# *Salmonella* Pathogenicity Island SPI-7 is an Integrative and Conjugative Element with a Close Relative in *Salmonella bongori*

**Helena M. B. Seth-Smith, Chinyere Okoro, Nicholas R. Thomson, Julian Parkhill**
**Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, CB10 1SA, UK**

## 1. SPI-7 background

•SPI-7 is a large pathogenicity island encoding virulence functions:
- •Vi antigen
- •SopE effector
- •Type IVB pili

•SPI-7 is found in most strains of *Salmonella* Typhi and *Salmonella* Paratyphi C, as well as some strains of *Salmonella* Dublin, ranging in size from 82kb to 120kb

## 2. SPI-7 shares features with ICEs

•SPI-7 shares features with characterised integrative and conjugative elements (ICEs):

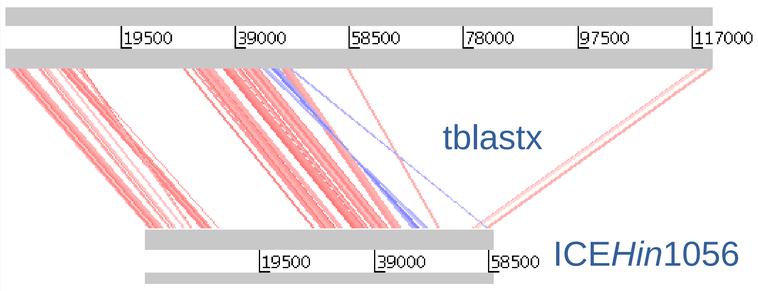


**Fig 1: ACT [Carver et al, 2005] comparison of SPI-7 and ICE*Hin*1056.** Red bars indicate regions of nucleotide identity. Blue bars indicate nucleotide identity with an inversion. Deeper colours indicate higher levels of identity.

•Comparing SPI-7 to ICEHin1056 from *Haemophilus influenzae* [Mohd-Zain, 2004], conservation of function and synteny is apparent.
•ICEs are large self-mobile factors, associated with a wide range of cargoes, stable within the host genome [Burrus, 2002].
•ICEs replicate through a series of steps:
- •precise excision of the element to form a double stranded circular intermediate and a reformed chromosomal integration site.
- •transfer of single stranded DNA to recipient through self-encoded conjugal structure
- •formation of a double stranded molecule in both cells, followed by integration into chromosome at the relevant target site (usually a tRNA locus).

## 3. A related ICE in *Salmonella bongori*

•A relative of SPI-7 has been identified within a strain of *Salmonella bongori* , isolated from a dog with diarrhoea
•This element, ICE*Sb*1, shares 98% nucleotide identity with SPI-7 along the ICE backbone.

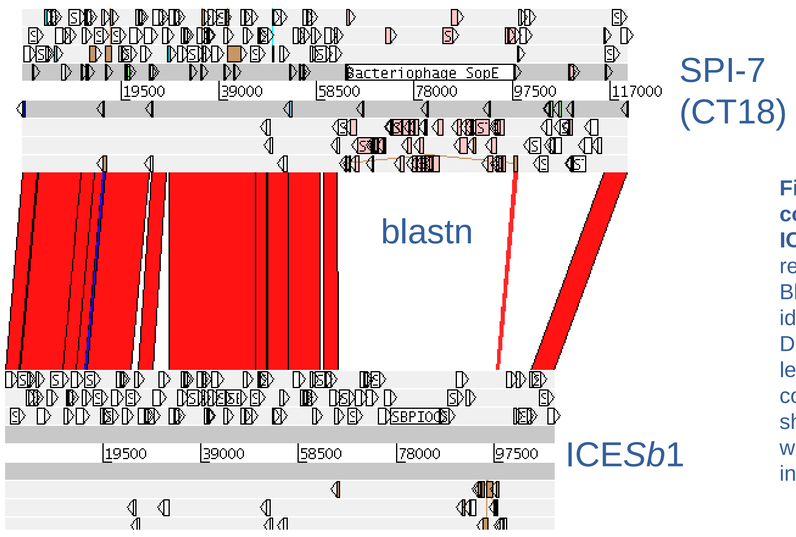


**Fig 2: ACT [Carver et al, 2005] comparison of SPI-7 and ICE*Sb*1.** Red bars indicate regions of nucleotide identity. Blue bars indicate nucleotide identity with an inversion. Deeper colours indicate higher levels of identity. Predicted coding sequences (CDSs) are shown in the relevant frame as white boxes, with brown boxes indicating pseudogenes.

