HYDROGEL AS BACTERIOPHAGE STORAGE, ASSAY AND TRANSPORTATION MATRIX

Sheetal Patpatia[1*], Lauri Paasonen[2], Mikael Skurnik[1,3], Saija Kiljunen[1]
1) Department of Bacteriology and Immunology, University of Helsinki, Finland
2) UPM-Kymmene, Helsinki, Finland
3) Division of Clinical Microbiology, Helsinki University Central Hospital, HUSLAB, Finland
*sheetal.patpatia@helsinki.fi

AIMS OF THE WORK

1) To use nanofibrillar cellulose (NFC) hydrogel as long-term storage matrix for bacteriophages.
2) To test rapid host-range assay with phages stored with NFC.
3) To create baseline for bacteriophage transportation and test a ready-to-screen plate format for it.

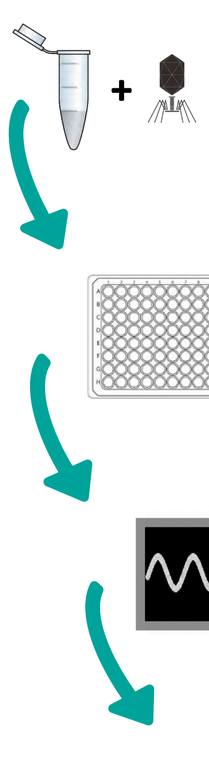
MATERIALS

- In this study four bacteriophages were used; fHoEco02 (vB_EcoM-fHoEco02, *Myoviridae*)[2], fTu-Eco01 (*Podoviridae*), fRuSau02 (vB_SauM-fRuSau02, *Myoviridae*)[1] and ΦEBHT (*Podoviridae*). Bacteriophages fHoEco02 and fTu-Eco01 were isolated from Finnish wastewater samples and phage fRuSau02 was isolated from the commercial *Staphylococcus aureus* cocktail. All three phages were selected from our own bacteriophage collection. *S. aureus* phage ΦEBHT was received from DSMZ, Germany.
- S. *aureus* phage fRuSau02 is virulent towards most clinical *S. aureus* strains, which are available in our collection. ΦEBHT is effective towards MRSA pig strains. Two strains were selected for the study; *S. aureus* 19A2, for fRuSau02 and *S. aureus* 13KP, for ΦEBHT. For *Escherichia coli* hosts, *E. coli* #123738 for fHoEco02 & *E. coli* #123789 for fTu-Eco01 were selected.
- As a supportive matrix, 1,5% nanofibrillar cellulose hydrogel, GrowDex® (UPM-Kymmene, Finland) was used in the study.

TRANSPORTATION

- Bacteriophage transportation can be challenging when multiple phages need to be transported. There is a need to create process for safe and reliable phage transportation between laboratories.
- The protocol that was designed for the study, allows shipping of NFC-embedded phages in ready-to-screen plate format.
- One of the test-plates were sent to DSMZ laboratories, Germany via courier service and the other plate was sent back to our own laboratory through local mail service.
- During transportation plates were exposed to different temperatures and harsh conditions.
- Results of the test-plate sent back to our own laboratory are shown in Fig.5.
- Similar results were obtained with test-plate which was sent to DSMZ, Germany.

EXPERIMENTAL SET-UP



2/3 VOL of 1,5% NFC hydrogel was mixed with 1/3 VOL of 10^8 PFU/ml bacteriophage lysate. Three different concentrations of NFC were used; 1,0%,0,5% and 0% (SM-buffer only). As a negative control, SM-buffer without bacteriophage lysate was used in the experiment.

The mixture was stored in three different conditions; 1) In the wells of 96-well microtiter plate as 10µl drops and dried before storage, Fig.1A. 2) in the wells of 96-well microtiter plate and kept as wet 10µl drops, Fig.1B. and 3) in 2ml tubes and 10µl drops were pipeted into the wells of 96-well plate prior of the use, Fig.2-4. Before measurement, 200µl diluted O/N bacterial culture was added to the wells and plate was incubated at +37°C.

Absorbance was measured at 600nm for up to 5 hours at 1 hour intervals.

