

Table S1. Materials, including strains and plasmids used in our study.

Strains	Description	Source
DH5 α	Host cells for plasmid amplification	TsingKe, Beijing, China
EcN Δ araBAD::FRT	Deletion of araBAD gene and insertion of FRT locus in <i>E. coli</i> Nissle 1917	Our lab
SP, <i>Salmonella</i> pullorum	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar pullorum ATCC9120	Our lab
SC, <i>Salmonella</i> choleraesuis	<i>Salmonella enterica</i> subsp. <i>Enterica</i> serovar choleraesuis ATCC13312	Our lab
EcN Δ slyD	Deletion of slyD gene in EcN	This study
EcN Δ dnaJ	Deletion of dnaJ gene in EcN	This study
Plasmids		
araC-ParaBAD-ID52-E	The template for constructing gene vectors for mutant genes	Our lab
pBV220- φ X174-E	The template for constructing expression vector of gene φ X174-E	Our lab
araC-ParaBAD-ID52-E-W4A	Lysis plasmid used in this study	This study
araC-ParaBAD-ID52-E-C61S	The mutant gene of ID52-E	Our lab
araC-ParaBAD-ID52-E-C93S	The mutant gene of ID52-E	Our lab
araC-ParaBAD-ID52-E-C61S&C93S	The mutant gene of ID52-E	Our lab
araC-ParaBAD- φ X174-E	Lysis plasmid used in this study	This study
pLysS-pHLIP	The template for constructing expression vector of mraY	Our lab
pCas	Plasmid for CRISPR-Cas9	Our lab
pTargetF	Plasmid for CRISPR-Cas9	Our lab

Table S2. Materials, including primers used in our study.

Primers	
φ X174-E-F	TCGAGCTCTAAGGAGGTATAAAAAATGGTACGCTGGACTTTGTG
φ X174-E-R	TCAAAC TGC GGATGAGACCACTCCTTCCGACGTAAATT
ID52E-F	ATGGAACGCTGGACCTTAAGC
ID52E-R	ACCGCCAGTACCGCGAC
E2V-F	GGTTATAAAAATGGTACGCTGGACCTTAAGCGGCATT
E2V-R	AATGCCGCTTAAGGTCCAGCGTACCATTTTATAACC
R3A-F	TATAAAAATGGAAGCGTGGACCTTAAGCGGCATTCTG
R3A-R	CAGAACGCCGTTAAGGTCACGCTCCATTTTATA
W4A-F	AAAAAAATGGAACGCCGACCTTAAGCGGCATTCTGGCG
W4A-R	CGCCAGAAATGCCGCTTAAGGTGCGCGTCCATT
S7W-F	ATGGAACGCTGGACCTTATGGGCATTCTGGCGTTCT
S7W-R	AGAAACGCCAGAATGCCCTAAAGGTCCAGCGTCCAT
G8D-F	GAACGCTGGACCTTAAGCGATATTCTGGCGTTCTGCT
G8D-R	AGCAGAAACGCCAGAATTCGCTTAAGGTCCAGCGTTC
I9T-F	CGCTGGACCTTAAGCGGCACCCGGCGTTCTGCTTCT
I9T-R	AGAACGAGAACGCCAGGGTGCCTTAAGGTCCAGCG
L10A-F	CCTTAAGCGGCATTGCGCGTTCTGCTTCTGCTGAGC
L10A-R	GCTCAGCAGAACGCCGCAATGCCGCTTAAGG
P21A-F	CTGCTGAGCCTGCTCTGGCGAGCCTGCTGATTATGTT
P21A-R	AACATAATCAGCAGGCTGCCAGAACGCAGGCTCAGCAG
R33K-F	TTATTCCGAGCACCTTAAACGCCCGTGCTGAGCTGG
R33K-R	CCAGCTCAGCACCGGGCGTTAAAGGTGCTCGGAATAA
L37S-F	ACCTTCGCCGCCGGTGTCAAGCTGAAAGTGCAGAG
L37S-R	CTCTGCACTTCCAGCTTGTGACACCGGGCGGAAAGGT
F66C-F	CTGCAGCCCCCTCTGTGAGCTTGTGCCGGAAA
F66C-R	TTTCCGGCACAAAGCTGACAGAACGCCGGCTGCG
S83C-F	GATGCCGAAACAGACCTGCGTAACAACATGCGC
S83C-R	GCGCATAGTTGTCACGCCAGGTCTTTCGGCATC
linearized	
araC-ParaBAD-ID52-E-F	TGGTCTCATCCGAGTTGA
linearized	TTTTTATAACCTCCTAGAGCTCGA
araC-ParaBAD-ID52-E-R	
α 3-F	GCTCTAAGGAGGTATAAAAAATGGAACGCTGGACCTTAC
α 3-R	TCAAAC TGC GGATGAGACCAACGCCAGTACCCAGTT
G4-F	GCTCTAAGGAGGTATAAAAAATGGAACATTGGACTTTAACG
G4-R	TCAAAC TGC GGATGAGACCAACGCCAGTACCCAGATCTT
mraY-F	TTAGTGGTGGTGGTGGTGTAAACCACACCTCGATGTAAAT

