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Genetic characteristics of toxigenic Clostridia and toxin gene evolution

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ABSTRACT

Clostridia comprise a heterogenous group of environmental bacteria containing 15 pathogenic species, which produce the most potent toxins. The origin of toxins is still enigmatic. It is hypothesized that toxins exhibiting an enzymatic activity have derived from hydrolytic enzymes, which are abundantly secreted by these bacteria, and that pore-forming toxins have evolved from an ancestor transmembrane protein. The presence of related toxin genes in distinct *Clostridium* species and the variability of some toxin genes support horizontal toxin gene transfer and subsequent independent evolution from strain to strain. *Clostridium perfringens* toxin genes involved in myonecrosis, mainly alpha toxin and perfringolysin genes, are chromosomally located, whereas toxin genes responsible for intestinal and food borne diseases are localized on plasmids except the enterotoxin gene which can be located either on the chromosome or plasmids. The distribution of these plasmids containing one or several toxin genes accounts for the diverse *C. perfringens* toxinotypes. *Clostridium difficile* strains show a high genetic variability. But in contrast to *C. perfringens*, toxin genes are clustered in pathogenicity locus located on chromosome. The presence of related toxin genes in distinct clostridial species like *Clostridium sordellii*, *Clostridium novyi*, and *C. perfringens* supports interspecies mobilization of this locus. The multiple *C. difficile* toxinotypes based on toxin gene variants possibly reflect strain adaptation to the intestinal environment. Botulinum toxin genes also show a high level of genetic variation. They have a diverse genetic localization including chromosome, plasmid or phage, and are spread in various *Clostridium* species (*Clostridium botulinum* groups, *Clostridium argentinense*, *Clostridium butyricum*, *Clostridium baratii*). Exchange of toxin genes not only include transfers between *Clostridium* species but also between *Clostridium* and other bacterial species as well as eukaryotic cells as supported by the wide distribution of related pore-forming toxins of the aerolysin family in various clostridial and non-clostridial species, animal, mushroom and plant.

1. Introduction

Toxins are the main virulence factors of Clostridia and are responsible for severe diseases in man and animals. Clostridia are the group of bacteria, which produces the largest number of toxins. About 20% of bacterial toxins are from *Clostridium*. The genus *Clostridium* is a vast and heterogenous group that contains more than 150 species. These anaerobic, fermentative spore-formers are however generally regarded as adapted to a long survival in the environment as per the production of spores. Metabolically, Clostridia are quite versatile and degrade an extremely wide range of organic materials that include carbohydrates, organic acids, alcohols, aromatic compounds, peptides, amino acids, amines, purines and pyrimidines. Thereby, they effectively participate in an important aspect of ecology involving biomass renewal. Therefore, the Clostridia

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synthesize and secrete numerous hydrolytic enzymes that degrade organic molecules in the microenvironment into more easily "digested" or assimilated compounds. The resulting monomeric compounds required for growth are brought into their cytoplasm by various transporters. Perhaps the toxins produced by some *Clostridium* species evolved from "ancestral" hydrolytic enzymes by acquisition of novel specific properties that include pore formation, translocation across lipid membranes, and/or recognition of crucial eukaryotic targets. So, one might ask whether bacterial toxins, like those produced by the Clostridia, represent an evolutionary protein "tool" derived from less toxic, food-gathering origins? But among the large number of *Clostridium* species, only 15 produce potent protein toxins (less than 10%). This raises the questions which is the advantage for these toxigenic species to produce so potent toxins and how such few environmental bacterial species have acquired the ability to produce such extremely active toxins directed toward eukaryotic cells. Perhaps this phenomenon is linked to acquisition of toxin genes from other organisms and/or an omnipresent and dynamic evolutionary process in some clostridial species?

Based upon DNA alignment of ribosomal RNA genes, *Clostridium* species belong to 16 different clusters that further illustrate the phylogenetic heterogeneity of this genus. Most of the toxigenic *Clostridium* species ($n = 10$) are classified into cluster I, which is considered as the only "true" representative of the genus *Clostridium*, and the other toxigenic species are scattered into 3 other clusters (Stackebrandt and Hippe, 2001) (Table 1). This indicates that the toxigenic Clostridia are not all related phylogenetically.

Genetic studies have been mainly focused in three toxigenic *Clostridium* species: *Clostridium perfringens*, *Clostridium difficile*, and *Clostridium botulinum*. This review concerns the main features of toxin genes in these three *Clostridium* species, and then the evolution of some clostridial toxin genes will be discussed.

