

INTRODUCTION

- The National Collection of Type Cultures (NCTC) as well as being the world's oldest bacterial collection, has a collection of over one hundred archived, historical bacteriophages and their corresponding bacterial hosts which were originally deposited primarily for their value in bacterial typing.
- Due to a growing interest in bacteriophages as a potential solution to tackle antibiotic resistance, NCTC set out as an objective, to re-authenticate and characterise these phages with a view to making them available to science.
- The collection has 62 Staphylococcus phages, 16 Campylobacter phages and 31 Streptococcus phages.

AIMS

To test the NCTC bacteriophage collection for viability and characterise the phages to make them available to science.

METHODS

The spot test was used to determine host range, phage titres were determined by plaque assay and phage ultrastructure by TEM. Phage purity (absence of bacterial contaminants) was confirmed by streaking on CBA and BPM plates.

Spot Test

- A lawn of the bacterial strain to be tested was prepared on solid media (Nutrient Agar, NZCYM Agar and Modified Todd Hewitt Agar for Staphylococcus, Campylobacter and Streptococcus phages respectively). 10µL spots of serially diluted phage were made on the bacterial lawn up to a final dilution of 1:1000. Following incubation at suitable conditions for the phage/host, plates were observed for the appearance of plaques and zones of complete lysis.

Plaque Assay

- Stationary or log cultures of host bacteria were used for phage propagation. The mixture of phage and host was incubated for 10 - 30 minutes followed by the addition of an agar overlay. Some phages required the addition of Ca²⁺ for plaque formation.
- Plates were observed following incubation, for plaques from which titres were determined.

Transmission Electron Microscopy

- Plaques were removed from agar plates and spun down to pellet cells. The supernatant was fixed onto a carbon-coated copper grid, twice washed and negatively stained with 1.5% PTA for viewing.

RESULTS

Bacteriophage Host Range

- Phages were tested against a panel of 10 – 16 bacterial strains of the same species as their host strain with results showing varying degrees of susceptibility to the phage. Table 1 represents a data set of the results including the phages with the broadest and narrowest host range identified in this study

Table 1: Host Range analysis of NCTC phages

Host Organism	Phage NCTC No	No of bacterial hosts tested	No of bacterial hosts susceptible to phage	Percentage susceptibility of host panel to phage
<i>S. aureus</i>	7814	13	10	76.9%
<i>S. aureus</i>	9316, 10969	13	9	69.2%
<i>S. aureus</i>	8289	16	10	62.5%
<i>S. aureus</i>	8401, 8402, 8403, 8419, 8424, 8290, 9314, 10032	13	1	7.7%
<i>C. Jejuni</i>	12673, 12674	11	6	54.5%
<i>C. Jejuni</i>	12671	11	5	45.5%
<i>C. Jejuni</i>	12675	11	1	9.1%
<i>S. agalactiae</i>	11267	10	7	70%
<i>S. agalactiae</i>	11275	11	8	72.7%
<i>S. agalactiae</i>	11261, 11262	10	1	10%

Phage Viability and Purity

- All 56 Staphylococcus phages and 30 Streptococcus historical phages tested were viable. 2 out of the 16 Campylobacter historical phages however showed no viability on all the assays giving an overall 98% viability of the phages tested. Only 1 out of 103 phages tested was found to have bacterial contaminants on non-selective media.

Bacteriophage Morphology and Classification

- TEM revealed 3 main phage morphologies: long noncontractile tailed, long contractile tailed and short tailed phages all of the order Caudovirales.
- Some of the long noncontractile tailed phages have elongated heads and some, icosahedral heads.
- Most of the Staphylococcus phages have long noncontractile tails placing them in the family Siphoviridae. Two were identified as Podoviridae with short tails and one as Myoviridae with long contractile tails surrounded by a tail sheath.
- 10 Campylobacter phages were identified as Myoviridae and the Streptococcal phages have been identified as Siphoviridae.
- Purity of the phages was further confirmed by uniformity of the structures across each grid.

