HYDROGEL AS BACTERIOPHAGE STORAGE, ASSAY AND TRANSPORTATION MATRIX

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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.

EXPERIMENTAL SET-UP

2/3 VOL of 1.5% NFC hydrogel was mixed with 1/3 VOL of 10⁸ 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 pipetted 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.

MATERIALS

In this study four bacteriophages were used; fHo-Eco02 (vB_EcoM-fHoEco02, Myoviridae)2, fTu-Eco01 (Podoviridae), fRuSau02 (vB_SamM-fRuSau02, Myoviridae)1 and fEBHT (Podoviridae). Bacteriophages fHoEco02 and fTu-Eco01 were isolated from Finnish wastewater samples and phage fRuSau02 was isolated from the commercial Staphylococcus aureus cocktail. Three phages were selected from our own bacteriophage collection. S. aureus phage fEBHT was received from DSMZ, Germany.

S. aureus phage fRuSau02 is virulent towards most clinical S. aureus strains, which are available in our collection. fEBHT is effective towards MRSA pig strains. Two strains were selected for the study; S. aureus 19A2, for fRuSau02 and S. aureus 19K2, for fEBHT. For Escherichia coli hosts, E. coli #123789 and 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.

RESULTS

Figure1. A. S.aureus phage fEBHT in 0.5% NFC and pipetted 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 both situations, phage was viable up to 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 fEBHT 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.

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REFERENCES