Genetic basis of phage-host interaction: Towards effective phage therapy of non-typhoidal Salmonella

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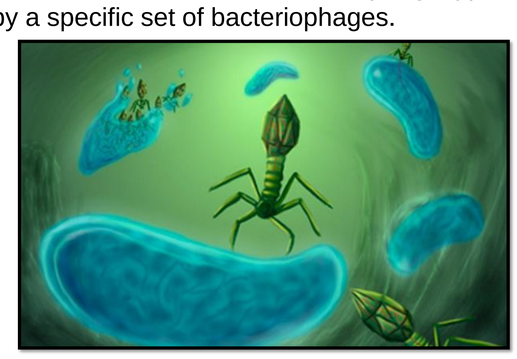
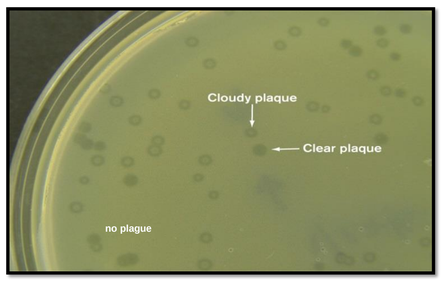
Complex dynamics of bacteria-phage interaction

Non-typhoidal *Salmonella* (NTS) including *S.* Typhimurium and *S.* Dublin have adapted to cause invasive illness in humans. It is estimated that invasive non-typhoidal *Salmonella* (iNTS) causes over 680,000 human deaths per year.

NTS have developed multi-drug resistance (MDR) against current antibiotics including the last resort; colistin (polymexins). Bacteriophage therapy is therefore the hope for the treatment of MDR bacterial infections however one of the key limitations to therapeutic use of phages, is the limited host range of many phages and the ease of development of bacterial resistance to phages. A solution is to develop one or a cocktail of engineered phage that overcome these limitations. An essential step towards this goal is understanding the complex dynamics of bacteria-phage interaction.

Phage typing has been used for decades as a rapid, low cost approach to sub typing *Salmonella enterica* in particular for serotypes Typhimurium and Enteritidis. S. Typhimurium can be differentiated into a number of phage types based on their pattern of susceptibility to lysis by a specific set of bacteriophages.





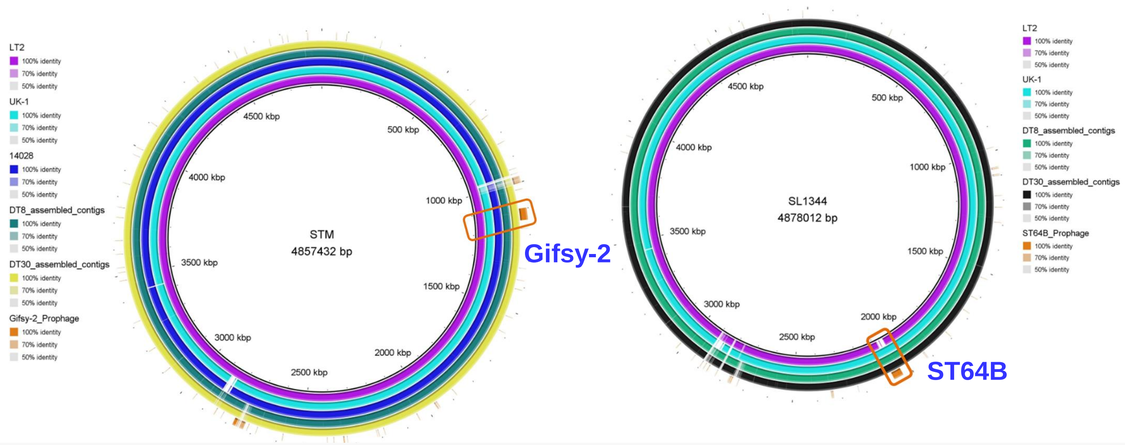
We therefore use Anderson phage typing scheme as a valuable model system for study of phage-host interaction to characterize all bacterial antiviral systems (including clustered regularly interspaced short palindromic repeat (CRISPRs) loci and CRISPR-associated (Cas) proteins (CRISPR-Cas) immune systems, superinfection exclusion (Sie) and restriction-modification (R-M) systems) as well as phage evasion strategies (including anti-CRISPR).

