TARGETING PHAGE THERAPY 2025

8th World Congress on Targeting Phage Therapy 2025

June 10-11, 2025 - Berlin, Germany

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2	BOURGINE, Baptiste	Development of a digital phagogram platform for accelerated matching of bacteriophages to multidrug resistant pathogens
3	CAN KURT, Kübra	Characterization and genomic analysis of a new bacteriophage klebsiella pneumoniae ctf-1
4	CASTILLO, Darwin Alexander	From data to the bench: identifying and testing phage therapy candidates to combat biofouling in seawater desalination plants
5	CHICHASHVILI, Maria	Characterisation of custom phages in a multidrug-resistant pseudomonas aeruginosa urinary tract infection case
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10	FUGLÍK, Vítězslav	Isolation of novel bacteriophages targeting clinical strains of klebsiella pneumoniae and acinetobacter baumannii and the development of phage-based therapeutic TRIOFAG®
11	GÓMEZ-CANO, Ignacio Samuel	Isolation and characterization of two lytic phages targeting salmonella enterica from european poultry environments
12	KASPRZAK, Hubert Oskar	Analysis of the profiles of pro- and anti-inflammatory response in patients subjected to phage therapy
13	KNEFEL, Miriam	Characteristics of selected environmental bacteriophage vb_sen-miriam1 infecting salmonella enterica serovars
14	KOROTANIA, Naina	Development of phage therapy to treat ash canker.
15	KOŠIARČIKOVÁ, Simona	Phage viability in cosmetic ingredients
16	LETHIN, Harper	More-than-human-kinships with phages: an artistic and microbiological approach
17	LIEW, Jun Hao	Addressing phage resistance: designing effective phage cocktails against multi-drug resistant mycobacterium abscessus
18	MARTINS, Filipa	The dsmz phage bank: a resource for phage research and therapy
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32	*LAUREANO REVILLA, Raúl Eduardo*	Microbiological, genomic, and structural mapping of two bacteriophages specific to salmonella enterica serovar typhimurium

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	SALAZAR-OSPINA, Lorena	High-specificity phage cocktails against the highly successful and disseminated s. Aureus clonal complex 8 (mrsa/mssa)
	KIZHEVA, Yoana Krasimirova	Characterization of escherichia coli bacteriophages isolated from wastewater in bulgaria

8th WORLD CONGRESS Targeting PHAGE THERAPY 2025

High-specificity phage cocktails against the highly successful and disseminated *S. aureus* Clonal Complex 8 (MRSA/MSSA)

UNIVERSIDAD DE ANTIOQUIA

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ABSTRACT

Staphylococcus aureus causes a wide variety of clinical manifestations, because it has a high number of virulence factors and resistant capacity. In particular, MRSA isolates belonging to the predominant clones CC8 and CC5 are great epidemiological importance in the community and the hospital settings (1). Furthermore, *S. aureus* is well known for the formation of biofilm, which has been linked to chronic infections (2). Bacteriophages are an alternative for the control of sensitive and resistant bacteria due their specificity and antibiofilm activity (3). This work describes the isolation, characterization, and performance of bacteriophages in combination, against *S. aureus* isolates (CC8) and biofilm.

METHODOLOGY

Study design: Experimental.

Host bacteria for the isolation of bacteriophages

Strain	Characteristic	Resistance gene	CC	Spa type	No. of strains
S. aureus (n=7)	Methicillin-Resistant	*mec*A	8	t024, t1610, t008	3
		*mec*A	5	t149	1
	Methicillin-susceptible	NA	8	t1610, t008	2
		NA	5	t002	1

