Article

Hemolysin Co-Regulatory Protein 1 Enhances the Virulence of Clinically Isolated *Escherichia coli* in KM Mice by Increasing Inflammation and Inducing Pyroptosis

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A ttgacagctagctcagtcctaggtataatactagtNNNNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAAT
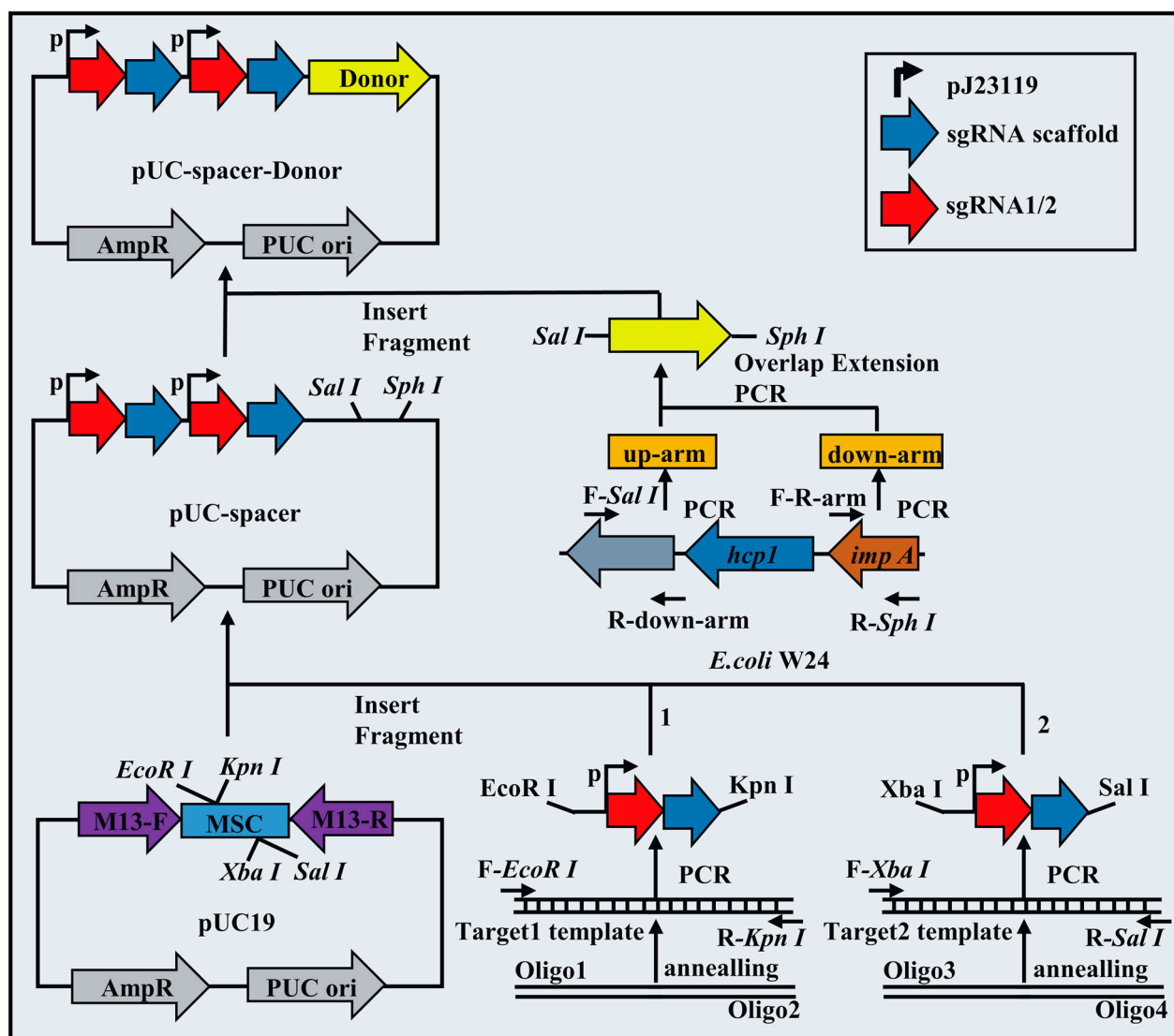AGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTG
B CTTTTTTT

Figure S1. sgRNA expression cassette and plasmid construction process. **(A)**. sgRNA expression cassette. **(B).** Plasmid construction process.
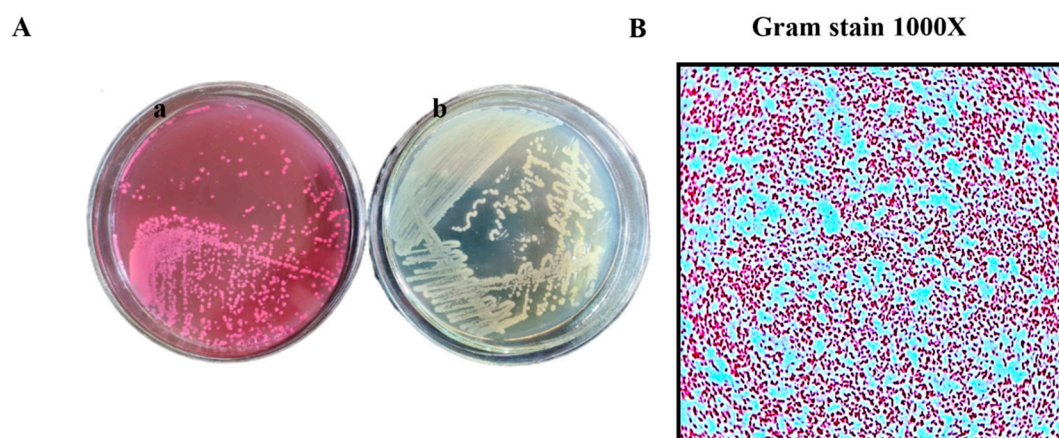
A **B** Gram stain 1000X

Figure S2. Clinical isolation of *E. coli*. **(A)**. a. *E. coli* is grown on MacConkey medium, b. *E. coli* is grown on LB medium. **(B)**. Gram staining of *E. coli*.

