The *Clostridium perfringens* enterotoxin gene is on a transposable element in type A human food poisoning strains

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The *Clostridium perfringens* enterotoxin gene (*cpe*) is rarely found in naturally isolated strains. In human food poisoning strains, *cpe* is found on the chromosome, and is located episomally in animal isolates. Observations that the gene was somewhat unstable and could be gained or lost suggested that the gene was on a mobile element. An IS200-like element, IS1469, is almost always upstream of *cpe*. A new insertion element was identified, IS1470, a member of the IS30 family, which is found both up- and downstream of *cpe* in the type A strain NCTC 8239. PCR results confirmed that this configuration was conserved in type A human food poisoning strains. The enterotoxin gene was on a 6.3 kb transposon which, in addition to the two flanking copies of IS1470, included IS1469 and two 1 kb stretches, one on each side of *cpe*, with no open reading frames. Results indicated that 14 bp was copied from the genome during insertion. Details of the configuration of DNA in this transposon are presented, and the possible connection of this transposon with the movement of the enterotoxin gene is discussed.

Keywords: *Clostridium perfringens*, enterotoxin, IS elements, *cpe*, transposon

INTRODUCTION

Clostridium perfringens is a Gram-positive, anaerobic, sporulating, ubiquitous bacterium which is important both in human and veterinary medicine. This species is divided into five subtypes (A–E) based on the major lethal toxins they produce. Type A can cause human food poisoning due to an enterotoxin produced during sporulation (Granum & Stewart, 1993; Rood & Cole, 1991). Other types of *C. perfringens* have been shown to carry the enterotoxin gene (Daube *et al.*, 1994) but have not been shown to cause food poisoning. The enterotoxin gene (*cpe*) can be chromosomally or plasmid-bound (Cornillot *et al.*, 1995), although in strains isolated from food poisoning outbreaks *cpe* is located on the chromosome. Observations that strains can suddenly lose or gain the ability to produce enterotoxin, along with the low prevalence of enterotoxin-positive strains from natural isolates (Van Damme-Jongsten *et al.*, 1989), has led to speculation that *cpe* is carried on a transposable element. We have previously sequenced

4.1 kb upstream of a chromosomally bound *cpe* and shown that it is associated with an IS200-like element (Brynstad *et al.*, 1994), IS1469. The close association of this insertion element to *cpe* in other strains has been confirmed (Cornillot *et al.*, 1995). The large episome to which plasmid-borne *cpe* has been mapped has been shown to harbour other toxin genes and IS elements (Daube *et al.*, 1993).

Prokaryotic transposons have been detected in essentially all species of bacteria that have been examined. Transposons always carry a gene encoding a transposase which mediates transposition. They can also carry other genes, most notably antibiotic resistance genes, catabolic genes, viral genes and genes encoding virulence factors (reviewed in Craig, 1996). Most IS elements have terminal inverted repeats, which are recognition sites for the transposase, with IS200 being a notable exception (Lam & Roth, 1986). There are certain 'hot spots' in the host DNA where insertion is more likely to occur. When the mobile element integrates into the target DNA it creates a duplication of a certain and characteristic number of base pairs from the host DNA (Galas & Chandler 1989). Mobile elements are an important vehicle for movement of genetic material both within and between species.

The EMBL/GenBank/DBJ accession numbers for the sequences reported in this paper are X71844, Y09556, Y0557 and Y0558.