<i>mraY</i> -R	AAAGGAAAGGAGGAAAGAAATAATGCTTGAGCAAGTCATTCTGTT
linearized pLysS-pHLIP-F	TATTCTTCCCTCCTTCCTT
linearized pLysS-pHLIP-R	GGTTACACCACCACCACTAATT
SN20-F	CCTAGGTATAACTAGTGTACCGGACCCCTGGTCGGTGTAGAAATAGC
SN20-R	ACTAGTATTACCTAGGACTGAGC
DH20-F	CCTAGGTATAACTAGTCATTCCGACTCTGGAAGAGTGTAGAAATAGC
DH20-R	ACTAGTATTACCTAGGACTGAGC
<i>slyD</i> -up1000-F	GGCACCGAGTCGGTGCACAGTGATTTCATCCATATCTCC
<i>slyD</i> -up1000-R	TTCCCATGCTCAGGAGATATCTATCGAAAAGGTGACAAAAAA
<i>slyD</i> -dn1000-F	TTTTGTCACCTTTCGATAGATATCTCTGAGCATGGAA
<i>slyD</i> -dn1000-R	TCGACTCTAGAGAACATTCAAAAAAACTCCGCATCAGGCAGCA
<i>dnaJ</i> -up1000-F	GCACCGAGTCGGTGCACCATGGGGGTGTGATGA
<i>dnaJ</i> -up1000-R	AGGTTTGGGGAGGCTTTTGATTGCCCTAG
<i>dnaJ</i> -dn1000-F	GGCAAATCAAAAAAGCTCCCCAAAGCCTGCCCG
<i>dnaJ</i> -dn1000-R	GAGAATTCAAAAAAAAGAATGACCAAGGCCAGTATA
Δ <i>slyD</i> -F	CGGGGATATCAGTGCCTTAA
Δ <i>slyD</i> -R	AGACTTCACCGCAGCTTCGA
Δ <i>dnaJ</i> -F	CGTAAAGATGTTAACCCGGAC
Δ <i>dnaJ</i> -R	GATAGATAGCTGTTATCAAGGTACAC

Red letters indicate homology arms representing unrestricted cloning (RF-cloning) and polymerase chain reaction (PCR).

