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Genetic Characteristics of Toxigenic Clostridium Species: A Review

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Abstract

Toxins are the most invasive virulence factors of Clostridium and are accountable for dangerous disorders in mammalians. Species of Clostridia include a broad group of anaerobic bacteria capable of producing some of the most potent toxins. Toxin-producing Clostridium displays high genomic plasticity due to the activity of diverse mobile genetic elements, frequent horizontal gene transfer, variable genomic contexts of toxin loci, and dynamic plasmid-chromosome interactions. This plasticity results in the emergence, diversification, and adaptation of virulent strains. Understanding the genetic characteristics of toxigenic Clostridium species is crucial for development of effective strategies to combat infections caused by these pathogens. The diversity of Clostridium perfringens toxinotypes is largely attributed to the distribution of plasmids that harbor one or multiple toxin-encoding genes. In contrast, Clostridium difficile exhibits substantial genetic variability, yet its toxin genes are organized within a chromosomal pathogenicity locus (PaLoc) rather than on plasmids. The detection of homologous toxin genes among different Clostridium species—such as C. sordellii, C. novyi, and C. perfringens—indicates the horizontal transfer and interspecies mobilization of this pathogenicity region. Furthermore, the existence of multiple C. difficile toxinotypes, arising from toxin gene polymorphisms, likely represents adaptive evolution to the intestinal niche. Similarly, botulinum toxin (BoNT) genes exhibit extensive sequence and positional variability, being located on chromosomes, plasmids, or bacteriophages, and are distributed across a range of Clostridium species, including C. botulinum groups I–IV, C. argentinense, C. butyricum, and C. baratii. This wide genomic distribution underscores the dynamic genetic exchange and evolutionary plasticity that characterize toxigenic clostridia.

Keywords: *Clostridium*, Toxin Genes, Horizontal Gene Transfer, PaLoc

Introduction

The genus Clostridium re[resents a physiologically and genetically diverse group of bacteria known for producing a variety of toxins, acids, and solvents. The wide variation in G+C content and findings from 16S rRNA analyses reveal a significant level of phylogenetic diversity, indicating that Clostridium is not a phylogenetically uniform group. According to Cato and Stackebrandt, this genus lacks a coherent evolutionary lineage. From an evolutionary perspective, Clostridium species are considered ancient organisms that likely emerged during the anaerobic stage of evolution. Members of this genus hold both medical and industrial importance. Originally described in 1880, the genus has since expanded to include nearly 100 species, reflecting its considerable diversity. Clostridium species are widely distributed in nature, inhabiting soil, marine sediments, and decaying organic material from plants and animals. They also commonly reside in the human intestinal tract and are frequently isolated from soft tissue infections in humans and animals. To qualify as a member of this genus, a bacterial isolate must be anaerobic or microaerophilic, form endospores, appear as Gram-positive or Gram-variable rods, and lack the ability to

perform dissimilatory sulfate reduction (Popoff & Brüggemann, 2017; Aktories & Papatheodorou, 2020).

Horizontal gene transfer (HGT) through conjugation, transduction, or transformation occurs frequently both within and between closely related bacterial species, and occasionally even among distantly related taxa. These processes play a crucial role in enabling microorganisms to adapt to new or changing environments. The rate of horizontal transfer is enhanced by genomic plasticity, often driven by transposable elements (transposons). Transposons can move within the genome using two main mechanisms: “copy and paste” (Class I) or “cut and paste” (Class II). Class I transposons typically involve an RNA intermediate, which allows them to increase their copy number over time. In Class II transposons, translocation during DNA replication—particularly when the donor and target sites lie on opposite sides of the replication fork—can also result in changes in copy number. Insertion sequences (IS elements) represent small transposable elements that encode only the proteins essential for their own movement. The transposases encoded by IS elements can act in trans, sometimes facilitating the mobilization of other genetic regions flanked by complete or partial IS elements. When two different replicons share identical IS elements, homologous recombination can occur, potentially leading to gene replacement events. IS elements are found in varying numbers across bacterial genomes, with strain-dependent differences in their abundance reflecting the dynamic nature of microbial evolution. (Kiu & Hall, 2018 ; Brüggemann & Pfohl-Leszkowicz, 2019; Rood et al., 2020).

