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Research article

Probiotic biogenics-mediated augmentation of phage therapy against multidrug-resistant Enterotoxigenic *Escherichia coli*

Abishek Mani[1], Nashath Kalangadan[1], Sharayu Magar[2], Barath Sivaraj[2], Gospel Gabriel Xavier[1], Thirumalai Diraviyam[3], Srivignesh Sundaresan[4], Sutharsan Govindarajan[2] and Kaushik Rajaram[1,*]

[1] Department of Microbiology, Central University of Tamil Nadu, Thiruvarur-610005, Tamil Nadu, India
[2] Department of Biological Sciences, SRM University, AP, Amaravati-522240, Andhra Pradesh, India
[3] Centre for Research in Infectious Diseases (CRID), Department of Bioengineering, School of Chemical & Biotechnology, SASTRA Deemed-to-be-University, Thanjavur-613401, Tamil Nadu, India
[4] Department of Horticulture, Central University of Tamil Nadu, Thiruvarur-610005, Tamil Nadu, India

* **Correspondence:** Email: rkaushik@cutn.ac.in; Tel: +914366277230.

Supplementary

Table S1. The tentative compounds list present in the postbiotic in LC-MS analysis with their m/z value, mode of charge, classification, mass error, Retention Time (RT), MSI level and Diagnostic structural rationale in the sample.

No.	Observed m/z	Theoretical m/z	Mass Error (ppm)	Ion Mode	RT (min)	Target Adduct	Assignment	MSI Level	Diagnostic Structural Rationale
1	89.06	89.0244	+4.0	ES−	1.926	[M−H]−	Lactic acid	Level 2	Confirmed major glycolytic end-product; completely absent in modified media baseline.
2	166.14	166.0868	+3.1	ES+	1.900	[M+H]+	Phenyllactic acid (PLA)	Level 2	Signature functional biomarker of phenylalanine catabolism; potent antimicrobial agent.
3	205.24	205.0766	−4.8	ES+	2.595	[M+H]+	Indole-3-lactic acid (ILA)	Level 2	Advanced tryptophan-derived postbiotic marker unique to the probiotic secretome.
4	212.19	—	—	ES+	4.018	[M+H]+	Unidentified Feature	Level 3	Mass matches Cyclo (Pro-Leu).
5	254.29	—	—	ES+	1.223	[M+H]+	Unidentified Feature	Level 3	Mass matches Cyclo (Phe-Pro).
6	304.09	—	—	ES−	1.197	[M−H]−	Unidentified Feature	Level 3	Mass matches Cyclo (Tyr-Pro).
7	456.56	—	—	ES+	2.872	[M+H]+	Unidentified Lipid Feature	Level 4	Pentacyclic core fragment or membrane lipid variant.
8	159.06	—	—	ES−	1.197	[M−H]−	Unidentified Aromatic Acid	Level 4	Purged of false-positive ‘ferulic acid’ database cross-match.
9	118.13	—	—	ES+	0.876	[M+H]+	Unidentified Polar Ion	Level 4	Nitrous amine breakdown fragment from media Tryptone core.
10	132.11	—	—	ES+	1.223	[M+H]+	Unidentified Peptidic Feature	Level 4	Dipeptide backbone segment resulting from protein auto-hydrolysis.

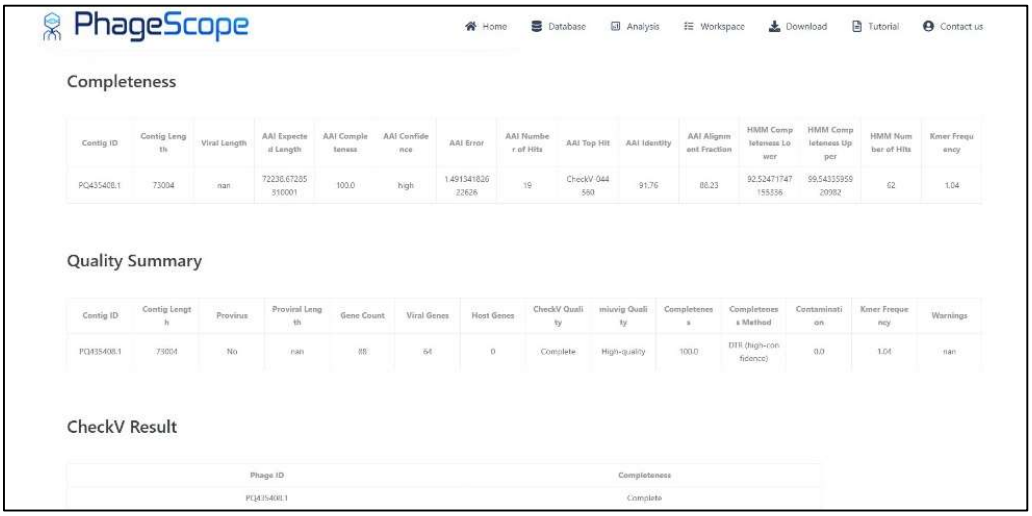


Figure S1. This analysis confirms that the genome is complete.