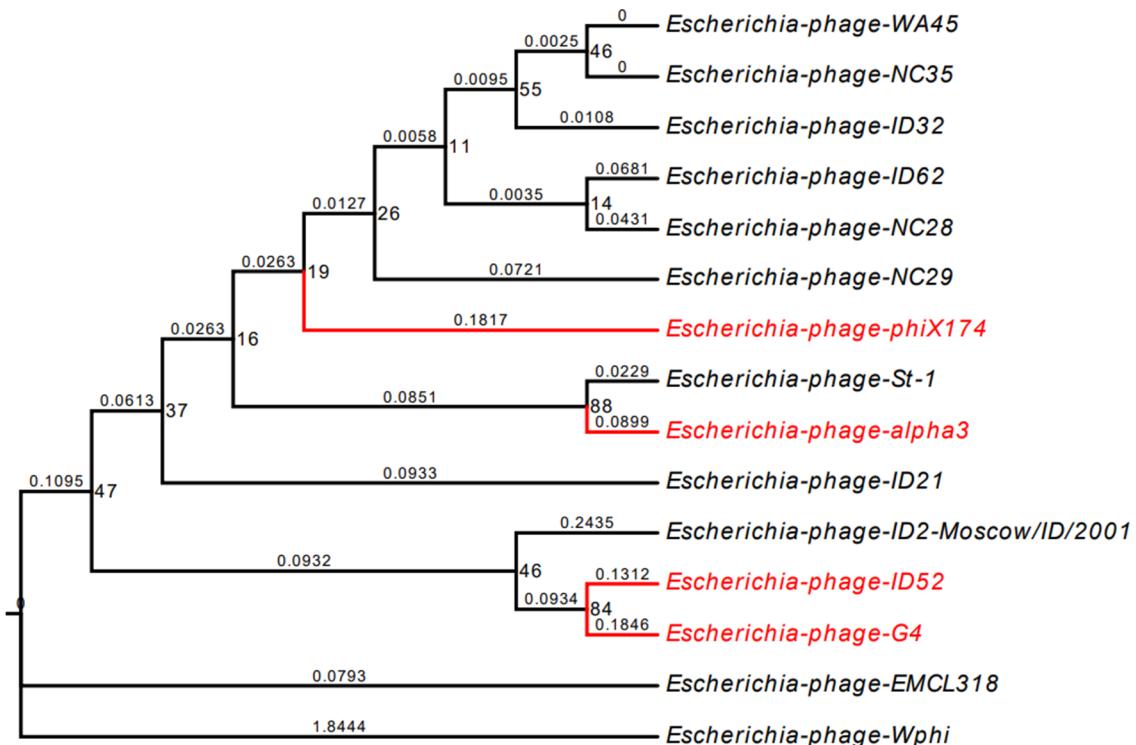


Figure S1. The evolutionary tree analysis of Enterobacteriaceae phage lysis gene E.

1. ID52-E	MERWTLSGILAFLLLSLLLPSSLIMFIPSTFRRPVLSWKVQSLPKTSLMVSNALRPPNCSPLLFFVPEETKTLVMPKTSVNNYALKELCNEKINSPLRRGTGG
2. ID52-E-E2V	MVRWTLSGILAFLLLSSLPSLLIMFIPSTFRRPVLSWKVQSLPKTSLMVSNALRPPNCSPLLFFVPEETKTLVMPKTSVNNYALKELCNEKINSPLRRGTGG
3. ID52-E-R3A	MEAWTLSGILAFLLLSSLPSLLIMFIPSTFRRPVLSWKVQSLPKTSLMVSNALRPPNCSPLLFFVPEETKTLVMPKTSVNNYALKELCNEKINSPLRRGTGG
4. ID52-E-W4A	MERATLWSGILAFLLLSSLPSLLIMFIPSTFRRPVLSWKVQSLPKTSLMVSNALRPPNCSPLLFFVPEETKTLVMPKTSVNNYALKELCNEKINSPLRRGTGG
5. ID52-E-S7W	MERWTLSGILAFLLLSSLPSLLIMFIPSTFRRPVLSWKVQSLPKTSLMVSNALRPPNCSPLLFFVPEETKTLVMPKTSVNNYALKELCNEKINSPLRRGTGG
6. ID52-E-G8D	MERWTLSGILAFLLLSSLPSLLIMFIPSTFRRPVLSWKVQSLPKTSLMVSNALRPPNCSPLLFFVPEETKTLVMPKTSVNNYALKELCNEKINSPLRRGTGG
7. ID52-E-I9T	MERWTLSGILAFLLLSSLPSLLIMFIPSTFRRPVLSWKVQSLPKTSLMVSNALRPPNCSPLLFFVPEETKTLVMPKTSVNNYALKELCNEKINSPLRRGTGG
8. ID52-E-L10A	MERWTLSGIAAFLLLSSLPSLLIMFIPSTFRRPVLSWKVQSLPKTSLMVSNALRPPNCSPLLFFVPEETKTLVMPKTSVNNYALKELCNEKINSPLRRGTGG
9. ID52-E-P21A	MERWTLSGILAFLLLSSLASLIMFIPSTFRRPVLSWKVQSLPKTSLMVSNALRPPNCSPLLFFVPEETKTLVMPKTSVNNYALKELCNEKINSPLRRGTGG
10. ID52-E-R3K	MERWTLSGILAFLLLSSLPSLLIMFIPSTFRRPVLSWKVQSLPKTSLMVSNALRPPNCSPLLFFVPEETKTLVMPKTSVNNYALKELCNEKINSPLRRGTGG
11. ID52-E-L37S	MERWTLSGILAFLLLSSLPSLLIMFIPSTFRRPVLSWKVQSLPKTSLMVSNALRPPNCSPLLFFVPEETKTLVMPKTSVNNYALKELCNEKINSPLRRGTGG
12. ID52-E-F66C	MERWTLSGILAFLLLSSLPSLLIMFIPSTFRRPVLSWKVQSLPKTSLMVSNALRPPNCSPLLFFVPEETKTLVMPKTSVNNYALKELCNEKINSPLRRGTGG
13. ID52-E-S83C	MERWTLSGILAFLLLSSLPSLLIMFIPSTFRRPVLSWKVQSLPKTSLMVSNALRPPNCSPLLFFVPEETKTLVMPKTSVNNYALKELCNEKINSPLRRGTGG
14. ID52-E-F66C S83C	MERWTLSGILAFLLLSSLPSLLIMFIPSTFRRPVLSWKVQSLPKTSLMVSNALRPPNCSPLLFFVPEETKTLVMPKTSVNNYALKELCNEKINSPLRRGTGG