Fig. 1. Schematic representation of the entire sequenced region in NCTC 8239. The white boxes show ORFs and the arrows indicate the coding strand and direction of transcription. Numbers under the IS elements indicate the total extent of the IS element including inverted repeats, while the length of the coding area of the putative transposase is given after ORF. Numbers under the other genes indicate the coding area. The *uapC* gene encodes purine permease, *cpe* encodes enterotoxin, and *nadC* encodes quinolinate phosphoribosyltransferase. Inverted repeats on each end of IS1470 are indicated. The black boxes show the up- and downstream areas of IS1469, with the stem-loop structures (s-l) and inverted repeats (ir) indicated. The location of the direct repeat (CATAATATACAAAT) is shown (dr). Restriction enzyme sites are indicated: B, *Bgl*II; C, *Cl*aI; E, *Eco*RI; H, *Hind*III; HP, *Hpa*I; K, *Kpn*I; N, *Nco*I; P, *Pst*I; X, *Xba*I. The lower part of the figure shows the location of primers used in PCR and sequencing reactions. The sequences of the primers are: 1470f, TCCTTCGGGATAAAACGC; 1470mr, TGAAAACCGTGAAGAATTGG; 1470ef, GCTTAGTAGTACCACGGCGTATTTTCG; 1470er, CACCATGGACTATCAAAATC; 1469f, ACATTTCTGGTGTATGGGA; 1469r, TGCTAGAGTAAAAAGTGTC; prof, AATACATACGACGGGTTAGAG; pror, ATTATAATTAACATCTCCTT; cpef, TCCAATGTGGTTCGAAAATG; cpeer, CATACTGTCTTTGTAAATT; dcpr, GTACATATCATTGGTGATAG; dcpr, GAATCTATCTAGGTAGCCAG; ppf, AAGCTATTGGAGAGACATCT; qpt, AAGTATTAGAGACATTAAGCTATG.

We were interested in defining a possible transposable unit that includes *cpe* to explain the sudden acquisition of enterotoxin-producing capabilities in food poisoning strains. We have previously mapped three open reading frames (ORFs) in the 3 kb region of the chromosome upstream of *cpe* and were interested in finding the gene organization downstream of *cpe*. By cloning the 2 kb DNA 3' to *cpe* we have mapped the entire transposon as well as a downstream 'housekeeping' gene.

METHODS

Bacterial strains and growth conditions. NCTC 8239 and 2154 have been described by Brynestad *et al.* (1994) and are human food poisoning strains. Strain 44071-C05 was supplied by S. Cole (Institut Pasteur, Paris, France) and was isolated from goat intestine (Cornillot *et al.*, 1995). A Norwegian hospital strain, 790-94, was obtained from the lungs of a patient who had died from complications after food poisoning. Strains were grown anaerobically in BHI medium (Difco). *Escherichia coli* XL1 Blue cells or JM109 cells (Stratagene) were used in cloning experiments. Clones were grown in LB broth or on LB agar with 100 µg ampicillin ml⁻¹.

Isolation of DNA. Plasmids from *Escherichia coli* were isolated using QIA prep or QIAGEN plasmid Maxi kits, or as described by Sambrook *et al.* (1989). DNA was isolated from gels with a QIAx gel extraction kit. Chromosomal DNA from *C. perfringens* was isolated using ADVAMAX beads and the

protocol supplied by the manufacturers for bacterial genomic DNA purification (Advanced Genetics Technologies).

PCR. PCR was carried out in an MJ Research Minicycler. The annealing temperature used was from 40 °C to 55 °C, depending on the primers used (see Fig. 1 legend). The standard programme was: 92 °C for 1 min, annealing temperature for 1 min and 72 °C for 1 min for 30 cycles. The low annealing temperatures are due to the high AT content of the *C. perfringens* genome. DYNAsZyme DNA polymerase and buffer and 10 mM dNTP from Finnzymes OY were used.

Hybridization. Various PCR products were used as probes. Blotting, the labelling of probes, and detection were performed as described in the ECL direct labelling and detection kit, using Hybond-N⁺ as the nylon membrane for blotting (Amersham).

PCR screening of clones. Clones from transformations were screened using PCR. Colonies were picked and transferred to new LB agar plates containing ampicillin. Small amounts of cells were suspended in 100 µl water, and microwaved for 3 min. Two to five microlitres from this tube was used in a PCR reaction. Initial screenings were done with 10 colonies in each tube. The 10 bacteria from positive tubes were tested individually in a new PCR.