Study the genomic correlates of the difference in phage susceptibility using WGS of the reference DT8 (PB469) and DT30 (MS57) strains.

Phage Type	Result for indicated phage																											
	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	32	35
DT8	-	-	-	-	-	-	-	CL	SCL	++/ SCL	-	-	-	-	+++	-	-	-	SCL	-	SCL	SCL	-	±	±	-	-	CL	CL	-
DT30	-	-	-	-	-	-	-	CL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Prophages integrated within DT8 and DT30 draft genomes:

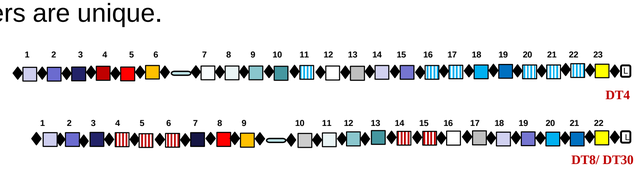
The draft genome of S. Typhimurium DT8 and DT30 contain 3 intact prophages; Gifsy-2, ST64B and one *S.* Enteritidis associated prophage (ELPhiS) named as RE_2010 that has not been detected previously in *S.* Typhimurium.



CRISPRs detected within DT8 and DT30 draft genomes:

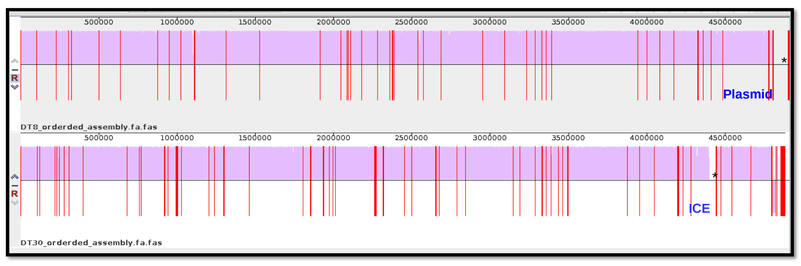
Both PB469 (DT8) and MS57 (DT30) contain highly similar palindromic repeats to other *S.* Typhimurium strains however their spacers are unique.

***CRISPR locus 1**



Comparative genomic analysis:

PB469 (DT8) and MS57 (DT30) differ from each other at 568 SNPs. MS57 carries a genomic island, absent from PB469, that is similar to putative ICE of Enterotoxigenic *Escherichia coli*. PB469 harbours a plasmid that is highly related to pSLT plasmid of *S.* Typhimurium strain LT2.



Restriction Modification System							
RMS Genes	Function	Recognition Sequence	DT1	DT4	DT44	DT8	DT30
Type I RMS:							
EcoKI	Restriction Enzyme	AACNNNNNNGTGC	+	+	+	+	+
M.Sen1736III	Methyltransferase	GAGNNNNNNRTAYG	+	-	+	+	+
S.Sen318I	Specificity Subunit		+	+	+	+	+
M.SenTFII	Methyltransferase	GAGNNNNNNRTAYG	-	+	-	-	-
Type II RMS:							
M.Sen1736V	Methyltransferase	GATC	+	-	+	+	+
M.Sen158IV	Methyltransferase	BATGCATV	+	+	+	+	+
M.Sen158III	Methyltransferase	GATC	-	-	-	+	+
M.SenAboDcm	Methyltransferase	CCWGG	+	+	+	+	+
Sen1736II	Restriction Enzyme/ Methyltransferase	GATCAG	+	+	+	+	+
M.EcoGIX	Methyltransferase	SAY	+	+	+	+	-
Type III RMS:							
SenAZII	Restriction Enzyme		+	+	+	+	+
M.Sen1736I	Methyltransferase	CAGAG	+	+	+	+	+
Type IV RMS:							
StyLT2Mrr	Methyl-Directed Restriction Enzyme		+	+	+	+	+

Genetic basis of virulence in invasive *Salmonella* Dublin

A high proportion of *S.* Dublin cases in humans are associated with systemic illness. Outbreaks of human infections by *S.* Dublin have been reported in several countries including high-income countries (Ireland 2013 and France 2016).

