

# Isolation, Characterization, and Comparative Genomic Analysis of Bacteriophage Ec\_MI-02 from Pigeon Feces Infecting *Escherichia coli* O157:H7

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## SUPPLEMENTARY INFORMATION

**Table S1:** Host range of the two bacteriophages Ec\_MI-02 and UAE\_MI-01 isolated from pigeon feces against different gram-positive and gram-negative bacteria.

Bacterial strains	Bacteriophage Ec_MI-02 (current study)	Bacteriophage UAE_MI-01 (Sultan-Alolama et al., 2022) [62]
<i>Escherichia coli</i> O157:H7 NCTC 12900	++	++
<i>Escherichia coli</i> ATCC 25922	–	–
<i>Escherichia coli</i> ATCC 8739	++	++
<i>Escherichia coli</i> ATCC 35218	+	–
<i>Escherichia coli</i> ATCC 15223	+	++
<i>Escherichia coli</i> ATCC 23227	+	–
<i>Escherichia coli</i> ATCC 9637	+	–
<i>Escherichia coli</i> ATCC 23224	+	–
<i>Escherichia coli</i> (patient isolate-urine)	+	–
<i>Escherichia coli</i> ESBL-producing (patient isolate-blood)	–	–
<i>Bacillus subtilis</i> ATCC 6051	–	–
<i>Pseudomonas aeruginosa</i> ATCC 25668	–	–
<i>Pseudomonas aeruginosa</i> ATCC 27853	–	–
Methicillin-resistant <i>Staphylococcus aureus</i> (patient isolate)	–	–
<i>Staphylococcus aureus</i> ATCC 6358	–	–
<i>Staphylococcus aureus</i> ATCC 29213	–	–
<i>Staphylococcus epidermidis</i> ATCC 12228	–	–
<i>Staphylococcus saprophyticus</i> ATCC-BAA 750	–	–
<i>Streptococcus pyogenes</i> ATCC 19615	–	–
<i>Enterococcus faecalis</i> ATCC 51299	–	–
<i>Enterococcus faecalis</i> (Patient isolate)	–	–
<i>Enterococcus casseliflavus</i> (Patient isolate)	–	–
<i>Enterobacter aerogenes</i> ATCC 13018	–	–
<i>Enterobacter hormaechei</i> (patient isolate)	–	–
<i>Klebsiella pneumonia</i> ESBL-producing ATCC 700603	–	–
<i>Klebsiella pneumonia</i> KPC 2 +ve (patient isolate)	–	–
<i>Haemophilus influenzae</i> ATCC 9007	–	–
<i>Stenotrophomonas maltophilia</i> ATCC 17666	–	–
<i>Salmonella enterica</i> ATCC 14028	–	–
<i>Salmonella</i> sp. (patient isolate)	–	–
<i>Proteus vulgaris</i> ATCC 29905	–	–
<i>Mycobacterium smegmatis</i> ATCC 607	–	–
NCTC: national collection of type cultures; ATCC: American type culture collection; ESBL: extended-spectrum beta-lactamase. (++) = host species susceptible to phage lysis with clear plaque, (+) = host species susceptible to phage lysis with turbid plaque, and (–) indicates no plaque. <i>Escherichia coli</i> O157:H7 NCTC 12900 was used as the propagation host.		

**Table S2:** Effect of different pH values on the viability of the two bacteriophages Ec\_MI-02 and UAE\_MI-01 isolated from pigeon feces.

