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Escherichia Coli CRISPR/CAS System Evolved with Bacteriophage: A Case Report

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Abstract

Background: Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a part of an adaptive immune system in prokaryotes and occurring in diverse forms of bacteria.

Case Presentation: We presented the results of sequence analysis which can help in understanding the presence of CRISPR-mediated antiphage ability in both pathogenic and non-pathogenic strains of Escherichia coli.

Conclusion: Endogenous CRISPR spacers with imperfect matches are the candidates of anti-phage function in genetically distant strains of E. coli.

Keywords: CRISPR/Cas System; Bacteriophage; Escherichia Coli; Spacers

Abbreviations: CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats; CAS: CRISPR-Associated Protein

Background

CRISPR is an acronym that stands for Clustered Regularly Interspaced Short Palindromic Repeats. It is an important part of an adaptive microbial immune system that occurs in wide varieties of bacteria [1]. CRISPR I and CRISPR II are two types of CRISPR elements that have been recognized in the Escherichia coli K-12 strain. CRISPR I is followed by eight cas genes namely, cas1, cas2, cas3, casA, casB, casC, casD and case in CRISPR element I. The last five genes encode the proteins to form a Cascade like complexes. Two genes cas1 and cas2 are responsible for adding exogenous spacers in to CRISPR region which lies near the leader sequence [2]. However, CRISPR II element is not found to be surrounded by such cas genes [3]. The procurement of new spacers provide host with an immunological memory to distinguish an invaded DNA molecules. The functional mechanism of CRISPR/Cas system is a hierarchical process. The results from our previous study indicated that two endogenous spacers; CR1-7 and CR2-6, which are relatively conserved can hinder replication of P1 phage in *E. coli* K12 strain [4]. However, the comprehensive correlation between the CRISPR spacers of pathogenic and non-pathogenic strains of *E. coli* and their phages still remains unclear.

Case Presentation

In this case study, we demonstrated the results of sequence analysis which can help understanding the presence of CRISPR-mediated anti-phage ability in pathogenic and non-pathogenic strains of *E. coli*. We collected *E. coli* spacers from CRISPRdb [5] and applied BLASTN program to compare the similarity between these spacers with the genome sequence of several Enterobacter phages, including P1, P2, T4, T5, T7, λ, ϕX147, MS2, Qβ, and M13. The different spacers targeting the same phage were found in genetically distant strains (Supplementary File 1). Moreover, multiple spacers against the same phage were found in many strains, which was similar to the presence of multiple P1 specific spacers in *E. coli* K12 strain (Supplementary File 2). The results indicated that the CRISPR/Cas system in *E. coli* had actively acquired spacers from the phage which infected them rather than a random event that occurred when searching for base pairing between short sequences.

Supplementary File 1:

E. Coli Strains\Phages	P1	P2	Lambda	T4	T7	T5	M13	Qbeta
UMN026	3			1				
42	2							
P12b	3	1						
ETECH10407	1		1					
ED1a	3							
O83:H1 str. NRG 857C	1	2						
LF82	1	1	1					
IAI1	2	1			1			
E24377A	1			1				
ATCC 8739	3	2				1	1	
MG1655	5		1	1	1			
BW2952	5		1	1	1			
DH1	5		1	1	1			
UMNK88	2	1		1				
O26:H11 11368	1	1	1					1
O111:H- 11128	1	1	2					
ETEC H10407	1							
BL21(DE3)	1	1						
B REL606	1	1						
UTI89		2	1					
UM146		1	1					
S88		1	1					
IHE3034		1	1					
55989		1	1	1				
APEC O1		1	1					
O152:H28 SE11		1						
O139:H28 E24377A		1						
O9 HS			1				1	
w				1		1		
KO11FL				1		1		
SMS-3-5								1
sum=	42	21	14	9	4	3	2	2

Strain=48

Strain with CRISRP=42

Number of Spacer=688

Selected Phages: P1, P2, Lambda, T4, T7, T5, M13, Qbeta

The parameters used in BLAST are the same as a default setting, but only matches have base-pairing region spanning greater than 15 base-pairs were reported here.