Cloning of genes from different strains

Strain NCTC 8239. The entire *cpe* gene with flanking areas was cloned into pUC18 as a 5.1 kb *Xba*I fragment (see Fig. 1) using essentially the same strategy as by Czczulin *et al.* (1993). The

entire transposon could not be cloned, despite repeated attempts, and the rest of the downstream DNA was PCR cloned using the 3' primer determined from 44071-C05 sequences described below.

Strain 44071-C05. ORF1 (purine permease) and IS1470 are on the chromosome, as in NCTC 8239, but with *cpe* and IS1469 located on a plasmid (Cornillot *et al.*, 1995). A Southern blot showed that the DNA of interest was contained on an approximately 1 kb *Hind*III fragment. Fragments from the appropriate size range were purified from a gel, and ligated into pUC18; PCR was carried out on this ligation solution, using universal primers and a primer internal to IS1470 (1470 ef). The resulting PCR product was cloned and sequenced.

Strain 790-94. DNA was cloned using PCR products resulting from the use of qpt and 1470 f primers (see Fig. 1).

Strain 2154. DNA was cloned using the product from qpt and ppf primers (see Fig. 1).

Cloning of PCR products. PCR products were cloned using the pMOSBlue T-vector kit (Amersham) following the manufacturer's instructions.

DNA sequencing. Sequencing was performed on a Perkin Elmer ABI Prism 377 automatic sequencer. ABI Prism Dye Primer or Dye Terminator Cycle Sequencing Ready Reaction kit were used following the manufacturer's instructions. Primers other than universal are listed in the legend to Fig. 1. All sequences from NCTC 8239 have been sequenced on both DNA strands. The cloned PCR products from the other strains were, in general, sequenced from both strands. In the cases where there was not complete overlap, the same strand was sequenced in separate reactions.

RESULTS AND DISCUSSION

Characterization of ORFs upstream of *cpe*

We had previously sequenced 4.1 kb 5' to the enterotoxin gene (*cpe*) in *C. perfringens* NCTC 8239 (Brynstad *et al.*, 1994). New entries in the databases, along with corrections in the sequence, gave identities to the two unidentified ORFs and a better total homology to IS200 for ORF3. ORF1 has high homology to the purine permease gene *uapC* in *Aspergillus nidulans* (Diallinas *et al.*, 1995). This is a housekeeping gene, and has a stable position on the chromosome of the strains that have been examined. ORF2 has been registered, according to convention, as IS1470 (Lederberg, 1987). ORF3 has been registered as IS1469. An overview of the region, including the 3' DNA, is given in Fig. 1.

Analysis of IS1469

IS1469 is an IS200-like element with a 453 bp ORF, encoding a 151 aa protein, corresponding to other known IS200-like elements with greater than 60% homology, both at amino acid and DNA levels, in the coding area. Most of the DNA differences are in the third 'wobble' position, with shifts to T or A, reflecting the high AT content of *C. perfringens*. This particular group of IS elements, which was first thought to be specific to *Salmonella* spp., appears to be highly conserved in a number of bacterial species (Fig. 2). The high degree of identity of IS1469 to IS200 elements found in

Salmonella typhimurium, *Yersinia pestis*, *E. coli* and *Vibrio cholerae* was rather surprising considering the relatively large phylogenetic distance between these bacteria and *C. perfringens*, and suggests horizontal transfer of IS1469. As both *C. perfringens* and *E. coli* are part of our normal intestinal flora, they should have had ample opportunity for exchanging genetic material, through transfer of naked DNA, plasmids or through phages. Several lines of evidence point to a phage origin: the acquisition pattern of *cpe*, the fact that the enterotoxin is a superantigen (Bowness *et al.*, 1992), and the absence of 6-base restriction endonuclease sites in the DNA surrounding the *cpe*.