Figure S2. This analysis confirms that the isolated phage does not encode genes involved in maintaining lysogeny, nor does it carry genes related to antimicrobial resistance (AMR) or virulence factors.

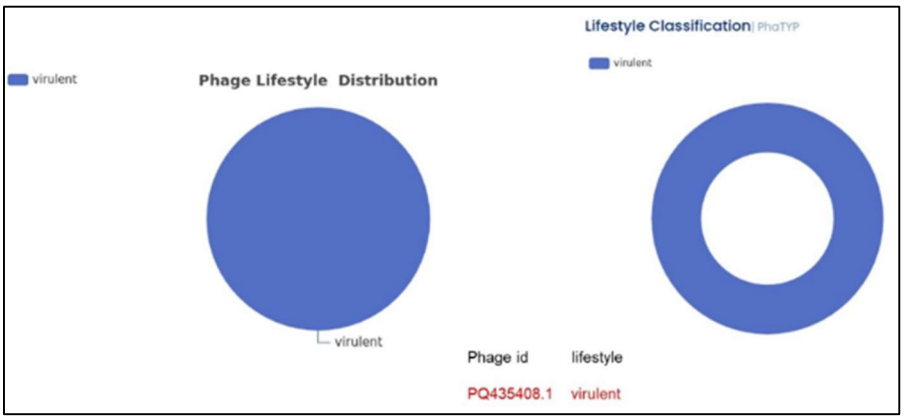


Figure S3. Phage lifecycle distribution determination using PhaTYP software showing that CU10EC phage is lytic in nature.

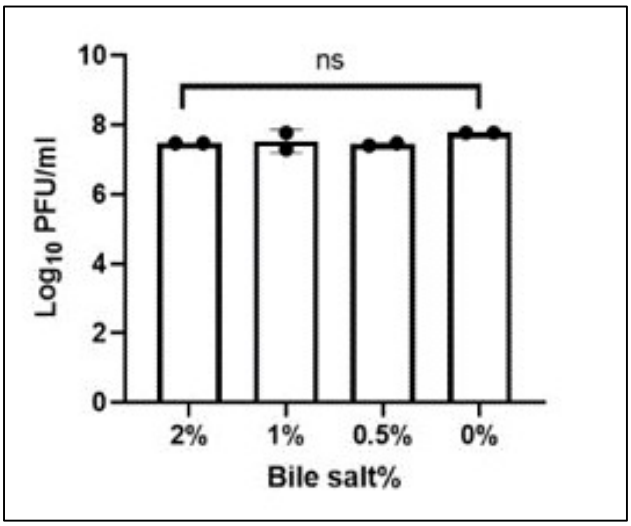


Figure S4. Phage stability analysis in the presence of different concentrations of bile salts. Statistical significance of the groups was determined using one way ANOVA and indicated as ns, no significance; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.005$; **** $p \leq 0.0001$.

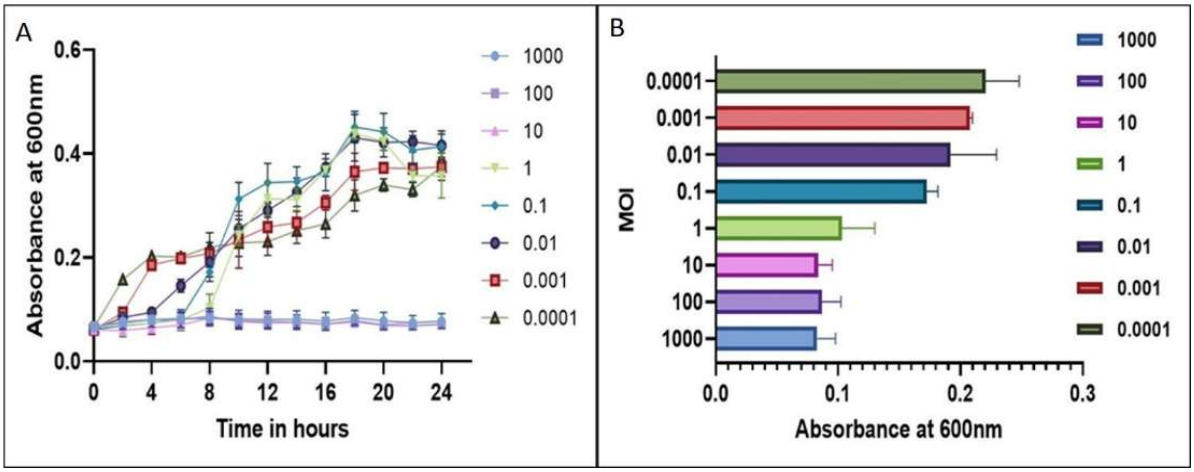


Figure S5. Optimum MOI determination based on antibacterial activity. (A) MOI optimization of CU10EC in growth curve assay using spectrometry analysis; (B) Different MOI's efficacy of biomass reduction at 8th hour.

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