Supplementary File 2:

Phage	E. coli strain	CRISPR ID	Spacer	Protospacer	
P1					
	UMN026	NC_011751_6	GCTGGTGGCGCGGGCAAACGGAACAATCCCGC	GCTGGTGGCGCGGGCGAACGGAACAATCCCGC	
		NC_011751_6	ACATGAATGTCGGTTCAGACCGTGTTTTTACC	ACATGAATGTCGGCTGTGACCGTGTTTTCACC	A
		NC_011751_3	TCGACGGGGTGCGGTAAAACCTTTGCGAACGC	AGCTCTTGGTGCGGTAAAACCATTCATTATCA	B
	042	NC_017626_6	CAAAGGACACCGGGAGGCACCCGGCACCGCA	TAAAGGACACCGGGAAGCACCCGGCACCGCA	
		NC_017626_6	ACATGAATGTCGGTTCAGACCGTGTTTTTACC	ACATGAATGTCGGCTGTGACCGTGTTTTCACC	A
	P12b	NC_017663_8	ACATGAATGTCGGTTCAGACCGTGTTTTTACC	ACATGAATGTCGGCTGTGACCGTGTTTTCACC	A
		NC_017663_9_4	TGGCTCTGCAACAGCAGCACCCATGACCACGTC	CCGCTCTGCAACAAGAGCACAACGACAACCGC	F
		NC_017663_9_1	GACAGAACGGCCTCAGTAGTCTCGTCAGGCTCC	CTTAACACAGGCTCAGGAAGCTCGTCAGGCTC	E
	ETEC H10407	NC_017633_7	AAAATTTGTTGCAAAACTCGCTGTAAAAATAG	TAATTTTGTTGCAAAACTCCCTACAATCATGA	
	ED1a	NC_011745_3	TGCAAACGCTACTTGCTCATTGACCACGTAAG	AAATTTCACCCCCAGCTTATTGACCACGTAAG	
		NC_011745_2	GTGGCGCTAGCAATGAGATTTGCGGAAAAAAG	ATTCGCGGCACACAGAGACTTGCGGAAAAAAG	
		NC_011745_2	AGGAACCGCGTCAGCCCCCTGCAAAGCTCATT	GAGCCGATCAATCTCCCCCTGCAAACGCTCATT	
	O83:H1 str. NRG 857C	NC_017634_2	AGCAGCTTTCCAGCGAGCGCGGTTAACTCACT	GTCGCAAGACCAGCAAGCGCGGTTAAAGTTGC	C
	LF82	NC_011993_2	AGCAGCTTTCCAGCGAGCGCGGTTAACTCACT	GTCGCAAGACCAGCAAGCGCGGTTAAAGTTGC	C
	IAI1	NC_011741_4	CCCGAAAGAGATTGCCAGCCAGCTTAATTTGC	CGTCAGAAATAGTGCCATCCAGCTTAATTGCA	
		NC_011741_2	CGACGGGGTGCGGTAAAACCTTTGCGAACGC	TCAGCTCTTGGTGCGGTAAAACCATTCATTAT	B
	E24377A	NC_009801_1	CGACGGGGTGCGGTAAAACCTTTGCGAACGC	TCAGCTCTTGGTGCGGTAAAACCATTCATTAT	B
	ATCC 8739	NC_010468_2	CACGGCTGGCCATTTGAAATACCTGTTGCTCT	CCGCATCCCCCATTTGAAATCCCTGTGGAGCA	D
		NC_010468_2	CACGGCTGGCCATTTGAAATACCTGTTGCTCT	CCGCATCCCCCATTTGAAATCCCTGTGGAGCA	D
		NC_010468_2	GACAGAACGGCCTCAGTAGTCTCGTCAGGCTCC	CTTAACACAGGCTCAGGAAGCTCGTCAGGCTC	E
	MG1655/ BW25113	NC_000913_5	TGGCTCTGCAACAGCAGCACCCATGACCACGTC	CCGCTCTGCAACAAGAGCACAACGACAACCGC	F
		NC_000913_5	GACAGAACGGCCTCAGTAGTCTCGTCAGGCTCC	CTTAACACAGGCTCAGGAAGCTCGTCAGGCTC	E
		NC_000913_4	GTAGTCCATCATTCCACCTATGTCTGAACTCC	CATATCCCTCATTCCACCTACACTGATTACCC	
		NC_000913_4	TCAACATTATCAATTACAACCGACAGGGAGCC	GCAACGTTAATGATTACAACCGAGCTATTAGC	
		NC_000913_4	AAGCTGGCTGGCAATCTCTTTCGGGGTGAGTC	AAGCTGGCTGGAATCACTCATCGAAAGTCAT	