Figure S2. Comparison of the amino acid sequences of wild-type and mutant of phage ID52 lysis protein E.

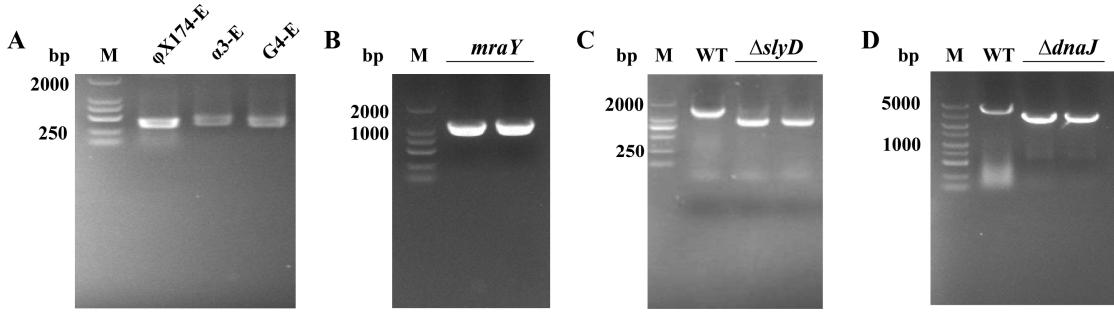


Figure S3. Colony analysis by agarose gel electrophoresis. (A) *E. coli* DH5 α colonies containing plasmids araC-ParaBAD- φ X174-E, araC-ParaBAD-G4-E, and araC-ParaBAD- α 3-E, respectively. (B) *E. coli* DH5 α colonies containing plasmids pLysS-*mraY*. (C) Colony PCR to identify the deletion of *slyD* gene in EcN. (D) Colony PCR to identify the deletion of *dnaJ* gene in EcN.

