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Synthetic DNA Probes for Detection of Enterotoxigenic *Clostridium perfringens* Strains Isolated from Outbreaks of Food Poisoning

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Four synthetic oligonucleotides encoding different parts of the *Clostridium perfringens* enterotoxin gene were used to test the enterotoxigenicity of *C. perfringens* strains isolated from confirmed outbreaks of food poisoning. Of the 245 strains isolated from food and feces originating from 186 separate outbreaks, 145 (59%) gave hybridization reactions with each of the four DNA probes used, while 104 strains did not hybridize with any of the probes. There was no correlation between serotype and the presence of the enterotoxin gene, although the *C. perfringens* enterotoxin gene was rarely detected among nontypable strains (17%). Results show that DNA hybridization is a suitable method for the identification of *C. perfringens* strains which have the potential to produce enterotoxin.

Clostridium perfringens food poisoning is caused by the ingestion of food that contains large numbers of vegetative cells of enterotoxigenic strains. These cells multiply in the intestine (12) and sporulate, releasing *C. perfringens* enterotoxin (CPE) (10). *C. perfringens* is implicated as the cause of a food poisoning outbreak by the appearance of typical symptoms (abdominal pain and diarrhea), incubation time (12 to 24 h), and the type and history of the food involved. Confirmation of an outbreak requires both the detection of high levels of *C. perfringens* in the food and feces of patients and the demonstration of a common serotype. In addition, fecal enterotoxin may be detected when appropriate samples are available (1, 3, 14).

There are reports of CPE synthesis by nonsporulating cultures of *C. perfringens* (8, 9); however, the CPE responsible for illness is generally thought to be synthesized during sporulation (5, 11) and has been described as a sporulation-specific gene product (5) and a structural component of the spore coat (7, 8).

Identification of strains which produce CPE is difficult because *C. perfringens* sporulates poorly in ordinary culture media (4, 11, 15); therefore, the production of CPE by isolated strains is not used routinely as a criterion for the confirmation of outbreaks. Alternative methods for rapid and reliable identification of enterotoxigenic *C. perfringens* strains which would also enable the environmental distribution of these strains to be established are needed.

A suitable technique is the use of DNA hybridization, using a probe encoding the CPE gene, the sequence of which has been determined recently by Van Damme-Jongsten et al. (22). The object of this study was to determine the enterotoxigenic potential of *C. perfringens* by DNA hybridization. To test whether the gene was well conserved, four different synthetic oligonucleotides encoding different parts of the CPE gene were used as probes.

***C. perfringens* strains.** Strains (n = 245) of *C. perfringens* were isolated from food and feces obtained from 186 separate confirmed outbreaks of *C. perfringens* food poisoning in the United Kingdom. Confirmation of these outbreaks was based on the serotyping of isolates and the detection of CPE in feces. From each outbreak, one or occasionally two strains of *C. perfringens* were tested. Four strains of *C. perfringens* isolated from a hospital kitchen (environmental isolates) were also tested. The reference strains NCTC 8239, NCTC 10239, F3686, ATCC 12916 (producing CPE), and RIVM-D_3 were used as controls.

Serotyping. The serotyping of strains was done as described by Stringer et al. (20).

Oligonucleotides. Four oligonucleotides were synthesized on a Pharmacia gene assembler, using phosphoamidite chemistry (17). The probes were all 40-mer probes located at four different regions of the CPE gene (22). Probe A consisted of a nucleotide sequence from base pairs 426 to 465 (5′-TGTAGAATATGGATTTGGAATAACTATAGGAGAA GAAAAT-3′). Probe B consisted of a nucleotide sequence from base pairs 768 to 807 (5′-TGCATTAAACTCAAACCC AGCTGGAAATTTATATGATTGG-3′). Probe C consisted of a nucleotide sequence from base pairs 1009 to 1048 (5′-TA TATGATGAATTAGCTTTCATTACAAGAACATATTGT CC-3′). Probe D consisted of a nucleotide sequence from base pairs 130 to 169 (5′-ATGCTTAGTAACAATTTAAA TCCAATGGTGTTCGAAAATG-3′).