	BW2952	NC_012759_5	TGGCTCTGCAACAGCAGCACCCATGACCACGTC	CCGCTCTGCAACAAGAGCACAACGACAACCGC	F
		NC_012759_5	GACAGAACGGCCTCAGTAGTCTCGTCAGGCTCC	CTTAACACAGGCTCAGGAAGCTCGTCAGGCTC	E
		NC_012759_4	GTAGTCCATCATTCCACCTATGTCTGAACTCC	CATATCCCTCATTCCACCTACACTGATTACCC	
		NC_012759_4	TCAACATTATCAATTACAACCGACAGGGAGCC	GCAACGTTAATGATTACAACCGAGCTATTAGC	
		NC_012759_4	AAGCTGGCTGGCAATCTCTTTCGGGGTGAGTC	AAGCTGGCTGGAATCACTCATCGAAAGTCAT	
	DH1	NC_017638_5	TGGCTCTGCAACAGCAGCACCCATGACCACGTC	CCGCTCTGCAACAAGAGCACAACGACAACCGC	F
		NC_017638_5	GACAGAACGGCCTCAGTAGTCTCGTCAGGCTCC	CTTAACACAGGCTCAGGAAGCTCGTCAGGCTC	E
		NC_017638_4	GTAGTCCATCATTCCACCTATGTCTGAACTCC	CATATCCCTCATTCCACCTACACTGATTACCC	
		NC_017638_4	TCAACATTATCAATTACAACCGACAGGGAGCC	GCAACGTTAATGATTACAACCGAGCTATTAGC	
		NC_017638_4	AAGCTGGCTGGCAATCTCTTTCGGGGTGAGTC	AAGCTGGCTGGAATCACTCATCGAAAGTCAT	
	UMNK88	NC_017641_5_4	TGGCTCTGCAACAGCAGCACCCATGACCACGTC	CCGCTCTGCAACAAGAGCACAACGACAACCGC	F
		NC_017641_5_1	GACAGAACGGCCTCAGTAGTCTCGTCAGGCTCC	CTTAACACAGGCTCAGGAAGCTCGTCAGGCTC	E
	O26:H11 11368	NC_013361_2_2	GACAGAACGGCCTCAGTAGTCTCGTCAGGCTCC	CTTAACACAGGCTCAGGAAGCTCGTCAGGCTC	E
	O111:H- 11128	NC_013364_2_1	GACAGAACGGCCTCAGTAGTCTCGTCAGGCTCC	CTTAACACAGGCTCAGGAAGCTCGTCAGGCTC	E
	ETEC H10407	NC_017633_7_1	GACAGAACGGCCTCAGTAGTCTCGTCAGGCTCC	CTTAACACAGGCTCAGGAAGCTCGTCAGGCTC	E
	BL21(DE3)	NC_012892_5_1	GACAGAACGGCCTCAGTAGTCTCGTCAGGCTCC	CTTAACACAGGCTCAGGAAGCTCGTCAGGCTC	E
	B REL606	NC_012967_5_1	GACAGAACGGCCTCAGTAGTCTCGTCAGGCTCC	CTTAACACAGGCTCAGGAAGCTCGTCAGGCTC	E
P2					
	ATCC 8739	NC_010468_3	CATCCGGCGCTGAACATCGCCACCTGCCTAAC	TGACAACGACTGAACATCGCCGCCTGCGGGCG	
		NC_010468_3	CGACGTTTTCTAATATCACCCAGCAATCAATT	TGCCACTTTCTCAATATCACCCAGCGACCCGAC	
		NC_010468_2	TTCTTGCGGGTGTTGCAAATATTCTTCACGTA	CGGCGGCGGGGGTTCCGAGATATTCTTCACATG	
	UTI89	NC_007946_3	GCGCGCAGTGCCTGATAATCAATTTTGCTCAT	AGGGGACGCGCCGGATAATCAATTTTCTTACC	
		NC_007946_3	ATGAGCAAAATTGATTATCAGGCACTGCGCGC	GGTAAGAAAATTGATTATCCGGCGCGTCCCCT	
	UMNK88	NC_017641_4	AATTGATTGCTGGGTGATATTAGAAAACGTCG	GTCGGGTCGCTGGGTGATATTGAGAAAGTGGCA	
	UM146	NC_017632_3	ATGAGCAAAATTGATTATCAGGCACTGCGCGC	GGTAAGAAAATTGATTATCCGGCGCGTCCCCT	G
	S88	NC_011742_3	ATGAGCAAAATTGATTATCAGGCACTGCGCGC	GGTAAGAAAATTGATTATCCGGCGCGTCCCCT	G
	LF82	NC_011993_3	ATGAGCAAAATTGATTATCAGGCACTGCGCGC	GGTAAGAAAATTGATTATCCGGCGCGTCCCCT	G
		NC_011993_3	GCGCACGGCGTGGCGACAGAGAGCACGCCCGC	GCGGACGGCCAGTGGCGA CAGATTGTCACCATTG	