Clostridium difficile is a significant bacterial pathogen accountable for most cases of hospital-acquired worldwide. In recent years, the incidence, severity, mortality rates, and healthcare burden linked to C. difficile infection (CDI) have increased substantially, establishing it as a major global public health concern. Standard CDI treatments rely on antibiotics such as metronidazole and vancomycin; however, approximately 30% of patients experience relapse, underscoring the urgent need for novel therapeutic approaches. The virulence of C. difficile is mainly driven by two potent glucosylating exotoxins— toxin B (TcdB)— and toxin A (TcdA) which belong to the large clostridial toxin (LCT) family. Certain hypervirulent strains also form a secondary toxin, known as C. difficile transferase (CDT), which further improves virulence and disease severity. The finding that A−B+ strains remain pathogenic in infected patients suggests that TcdB alone can cause disease in humans. Supporting this conclusion, experimental studies have demonstrated that TcdB disrupts epithelial barrier integrity and induces tissue injury in human colon explants as well as in a chimeric mouse model containing human intestinal xenografts transplanted into immunodeficient mice. (Popoff & Brüggemann, 2017; Curry et al., 2019).

The capacity to synthesize botulinum neurotoxin (BoNT) is limited to six phylogenetically and physiologically distinct bacterial groups, including Clostridium botulinum Groups I–IV, along with specific strains of C. baratii and C. butyricum. This neurotoxin is regarded as the most poisonous biological toxin known, is the bacterial agent of botulism, a severe neuroparalytic disorder affecting humans, animals, and birds. To mitigate the risks associated with BoNT-producing clostridia, a comprehensive understanding of their biological characteristics is essential.Advances in genomic sequencing combined with physiological investigations have begun to unveil novel insights into the molecular and evolutionary biology of these hazardous microorganisms. Comparative genomic analyses have demonstrated marked diversity among the six BoNT-producing clostridia, highlighting their evolutionary separation. For instance, the genomes of all groups of botulinum strains exhibit significant divergence, lacking both synteny and homologous regions, which reflects their distinct evolutionary lineages and adaptive specializations. (Williamson et al., 2016; Smith et al., 2021).

Evidence indicates that Clostridium difficile is a significant pathogenic bacteria accountable for most cases of dangerous and life-threatening infections. In recent years, the incidence, severity, mortality rates, and healthcare burden linked to C. difficile infection (CDI) have increased substantially, establishing it as a major global public health concern (Sakurai et al., 2020).

Genetic Diversity and Pathogenic Potential

Among the master regulators in Clostridium, alternative sigma factors play a crucial part in determining when toxin genes are transcribed. For example, in neurotoxin-producing species such as Clostridium botulinum and Clostridium tetani, sigma factors like BotR and TetR (located immediately upstream of neurotoxin operons)

are essential for initiating transcription of neurotoxin genes (Popoff & Brüggemann, 2022). These sigma factors bind to promoters of toxin genes and recruit RNA polymerase to enable transcription under favorable growth conditions (Popoff & Brüggemann, 2022). Global metabolic regulators also exert major influence. CodY, a nutrient-sensing transcriptional regulator conserved across many decreased-G+C bacteria that react positively with Gram staining, has been revealed to intensively control botulinum neurotoxin gene transcription in C. botulinum ATCC 3502. When codY was inactivated, both transcript levels of botA (encoding the toxin) and actual toxin production fell; overexpression of codY restored toxin synthesis (Zhang, Dahlsten, Korkeala, & Lindstr̤m, 2015). CodY appears to bind directly to promoter elements of toxin genes, its activity is modulated by signals such as GTP and multiple chain amino acids that reflect the nutritional status of the cell Further genomic organization analyses revealed a conserved chromosomal structure across lineages, accompanied by a large number of dynamic plasmids. These plasmids play a critical role in horizontal gene transfer, allowing toxin genes and other mobile genetic elements to move across species and lineage boundaries. Consequently, *C. botulinum*, *C. novyi*, and *C. haemolyticum* exhibit interconnected genomic architectures, shaped by extensive plasmid exchange and toxin gene recombination, which underpin their evolutionary plasticity and pathogenic diversity (Popoff, 2013).