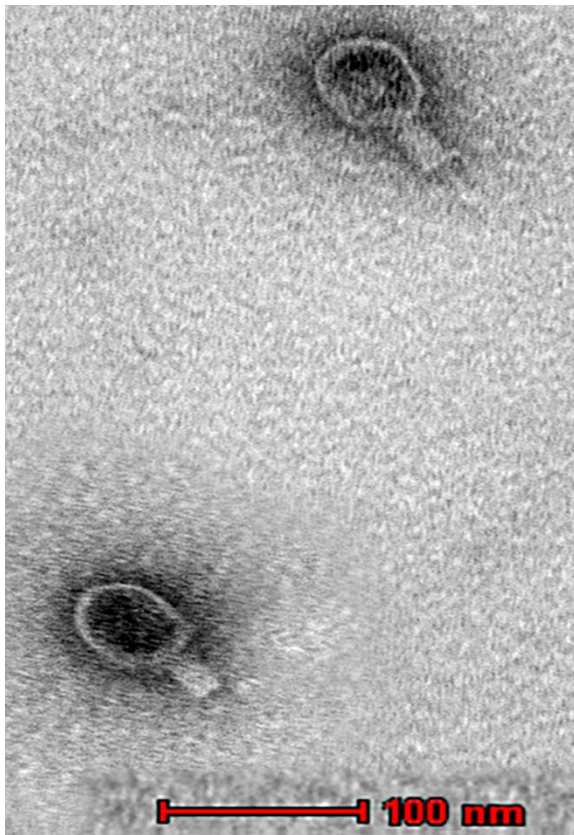
pH Value	Log <sub>10</sub> pfu/mL of bacteriophage Ec_MI-02 (current study)	Log <sub>10</sub> pfu/mL of bacteriophage UAE_MI-01 (Sultan-Alolama et al., 2022) [62]
3	5.93 ± 0.048 aA	0.00 ± 0.00 aB
4	5.96 ± 0.047 aA	5.72 ± 0.12 bB
7	5.91 ± 0.10 aA	7.79 ± 0.11 cB
9	5.89 ± 0.10 aA	7.76 ± 0.15 cB
10	5.92 ± 0.13 aA	7.78 ± 0.16 cB
<p>Values of the number of phages (log<sub>10</sub> pfu/mL) are means of four independent replicates ± standard deviation. Values with the same lower-case letters are not significantly (<math>p &gt; 0.05</math>) different within columns according to Fisher's Protected LSD Test. Values with the same upper-case letters are not significantly (<math>p &gt; 0.05</math>) different within rows according to Fisher's Protected LSD Test. pfu = plaque forming units. <i>Escherichia coli</i> O157:H7 NCTC 12900 was used as the propagation host.</p>		

**Table S3:** Effect of different temperatures on the viability of the two bacteriophages Ec\_MI-02 and UAE\_MI-01 isolated from pigeon feces.

Temperature	Log <sub>10</sub> pfu/mL of bacteriophage Ec_MI-02 (current study)	Log <sub>10</sub> pfu/mL of bacteriophage UAE_MI-01 (Sultan-Alolama et al., 2022) [62]
25 °C for 15 minutes	5.75 ± 0.078 aA	7.46 ± 0.13 aB
25 °C for 30 minutes	5.61 ± 0.055 aB	7.42 ± 0.15 aB
45 °C for 15 minutes	5.46 ± 0.055 bA	7.49 ± 0.14 aB
45 °C for 30 minutes	5.43 ± 0.032 bA	7.48 ± 0.21 aB
55 °C for 15 minutes	5.23 ± 0.087 cA	7.43 ± 0.18 aB
55 °C for 30 minutes	5.19 ± 0.114 cA	7.46 ± 0.12 aB
65 °C for 15 minutes	4.91 ± 0.080 dA	7.47 ± 0.19 aB
65 °C for 30 minutes	4.85 ± 0.067 dA	7.43 ± 0.26 aB
75 °C for 15 minutes	0.00 ± 0.00 eA	3.29 ± 0.09 bB
75 °C for 30 minutes	0.00 ± 0.00 eA	0.00 ± 0.00 bA
100 °C for 15 minutes	0.00 ± 0.00 eA	0.00 ± 0.00 cA
100 °C for 30 minutes	0.00 ± 0.00 eA	0.00 ± 0.00 bA
<p>Values of the number of phages (log<sub>10</sub> pfu/mL) are means of four independent replicates ± standard deviation. Values with the same lower-case letters are not significantly (<math>p &gt; 0.05</math>) different within columns according to Fisher's Protected LSD Test. Values with the same upper-case letters are not significantly (<math>p &gt; 0.05</math>) different within rows according to Fisher's Protected LSD Test. pfu = plaque forming units. <i>Escherichia coli</i> O157:H7 NCTC 12900 was used as the propagation host.</p>		

