

1 Title: Genome sequence and characterisation of coliphage vB\_Eco\_SLUR29  
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3 Running title: coliphage vB\_Eco\_SLUR29  
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18 Authorship statement

19

20 JH and AM conceived the idea. IB, PS, CH, LG, TR, SM, SH were involved in practical aspects of ,  
21 sample collection, phage isolation, genome sequencing, one step growth experiments, virulence  
22 assays and analysis of raw data . LG, CH, TR and AM wrote the manuscript. All authors have  
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24

25 Author Disclosure statements

26

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28 **Abstract**

29

30 Bacteriophage that infect *Escherichia coli* are relatively easily isolated, with greater than 600  
31 coliphage genomes sequenced to date. Despite this there is still much to be discovered about the  
32 diversity of coliphage genomes. Within this study we isolated a coliphage from cattle slurry  
33 collected from a farm in rural England. Transmission electron microscopy identified the phage as  
34 member of the *Siphoviridae* family. Phylogenetic analysis and comparative genomics further placed  
35 it within the subfamily *Tunavirinae* and forms part of a new genus. Characterisation of the lytic  
36 properties reveals that it is rapidly able to lyse its host when infected at high multiplicity of  
37 infection, but not at low multiplicity of infection.

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40

41 **Introduction**

42

43 Bacteriophages infecting *Escherichia coli* (coliphages) are readily isolated from a variety of  
44 sources, with > 600 complete or near complete coliphage genomes publicly available (May 2019).  
45 Coliphage genomes range in size from 3.39 kb <sup>1</sup> to 386.44 kb <sup>2</sup> and are represented in a number of  
46 phage families including the *Leviviridae*, *Siphoviridae*, *Podoviridae*, *Myoviridae* and the  
47 *Microviridae*. Currently within these families, there are 37 genera and 157 species that contain  
48 coliphages, with the many taxa being poorly sampled <sup>3</sup>. Therefore, there is still much to be  
49 discovered by the continued isolation and sequencing of coliphage, with many new phage types and  
50 taxa still to be discovered <sup>3</sup>. Building on our previous research that has isolated coliphages from  
51 animal slurries <sup>4,5</sup>, we aimed to further isolate phage from this system and characterise the  
52 phenotypic properties.

53

54 **Materials and Methods**

55 Bacteriophage vB\_Eco\_SLUR29 was isolated from cattle slurry that was collected from a farm in  
56 the East Midlands, in the United Kingdom. The double-agar overlay method was used for phage  
57 isolation and the subsequent three rounds of purification using *E. coli* K-12 MG1655 as host, as has  
58 been described previously. High titre lysate was produced by infection of ~50 ml of exponentially  
59 growing *E. coli* MG1655 and incubated at 37°C with shaking at 300 rpm, until lysis had occurred.  
60 Phage growth parameters (burst size, eclipse and latent period) were determined by performing one-  
61 step growth experiments as described by Hyman and Abedon (2009), with free phages being  
62 removed from the culture by pelleting the host cells via centrifugation at 10,000 g for 1 min,  
63 removing the supernatant and re-suspending cells in fresh medium <sup>6</sup>. Three independent replicates  
64 were carried out for each experiment. The virulence index was calculated using the method  
65 described by Storms and Sauvageau (2019) using a SPECTROstar Omega (BMG) plate reader. For  
66 genome sequencing

67 DNA was extracted from 1 ml of bacteriophage lysate as previously described <sup>7</sup>. One nanogram of  
68 DNA was used as input for Nextera XT library preparation, following the manufacturer's  
69 instructions. Sequencing was performed on an Illumina MiSeq (250 bp paired-end) with both the  
70 genome sequence (accession: LR596614) and raw reads (accession: ERR3385641) submitted to the  
71 ENA under Project accession: PRJEB32519

72

73 **Bioinformatics and comparative genomics**

74 Reads from sequencing were trimmed with Sickle v1.33 using default parameters <sup>8</sup>. Assembly was  
75 carried out with SPAdes v.3.6.0 using “--only-assembler” option <sup>9</sup>. Genome assembly errors were

