) (yeast autolysate and pancreatic digest of casein, 6%, Becton Dickinson Microbiology Systems, Cockeysville, MD), Biosate, 6%, plus 2% yeast extract (Biosate+YE).

Production of SE.

The sac culture method of Donnelly et al. (*1*) was used in order to produce increased amounts of SEA, SED, and SEE. The effect of volume per sac (50, 75, and 100 ml/sac) with cellulose sausage casing of 10,000 molecular weight cut-off, 3.5 cm flat width (Union Carbide Corp., Chicago, IL)), and different shaking speeds (100, 150, and 200 rpm) was investigated. Cellulose dialysis tubing from Thomas Scientific (Swedesboro, NJ) of 10,000 molecular weight cut-off, 10.0 cm flat width was used in the experiment to determine the effect of volume of medium on the production of SE.

Two milliliters of inoculum (5 ml of BHI in a 15 by 125 mm screw-cap test tube inoculated with the appropriate staphylococcal culture and incubated at 37°C for 18-20 h) was added to 18 ml of 0.02 M sodium phosphate, pH 7.4, in 0.9% NaCl placed in the flasks outside the tubing containing the medium. Incubation was at 37°C for 24 h.

Detection of SE

The single gel diffusion tube method was used to quantify the SE (*2*).

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RESULTS

Volume of media

The production of SEA by *S. aureus* strains FRI-100 and FRI-722 was determined quantitatively in three media using 50, 75, and 100 ml of medium/sac. The most enterotoxin was produced with 100 ml/sac (Table 1). This amount of medium was used for the remainder of the experiments.

Shaking speed

More SE was produced with a shaking speed of 200 rpm (Table 2), but because of occasional breakage of the tubing at this speed, subsequent experiments were done with a shaking speed of 150 rpm.

Media

Results of the production of SEA by strain FRI-722, of SED by strain 1151m, and of SEE by strain FRI-326 in the different media are presented in Table 3.

DISCUSSION

A number of methods have been used to produce staphylococcal SE. Robbins et al. (*4*) concluded that the sac culture method of Donnelly et al. (*1*) was superior to other methods for the production of concentrated amounts of SE. In this study we determined the effect of volume of medium per sac and the shaking speed during incubation on SE production. Robbins et al. (*4*) used a gyrotory shaking speed of 200 rpm, but we found that at this speed frequent breaking of the sac occurred. Although less SEA was produced at 150 rpm, this shaking speed was used for most of the experiments to avoid the breakage problem.

The medium used for several years for production of the SEs was NZ-Amine NAK and PHP (protein hydrolyzate powder), pancreatic digests of casein, but these materials are no longer available. In this investigation, several available media were investigated, including BHI, as the latter medium had proved to be useful for SE production (*3,4*). Production of SEB in a defined medium composed of 18 amino acids, inorganic salts, and vitamins revealed that arginine was essential for the production of this toxin (*5*). The effect of supplementing BHI with arginine resulted in only a slight increase in the production of the SED and SEE (Table 3).

Although strain variation makes it impossible to select a medium that will yield the largest amount of SE by all strains, it is important for production of SE for purification to select a medium that will give the highest average amount of SE with the strains selected for this purpose. In this case the medium of choice is BHI has strains FR1-722 and 1151m produced the largest average amounts of SEA and SED in BHI, respectively. In the shake flask method, strain FRI-722 produced 15 µg/ml and strain 1151m produced 2 µg/ml (unpublished data) vs over 100 µg/ml of SEA and 20 µg/ml of SED, respectively, by the sac culture method.

The largest average amount of SEE was produced in Biosate+YE (13.3 µg/ml, Table 3), which would be the medium of choice for production of SEE for purification purposes by strain FRI-326. Although there were considerable differences in the amounts produced in any one medium, the average amount is of importance because several flasks would be used to produce the SE for purification.

TABLE 1. *Production of SEA by the sac culture method.*[a]

Strain	Medium	Quantity of medium (ml) 50	75	100
		SEA produced (µg/ml)[b]		
FRI-100	BHI	3.6	2.9	8.2
	BHI+YE	6.1	6.4	8.2
	TSB	1.7	1.8	3.0
FRI-722	BHI	40.3	69.3	89.3
	BHI+YE	44.0	54.0	69.3
	TSB	23.8	32.7	40.7

[a]Dialysis tubing from Thomas Scientific; shaking speed, 200 rpm.
[b]Average of three experiments.

TABLE 2. *Effect of shaking speed on production of SE by sac culture method.*[a]

Culture Medium	Strain FRI-100 (SEA) 100[b]	150	200	Strain FRI-326 (SEE) 100	150
BHI	4.9[c]	8.2	13.3	7.6	9.3
BHI+Arg	5.2	7.1	12.5	7.6	7.6
TSB	1.2	3.6	8.3	1.8	3.5
Biosate+YE	3.4	9.1	7.1	6.7	7.8

[a]Sausage casing from Union Carbide; 100 ml of medium per sac.
[b]Rpm, gyrotory shaker.
[c]µg/ml of SEA.

TABLE 3. *Production of SE in different media.*[a]

Culture Medium	SE produced (µg/ml) Strain FRI-772 (SEA)	Strain 1151m (SED)	Strain FRI-326 (SEE)
BHI	122.0[b]	18.7	8.6
BHI+Arg	88.2	20.0	9.7
TSB+YE	94.0	9.7	11.0
Biosate+YE	116.0		13.3

[a]Sac culture method, Union Carbide sausage casing, 100 ml of medium; 150 rpm shaking speed.
[b]Average of five experiments for strains FRI-722 and FRI-326;

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