Investigating the molecular basis of *Clostridioides difficile* – gut commensal interactions within biofilms with TraDIS

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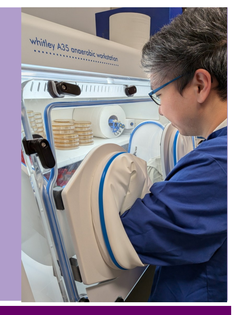
References:

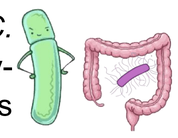


Introduction

Clostridioides difficile infection (CDI) is a leading cause of healthcare-associated diarrhoea and life-threatening pseudomembranous colitis.

Single and/or mixed species biofilm formation with microbiota species contributes to *C. difficile* persistence, leading to recurrent CDI[3,5].

Dominant gut microbiota species, ***Bacteroides spp.***, are known to inhibit *C. difficile* growth in both planktonic form and within biofilms[4,6], yet the underlying molecular mechanisms for *C. difficile* persistence in its mixed biofilms remain elusive.



Transposon Directed Insertion site Sequencing (**TraDIS**) is a useful, high-throughput functional genomic technique to study bacterial fitness under specific conditions[1,2].

Project aim: Screening of conditionally essential genes for *C. difficile* survival in dual-species biofilm co-cultured with *Bacteroides species* (*Bacteroides vulgatus*).

Results

Reduced *C. difficile* inhibition by *B. vulgatus* in 18 h biofilm

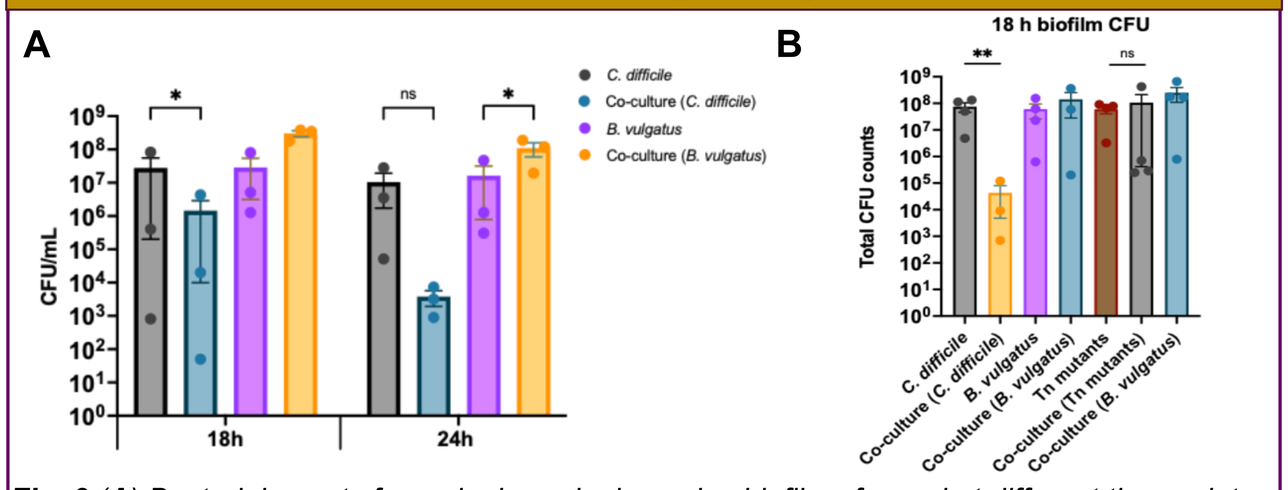


***Fig. 3**:(**A**) Bacterial counts from single or dual-species biofilms formed at different time points. (**B**) Enumeration of bacteria derived from optimised dual-species biofilm assay used for TraDIS (N = 4).*

