Characterisation of the prophage of *Clostridium difficile* NCTC12727

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Introduction

Sequenced *Clostridium difficile* strains contain an array of mobile genetic elements including plasmids, conjugative transposons and temperate bacteriophages. One such temperate bacteriophage, ΦCD27, was previously identified and sequenced from NCTC12727 during a screen of *C. difficile* strains (1) (Fig 1).

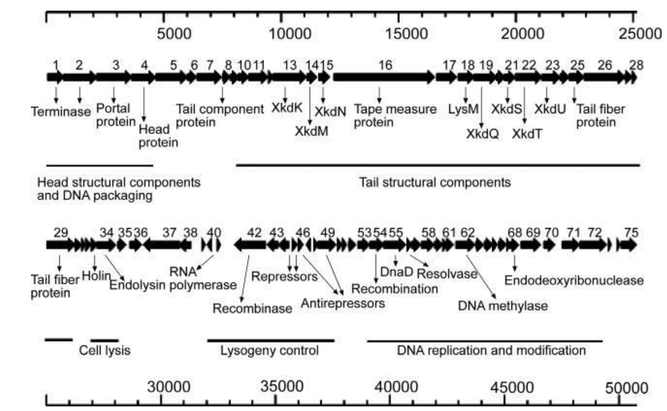

Fig 1: ΦCD27 genome map showing predicted ORFs

Aims

To determine the genomic location and activity of ΦCD27, and to investigate its regulation of lysogeny.

Results

Integration of prophage in *C. difficile* NCTC12727

We have shown that the region encoding the lysogengy control module was most similar to sequences from prophage 2 in *C. difficile* strain 630. We therefore hypothesised that the *attP* site would be within the intergenic region upstream of the putative site-specific recombinase (ORF42), as it is for prophage 2. A PCR-based strategy was used to determine the left-hand (LH) and right-hand (RH) prophage:chromosomal DNA junctions confirming that a prophage is integrated in the genome of NCTC12727 at the same chromosomal location as prophage 2 in 630; within an ORF encoding a putative ATP-binding protein of an ABC transporter. The integrated phage is flanked by 10bp direct repeats (1bp mismatch) (Fig. 2A). A second integrated phage was identified using this strategy within a homolog of *fliI* encoding a flagellum-specific ATP synthase, and is flanked by 7bp direct repeats (Fig. 2B). Although a homolog of *fliI* is present in 630 (CD_0251), no prophage is present within this ORF.

Results (cont'd)

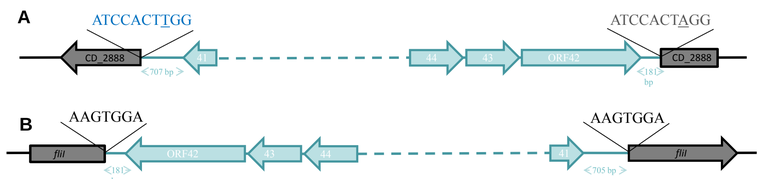

Fig 2: Location of prophages in NCTC12727. Chromosomal DNA is shown in grey and phage DNA in blue. Arrows indicate the direction of transcription. The phage ORFs are numbered according to figure 1. The direct repeats flanking the integrated phage are shown (1bp mismatch at integration site 1 is underlined). Not drawn to scale.

We have yet to determine if the prophages within NCTC12727 are ΦCD27. However, the LH and RH ends of these phage are identical to ΦCD27 and only one phage which produced plaques on 'sensitive' *C. difficile* strains was produced from NCTC12727 upon induction with mitomycin C(1).

The NCTC12727 prophage forms circular molecules upon excision from the chromosome

Using primers to the LH and RH ends of ΦCD27 and to the interrupted NCTC12727 ORFs, we have been able to amplify the joint of a circular form of ΦCD27 together with regenerated genomic sites from colonies of NCTC12727 (Fig 3). This indicates that these phage form a circular molecule upon excision from the chromosome and that prophage spontaneously excise from the genome within a subset of cells. In agreement with this, low levels of phage were detected in supernatants of uninduced cultures of NCTC12727 as has been seen with phages MMP02 and MMP04(2).

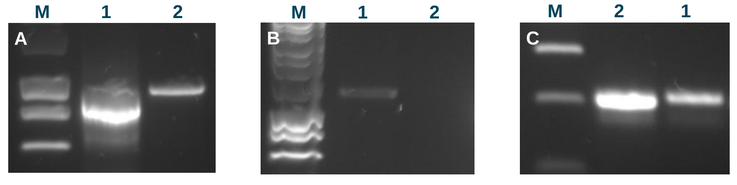

Fig 3: Amplified fragments of the circularised molecule of ΦCD27 and empty genomic sites.
A - circularised molecule; B – empty genomic site 1 (ABC transporter); C – empty genomic site 2 (*fliI*). M= markers; 1=12727; 2=630

Identification of a putative repressor of lytic activity in lysogens of sensitive strains and demonstration of the effect on toxin production

Lysogens of ΦCD27 in four sensitive strains; NCTC11204, 11205, 11207 and 11209 were produced by culturing samples from the centre of plaques, and the integration of ΦCD27 into the genome was confirmed by mitomycin C induction. RNA extracted from lysogens and the original strains demonstrated expression of the putative repressor, *orf44,* only in lysogens (Fig 4A). Lysogeny led to a reduction in toxin levels in 3 strains, but the lysogen of NCTC11207 showed increased toxin production (Fig 4B).

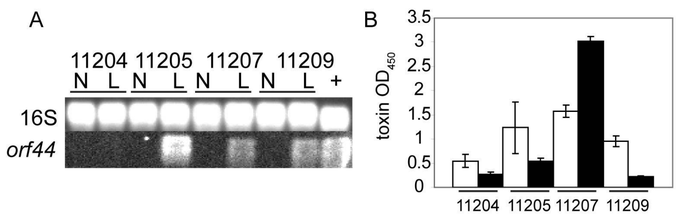

Fig 4: Gene and toxin expression in lysogens. A - RT-PCR analysis of lysogens (L) and wild type parents (N) showing differential expression of the putative repressor *orf44* compared to the 16S rRNA control (+, genomic DNA control); B – toxin production in wild type (white bars) and lysogens (black bars). Results are the means of triplicate assays +/- SD.

Summary

- At least two prophage are present in *C. difficile* NCTC12727.
- Both of these phage integrate into ORFs predicted to encode proteins which bind ATP.
- Prophage in NCTC12727 spontaneously excise from the genome, form a circular molecule upon excision and can be detected in the supernatant of uninduced cultures.
- The integrated prophage expresses *orf44* in some strains and affects toxin production.
- Prophage 2 from strain 630 can also spontaneously circularise.

References

(1) Mayer, M.J. *et al*. (2008). J. Bacteriol. 190: 6734-6740; (2) Meessen-Pinard, M. *et al.* (2012) Appl. Environ. Microbiol. 2012. (Epub ahead of print)

Acknowledgments: The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 223585 and the UK Medical Research Council (grant no. G0601176). EM is supported by a studentship from the Biotechnology and Biological Science Research Council and the University of East Anglia; RW gratefully acknowledges the receipt of an ESCMID attendance grant to attend the ICDS 2012