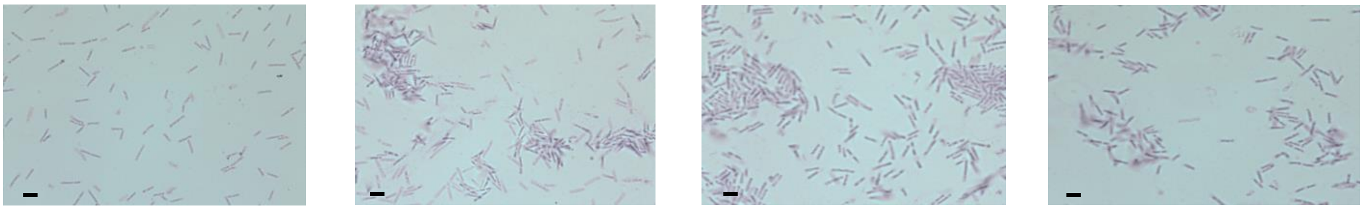
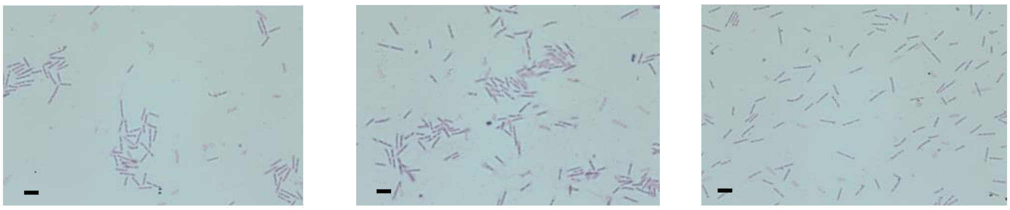
Role of glycosyltransferases modifying type B flagellin of emerging hypervirulent *Clostridium difficile* lineages and their impact on motility and adhesion

Esmeralda Valiente[1], Laura Bouché[2], Paul Hitchen[2], Alexandra Faulds-Pain[1], Mario Songane[3], Lisa F. Dawson[1], Elizabeth Donahue[1], Richard A. Stabler[1], Maria Panico[2], Howard R. Morris[2,5], Mona Bajaj-Elliott[3], Susan M. Logan[4], Anne Dell[2] and Brendan W. Wren[1*]

