

Table S1: *Proteus\_virus\_309* host specificity and identification based on 16s rDNA sequences

Isolate	Top two BLASTn hits	Coverage%	Identity%	Accession	Source	Infectivity
<i>P. mirabilis</i> B16	<i>Proteus mirabilis</i> strain MPE4069 chromosome, complete genome	100	100	CP053718.1	Human urine isolate (Tygerberg Hospital)	+
	<i>Proteus mirabilis</i> strain P8 16S ribosomal RNA gene, partial sequence	100	100	MT276305.1		
<i>P. mirabilis</i> B15	<i>Proteus mirabilis</i> strain MPE5203 chromosome, complete genome	99	100	CP053685.1	Human urine isolate (Tygerberg Hospital)	-
	<i>Proteus mirabilis</i> strain AGMDRUC5 16S ribosomal RNA gene, partial sequence	99	100	MN795608.1		
<i>P. mirabilis</i> B14	<i>Proteus mirabilis</i> strain 1701092 chromosome, complete genome	100	100	CP042857.1	Human urine isolate (Tygerberg Hospital)	-
	<i>Proteus mirabilis</i> strain VS-26 16S ribosomal RNA gene, partial sequence	100	100	MK785117.1		
<i>P. vulgaris</i> B24	<i>Proteus vulgaris</i> strain EX14 16S ribosomal RNA gene, partial sequence	100	99,85	MT066063.1	Human urine isolate (Tygerberg Hospital)	-
	<i>Proteus vulgaris</i> strain FDAARGOS_556 chromosome, complete genome	100	99,78	CP033736.1		
<i>P. mirabilis</i> ATCC 25933					American Type Culture Collection	-
<i>E. coli</i> B8	<i>Escherichia coli</i> strain EC 16S ribosomal RNA gene, partial sequence	100	99,78	MN083306.1	Human urine isolate (Hospital)	-

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<i>Escherichia coli</i> strain LWY24 chromosome, complete genome	100	99,71	CP054556.1
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Table S2A: Comparison of physiological characteristics of *Novosibovirus* members

Phage	Susceptible Hosts	Latent period (min)	Plaque forming units per cell	Source	Reference
309	<i>P. mirabilis</i> strain B16	5	39	Cape Town, South Africa	Current study
PM16	<i>P. mirabilis</i> strain CEMTC 73	15	~100	Novosibirsk, Russia	[4]
PM75	<i>P. mirabilis</i> strain CEMTC 821	20	32	Novosibirsk, Russia	[4]
<i>vB_PmiP_RS1pmA</i>	<i>P. mirabilis</i> strain RS1 & RS3	unavailable	unavailable	United Kingdom	[4]
<i>vB_PmiP_RS8pmA</i>	<i>P. mirabilis</i> strain RS8	unavailable	unavailable	United Kingdom	[4]

Table S2B: Comparison of the lysis profiles of *Proteus* infecting phages

Phage	Burst (min)	Plaque forming units per cell	Reference
309	15	39	Current study
<i>vB_PmiP_5460</i>	10-15	46	[29]
<i>vB_PmiM_5461</i>	25	11	[29]
<i>vB_PmiS-TH</i>	20	260	[46]
<i>Privateer</i>	40	140	[47]
PM85	7	18	[44]
PM93	7	75	[44]
PM116	7	70	[44]

Table S3: Sequencing analysis report for the assembly of phage *Proteus\_virus\_309*

Feature	Value
Total number of reads	9909485
Average Read length (bp)	194.49
Genome size (bp)	41740
DNA G+C (%)	41.3
Average Coverage	45595x
Number of ORFs	59
Accession number	OL416096
Bacphlip virulence score	0.99
Highest percent nucleotide similarity with a species in the same genus	89.5
Lineage	<i>Duplodnaviria, Heunggongvirae, Uroviricota, Caudoviricetes, Caudovirales, Autographiviridae, Slopekovirinae, Novosibovirus</i>