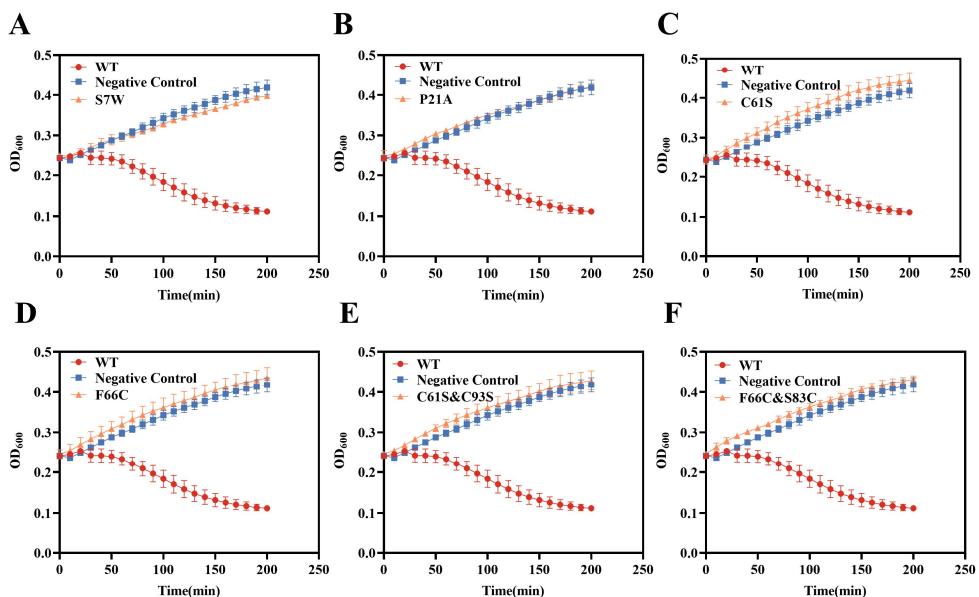


Figure S4. The lysis curves of EcN mutated to lose activity. The growth curves of at least five replicates of each mutant were monitored using OD₆₀₀.

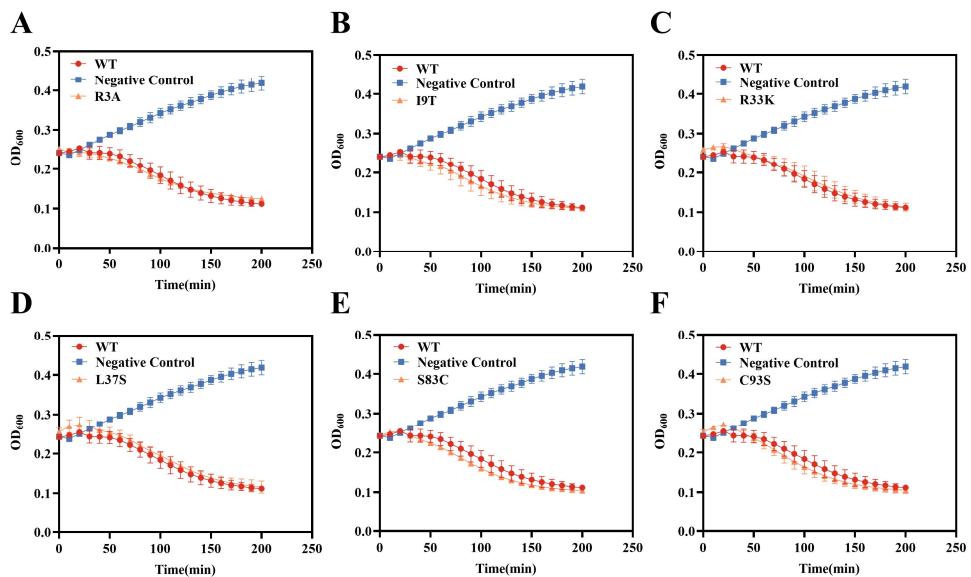


Figure S5. The lysis curves of the EcN mutant were not significantly affected. The growth curves of at least five replicates of each mutant were monitored using OD₆₀₀.

Table S3. Agricultural productivity and yield of two lysis proteins.

Lysis Protein E	Induction of OD600	Agricultural Productivity	Yield
ID52-E-W4A	6.0	60.39%	0.223 g/L
ID52-E-W4A	4.0	44.08%	0.185 g/L
φX174-E	4.0	3.09%	0.014 g/L

Western blot of the samples manifested that the lysis protein ID52-E-W4A could be expressed in EcN. The lysis protein ID52-E-W4A encoded 103 amino acids with the addition of the Strep-tag II tag and a molecular weight size of 12.93 kDa.

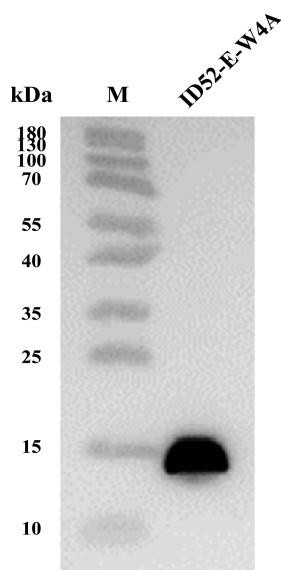


Figure S6. The expression of lysis protein ID52-E-W4A was verified by Western blot. M: 26616 pre-stained protein marker.

Table S4. The inactivated efficiency of different lysis protein E in EcN.

Initial Induction OD ₆₀₀ values	Inactivated Efficiency (%)	
	ID52-E	φX174-E
0.8	99.994	92.727
1.2	99.950	97.142
1.6	99.952	98.500
2.0	99.428	99.883