

Comparative Analysis of Phenotypic and Genetic Changes in Felixounavirus Phages Under Bacterial Challenge

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Abstract: *Salmonella* Infantis is considered in recent years an emerging *Salmonella* serovar, as it has been associated with several outbreaks and multidrug resistance phenotypes. Phages appear as a possible alternative strategy to control *Salmonella* Infantis (SI). The aims of this work were to characterize two phages of the *Felixounavirus* genus, isolated using the same strain of SI, and to expose them to interact in challenge assays to identify genetic and phenotypic changes generated from these interactions. These two phages have a shared nucleotide identity of 97% and are differentiated by their host range: one phage has a wide host range (lysing 14 serovars), and the other has a narrow host range (lysing 6 serovars). During the 12 h challenge we compared: (1) optical density of SI, (2) proportion of SI survivors from phage-infected cultures, and (3) phage titer. Isolates obtained through the assays were evaluated by efficiency of plating (EOP) and by host-range characterization. Genomic modifications were characterized by evaluation of single nucleotide polymorphisms (SNPs). The optical density (600 nm) of phage-infected SI decreased, as compared to the uninfected control, by an average of 0.7 for SI infected with the wide-host-range (WHR) phage and by 0.3 for SI infected with the narrow-host-range (NHR) phage. WHR phage reached higher phage titer (7×10^{11} PFU/mL), and a lower proportion of SI survivor was obtained from the challenge assay. In SI that interacted with phages, we identified SNPs in two genes (*rfaK* and *rfaB*), which are both involved in lipopolysaccharide (LPS) polymerization. Therefore, mutations that could impact potential phage receptors on the host surface were selected by lytic phage exposure. This work demonstrates that the interaction of *Salmonella* phages (WHR and NHR) with SI for 12 h in vitro leads to emergence of new phenotypic and genotypic traits in both phage and host. This information is crucial for the rational design of phage-based control strategies.

Dataset:

Keywords: *Salmonella virus FelixO1*; *Salmonella* Infantis; selective challenge assay.

1. Summary

This work demonstrates that the interaction of *Salmonella* phages (WHR and NHR) with SI for 12 h in vitro leads to emergence of new phenotypic and genotypic traits in both phage and host. This information is crucial for the rational design of phage-based control strategies.

2. Data Description

Supplementary material, this material contains supplementary table and figures that may be relevant to understanding the work

2.1.1. Subsubsection

1. Figure S1
2. Figure S2
3. Figure S3
4. Figure S4

5. Table S1

2.2. Figures, Tables and Schemes

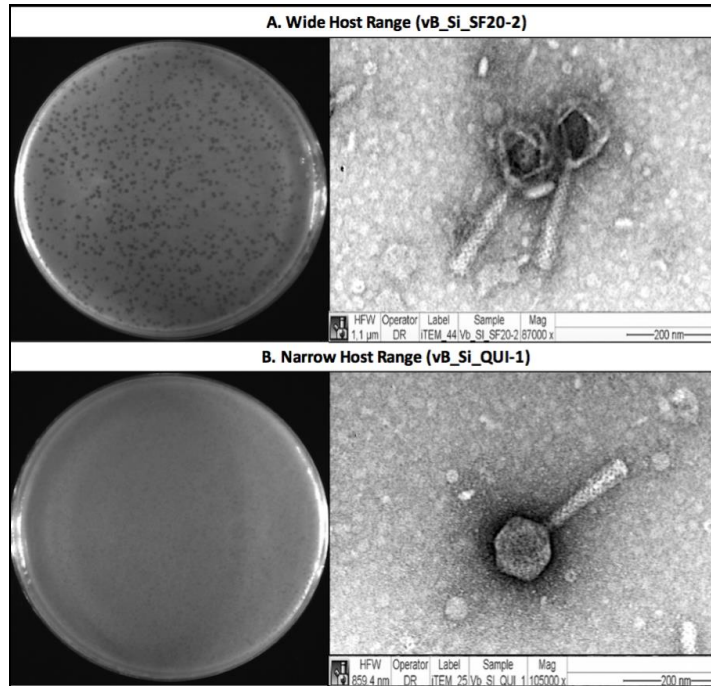


Figure S1. Morphology of lysis plaques and electron microscopy of wide host range and narrow host range phages. Morphology of lysis plaques and electron microscopy of transmission in (A) wide host range phage (B) narrow host range phage.

