Supplementary Methods

Strains

CD630 (or 630) was isolated from a patient with pseudomembranous colitis during an outbreak of *C. difficile* infection (CDI)[1]. The strain is ribotype 012 and produces toxins A (*tcdA*$^+$) and B (*tcdB*$^+$). Ribotype 078 (SH11) and 027 (R2091) strains were obtained from Public Health England or B. Wren respectively.

Construction of *srfAA* mutant

The genome of Bv277 (HELM$^+$) was sequenced in its entirety revealing the four cistrons of the *srfA* operon. Primers (F: AAAAAGCTTCGGGGTTCGGGAAACTCATTTC; R: TTTGAATTCGCATGCGCAGGGGTTCCGTCAG) were used to amplify a 2007 bp internal segment of the *srfAA* cistron of Bv277. Restriction endonuclease sites incorporated in the PCR primers enabled direct cloning of the amplified DNA into pSGMU2, a plasmid able to replicate autonomously in *E.coli* but not *Bacillus*[2]. Protoplasts of Bv277 cells were made using lysozyme degradation in an isotonic solution as described elsewhere[3] and DNA of the recombinant plasmid introduced followed by two-step phased selection of chloramphenicol resistance (5 μg ml^{-1}). Transformants would only arise if plasmid DNA had integrated at the *srfAA* cistron by virtue of homologous DNA between genome and plasmid. Integration by a Campbell-type recombination disrupts the *srfA* operon resulting in failure to transcribe all four cistrons.

Analysis of toxins using ELISA

Toxins A and B were recovered from fecal or cecal samples and diluted 1:5 (w/v) in extraction buffer (PBS containing 2% (v/v) fetal calf serum, penicillin-streptomycin (Sigma P4333; 10ml/L) and Pierce protease inhibitor tablets (Thermo 88265). Samples were homogenized in extraction buffer and incubated for 2h at 4^{o}C. The supernatant was harvested after centrifugation (12,000 g, 5 min.) and filtered (0.2 μm). Rabbit anti-toxin A and toxin B (in house reagents) were used to coat ELISA plates (1/6,000) and left overnight at RT. Subsequently, plates were blocked for 1h at 37^{o}C with 2% BSA. Toxin extracted samples were incubated for 2h at RT. Replicate samples were used together with a negative control (toxin extract from a non-infected animal). Detection antibodies were mouse anti-toxin A and anti-toxin B (in house reagents; 1/1000) and were incubated for 1h at 30^{o}C. HRP-conjugated anti-mouse IgG (Dako; 1/2000) was used as the detection antibody for 1h at RT. Reactions were developed using TMB substrate and stopped by 2M H2SO4 and OD read at 450nm.

References

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2. Fort, P. & Errington, J. Nucleotide sequence and complementation analysis of a polycistronic sporulation operon, *spoVA*, in *Bacillus subtilis*. *J Gen Microbiol* **131**, 1091-1105, doi:10.1099/00221287-131-5-1091 (1985).

3, Vehmaanpera, J. Transformation of *Bacillus amyloliquefaciens* protoplasts with plasmid DNA. *FEMS Microbiology Letters* **49**, 101-105 (1988).