#203 Development of Bacteriophage Cocktails with Expanded Host Range against MDR *Pseudomonas aeruginosa*

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Abstract

Background: We found that co-coating catheters with a Pseudomonas (Psa)-specific bacteriophage and a biofilm of benign E.coli HU2117 synergistically prevented Psabiofilm formation on urinary catheters. We are working on the development of broad host-range Psa-specific phage cocktails to use in this application.

Methods: Co-incubation of a mixture of 4 Psa-specific bacteriophage on 16 drug-resistant Psa isolates from human urine was performed in cycles, with the supernatant (phage cocktail) from each cycle being used to inoculate the 16 Psa isolates in the subsequent cycle. Lytic assays on the original 16 strains (development strains) were performed using the phage cocktail from cycles 0, 5, 10, 15, and 20 to measure expansion of the phage host range. These cocktails were also tested on 10 fresh Psa clinical isolates that had not been used in cocktail development (test strains). 10 plaques (subclones) isolated from each of the cycle 5 and 20 cocktails were subjected to host range and restriction analyses.

Results: Initially, 7 of the Psa development strains were sensitive to the 4 phages, and 9 were resistant (44% sensitive). By cycle 5, 12 of the Psa isolates were sensitive to the phage cocktail (75%), 4 were resistant; % sensitivity did not increase with additional cycles. On the 10 test strains, the initial combination of 4 phages lysed only a single Psa isolate (10%), while 9 Psa were resistant. Susceptibility to the cocktail increased with cycle number until at cycle 20, 6/10 (60%) of the test Psa strains were sensitive to the cocktail. Restriction analysis of individual phage subclones isolated from cocktails did not detect chimeric patterns indicative of recombination.

Conclusions: The spectrum of Psa killing was expanded for both the development strains and the test strains after several cycles of co-incubation. We did not detect genetic recombination in individual phage from the phage cocktails, suggesting that host range expansion occurs by mutation. Alternatively, recombinants and/or mutants that contribute to the expanded host range may be minor components of the cocktail. The phage cocktail method can potentially be applied to other multi-drug resistant uropathogens.

Introduction

We have shown that a pre-existing catheter biofilm of benign *Escherichia coli* HU2117 plus a single anti-pseudomonal phage interfered with catheter colonization by *P. aeruginosa* in vitro.

However, we observed emergence of phage-resistant *P. aeruginosa* variants to this single phage. Thus, we aimed to develop a phage cocktail with broad coverage through cycles of co-incubation of several phage with multiple strains of *P. aeruginosa*.

Methods

Co-Incubation protocol: 96-well plate assay

P. aeruginosa strain	Serial dilutions of phage cocktail										
	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	Control (no phage)
PAO1	○	○	○	○	○	○	○	○	○	●	●
Psa4	○	○	○	○	○	○	○	○	○	●	●
VA1	○	○	○	○	○	○	●	●	●	●	●
VA2	○	○	○	○	○	○	○	●	●	●	●
VA3	○	○	○	○	○	○	○	●	●	●	●
VA4	○	○	○	○	○	○	○	○	●	●	●
VA5	○	○	○	○	○	○	○	○	○	●	●
VA6	○	○	○	○	○	○	●	●	●	●	●
VA7	○	○	○	●	●	●	●	●	●	●	●
VA8	●	●	●	●	●	●	●	●	●	●	●

Results

I. Increased lysis observed in 96-well plates

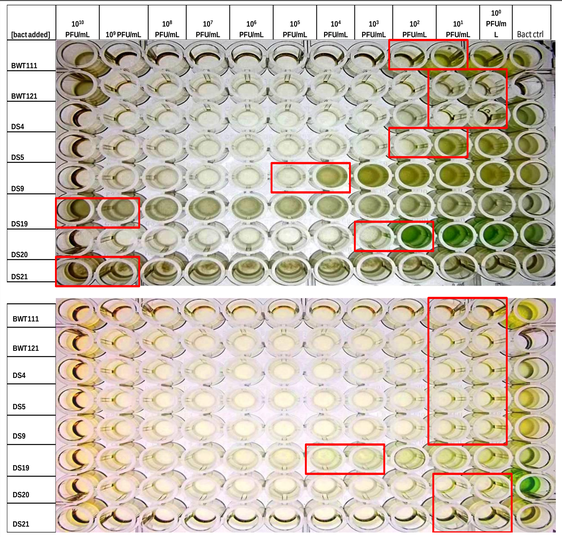


Figure 1: Comparison of a 96-well plate assay of 8 *P. aeruginosa*isolates using round 1 phage cocktail and round 20 phage cocktail. Wells marked by red boxes were harvested and purified to produce the phage cocktail used to inoculate the following round. A shift of the red boxes to the right in round 20 indicates increased lytic ability of phage lysate compared to that in round 1 (lysis at lower phage concentrations).