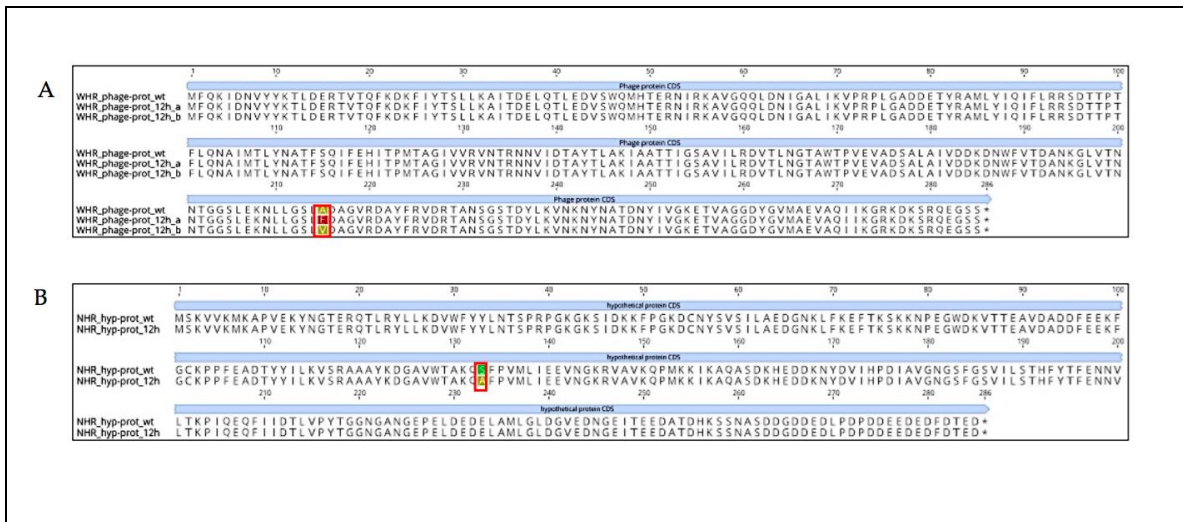


Figure S2. Amino acid alignment of phage protein and hypothetical protein in wild-type phage compared to WHR or NHR phage exposed to *S. Infantis* for 12-h. (A) Amino acid alignment of phage protein in wild-type WHR phage and WHR phage exposed to *S. Infantis* for 12-h. Sequences a and b represent two different mutations observed at the same position. (B) Amino acid alignment of hypothetical protein in wild-type NHR phage and NHR phage exposed to *S. Infantis* for 12-h. The nucleotide sequence of the gene from the wild type genome was extracted and modified to reflect the mutation, then the wild type and mutation-containing nucleotide sequences were translated and aligned with ClustalW[75] in Geneious Prime[76]. Amino acids labeled with different

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colors represent differences among sequences of wild-type phages and the 12-h exposed phages. Amino acid changes caused by SNPs are marked in red frames.

