Phage Activity Enumeration: Comparison of MD/SP, PLC and Tetrazolium Reduction

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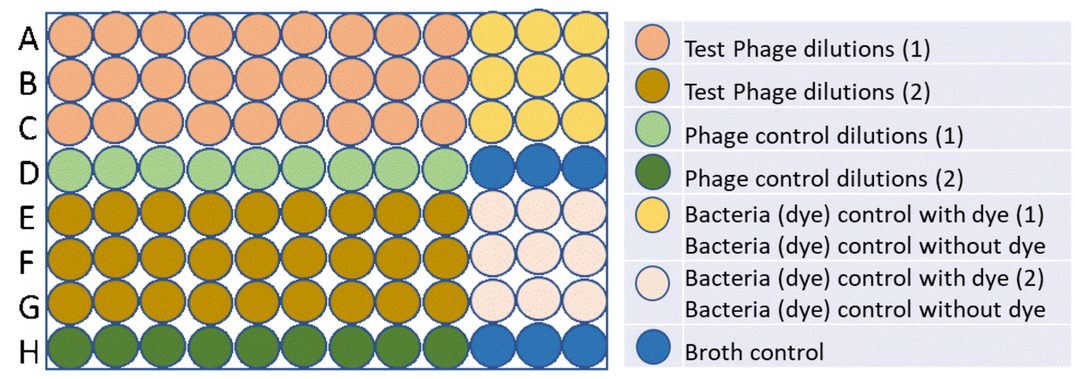
INTRODUCTIONS & OBJECTIVES.

Prevention of rapidly spreading of antibiotic-defeating germs is major challenge today. Using of phages as the adjuvants to antimicrobials to address the threat of antibiotic resistance (AR) is very promising. But developing of simple and sensitive methods for phage activity evaluation in a quick manner is limited, because of phages are "living organisms" with high specificity to bacteria. The optimal management of phage activity evaluation is highly important to prevent infections in the way to improve effectiveness of antibiotic-phage synergistic treatment and slow down the resistance development to both bacteria and phage.

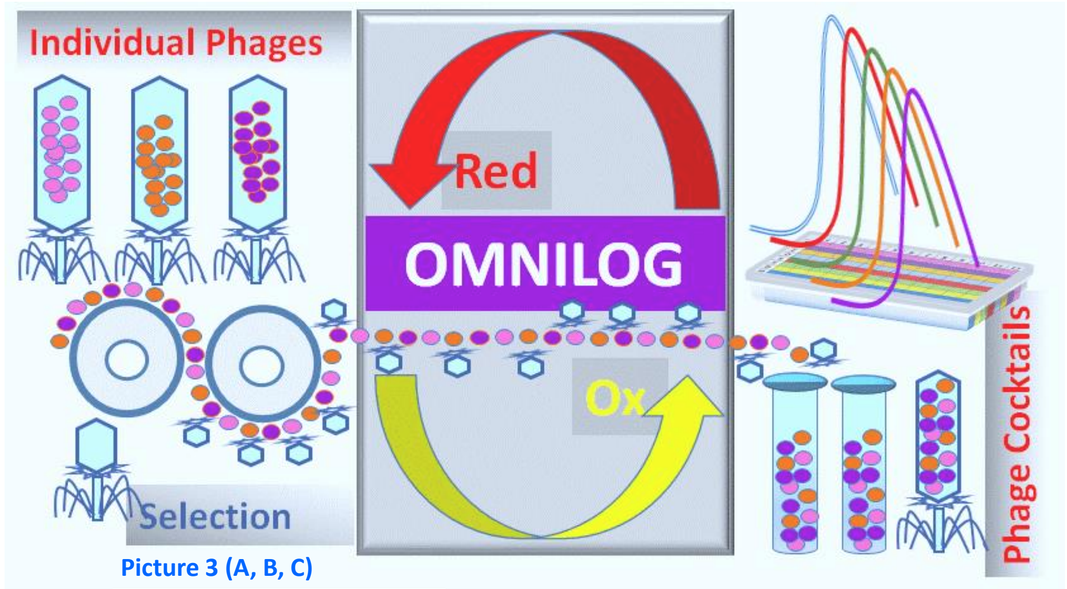
METHODS & WORKFLOW.

