

Article

Isolation and characterization of a novel lytic phage, vB_PseuP-SA22, and its efficacy against carbapenem-resistant *Pseudomonas aeruginosa*

Addisu D. Teklemariam¹, Rashad R. Al-Hindi^{1*}, Mona G. Alharbi¹, Ibrahim Alotibi², Sheren A. Azhari¹, Ishtiaq Qadri¹, Hatoon A. Niyazi³, Hanouf A. Niyazi³, Turki Alamri⁴, Ahmed Esmael^{5,6*} and Steve Harakeh^{7*}

¹Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia; ademeke16@yahoo.com (A.D.T.); mgalharbi@kau.edu.sa (M.G.A.); sazhari@kau.edu.sa (S.A.A.); ishtiaq80262@yahoo.com (I.Q.)

²Health Information Technology Department, Applied College, King Abdulaziz University, Jeddah 21589, Saudi Arabia; ialotibi@kau.edu.sa (I.A.)

³Department of Medical Microbiology and Parasitology, Faculty of Medicine, King Abdulaziz University, Jeddah 21589, Saudi Arabia; hniyazi@kau.edu.sa (Hat.A.N); hneiazi@kau.edu.sa (Han.A.N).

⁴Family and Community Medicine Department, Faculty of Medicine in Rabigh, King Abdulaziz University, Jeddah, Saudi Arabia; olbalamri7@kau.edu.sa (T.A.)

⁵Botany and Microbiology Department, Faculty of Science, Benha University, Benha 13518, Egypt

⁶Nebraska Center for Virology, University of Nebraska-Lincoln, Lincoln, NE 68583, USA

⁷King Fahd Medical Research Center, Yousef Abdullatif Jameel Chair of Prophetic Medicine Application, Faculty of Medicine, King Abdulaziz University, Jeddah 21589, Saudi Arabia; sharakeh@gmail.com (S.H.)

*Correspondence: R.R.A., rhindi@kau.edu.sa; A.E., a7medesmael@gmail.com; S. H., sharakeh@gmail.com

Supplementary materials

Supplementary Table S1. Antibiotic disks used to test *P. aeruginosa* and their respective concentrations.

No.	Antibiotic disks	Disk code	Concentration
1	Colistin	COL	10 µg
2	Ciprofloxacin	CIP	5 µg
3	Streptomycin	STR	10 µg
4	Gentamicin	GM	10 µg
5	Neomycin	NEO	15 µg
6	Piperacillin	PRL	100 µg
7	Cefuroxime	CXM	30 µg
8	Cefotaxime	CTX	30 µg
9	Ceftriaxone	CRO	30 µg
10	Ceftazidime	CAZ	30 µg
11	Levofloxacin	LEV	5 µg
12	Imipenem	IPM	10 µg
13	Meropenem	MEM	10 µg
14	Amikacin	AMK	30 µg
15	Tobramycin	TOB	10 µg
16	Ertapenem	ERT	10 µg

Supplementary Table S2: Annotated open reading frame (58 CDC) of vB_PseudoP-SA22

ORF	Region	Length	Direction	Function
ORF #1	452-757	306	-	Hypothetical protein
ORF #2	2812-3270	459	+	Hypothetical protein
ORF #3	3700-5148	1449	+	Terminase Large Subunit
ORF #4	5149-7266	2118	+	Cell envelope integrity protein TolA
ORF #5	7269-7511	243	+	Hypothetical protein
ORF #6	7511-8503	993	+	Putative capsid and scaffold protein
ORF #7	8402-9361	960	-	Capsid and scaffold protein
ORF #8	8522-9475	954	+	Major Capsid Protein
ORF #9	9524-9844	321	+	Hypothetical protein
ORF #10	9848-10474	627	+	Putative structural protein
ORF #11	10317-10676	360	+	Hypothetical protein
ORF #12	10660-10908	249	+	Hypothetical protein
ORF #13	10898-11545	648	+	Tail fibers protein