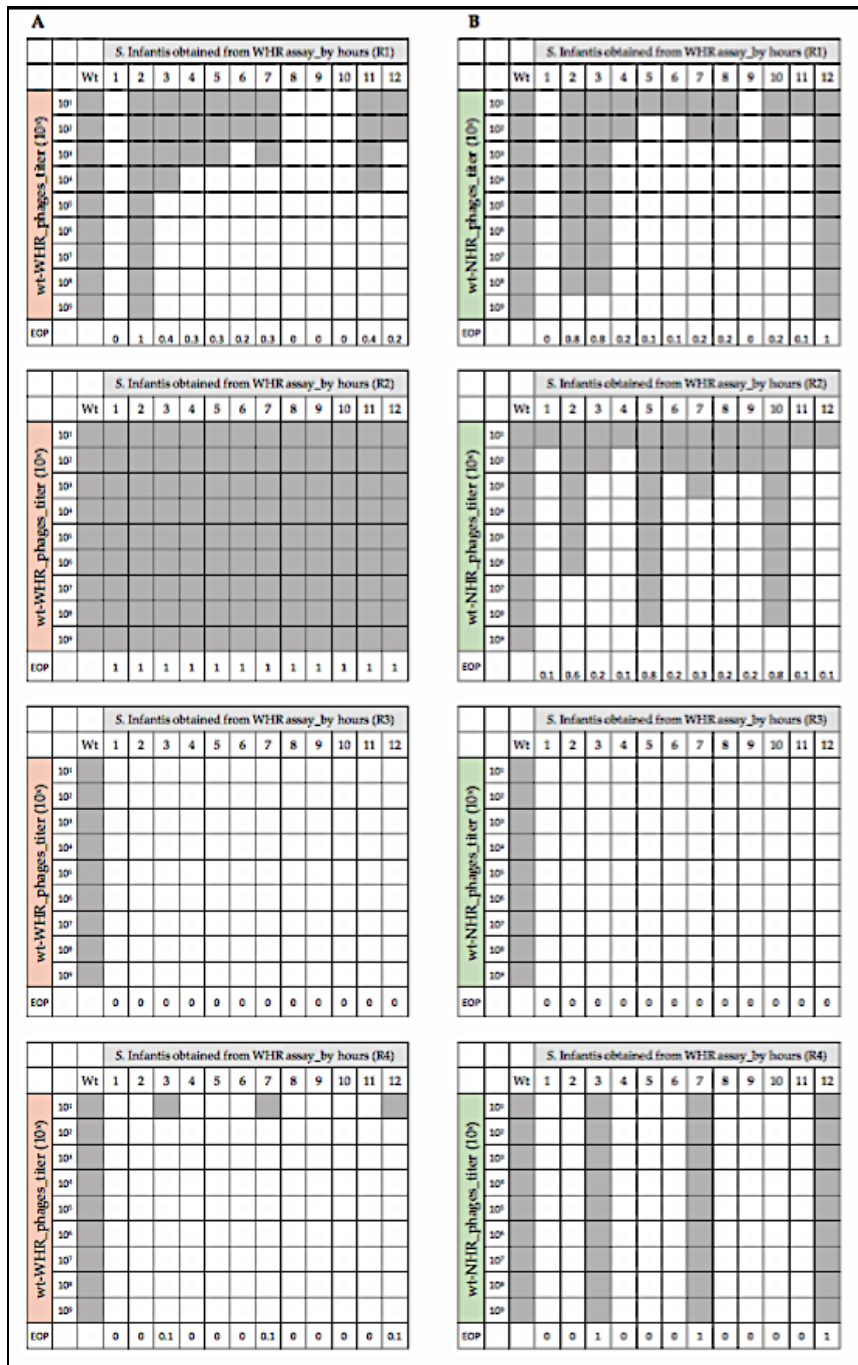


Figure S3. Evaluation of phage sensitivity by efficiency of plating (EOP) on isolated *S. Infantis* SI survivors to WHR phage and cross-resistance assays. It shows the titer of the wild type phage on *S. Infantis* obtained from challenge assay. Lysis is marked in grey and no lysis in white. A. Evaluation of phage sensitivity of EOP in *S. Infantis* obtained every hours of wide assay, exposed to wild type (wt)-WHR phage. B. Cross-resistance assay of EOP in *S. Infantis* obtained every hour of wide assay, exposed to wild type (wt)-NHR phage.