The entire IS200 element from *Salmonella* spp. is 709 bp (153 aa transposase) (Lam & Roth, 1983, 1986; O'Reilly *et al.*, 1990). IS1469 appears to be somewhat larger (789 bp) based on sequencing of the IS1469 from strain 44071-C05. The elements in the IS200 family are unusual in that they do not contain inverted repeats on each end of the IS element. A comparison in four species of bacteria of regions up- and downstream to the ORF showed two interesting features. *E. coli*, *Y. pestis* and *S. typhimurium* all have >90% homology in the upstream region, which contains a potential stem-loop structure. In *C. perfringens*, there is minimal homology to the other bacteria in the upstream region, but it does contain a potential stem-loop structure (Figs 1 and 2), which is positioned closer to the ORF than in the other species. Downstream to the ORF there are two blocks of highly conserved DNA regions in all four species, one of which potentially forms a stem-loop structure and defines the end of the IS element.

We have not yet performed studies on expression or transposition on IS1469. Studies performed on the IS200 from *S. typhimurium* (Haack, 1995) indicated that the element transposes through a replicative cointegrative RecA-dependent mechanism. The *recA* gene from *C. perfringens* has been cloned (Johnston *et al.*, 1997) and complements a *recA* mutant in *E. coli*, so theoretically there should be no hindrance to active transposition. The transposition studies also showed that expression was undetectable unless the upstream stem-loop structure was removed. The stem-loop upstream of IS1469 is closer to the start codon of the transposase than in the other bacteria (Fig. 2), but there is a potential promoter immediately upstream which could be used after the removal of the stem-loop structure. The fact that there is only a single copy of IS1469 suggests that this is not a very active transposon and probably does not play an important role in the types of genome rearrangements described by Haack & Roth (1995). IS1469 is found 1 kb upstream from almost all *cpe* genes, and in all type A strains tested, on both plasmids and chromosomes.

Analysis of the 5' copy of IS1470

IS1470 has been shown, using FASTA, to have 36% amino acid homology with IS1161 from *Streptococcus salivarius*, and 30% homology with IS30 from *E. coli*, and it is considered to be part of the IS30 family, which

Fig. 2. PILEUP figure of various IS200 sequences. Up- and downstream DNA and amino acids of the transposase are shown. The DNA is shown for *E. coli* (ec200), *S. typhimurium* (sa200), *Y. pestis* (yp200) and *C. perfringens* (1469). The amino acid sequence is also shown for *E. coli* (ec200) and *V. cholerae* (vc). Start and stop codons are shown in bold type. The potential stem-loop structures are underlined.

presently has 19 members (J. Mahillon & M. Chandler, unpublished). The 'DDE signature' (Kulkosky *et al.*, 1992; Rezsöházy *et al.*, 1993) and other conserved amino acids in the integrase domain are present (Fig. 3). There are inverted repeats of 26 bp, with four mismatches, flanking this element.

IS1470, a member of the IS30 family of transposases, is found in multiple copies (at least eight) in NCTC 8239, but the number per genome can vary from 0 to more than 10 in different strains of *C. perfringens* (Brynestad *et al.*, 1994; Cornillot *et al.*, 1995). It is tempting to postulate that IS1470 transposes by the same mechanism as the well-studied IS30, but we have no experimental evidence yet that shows the mode of transposition. What we have noticed is that the configuration which is very stable in the *C. perfringens* chromosome becomes very unstable when placed in a cloning vector in *E. coli*. We have never successfully cloned DNA with both copies of IS1470. Those who have isolated such a clone found that the plasmid had always undergone rearrangements and was present in high copy numbers, despite being cloned in a low copy number *E. coli* vector (G. Stewart, personal communication).

Studies on the transposition mechanism of IS30 show that the formation of a tandem repeat (IS30)₂ with an unstable circular intermediate plays an important role in transposition, and possibly gene rearrangement (Olasz *et al.*, 1993). Since we see the same type of organization of IS1470 on the genome of *C. perfringens* we can speculate that the same type of reactions occur in this bacterium. The existence of circular transposons in *C. perfringens* has been shown (Bannam *et al.*, 1995), and

these were shown to be able to transpose into the *E. coli* genome.

