

Supplementary Materials

Table S1. List of annotated ORFs with the size, location and predicted functions in the genome of Ro145clw.

| ORFs | Predicted Function ^a | Functional Category ^b | Starting bp | Ending bp | Length of ORF (bp) | Direction |
|------|--|----------------------------------|-------------|-----------|--------------------|-----------|
| 1 | Major capsid protein CDS | Structural protein | 299 | 1348 | 1050 | forward |
| 2 | hypothetical protein CDS | n/a | 1408 | 1707 | 300 | forward |
| 3 | Decoration protein CDS | Structural protein | 1720 | 2070 | 351 | forward |
| 4 | hypothetical protein CDS | n/a | 2107 | 2286 | 180 | forward |
| 5 | hypothetical protein CDS | n/a | 2290 | 2802 | 513 | forward |
| 6 | hypothetical protein CDS | n/a | 2805 | 3419 | 615 | forward |
| 7 | hypothetical protein CDS | n/a | 3419 | 3778 | 360 | forward |
| 8 | hypothetical protein CDS | n/a | 4173 | 4586 | 414 | forward |
| 9 | Tail protein CDS | Structural protein | 4589 | 5755 | 1167 | forward |
| 10 | hypothetical protein CDS | n/a | 5781 | 6269 | 489 | reverse |
| 11 | hypothetical protein CDS | n/a | 6313 | 6543 | 231 | reverse |
| 12 | hypothetical protein CDS | n/a | 6557 | 7027 | 471 | reverse |
| 13 | Putative calcineurin-like phosphoesterase superfamily domain protein CDS | DNA replication | 7024 | 8154 | 1131 | reverse |
| 14 | hypothetical protein CDS | n/a | 8193 | 8384 | 192 | reverse |
| 15 | hypothetical protein CDS | n/a | 8552 | 8971 | 420 | forward |
| 16 | hypothetical protein CDS | n/a | 9070 | 9336 | 267 | forward |
| 17 | Tape measure protein CDS | Structural protein | 9329 | 11599 | 2271 | forward |
| 18 | hypothetical protein CDS | n/a | 12094 | 12609 | 516 | forward |
| 19 | hypothetical protein CDS | n/a | 12606 | 12971 | 366 | forward |

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|----|--|--------------------|-------|-------|------|---------|
| 20 | Putative tail assembly chaperone CDS | Structural protein | 12962 | 15517 | 2556 | forward |
| 21 | Putative tail fiber protein CDS | Structural protein | 15530 | 17620 | 2091 | forward |
| 22 | hypothetical protein CDS | n/a | 17649 | 17789 | 141 | reverse |
| 23 | hypothetical protein CDS | n/a | 17786 | 17974 | 189 | reverse |
| 24 | putative helicase CDS | DNA replication | 17971 | 19395 | 1425 | reverse |
| 25 | hypothetical protein CDS | n/a | 19648 | 19842 | 195 | reverse |
| 26 | hypothetical protein CDS | n/a | 19842 | 20045 | 204 | reverse |
| 27 | hypothetical protein CDS | n/a | 20078 | 20362 | 285 | reverse |
| 28 | hypothetical protein CDS | n/a | 20362 | 20511 | 150 | reverse |
| 29 | hypothetical protein CDS | n/a | 20594 | 20725 | 132 | reverse |
| 30 | Putative thermostable DNA polymerase I CDS | DNA replication | 20738 | 22915 | 2178 | reverse |
| 31 | hypothetical protein CDS | n/a | 22974 | 23603 | 630 | reverse |
| 32 | hypothetical protein CDS | n/a | 23690 | 23845 | 156 | reverse |
| 33 | Putative PD-(D/E)XK nuclease superfamily protein CDS | DNA replication | 23842 | 25083 | 1242 | reverse |
| 34 | hypothetical protein CDS | n/a | 25055 | 25351 | 297 | reverse |
| 35 | hypothetical protein CDS | n/a | 25395 | 25895 | 501 | reverse |
| 36 | Transcriptional repressor Dica CDS | DNA replication | 26021 | 26236 | 216 | forward |
| 37 | Putative helicase-primase CDS | DNA replication | 26254 | 28494 | 2241 | reverse |
| 38 | hypothetical protein CDS | n/a | 28553 | 28786 | 234 | reverse |
| 39 | hypothetical protein CDS | n/a | 28783 | 28953 | 171 | reverse |
| 40 | hypothetical protein CDS | n/a | 29448 | 29633 | 186 | forward |
| 41 | hypothetical protein CDS | n/a | 29633 | 29887 | 255 | forward |

