

Sequencing results of the VTEC O157 typing phages

Lauren Cowley, PhD student





VTEC O157 phage typing scheme

- Used in the UK to type the different strains and some phage types(PT) are associated with more severe disease than others.
- Scheme based on the use of 16 bacteriophage that produce a profile of phage infection for the strain based on what level of lysis is achieved by each phage (Ahmed et al. 1987) and has been used to categorize outbreaks and sporadic cases.





Phage type prevalence 1996-2010

Today 80% of all strains typed are PT 8, 21/28, 2, 4 or 32.



Claire Jenkins, 2012



Predominant PTs and country: human isolates



4 Sequencing results of the VTEC O157 typing phages

Two-way cluster Euclidian

 The 14 Myoviridae(T4) and 2 Podoviridae(T7) typing phages cluster into groups based on their reactivity profiles.

Courtesy of Margo Chase-Toppin

5

Sequencing the typing phages

- 12 (phages 1, 3, 4, 5, 6, 7, 8, 9, 11,12, 13 and 14) of the 16 phages were extracted using the QIAamp UltraSens Virus kit that uses a spin column.
- Then sequenced at The Genome Analysis Centre on a run of the Illumina MiSeq. Illumina TruSeq DNA library construction was performed and sequencing of the libraries was pooled on one run using 150 bp paired-end reads.
- The remaining phages(2,10,15 and 16) were more difficult to extract enough DNA out of and sequencing is still underway.
- The sequenced phages were *de novo* assembled using Velvet and annotated using Prokka.

Sequencing results of the VTEC O157 typing phages

Phages that are very similar have similar resistance patterns

CRISPR and SIE analysis

CRISPRs in bacteria can provide Phage genomes were also BLASTed against strains of VTEC to look for regions of prophage that may provide immunity by super infection CRISPR preserved in certain strains of VTEC reubignised by COIRESTR Withder acceptibility and resistance to the sphage. sequences were extracted and put into a python script that parses phage sequences to find corresponding protospacer sequences. However no protospacer regions were found.

CRISPR-associated

(Cas) genes

CRISPR

Asako Miyakawa and Sam Sternberg

Conclusions and future work

- The negative result in CRISPR analysis indicates that CRISPR systems may not be involved in phage susceptibility and resistance in the phage typing scheme.
- The negative result in prophage analysis indicates that super infection exclusion mechanisms may not be responsible for immunity in the phage typing scheme.
- Analysis of the regions within groups that are heterologous could help determine the genes responsible for the subtle differences in the reaction profiles of those phages and correlate them with the phage types that particular region is responsible for.
- Plans to carry out TraDIS (Pickard et al., 2013) on strains of VTEC to look for mutants that change their susceptibility to phages and identify host factors in infection.

VTEC O157 Sequencing project

All strains used in host strain analysis were taken from the PHE sequencing project (NIHR funded). There have been ~800 strains sequenced so far.

Representative tree of 250 strains.

Acknowledgements

Claire Jenkins, Tim Dallman, Margo Chase-Toppin, Matthew Hannah, Neil Perry and David Gally

Questions?