Cloning of DNA 3' to *cpe*

A 5.1 kb *Xba*I fragment containing *cpe*, which includes 2.4 kb upstream and 1.7 kb downstream of *cpe*, was cloned and the downstream DNA was sequenced. Analysis of the sequence showed that there was another copy of IS1470, 1.1 kb downstream of *cpe*. The intervening 1.1 kb DNA was, as the DNA upstream of *cpe*, devoid of 6-base cutting restriction enzymes and ORFs, and showed no significant similarity with other sequences in the databases.

Cloning of a partial putative *nadC* (quinolinate phosphoribosyltransferase) gene from strain 44071-C05

Since *Xba*I cuts internally in the transposon gene, the sequence of the entire gene was not obtained. We reasoned that genes linked to ORF1 in strains without or with episomally located *cpe* would be located downstream of *cpe* in NCTC 8239. Strain 44071-C05 was, therefore, utilized (see Methods). Sequencing of the PCR product showed two putative partial ORFs 3' to IS1470, one on each strand in an overlapping area. In BLAST and FASTA searches, one of these putative ORFs with a length of 77 aa gave 48 % homology with the 3' end of the *nadC* gene from *E. coli*, and had homology with *nadC* genes from other species. The other ORF had no homology to known genes. The *nadC* gene encodes quinolinate phosphoribosyltransferase, which is part of the pyridine

	1		50
IS1086		M	TRTKYQQLQP
IS30	MRRITITAEK	ASVFELWKMG	TGFSEITNIL GSKPGTIFTM LRDT.GGKPK
IS1470			MDY.QNH
IS1161			MNMSTNY
	51		100
IS1086	BERMRI....	EIWK A..EDVSLRA	MARRLGRAPS TLMRELRRNA
IS30	HERKRAVAHL	TLSEREIRA	GLSAKMSIRA IATALNRSPS TISREVRQNR
IS1470	NTESRKNKHL	NMKERMIVEI	RLKDGFSAYK NTKELNRPIN TVLNEIRRG
IS1161	STTNQSYKHL	SEAEERGEIA	YLSVGLKPAE IARRLGRNRS TTITREINRGS
	101		150
IS1086	T.....A	RGGYGAMSAQ	ACRTQRLKAS RPKVAKL.....APDGVLMGV
IS30	R.....G	RRYKAVDAN	N.RANRMAKR PKPCLL.....DQNLPLRKL
IS1470	TKQIKQKKEF	HVYFADTGEA	VYKKNRLKSN RK.....YKLL ECS.DFIKYV
IS1161	ITQVKKVNGA	KGLLPPLLRC	CCSITVIRHA REASYLLKLD SVSDDFMRAF
	151		200
IS1086	VRHFLDQKWS	PQESIGTLKR	AFDPQDLNV SHETIYNAYI AYPGELRRQ
IS30	VLEKLEMKWS	PEQISGWLRR	TKPRQKTLRI SPETIYKTLI FRSREALHHL
IS1470	VDKVNKHWS	LDACVGEALH	SSRFSPSQII ..ST..KTLI NYVDLGLLLPI
IS1161	TDAMREKPRV	..HSVDTFVH	TYRLQHVDAV VPST..KTLI NYIHQGLLEI
	201		250
IS1086	LIACLRQART	KRLPRSRGTD	RRGQ.....I PDMVSIHVRP PEVNDRLMPG
IS30	NIQHLR..RS	HSLRHGRHRT	RKRGERTINI VNGTPIHERS RNIDNRSLG
IS1470	KNIDLPAKLH	RNKKSTRVRN	NKKKLG.... TSISDRP NSIENREEFG
IS1161	KVIDLPRVR	IRKKFTKRP	TKKHLG.... KSIEERP EENNRSRFG
	251		300
	* * *	*** * *	* * *
IS1086	HWEGDLIKGA	G..NQSAVGV	LVERMSRAVL LVKMPDATAA SALAGFTGKL
IS30	HWEGDLVSGT	K..N.SHIAT	LVDRKSRYTI ILRLRGKDSV SVNQALTDKF
IS1470	HWIEDCVLGE	KSNKDKVLLT	LVERKTRYAI ISEMSSHSTI SVTKALDKIK
IS1161	DWEIDSVLGG	KTIGEPSILT	LVERQTRYAV TKKLVEKAE YVQAVLECM
	301		350
IS1086	QSLVAPLR..	*.QLTYDQGR	EMARHAELSA ATGVVYFCD PHSPWQRTG
IS30	LSLPSELRL	..KSLTWDRGM	ELARHLEFTV STGVKVFCD PQSPWQRTG
IS1470	EFLGSKFSEV	FKSITADNGS	EFADLSEFEL KTKTKVYFTH PYSSFEKGTN
IS1161	KLYP.....	IKSITADNGN	EFSSLSKIE..GLDVYFAH AYSSYERGTN
	351		400
	* ..		
IS1086	ENTNGLLRQY	LPGKTDLSVY	SQEELDAIAD SLNGRPRKTL NWHSPLOVLA
IS30	ENTNGLLRQY	FPKKTCLAQY	TQHELDLVAA QLNNRPRKTL KFKTPKEIIE
IS1470	ERNHGLIRRF	IPKGRISDY	SLETISFIEN WMNTLPR...DYKTPPELFE
IS1161	ENFNGLLREF	IPKGCSELKEL	NQNLLDYTK AINERPRRIH GYQSAKKLF
	401	413	
IS1086	QVLANPTDRL	PVQ	
IS30	RGVALTD...		
IS1470	IHLDKIYSLY		
IS1161	LTQTA.....		