76 corrected with two rounds of checking and polishing with Pilon v1.23 using default parameters <sup>10</sup>.  
77 The genome was annotated with Prokka 1.12 using a protein database constructed from accession  
78 LR027385 <sup>11</sup>. The start of the genome was arbitrarily set at the gene encoding for the large  
79 terminase subunit, for ease of comparison. For rapid comparison against all other phage genomes a  
80 Mash database was constructed of all complete bacteriophage genomes available at the time of  
81 analysis (~ 11,000, April 2019) using the following Mash setting: “–s 10000” using a previously  
82 described method <sup>12</sup>. Closely related genomes were identified using this database and the `dist`  
83 function. From this initial set of genomes, the *terL* gene was used to construct a phylogenetic tree  
84 using IQ-TREE<sup>13</sup>. Following this, a more detailed analysis of the most closely related genomes was  
85 carried out. Phage genomes that were found to be similar were re-annotated with Prokka to ensure  
86 consistent gene calling between genomes for comparative analysis <sup>11</sup> and the  
87 GET\_HOMOLOGUES pipeline used to identify core genes <sup>14</sup>. For calculation of phage average  
88 nucleotide identity, pyani was used with default settings <sup>15</sup>. Core gene analysis for phages within  
89 the putative genus *Swanvirus* was carried out with ROARY using “--e --mafft -p 32 -i 90” <sup>16</sup>.

90

## 91 **Transmission Electron Microscopy**

92 TEM was carried out at the University of Leicester Core Biotechnology Services Electron  
93 Microscopy using a previously described method <sup>12</sup>. Digital images were collected with a  
94 Megaview III digital camera using iTEM software. Phage images were processed in ImageJ using  
95 the measure tool, with the scale bar present used as a calibration to measure phage particle size <sup>17</sup>.  
96 The data presented in the mean of 15 phage particles.

97

## 98 **Results**

99 Bacteriophage vB\_Eco\_SLUR29 was isolated from animal slurry collected from a farm in the East  
100 Midlands, United Kingdom using *E. coli* K-12 MG1655 as a host. The plaque morphology was  
101 small (< 3 mm) clear plaques, suggestive of an obligately lytic phage. Imaging of phage  
102 vB\_Eco\_SLUR29, using transmission electron microscopy, revealed a polyhedral head with a long  
103 flexible non-contractile tail. The head was 57 nm ( $\pm$  2.8) and 56.7 nm ( $\pm$  6.7) in width and length  
104 respectively, with a tail that was 12 nm ( $\pm$  1.4) wide and 142 nm ( $\pm$  23.2) long. The long non-  
105 contractile tail allowed its classification within the *Siphoviridae* and the head length:width ratio  
106 further classified vB\_Eco\_SLUR29 within subgroup B1 <sup>18</sup> (Fig. 1A).

107

108

109 The lytic properties of the phage vB\_Eco\_SLUR29 were determined using a one-step experiment  
110 (Fig 1B). The latent period was determined to be ~ 21 mins, the eclipse period 17 mins, with a burst

111 size of 25 (+/-6). To further characterise the infection properties, killing curves were used to  
112 investigate the ability of vB\_Eco\_SLUR29 to kill its host over a range of MOIs and also determine  
113 the recently described virulence index <sup>19</sup>. At an MOI of 1, there was rapid lysis of the culture with  
114 near complete lysis after 100 minutes, followed by a steady increase in growth after 300 minutes.  
115 When a very low MOI of  $1 \times 10^{-5}$  or lower was used, these cultures displayed growth comparable to  
116 an un-infected control until the onset of lysis was observed at ~300 minutes (MOI  $1 \times 10^{-7}$ ). Post 300  
117 minutes a decrease in growth was observed, before a steady increase from 700 minutes onwards.  
118 For calculation of the virulence index, the local virulence values at MOIs from 1 to  $10^{-7}$  were  
119 determined (Fig 1C). This further highlighted that phage vB\_Eco\_SLUR29 was inefficient at lysing  
120 its host at very low MOIs (Fig 1C). The virulence index of phage vB\_Eco\_SLUR29 was calculated  
121 to be 0.37 at 37 °C in LB medium.

