

Supplementary Information

Characterization of a new podovirus infecting *Paenibacillus larvae*

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Table S1: PCR conditions for 16S-PCR of *P. larvae* identification and rep-PCR genotyping.

Target gene	Sequence (5'-3')	PCR conditions
16S rRNA	Fw: CTTGTGTTTCTTTTCGGGAGACGCC Rev: TCTTAGAGTGCCACCTCTGCG	10 min 95 °C 15 sec 95 °C; 15 sec 60 °C; 15 sec 72 °C (30 cycles) 3 min 72 °C
rep-PCR	ERIC2: AAGTAAGTGACTGGGGTGAGC ERIC1R: ATGTAAGCTCCTGGGGATTCA	3 min 95 °C 45 sec 95 °C; 30 sec 45 °C; 3 min 72 °C (30 cycles) 10 min 72 °C

Table S2: API480 genome annotation and BLASTP or HHpred homology. CDSs unique to API480 are in white; CDSs homologue to bacteria are coloured in light grey; CDSs homologue to phages are coloured in dark grey; proteins with an identified function are highlighted in bold. ¹ indicates proteins with assigned function validated by ESI-MS/MS.

gp	Ni	Nf	NN	+/-	AA	Product	Blastp/HHpred	E-value
1	1	426	426	+	141	Hypothetical protein	Hypothetical protein [Bacillus thuringiensis]	3E-13
2	404	658	255	+	84	Hypothetical protein		
3	655	963	309	+	102	Hypothetical protein	Hypothetical protein [Bacillus phage Mgbh1]	6E-18
4	956	2407	1452	+	483	Terminase large subunit ¹	Phage terminase, large subunit, PBSX family (TIGR01547) [uncultured Mediterranean phage uvMED]	2E-43
5	2420	2611	192	+	63	Hypothetical protein		
6	2627	4405	1779	+	592	Portal protein ¹	Portal protein [Enterobacteria phage P22]	1E-46
7	4562	5371	810	+	269	Hypothetical protein	Hypothetical protein [Bacillus cereus]	1E-21
8	5462	6403	942	+	313	Major capsid protein ¹	Major capsid protein [Bacillus phage BM5]	1E-39
9	6470	6733	264	+	87	Hypothetical protein	Hypothetical protein [Bacillus thuringiensis]	1E-15
10	6772	7152	381	+	126	Hypothetical protein	Hypothetical protein [Bacillus sp. J37]	2E-06
11	7161	9152	1992	+	663	Hypothetical protein		
12	9164	10342	1179	+	392	Hypothetical protein		
13	10354	10647	294	+	97	Hypothetical protein		
14	10649	11578	930	+	309	Hypothetical protein		
15	11591	12559	969	+	322	Hypothetical protein		
16	12564	17459	4896	+	1631	Hypothetical protein		
17	17841	18083	243	+	80	Holin ¹	Holin_BhIA [PF10960.8 hit]	8E-23
18	18101	18772	672	+	223	Endolysin	N-acetylmuramoyl-L-alanine amidase [Paenibacillus phage Vegas]	7E-159
19	18778	19248	471	+	156	Hypothetical protein	Hypothetical protein [Paenibacillus larvae]	2E-109
20	19266	19541	276	+	91	Hypothetical protein	Hypothetical protein WANDERER_22 [Paenibacillus phage Wanderer]	9E-53
21	19554	19793	240	+	79	Holin ¹	Phage_holin_5_1 [PF06946.11 hit]	2E-17
22	19881	20150	270	-	89	Hypothetical protein		
23	20190	20432	243	-	80	Hypothetical protein	Nucleotide binding domain [Escherichia coli K12]	1E-12
24	20425	21408	984	-	327	DNA-binding protein ¹	ParM/StbA family protein [Bacillus mycoides]	3E-59
25	21603	21893	291	-	96	Hypothetical membrane protein	Hypothetical protein [Paenibacillus larvae]	7E-47