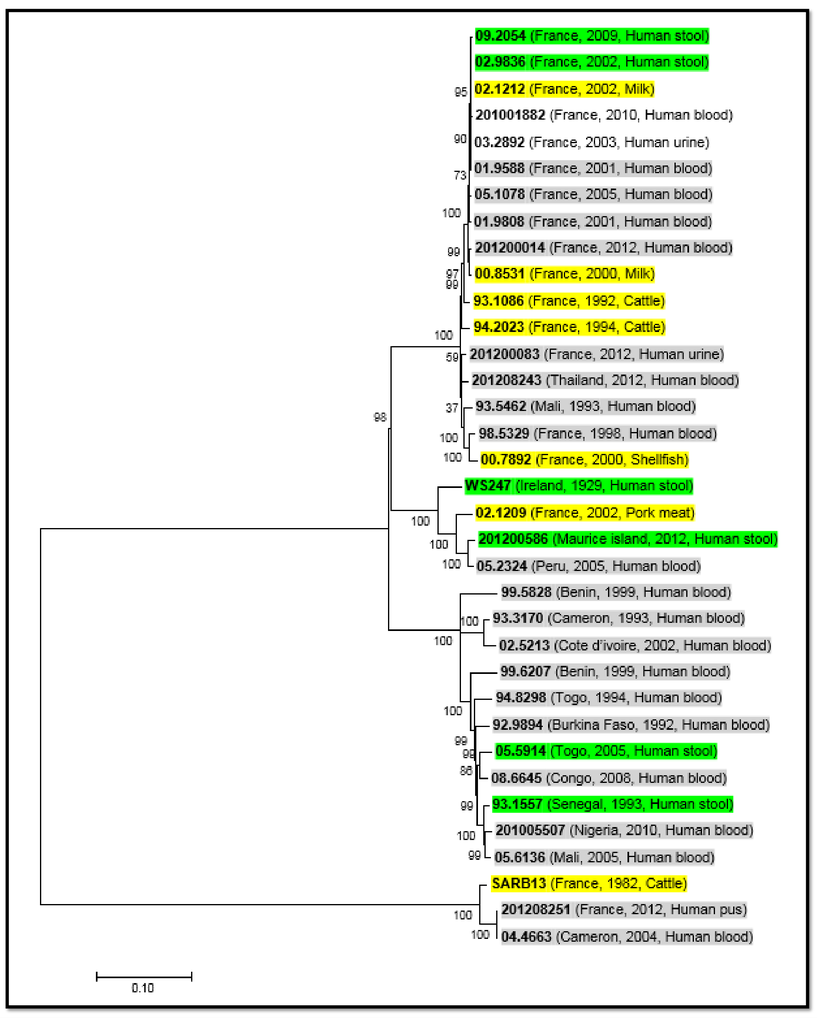
There is no vaccine against NTS. We therefore apply next generation sequencing (NGS) technologies and associated bioinformatics analyses tools to understand the genetic basis of invasive NTS Dublin.

Morbier cheese

Vacherin Mont d'Or cheese

Phylogenetic relationship among *S.* Dublin

Invasive and gastroenteritis isolates were intermixed as SNPs were randomly distributed around the chromosome of *S.* Dublin.

