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Diversified Therapeutic Phage Cocktails from Close Relatives of the Target Bacterium

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ABSTRACT

This project tackles the antibiotic resistance crisis, developing a new method for discovering numerous efficacious bacteriophages for therapeutic cocktails against bacterial pathogens. The phage therapy approach to infectious disease, recently rekindled in U.S. medicine, requires numerous phages for each bacterial pathogen. Our approach 1) uses Sandia-unique software to identify dormant phages (prophages) integrated into bacterial chromosomes, 2) identifies prophage-laden bacteria that are close relatives of the target pathogenic strain to be killed, and 3) engineers away properties of these phages that are undesirable for therapy. We have perfected our phage-finding software, implemented our phage therapy strategy by targeting the pathogen *Pseudomonas aeruginosa*, and prepared new software to assist the phage engineering. We then turned toward *Burkholderia* pathogens, aiming to overcome the difficulty to transform these bacteria with a novel phage conjugation approach. Our work demonstrates the validity of a new approach to phage therapy for killing antibiotic resistant pathogens.

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ACRONYMS AND DEFINITIONS

Abbreviation	Definition

1. SOFTWARE FOR DETECTING PROPHAGES INTEGRATED WITHIN BACTERIAL CHROMOSOMES

Please see our recent publication describing the combined use of our Islander and TIGER software for finding and precisely mapping prophages: [1] Mageeney CM, Lau BY, Wagner JM, Hudson CM, Schoeniger JS, Krishnakumar R, Williams KP. 2020. New candidates for regulated gene integrity revealed through precise mapping of integrative genetic elements. Nucleic Acids Res. doi: 10.1093/nar/gkaa156.

2. NEW APPROACH TO PHAGE THERAPY

Please see our recent publication describing our new method for developing phage cocktails against virtually any bacterial pathogen: [2] Mageeney CM, Sinha A, Mosesso RA, Medlin DL, Lau BY, Rokes AB, Lane TW, Branda SS, Williams KP. 2020. Computational basis for on-demand production of diversified therapeutic phage cocktails. mSystems 5:e00659-20.

3. PHAGE THERAPY FOR A SECOND GROUP OF PATHOGENS, THE *BURKHOLDERIA CEPACIA* COMPLEX

Having demonstrated our approach in Pseudomonas (section 2), we turned to another group of pathogens, the *Burkholderia cepacia* complex. This target group was recommended to us by our collaborator Robert Schooley, (Professor of Medicine in the Division of Infectious Diseases, Vice Chair of the Department of Medicine, Co-Director of the International Core of the Center for AIDS Research at UCSD and Senior Director for International Affairs for UCSD). Dr. Schooley is a seminal figure in the reintroduction of phage therapy to U.S. medicine, and identified these bacteria as a group in need of new phages for therapy, in cystic fibrosis patients and others. This target also fit with the experience of team member Steve Branda in studying *Burkholderia*.

We identified phages from numerous *Burkholderia* strains and tested these phages for activity on the same set of strains (Fig. 3-1). Attempting to engineer these phages as we had those for *Pseudomonas* (section 2), we encountered difficulty transforming *Burkholderia*. We have devised a new approach to deliver the engineered phage into Burkholderia, that we call phage conjugation (Fig. 3-2). We will use the E. coli strain MFDpir [3] as a conjugation donor. Currently we have recently obtained MFDpir, prepared repressor plasmid for a particular Burkholderia phage (40R) identified in Fig. 3-1, and prepared the phage-cassette construct. When our lab shutdown is over, we will be able to test this conjugation system.

								Susceptibility to lysate from strain ID:																				
ID	Species	Strain	Isolation	Source	GIs	Phages	OTUs	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	cenocepacia	LMG 16656	CF patient	BEI	11	2	1, 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	cenocepacia	LMG K56-2	CF patient	BEI	9	1	3	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
3	cepacia	UCB 717	Soil	BEI	11	3	1, 2, 8	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
4	cepacia	MSMB1829	Soil	D. Wagner	10	4	1, 2, 9, 13	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-
5	cepacia	MSMB1824	Soil	D. Wagner	6	4	2, 3, 5, 19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	cepacia	MSMB1061	Onion	D. Wagner	9	5	5, 12, 16, 17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	cepacia	MSMB1063	Soil	D. Wagner	9	6	2, 3 ,6, 7, 30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
8	cepacia	MSMB2211	Soil	D. Wagner	7	3	10, 25, 38	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
9	cepacia	MSMB648	Soil	D. Wagner	7	5	1, 10, 33, 39	-	-	-	+	+	+	+	+	-	+	-	-	-	-	-	-	-	+	+	-	+
10	cepacia	MSMB1533	Soil	D. Wagner	12	3	1, 18, 40	-	-	-	+	-	+	-	+	-	-	-	-	-	-	+	-	-	+	+	+	-
11	cepacia	INT3-BP177	Soil	D. Wagner	4	1	34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	multivorans	249	Soil	ATCC	11	3	1, 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	mutivorans	LMG 13010	CF patient	ATCC	7	3	2, 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	pyrrocinia	LMG 14191	Soil	ATCC	0	0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	stabilis	LMG 14294	CF patient	ATCC	6	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	vietnamiensis	LMG 10929	Rice field soil	BEI	9	2	5, 28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	Bcc?	UCSD_1	CF patient	R. Schooley	?	?	N.A.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	thailandensis	E264	Rice field soil	LLNL	8	2	N.A.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
19	thailandensis	E421	Rice field soil	LLNL	8	3	N.A.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
20	thailandensis	E426	Rice field soil	LLNL	5	1	N.A.	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	thailandensis	DW503	E264 mutant	LLNL	7	1	N.A.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-

