Metagenomic Mining: Evolution of 'Single-Gene Lysis' Systems in Small Phages

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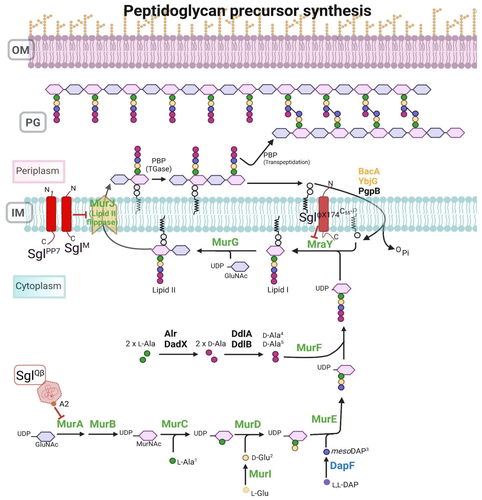
Background

- Small bacteriophages (ssDNA and ssRNA) encode a single gene (called ***sgl***) that induces the host to undergo autolysis and liberate progeny virions [1].
- The number of discovered ssRNA phages are limited. But recent meta-transcriptomics studies have uncovered thousands of genomes [2].
- Previous study identified 35 new Sgls from metagenomes exhibiting activity in *E. coli* [2].
- From the nine known ssRNA phages: the activity of Sgls was classified into **type I** (inhibit PG synthesis) and **type II** (L-like) [3].



Objective

- Functional analysis of Sgls in *P. aeruginosa* (based on coat protein GC content)
- Lysis profiles of discovered Sgls in *E. coli*
- Distinguish the activity of new Sgls based on physiological analysis (type I -septal collapse and type II –random blebbing)

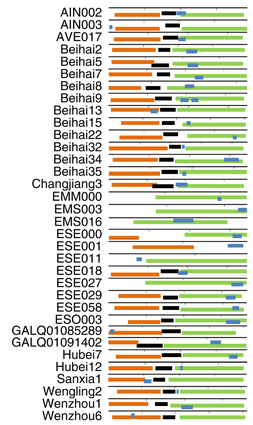
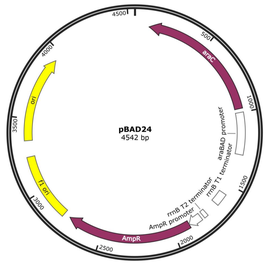


Fig. Genome organization of discovered Sgls[2]

No.	Virus Name	TMD	Codon length	Relative Location
1	EMS016	Y	45	Rep
2	ESE011	Y	22	Junction 2
3	ESE027	Y	53	Rep
4	ESE001	Y	72	Junction 2
5	EMS003	Y	52	Rep
6	EMM000	Y	27	Rep
7	GALQ01091402	N	74	Rep
8	Beihai8	Y	75	Rep
9	Beihai35	Y	36	Rep
10	ESE000_1	Y	78	Rep
11	Beihai34	Y	101	Rep
12	Beihai13	Y	62	Mat
13	AVE017	N	63	Junction 2/Rep
14	ESE018	Y	85	Junction 2/Rep
15	Beihai9	N	53	Rep
16	Beihai9	Y	63	Rep
17	Changjiang3	Y	103	Junction 2/Rep
18	Beihai7	Y	75	Rep
19	ESE058	Y	61	Rep
20	Wenzhou1	N	103	Rep
21	ESE029	Y	88	Rep
22	ESO003	Y	128	Rep
23	Beihai5	Y	100	Rep
24	Hubei12	Y	30	Rep
25	AIN002	Y	91	Junction 2/Rep
26	Hubei7	Y	83	Rep
27	Sanxia1	Y	74	Mat/Junction 1
28	AIN003	Y	27	5'
29	Beihai22	Y	40	Rep
30	Beihai2	Y	108	Rep
31	Beihai15	Y	41	Rep
32	GALQ01085289	Y	43	Mat
33	Wenzhou6	Y	39	Mat
34	Wenling2	N	25	Junction 2
35	Beihai32	Y	33	Junction 2

Methods and Strategy

- Bacterial strains: *E. coli* XL1Blue and *P. aeruginosa* PAO1
- Plasmids: pBAD24 and pQFT (cumate inducible)