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|----|----------------------------------|--------------------|-------|-------|------|---------|
| 42 | hypothetical protein CDS | n/a | 30096 | 30305 | 210 | forward |
| 43 | hypothetical protein CDS | n/a | 30430 | 30606 | 177 | forward |
| 44 | hypothetical protein CDS | n/a | 30673 | 30822 | 150 | forward |
| 45 | hypothetical protein CDS | n/a | 30812 | 31081 | 270 | forward |
| 46 | hypothetical protein CDS | n/a | 31101 | 31211 | 111 | forward |
| 47 | hypothetical protein CDS | n/a | 31217 | 31579 | 363 | forward |
| 48 | hypothetical protein CDS | n/a | 31576 | 31779 | 204 | forward |
| 49 | hypothetical protein CDS | n/a | 31782 | 31964 | 183 | forward |
| 50 | hypothetical protein CDS | n/a | 31964 | 32257 | 294 | forward |
| 51 | Putative holin-like class II CDS | Cell lysis | 32333 | 32641 | 309 | forward |
| 52 | Putative holin-like class I CDS | Cell lysis | 32634 | 32906 | 273 | forward |
| 53 | Endolysin CDS | Cell lysis | 32884 | 33372 | 489 | forward |
| 54 | hypothetical protein CDS | n/a | 33628 | 33777 | 150 | forward |
| 55 | hypothetical protein CDS | n/a | 33774 | 33944 | 171 | forward |
| 56 | hypothetical protein CDS | n/a | 33941 | 34186 | 246 | forward |
| 57 | hypothetical protein CDS | n/a | 34218 | 34457 | 240 | forward |
| 58 | hypothetical protein CDS | n/a | 34992 | 35228 | 237 | forward |
| 59 | hypothetical protein CDS | n/a | 35225 | 35407 | 183 | forward |
| 60 | hypothetical protein CDS | n/a | 35627 | 36172 | 546 | forward |
| 61 | putative terminase CDS | Packaging | 36169 | 37419 | 1251 | forward |
| 62 | Putative structural protein CDS | Structural protein | 37432 | 38907 | 1476 | forward |
| 63 | Putative head protein CDS | Structural protein | 38904 | 39959 | 1056 | forward |
| 64 | Putative tail protein CDS | Structural protein | 39959 | 40420 | 462 | forward |
| 65 | hypothetical protein CDS | n/a | 40449 | 40673 | 225 | reverse |
| 66 | putative spanin protein CDS | Cell lysis | 41048 | 41434 | 387 | forward |
| 67 | putative spanin protein CDS | Cell lysis | 41397 | 41543 | 147 | forward |

^a CDS means coding sequence.

^b n/a means the ORFs do not have any functional category.

Table S2. Storage evaluation of phage Ro145clw in 25% glycerol at -80°C for 5 months.

| Temperature | Before storage (Log PFU/ml)* | After storage (Log PFU/ml) ^α |
|-------------|------------------------------|---|
| -80°C | 10.43 | 10.22 |

* Phage titers were obtained prior to addition of glycerol for frozen storage.

^α Phage titers were obtained right after thawing the phage solution.

Table S3. Comparison of the antimicrobial activities of phage Ro145clw (MOI of 100) against different EOP bacterial strains* (initial concentration= 7 log CFU/ml) in LB broth at 25°C.

| Incubation time (h) | Bacterial reduction (Log CFU/ml) ^α | |
|---------------------|---|-------------------------|
| | <i>E. coli</i> O145:NM | <i>E. coli</i> O145:H28 |
| 0 h | 0.40 ± 0.13 | 0.27 ± 0.05 |
| 6 h | 6.20 ± 0.26 | 3.51 ± 0.30 |
| 24 h | 1.65 ± 0.07 | 1.05 ± 0.16 |

* Two strains, including *E. coli* O145:NM and *E. coli* O145:H28 (RM13514), were used; The *E. coli* O145:NM strain has EOP=1.05 and *E. coli* O145:H28 strain has EOP < 0.001.