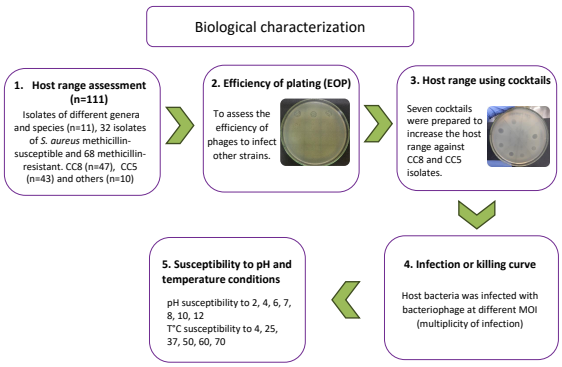
Sample collection

Medellín, Antioquia, Colombia

Wastewater from a tertiary hospital

Human nasal swab

Mouth and back swab of pets



RESULTS

Host range assessment

Inter-species and inter-genera evaluation: Five phages were isolated and all them showed a gender specificity of 100%

Intra-species evaluation: Bacteriophage activity ranged between 10 and 33%. These percentages of activity do not differ in sensitive (between 6,25% to 31,25%) and resistant (between 11,76% to 33,82%) isolates.

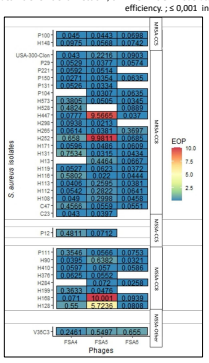
Figure 1. Intraspecies host range using individual bacteriophages and cocktails

Figure 1A shows the results of the intraspecies host range of individual bacteriophages. FSA1 and FSA2 were obtained from MRSA-CC8-t1610 isolate, phages FSA4 and FSA5 were obtained from MRSA-CC8-t024 and FSA6 was obtained using MSSA-CC5-t002. Figure 1B shows the results of the intraspecies host range of the evaluated cocktails. Eight cocktails were prepared. Cocktails 1, 2, 3, 5, 6 and 8 were preparate to increase host range against strains belonging to CC8 and CC5. Cocktails 4 and 7 were prepared to increase the host range against strains belonging to CC8. Cocktail 1: phages FSA4, FSA5 and FSA6; Cocktail 2: phages FSA2,FSA4 and FSA6; Cocktail 3: phages FSA2,FSA5 and FSA6; Cocktail 4: phages FSA2, FSA4 and FSA5; Cocktail 5: phages FSA2, FSA4, FSA5, and FSA6; Cocktail 6: phages FSA5, FSA6; Cocktail 7: phages FSA2 and FSA5; Cocktail 8: FSA2 and FSA6

Phages were more active against *S. aureus* (MSSA - MRSA) strains belonging to CC8 up to 61,7%
The evaluation of the cocktails show activity between 22% to 38% of the strains .
Cocktails increased the formation of completely translucent zones in up to 72.7% of the susceptible strains, compared to the activity observed with individual phages, which presented slightly opaque zones.

Figure 2. Efficiency of plating (EOP) of *S. aureus* phages.

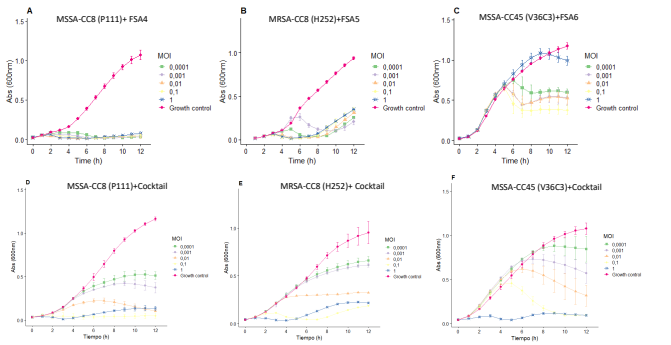
values ≥ 0.5: efficient infection ; 0.1 ≤ EOP < 0.5: moderately efficient infection ; 0.001 ≤ EOP < 0.1: infection low efficiency. ; ≤ 0,001 inefficient infection



The infection efficiency of the bacteriophages that made up the cocktail was low (< 0.1).
A high infection efficiency occurs between 3.7% and 18.1% of the isolates.