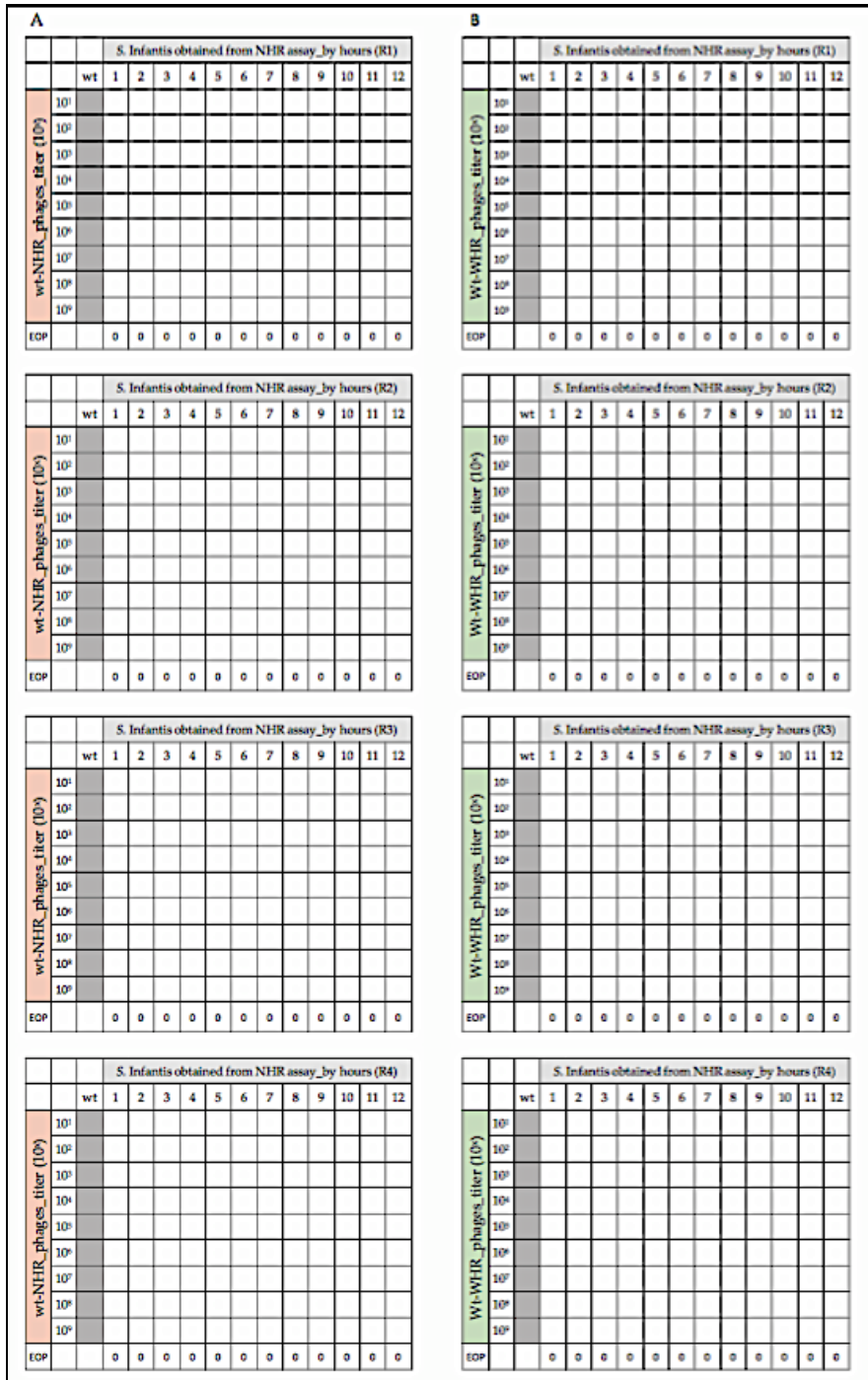


Figure S4. Evaluation of phage sensitivity by efficiency of plating (EOP) on isolated SI survivors to NHR phage cross-resistance assays. It shows the titer of the wild type phage on *S. Infantis* obtained from challenge assay. Lysis is marked in grey and no lysis in white. **A.** EOP in *S. Infantis* obtained every hour of narrow assay, exposed to (wt) wild type-NHR phage. **B.** Cross-resistance assay of EOP in *S. Infantis* obtained every hours of narrow assay, exposed to wild type (wt)-WHR phage.

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Table 1. Antigenic somatic formulae (O) and presence of *rfaB* and *rfaK* gene in serovars of *Salmonella enterica* susceptible to wide and narrow host range phages obtained from challenge assays.