Colony hybridization. Strains were grown in cooked meat medium and inoculated onto brain heart infusion agar plates in fourfold, with 46 strains patterned out per plate. The inoculated plates were incubated anaerobically at 37°C overnight. Colonies were transferred onto GeneScreen Plus filters (Dupont, NEN Research Products), and the cells were lysed by an alkaline steaming procedure, as previously described (22). Positive and negative controls were placed on each filter. The reference strains NCTC 8239 (22), NCTC 10239, F3686 (22), and ATCC 12916 were used as positive controls. Negative controls were *C. perfringens* RIVM-D_3 and *Escherichia coli* and *Staphylococcus aureus* strains isolated and characterized in our laboratory. Filters (one set per probe) were prehybridized for 4 h at 40°C in 15 ml of a solution containing 50 mM Tris hydrochloride (pH 7.5), 10 mM EDTA, 1 M NaCl, 10× Denhardt solution (1× Denhardt solution contains 0.02% Ficoll [Pharmacia Fine Chemicals], 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albu-

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TABLE 1. Hybridization reactions of *C. perfringens* strains with four synthetic oligonucleotides encoding different parts of CPE

Origin of strains[a]	No. of strains tested	No. of strains with hybridization reactions with probe:			
		A	B	C	D
Food	53	35	34	35	34
Feces	192	110	110	110	110
Environment	4	0	0	0	0

[a] Strains were isolated in cases of food poisoning.

min fraction v), 100 μg of denatured herring sperm DNA per ml, 0.1% sodium PP_i, and 1% sodium dodecyl sulfate. 5′-Terminal-^{32}P-labeled oligonucleotide (200 ng) (13) was then added and allowed to hybridize for 18 h at 40°C. Afterwards, the filters were washed twice in 5× SSC (1× SSC contains 0.15 M NaCl and 0.015 M sodium citrate) and twice in 0.2× SSC for 30 min at 60°C. In all of the washing solutions, 1% sodium dodecyl sulfate and 0.1% sodium PP_i were included. The filters were exposed to Konica A2 film with an intensifying screen at −70°C for 24 h. To test for the transfer of colonies from the agar plates to the hybridization filters, one set of filters was then hybridized with the total DNA of strain NCTC 8239 (22) labeled with [α-^{32}P]ATP, using the random primed labeling method (Boehringer Mannheim Biochemicals) (6). The filters were prehybridized as described above, washed twice in 0.2× SSC for 30 min at 68°C, and then autoradiographed as described above. Only those colonies which gave a positive reaction with total DNA were examined.

Enterotoxin production. For enterotoxin production, strains were grown as described by Stringer et al. (20) in the sporulation medium of Tsai et al. (21) and the medium of Duncan and Strong (4), with raffinose or starch as the energy source, and in a sporulation medium as described by Phillips (15). Culture supernatants were tested for the presence of CPE by an enzyme-linked immunosorbent assay (1).

The hybridization reactions of all *C. perfringens* strains tested with each of the four probes are presented in Table 1. Identical results were obtained by using these four probes, which encode different parts of the CPE gene. (However, there was one strain with which probes A and C gave a hybridization reaction while probes B and D did not, possibly because of inoculation failure.) These results demonstrate that the CPE gene is highly conserved and support the findings of Richardson and Granum (16), who reported the absence of microheterogeneity in the amino acid sequence of CPE. Of the strains isolated from food and feces, only 59% (food, 66%; feces, 57%) contain the gene encoding CPE. Since there is no evidence that *C. perfringens* produces more than one enterotoxin (19), it must be concluded that strains which cannot produce enterotoxin have been isolated. Normally, *C. perfringens* is a ubiquitous organism and is frequently present in the intestinal tracts of humans (2, 18). Food may also be contaminated with *C. perfringens* from various sources, and food causing food poisoning may be contaminated with several different strains of *C. perfringens*. Therefore, it is obvious that strains which are not related to the infection have been isolated.

The serotyping of *C. perfringens* is often used to identify the food responsible for food poisoning and to confirm outbreaks (21). The 245 strains tested in this study belonged to 81 different serotypes. The incidence of the CPE gene among several serotypes showed that the gene is present in 50 to 89% of the individual serotypes tested. Statistical analysis (with the χ^2 test) showed that the CPE gene is randomly distributed among the different serotypes. However, in the nontypable strains, the gene was only found in 4 of the 24 strains tested.

Finally, of the 20 strains containing the CPE gene, 19 produced detectable quantities of CPE in vitro. One of the 18 strains lacking the CPE gene gave positive results in an enzyme-linked immunosorbent assay. The strain which did not produce CPE showed no sporulation in the culture medium. Experience with using the enzyme-linked immunosorbent assay for the detection of CPE has shown that false-positive results cannot be excluded (1, 14). Therefore, the results described above confirm the reliability of DNA hybridization in the detection of potentially enterotoxigenic *C. perfringens* strains.

From the results presented here, it can be concluded that the gene encoding CPE is highly conserved. Furthermore, only a proportion (57%) of *C. perfringens* strains contains the CPE gene. The existence of CPE-negative strains of *C. perfringens* contrasts with the theory that the enterotoxin is a structural part of the spore coat (7, 11). Therefore, further studies will be done in an attempt to establish the distribution of enterotoxigenic *C. perfringens* strains from feces and environmental samples.

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