122

### 123 **Genome Sequencing**

124 To further classify vB\_Eco\_SLUR29 beyond morphological similarity to other Siphoviruses, the  
125 genome of vB\_Eco\_SLUR29 was sequenced. Genome sequencing resulted in a single chromosome  
126 with an average coverage of 426x. The genome was 48,466 bp in length with a G+C content of 44.7  
127 %, with 78 predicted genes and no tRNAs. Of the 78 predicted genes, functions could only be  
128 predicted for 25 of the proteins they encode and the majority of these were phage structural  
129 proteins. Comparing the genome sequence of vB\_Eco\_SLUR29 against current phage genomes  
130 identified that it had the greatest similarity (Mash distance < 0.05) to the coliphages SECphi27,  
131 vB\_Eco\_swan01, vB\_EcoS-95, vB\_Eco\_mar001J1 and vB\_Eco\_mar002J2. These phages have  
132 previously been found to form a monophyletic cluster, which represents a putative genus within the  
133 subfamily Tunavirinae <sup>12</sup>. In addition, vB\_Eco\_SLUR29 showed some similarity to a group of  
134 phages infecting *Campylobacter* (Mash distance < 0.25), that are not currently classified by the  
135 ICTV.

136

137 A phylogeny was reconstructed using *terL*, with the top 100 hits to the vB\_Eco\_SLUR29 *terL* based  
138 on BLASTP analysis. The resultant phylogeny placed vB\_Eco\_SLUR29 within the subfamily  
139 Tunavirinae, forming a clade with the coliphages vB\_Eco\_swan01 (acc: LT841304)<sup>20</sup> , SECphi27  
140 (acc: LT961732) , vB\_Eco\_mar001J1 (acc: LR027388) , vB\_Eco\_mar002J2 (acc: LR027385) and  
141 vB\_EcoS-95 (acc: MF564201)<sup>21</sup> which is a sister group to the clade containing phage pSF-1, the  
142 sole representative of the genus *Hanrervirus* <sup>22</sup>. To further clarify the position of  
143 vB\_Eco\_SLUR29 within the subfamily Tunavirinae, a core-gene phylogeny was constructed for  
144 phages that were closest to vB\_Eco\_SLUR29. This included phages of the genera *Tlsvirus*,  
145 *Webervirus*, *Hanrervirus* and the large group of unclassified phages that infect *Campylobacter*

146 (Fig 2). Four core genes were identified in this set of phage (representative homologues are:  
147 SLUR29\_0019, SLUR29\_0039, SLUR29\_0053 and SLUR29\_0059) using the  
148 GET\_HOMOLOGUES pipeline<sup>14</sup>. The resultant four genes were concatenated and used in further  
149 phylogenetic analysis. This phylogeny confirmed that phage vB\_Eco\_SLUR29 formed a clade with  
150 the coliphages vB\_Eco\_swan01 SECphi27, vB\_EcoS-95, vB\_Eco\_mar001J1 and  
151 vB\_Eco\_mar002J2 all of which were isolated on *E.coli* and likely represent a new genus. The  
152 *Shigella* infecting phage pSf-1 again grouped with phage A16a, with this cluster being more closely  
153 related to the group of unclassified phages infecting *Campylobacter* than it is to vB\_Eco\_SLUR29.  
154

### 155 Comparative Genomics

156 Phages vB\_Eco\_SLUR29, vB\_Eco\_swan01, SECphi27, vB\_EcoS-95, vB\_Eco\_mar001J1,  
157 vB\_Eco\_mar002J2 all have average nucleotide identity (ANI) above 90% with each other and an  
158 ANI of < 80% with pSF1 (Fig 3). Whole genome comparisons of vB\_Eco\_SLUR29,  
159 vB\_Eco\_swan01, SECphi27, vB\_EcoS-95, vB\_Eco\_mar001J1, vB\_Eco\_mar002J2 reveals they  
160 have 51core genes (Fig 4) and have high degree of synteny across the genomes (Fig 4). Inclusion of  
161 the phage pSF-1 in this analysis, results in only 1 core-gene.  
162

### 163 Discussion

164  
165 We have previously isolated coliphages from the same slurry tank and have found phages that are  
166 representatives of the genera *T4virus* and *Seuratvirus*<sup>4,5</sup>. This is the first report of phage from  
167 within the subfamily *Tunavirinae* from this particular slurry tank environment. Whether this is a  
168 reflection of their abundance or due to small sample sizes remains to be seen. Comparison of  
169 vB\_Eco\_SLUR29 with its closest relatives, reveals they have all been isolated on *E.coli* K-12  
170 MG1655, although many can infect other bacteria within the Enterobacteriaceae (Fig 3)<sup>12,21</sup>.  
171