26	21920	22249	330	-	109	mRNA-degrading endonucleas 1	mRNA-degrading endonuclease [Paenibacillus larvae]	2E-73
27	22246	22515	270	-	89	Antitoxin MazE	Antitoxin MazE [Paenibacillus larvae subsp. larvae]	3E-54
28	22580	22777	198	+	65	Hypothetical membrane protein		
29	22906	23064	159	-	52	Hypothetical protein		
30	23057	23431	375	-	124	Hypothetical protein	Hypothetical protein [Paenibacillus larvae]	6E-75
31	23415	23603	189	-	62	Hypothetical protein	Hypothetical protein [Paenibacillus larvae]	4E-31
32	23600	23800	201	-	66	Hypothetical membrane protein		
33	23805	23996	192	-	63	Hypothetical membrane protein		
34	23981	24241	261	-	86	Hypothetical protein	Hypothetical protein TRIPP_91 [Paenibacillus phage Tripp]	2E-37
35	24243	24428	186	-	61	Hypothetical membrane protein		
36	24484	24864	381	-	126	Hypothetical protein	Hypothetical protein [Paenibacillus larvae]	3E-71
37	24871	25161	291	-	96	Hypothetical protein	Hypothetical protein WANDERER_61 [Paenibacillus phage Wanderer]	8E-60
38	25154	25477	324	-	107	Hypothetical protein	Hypothetical protein C7CDELTA_68 [Paenibacillus phage C7Cdelta]	6E-58
39	25507	26010	504	-	167	dUTP pyrophosphatase 1	dUTP pyrophosphatase [Paenibacillus phage C7Cdelta]	9E-72
40	26022	26282	261	-	86	Hypothetical protein	Hypothetical protein VEGAS_60 [Paenibacillus phage Vegas]	7E-12
41	26298	26417	120	-	39	Hypothetical protein	Hypothetical protein VEGAS_59 [Paenibacillus phage Vegas]	3E-20
42	26414	26602	189	-	62	Hypothetical protein	Hypothetical protein [Paenibacillus larvae]	1E-22
43	26605	26805	201	-	66	Hypothetical protein	Hypothetical protein [Paenibacillus larvae]	4E-34
44	26798	27301	504	-	167	Resolvase 1	Holliday junction resolvase [Escherichia coli]	2,80E-24
45	27303	27497	195	-	64	Hypothetical protein		
46	27613	29838	2226	-	741	DNA polymerase I 1	DNA polymerase I [Geobacillus stearothermophilus]	3E-76
47	29870	32662	2793	-	930	DNA primase 1	DNA primase small subunit [Bacillus phage vB_BboS-125]	2E-12
48	32686	33147	462	-	153	Single-stranded DNA-binding protein 1	Single-stranded DNA-binding protein [Paenibacillus larvae]	9E-86
49	33214	33756	543	-	180	Hypothetical protein		
50	33807	34355	549	-	182	Host-nuclease inhibitor 1	Host-nuclease inhibitor [Desulfovibrio vulgaris]	3E-28
51	34336	34542	207	-	68	Hypothetical protein	Hypothetical protein WANDERER_58 [Paenibacillus phage Wanderer]	3E-17
52	34532	34774	243	-	80	Hypothetical protein	Hypothetical protein [Paenibacillus larvae]	1E-49
53	34767	34997	231	-	76	Hypothetical protein	Hypothetical protein [Paenibacillus larvae]	3E-38
54	35004	35312	309	-	107	Hypothetical protein	Hypothetical protein [Paenibacillus larvae]	6E-69
55	35359	35541	183	-	60	Hypothetical protein	Hypothetical protein WANDERER_53 [Paenibacillus phage Wanderer]	7E-26