^α Reduction data are represented as means ± standard deviation and consist of three replicates compared to the bacterial population (log CFU/ml) recovered from untreated controls (no phages) at each time point.

Table S4. Bacterial strains, including *E. coli* and *Salmonella*, used in current study for either phage isolation or host range test of the isolated phage.

| Internal Ref. No. | Strain | Source ^a | Serogroup | <i>eae</i> | <i>Stx1</i> | <i>Stx2</i> | Note ^b |
|-------------------|-------------------------|---------------------|-----------|------------|-------------|-------------|-------------------|
| RM17857 | <i>E. coli</i> O26:H18 | water | 26 | - | + | - | |
| RM18118 | <i>E. coli</i> O26 | water | 26 | + | + | - | |
| RM18132 | <i>E. coli</i> O26 | water | 26 | + | + | - | Host panel |
| RM17133 | <i>E. coli</i> O26 | water | 26 | - | + | - | Host panel |
| RM12551 | <i>E. coli</i> O103:H2 | water | 103 | + | + | - | |
| RM13322 | <i>E. coli</i> O103:H2 | cattle feces | 103 | + | + | - | Host panel |
| RM8356 | <i>E. coli</i> O103 | water | 103 | - | - | + | |
| RM10744 | <i>E. coli</i> O103 | cattle feces | 103 | + | - | + | Host panel |
| RM10046 | <i>E. coli</i> O121:H19 | cattle feces | 121 | + | - | + | |
| RM10068 | <i>E. coli</i> O121:H19 | trough water | 121 | + | - | + | |
| RM8082 | <i>E. coli</i> O121 | cattle feces | 121 | - | + | + | Host panel |
| RM12997 | <i>E. coli</i> O121 | pig feces | 121 | - | + | - | Host panel |
| RM13483 | <i>E. coli</i> O111:H2 | cattle feces | 111 | + | + | - | |
| RM13789 | <i>E. coli</i> O111 | water | 111 | + | + | - | |
| RM11765 | <i>E. coli</i> O111 | water | 111 | + | + | - | Host panel |
| RM14488 | <i>E. coli</i> O111 | water | 111 | + | - | + | Host panel |
| RM8732 | <i>E. coli</i> O145 | water | 145 | + | + | - | |
| RM11691 | <i>E. coli</i> O145 | water | 145 | + | + | - | |
| RM12367 | <i>E. coli</i> O145 | water | 145 | + | - | + | Host panel |
| RM10808 | <i>E. coli</i> O145 | cattle feces | 145 | + | + | - | Host panel |
| RM9872 | <i>E. coli</i> O145:H28 | Cattle feces | 145 | - | - | + | |
| RM13514 | <i>E. coli</i> O145:H28 | Outbreak strain | 145 | + | - | + | |
| RM13516 | <i>E. coli</i> O145:H28 | Outbreak strain | 145 | + | - | + | |
| RM12761 | <i>E. coli</i> O145:H28 | Outbreak strain | 145 | + | - | + | |

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|--------------|---|-----------------|------|---|---|---|------------|
| RM12581 | <i>E. coli</i> O145:H28 | Outbreak strain | 145 | + | - | + | |
| SJ23 | <i>E. coli</i> O145:NM | environment | 145 | - | + | + | |
| 94-0491 | <i>E. coli</i> O145:H- | environment | 145 | - | + | + | |
| RM10729 | <i>E. coli</i> O45 | cattle | 45 | - | + | - | Host panel |
| RM13726 | <i>E. coli</i> O45 | cattle | 45 | - | + | - | |
| RM13745 | <i>E. coli</i> O45 | cattle | 45 | - | + | - | |
| RM13752 | <i>E. coli</i> O45 | cattle | 45 | - | + | - | Host panel |
| RM18959 | <i>E. coli</i> O157:H7 | water | O157 | + | - | + | Host panel |
| RM18961 | <i>E. coli</i> O157:H7 | water | O157 | + | - | + | |
| RM18972 | <i>E. coli</i> O157:H7 | water | O157 | + | + | + | |
| RM18974 | <i>E. coli</i> O157:H7 | water | O157 | + | - | + | Host panel |
| DH5 α | Generic <i>E. coli</i> | | | | | | Host panel |
| ATCC 13706 | Generic <i>E. coli</i> | | | | | | Host panel |
| ATCC 43888 | <i>E. coli</i> O157:H7 (without <i>stx</i> gene) | | O157 | | | | Host panel |
| n/a | <i>Salmonella</i> Montevideo 51 | | | | | | |
| n/a | <i>Salmonella</i> Newport H1073 | | | | | | |
| n/a | <i>Salmonella</i> Heidelberg 45955 | | | | | | |
| n/a | <i>Salmonella</i> Enteritidis PT30 | | | | | | |
| n/a | <i>Salmonella</i> Typhimurium 14028 | | | | | | |