172 Given the high sequence identity between vB\_Eco\_SLUR29 and its closest relatives, it was  
173 unsurprising that it has similar morphological properties when examined by transmission electron  
174 microscopy. Phylogenetic analysis clearly places vB\_Eco\_SLUR29 within the subfamily  
175 *Tunavirinae*, in a clade that is sister to a clade that contains phages of the genus *TLsvirus* and  
176 contains the phages vB\_Eco\_mar001J1, vB\_Eco\_mar002J2, vB\_EcoS-95, vB\_Eco\_swan01 and  
177 SECphi27. Previously, we have suggested SECphi27, vB\_Eco\_mar001J1, vB\_Eco\_mar002J2,  
178 vB\_Eco\_swan01 and pSf1 are members of the same genus<sup>12</sup>. Since then *Shigella* phage pSf1 has  
179 been formally classified as the sole member of the genus *Hanrivervirus*. The addition of phages  
180 vB\_Eco\_SLUR29, vB\_EcoS-95 and *Campylobacter* infecting phages to the database further

181 clarifies the position of *Shigella* phage pSf1, into a separate clade from vB\_Eco\_mar001J1,  
182 vB\_Eco\_mar002J2 and SECphi27. The current starting point for classification of phage species is  
183 >95 % ANI<sup>23</sup>. Using this criterion, vB\_Eco\_SLUR29, vB\_Eco\_mar001J1, vB\_Eco\_mar002J2,  
184 vB\_EcoS-95, vB\_Eco\_swan01, SECphi27 and vB\_EcoS-95 would form a single species. This is  
185 inconsistent with the phylogeny observed and a higher ANI cut-off of >97% might be more suitable  
186 for this group of phages, as has previously been suggested for this and other phage groups<sup>5,12</sup>. Thus  
187 we propose this group of phages represents a new genus with four species, represented by the type  
188 phages vB\_Eco\_mar001J1, vB\_EcoS-95, vB\_Eco\_swan01 and SECphi27. With vB\_Eco\_SLUR29  
189 being the same species as phage SECphi27, which was isolated first. We propose the genus is  
190 named *Swanvirus* after the phage vB\_Eco\_swan01, which was the first isolated phage in the genus.  
191

192 All phages of the proposed genus *Swanvirus*, have a conserved and syntenous genome structure. In  
193 contrast to the conservation of genes between phages, there is greater variation in phenotypic  
194 properties. The burst size of vB\_Eco\_SLUR29 is 22, which is smaller than the reported values of  
195 115, 78 and 51 for vB\_Eco-S59, vB\_Eco\_swan01 and vB\_Eco\_mar002J2 respectively<sup>12,21</sup>. There is  
196 also considerable variation in the latent period for these phages, with vB\_Eco\_SLUR29 having a  
197 substantially longer latent period (21 min) than the reported 4, 12 and 15 min for vB\_Eco-S59,  
198 vB\_Eco\_swan01 and vB\_Eco\_mar002J2<sup>12,21</sup>. However, the reported 4 min latent period of  
199 vB\_Eco-S59 seems extraordinarily rapid and maybe an artefact of the method used.  
200

201 Phage vB\_Eco\_SLUR29 was found to rapidly lyse its host when used at an MOI of 1, but was less  
202 effective at lower MOIs. This was apparent in the local virulence index which is very close to zero  
203 at MOIs of  $1 \times 10^{-6}$  and  $1 \times 10^{-7}$  when the integrated area of the curve of infected and control samples  
204 are compared. To overcome this, we could have chosen a later point where lysis had occurred for  
205 cells infected at low MOIs. However, this would be well into the stationary phase of growth in the  
206 uninfected control so we chose to integrate from time zero to the onset of stationary phase as  
207 described in the original method<sup>19</sup>. When compared to the virulence index of phages T7, T5 and  
208 T4, it has a lower virulence index of 0.37<sup>19</sup> than any of these phages under any condition,  
209 suggesting it is not a particularly virulent phage.  
210