	IHE3034	NC_017628_3	ATGAGCAAAATTGATTATCAGGCACTGCGCGC	GGTAAGAAAATTGATTATCCGGCG-CGTCCCCT	G
	IAI1	NC_011741_4	TTTGCCGCTGTCAGCATTGCTGGCGCGTAATA	AATGCCGGTGTCAGCATTGAAGAAAC-CGCCGC	
	BL21(DE3)	NC_012892_4	AGCTGGGCGAAATTTTGATTCATCGTGATGAC	AACTGGGGAAATTTTGATTCCCGCTAT-GTGA	H
	B REL606	NC_012967_4	AGCTGGGCGAAATTTTGATTCATCGTGATGAC	AACTGGGGAAATTTTGATTCCCGCTAT-GTGA	H
	55989	NC_011748_4	TACCGGTGTGAATTACAGCACCACCGCCACCC	ATGGGATACGGTCACCAGTACCACCGC-CACCG	
	APEC O1	NC_008563_3	ATGAGCAAAATTGATTATCAGGCACTGCGCGC	GGTAAGAAAATTGATTATCCGGCG-CGTCCCCT	G
	O152:H28 SE11	NC_011415_4	AATAACTCGCGTAAATGCTCTGCGGCGCTACG	TCAGGAGATAGTAAATGCTCTGCTGCT-GTAAA	
	P12b	NC_017663_8	AGCCGGGCGAAATTTTGATTCATCGTGATGAC	CAACTGGGGAAATTTTGATTCCCGCTAT-GTGA	
	O83:H1 str. NRG 857C	NC_017634_3	TCATTCTGTACAGAAAGATCAACCATAATATT	TGATTCTGTACAGGAAGACGCACGT-TAGCTAT	
		NC_017634_3	GCGCACGGCGTGGCGACAGAGAGCACGCCCGC	GCGGACGGCCAGTGGCGACAGATTGT-CACCATTG	
	O26:H11 11368	NC_013361_2	TACGTGAAGAATATTTGCAACACCCGCAAGAA	CATGTGAAGAATATCTCGGAACCCCCGC-CGCCG	H
	O111:H- 11128	NC_013364_2	TACGTGAAGAATATTTGCAACACCCGCAAGAA	CATGTGAAGAATATCTCGGAACCCCCGC-CGCCG	H
	O139:H28 E24377A	NC_009801_3_15	TTCAGGGGAGTTCGCAAGACCAGCGGAATCGG	ATTGAGGGATTCCGCAAGACCAGTGCA-CAGCT	
lambda					
	UTI89	NC_007946_3_4	GCGCGCAGTGCCTGATAATCAATTTTGCTCAT	TCACGCAGTGCCTGAGAGTTAAT-TTCGCTCAC	I
	UM146	NC_017632_3_2	GCGCGCAGTGCCTGATAATCAATTTTGCTCAT	TCACGCAGTGCCTGAGAGTTAAT-TTCGCTCAC	I
	S88	NC_011742_3_5	GCGCGCAGTGCCTGATAATCAATTTTGCTCAT	TCACGCAGTGCCTGAGAGTTAAT-TTCGCTCAC	I
	LF82	NC_011993_3_15	GCGCGCAGTGCCTGATAATCAATTTTGCTCAT	TCACGCAGTGCCTGAGAGTTAAT-TTCGCTCAC	I
	IHE3034	NC_017628_3_5	GCGCGCAGTGCCTGATAATCAATTTTGCTCAT	TCACGCAGTGCCTGAGAGTTAAT-TTCGCTCAC	I
	APEC O1	NC_008563_3_5	GCGCGCAGTGCCTGATAATCAATTTTGCTCAT	TCACGCAGTGCCTGAGAGTTAAT-TTCGCTCAC	I
	O26:H11 11368	NC_013361_2_8	CTGCCGGGTGAAACCACTCGCGGCAGATCTTG	ATGCAGGGTGAAACCATTCCCGGCAT-TCATCG	J
	O111:H- 11128	NC_013364_2_6	CTGCCGGGTGAAACCACTCGCGGCAGATCTTG	ATGCAGGGTGAAACCATTCCCGGCAT-TCATCG	J
		NC_013364_2_3	TCCAACCTTCCATGAGATACGCGCATTAGCGG	TAAAACCTTCCATGTGATACGAGGGCG-CGTAG	
	O9 HS	NC_009800_6_17	TCAGGGATTTTTAATTGATGATATGCAGATAC	TTCATCACTTTTAATTGATG-TATATGCTCTCTT	
	ETEC H10407	NC_017633_7_5	TCGCGCCAGCACGTCGTCCGGAATCATGATTC	CGCCGCCAGCACGTCCGCCGGA-CAGGCTGCAT	
	MG1655	NC_000913_4_11	AGCGTGTTCGGCATCACCTTTGGCTTCGGCTG	CCCATCTCTCCGCATCACCTTTGGTAAA-GGTTC	K
	BW2952	NC_012759_4_11	AGCGTGTTCGGCATCACCTTTGGCTTCGGCTG	CCCATCTCTCCGCATCACCTTTGGTAAA-GGTTC	K