^a The source is the type of environmental samples where the strain was originally isolated from.

^b Host panel means the strains were used for the enrichment of the environmental samples for phage isolation.

“-” is the negative and “+” is positive of the PCR results.

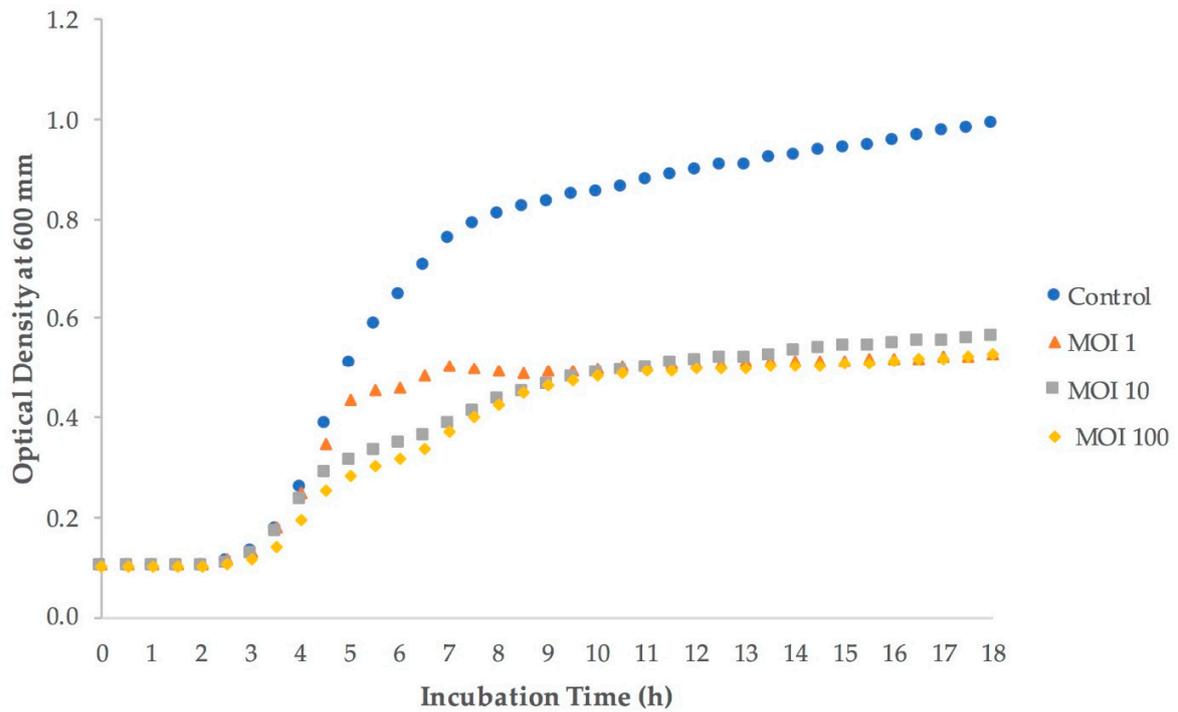


Figure S1. Bacterial challenge assay of the *E. coli* O145:H28 strain (RM13514) treated with phage Ro145clw in 96-well plate. The OD₆₀₀ was measured by spectrophotometer every 0.5 h for the period of 18-h incubation at 37°C. The control (circles) contains only bacterial culture. MOI 1 (triangles) contains bacterial culture treated with the equal concentration of phage Ro145clw; MOI 10 (squares) contains bacterial culture treated with 10-fold more concentration of the phage; MOI 100 (diamonds) contains bacterial culture treated with 100-fold more concentration of the phage.