Methods

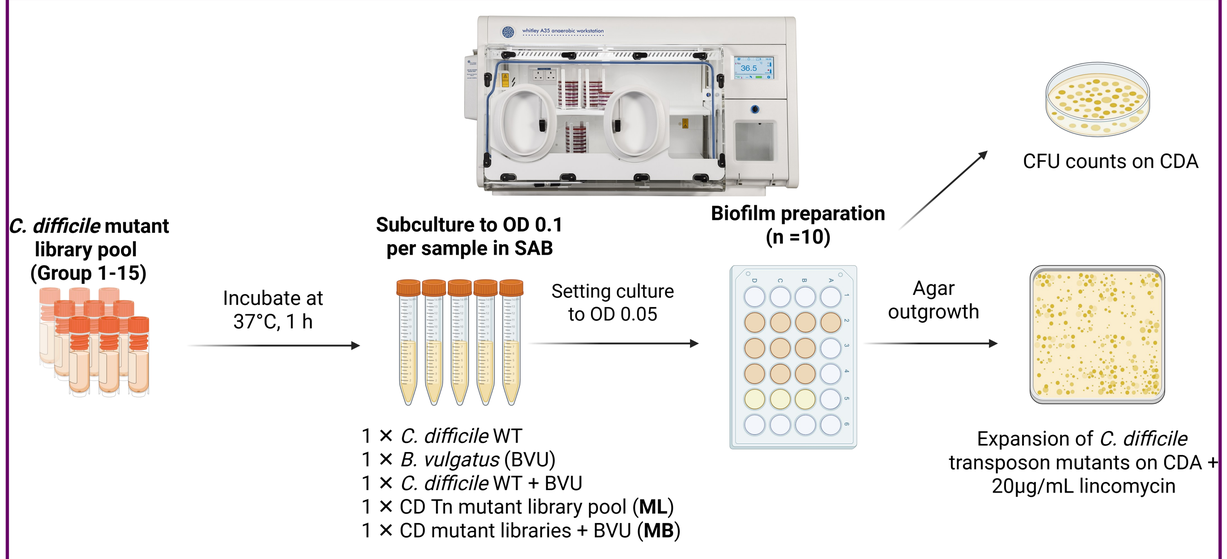


***Fig. 1**: Optimised workflow of dual-species 18 h biofilm assay, conducted within Whiteley A35 anaerobic workstation, with C. difficile WT, C. difficile transposon mutant library pool and B. vulgatus.*