Supplementary materials

ORF #14	11554-13095	1542	+	Tail protein
ORF #15	13092-13781	690	+	Hypothetical protein
ORF #16	13778-14212	435	+	Hypothetical protein
ORF #17	14193-15137	945	+	Hypothetical protein
ORF #18	15137-15520	384	+	Putative structural protein
ORF #19	15525-17048	1524	+	Putative structural protein
ORF #20	17204-20374	3171	+	Putative structural protein
ORF #21	20268-21272	1005	+	Putative structural protein
ORF #22	21287-21643	357	+	Putative capsid decoration protein
ORF #23	22021-22260	240	-	Hypothetical protein
ORF #24	22642-23403	762	-	Putative endonuclease
ORF #25	23396-23746	351	-	Putative endonuclease
ORF #26	23628-24575	948	-	Endonuclease
ORF #27	24550-25434	885	-	DNA polymerase I
ORF #28	25434-25718	285	-	Hypothetical protein
ORF #29	25690-26259	570	-	Hypothetical protein
ORF #30	26171-26725	555	-	DNA binding protein
ORF #31	26795-28273	1479	-	DNA polymerase II
ORF #32	28635-29144	510	-	Tail length tape measure protein
ORF #33	29221-29508	288	-	Putative holin
ORF #34	29471-29773	303	+	Hypothetical protein
ORF #35	29813-30046	234	-	Hypothetical protein
ORF #36	30082-30393	312	-	Hypothetical protein
ORF #37	30360-30869	510	-	DNA polymerase III
ORF #38	30853-32094	1242	-	DNA primase
ORF #39	32563-32940	378	-	Hypothetical protein
ORF #40	32940-33338	399	-	Putative glutamyl-tRNA amidotransferase
ORF #41	33338-34219	882	-	Hypothetical protein
ORF #42	34578-36110	1533	-	Putative L-glutamine-D-fructose-6- phosphate amidotransferase
ORF #43	36122-37297	1176	-	Hypothetical protein
ORF #44	37273-37842	570	-	Hypothetical protein
ORF #45	37796-38803	1008	+	Putative ligase-like domain containing protein
ORF #46	38632-39588	957	-	Hypothetical protein
ORF #47	39568-40122	555	+	Hypothetical protein
ORF #48	39607-40569	963	-	Hypothetical protein

Supplementary materials

ORF #49	40791-41615	825	-	Hypothetical protein
ORF #50	41850-42302	453	-	Hypothetical protein
ORF #51	42247-42564	318	+	Hypothetical protein
ORF #52	42653-43078	426	-	Hypothetical protein
ORF #53	43078-43332	255	-	Hypothetical protein
ORF #54	43338-43610	273	-	Hypothetical protein
ORF #55	43610-43891	282	-	Hypothetical protein
ORF #56	43881-44144	264	-	Hypothetical protein
ORF #57	44058-44330	273	+	Hypothetical protein
ORF #58	44460-44810	351	+	Hypothetical protein

Supplementary Table S3. Vitek 2 identification results

Well	Test name	Amount/well (mg)	Mnemonic	Test result
2	Ala-Phe-Pro-ARYLAMIDASE	0.384	APPA	-
10	Hydrogen Sulfide	0.0024	H2S	-
17	BETA-GALACTOSIDASE	0.036	BGLU	-
23	L-Proline ARYLAMIDASE	0.0234	ProA	+
33	SACCHAROSE/SUCRALOSE	0.3	SAC	-
40	L-LACTATE alkalinisation	0.15	ILATK	+
46	Glycine Arylamidase	0.012	GlyA	-
58	O/129 RESISTANCE	0.0105	O129R	+
3	ADONITOL	0.1875	ADO	-
11	BETA-N-ACETYL-GLUCOSAMINIDASE	0.0408	BNAG	-
18	D-MALTOSE	0.3	dMAL	-
26	LIPASE	0.0192	LIP	-
34	d-TAGATOSE	0.3	dTAG	-
4	L-Pyrrolydonyl-ARILAMIDASE	0.018	PyrA	-
47	ORNITHINE DECARBOXYLASE	0.3	ODC	-
59	Glu-Gly-Arg-ARYLAMIDASE	0.0576	GGAA	-
12	GlutamylArylamidase pNA	0.0324	AGLTp	+
19	D-MANNITOL	0.1875	dMAN	+