Newly isolated phages of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* from different natural and industrial sewage environments (picture 4) and with sequenced genome (picture 5) were applied to study comparison of results produced from the methods of MD/SP (Multiple Dilutions on Single Plates) (picture 3B) , PLC (Phage Liquid Culturing) (picture 1) and BTR (Bacterial Tetrazolium Reduction). OmniLog™ system of redox chemistry to automatically measure cell respiration (picture 2 , a marker for bacterial growth.

Picture 1. PLC plate outline for OMNILOG



Picture 2. OMNILOG System

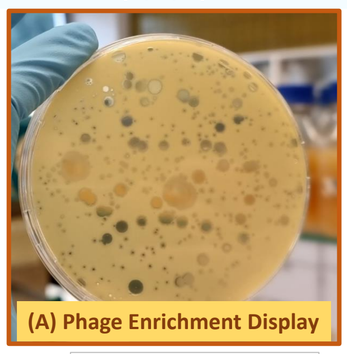


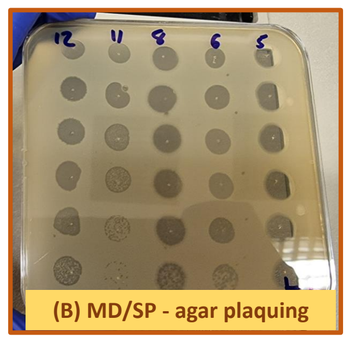
The phage lytic activity was tested against a large bacterial matrix (103 *P. aeruginosa* and 172 *K. pneumoniae strains*) (Graphs 7-8). Efficiency of plating (EOP) (Graphs 1-6) was determined using both the agar plaquing (Picture 3B) and kinetic clearing methods and the results were correlated in parallel with TR and colony-forming reduction (CFR) (Picture 3C) in a multi-well-spot format.

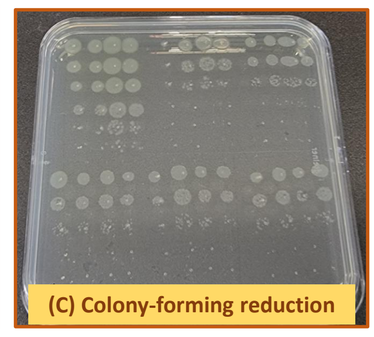
RESULTS.

Based on EOP results, the host coverage of the selected phages of both *P. aeruginosa* and *K. pneumoniae* was about 64-87% (Graphs 1-6) . The given approach of phage lytic activity evaluation included pfu/ml (picture 3B) and cfu/ml enumerations (Picture 3C) with correlation-adjustment to TR (Graphs 7-8 and 9-10) for a given timepoint.

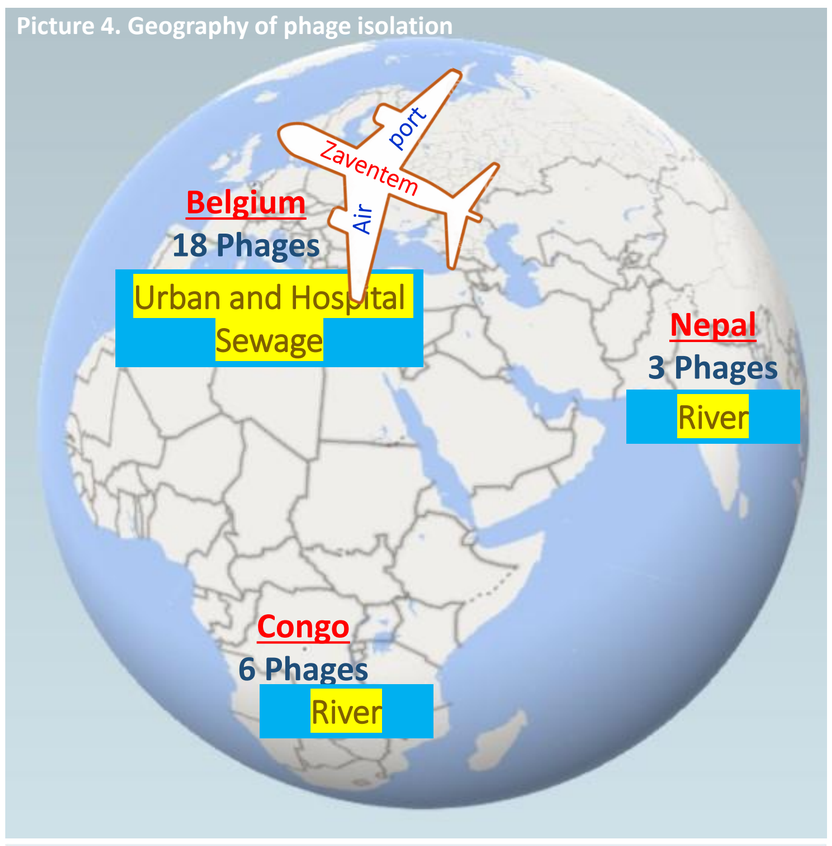
Picture 3 (A, B, C)


