A Research Note

Examination of Enterotoxin Production at Low Temperature by Yersinia spp. in Culture Media and Foods

DONALD A. SCHIEMANN

Department of Microbiology, Montana State University, Bozeman, Montana 59717

(Received for publication October 5, 1987)

ABSTRACT

Sixteen of 27 (59%) cultures of Yersinia spp. produced enterotoxin measured by the suckling mouse assay at 25°C in aerated broth culture media. Only one of 15 of these cultures, which was identified as Yersinia kristensenii, produced enterotoxin at 6°C. No enterotoxin was detected in water and methanol extracts of 6 food slurries inoculated with this toxigenic culture after incubation at 9.8°C for 4 d.

Certain strains of Yersinia spp., particularly those of the species Yersinia kristensenii, have been reported to be capable of producing a heat-stable enterotoxin detectable with the suckling mouse assay in aerated laboratory culture media at both room and refrigeration temperatures (7,8,9). It has been suggested that refrigerated foods contaminated with these toxigenic strains of Yersinia might cause intoxication by ingestion of preformed enterotoxin. However, all reported studies on enterotoxin production in refrigerated milk inoculated with toxigenic strains of Yersinia have described negative results even though growth of the bacteria is good under these conditions and toxin production occurs in milk at 25°C (2,5,9). Fukushima et al. (4) examined some components of milk that may account for suspension of toxin production at 4°C and concluded that it was not lactose nor fat.

This study found that only one of 15 strains of Yersinia that was toxigenic in aerated broth medium at 25°C could also produce enterotoxin at 6°C. No enterotoxin could be detected in aqueous and methanol extracts of 6 food slurries inoculated with this toxigenic strain after incubation at 9.8°C for 4 d.

MATERIALS AND METHODS

Experimental bacterial strains

Twenty-seven cultures of Yersinia were used in this study: 16 of Yersinia enterocolitica, 1 of Yersinia frederiksenii, 4 of Yersinia intermedia, and 6 of Yersinia kristensenii. Six of these cultures were human stool isolates, 2 were from raw milk, and 19 were originally isolated from porcine throats. All cultures were preserved in 1% peptone-40% glycerol at -20°C.

Enterotoxin production in broth media

Subcultures in broth media for production of enterotoxin were made by direct inoculation from preserved stock cultures. All broth cultures incubated at 25°C except 2 (E709 and E866, both Y. kristensenii) which used brain heart infusion (BHI) broth, were prepared with Trypticase soy-0.06% yeast extract (TSY) broth. All broth cultures incubated at low temperature were prepared with this latter medium only. Aeration was provided by incubation at 200 rpm on a rotary shaker (Psycro-Therm, New Brunswick Scientific Co., Inc., Edison, NJ). Temperature was monitored by a water-immersed thermometer meeting requirements of the National Bureau of Standards and having divisions of 0.1°C. Cultures at 25°C were incubated for 2 d, and those at low temperatures for 4 or 5 d.

Bacteria were removed from broth cultures by centrifugation (4500 x g 20 min), and the supernatant was sterilized by filtration (0.22 µm). Filtrates were held at 4°C until examined for enterotoxin by the suckling mouse assay.

Enterotoxin production in foods

The following fresh foods were purchased from a retail outlet: Lettuce, ground beef, boneless pork chops, boneless chicken thighs, pasteurized homogenized whole milk, and fish (cod fillets). A quantity of each food was mixed with a small volume of sterile saline and a slurry prepared in a Stomacher (Lab-Blender 400, Tekmar Co., Cincinnati, OH). The thick slurry was divided into two portions, one of which was inoculated with about 10⁵ bacteria of the toxigenic test strain of Y. kristensenii (E866). The inoculated and uninoculated slurries were divided and spread over the bottom of two sterile Petri plates. The plates were incubated at 9.8 ± 0.1°C for 4 d.

One inoculated and one uninoculated food slurry was extracted with sterile distilled water and a second set of slurries with methanol. The slurries were transferred to plastic bags along with an equal volume of either water or methanol and placed on the Stomacher. The homogenate was transferred to a centrifuge bottle the solids removed by centrifugation (3700 x g...
TABLE 1. Effect of aeration on enterotoxin production at 25°C by Yersinia enterocolitica in Trypticase soy-yeast extract broth.

<table>
<thead>
<tr>
<th>Culture no.</th>
<th>Gut:carcass weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60 rpm</td>
</tr>
<tr>
<td>E668</td>
<td>0.066</td>
</tr>
<tr>
<td>E694</td>
<td>0.069</td>
</tr>
</tbody>
</table>

rpm = revolutions per min on a rotary shaker.