56	35538	35798	261	-	86	Hypothetical protein		
57	35802	37370	1569	-	522	DEAD/DEAH box helicase ¹	DEAD/DEAH box helicase [Bacillus thuringiensis]	3E-129
58	37363	37515	153	-	50	Hypothetical protein		
59	37796	38482	687	-	228	Hypothetical protein	Hypothetical protein [Bacillus cereus]	8E-44
60	38495	39013	519	-	172	Hypothetical protein	Hypothetical protein [Bacillus cereus]	2E-20
61	39031	39198	168	-	55	Hypothetical membrane protein		
62	39188	39346	159	-	52	Hypothetical protein		
63	39387	39638	252	-	83	Hypothetical protein	Hypothetical protein [Paenibacillus larvae]	2E-43
64	39673	39876	204	-	67	Hypothetical protein		
65	39889	40290	402	-	133	Hypothetical protein		
66	40355	40981	627	-	208	Hypothetical protein		
67	40968	41144	177	-	58	Hypothetical membrane protein		
68	41189	41380	192	-	63	Hypothetical protein		
69	41402	41584	183	-	60	Hypothetical protein		
70	41614	42114	501	-	166	Antirestriction protein ArdA ¹	Antirestriction protein ArdA [Paenibacillus larvae]	6E-26
71	42165	42347	183	-	60	Hypothetical protein	Hypothetical protein [Paenibacillus larvae]	5E-24
72	42358	42618	261	-	86	Hypothetical protein		
73	42742	42987	246	-	81	Hypothetical protein	Hypothetical protein [Brevibacillus laterosporus]	1E-22
74	42992	43219	228	-	75	Hypothetical protein	Hypothetical protein [Paenibacillus larvae]	1E-36
75	43546	43677	132	-	43	Hypothetical protein		
76	43704	44159	456	-	151	Hypothetical protein	Hypothetical protein [Paenibacillus larvae]	1E-32
77	44461	44676	216	-	71	Hypothetical protein	Hypothetical protein JENST_60 [Brevibacillus phage Jenst]	5E-07

Table S4: Function and distribution of all API480 genes (n=19) shared with *P. larvae* phages, according to Phamerator. For each API480 gene product (pham) that is shared, the number of phages per cluster that have protein homologs are listed. The total number of phages that belong to each cluster are in brackets. Below, the percentage of phages for each cluster that shared proteins with API480 is provided.

API480 GENOME						
Protein	Function	Fern (n=30)	Harrison (n=2)	Vegas (n=7)	Lily (n=1)	Halcyone (n=8)
gp17	Holin	30	2	7	1	0
gp18	Endolysin	30	2	7	1	0
gp19	Hypothetical protein	0	0	4	0	0
gp20	Hypothetical protein	1	2	2	0	0
gp21	Holin	30	2	7	1	0
gp30	Hypothetical protein	0	0	4	0	0
gp34	Hypothetical protein	0	0	0	0	8
gp38	Hypothetical protein	6	0	0	3	3
gp37	Hypothetical protein	0	0	2	0	7
gp39	dUTP diphosphatase	0	0	0	0	7
gp40	Hypothetical protein	0	0	4	0	0
gp41	Hypothetical protein	0	0	4	0	0
gp42	Hypothetical protein	0	0	0	0	1
gp48	Dingle stranded binding protein	0	2	0	0	0
gp50	Host-nuclease inhibitor	0	2	0	0	0
gp52	Hypothetical protein	1	0	2	0	0
gp53	Hypothetical protein	0	2	2	0	1
gp55	Hypothetical protein	11	2	2	1	0
gp73	Hypothetical protein	1	2	6	0	0
		19 %	47 %	39 %	37 %	18 %