	DH1	NC_017638_4_11	AGCGTGTTCGGCATCACCTTTGGCTTCGGCTG	CCCATCTCTCCGCATCACCTTTGGTAAAGGTTC	K
T4					
	W	NC_017635_3_13	TAAAGTAGAATAAAAATATTCGCATAACAGAC	ATCTTTAGAATAAAAATATTCATCAAGATATC	
	UMNK88	NC_017641_5_7	TTTGCCACCCGAGTCCATAAATTCTTGATATGC	CACTCGCGCTTCGGCAATAAATTCTTGATATGT	
	UMN026	NC_011751_7_1	ACAAATGATGCGCCAAAACCAAGACTTTTACA	ACAAATGATGCGCCAATAATTTCAATAGTTTT	
	KO11FL	NC_016902_3_2	GTCTGTTATGCGAATATTTTTATTCTACTTTA	GATATCTTGATGAATATTTTTATTCTAAAGAT	
	E24377A	NC_009801_3_7	CTCAAAAAACTTGAAATCAAAACCGGTGAAGA	ATTCAAAAACTTGAATCAAAACCAATGAATA	
	55989	NC_011748_4_10	ATTAAAGGATTATTTTGATGAGTCTGAAAAAT	GTTAAAGCATTATTTTGATCTACTATAAAAGA	
	MG1655	NC_000913_4_10	TCAACATTATCAATTACAACCGACAGGGAGCC	ACAACATTATCACTTAAAAATTTAAAATTACT	N
	BW2952	NC_012759_4_10	TCAACATTATCAATTACAACCGACAGGGAGCC	ACAACATTATCACTTAAAAATTTAAAATTACT	N
	DH1	NC_017638_4_10	TCAACATTATCAATTACAACCGACAGGGAGCC	ACAACATTATCACTTAAAAATTTAAAATTACT	N
T5					
	W	NC_017635_3_13	TAAAGTAGAATAAAAATATTCGCATAACAGAC	TAAAGGAGAATAAAAATGATCCGCAACGTTTCT	
	KO11FL	NC_016902_3_2	GTCTGTTATGCGAATATTTTTATTCTACTTTA	AGAAACGTTGCGGATCATTTTTATTCTCCTTTA	
	ATCC 8739	NC_010468_2_20	CTCGATCAGGAAAATGAATTCCTGGAAAAAAA	CATTTACAGGAAAATGATATTCCTGACGTATGG	
T7					
	IAI1	NC_011741_3_8	TAAACCACCAGCCAGACCACCAATTACCACAC	GAAACCACCAGCGAGACCCATAGAGGTGATGA	O
	MG1655	NC_000913_4_6	AAGCTGGCTGGCAATCTCTTTCGGGGTGAGTC	GCGCTGGCTGGCATCTCTCCGATGTTCCAAC	O
	BW2952	NC_012759_4_6	AAGCTGGCTGGCAATCTCTTTCGGGGTGAGTC	GCGCTGGCTGGCATCTCTCCGATGTTCCAAC	O
	DH1	NC_017638_4_6	AAGCTGGCTGGCAATCTCTTTCGGGGTGAGTC	GCGCTGGCTGGCATCTCTCCGATGTTCCAAC	
M13					
	O9 HS	NC_009800_6_16	GAGAGACGAAGCATGAAAAAATTAATAATATC	GAGATTTTCAACATGAAAAAATTATTATTCGC	
	ATCC 8739	NC_010468_2_15	ATTACGCCGCCTCGCGTTTTTAGTCATTTCTA	GCTAAAACGCCTCGCGTTCTTAGAATACCGGA	
Qbeta					
	SMS-3-5	NC_010498_3_13	TCATACTGCTCCCACACCGAAAGCGCGGCAGC	AGGAACTGCACCCGCTTCTGAAAGCGCGGCAAC	
	O26:H11 11368	NC_013361_2_5	AATCGTGTGTAAATTCGCGGCGGCTCCACTGG	TAACTACCTGTCCCTGGCGGCGGCTCCAGTGG	