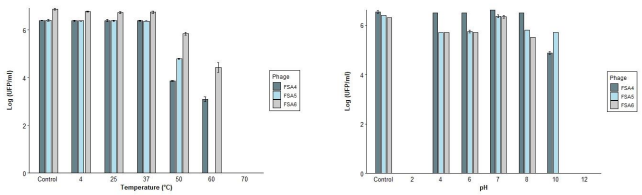
Figure 3. Infection or killing curve

The infection curves show the capacity of the individual bacteriophage (A, B and C) and cocktail 1, the one with the best performance, (D, E and F) to control bacterial growth for 12 hours.



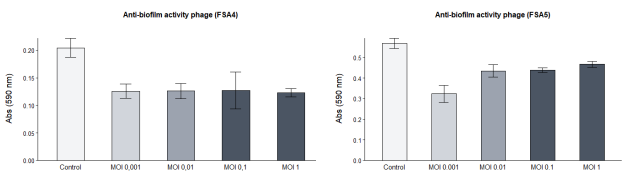
Phage FSA4 controlled bacterial growth (MRSA-CC8) for 12 hours with all MOIs evaluated (A); however, when the bacteriophage cocktail was used, only MOIs of 1, 0.1 and 0.01 were effective (D)
On the other hand, the cocktail performed better at MOIs of 1 and 0.1 (E) to avoid the observed regrowth (MRSA-CC8) to 8 hours when the individual bacteriophage was used (FSA5)(B). Finally, the cocktail performed better at 12 hours at MOIs of 1 and 0.1 (MSSA-CC5)(F) compared to the performance of the individual phage (FSA6)(C)

Figure 4. susceptibility to pH and temperature conditions



Bacteriophages were stable at temperatures between 4 and 37°C and at pH between 4 and 10

Figure 5. Anti-biofilm activity



Antibiofilm assays have preliminarily demonstrated that FSA4 and FSA5 bacteriophages decrease biomass formation in biofilms after 12 hours of treatment using low MOIs of 0.001.

CONCLUSIONS

These results show the isolation of highly specific bacteriophages against *S. aureus* belonging to CC8. Furthermore, the use of cocktails enhances the action, eliminating bacterial growth more efficiently in some strains. In addition, bacteriophages have antibiofilm activity.

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CHARACTERIZATION OF *ESCHERICHIA COLI* BACTERIOPHAGES ISOLATED FROM WASTEWATER IN BULGARIA.

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Introduction: Phage research in Bulgaria, in terms of phage therapy, is in its early stage but has been grown rapidly in recent years. Thus, the aim of this study is the isolation and characterization of phages infecting *Escherichia coli* in Bulgaria.

Materials and Methods: Wastewater was use as source for phages isolation. Seven *E. coli* strains were used as initial hosts. Phages isolation, host range determination (HRD) and plaques characterization were done via double agar overlay assay. Twenty-one bacterial strains (*E. coli* and related species) were used as hosts for HRD. The virion morphology of phage SfEc8432/3 was established via TEM.

Results: The initial isolation of the phages was done using wastewater sample obtained from WWTF (Waste water treatment facility) near Sofia, Bulgaria. The sample was collected at the entrance of the WWTF (**Fig. 1**). A total of eight bacteriophages were isolated and designed as shown in **Fig. 2**, **Table 1**, respectively.

Fig. 1 Wastewater at the entrance of WWTF near Sofia, Bulgaria

Table 1 Newly isolated bacteriophages.

Target bacteria	Phage isolate	Plaque dimensions, d, mm	Plaque type
E. coli	SfEc8432/1	3.23/8.81	turbid, halo
	SfEc8432/2	3.85	turbid
	SfEc8432/3	1.95	clear
	SfEcC2987/1	4.29	turbid
	SfEcC2987/2	2.56	clear
	SfEcC2987/3	1.79	turbid
	SfEcTs6/1	0.78	turbid
	SfEc2/1	2.74	turbid

Fig. 2 *E. coli* strains used as initial host for phage isolation. Arrows indicates different plaques formed.

