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Variation in spore properties and growth of two groups of *Clostridium perfringens*

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Introduction

Clostridium perfringens is a spore-forming anaerobe and a major cause of foodborne illness worldwide. Some strains of *C. perfringens* have the ability to produce the enterotoxin CPE (a major virulence factor of food poisoning) which is produced upon sporulation. The *cpe* gene can be either chromosome (C-*cpe*) or plasmid-based (P-*cpe*). The different locations of *cpe* associate with two phylogenetically distinct groups that show niche adaptation for the gut or the food environment, and strains in these groups generally have different spore properties, including heat and chemical resistance[1]. The *cpe* gene itself does not affect spore properties, although various factors have been identified, including variations in spore-associated proteins, and the composition of peptidoglycan and spore contents. However, the factors underlying spore properties are not fully understood.

Aims

- **Characterise the phenotypic and genomic differences seen between *C. perfringens* strains**
- **Optimise genetic tools for use in *C. perfringens***
- **Identify genetic factors contributing to spore properties**
- **Formulation of an easy-to-use and effective method to identify and distinguish *C. perfringens* strains**

Comparative genomics

The genomes of 22 Type A *C. perfringens* strains were recently sequenced and, alongside two publically available genomes, used for comparative genomic studies. The strains were grouped based on spore heat resistance properties or phylogenetic distance and used as input files for an in-house bioinformatics program. This program uses OGs generated by OrthoMCL, correlates them with the assigned phenotypic groups and scores them.

Genes that scored highly were viewed with Artemis to check for pseudogenes and to confirm the observed correlations, and subsequently analysed with BLAST to determine putative functions. A list of the types of genes linked to either grouping is in **Table 1**. A large number of genes are uncharacterised or hypothetical, and several are associated with metabolism, transport and host interactions.

Table 1: General gene categories identified from gene-trait matching, based on spore heat resistance and phylogenetic grouping,

Hypothetical/uncharacterised
Metabolism
Transport
Energy production
Transcription regulation
Biosynthesis
Stress response
Host tissue interactions
Quorum sensing

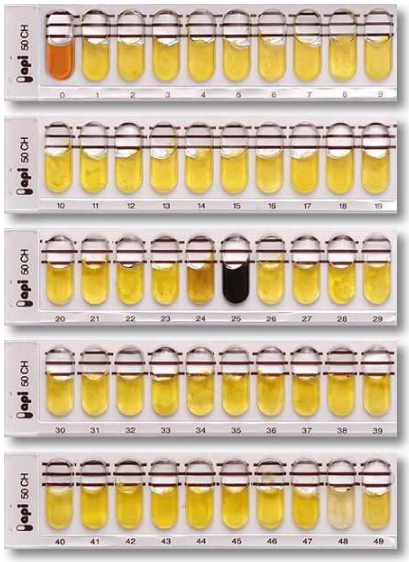

Figure 1: Example result of API 50 CH strips (BioMérieux, France). Use of these strips will allow for a visual record of metabolic variation between *C. perfringens* strains.

Gene characterisation

API strips and carbohydrate growth experiments will be used to characterise metabolism differences between the strains further, based on previous work by Lahti *et al*[2] and our comparative genomics analysis (**Figure 1**). In addition, spore resistance assays will be performed on the available strains, to identify further phenotypic differences.

Mutants will be created of genes identified from gene-trait matching, using ClosTron and allele-coupled exchange (ACE), and the resulting phenotypes determined. ClosTron uses a Group II intron to create insertion mutations in the targeted gene, disrupting function[3] (**Figure 2**). ACE exploits the phenotype of *pyrE* mutants to easily generate and screen for in-frame deletion mutants, as well as allowing the introduction of cargo downstream of *pyrE*[4].

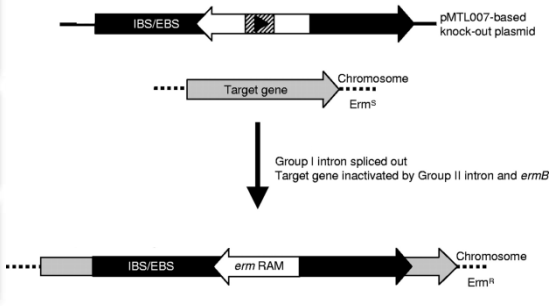

Figure 2: ClosTron gene knockout strategy. A group II intron is located on a pMTL007-based plasmid, which contains an internal retrotransposition-activated marker (RAM) conferring erythromycin resistance, which itself contains a group I intron. Upon transformation into *C. perfringens*, the group II intron inserts into the target gene and the group I intron is spliced out, enabling positive selection of mutants and disruption of the gene. Modified from [5].

Impact

- Identifying the causes of growth and spore resistance variation will aid the understanding of the origins and evolution of niche specialisation between the gut and environmental strains.
- Distinguishing between *C. perfringens* strains will allow better identification of strains from food, clinical and soil samples
- Understanding differences between strains will help improve heating and storage regimes

References

[1]Xiao, Y. et al (2012). *App Environ Microbiol*, *78*(19), 7060-7068.
[2]Lahti, P. et al (2012). *PLoS One*, *7*(10), e46162.
[3]Kuehne, S.A. & Minton, N.P. (2012). *Bioengineered*, *3*(4), 247-254.
[4]Ng, Y.K. et al (2013). *PLoS One*, *8*(2), e56051.
[5]Underwood, S. et al (2009). *J Bacteriol*, *191*(23), 7296-7305.

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