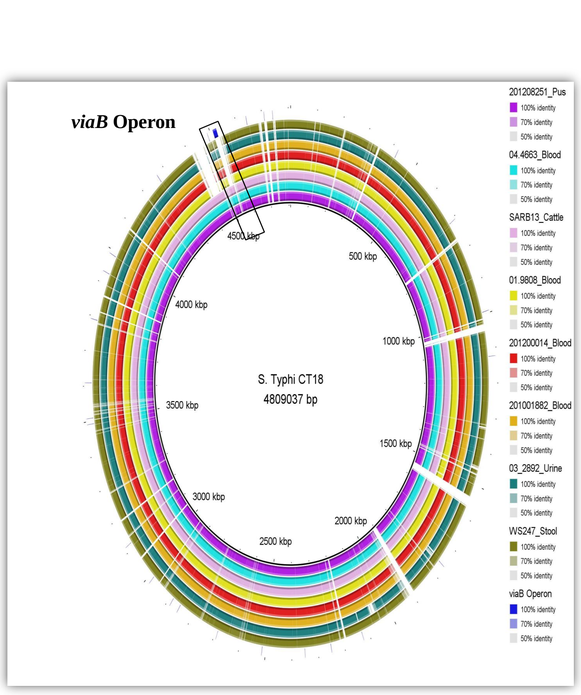


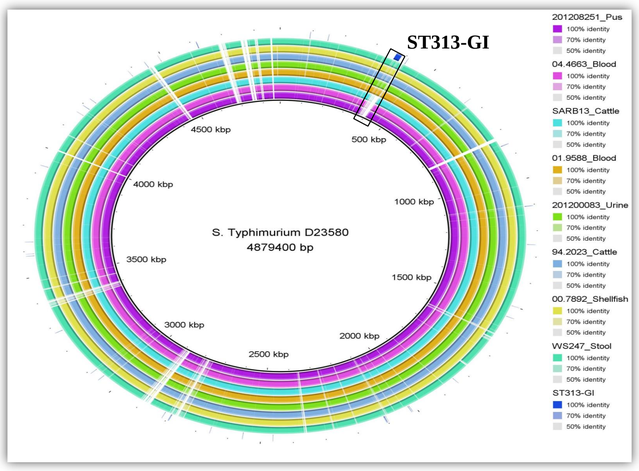
Putative virulence regions in *S.* Dublin

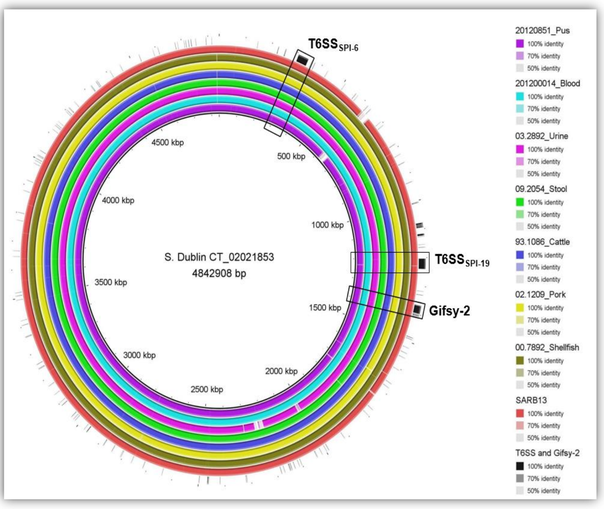
Vi-coding genes harboured by *Salmonella* Pathogenicity Island (SPI); SPI-7 were absent from all *S.* Dublin isolates except three isolates including the reference cattle isolate; SARB13 and two clinical isolates; 04.4663 from blood and 201208251 from pus.

All *S.* Dublin isolates except these three isolates; SARB13, 04.4663 and 201208251 harbour the putative virulence gene *st313-td* on the degraded pathogenicity island ST313-GI (Figure 2) which is entirely absent from the Vi positive three isolates (SARB13, 04.4663 and 201208251).

All *S.* Dublin isolates sequenced in this study harbour pathogenicity islands SPI-6 and SPI-19 that encode type VI secretion system (T6SS); $T6SS_{SPI-6}$ and $T6SS_{SPI-19}$ respectively and they are all lysogenic for Gifsy-2 prophage (Figure 3) that harbor the gene encoding Gifsy-2 prophage attachment and invasion protein.







Interestingly, no genomic markers were detected that differentiate among invasive and non-invasive isolates therefore comparative transcriptomics will be carried out to identify differentially expressed genes in invasive strains compared with non-invasive strains aiming to identify novel virulence-attenuated strains with a potential for use as vaccine candidates for high-risk groups including children, elderly and immunocompromised patients.