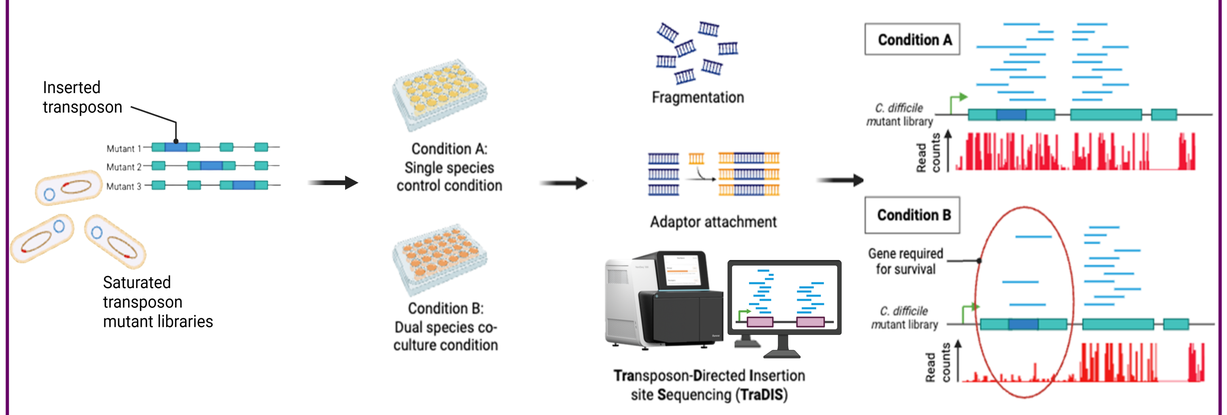


***Fig. 2**: Illustrations of TraDIS workflow.*

Distinct bacterial fitness signatures revealed by TraDIS analysis

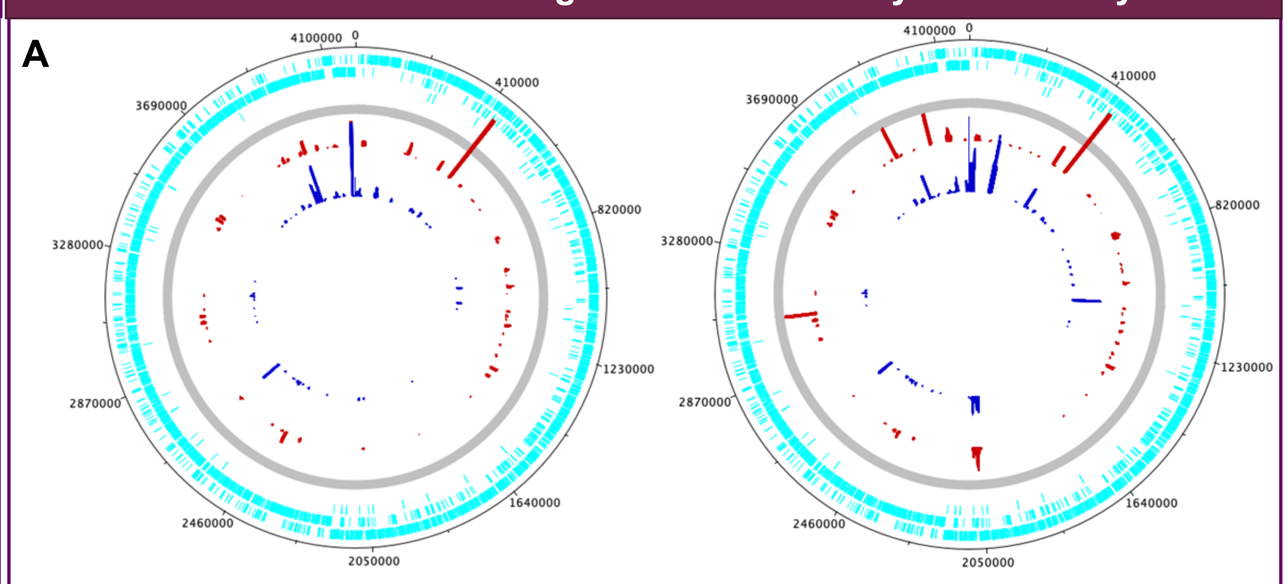
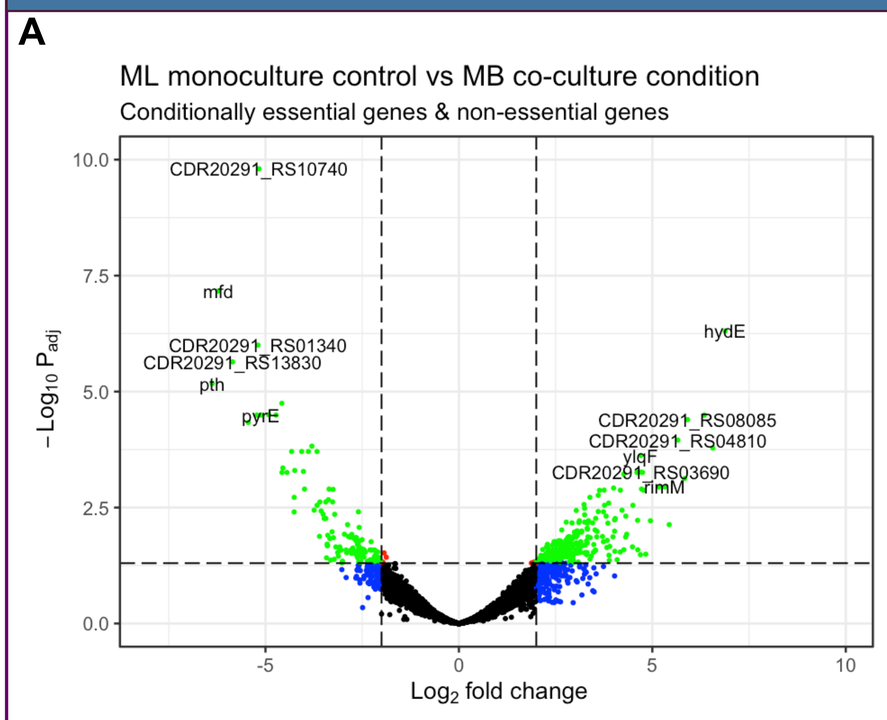