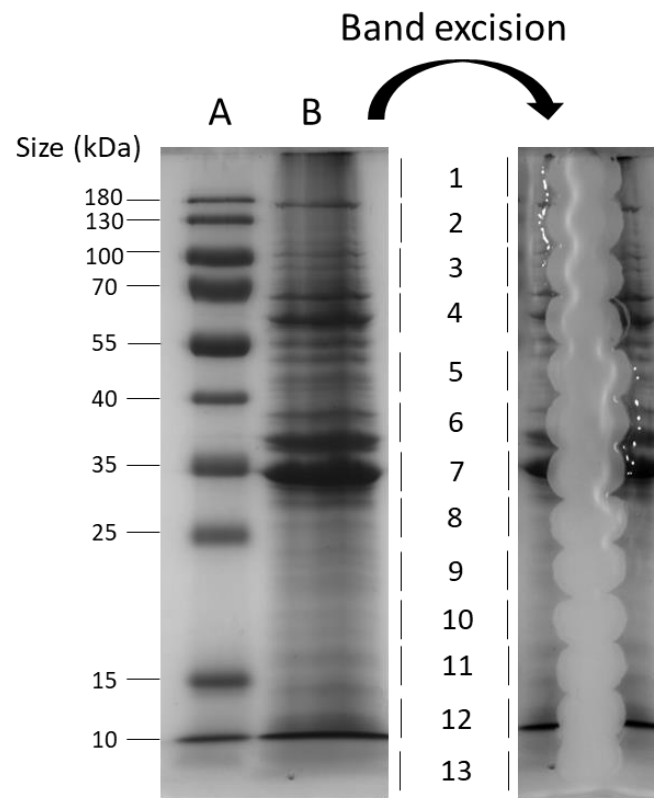


Figure S1: SDS-PAGE analysis of the API480 phage proteins (lane B) on a 12% SDS-PAGE separation gel. Lane A shows the protein ladder bands from 10 to 180 kDa. The entire lane B was cut into 13 slices, numbered on the right. Band excision is illustrated in the cropped image from the same gel, as indicated.

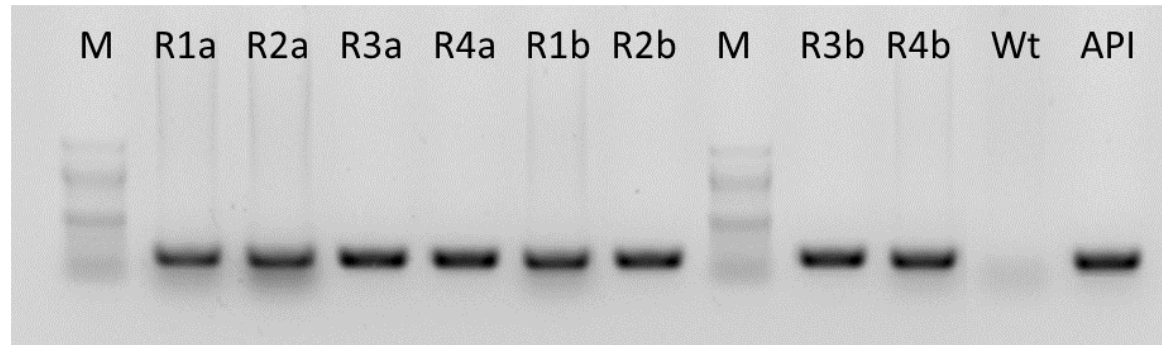


Figure S2: Amplification of CDS₁₂ fragment. Agarose gel revealing 227 bp bands in R-PI27 strains tested (R1, R2, R3 and R4 in duplicate (a and b), in API480 (API) and its absence in the original PI02-27 strain (Wt). DNA ladder 100 bp NEB (M).

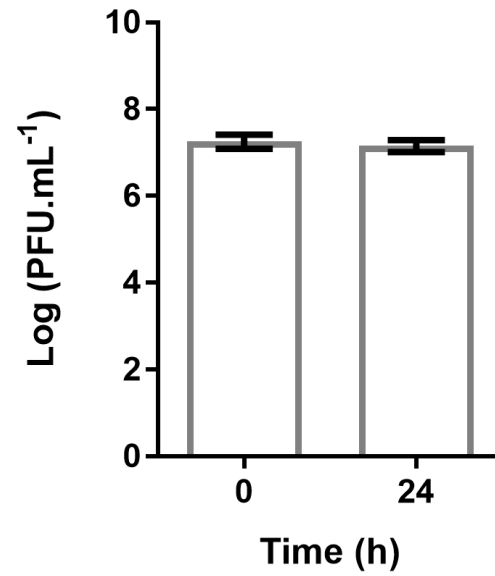


Figure S3: Effect of 50% (w/v) sucrose on API480 phage concentration (PFU.mL⁻¹). Data shows represents the average of three independent assays and error bars indicate the standard deviation. LOD (Limit of Detection) = 3 Log. Statistical significance, $p < 0.05$.