Molecular Mechanisms of Toxin Production

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Horizontal Gene Transfer and Evolution

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Genetic Regulation of Toxin Production

Toxin production is one the most invasive virulence factors in the pathogenesis of many Clostridium species, and tightly regulated genetic networks ensure that toxins are expressed under appropriate environmental and physiological conditions. At a broad level, toxin gene expression is controlled by interactions among environmental cues, small noncoding RNAs (sRNAs), quorum sensing, two-component systems, global metabolic regulators, and alternative sigma factors such as nutrient availability, growth phase, and stress conditions (Dupuy, 2023; Popoff & Brüggemann, 2022). Plasmids are widespread genetic elements found across bacterial species and play crucial roles in bacterial physiology, particularly in facilitating horizontal gene transfer. However, information regarding plasmids in the enteropathogen Clostridioides difficile remains scarce, and for many identified plasmids, no clear phenotypic effects have been observed. Nonetheless, recent studies indicate that plasmids are more prevalent in C. difficile than previously recognized, potentially carrying genes associated with pathogenic traits, including antimicrobial resistance and toxin synthesis. A study of 464 genomes revealed toxins encoded by plasmids and chromosomes and correlated with source and disease phenotype; isolates from animal or environmental sources tend to differ in their toxin combinations and plasmid contents

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Role of Global Regulators

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Integration with Metabolism

Regulation of toxins is not independent of metabolism. In Clostridium difficile, multiple regulators link toxin gene expression to metabolic factors. For example, the regulators CodY and CcpA mediate repression of toxin genes under conditions that interfere with promoter activity of the sigma factor tcdR, which itself is needed for expression from the toxin promoters tcdA and tcdB (Chandra et al., 2023).

Growth phase is another critical input. In many Clostridium species, maximal toxin gene transcription occurs during early stationary or late exponential phase. For instance, in C. botulinum strain ATCC 3502, toxin expression peaks at the transition into stationary phase, and then declines as cells enter deeper stationary phase (Zhang et al., 2015). Similar phase-dependent patterns have been observed in other pathogenic Clostridium spp., where stationary phase or stress conditions relieve repression mediated by global regulators and allow alternative sigma factors to drive toxin transcription (Popoff & Brüggemann, 2022).

Small RNAs

Beyond sigma factors and metabolic regulators, smaller-scale regulatory modules contribute specificity and environmental sensitivity. Two-component systems (TCS) are involved in C. perfringens, for example via the VirR/VirS system, which regulates multiple toxin genes. Other regulatory RNAs—including VR-RNA in C. perfringens—feed into cascades downstream of TCS components to mediate fine control in response to cell-cell signaling. Growth phase is another critical input. In many Clostridium species, maximal toxin gene transcription occurs during early stationary or late exponential. For instance, in C. botulinum strain ATCC 3502, toxin expression peaks at the transition into stationary phase, and then declines as cells enter deeper stationary phase (Ohtani & Shimizu, 2015 ; Popoff & Brüggemann, 2022).