Fig. 3. PILEUP figure of IS1470 and three of the transposases in the IS30 family. IS1086 was isolated from *Alcaligenes eutrophus*, IS30 from *E. coli* and IS1161 from *Strep. salivarius*. As in the whole IS30 family, the 5' recognition end is less conserved than the 3' integrase domain. The amino acids which are highly conserved in the family are indicated by an asterisk (Rezsöházy *et al.*, 1993). The essential DDE binding motif amino acids are shown in bold type.

nucleotide synthesis pathway. PCR results showed that 'nadC' is proximal to IS1470 in NCTC 8239, and is proximal to the *uapC* gene in enterotoxin-negative strains, and in strains where *cpe* is plasmid-bound.

Cloning of the downstream copy of IS1470 in NCTC 8239

The sequence of the downstream copy of IS1470 was completed using 1470 f and qpt primers on DNA from NCTC 8239. It has 98% identity on the DNA level, and a 96% identity (97% similarity) with 12 aa changes as compared to the upstream copy. The putative inverted repeats were conserved on both ends of the downstream copy. There is 26 bp between the putative inverted repeats and the stop codon in *nadC*, which is 204 bp less than was found in 44071-C05. A 14 bp directly repeated region was found directly before the initial inverted

repeats of the first copy of IS1470 and immediately after the inverted repeats on the 3' end of the second copy (Fig. 1).

Comparison of DNA between *uapC* or IS1470 and *nadC* in various strains

The region between *uapC* and *nadC* should contain the target sequence for insertion. As the results indicated that NCTC 8239 had lost 204 bp compared to 44071-C05, PCR products from two other strains were sequenced (see Methods). In strain 2154, which has lost *cpe*, the 317 bp PCR product using ppf and qpt primers was homologous to the first 87 bp of the *uapC*-IS1470 sequence in NCTC 8239. The 14 bp direct repeat (see above) was found once, and the rest of the sequence was identical to that in 44071-C05, which indicates that IS1470 duplicated 14 bp from the host genome upon insertion. The 1470ef-qpt PCR product from strain 790-94 was identical to 44071-C05 (Fig. 4). As IS1470 is part of the IS30 family, the consensus sequence for insertion of IS30 was searched for, but not found (Stalder *et al.*, 1987). PCR of the area between *uapC* and *nadC* in enterotoxin-negative strains showed that this region is somewhat variable in size (not shown). A FASTA search of the DNA between *nadC* and IS1470 showed that there was 88% identity over 97 bp to the DNA found downstream of the gene *sspC1* encoding the acid-soluble spore protein from *C. perfringens*. The loss of this region has no effect on *cpe* transcription, as judged from the amounts of enterotoxin produced during sporulation (unpublished observations). It is possible that recombination of homologous DNA has deleted the region.