211 A further observation of the properties of vB\_Eco\_SLUR29 infection, was the rapid recovery of  
212 infected cells. When infected at high MOIs there was rapid lysis of host cells, with recovery of cell  
213 numbers close to an un-infected control after 13 hrs. Whilst we did not explicitly test cells at the  
214 end of the killing assay, it is most probable that this recovery in cell growth is due to the rapid  
215 selection of resistant cells. The rapid development of resistance in *E. coli* to the closely related

216 phage vB\_Eco\_S59 has been reported, suggesting there might be a minimal cost to developing  
217 resistance for this type of phage <sup>21</sup>. However, the mechanism behind the development of resistance  
218 still remains to be elucidated.

219

220 The sequencing of vB\_Eco\_SLUR29 has expanded and helped to further clarify the phylogeny of  
221 phages within the genus *Tunavirinae*, with vB\_Eco\_SLUR29 a member of putative new genus that  
222 is clearly separate from the phage pSf-1 (Fig 4). Furthermore, the characterisation of phenotypic  
223 properties reveals that phages which are similar at the genomic level, have very different  
224 phenotypic properties. This highlights the need to assess the lytic properties of phage that are  
225 genetically similar, as it cannot always be assumed they will have similar infection properties. With  
226 increasing interest in the use of phages as therapeutics, the lytic properties are important factors that  
227 will need to be considered. In part, differences in lytic properties may result from the method used  
228 for carrying out one-step experiments. Therefore, we utilised the recently developed virulence index  
229 <sup>19</sup> which should allow more consistent comparison of the lytic properties of phages from different  
230 laboratories in the future.

231

232

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234

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238 DTP

239

240 **Figure 1.** Phenotypic properties of phage vB\_Eco\_SLUR29. A) TEM image of phage  
241 vB\_Eco\_SLUR29, identifying the morphological features representative of a siphoviruses. B). One-  
242 step growth experiment (n=3), samples treated with CHCl<sub>3</sub> are closed black circles, untreated  
243 samples are stars. C) Local virulence index of vB\_Ecol\_SLUR29 at MOIs ranging from 1 - 1 x10<sup>-7</sup>.  
244 D) Killing curves of vB\_Eco\_SLUR29 against *E.coli* K-12 MG1655 (n=3).

245

246 **Figure 2.** Phylogenetic analysis of vB\_Eco\_SLUR29. The phylogeny was created using the *terL*  
247 gene as marker. Sequences were aligned with MAFFT<sup>24</sup> and trees constructed with IQ-TREE with  
248 1000 bootstrap replicates<sup>13</sup>. Bootstrap values > 70 are marked with a black circle, with increasing  
249 size proportional to the bootstrap values. The clades containing phages of the genera *Tlsvirus* and  
250 *Webervirus* were collapsed for clarity.

251

252 **Figure 3.** Phylogenetic analysis of vB\_Eco\_SLUR29. The phylogeny was created using four  
253 concatenated core-genes. Genes were aligned with MAFFT<sup>24</sup> and trees constructed with IQ-TREE  
254<sup>13</sup>, using a SYM+R4 model of evolution. Bootstrap values > 70 are marked with a black circle, with  
255 increasing size proportional to the bootstrap values. The clades containing phages of the genera  
256 *Tlsvirus* and *Webervirus* were collapsed for clarity in presenting the tree.

257

258 **Figure 4.** Comparative genomics of vB\_Eco\_SLUR29 with phages SECphi27, vB\_Eco\_swan01,  
259 vB\_EcoS-95, vB\_Eco\_mar001J1 and vB\_Eco\_mar002J2. Alignments were constructed with  
260 EasyFig using blastn<sup>25</sup>. Selected genes are annotated for vB\_Eco\_SLUR29. Genes that are  
261 coloured purple and green are core-genes and accessory respectively, and are only marked on phage  
262 vB\_Eco\_SLUR29.