Gene Transfer and Genomic Plasticity in Toxigenic Clostridia

Genomic plasticity is a distinctive feature of many pathogenic bacteria. In toxigenic *Clostridia*, this plasticity is tightly linked to virulence including: horizontal gene transfer (HGT), mobile genetic elements (MGEs), plasmids, phage-borne loci, genomic islands, and recombination events. Understanding of these processes provides insight into how antibiotic-resistant strains emerge, and how epidemic potential might shift over time. Plasmids are ubiquitous in the bacterial world. In many microorganisms, Plasmids are widespread genetic elements found across bacterial species and play crucial roles in bacterial physiology, particularly in facilitating horizontal gene transfer. However, information regarding plasmids in the enteropathogen Clostridioides difficile remains scarce, and for many identified plasmids, no clear phenotypic effects have been observed. Nonetheless, recent studies indicate that plasmids are more prevalent in C. difficile than previously recognized, potentially carrying genes associated with pathogenic traits, including antimicrobial resistance and toxin synthesis. Emerging evidence suggests, however, that plasmids are common in *C. difficile* and may encode

functions relevant to pathogenesis, such as antimicrobial resistance and toxin production. A study of 464 genomes showed that plasmid and chromosomally encoded toxins correlate with source and disease phenotype; isolates from animal or environmental sources tend to differ in their toxin combinations and plasmid contents compared to human disease isolates. The presence of IS elements at flanking regions of toxin-encoding loci suggests that these elements may mediate gene mobilization, recombination, or even gene duplication (Moore & Lacey, 2019).

Mechanisms of Gene Transfer

The pangenome or genomic plasticity is the full complement of genes found across all strains of Clostridioides difficile and C. perfringens. In C. difficile, strains are grouped into clades with extensive variation in accessory genes, mobile elements, prophages, and plasmids. Toxin loci are only part of a broader set of adaptive genes that differentiate strains in terms of virulence, antibiotic resistance, and colonization potential (Moore & Lacey, 2019). On the side, the accessory genome includes plasmids, phage-like contigs, prophages, and toxin loci which makes up a large proportion of phenotypic variability. A study of 464 genomes showed that plasmid and chromosomally encoded toxins correlate with source and disease phenotype; isolates from animal or environmental sources tend to differ in their toxin combinations and plasmid contents compared to human disease isolates (Williamson et al., 2016; Williamson et al., 2016; Moore & Lacey, 2019).

Role of Plasmids

Plasmids are widespread genetic elements found across bacterial species and play crucial roles in bacterial physiology, particularly in facilitating horizontal gene transfer. However, information regarding plasmids in the enteropathogen Clostridioides difficile remains scarce, and for many identified plasmids, no clear phenotypic effects have been observed. Nonetheless, recent studies indicate that plasmids are more prevalent in C. difficile than previously recognized, potentially carrying genes associated with pathogenic traits, including antimicrobial resistance and toxin synthesis. A study of 464 genomes showed that plasmid and chromosomally encoded toxins correlate with source and disease phenotype; isolates from animal or environmental sources tend to differ in their toxin combinations and plasmid contents compared to human disease isolates. The presence of IS elements at flanking regions of toxin-encoding loci suggests that these elements may mediate gene mobilization, recombination, or even gene duplication (Moore & Lacey, 2019).

Plasticity in Toxin Gene Loci and Diversity

Comparative genomics has revealed that toxin loci are highly variable in both sequence and genomic location. In *C. botulinum*, different groups have shown variation in where toxin genes reside: some on chromosomes, others on plasmids, some on phage-like replicons. The same BoNT subtype may be found on plasmids in some strains and on chromosomal loci in others. Also, novel or hybrid toxin variants have been identified in recent years. These may arise through recombination between different toxin gene clusters. (Williamson et al., 2016).

Pangenome

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Conclusion

Toxin-producing *Clostridiu*m displays high genomic plasticity due to the activity of diverse mobile genetic elements, frequent horizontal gene transfer, variable genomic contexts of toxin loci, and dynamic plasmid-chromosome interactions. This plasticity results in the emergence, diversification, and adaptation of virulent strains. Understanding the genetic characteristics of toxigenic *Clostridium* species is crucial for development of effective strategies to combat infections caused by these pathogens.

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