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Investigaton of novels genes expressed during sporulation in *Clostridium perfringens*

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Introduction

Clostridum perfringens is a rod shaped, non-motile, strict anaerob firmicute. It is ubiquitous in nature and is one of the most common causes of food spoilage and food poisoning in the Western world. It is regarded as an opportunistic pathogen and produces a wide range of toxins.

One of these toxins is called "Clostridium Perfringens Enterotoxin" (CPE), which causes food poisioning in the form of diarrhea. CPE is produced in the mother cell as it undergoes sporulation, and is released upon cell lysis. In other spore forming bacteria, the trigger(s) and or mechanisms of initation of sporulation are understood, but that is not the case for *C. perfringens*. Given the scale at which it causes food poisoning, a better understanding of the sporulation process is needed.[1,2]

Aim

The aim of this project is to look into novel genes that are upregulated specifically during sporulation and may determine spore properties, sporulation and/or germination behavior.

Selection of target genes

Whole-genome expression profiling rendered genes that are upregulated during sporulation[1]. We selected genes with unknown function that were upregulated by 3-7 $\log_2$ -fold[3] (from cluster 1, Figure 1). These genes were checked for homologues genes present in the genomes of 23 newly sequenced *C. perfringens* strains (NIZO), other clostridia and firmicutes. Genes specific to *C. perfringens* and other clostridia were chosen for further investigations. Figure 3 visualizes the selection process used to select these genes.

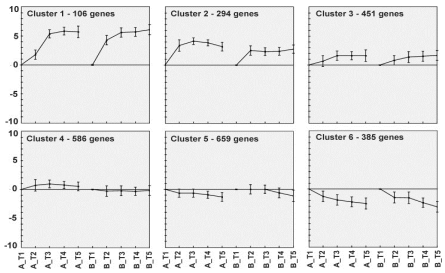

Figure 1: Clusters generated based on similarity of genome wide expression during sporulation of *C. perfringens* in two separate experiments (A)(B). The data show average $\log_2$ -fold change in expression against 5 timepoints from exponential to stationary phase. This work was performed by Yinghua Xiao[3]

The upstream regions of selected upregulated genes were checked for possible sporulation-specific sigma factor binding sites, as the upregulation of genes during sporulation alone does not tell us whether it is sporulation related, or stationary phase related.

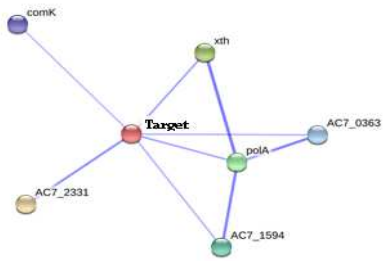

Figure 2: STRING[5] protein network with an example target gene. Thickness of lines indicate evidence of interaction/relation.

Genes that possibly do have a sigma factor binding site are then checked by using STRING to see the predicted protein interactions (Figure 2). Mutants will subsequently be constructed using the ClosTron mutagenesis system which has been designed to be an easy and efficient genetic tool to use in clostridia. An intron will via the shuttle vector pMTL007C-E2 be inserted into the target gene, effectively disrupting it[4]. Southern blotting will confirm that there is only one insertin of the inton into the genome, and a RT-PCR of genes downstram of the target will reveal if the inton has disrupted the transcription of those genes. When screening for a phenotype, we will look for the ability to sporulate/germinate, spore yield, UV resistance and other chemical agents effect on spores.

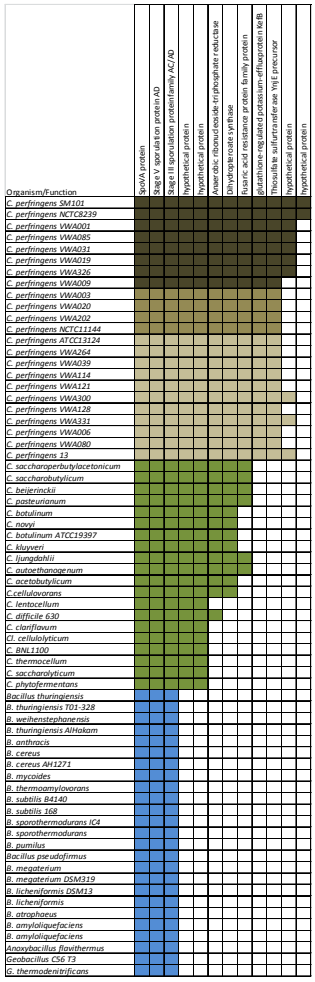

Figure 3: Present (filled) and absent (blank) genes in a orthology group matrix. Brown (light to dark) squares: *C. perfringens*; Green squares: Other clostridia; Blue squares: other firmicutes. Selected genes are just to illustrate an example selection.

Impact

- Understanding the activation of sporulation in *C. perfringens* is of importance as it is during sporulation that the enterotoxin CPE is produced.
- Further knowledge about sporulation and spore properties may help the industry improve food safety

References:
[1]Xiao, Y. et al, AEM 2012, v.78 p.7060-7068
[2]Xiao, Y et al, IJFM, v 194 p 40-45
[3]Xiao, Y et al, PLos One 2015, v 10, e0127036
[4]Heap, J.T et al, JMM 2010, v 80, p.49-55
[5]Sne, Bl et al, NAR 2000, V15, p3442-4

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