Result interpretation: If phages were viable, bacterial growth was inhibited. In control samples bacterial growth should be normal. Fig.1.-4.

RESULTS

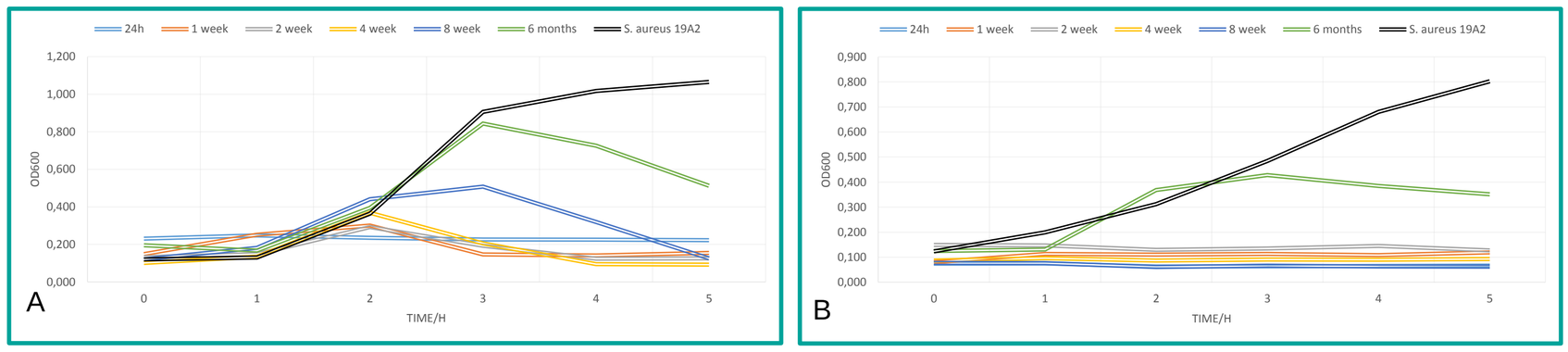


Figure1. **A.** *S.aureus* phage ΦEBHT in 0,5% NFC and pipeted as 10µl drops in the wells of 96-well plate. **B.** Same method was applied as in situation **A.** but the phage and NFC drops were dried and then the procedure was continued. In the both situations, phage was viable up till 8 weeks of storage. After 6 months, phage viability decreased but the phage was still infective towards its host strain *S.aureus* 19A2.

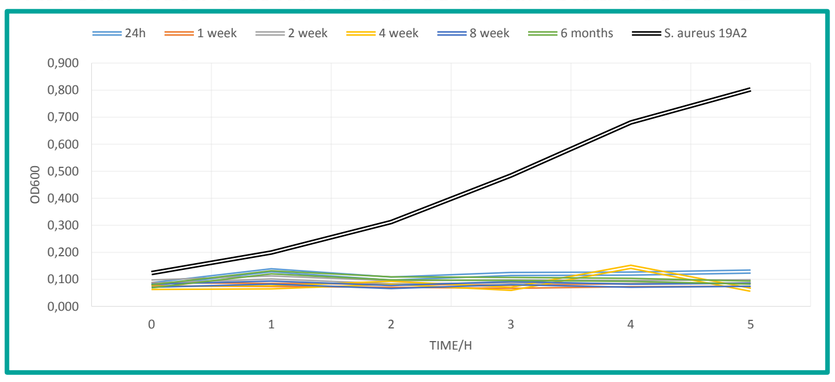


Figure2. *S.aureus* phage ΦEBHT in 0,5% NFC. The phage was viable after 6 months of storage in a 2ml tube and inhibited bacterial (*S.aureus* 19A2) growth. Host strain is shown as a control in the graph.