(A) Phage Enrichment Display

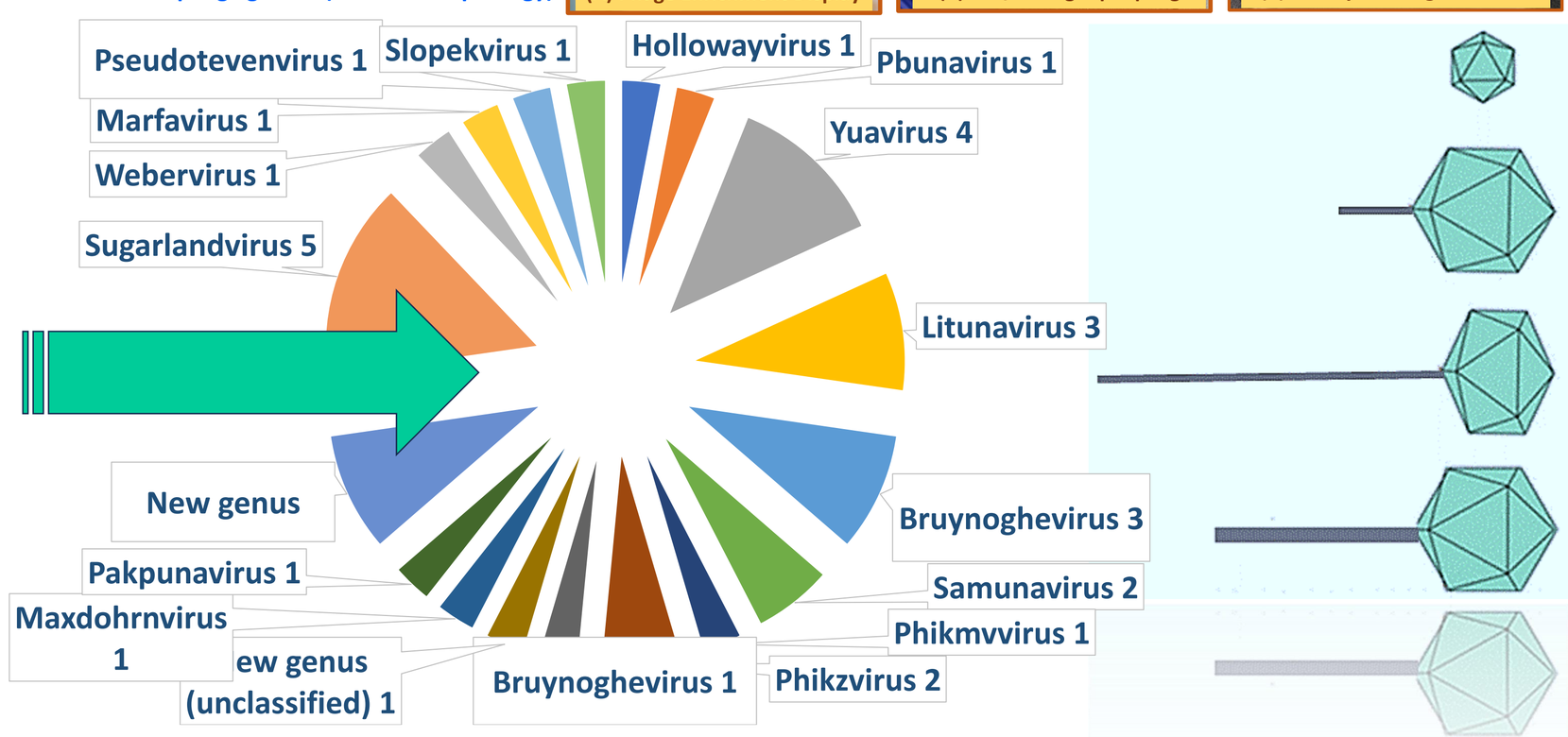

(B) MD/SP - agar plaquing


(C) Colony-forming reduction

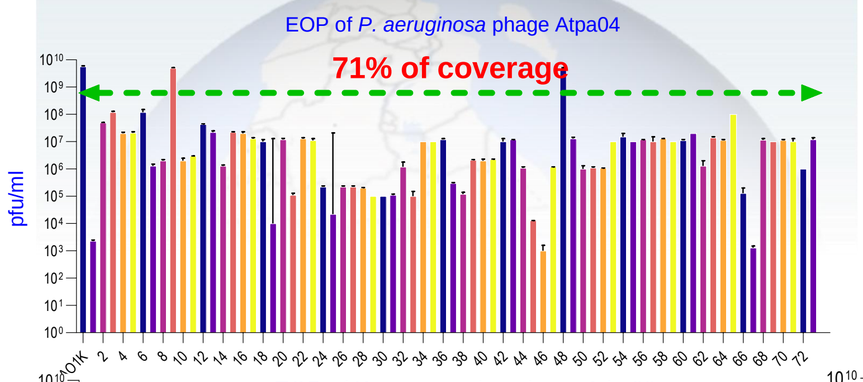
Picture 4. Geography of phage isolation