Results: The morphology of plaques varied from clear to turbid (d= 0.78 to 4.98 mm). Clear plaques were observed for two phage isolates - SfEc8432/3 and SfEcC2987/2 (**Fig. 3**).

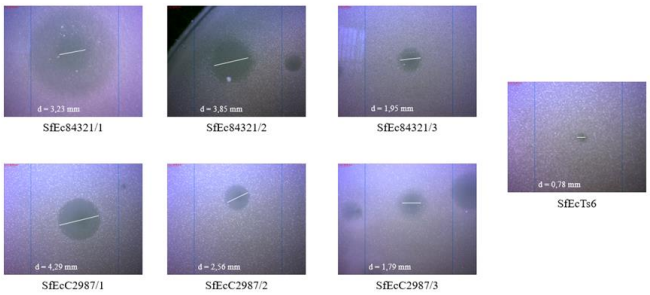

Fig. 3 Morphology of the plaques of the newly isolated phages.

Results: The phages demonstrated diverse but narrow host range i.e. capable to infect different but only *E. coli* strains (**Table 2**). The TEM micrographs revealed virion with capsid of approximately 50 nm in diameter; probably contractile tail - over 50 nm long and total length of the phage - about 100 nm (**Fig. 4**).

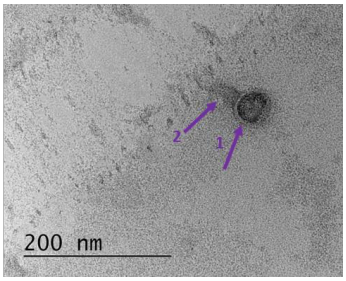

Fig. 4 TEM image of bacteriophage SfEc8432/3. Arrow 1 – capsid; Arrow 2 – tail.

Table 2 Host range of the newly isolated phages.

Phage isolate / Bacterial strains	*SfEc8432/1*	*SfEc8432/2*	*SfEc8432/3*	*SfEcC2987/1*	*SfEcC2987/2*	*SfEcC2987/3*	*SfEcTs6*
E.coli C1	-	-	-	-	-	+	-
E.coli C2	-	-	-	-	+	-	-
E.coli C3	-	-	-	-	+	-	-
E.coli C2987	-	-	+	+	+	+	+
E.coli ATCC 8739	+	-	+	+	+	+	+
E.coli 8432	+	+	+	+	+	+	+
E.coli Ts6	-	-	+	+	+	+	+
Total phage activity against E. coli, %	**28,6%**	**14,3%**	**57%**	**57%**	**85,7%**	**71%**	**57%**
Enterobacter cloacae M1	-	-	-	-	-	-	-
Enterobacter asburiae M3	-	-	-	-	-	-	-
Enterobacter kobei M4	-	-	-	-	-	-	-
Enterobacter asburiae M8	-	-	-	-	-	-	-
Acinetobacter guillouiae M2	-	-	-	-	-	-	-
Acinetobacter baumannii M9	-	-	-	-	-	-	-
Klebsiella oxytoca M5	-	-	-	-	-	-	-
Klebsiella pneumoniae M6	-	-	-	-	-	-	-
Klebsiella pneumoniae M7	-	-	-	-	-	-	-
Proteus mirabilis M11	-	-	-	-	-	-	-
Proteus mirabilis M12	-	-	-	-	-	-	-
Proteus mirabilis M13	-	-	-	-	-	-	-
Staphylococcus aureus ATCC 6538	-	-	-	-	-	-	-
Pseudomonas aeruginosa ATCC 9027	-	-	-	-	-	-	-

Conclusion: This study reveals diversity among the isolated *E. coli* phages and is a solid bases for continuation of phage research in Bulgaria.

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