Characteristics of susceptible serovars of both wide and narrow host range phages			
Serovars (Serogroup)	Somatic antigenic (O) formulae	Presence <i>rfaB</i> ¹ (ID) ²	Presence <i>rfaK</i> ³ (ID) ²
Typhimurium (Group O:4 [B])	1,4,[5],12	TMV96147.1	ASF66624.1
Virchow (Group O:7 [C ₁])	6,7,14	AZH72471.1	AZH72476.1
Oranienburg (Group O:7 [C ₁])	6,7,14	THC06534.1	SUH68841.1
Braenderup (Group O:7 [C ₁])	6,7,14	Not found ⁴	SUH55395.1
Infantis (Group O:7 [C ₁])	6,7,14	TMV47934.1	SQI71138.1
Newport (Group O:8 [C ₂])	6,8,20	TMM69866.1	AGS27141.1
Corvallis (Group O:8 [C ₃])	8,20	TLC03182.1	Not found ⁴
Kentucky (Group O:8 [C ₃])	8,20	TMW25308.1	KUD18860.1
Javiana (Group O:9 [D ₁])	1,9,12	Not found ⁴	KUD61483.1
Dublin (Group O:9 [D ₁])	1,9,12	TKX13464.1	SUI05380.1
Panama (Group O:9 [D ₁])	1,9,12	Not found ⁴	SUH76918.1
Characteristics of susceptible serovars of only wide host range phage			
Muenster (Group O:3,10 [E ₁])	3,{10}{15}{15,34}	Not found ⁴	KTW46212.1
Agona (Group O:4 [B])	1,4,[5],12	TLA84965.1	RKD15901.1
Saintpaul (Group O:4 [B])	1,4,[5],12	QCW59584.1	KUD72187.1
Heidelberg (Group O:4[B])	1,4,[5],12	TMV96713.1	RFN36928.1
I 4,5,12:i:- (monophasic variant of Typhimurium) (Group O:4 [B])	4,5,12	BBH80090.1	BBH80095.1
Stanley (Group O:4 [B])	1,4,[5],12,[27]	TLC20503.1	SQH42842.1
Choleraesuis (Group O:7 [C ₁])	6,7	Not found ⁴	Not found ⁴
Mbandaka (Group O:7 [C ₁])	6,7,14	RJJ85429.1	RAN13577.1
Montevideo (Group O:7 [C ₁] or O:54 ⁵)	6,7,14 or {6,7,14}{54} ⁵	TMM93554.1	AJQ76118.1
Enteritidis (Group O:9 [D ₁])	1,9,12	TLC90283.1	SUI12981.1

¹(*rfaB*) gene encoding for the enzyme waaB engaged in the polymerization of core LPS adding UDP-D-galactose lipopolysaccharide

1,6 glucosyltransferase or Lipopolysaccharide 1,6-galactosyltransferase.

²GenBank ID: Accession number.

³(*rfaK*) gene encoding for the enzyme waak engaged in the polymerization of core LPS adding

UDP-D-glucose:lipopolysaccharide α -1,2 glucosyltransferase or lipopolysaccharide 1,2-N acetylglucosaminetransferase.

⁴Not Found: These genes were not found in the mentioned serovars but have been described for *Salmonella enterica*.

⁵Factor O:54 is plasmid-controlled. In serovar Montevideo, factors O:6,7,14 are expressed in the absence of O:54.

Reference of Somatic antigenic (O) formulae: Grimont et al,[82].

3. Methods

Two phages have a shared nucleotide identity of 97% and are differentiated by their host range: one phage has a wide host range (lysing 14 serovars), and the other has a narrow host range (lysing 6 serovars). During the 12 h challenge we compared: (1) optical density of SI, (2) proportion of SI survivors from phage-infected cultures, and (3) phage titer. Isolates obtained through the assays were evaluated by efficiency of plating (EOP) and by host-range characterization. Genomic modifications were characterized by evaluation of single nucleotide polymorphisms (SNPs).

Author Contributions:

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Author contributions: Conceptualization, D.R., L.K.H., T.G.D. and A.I.M.S.; methodology, D.R., L.K.H., T.G.D., C.H.W. and D.P.; software, D.R., L.K.H. and C.H.W.; validation, D.P.; formal analysis, D.R., L.K.H., and T.G.D.; investigation, D.R.; resources, T.G.D.; data curation, L.K.H. and T.G.D.; writing - original draft preparation, D.R., L.K.H., T.G.D. and A.I.M.S.; writing - review and editing, D.R., LKH, T.G.D. and A.I.M.S.; visualization, D.R. and L.K.H.; supervision, T.G.D. and A.I.M.S.

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Conflicts of interest: The authors declare that there are no conflicts of interest.

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