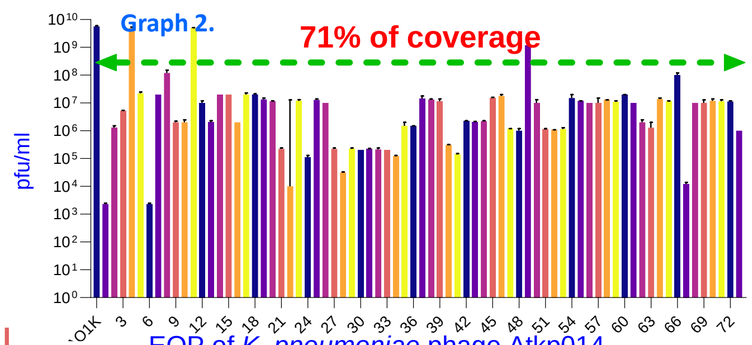
Picture 5. New phage genera (Particle Morphology)



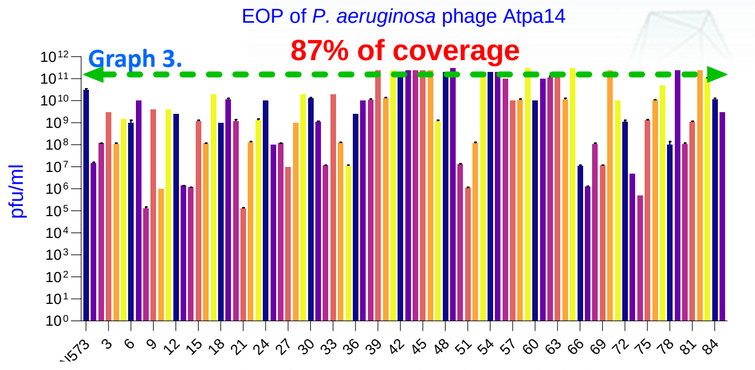
Graph 1.



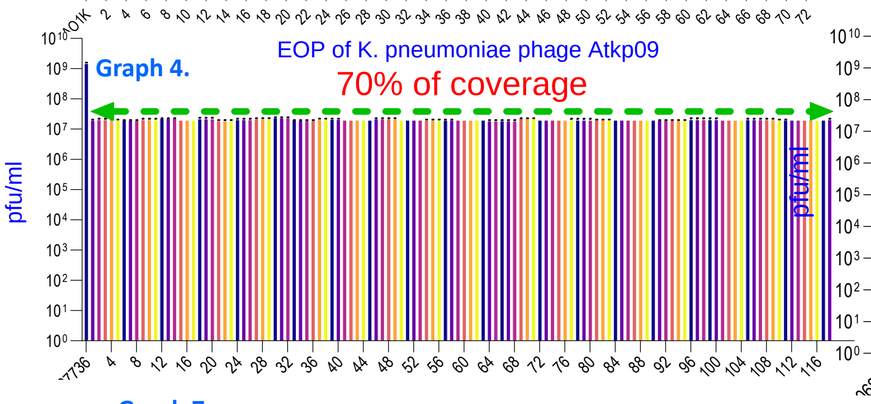
Graph 2.