Figure 3-1. Phages from *Burkholderia* strains, tested on all *Burkholderia* strains

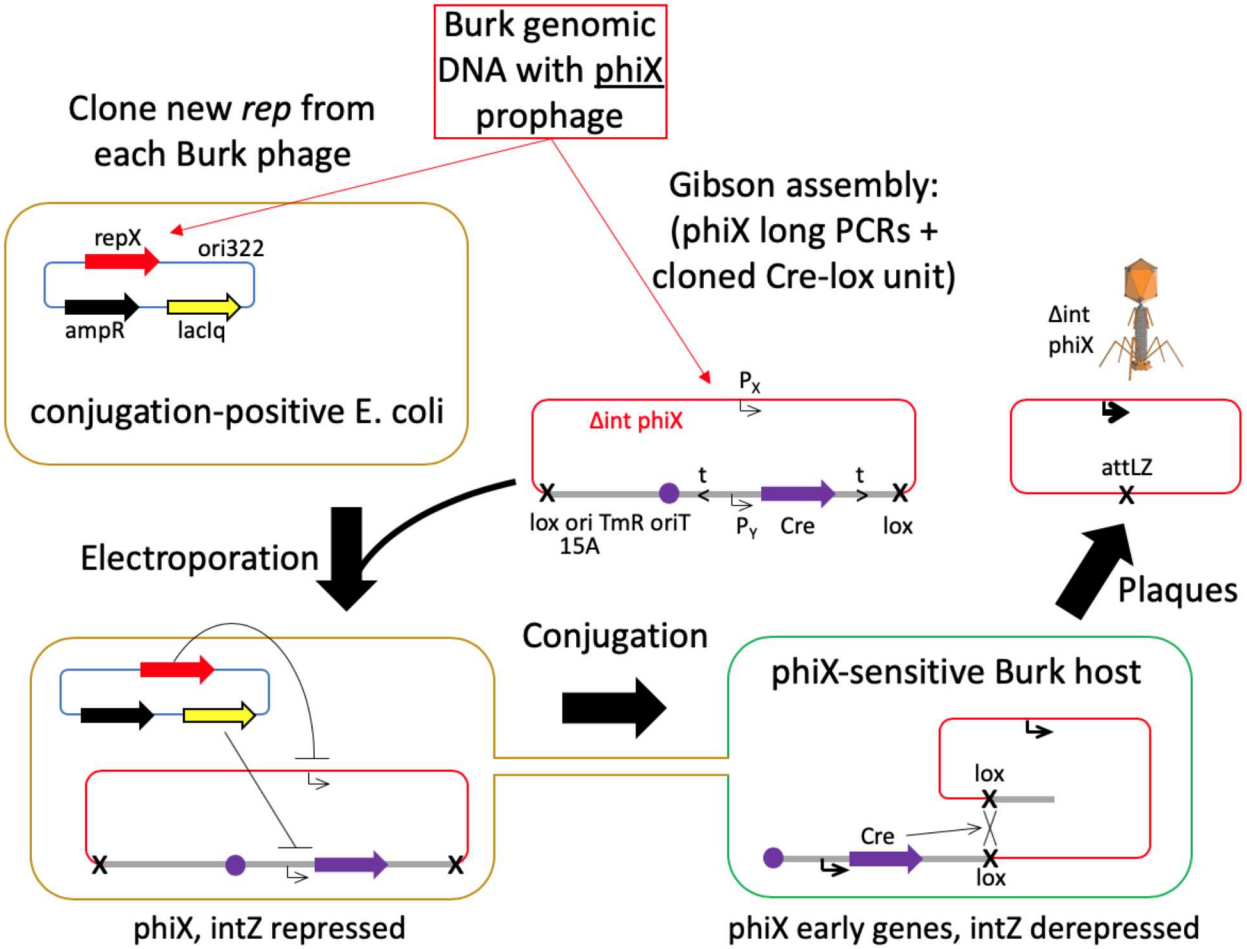

Figure 3-1. Scheme for conjugating phages into *Burkholderia*. To protect the *E. coli* conjugation donor from killing by each phage (phiX) to be transferred, the phiX repressor will be cloned into a repressor plasmid. The body of the engineered phage genome will be constructed with a removeable cassette contained an origin-of-transfer so that the phage can be transferred into a Burkholderia recipient strain by conjugation. Due to the lack of relevant repressors in the cytoplasm of the recipient, the Cre gene in the cassette will act on the cassette-flanking *lox* sites, to remove the entire cassette and leave only the phage genome; moreover the unrepressed phage will grow on the new host for isolation.

REFERENCES

[1] Mageeney CM, Lau BY, Wagner JM, Hudson CM, Schoeniger JS, Krishnakumar R, Williams KP. 2020. New candidates for regulated gene integrity revealed through precise mapping of integrative genetic elements. Nucleic Acids Res. doi: 10.1093/nar/gkaa156.

[2] Mageeney CM, Sinha A, Mosesso RA, Medlin DL, Lau BY, Rokes AB, Lane TW, Branda SS, Williams KP. 2020. Computational basis for on-demand production of diversified therapeutic phage cocktails. mSystems 5:e00659-20.

[3] Ferrières L, Hémery G, Nham T, Guérout A-M, Mazel D, Beloin C, Ghigo J-M. 2010. Silent mischief: Bacteriophage Mu insertions contaminate products of Escherichia coli random mutagenesis performed using suicidal transposon delivery plasmids mobilized by broad-host-range RP4 conjugative machinery. J Bacteriol. 192: 6418–6427.

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