ELSEVIER

Institut Pasteur

Research in Microbiology xx (2014) 1–16

www.elsevier.com/locate/resmic

Clostridium perfringens type A–E toxin plasmids

John C. Freedman [a], James R. Theoret [a], Jessica A. Wisniewski [b], Francisco A. Uzal [c], Julian I. Rood [b], Bruce A. McClane [a,*]

[a] *Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA*
[b] *Department of Microbiology, Monash University, Clayton, Victoria, Australia*
[c] *California Animal Health and Food Safety Laboratory, San Bernadino Branch, School of Veterinary Medicine, University of California—Davis, San Bernadino, CA, USA*

Received 11 August 2014; accepted 9 September 2014

Abstract

Clostridium perfringens relies upon plasmid-encoded toxin genes to cause intestinal infections. These toxin genes are associated with insertion sequences that may facilitate their mobilization and transfer, giving rise to new toxin plasmids with common backbones. Most toxin plasmids carry a transfer of clostridial plasmids locus mediating conjugation, which likely explains the presence of similar toxin plasmids in otherwise unrelated *C. perfringens* strains. The association of many toxin genes with insertion sequences and conjugative plasmids provides virulence flexibility when causing intestinal infections. However, incompatibility issues apparently limit the number of toxin plasmids maintained by a single cell.

Keywords: Plasmid-encoded toxin; Clostridia; Gastrointestinal disease; Conjugation; Plasmid evolution

1. Introduction to *Clostridium perfringens*

C. perfringens has a ubiquitous environmental distribution but also ranks amongst the most important pathogens of humans and domestic animals. The virulence of this bacterium is largely attributable to its ~17 toxin arsenal. However, individual strains produce only subsets of this toxin repertoire, which forms the basis for a toxinotyping classification scheme that consigns isolates to five types (A–E), based upon their production of four typing toxins (Table 1). In addition, several toxins not used for toxinotyping are important for pathogenicity, as will be discussed later [1,2].

C. perfringens causes a panoply of illnesses ranging from histotoxic infections, such as clostridial myonecrosis (gas gangrene), to intestinal infections. The ability of *C. perfringens* to cause infections originating in the intestines is often dependent upon possession of toxin plasmids, which are the main focus of this review.

2. *C. perfringens* toxin plasmids and intestinal disease

When producing certain plasmid-encoded toxins, each *C. perfringens* type (and sometimes even specific subtypes) can cause intestinal infections, as shown in Table 2. These infections include enteritis and enterotoxemias, the latter characterized by toxins produced in the intestines, which then transit into the circulation to affect extra-intestinal organs. The ability of each *C. perfringens* type/subtype to cause intestinal diseases will now be briefly reviewed, along with a brief description of the plasmids relevant to those illnesses.

* Corresponding author. 420 Bridgeside Point II Building, 450 Technology Drive, Pittsburgh, PA 15219, USA. Tel.: +1 412 648 9022.
E-mail address: bamcc@pitt.edu (B.A. McClane).

http://dx.doi.org/10.1016/j.resmic.2014.09.004
0923-2508/

Table 1
Classification of *Clostridium perfringens* based on the production of the four major typing toxins.

Type	Typing toxin produced:			
	Alpha	Beta	Epsilon	Iota
A	+	–	–	–
B	+	+	+	–
C	+	+	–	–
D	+	–	+	–
E	+	–	–	+

2.1. *Type A* C. perfringens

2.1.1. C. perfringens *enterotoxin (CPE) plasmids*

Type A strains producing CPE are the second most common cause of bacterial food poisoning in the United States, with ~1,000,000 cases/yr at an estimated economic cost of >$300 million USD/yr [3,4]. Additionally, CPE-producing type A strains are associated with 5–15% of nonfoodborne human intestinal diseases, including antibiotic-associated diarrhea (AAD) and sporadic diarrhea (SD) [5]. The enterotoxin gene (*cpe*) can be located chromosomally or on plasmids, with ~70% of food poisoning strains harboring a chromosomal copy of *cpe*, whereas the remaining ~30% of food poisoning strains, and virtually all AAD/SD strains, carry a plasmid-borne *cpe* gene [6,7]. All of these strains cause disease when *C. perfringens* sporulates in the intestine and produces CPE (see below). During this *in vivo* sporulation, CPE accumulates in the cytoplasm and is finally released into the intestinal lumen when the mother cell lyses [6].