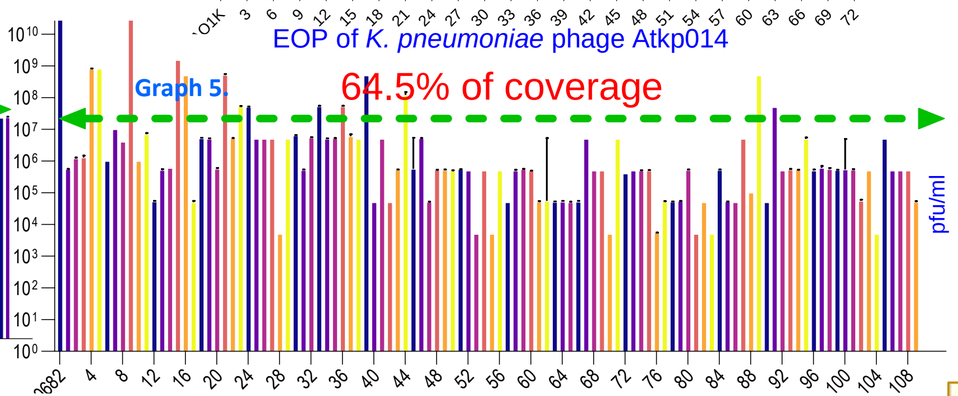
Graph 3.



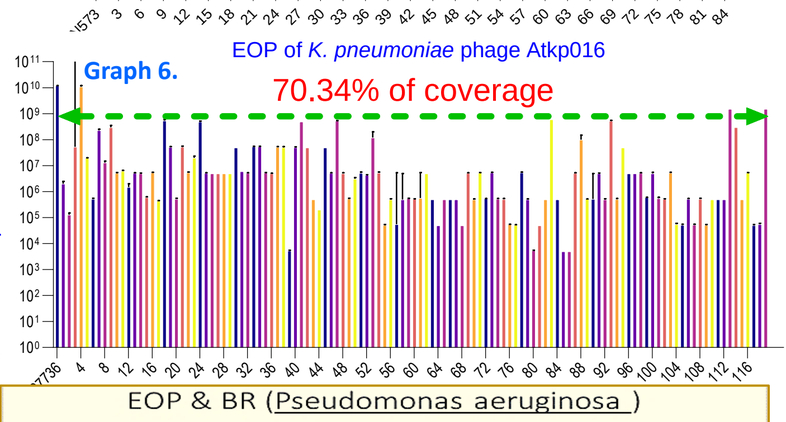
Graph 4.



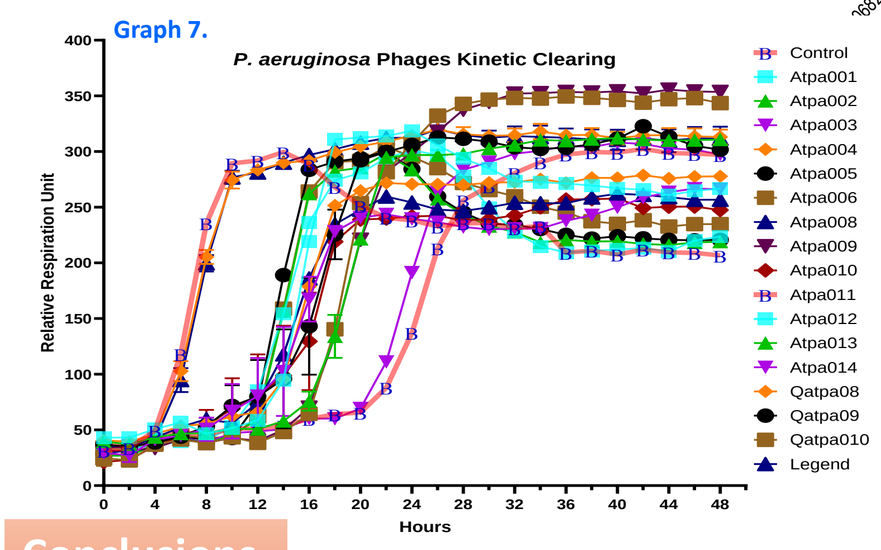
Graph 5.