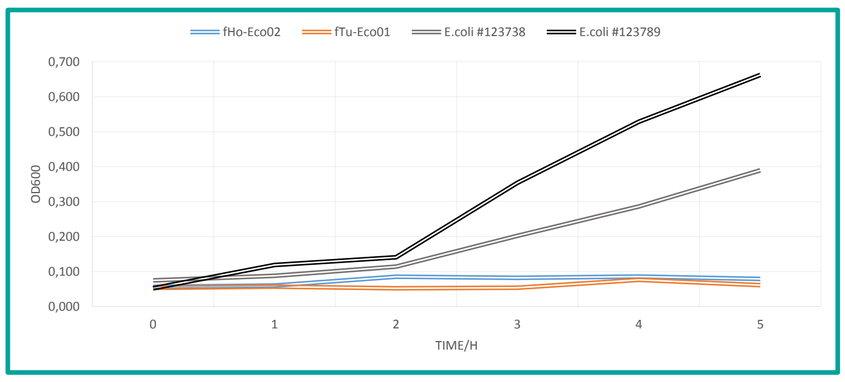


Figure3. Both *E.coli* phages were viable and inhibited bacterial growth after 24 hours of storage with 0,5% NFC.

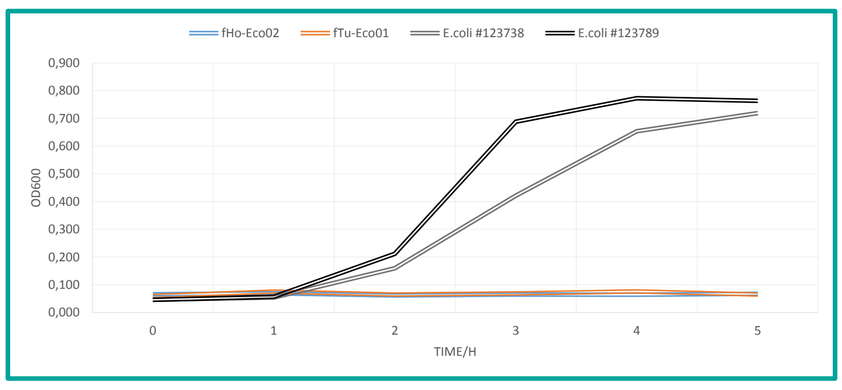


Figure4. Both *E.coli* phages were viable and inhibited bacterial growth after 6 months of storage with 0,5% NFC.

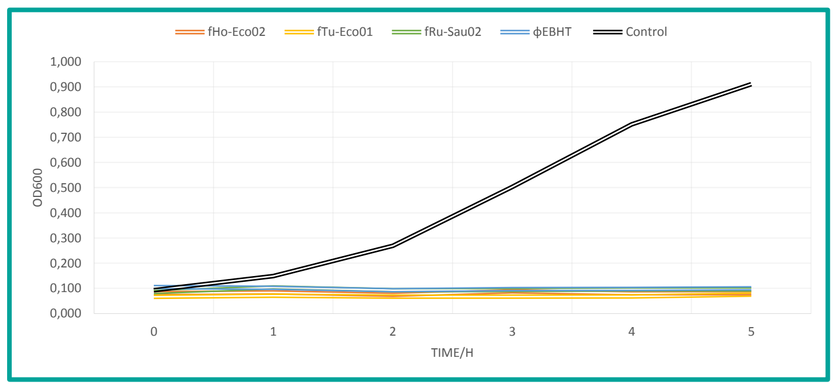


Figure5. All tested phages were still viable after transportation and infected their host strains accordingly. Host strain results shown as control in the graph.

CONCLUSIONS

- Phages can be stored with NFC for at least 6 months in 2ml tubes.
- Podoviruses were more viable after 6 months than myoviruses, when dried with NFC.
- Phages can be stored and transported via mail across laboratories as 10µl drops on 96-well plate and NFC as supportive matrix.
- Transporting phages in 96-well plate is safe and reliable as temperature doesn't affect phage viability.

ACKNOWLEDGEMENTS

- Dr. Johannes Wittmann (DSMZ, Germany) is acknowledged for testing the ready-to-screen plate format and transportation method for the study.

REFERENCES
1. Leskinen,K. *et al*.(2017) Characterization of vB_SauM-fRuSau02, a Twort-Like Bacteriophage Isolated from a Therapeutic Phage Cocktail. Viruses 9:258.
2. Kiljunen,S. *et al*.(2018) Complete Genome Sequences of Two *Escherichia* Phages Isolated from Wastewater in Finland. Viruses Genome Announc. 6:e00401-18.