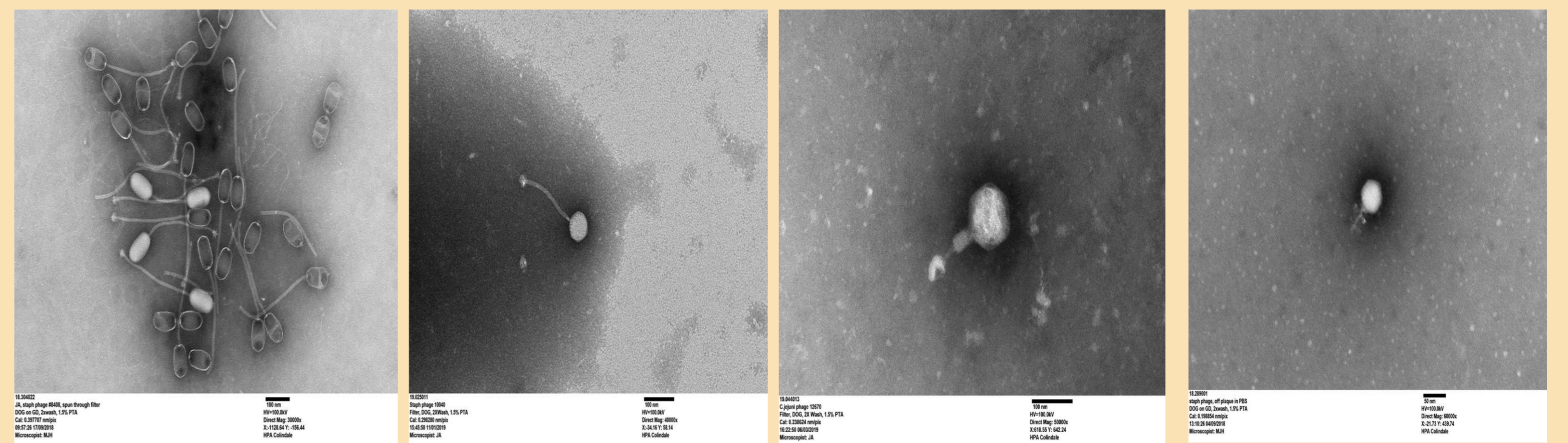


Fig. 1: Four distinct phage morphologies (left to right), Siphoviridae with elongated head, Siphoviridae with icosahedral head, Myoviridae with contractile tail and Podoviridae with short tail

CONCLUSIONS

- This study highlights the fact that bacteriophages can remain viable even after several years following freeze drying.
- NCTC 7814, 8289, 9316, 10969, 11267 and 11275 were identified as having the broadest host range, lysing between 62.5% and 76.9% of the strains they were tested against.
- The molecular characterisation done in this study has unravelled the diversity of NCTC's bacteriophage collection.
- The re-authentication of NCTC's bacteriophage collection is opening opportunities for further research using these phages, with PHE aiming to make it as accessible to the scientific community as possible.
- The NCTC bacteriophage collection once fully operational will be a dynamic repository into which microbiologists can both source and deposit strains to support accessibility and reproducibility in science and industry.
- NCTC is also a long established International Depository Authorities (IDAs) for the deposit of pathogenic bacteria (both clinical and veterinary) and their associated bacteriophage for the purposes of patenting under the Budapest Treaty.

REFERENCES

- Blair, J. E. and R. E. Williams (1961). "Phage typing of staphylococci." *Bull World Health Organ* **24**(6): 771-784.
- Domelier, A. S., et al. (2009). "Molecular Characterization and Lytic Activities of Streptococcus agalactiae Bacteriophages and Determination of Lysogenic-Strain Features." *Journal of bacteriology* **191**(15): 4776-4785.
- Sails, A. D., et al. (1998). "Characterisation of 16 Campylobacter jejuni and C. coli typing bacteriophages." *J Med Microbiol* **47**(2): 123-128.

COMMUNICATIONS

- ❖ www.pheculturecollections.org.uk/collections/nctc-3000-project
- ❖ Follow us on Twitter:  @NCTC_3000
- ❖ If you would like to deposit strains with NCTC please contact: sarah.alexander@phe.gov.uk