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Inactivation of *st313-td* in *Salmonella* Dublin increases virulence in the mouse infection model

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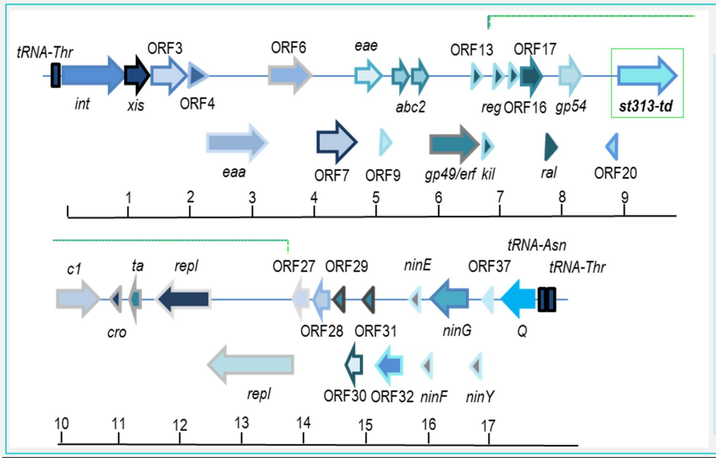
INTRODUCTION

Salmonella enterica (*S. enterica*) serovar Dublin is a host-restricted serovar causing typhoidal disease in cattle. *S.* Dublin can occasionally infect humans leading to invasive disease (1).
In previous studies, we discovered a new virulent gene, *st313-td*, of unknown function, in the pathogenic and multidrug-resistant lineage *S.* Typhimurium ST313 (2). *st313-td* was found to be conserved in the *S.* Dublin genomes (2).

In ST313, the gene is harbored is a new potential pathogenicity island (ST313-GI of ca. 17,7 kb) while in Dublin, it is contained in a region of ca. 6.8 kb matching a part of ST313-GI (99% identity) (Fig. 1).

st313-td encodes a protein, which precise function in S. Typhimurium ST313 is unknown. The gene was classified as a virulence gene, since deletion of the gene led to significantly decreased macrophage survival and virulence in a mouse model of infection (2).

PURPOSE: To analyze the role of *st313-td*, apparently conserved in all the *S.* Dublin genomes, in the virulence of this serovar through infection of cell lines and mouse model of infection.



GENOMIC STRUCTURE OF ST313-GI

Figure 1. Region of the island present in the *S.* Dublin genomes highlighted with a discontinued line in green) (2, modified)

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MATERIAL AND METHODS

Bioinformatics analyses: NCBI (www.ncbi.nlm.nih.gov) and Uniprot database (www.uniprot.org): Analysis of presence of *st313-td* homologs in non-*Salmonella* bacteria and study of the putative role of *st313-td* encoded protein.

Bacterial strains and growth conditions: Wild type (WT) control strains: *S.* Dublin 3246 (*st313-td*-positive) and *S.* Typhimurium 4/74 (non *st313-td*) (3,1).
Isolates incubated in LB for 16 h at 37 ºC with shacking. LB was supplemented with antimicrobials where appropriate: kanamycin (50 µg/ml), apramycin (75 µg/ml).

Mutagenesis experiments
Table 1. WT strains and plasmids used for the mutagenesis experiments and derived isogenic strains (obtained as previously described; 2)

Strains and plasmids	Relevant features	Reference
Salmonella strains		
S. Dublin 3246	WT, *st313-td*-positive strain	3
4/74	WT. Virulent reference strain	1
KP1274	Restriction deficient strain	5
ΔSDst313-td	Δ*st313-td*, Apr^R	This work
SD3246-C	Δ*st313-td*, pACY177+*st313-td* (pAHF4), Apr^R, Kn^R	This work
invH201::TnphoA	*invH* mutant, Kn^R	6
ΔssaV	*ssaV* mutant, SPI2-T3SS defect, Kn^R	4
Plasmids		
pACY177	Cloning vector, Ap^R, Kn^R	7
pAHF4	pACY177 expressing st313-td	2
pKD46	Plasmid with λ red recombinase expressed from arabinose inducible promoter	8
pUO9090	Apr^R	Unpublished

Infection of mouse and cattle macrophages: Analysis of *st313-td* role in intracellular survival and replication within mouse and cattle macrophages (J774A.1 and BoMac) (2). Controls: WT 4/74 and Δ*ssaV* (1,4). Experiments performed in quadruplicates.

Mouse mixed infections: Infections of six-week old female C57/BL6 mice performed (2). Groups of five mice each were inoculated i.p. with 0.1 ml of a 1:1 mixture of the WT and the isogenic strains (stationary phase) suspended in physiological saline. Competitive index (CI) was calculated (Table 2)

RESULTS

st313-td encoded protein have homologs in other pathogens: i.e; *Kleibsiella pneumoniae* and *Cronobacter sakazakii* (95 % identity). Region of St313-td with 26-32.8 % identity with KilA N domain (9) (may be enzymes, such as nuclease domains, or may mediate specific interactions with nucleic acids or proteins)

st313-td influences uptake by macrophages in S. Dublin but not intracellular survival within murine and cattle macrophages

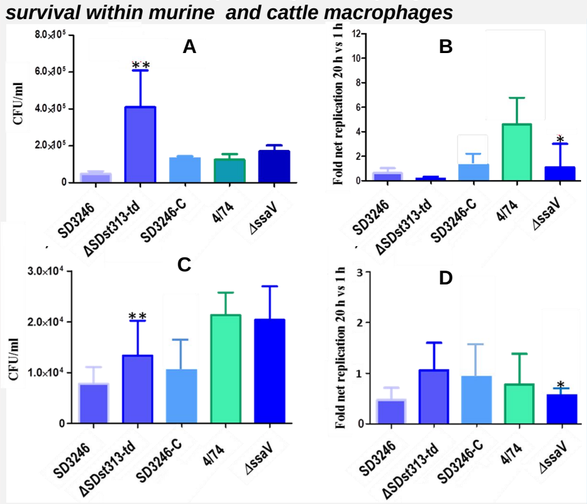

Figure 2. Intracellular survival/replication, A and B) at t=1 h and t=20h post-infection (in J774A.1) and with regards to t=1 h post-uptake (the intracellular survival at t=1 h for all the tested isolates is regarded as 1), C and D) at t=1 h and t=20h post-infection (in BoMac) and with regards to t=1 h post-uptake. Isolates: *S.* Dublin 3246 (SD3246), *S.* Typhimurium 4/74 and mutants: ΔSD*st313-td*, SD3246-C (pAHF4, complemented strain) and Δ*ssaV*.

Deletion of st313-td leads to a virulence increase in systemic mice infection

Salmonella str	Competitive index (CI)
WT SD3246	
versus	
ΔSDst313-td (4)	1.56±0.47[a]
SD3246-C (4)	0.68±0.07[b]

Table 2. CI for *S.* Dublin 3246 mutants in mice. CI=1: the virulence of the strains tested is equal. CI<1: the mutant is less virulent than the WT. [a]CI was significantly different from 1.0 (P<0.001). [b]CI was significantly different from corresponding mutant (P>0.05).

CONCLUSIONS

- Contrary to results observed in ST313, the gene *st313-td* in *S.* Dublin, influences uptake by macrophages but not survival within the cells
- The absence of *st313-td* in *S.* Dublin enhances virulence in a murine systemic infection, in contrast to *S.* Typhimurium ST313 results
- *st313-td* might be a regulator gene and may act in a serovar-specific manner (mediate opposite regulatory functions)