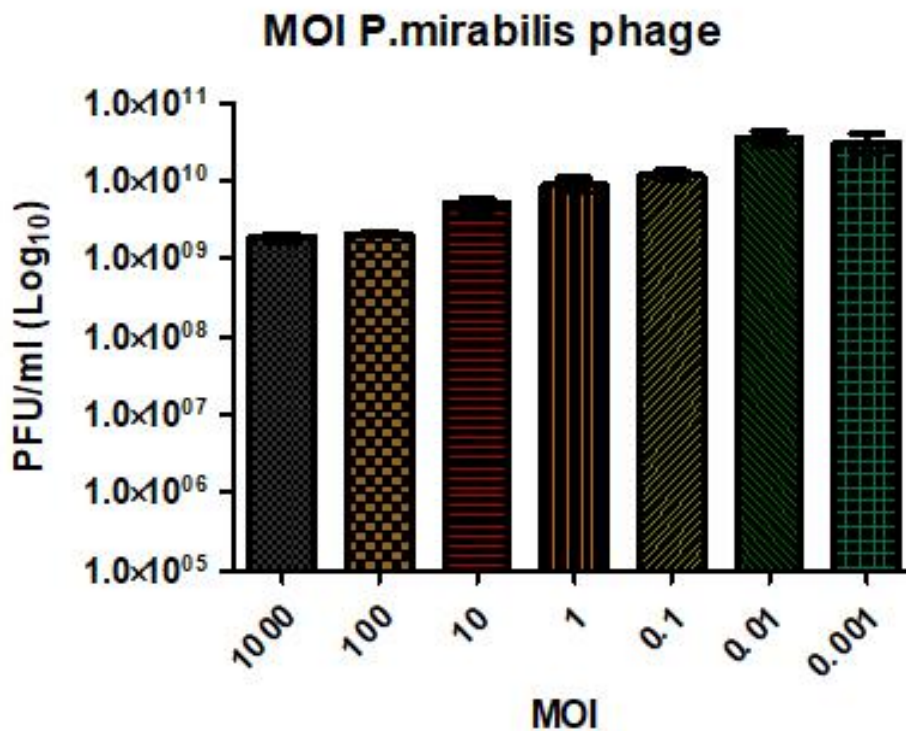


Figure S1: The effects of different MOIs on the production of phage production. A one-way ANOVA showed significant difference between an MOI of 0.01 and MOI's  $\geq 0.1$  with no significant difference between 0.01 and 0.001 with higher titre lysates produced from MOI= 0.01