**Table S4:** Effect of different common laboratory disinfectants on the viability of the two bacteriophages Ec\_MI-02 and UAE\_MI-01 isolated from pigeon feces.

Disinfectant	(log <sub>10</sub> pfu/mL) of bacteriophage Ec_MI-02 (current study)	(log <sub>10</sub> pfu/mL) of bacteriophage UAE_MI-01 (Sultan-Alolama et al., 2022) [62]
Ethanol 70% Initial Titer	6.19 ± 0.028 aA	7.48 ± 0.11 aB
Ethanol 70% After 2 min	5.91 ± 0.155 bA	6.62 ± 0.06 bB
Sodium hypochlorite 2% Initial Titer	6.18 ± 0.075 aA	7.51 ± 0.15 aB
Sodium hypochlorite 2% After 2 min	0.00% ± 0.00 cA	6.09 ± 0.10 cB
Commercial disinfectant 20% Initial Titer	6.20 ± 0.057 aA	7.45 ± 0.10 aB
Commercial disinfectant 20% After 2 min	3.14 ± 0.240 dA	7.09 ± 0.06 dB
Values of the number of phages (log <sub>10</sub> pfu/mL) are means of four independent replicates ± standard deviation. Values with the same lower-case letters are not significantly ( $p > 0.05$ ) different within columns according to Fisher's Protected LSD Test. Values with the same upper-case letters are not significantly ( $p > 0.05$ ) different within rows according to Fisher's Protected LSD Test. pfu = plaque forming units. <i>Escherichia coli</i> O157:H7 NCTC 12900 was used as the propagation host.		