Supplementary materials

27	PALATINOSE	0.3	PLE	-
35	TREHALOSE	0.3	dTRE	+
42	SUCCINATE alkanisation	0.15	SUCT	+
48	LYSINE DECARBOXYLASE	0.15	LDC	-
5	L-Arabitol	0.3	IARL	-
13	D-Glucose	0.3	dGLU	+
20	D-MANNOSE	0.3	dMNE	+
53	L-HISTIDINE assimilation	0.087	IHISa	-
36	CITRATE (SODIUM)	0.054	CIT	+
43	Beta-N-ACETYL- GALACTOSAMINIDASE	0.0306	NAGA	-
7	D-CELLOBIOSE	0.3	dCEL	-
14	GAMMA-GLUTAMYL- TRANSFERASE	0.0228	GGT	+
21	BETA-XYLOSIDASE	0.0324	BXYL	-
31	UREASE	0.15	URE	-
37	MALONATE	0.15	MNT	+
44	ALPHA-galactosidase	0.036	AGAL	-
56	COUMARATE	0.126	CMT	+
64	L-LACTATE assimilation	0.186	ILATa	+
9	BETA-GALACTOSIDASE	0.036	BGAL	-
15	FERMENTATION GLUCOSE	0.45	OFF	-
22	BETA- ALANINEARYLAMIDASE pNA	0.0174	BAlap	+
32	D-SORBITOL	0.1875	dSOR	-
39	5-KETO-D-GLUCONATE	0.3	5KG	-
45	PHOSPHATASE	0.0504	PHOS	-
57	BETA-GLUCORONIDASE	0.0378	BGUR	-