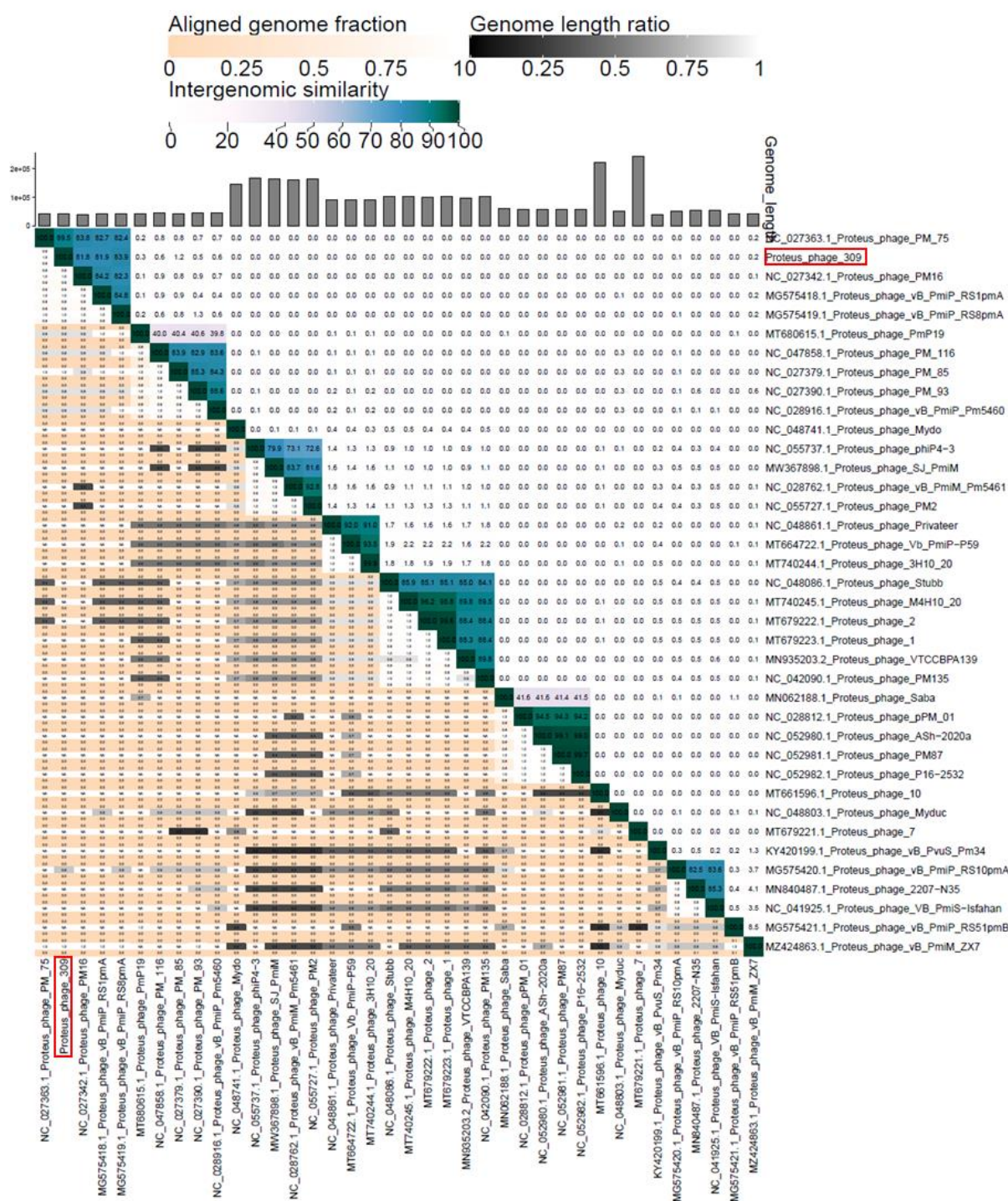


Figure S2: A) Intergenomic similarity between all *Proteus* infecting phages determined using VIRIDIC. *Proteus\_virus\_309* is highlighted with a red border.



Figure S2 continued: B) Protein cluster clustering between all *Proteus* infecting phages determined using VirClust