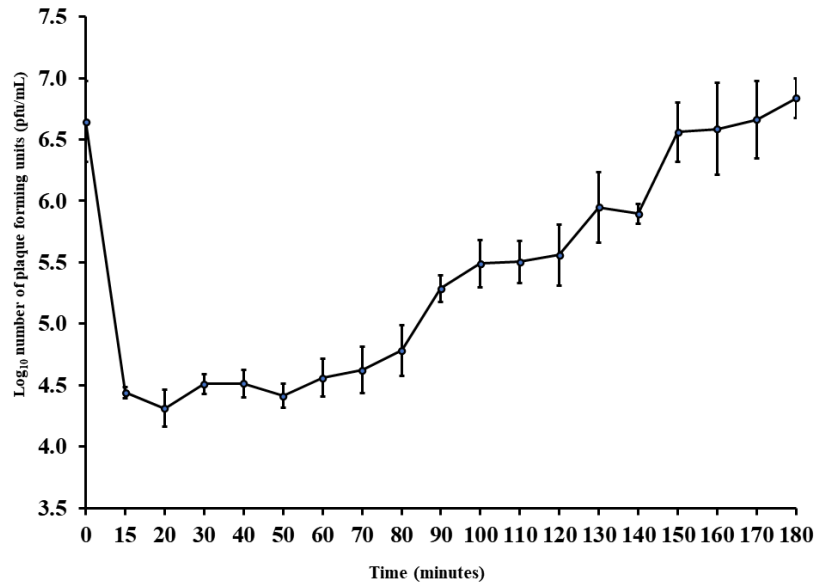


Ec\_MI-02

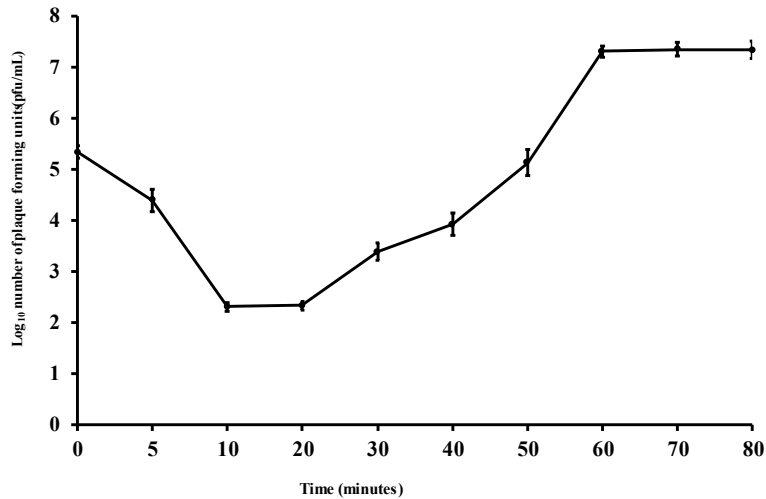


UAE\_MI-01

**Figure S1.** Transmission electron micrograph of the two bacteriophages, Ec\_MI-02 (current study, left) and UAE\_MI-01 (Sultan-Alolama et al., 2022) [62], right) isolated from pigeon feces Scale bar = 100 nm.