Supplementary materials

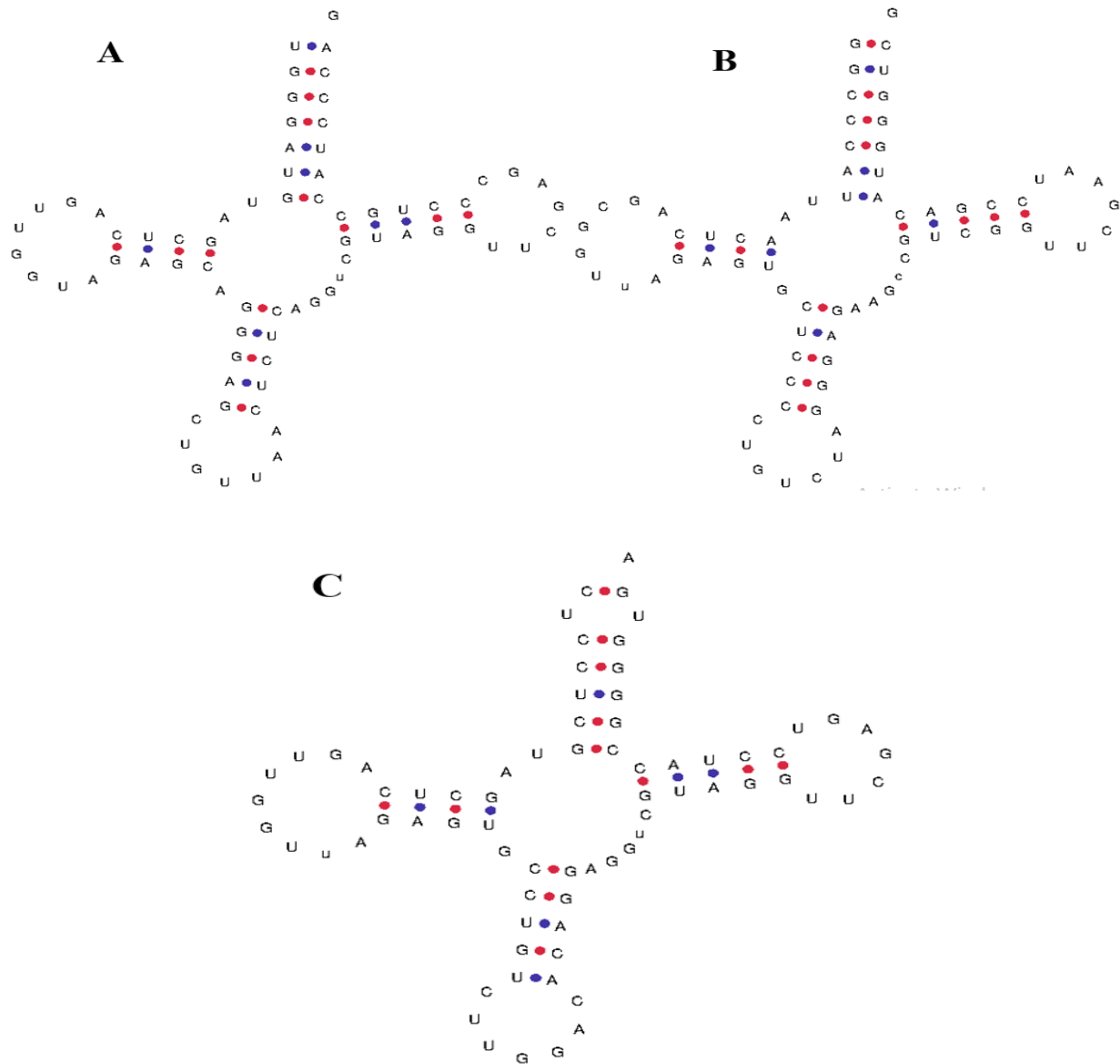
Supplementary Figure S1. Genomic feature of the predicted rho-independent terminators. The terminators were composed of loops (**red**) and stems (**blue**) located at different regions of the genomic sequence.

```
>ccttgatctgcaagataacgctcgctcgctcttccagttggcccttgctctatggcgcggtt
1 3358 Both + GAAAGATAGTAAGGCAGCCTATGATGCAGCCCAAGGGCAAGTATCTCAGCTGCCTTTTTCGA
2 9412 Both + CTAACCCAACGGGGCCCTTCGGGGCCCTTTGTTTCCCCT -13.90
3 21577 Erpin - ACCAAAAAAGGGCCCAAGGGTATCATCCCAAGGGGCCTTATCTTTAGCTC NA
4 21579 Both + GCTAAAGATAAGGCCCTTGGGATGATACCCTTGGGGCCTTTTTTTTGGTTA NA
5 22084 Rnamotif - GCTCTTGCGAAGGCTGAGGCGAAGGCCTTTTCCTTTGAA -5.20
6 39660 Rnamotif - GTAGATAGCGAGGTGGGAGGTTTCATCCGCCTTTATCTTCATA -7.30
7 41625 Rnamotif - CACCCAGGTAAGCCCCCGCATGATTGGGGCTTCTATGTACTG -8.70
8 41650 Rnamotif + GGGGGCTTACCTGGGTGGCCAGCAGGCCGTCTATCTTATGTATGT -6.70
```



Supplementary materials

Supplementary Figure S2. Predicted tRNA structure. (A) Asparagine (Asn) (2461 - 2533), (B) Aspartic acid (Asp) (2541 - 2613), (C) Proline (Pro) (2713 - 2786).



Supplementary materials

Supplementary Figure S3. API-20E test showing the 7-digit profile number which indicated that the organism confirmed to be 97% *P. aeruginosa* with reference to the API-20E analytical profile index book.



Supplementary Figure S4. Photographs showing qualitative detection of biofilm production. (A) Test tube biofilm production assay (test tube ring test) - the right three

Supplementary materials

tubes are showing positive triplicated results and the left tube serves as a negative control. (B) Biofilm visualized under UV light (arrow indicating the biofilm formed a clear white ring). (C) Biofilm stained using 0.1% CV. (D) CV assay in 96 well plates.