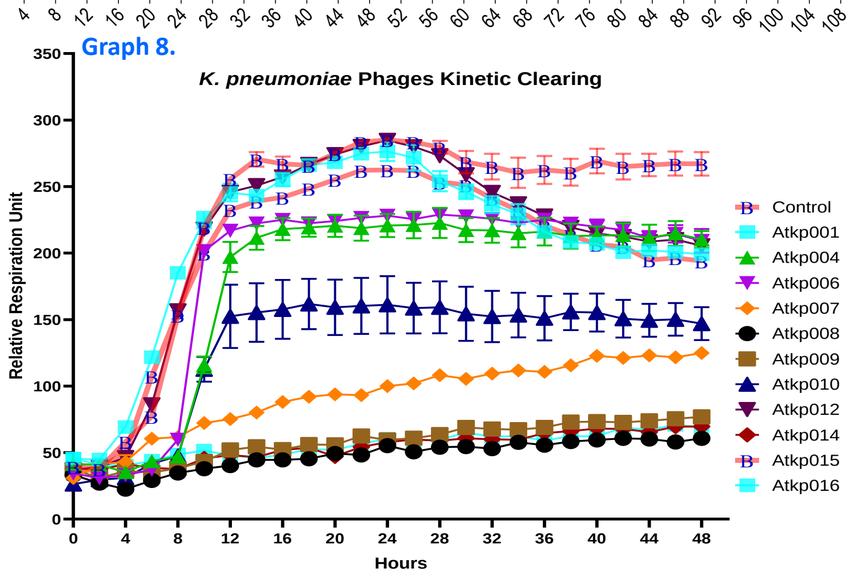
Graph 6.



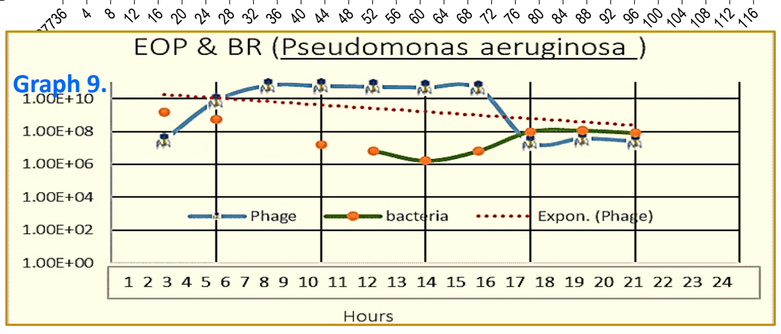
Graph 7.



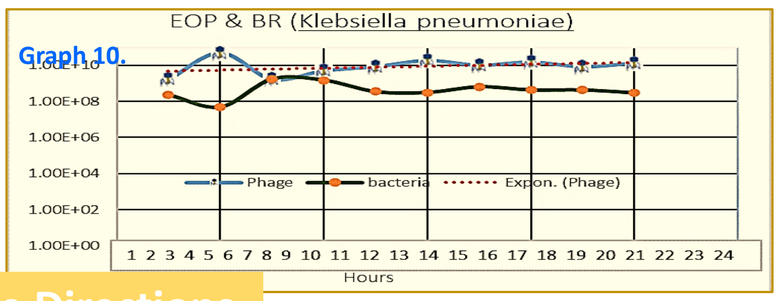
Graph 8.



Graph 9.



Graph 10.



Conclusions.

- Six phages of *P. aeruginosa* (Atpa004, Atpa005 and Atpa014) and *K. pneumoniae* (Atkp009, Atkp014 and Atkp016) revealed coverage of about 64-87% for host range (Graphs 1-6).
- EOP of Most of phages was about two logs less on the test bacterial strains than on the propagating strains.
- Based on results obtained from Appelmans PLC corelating with plaquing and BR ones, were Identified effective phage/bacteria ratios for each test phage (Graphs 7-8).
- Phage lytic activity evaluation including pfu/ml and cfu/ml enumerations with correlation-adjustment to TR (Graphs 9-10) for a given timepoint, allowed evaluation of phage mutant formation.

Future Directions.

This described approach here, will facilitate further development of phage lytic activity and emerging phage-resistance mutants' determination, and selection of candidate therapeutic phages.

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