•ICE*Sb*1 carries an alternative cargo, which includes:
- •putative autotransporter
- •putative antibiotic resistance determinants and drug efflux
- •putative immunoglobulin binding regulators IbrAB
- •Von Willebrand A homologue

## 4. Mobility of SPI-7 and ICE*Sb*1

•SPI-7 from *Salmonella* Typhi is not able to transfer itself into new hosts, although it can promote the conjugation of other resident plasmids [Baker, 2008].
•Nested PCR shows that SPI-7 from *S*. Typhi strains is not able to excise from the chromosome and circularise, whereas SPI-7 from strains of *S*. Dublin and *S*. Paratyphi C is able to do this (data not shown).
•ICE*Sb*1 is mobile: it has been shown to conjugate into strains of *Salmonella* Typhimurium at a rate of 1.8x10-6 ± 4.0x10-7 transconjugants per donor.
•Thus ICE*Sb*1 is a good model for SPI-7 mobility.

## 5. ICE comparisons identify homologues and candidate knockouts

•Many genes are conserved between SPI-7, ICE*Sb*1 and ICE*Hin*1056.
•Some genes have putative assigned functions:
- •the "int" region is involved in the integration/excision of the ICE
- •the "replication region" is involved in replicating the circular intermediate
- •the "transfer" region is involved in the formation of the novel "GI" (Genomic Island) Type 4 Secretion System (T4SS) [Juhas, 2007].

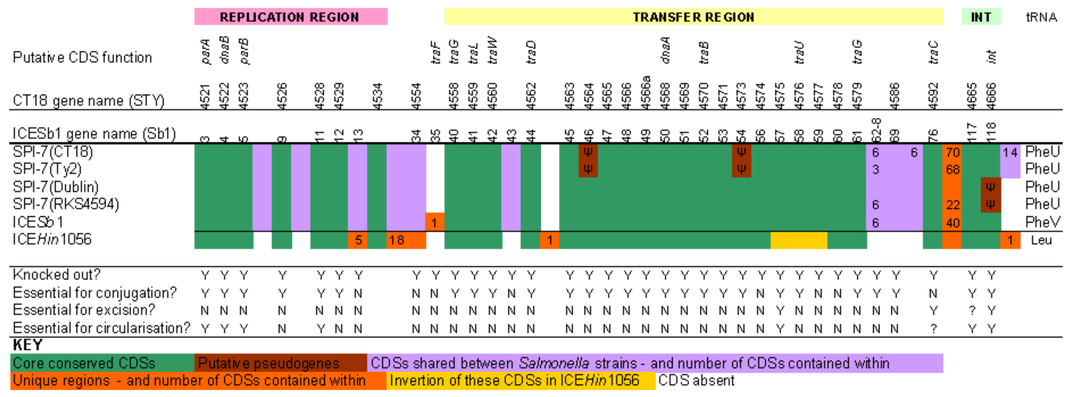


**Fig 3: Distribution of core and cargo CDSs.** Core conserved CDSs are indicated by green boxes, and location and number of cargo CDSs are shown in orange boxes. White boxes indicate the absence of a CDS in that ICE. Some CDSs are shared between the *Salmonella* strains (purple); pseudogenes (Ψ) are shown in brown; one inversion exists (yellow). Involvement of each gene in conjugation, excision and circularisation is indicated below.