Substantial evidence supports CPE involvement in human intestinal disease. For example: 1) administration of CPE to human volunteers caused the classical diarrhea observed during natural disease [8]; 2) CPE is detectable in the feces of individuals with *C. perfringens* type A infection [9]; 3) CPE antisera can inhibit intestinal pathology in experimental animal models [10]; and 4) purified CPE damaged human ileal tissue *ex vivo* [11]. Perhaps the most persuasive evidence for the pathogenic role of CPE was provided by fulfilling molecular Koch's postulates for strain SM101 (a type A, chromosomal *cpe*, food poisoning strain) and F4969 (a type A, plasmid *cpe*, SD strain), which showed that CPE is essential for these two strains to cause histological damage and fluid accumulation in rabbit ileal loops [12].

CPE, an ~35 kDa single polypeptide, consists of a C-terminal binding domain and an N-terminal domain that mediates oligomerization and membrane insertion [6]. CPE action starts when this toxin binds to claudins, including claudin-3, -4, -6, -7, -8, -14, on the apical surface of small intestinal or colonic cells [13–19]. This binding localizes CPE in a small ~90 kDa complex, which then oligomerizes [20] into an ~500 kDa hexameric prepore named CH-1 that forms on the plasma membrane surface [17,21,22]. The toxin then uses its amphipathic region named TM1 to insert into membranes and form a pore of 0.5–1.0 nm [23]. Both the small complex and CH-1 contain receptor and nonreceptor claudins, as well as CPE [17]. A secondary CPE large complex, named CH-2, can form that contains receptor and nonreceptor claudins, as well as another tight junction protein named occludin [17]. Formation of the CH-1 pore leads to an influx of Ca^{2+} into the cell and a K^+ efflux. The Ca^{2+} influx activates calpain, which can lead to apoptosis (low toxin dose) or necrosis (high toxin dose) [24,25]. During *in vivo* disease, CPE-induced cell death leads to the intestinal lesions that trigger fluid accumulation and diarrhea [10,18]. Upon prolonged contact with the intestines, CPE can be absorbed into the circulation and cause enterotoxemia, affecting organs such as the liver or kidneys [26]. This enterotoxemia may explain fatalities that occurred during two food poisoning outbreaks in psychiatric hospitals [27,28]. In mouse models of CPE enterotoxemia, this leads to increased serum K^+ and hyperkalemia, which then causes cardiac arrhythmia and death [26].

During type A foodborne illness involving CPE, *C. perfringens* spores that survive the cooking process germinate in food, multiply and then are ingested [6]. Spore resistance against cooking and other stresses is influenced by which Ssp4 small acid-soluble protein variant is produced by the infecting strain [29,30]. Foodborne strains carrying a chromosomal *cpe* gene typically make a Ssp4 variant that binds strongly to spore DNA and thus imparts exceptional heat and chemical

Table 2
C. perfringens toxinotypes, plasmid-encoded toxins, and associated diseases.

Type	Toxin(s)	Human disease(s)	Animal disease(s)
A	CPE[a]	Human food poisoning; non-food-borne GI diseases	Possible enteritis in dogs, pigs, horses, and goats
	NetB	Not reported	Necrotizing enteritis in chickens
	CPB2	Not reported	Possible enteritis in pigs; possible enterocolitis in horses
	BEC	Possible human food poisoning	Not reported
B	Beta toxin, Epsilon toxin	Not reported	Necrotizing enteritis and enterotoxemia in sheep, cattle, and horses. Rare focal symmetrical encephalomalacia in sheep.
C	Beta toxin, CPE	Human enteritis necroticans	Necrotizing enteritis and enterotoxemia in pigs, sheep, cattle, horse, and other spp. (usually neonatal)
D	Epsilon toxin	Not reported	Enterotoxemia in sheep and goats; occasionally cattle and other species
E	Iota toxin	Not reported	Possible enteritis in rabbits, sheep and cattle

Modified with permission from Ref. [120] and incorporating newly discovered BEC toxin [71].
[a] CPE is usually chromosomally-encoded in food poisoning strains but plasmid-encoded in non-foodborne GI disease strains (see text).

Please cite this article in press as: Freedman JC, et al., *Clostridium perfringens* type A–E toxin plasmids, Research in Microbiology (2014), http://dx.doi.org/10.1016/j.resmic.2014.09.004