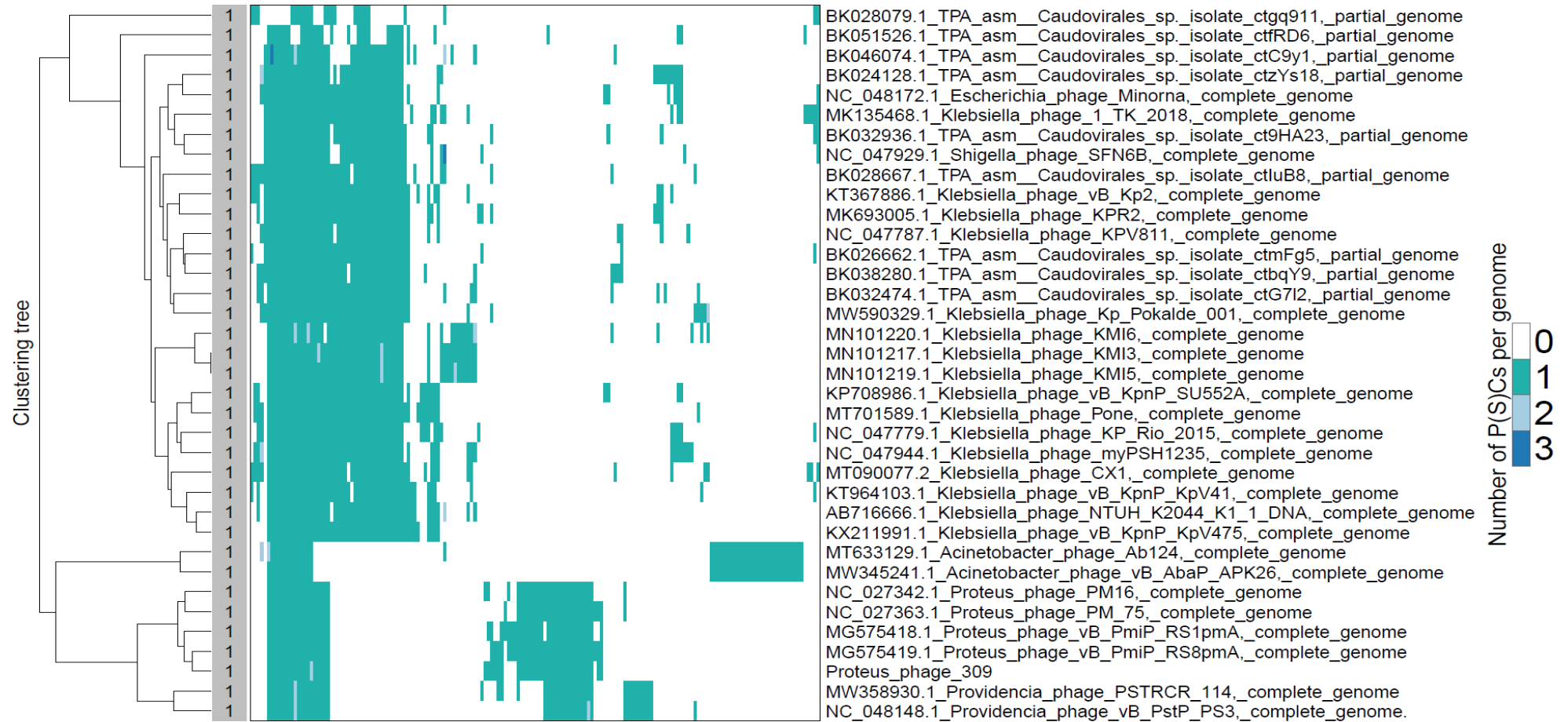


Figure S3: Protein cluster clustering (a) between all phage that show significant nucleotide similarity to *Proteusvirus309* determined by VirClust

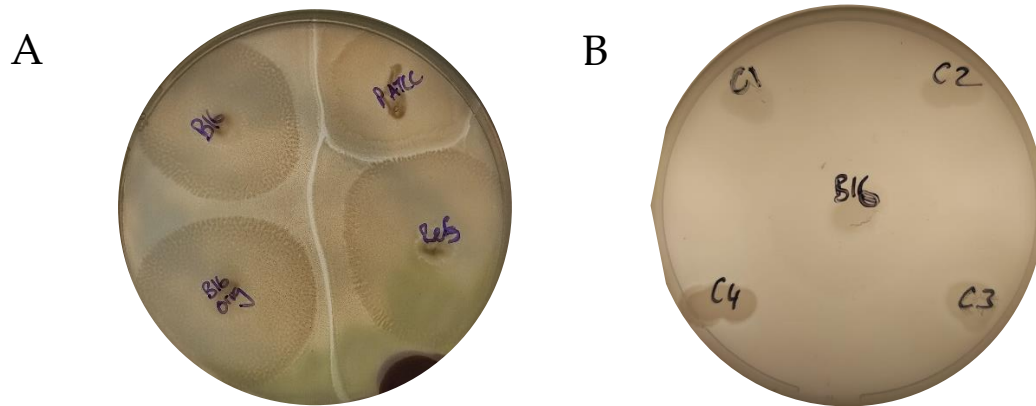


Figure S4: A) Dienes phenomenon observed across different strains of *Proteus mirabilis* unrecognisable to self, as *P. mirabilis* B16 does form a dienes line between the *P. mirabilis* ATTC 25933. The phage resistant mutants (C1-C4) that arose after the bactericidal assay are still recognized by the mother strain (phage-unexposed).