This idea is further supported with by the fact that *E. coli* 42, UMN026, and P12b display almost nearly ideal matched spacers against P1 phage. We also found that several strains carried the same anti-P1 phage spacer as that of *E. coli* K12. *E. coli* DH1 and BW2952 strain showed the same CRISPR sequence as those of K12 strains. A spacer found in *E. coli* IAI1 was similar to the seventh spacer in CRISPR I locus of K12 (25/25, 100% identical), but it can form long base-pairing region to P1 genome. Similarly, the last spacer in CRISPR II region was widespread in 11 strains (Supplementary File 2). The abundance of CRISPR spacers is shown in Figure 1. These spacers could pair with 16 nucleotides of phage sequence, which is as half the size of an *E. coli* type spacer. The spacers against P1 were the most abundant. On the contrary, only few spacers matched the genomes of M13 and Qβ with a statistically significant number of base pair. The general trend for all *E. coli* derived-CRISPR spacers was similar to that observed in the CRISPR region of *E. coli* K12 BW25113, which was used to conduct most of the experiments in the present case.

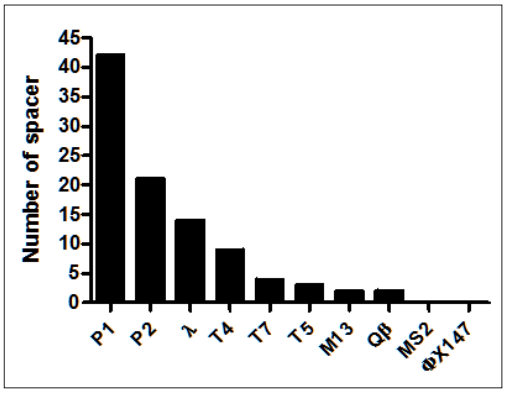

Figure 1: Distribution of antiphage spacers in *E.coli* in CRISPR database [5].

Discussion

Formerly it was disclosed that the high interrelationship between multilocus sequence typing tree and spacer repertoire was an indication that the CRISPR system in various strains of *E. coli* has lost immune function [6]. However, our results not only demonstrated that several endogenous CRISPR spacers in *E. coli* K12 strains still provide anti-phage function but also found the distribution of potential anti-phage spacers is more diverse than the genetic distance indicated by a phylogenic tree[7]. Therefore, the lack of significant immune effect in prior studies might be due to the accruement of plentiful of escape mutations in bacteriophage they studied [2,8]. Provided that phages with escape mutations might be used for replication, the rivalry between phage and bacteria establishes a pressure of natural selection to both species, which is an essential compelling force for phage diversification. In addition, a CRISPR deletion *E. coli* protein expression strain can be used as competent cell because the plasmids are highly stable in these mutant strains due to less interference on the replication and expression of protein expression plasmids.

Conclusion

The results of this case report revealed that endogenous spacer with flawed matches as the candidates of anti-phage function in genetically distant strains of *E. coli*. Furthermore, endogenous spacers with imperfect matches showing anti-phage function can be validated using spacer deletion strains.

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