II. Increased lytic ability confirmed via spot testing

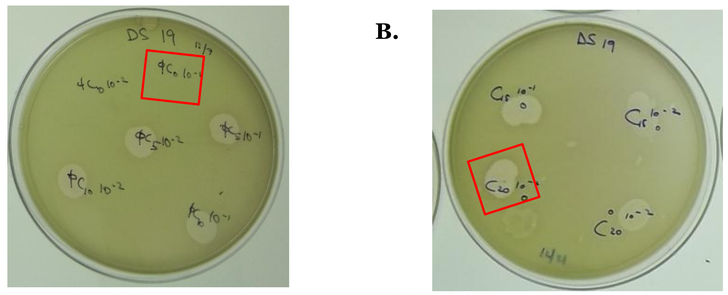
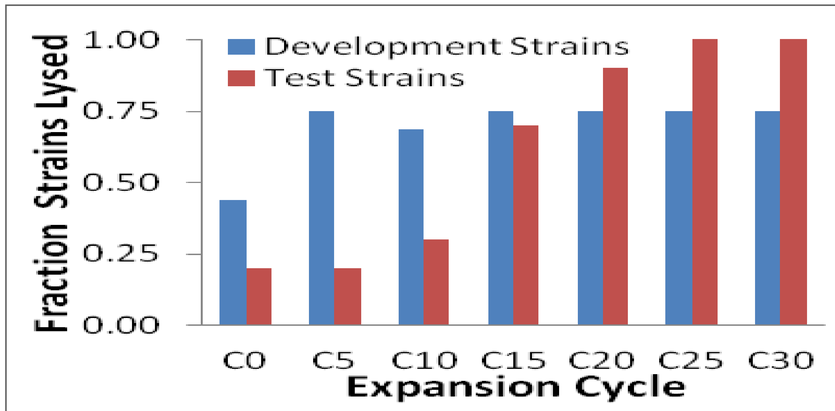


Figure 2: Visual demonstration of increased lytic ability of phage cocktail from rounds 15 and 20 (**B**) compared to the initial phage cocktail rounds 0, 5, and 10 (**A**) against one of the input host strains (*P. aeruginosa*DS19). Initial phage cocktail (ΦC0) did not lyse bacterial lawn (red box in **A**), while phage lysate harvested after 20 rounds (ΦC20) lysed the bacterial lawn (red box in **B**).

III. Percent P. aeruginosa *strains lysed by phage cocktail following repeated co-incubation cycles*



Results, continued

IV. Spectrum of bacterial lysis

Table 1: Lysis of *Psa* strains by parental phages, parental cocktails, and clones from parental cocktails.

Phage*	*Psa* Development Strains																*Psa* Test Strains										
	BWT121	BWT111	DS4	DS5	DS9	DS19	DS20	DS21	DS22	DS23	DS24	DS25	DS27	DS28	DS30	DS38	% Lysed	DS18	DS26	DS29	DS31	DS32	DS33	DS34	DS35	DS36	DS37	% Lysed
ϕKMV parent	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	12	-	-	-	-	+	-	-	-	-	-	10
ϕPA2 parent	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	31	-	-	-	-	-	-	-	-	-	-	0
ϕPaer4 parent	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	12	-	-	-	-	+	-	-	-	-	-	10
ϕE2005parent	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	12	-	-	-	-	+	-	-	-	-	-	10
C^{0}-Cocktail	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	+	44	-	-	+	-	+	-	-	-	-	-	20
C^{5}-Cocktail	+	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+	75	-	-	-	-	+	-	-	-	-	+	20
C^{10}-Cocktail	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	69	-	-	+	-	+	-	-	-	-	+	30
C^{20}-Cocktail	+	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+	75	+	+	+	-	+	+	-	+	+	+	80
C^{20}-Clone 1	+	-	+	+	-	-	-	-	+	-	-	-	-	-	+	-	31	-	-	-	-	-	+	-	-	-	-	10
C^{20}-Clone 2	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	19	-	-	-	-	+	+	-	-	-	-	20
C^{20}-Clone 3	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	+	44	-	-	-	-	+	-	-	-	-	-	10
C^{20}-Clone 4	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	+	44	-	-	-	-	+	-	-	-	-	-	10
C^{20}-Clone 5	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	+	44	-	-	-	-	+	+	-	-	-	-	20
C^{20}-Clone 6	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	+	44	-	-	-	-	+	-	-	-	-	-	10
C^{20}-Clone 7	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	+	44	+	-	-	-	+	-	-	-	-	-	20
C^{20}-Clone 8	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	38	-	-	-	-	-	-	-	-	-	-	0
C^{20}-Clone 9	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	19	-	-	-	-	-	-	-	-	-	-	0
C^{20}-Clone 10	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	+	44	-	-	-	-	-	-	-	-	-	-	0
Reconstit. C^{20}	+	+	+	+	-	-	+	-	+	-	-	-	-	-	+	+	50	+	-	-	-	+	+	-	-	-	-	30
C^{30}-Cocktail	+	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+	75	+	+	+	+	+	+	+	+	+	+	100

* The host range determined by spot tests for the parental phages, the uncloned cocktails at cycles 10 (C^{10}), 20 (C^{20}) and 30 (C^{30}), of 10 clones derived by plaque isolation of the C^{20} cocktail, and a mix of clones 1-10 (Reconst. C^{20}).

Table 1: Lytic activities of phage cocktails 0, 5, 10, 15, 20,30. ("-" indicates minimal or no lysis and "+" indicates substantial clearance). (**A**) Host range of the phage cocktail expanded from 44% of the input *P. aeruginosa* strains in round 0 to 75% in round 30. (**B**) When testing the phage cocktails against 10 novel clinical *P. aeruginosa* strains not used in cocktail development, the lytic spectrum broadened from 20% to 100%.

Summary

30 rounds of the co-incubation protocol led to a phage cocktail with broadened lytic spectrum.

- Development *P.aeruginosa* strains: 44% to 75%
- Test (novel) strains of *P. aeruginosa:* 20% to 100%

Next we will investigate whether host range expansion occurs through mutation, recombination, or both.

Acknowledgements

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