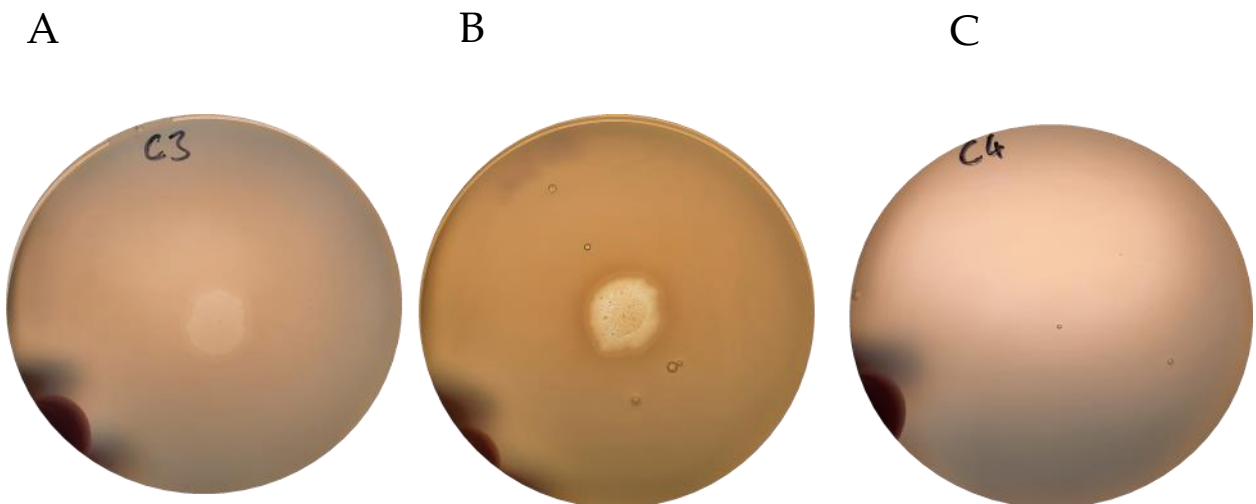


Figure S5: A) is a positive spot test of colony 3. B) is the spot assay of the *P. mirabilis* B16 (used to generate the mutants). C) is Colony 4 a phage resistant mutant. Notable that colonies C1, C2 and C4 are all resistant to the phage whilst a cloudy zone appears for C3.

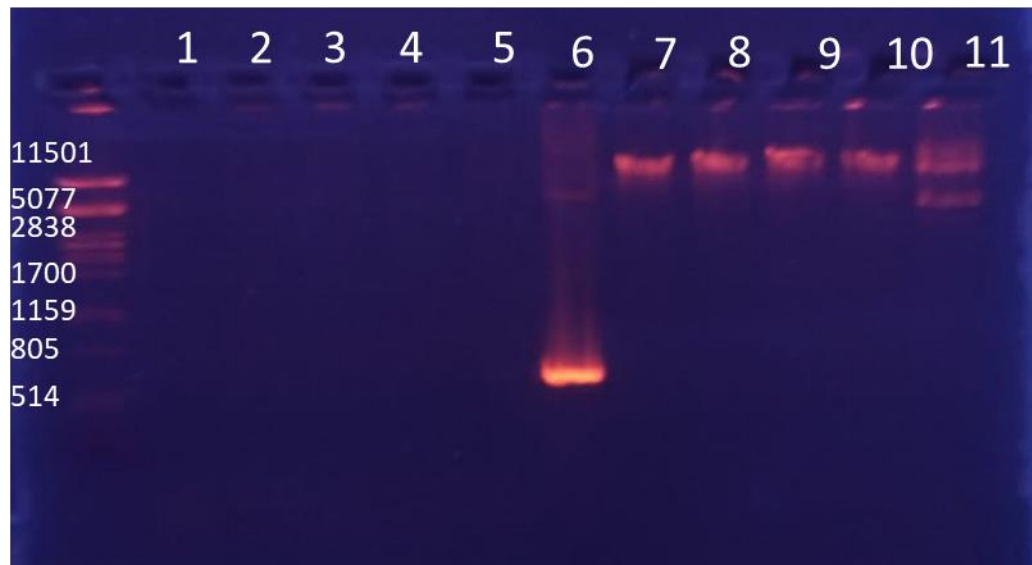


Figure S6: Figure S6: PCR assay for the presence of lipase gene (ORF 3, 633bp) in host DNA to determine lysogeny. From left to right: Lambda PstI ladder; Lane 1 negative control; Lane 2-5 PCR amplicons from phage resistant mutants (C1 - C4); Lane 6 pRSF\_lipase construct positive control; Lane 7-10 One microliter of gDNA of each of the mutants; Lane 11 contains 1µl of the pRSF\_lipase construct