***Fig. 4**:(**A**) Distribution of insertion sites in C. difficile mutant library monoculture control (ML, left), and those in C. difficile mutant library-B. vulgatus co-culture experimental condition (MB, right). (**B**) PCA plot of normalised sequencing read counts for sample clustering.*

Candidate conditionally essential genes identified in dual-species biofilm



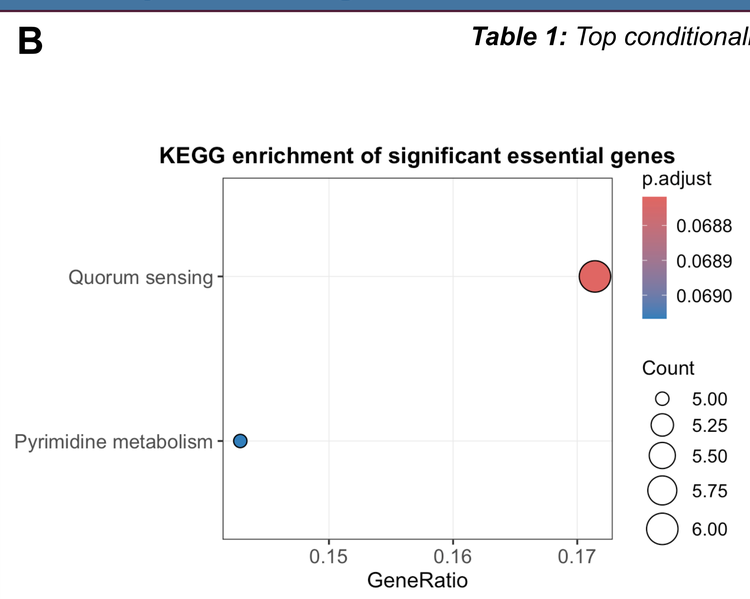


***Fig. 5**: (**A**) Volcano plot of screened genes with log_2 Fold change thresholds at 2 or –2 and adjusted p-value (or q-value) cutoff at 0.05. Upper left sector highlights conditionally essential genes; upper right sector highlights conditionally non-essential genes. (**B**) Overrepresented biological pathways were identified by KEGG pathway enrichment analysis of conditionally essential genes, with significance level of < 0.1.*

***Table 1:** Top conditionally essential genes ranked based on log2Foldchange and adjusted P-value.*

Gene names	Product description	Log2Fold-Change	Padj
pth	aminoacyl-tRNA hydrolase	-6.38	6.73e-06
mfd	transcription-repair coupling factor	-6.22	6.77e-08
CDR20291_RS13830	cation:proton antiporter domain contain-	-5.85	2.29e-06
CDR20291_RS04980	ABC transporter permease	-5.45	4.63e-05
CDR20291_RS08710	NifB/NifX family molybdenum-iron cluster-binding protein	-5.23	3.21e-05
CDR20291_RS01340	dihydroorotate dehydrogenase electron transfer subunit	-5.19	9.97e-07
CDR20291_RS10740	MurR/RpiR family transcriptional regulator	-5.17	1.57e-10
pyrE	**orotate phosphoribosyltransferase**	**-5.11**	**3.21e-05**
CDR20291_RS05290	hypothetical protein	-4.92	3.15e-05
CDR20291_RS04370	isocitrate/isopropylmalate dehydrogen-	-4.73	3.21e-05
licT	BglG family transcription antiterminator LicT	-4.58	1.79e-05
CDR20291_RS19300	hypothetical protein	-4.57	0.00055
pyrB	**aspartate carbamoyltransferase**	**-4.55**	**0.00044**
CDR20291_RS02605	HAMP domain-containing sensor histidine kinase	-4.45	0.00055
pyrF	**orotidine-5'-phosphate decarboxylase**	**-4.32**	**0.00020**

Conclusions

Using TraDIS technique, a total of 122 conditionally essential genes required for *C. difficile* survival during dual-species biofilm growth with *B. vulgatus* were identified. These genes were associated with enriched pathways involved in quorum sensing and pyrimidine metabolism, suggesting the importance of interspecies communication and metabolic adaptation within mixed biofilms. Overall, TraDIS successfully identified candidate genetic determinants contributing to *C. difficile* fitness and persistence in polymicrobial biofilm environments.

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