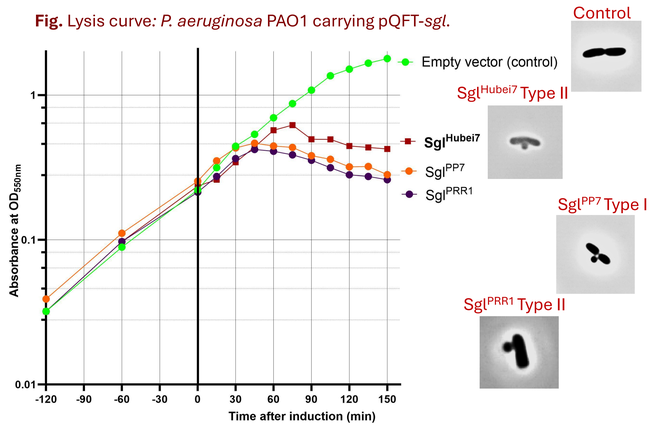


- Lysis curve: Lysis profiles were obtained by taking 125 µL of overnight cultures and adding them into respective 250 mL culture flasks with 25 mL of LB supplemented with amp (100 µg/mL)/ tet (50 µg/mL). The flasks were incubated in a 37°C water bath shaker and induced at $A_{550} = 0.2$ with arabinose or cumate. After induction, the optical density was determined at regular intervals.
- Microscopy: Samples were taken 10 min prior, 5 min prior, and at the time of expected lysis. At each time point, 5 µL samples were put onto a glass slide and covered with cover slip and imaged.

Results

- One Sgl was discovered to possess activity beyond *E. coli* for the first time. Sgl^{Hubei7} (of 14 examined) exhibited the capacity to lyse *P. aeruginosa*, a type II (random blebs).
- Two ssRNA phages infecting *P. aeruginosa* (T4P) exhibit distinct lysis morphotypes; PP7 is classified as type I (targeting MurJ), whereas PRR1 is classified as type II (characterized by scattered blebs).

Fig. Lysis curve: *P. aeruginosa* PAO1 carrying pQFT-*sgl*.



- The *P. aeruginosa* genome has a high GC content of 65-67%, suggesting that phages utilizing *P. aeruginosa* as a host may also possess high GC content, particularly in their coat protein. Among the 35 previously identified Sgls, the GC content of the coat protein was classified as follows: 1 with >60%, 13 with >50%, 15 with <50%, and 6 with no coat.

Fig. Lysis curve: *E. coli* XL1Blue carrying pBAD-*sgl*.

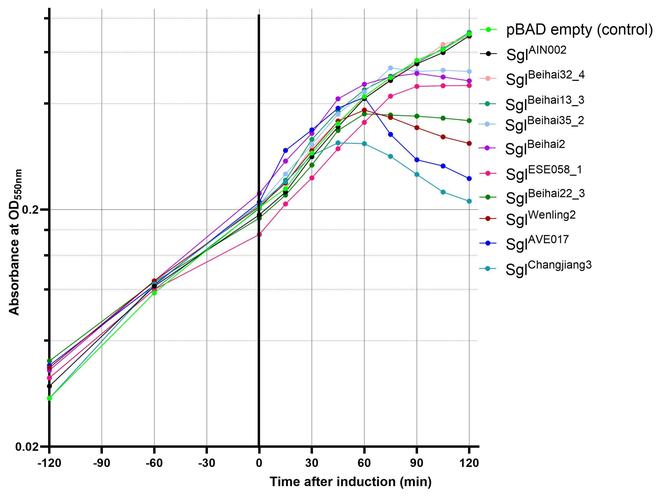
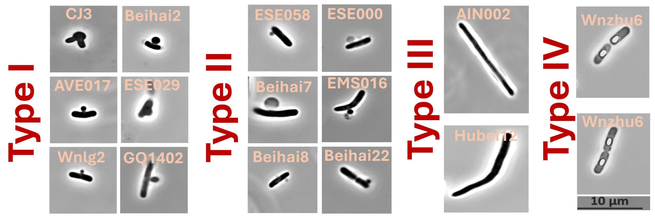


Fig. Type I lysis (septal blebs), type II lysis (random blebs), type III (filamentation), and type IV (hole-formation). Shown are phase contrast images of *E. coli* after induction of different *sgl* genes.



