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Genomic analyses of *Clostridium perfringens* isolates from five toxinotypes

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Abstract

Clostridium perfringens can be isolated from a range of environments, including soil, marine and fresh water sediments, and the gastrointestinal tracts of animals and humans. Some *C. perfringens* strains have attractive industrial applications, e.g., in the degradation of waste products or the production of useful chemicals. However, *C. perfringens* has been most studied as the causative agent of a range of enteric and soft tissue infections of varying severities in humans and animals. Host preference and disease type in *C. perfringens* are intimately linked to the production of key extracellular toxins and on this basis toxigenic *C. perfringens* strains have been classified into five toxinotypes (A–E). To date, twelve genome sequences have been generated for a diverse collection of *C. perfringens* isolates, including strains associated with human and animal infections, a human commensal strain, and a strain with potential industrial utility. Most of the sequenced strains are classified as toxinotype A. However, genome sequences of representative strains from each of the other four toxinotypes have also been determined. Analysis of this collection of sequences has highlighted a lack of features differentiating toxinotype A strains from the other isolates, indicating that the primary defining characteristic of toxinotype A strains is their lack of key plasmid-encoded extracellular toxin genes associated with toxinotype B to E strains. The representative B–E strains sequenced to date each harbour many unique genes. Additional genome sequences are needed to determine if these genes are characteristic of their respective toxinotypes.

Keywords: *Clostridium perfringens*; Genomics; Toxin; Pathogenesis; Plasmid; Horizontal gene transfer

1. Background

Clostridium perfringens is a spore-forming, anaerobic rod-shaped bacterium that is classified within the phylum Firmicutes. *C. perfringens* can be isolated from a broad range of environments including soil, marine sediments, fresh water sediments, and the gastrointestinal tract of humans and animals. Some *C. perfringens* isolates have been recognised for their potential industrial utility; for example, in the synthesis of valuable chemical compounds such as acetate, butyrate, lactate, ethanol, hydrogen, and carbon dioxide [1]. However, *C. perfringens* is better known for its capacity to cause a range of histotoxic and enteric diseases of varying severity in humans and animals. *C. perfringens* is a major cause of human gas gangrene or clostridial myonecrosis [2], food poisoning, and sporadic and antibiotic-associated diarrhoea [3,4]. Additionally, some strains can cause the more serious enteric infection in humans, enteritis necroticans (pigbel or Darmbrand) [5,6]. In wild and domesticated animals and livestock

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C. perfringens also causes several significant diseases, including necrotic enteritis, enterotoxemia, dysentery and intestinal clostridiosis [7,8]. Importantly, the pathogenic potential of *C. perfringens* is facilitated by extracellular toxins and hydrolytic enzymes, which are often encoded in the *C. perfringens* accessory genome, i.e., these toxins are not conserved in all strains [9].

C. perfringens displays a vast toxin producing capacity. At least 17 different toxins or extracellular hydrolytic enzymes are encoded within the *C. perfringens* pan-genome [10–12]. Toxin producing strains have been divided into five distinct groups or toxinotypes (A to E) on the basis of their differential production of the four *C. perfringens* extracellular typing toxins, α, β, ε and ι (Table 1). This typing system has some utility in determining the disease-causing potential of *C. perfringens* isolates [13]. For example, in humans, food poisoning and gas gangrene are attributed to toxinotype A strains, whereas the development of enteritis necroticans involves β-toxin and is therefore associated with toxinotype C strains [14]. The major toxin involved in gas gangrene is α-toxin and the chromosomal structural gene, *plc* or *cpa*, is conserved in the *C. perfringens* core genome. However, most *C. perfringens* toxin genes are carried on mobile genetic elements, commonly on large conjugative plasmids, and as such they may be transferred between strains, potentially altering the virulence capacity of the recipient strain [15]. Indeed, the β, ε and ι typing toxins are encoded exclusively on plasmids [10]. Many toxigenic *C. perfringens* isolates encode more than one significant toxin and the specific subset of toxins carried by each isolate is the major determinant of disease type and host preference [10,11,16].