Configuration of *cpe*

NCTC 8239 is a type A strain originally from the UK which has been used since 1952 by laboratories all over the globe. Strain 790-94 was isolated recently from a patient in Norway. Only a 204 bp difference was observed between these two strains. The NCTC strain has 'lost' these bases between IS1470 and *nadC* (Fig. 4). There are two regions of about 1 kb flanking *cpe* on this transposon which have no ORFs, and almost no restriction enzyme sites. Only the promoter area 5' of *cpe* would seem to have any vital function. Yet this DNA is conserved. Such large chromosomal DNA domains without ORFs are atypical. A look at the databases of the genomes of *Mycoplasma* (Fraser, 1995) and *Haemophilus* (Fleischmann *et al.*, 1995), which are entirely sequenced, and *E. coli*, much of which is sequenced, shows that the maximum length of DNA without a coding function is about 1 kb. Such non-coding regions are, however, always located on one side of an operon, and do not flank a single gene. The same *cpe*-flanking DNA is also found in the strain 44071-C05, which has *cpe* on a plasmid. On this plasmid, *cpe* is linked to IS1469 but not coupled to IS1470. An explanation for the conservation of this flanking DNA can be that this region contains no sequences which would give a basis for homologous recombination and excision. In ad-

Fig. 4. A comparison of the region between *uapC* and *nadC* in the four strains where this area was examined. The zig-zag indicates where IS1469 and *cpe* are located in the two strains. The dotted lines indicate which DNA is deleted in comparison to 790-94. All sequences are continuous in the bacteria.

dition, several pyrimidine tracts are found in this DNA, and these are known to give a rigid structure (Calladine & Drew, 1992) which could impair recombination.

The physical map of the chromosome of *C. perfringens* has almost 100 markers. The enterotoxin gene has been shown to be carried on a 5 kb *NruI* fragment when found on the chromosome (Canard *et al.*, 1992; Cornillot *et al.*, 1995). The larger 370 kb I-CeuI fragment which encompasses the gene shows signs of rearrangement involving both insertion and deletion in strains where the enterotoxin is on the chromosome. In enterotoxin-negative strains, and strains where *cpe* is plasmid-borne, the arrangement of this region is constant. The majority of enterotoxin-positive strains carry the gene on a plasmid, and almost all of these strains have IS1469 upstream from *cpe*, but do not have IS1470 on the plasmid. Plasmid-borne copies of *cpe* are often found in association with another IS element, IS1151 (Daube *et al.*, 1993), and the episome also carries other toxin genes. Most of these strains with episomally located *cpe* are of animal origin, and enterotoxin production is still regulated by sporulation. Human food poisoning strains have *cpe* on the chromosome in the transposon described here. Since the IS elements associated with, and the genetic localization of, *cpe* seem to vary from human food poisoning to animal strains, it is interesting to speculate when and why these changes in genetic structure occur. The major difference in the environments between the strains isolated from animals and those from human food poisoning cases is the exposure to heat treatment in food for the human strains. Strains which are enterotoxin-positive and are passed repeatedly on medium without heat shocking can lose the enterotoxin gene (P. E. Granum, unpublished observations). How heat treatment affects the movement and expression of *cpe* is one aspect of the production of enterotoxin which should be examined further.

ACKNOWLEDGEMENTS

This work has been supported by the Research Council of Norway, grant 105064/110.

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Received 4 November 1996; revised 24 February 1997; accepted 4 March 1997.