Type I	Type II	Type III
Beihai2, Wenling2, AVE017, Changjiang3, GALQ1091402, ESE029_1, Behai5, Wenzhou1_2	ESE058_1, Beihai22_3, Beihai32_4, Beihai13_3, Beihai35_2, ESE000_1, EMS003_2, EMS016_2, Beihai34, Beihai8, Beihai9_1, Beihai7	AIN002, Hubei12_3, ESE001_1, ESE027_1, EMM000_2, Sanxia1, ESE011, ESO003_1, Beihai15_1, ESE018, AIN003, GALQ1085289_1
Type IV		
Wenzhou6_2		

Fig. Lysis curve: *E. coli* XL1Blue carrying pBAD-*sgl*

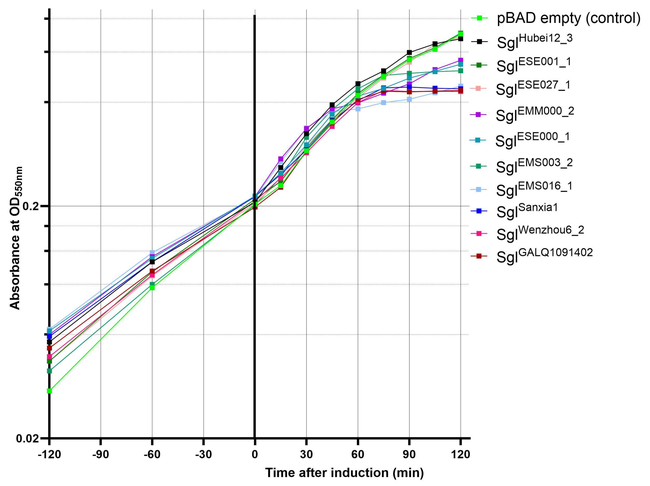
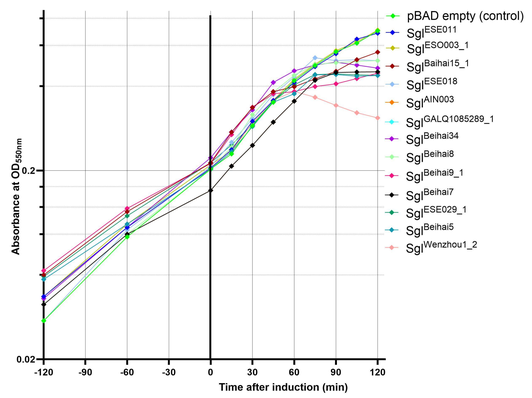


Fig. Lysis curve: *E. coli* XL1Blue carrying pBAD-*sgl*.



Discussion

- The natural hosts of these metagenomic phages remain unknown; nevertheless, prior research indicated that 10% (35) of the examined Sgls had lytic activity in *E. coli*, none of which inhibited *P. aeruginosa*. It is essential to emphasize that there is no evidence indicating that these ssRNA genomes extracted from metatranscriptomes originate from plaque-forming lytic phages. RNA phages might induce chronic infections in which virions accumulate indefinitely while the host cell persists in growth and division. Consequently, numerous ssRNA phages may persist in an endemic carrier state.
- Our findings encourage additional investigation into utilizing these peptides for target identification in antibiotic development.

- **Future**: To explore more Sgls and identify the new PG targets (type I Sgls) which can serve as a 'protein antibiotic' in future.

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References:

1] Chamakura KR, Young R. Single-gene lysis in the metagenomic era. Current opinion in microbiology. 2020; 56:109-17.

2] Chamakura KR, Tran JS, O'Leary C, Lisciandro HG, Antillon SF, Garza KD, Tran E, Min L, Young R. Rapid de novo evolution of lysis genes in single-stranded RNA phages. Nature communications. 2020; 11(1):6009.

3] Antillon SF, Bernhardt TG, Chamakura K, Young R. Physiological characterization of single-gene lysis proteins. Journal of Bacteriol. 2024; 206(3):e00384-23.

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