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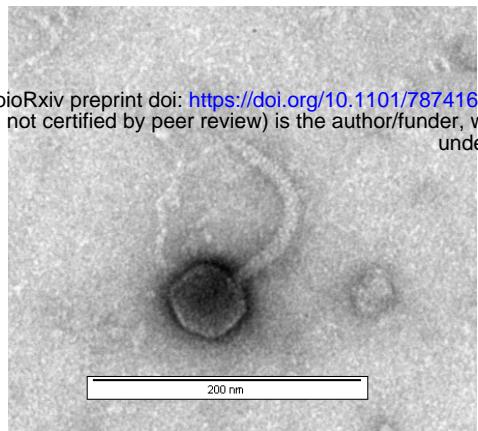
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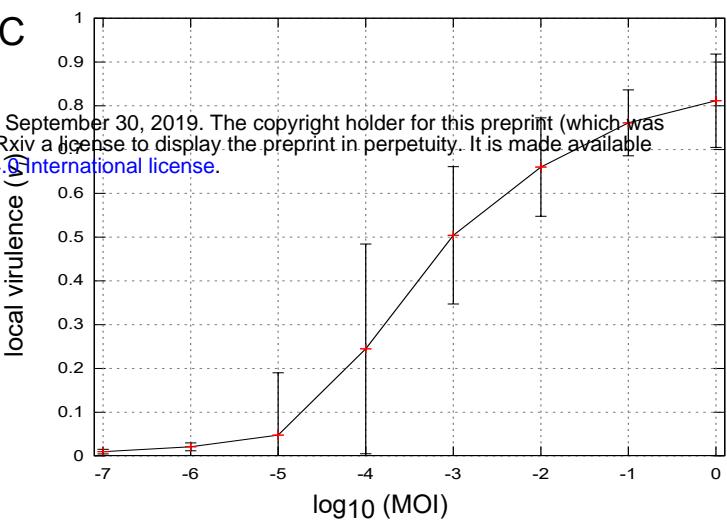
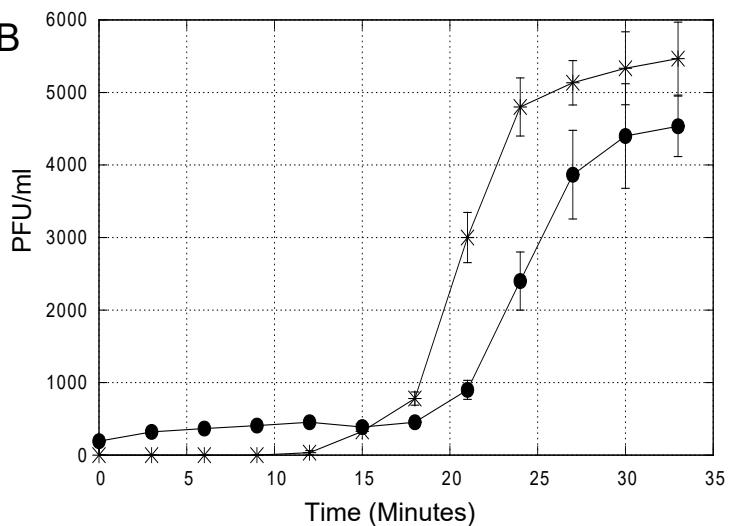
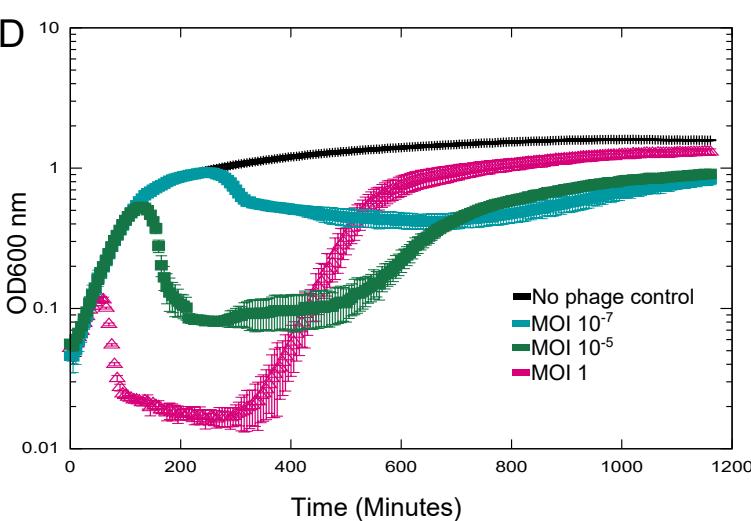
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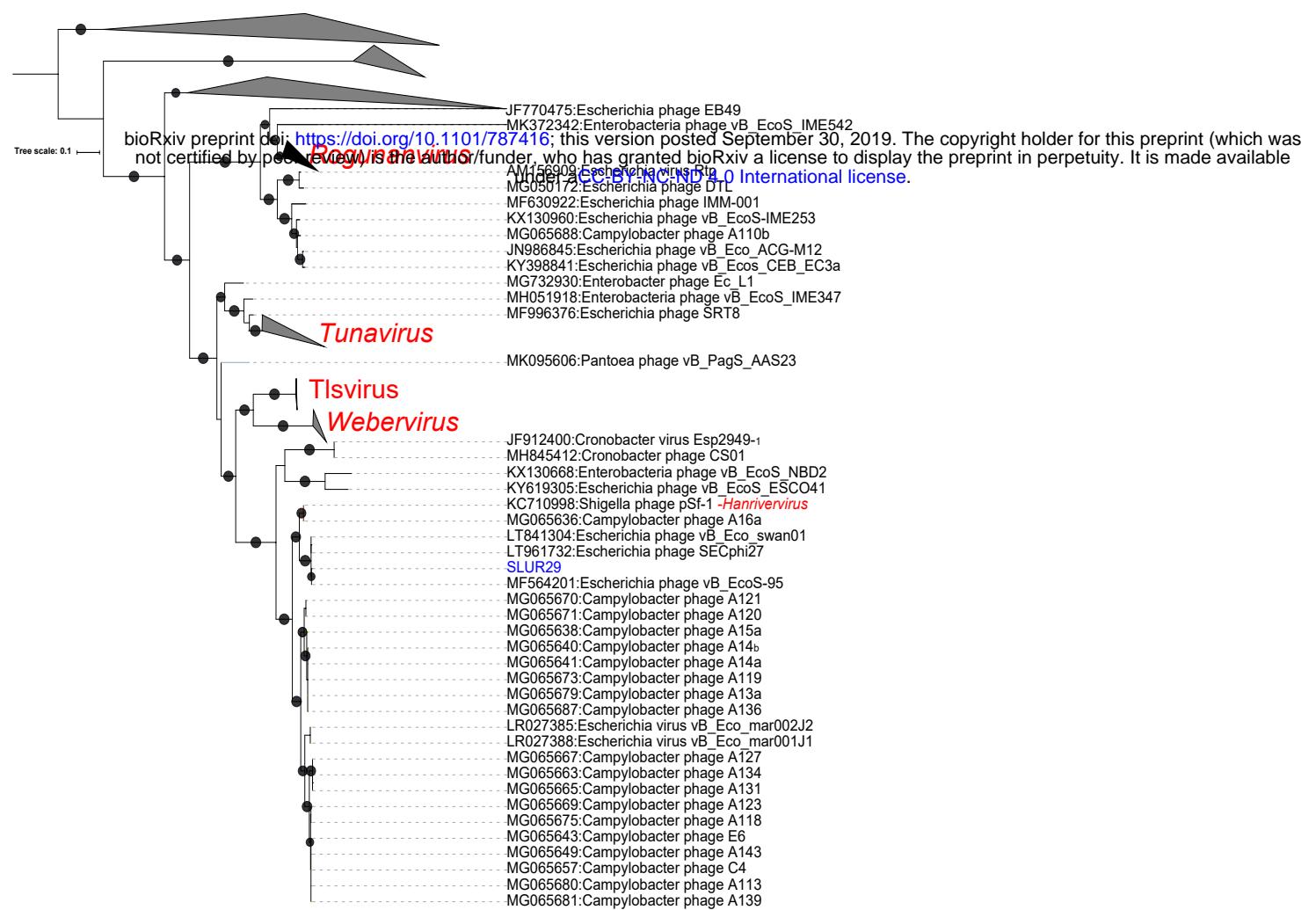
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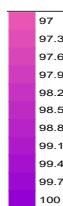
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**C****B****D**



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Host

Legend for host bacteria:

- Escherichia
- Shigella
- Campylobacter
- Chronobacter

