RESEARCH NOTE

Conditions for the Production and Detection of *Aeromonas* Enterotoxins

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Aeromonas species have been associated with human infections specially those from the gastrointestinal tract. Virulence factors such as enterotoxins have been detected in some of these species (JM Janda 1991 *Clin Microbiol Rev 4:* 397-410). These enterotoxins, heat labile or heat stable, are products that stimulate biochemical events in the intestine leading to diarrhoea (MM Cahill 1990 *J Appl Bacteriol 69:* 1-16). The suckling mouse test modified by V Burke et al. (1981 *J Med Microbiol 14:* 401-408) has been used as a model for the detection of *Aeromonas* enterotoxins. The purpose of this study was to evaluate the production of enterotoxins by *Aeromonas* cultured in three different media as well as to evaluate the best time to detec the action of enterotoxins by using the suckling-mouse test.

Ten *Aeromonas* (five *A. hydrophila*, three *A caviae*, and two A. sobria) recovered from clinical and environmental sources were cultured in TSB-YE: Tryptone Soya Broth (Oxoid) supplemented with 0.6% (w/v) of Yeast Extract (Oxoid), as recommended by Burke et al. (*loc. cit.*). Aeromonas were cultured into 5 ml of the broth dispensed in 25 ml Erlenmeyer flasks incubated at 37°C for 24 hr in shaker at 100 rpm. The supernates were obtained after centrifugation of the growth cultures at 10,000 X g for 20 min. Supernates containing only TSB-YE served as negative controls and *A. hydrophila* (CIP7614) as a positive control. The supernates (100 µl containing a drop of a 2% Evans Blue solution) were inoculated with syringes intragastically into three or more suckled Swiss albino mice (2-6 days old). After inoculation the mice were maintained at 28°C.

In attempt to evaluate the best time for the detection of the enterotoxins action groups of mice were killed at intervals of 1, 2 and 3 hr respectively. Intestines were removed from the remaining bodies and both intestinal weights (IW) and remaining body weights (BW) were measured for each mice group and the ratios (IW/BW) calculated. We considered as enterotoxigenic strains the ones that originated ratios ≥ 0.08 (Burke et al. *loc. cit.*). Culture supernates from strains considered as toxigenic were heated at 56°C for 10 min in water bath and tested in mice as described earlier to verify the effect of temperature in the enterotoxic activity.

After the preliminary experiments, it was established that the best time to detect the enterotoxins action was 2 hr after the inoculation of the mice, so, we have also decided to evaluate the enterotoxins production in the medium proposed by CH Pai et al. (1978 *Infect Immun 19:* 908-911) used for *Yersinia* spp. and in the Evans medium for *Escherichia coli* (DJ Jr Evans 1973 *Infect Immun 8:* 725-730) under the same conditions exposed above. Bacterial positive controls for these media included *Y. enterocolitica* WA, serotype 8 (NCTC10938) and *E. coli* (strain TR22/4, serotype 0128a, 128c:H12) as well as negative controls consisting of non-inoculated media. All the bacterial positive controls produced enterotoxins in their respective media originating ratios (IW/BW) higher than 0.10 in the suckling-mose assay. Supernates without bacteria, acting as negative controls, originated always ratios (IW/BW under 0.06).

The results of all experiments are inserted in the Table. All Aeromonas toxigenic supernates lost their activities after heating at 56°C-10 min.

We concluded that the best time to detect the action produced by the enterotoxins in the suckling-mouse assay was 2 hr since 60% of the *Aeromonas* strains showed positivity. Burke et al. (*loc. cit.*) considered possible to identify known positive and negative *Aeromonas* strains correctly by as early as 2 hr when the suckling-mouse test was made at 28°C, however, they recommend 3 hr because they have found a better discrimination at this time. If our suckling-mouse assays were observed only 3 hr after inoculation, only 40% of our strains would be positive since the enterotoxins would not be detected in the two *A. sobria* species (species that were not tested by Burke et al. (*loc. cit.*) that showed positivity only after 2 hr. Those investigators also observed that enterotoxins lost their activities at 56°C-10 min. AG Dean et al. (1972 *J Infect Dis 125:* 407-411) described the suckling- mouse test to detect *E. coli* heat-stable enterotoxin. In relation to Aeromonas enterotoxins, those detected by this

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TABLE

Ratios (intestinal weights/remaining body weights) obtained after inoculation of mice with *Aeromonas* supernates in relation to time and culture media

Strains	Origin	Ratios obtained from supernates cultured in the medium proposed by Burke et al. in relation to time (hr)[a]			Ratios obtained in mice inoculated 2 hr with supernates cultured in two media[a]	
		1	2	3	CH Pai	Evans
A. hydrophila (AHPA)	water	0.076	0.070	0.055	0.075	0.067
A. hydrophila (AHU)	urine	0.080	0.080	0.097	0.067	0.086
A. hydrophila (AH1031-2)	feces	0.086	0.090	0.087	0.080	0.070
A. hydrophila (AH0712-1)	feces	0.075	0.080	0.097	0.090	0.066
A. hydrophila (AHScut)	cutaneous infection	0.060	0.066	0.077	0.070	0.078
A. caviae (ACU)	urine	0.070	0.060	0.055	0.067	0.064
A. caviae (ACO121-1)	feces	0.078	0.067	0.056	0.067	0.070
A. caviae (ACO32-2)	feces	0.098	0.086	0.100	0.097	0.082
A. sobria (ASO42-1)	feces	0.070	0.090	0.070	0.080	0.072
A. sobria (AS4831-2)	feces	0.090	0.086	0.075	0.083	0.076

[a]: strains considered as toxigenic originated ratios ≥ 0.08.

enterotoxin heat-stable (100°C-30 min) was discovered and detected in the rat perfusion system (CW Houston et al. 1991 *Experientia 47:* 424-426).

Like in the experiments of Burke et al. (*loc. cit.*), we also concluded that TSB-YE was the best medium for the production of enterotoxins since 60% of the *Aeromonas* strains were positive if compared to the other media analyzed. Our findings clearly show that the incubation for the detection of enterotoxins in the suckling-mouse assay proposed by Burke et al. (*loc. cit.*) would give negative results specially for *A. sobria* strains that showed positivity in 2 hr. Based on these results we recommend TSB-YE for the production of *Aeromonas* enterotoxins and 2 hr as the time necessary to detect them in the suckling-mouse assay.

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