Table S1. Strains and plasmids used in this study.

Strain or plasmid	Characteristic(s)	Source
Strains		
W24	A clinical isolate from the faeces of a piglet with diarrhea; EPEC	Laboratoy stock
DH5α	F$^-$, φ80d*lacZ* ΔM15, Δ(*lacZYA-argF*)U169, *deoR* , *recA1* , *endA1* , *hsdR17* (rκ^- , mκ^-), *phoA*, *supE*44 , λ^- , *thi*-1, *gyrA*96 , *rel*A1	TaKaRa
W24Δ*hcp1*	W24 mutant with hcp1 deletion	This study
Plasmids		
pUC19	Amp^r, pMBI origin ,DNA sequencing using M13 series primers	TaKaRa
pCas	Kan^r,ParaBAD-λ-Red recombinase, Pcas-cas9,Ptrc-pMBI-sgRNA,pSC101ts origin	Laboratoy stock
pUC-spacer-Donor	Based on pUC19 skeleton,sgRNA1-Hcp1, sgRNA2-Hcp1,Donor	This study

Table S2. Primers used for the PCR amplification.

Primers	Primer sequences
up-arm-F-*Sal I*	*ACGCGTCGAC*AACTAACCAGGCGAGTGTAT
up-arm -R	GCCAGAGGCGGCAACCGGTACTTACTGTAATAACATAATA
down-arm-F	TATTATGTTATTACAGTAAGTACCGGTTGCCGCCTCTGGC
down-arm-R *Sph I*	*ACATGCATGC*CAGCAACAGGCCTCGACACAATG
Oligo1-F	tcctaggtataatactagt**GAATGCAATGAGTAAATGGC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAA GGCTAGTCCGT
Oligo1-R	ACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC**GCCATTTACTCATTGCATTC**actag tattataacctagga
Oligo2-F	tcctaggtataatactagt**ATAAAGGCAATTCTCTCCTG**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAA GGCTAGTCCGT
Oligo2-R	ACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC**CAGGAGAGAATTGCCTTTAT**acta gtattataacctagga
F-*EcoR I*	*CCGGAATTC*ttgacagctagctcagtcctaggtataatactagt
R-*Kpn I*	*CGGGGTACC*AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATT TA
F- *Xba I*	*CTAGTCTAGA*ttcttgacagctagctcagtcctaggtataatactagt

R- *Sal I*	***ACGCGTCGAC***AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTAT TTTA
pCas-JD-F	TCCGCCTGTCTGTACTTCTG
pCas-JD-R	CGTCGTTGGAACTGCTTTGA
M13-F	GTAAAACGACGGCCAGT
M13-R	CAGGAAACAGCTATGAC
QSJD-F	CAATAGCGAGTTCCAGAACT
QSJD-R	AGATGATGCGTGTTGCCGAC

Table S3. Primers used for qPCR analysis.

Name	Sequences
β-actin	CACCACACCTTCTACAATGA
NM_007393.5	GTCTCAAACATGATCTGGGT
NLRP3	GAGTCTAGCAGACCTGATTG
KF032621.1	TTGTAGCTCATCAAAGCCAT
ASC	GCTCTTGAAAACTTGTCAGG
AB059327.1	CATGTCTCTAAGCACAGTCA
Caspase-1	GTACACGTCTTGCCCTCATT
NM_009807.2	TCACGGTATACCCCAGATCC
IL-1β	GTGATATTCTCCATGAGCTTTGTAC
NM_008361.4	CATTACACAGGACAGGTATAGATTC
IL-18	TGTCTACCCTCTCCTGTAAG
NM_008360.2	AAGCAAGAAAGTGTCCTTCA
GSDMD	TGACAGTTCCAGTGCCT
NM_026960.4	CCTCGGTCACCACAAAC

Table S4. Docking scores and confidence scores.

Rank	1	2	3	4	5	6	7	8	9	10
Docking Score	-266.70	-264.71	-260.41	-258.40	-253.89	-253.57	-230.70	-228.65	-224.58	-223.83
Confidence Score	0.9117	0.9084	0.9010	0.8973	0.8887	0.8881	0.8340	0.8282	0.8163	0.8141
Ligand rmsd (Å)	87.10	107.29	92.66	102.68	102.67	93.32	119.23	121.56	94.18	137.16

Table S5. Reaction system and procedure for qPCR analysis.

constituents	reaction system	temperature	time	
SYBR Premix Ex Taq Ⅱ	10.0 μl	37℃	10 min	⎫ 10 cycles
Forward/Reverse Primer（10μM）	0.8 μl	16℃	10 min	⎭
Template cDNA	2.0 μl	50℃	5 min	
dH2O	6.4 μl	65℃	20 min	
Total	up to 20 μL	4℃	∞	