Supplementary figure 1

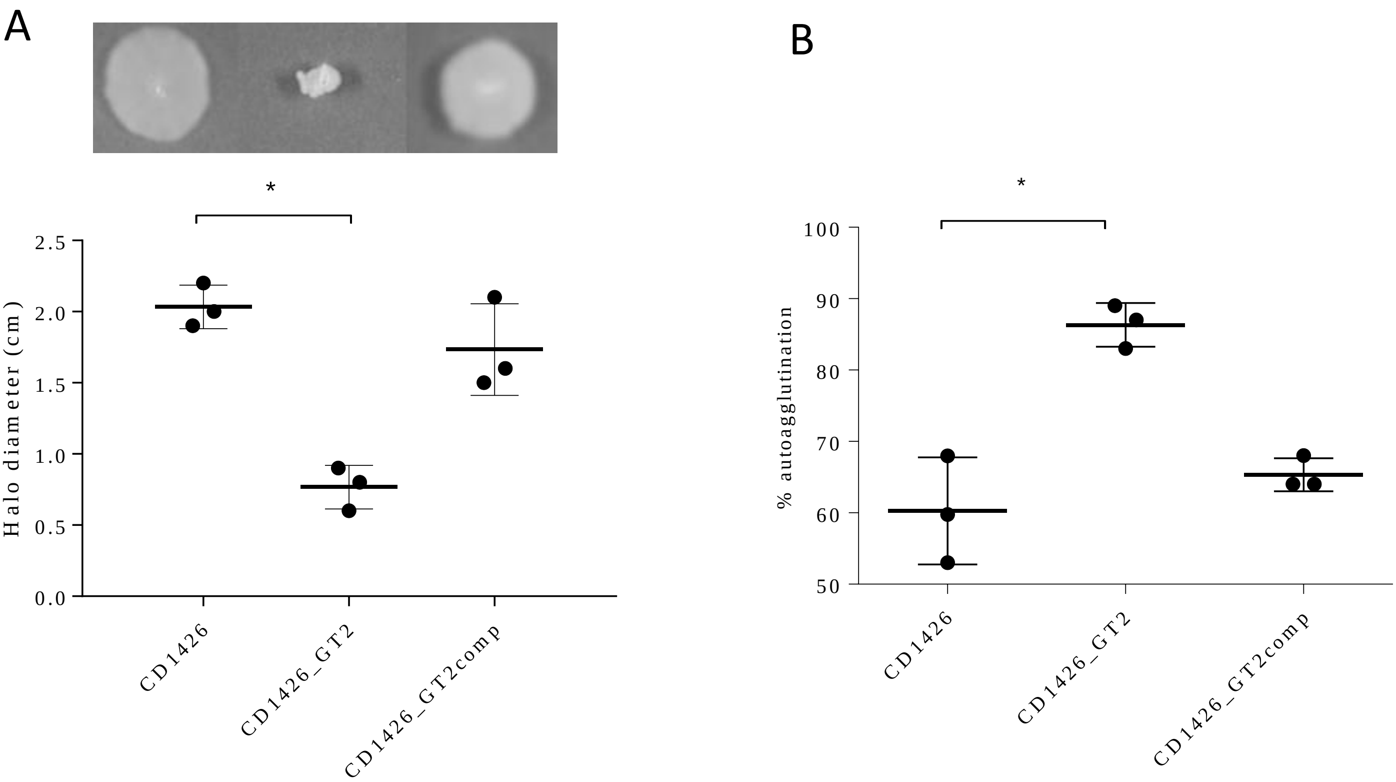


R20291 GT1 GT2 GT3



CDR20291_0245 CDR20291_0246 R20291*fliC*

Supplementary figure 2



Supplementary table 1. List of oligonucleotides used in this study

Primers used for screening mutants and make complements	Nucleotide sequence (5’—3’)
*fliC*F	GGGAAGAAACGTAAATGCACA
*fliC*R	TTGTGCCCCTAATTTTGCTC
GT1F	TCCTATGCTAAAGGTGAATGGTT
GT1R	TCCCTAAATCTACTTGTGCTTGT
GT2F	ACACCAATAGTGGTTAATGA
GT2R	TATGTTTTCACCAGTAGCCA
GT3F	ACCTGTGTAAATGATGAAAG
GT3R	GCATCTTGATGTAAGTATACCT
CDR20291_0245F	TGCGAAAAAGATTTGTGATGA
CDR20291_0245R	CCATCTTGTGAGGCTTCCTT
CDR20291_0246F	CAGTAGAAATAACTACATC
CDR20291_0246R	CAGTAGAAATAACTACATC
GT2-EBS1δ	CAGATTGTACAAATGTGGTGATAACAGATAAGTCGATCCAACTAACTTACCTTTCTTTGT
GT2-EBS2	TGAACGCAAGTTTCTAATTTCGGTTGAAGATCGATAGAGGAAAGTGTCT
GT2-IBS	AAAAGCTTATAATTATCCTTATCTTCCGATGGAGGTGCGCCCAGATAGGGTG
GT3-EBS1δ	CAGATTGTACAAATGTGGTGATAACAGATAAGTCGAGGAATGTAACTTACCTTTCTTTGT
GT3-EBS2	TGAACGCAAGTTTCTAATTTCGGTTTAAAGTCGATAGAGGAAAGTGTCT
GT3-IBS	AAAAAGCTTATAATTATCCTTACTTTACGAGGAAGTGCGCCCAGATAGGGTG
EBS universal	CGAAATTAGAAACTTGCGTTCAGTAAAC
RAM-F	ACGCGTTATATTGATAAAAATAATAATAGTGGG
RAM-R	ACGCGTGCGACTCATAGAATTATTTCCTCCCG
GT1EcoRI_F	ATAGAATTCATTGGATATAGAAGAAAGGG
GT1XbaI_R	ATATCTAGATCTATTTGATTATATGTC
GT2NdeI_F	TATGAATACACCAATAGTGGT *
GT2 EcoRI_R	ATCGAATTCACAAGACATTCCTCATAAAGT
GT3_EcoRI_F	ATAGAATTCAATGAATCACCAATAGTG
GT3_XbaI_R	ATATCTAGAGTCCTCAGTGAAT

	TTTCC
CDR20291_0245EcoRI_F	ATAGAATTCATGAAAAGATTAATGATTTTAGG
CDR20291_0245XbaI_R	ATATCTAGATTAAAAATTTTCTATCAT
CDR20291_0246EcoRI_F	ATAGAATTCTATGATAGATATGAAGCTA
CDR20291_0246XbaI_R	ATATCTAGATTGCTTTATTTGATTCTA
Primers used for RT-PCR	
1F	GTCTCAAAGACCACTATATTCTG
1R	GGTAATATATCATTGGTTTATGG
2F	CCAAGCCACTGATAAAAACTG
2R	CAAGACCCTGCTCTAT
3F	GGTTTATATGGAATGACAAGC
3R	ACCTTTCTTCTTCAAATAGCT
4F	GACAGTATTAGAGATGGATAGAG
4R	CAATAGAATCGGCTCCT
5F	GTGAATTAGAAGAATTTGATACTG
5R	GCAATAGAATCGGCTCCTCT
6F	GATGAAATCGAAGACTATCAGC
6R	CATAAGTTTCACATGCAGG
7F	GGCAAATACTTAGACGAGG
7R	GAGTATCAATTCTATTATCAC
8F	GATGAGAGACAGGATTGTGG
8R	CTCCCTGGCAATCTTGTAACC
9F	GATAGAATTAAAAGAACC
9R	GCCTTACTTCATTTCCAC
luxS-F	GTACTTGATGGAGTAAAGG
luxS-R	TTGCATATTCCTTTGCC
Int-F	GTGTCTTGTATTGGGGG
Int-R	CCAGTATCAAGCCAAGCC
*fliC*F	GGGAAGAAACGTAAATGCACA
*fliC*R	TTGTGCCCCTAATTTTGCTC

*5’end is phosphorilated

SUPPLEMENTARY FIGURE 1. Optical microscopy images of R20291 and its corresponding flagellin modification mutants showing cell aggregation in the mutants. The bar corresponds to 1 µm.

SUPPLEMENTARY FIGURE 2. Motility and cell aggregation assays in RT 023 and GT2 mutant. A. Motility in CD1426 (RT023) and corresponding modification mutant and complement; B. Cell aggregation in CD1426 and corresponding modification mutant and complement. Data presented as the mean ± SEM from 3 independent experiments with three technical replicates each. Significant differences are marked with asterisk; $p < 0.05$.