•Knockouts of key conserved genes has been performed using the lambda-red system [Datta et al, 2006; Datsenko and Wanner, 2000; Cherepanov and Wackernagel, 1995].
•Nested PCR was performed on extracted genomic DNA from each knockout strain.
•Conjugation assays were performed on each knockout strain.

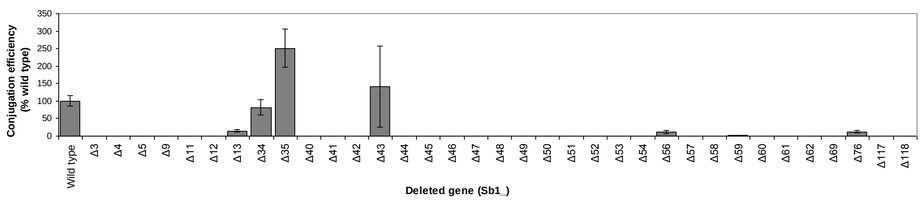


**Fig 4: Conjugation assays on knockouts.** Conjugation into a recipient Salmonella Typhimurium strain was assayed in triplicate and is displayed as % of wild type activity.

•This phenotypic analysis indicates which genes are involved in excision, circularisation and conjugation.

## 6. Knockouts imply function

•Excision is the first step in the process: if this is abolished, no further steps occur (as seen in Δ57, Δ76 (to a large degree), Δ117 (preliminary data) and Δ118).
•Circularisation is the next step and is non-functional in mutants Δ3, Δ4, Δ5 and Δ11.
•Conjugation requires many gene products, forming the GI T4SS and controlling DNA transfer. This is abolished in: Δ9, Δ12, Δ40-42, Δ44-54, Δ58 and Δ61-69.
•Genes not involved in the process include Sb1_34, Sb1_35 and Sb1_43.
•Mutants Δ56 and Δ59 demonstrate a reduction in conjugation efficiency, but these genes are not essential for conjugation.

## 7. Why is SPI-7 immobile?

•SPI-7 from *S*. Typhi has been shown to promote conjugation, thus must contain all the genes essential for conjugation.
•Thus several of the perceived pseudogenes within SPI-7 must be functional. Closer analysis of the sequence indicates that these genes may be expressed from alternative start sites without compromising the putative functional domains of the proteins.
•SPI-7 from *S*. Typhi cannot excise, yet carries apparently functional genes for all excision functions identified in this work. Regulatory mutations must be considered.
•The most obvious barrier to SPI-7 excision is the phage-like 14 CDS insertion adjacent to the $tRNA^{Phe}$. Perhaps this integration has rendered SPI-7 immobile.

## 8. References

Baker, S. et al. 2008. Mobilization of the *incQ* plasmid R300B with a chromosomal conjugation system in *Salmonella enterica* serovar Typhi. J. Bacteriol. 190:4084-7
Burrus, V. et al. 2002. Conjugative transposons: the tip of the iceberg. Mol. Microbiol. 46:601-610
Carver, T. J. et al. 2005. ACT: the Artemis Comparison Tool. Bioinformatics 21:3422-3423
Cherepanov, PP and Wackernagel, W. 1995. Gene Disruption in *Escherichia coli* – Tc(R) and Km(R) Cassettes with the Option of FLP-Catalysed Excision of the Antibiotic Resistance Determinant. Gene 158 (1): 9-14
Datsenko, K.A. and Wanner, B.L. 2000. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. PNAS 97 (12): 6640-6645
Datta, S. et al. 2006. A set of recombineering plasmids for gram-negative bacteria. Gene 379: 109-115
Juhas, M. et al.. 2007. Novel type IV secretion system involved in propagation of genomic islands. J. Bacteriol. 189:761-771
Mohd-Zain, Z. et al. 2004. Transferable Antibiotic Resistance Elements in *Haemophilus influenzae* Share a Common Evolutionary Origin with a Diverse Family of Syntenic Genomic Islands, J. Bacteriol 186 (23): 8114-8122

## 9. Acknowledgement

This project is funded by the Wellcome Trust through its core funding for the Sanger Institute

wellcome trust sanger institute