2. Toxin genes in *C. perfringens*

2.1. Toxin gene localization and variability

C. perfringens is the most prolific toxin-producer (Table 2) among known microorganisms via toxin genes that are present either on the chromosome or plasmids. The genome sequence of *C. perfringens* type A strains reveals a low G + C content (28.6%) equally distributed throughout the chromosome without any region exhibiting a remarkably higher, or lower, G + C content. The chromosomal genes for toxins and extracellular enzymes do not form pathogenicity islands or possess insertion, transposon, or phage-related sequences with one known exception being the *C. perfringens* enterotoxin (CPE) gene (Hassan and Paulsen, 2011; Myers et al., 2006; Shimizu et al., 2002).

The gene for alpha toxin (*plc*), which is the main virulence factor responsible for a life-threatening form of myonecrosis often associated with soiled wounds and commonly known as gangrene, is by classic definition produced by all *C. perfringens* strains and localized at the, same site on a variable region of the chromosome near the origin of replication (Justin et al., 2002; Rood, 1998; Tsutsui et al., 1995). Sequencing of the alpha toxin gene from different strains reveals variations, which most often are conservative and do not affect enzymatic activity, but can impact on certain biological properties like resistance to degradation (Titball et al., 1999). The chromosomal genes of other toxins, such as perfringolysin O (PFO) (also known as θ-toxin), collagenase (κ-toxin), and extracellular enzymes (i.e. hyaluronidase and neuraminidase) are also localized within a variable region near the alpha toxin gene locus.

Table 1
Phylogenetic heterogeneity, number of toxins, and subsequent main diseases of toxigenic *Clostridium* species.

Clostridium species	Toxin number	16s rDNA cluster	Main disease
C. argentinense	1	I	Botulism
C. baratii	2	I	Botulism
C. botulinum	3	I	Botulism
C. butyricum	1	I	Botulism
C. bifermentans	3	I	Gangrene
C. chauvoei	4	XI	Gangrene
C. difficile	3	XI	Colitis
C. haemolyticum	3	I	Hemoglobinuria
C. histolyticum	5	II	Gangrene
C. novyi	8	I	Gangrene
C. perfringens	14	I	Gangrene, enteritis
C. speticum	4	I	Gangrene, enterotoxemia
C. sordellii	4	XI	Gangrene
C. spiroforme	1	XVIII	Enteritis
C. tetani	2	I	Tetanus

In contrast, the other toxin genes (*cpb* for beta toxin, *cpb2* for beta2, *etx* for epsilon, *ia* and *ib* for iota, *netB* for NetB, necrotic enteritis toxin B-like, *tpeL* for TpeL, toxin *C. perfringens* large cytotoxin) are localized on plasmids of varying sizes. These plasmids can be lost or transferred, thus accounting for the various *C. perfringens* toxinotypes as classically defined by the production of one or several toxins. Therefore, *C. perfringens* type A that produces only one major toxin (alpha) represents the basic toxinotype for this species, which upon acquisition of a plasmid encoding for another specific toxin (beta, epsilon, or iota) yields another distinct toxinotype (B, C, D or E) (Petit et al., 1999; Tsutsui et al., 1995). The same toxin gene can be distributed on distinct plasmids and a plasmid can harbor several toxin genes. Indeed, *etx* can be found in at least five different plasmids ranging from 48 to 110 kb (Sayeed et al., 2007), and *cpb* is localized in a 65, 90, or 110 kb plasmid (Gurjar et al., 2010; Sayeed et al., 2010). Although *etx* and *cpb* are harbored by different plasmids, *etx* plasmids can contain *cpe* and *cpb2*, and *cpb* plasmids can also carry *cpe* and/or *tpeL*. But, *cpb* and *cpb2* are localized on distinct plasmids, as well as urease and lambda toxin (or lambda protease) genes, which are on separate plasmids than those containing the other toxin genes. The same *C. perfringens* strain can possess several plasmid types. For example, *C. perfringens* type B strains may contain three virulence plasmids including *etx* plasmid, *cpb* plasmid with or without *tpeL*, and urease/lambda plasmid. Similarly, a single *C. perfringens* type D strain may possess a *etx* plasmid and additional plasmids containing *cpe* and *cpb2*, together or separately, or a unique plasmid type harboring the three toxin genes (Gurjar et al., 2010; Sayeed et al., 2007, 2010).

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