Despite its utility, the classical *C. perfringens* toxin typing system is somewhat out-dated due to the discovery of several new toxins that can play equally important roles in dictating virulence potential as those used in the typing scheme. For example, since the typing system was developed, the *C. perfringens* enterotoxin (CPE) has been shown to be a determinant of food poisoning and non-foodborne gastrointestinal infections [17]. CPE, which is produced during sporulation, binds to claudin receptors on enterocytes lining the surface of the intestine and subsequently oligomerises to form pores that facilitate the passage of small cationic molecules, ultimately leading to cell death via apoptosis [18–20]. Notably, the genomic location of the gene encoding CPE is also linked to disease type in the encoding strain. This gene, *cpe*, can be carried either on a plasmid or chromosomally by transposon Tn*5565* [21,22]. Strains that carry the Tn*5565*-linked *cpe* gene are associated with food poisoning, whereas strains carrying *cpe* on a plasmid are more commonly associated with non-foodborne enteric infections [3,23]. Non-typing toxins are also essential determinants of several animal infections. For example, *C. perfringens* NetB toxin is a plasmid-encoded β-pore-forming toxin that is essential for necrotic enteritis in chickens [24]. Other plasmid-encoded *C. perfringens* toxins include β2-toxin [25] and the recently discovered BEC toxin [26]. *C. perfringens* strains producing CPE or NetB are

Table 1
Major *C. perfringens* toxin genes in sequenced strains.

	Major toxins				CPE
	α-Toxin[a]	β-Toxin	ε-Toxin	ι-Toxin	
Gene name	*plc*	*cpb*	*etx*	*iap ibp*	*cpe*
Location	Chromosome	Plasmid	Plasmid	Plasmid	Chromosome/plasmid
Genes in sequenced strains[b]					
Toxinotype A					
ATCC 13124	CPF_0042[c]	–	–	–	–
SM101	CPR_0041	–	–	–	CPR_0381 (chr)
Strain 13	CPE0036	–	–	–	–
F4969	AC5_0057	–	–	–	AC5_A0094 (plasmid)
NCTC 8239	AC7_0038	–	–	–	(chr)[d]
JJC	VBICloPer314657_2847	–	–	–	–
F262	HA1_00170	–	–	–	–
WAL-14572	HMPREF9476_02964	–	–	–	–
Toxinotype B					
ATCC 3626	AC1_0063	AC1_A0493	AC1_A0111	–	–
Toxinotype C					
JGS1495	CPC_0066	CPC_A0266	–	–	–
Toxinotype D					
JGS1721	CJD_A0728	–	CJD_A0448	–	–
Toxinotype E					
JGS1987	AC3_0065	–	–	AC3_A0575 (Ib); AC3_A0576 (Ia)	–

[a] Toxinotype A strains may produce higher levels of α-toxin than other strains [11,46,47].
[b] Genome sequences can be obtained from Genbank under accession numbers CP000246.1 (ATCC 13124), CP000312.1, CP000313.1, CP000314.1 and CP000315.1 (SM101), BA000016.3 and AP003515.1 (strain 13), ABDX00000000 (F4969), ABDY00000000 (NCTC 8239), AWRZ00000000 (JJC), AFES00000000 (F262), ADLP00000000 (WAL-14572), ABDV00000000 (ATCC 3626), ABDU00000000 (JGS1495), ABOO00000000 (JGS1721), ABDW00000000 (JGS1987). The JJC annotation used in this study was obtained from the PATRIC database (genome accession number: 314657).
[c] Locus tags from the NCBI or PATRIC (JJC only) databases are given for the genes encoding each toxin.
[d] The *cpe* gene encoded by *C. perfringens* NCTC 8239 [21] was lost from the sequenced isolate prior to sequencing.