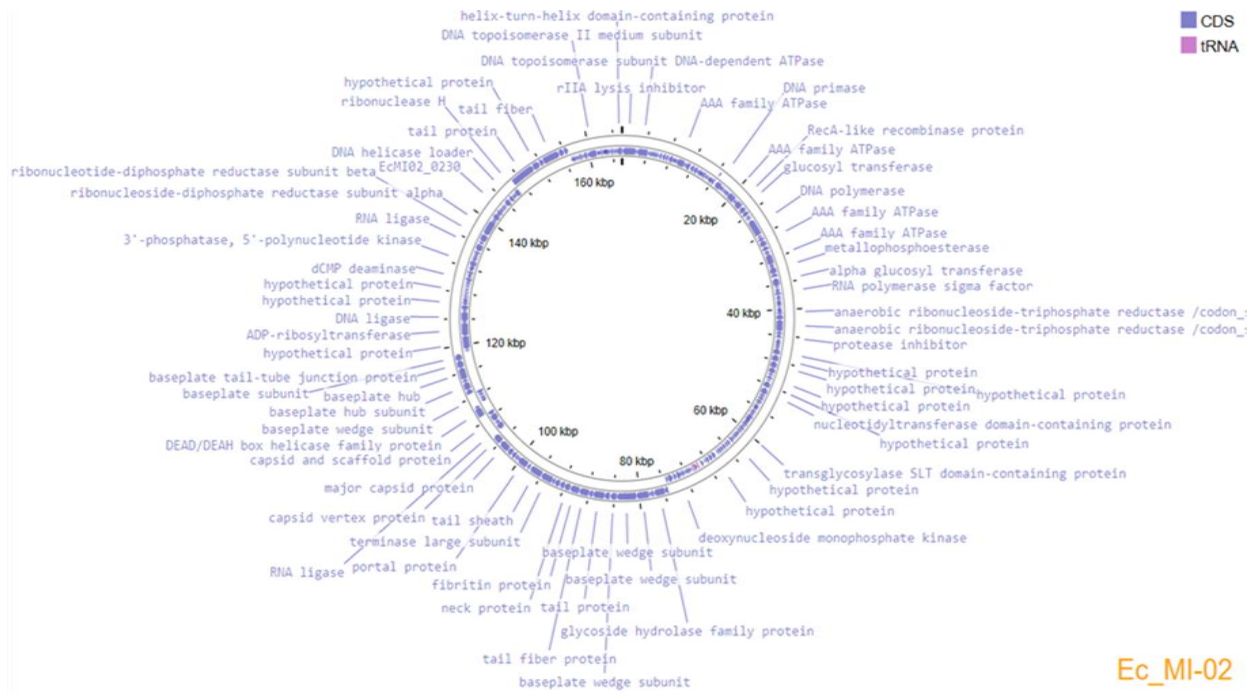


**Ec\_MI-02**

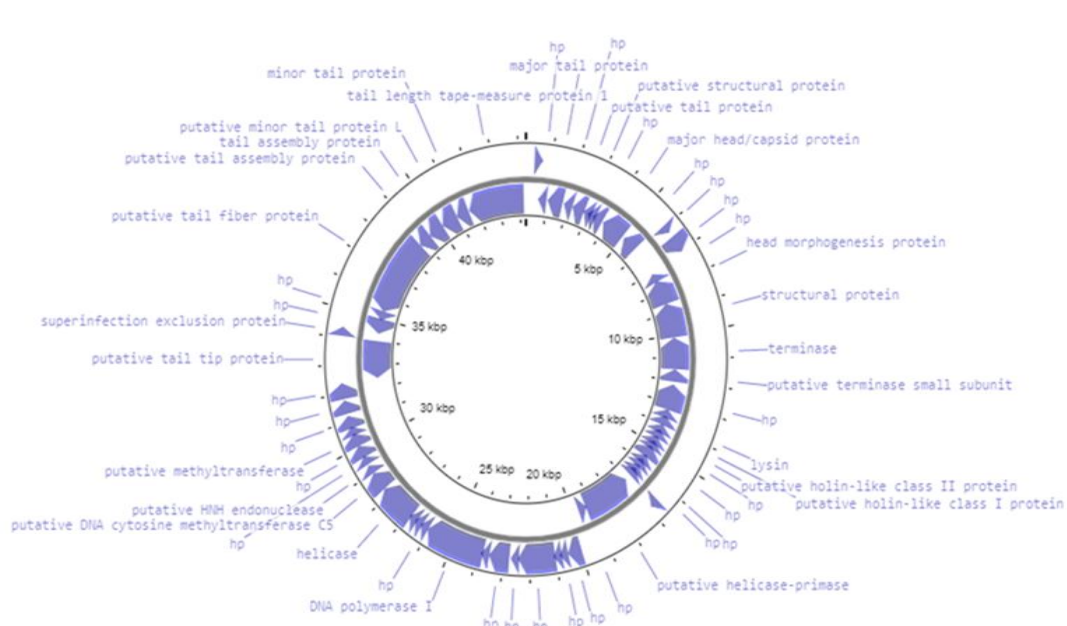


**UAE\_MI-01**

**Figure S2.** One-step growth curve of the two bacteriophages, Ec\_MI-02 (current study, top) and UAE\_MI-01 (Sultan-Alolama et al., 2022) [62], bottom) isolated from pigeon feces with *Escherichia coli* O157:H7 NCTC 12900 as the propagation host. Values are means  $\pm$  standard deviation of four replicates for each timing. Bars represent standard deviation. pfu = plaque forming units.



Ec\_MI-02



Escherichia phage UAE\_MI-01, complete genome.

**Figure S3.** Genome organization map of the two bacteriophages, Ec\_MI-02 (current study, top) and UAE\_MI-01 (Sultan-Alolama et al., 2022) [62], bottom) isolated from pigeon's feces with *Escherichia coli* O157:H7 NCTC 12900 as the propagation host.



## Reference

62. Sultan-Alolama, M.I.; Amin, A.; El-Tarabily, K.A.; Vijayan, R. Characterization and genomic analysis of *Escherichia coli* O157:H7 phage UAE\_MI-01 isolated from birds. *Int. J. Mol. Sci.* **2022**, *23*, 14846. doi:10.3390/ijms232314846