Yersinia enterocolitica in Trypticase soy-yeast extract broth.

RESULTS

A requirement for aeration for production of heat-stable enterotoxin in TSY broth at 25°C was confirmed with two strains of Yersinia (Table 1). Sixteen of 27 (59%) cultures of Yersinia produced enterotoxin at 25°C in aerated TSY (14 cultures) or BHI (2 cultures) broth (Table 2). Only one culture (E866, Y. kristensenii) could also produce enterotoxin in TSY broth at 6°C.

None of the aqueous or methanol extracts from 6 different types of food slurries inoculated with toxigenic Y. kristensenii showed the presence of enterotoxin after incubation at 9.8°C for 4 d (Table 3). In fact, the gut:carcass weight ratios were usually lower than seen with negative broth cultures suggesting that the food extract caused some dehydration of the intestinal tissues.

DISCUSSION

The ability to produce a heat-stable enterotoxin is widespread among species of Yersinia and strains of Y. enterocolitica that are not otherwise recognizable as pathogens (10). Naturally-occurring strains of Y. enterocolitica possessing other virulence markers except toxigenicity further suggest that this heat-stable enterotoxin is not widely important in pathogenesis (9). However, the ability to produce this enterotoxin at refrigeration temperature, and the common occurrence of species such as Y. kristensenii in foods, is new reason for concern with toxin production by Yersinia. This assumes that the ingestion of preformed heat-stable enterotoxin alone would induce disease symptoms in humans, something that has not yet been demonstrated with heat-stable enterotoxins from any gram-negative bacteria.

The results from this study are not definitive proof that Yersinia is unable to produce enterotoxin in foods at refrigeration temperature. It is not known, for example, whether water or methanol extraction was an efficient technique for separating the enterotoxin from food components. This does not explain, however, the negative results with milk where the product was administered directly to the suckling mice. The lack of a selective quantitative enumeration method prevented determination of growth of the inoculated strain of Y. kristensenii among the mixed bacterial flora of the foods selected for study. The inability to grow would obviously account for the absence of enterotoxin in the foods. There were some obvious organoleptic changes during incubation, especially in the fish, indicating the multiplication of psychrotrophic spoilage organisms. Fukushima (3) and Fukushima and Gomyoda (4) found that Y. enterocolitica was inhibited by the microbial flora in raw ground pork held at 6°C.
TABLE 3. Examination of extracts from food slurries inoculated with toxigenic Yersinia kristensenii for enterotoxin after incubation at 9.8°C for 4 d.

<table>
<thead>
<tr>
<th>Food</th>
<th>Gut:carcass weight ratio</th>
<th>Aqueous extract</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>0.051</td>
<td>0.050</td>
<td></td>
</tr>
<tr>
<td>Fish</td>
<td>0.051</td>
<td>0.060</td>
<td></td>
</tr>
<tr>
<td>Lettuce</td>
<td>0.057</td>
<td>0.062</td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td>0.074</td>
<td>0.057</td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td>0.050</td>
<td>0.060</td>
<td></td>
</tr>
<tr>
<td>Pork</td>
<td>0.057</td>
<td>0.050</td>
<td></td>
</tr>
</tbody>
</table>

Aeration is important for production of enterotoxin in laboratory broth media. This was demonstrated originally by Gianella (6) with Escherichia coli, and aerated cultures have been standard for producing heat-stable enterotoxin. It may be that the oxidation reduction (Eh) potential of foods held under static conditions, and especially after the growth of other bacteria, is inappropriate for production of enterotoxin. Neither of these hypotheses is, however, supported by the observations of Fukushima et al. (5), who found that enterotoxin production was suppressed in sterile and aerated milk when incubated at 4°C, and suggested the reason was a chemical component of milk other than fat or lactose.

The data now available, including that derived from this study, do not bolster the health concern expressed by the investigators who first reported the production of enterotoxin at refrigeration temperature (7,8,9). The incidence of toxin production at low temperature observed in this study was also far less than that reported by the original observers. However, considering the compositional and microbiological variety of refrigerated foods that may be contaminated with Yersinia, this concern can not yet be summarily dismissed. Enterotoxin production at low temperatures will remain both a public health and scientific concern so long as we do not understand the genetic and/or physiological basis for suppression of enterotoxin production that accompanies a change in the growth medium.

ACKNOWLEDGMENT

Contribution no. J-2048 from the Montana Agricultural Experiment Station.

REFERENCES


JOURNAL OF FOOD PROTECTION, VOL. 51, JULY 1988