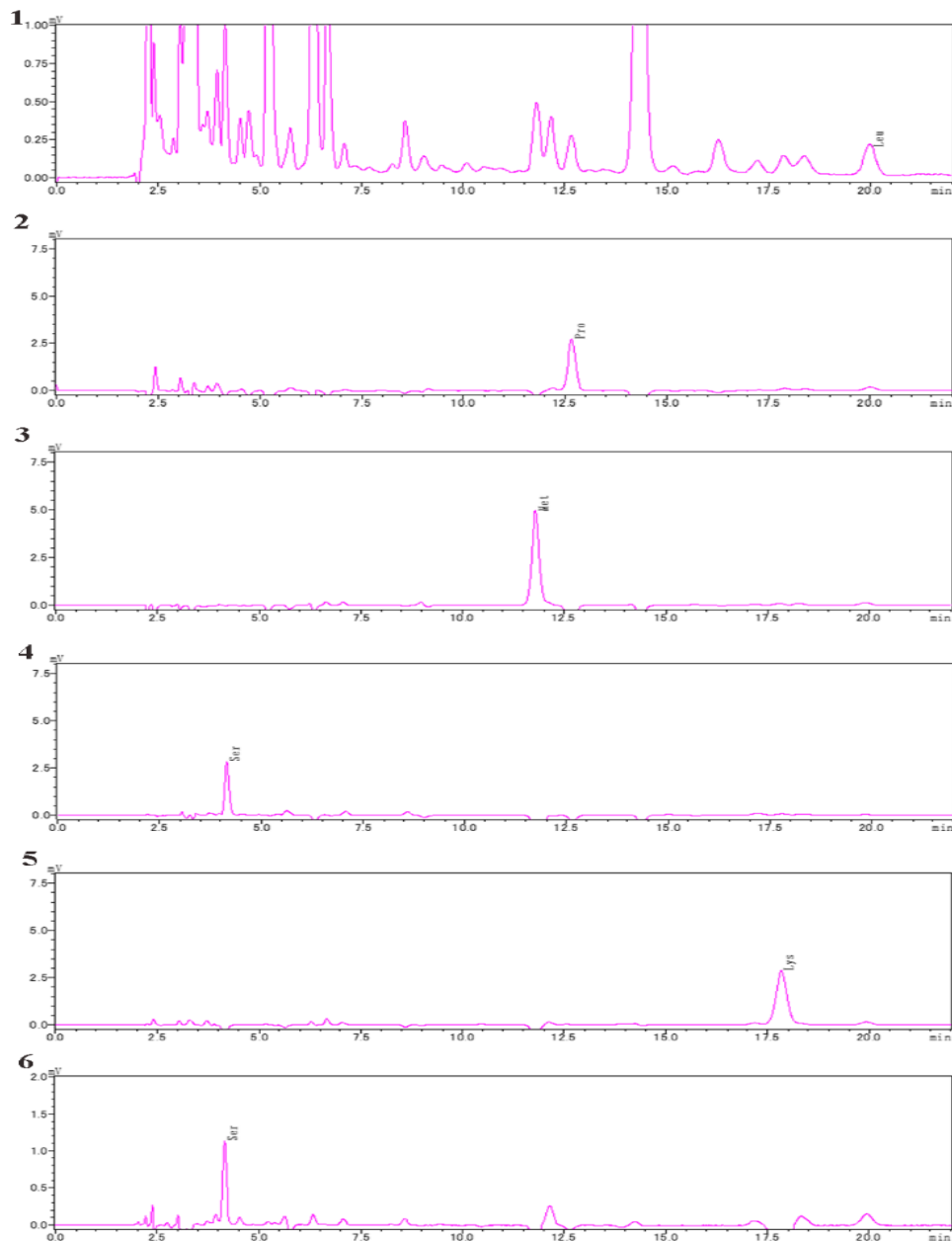
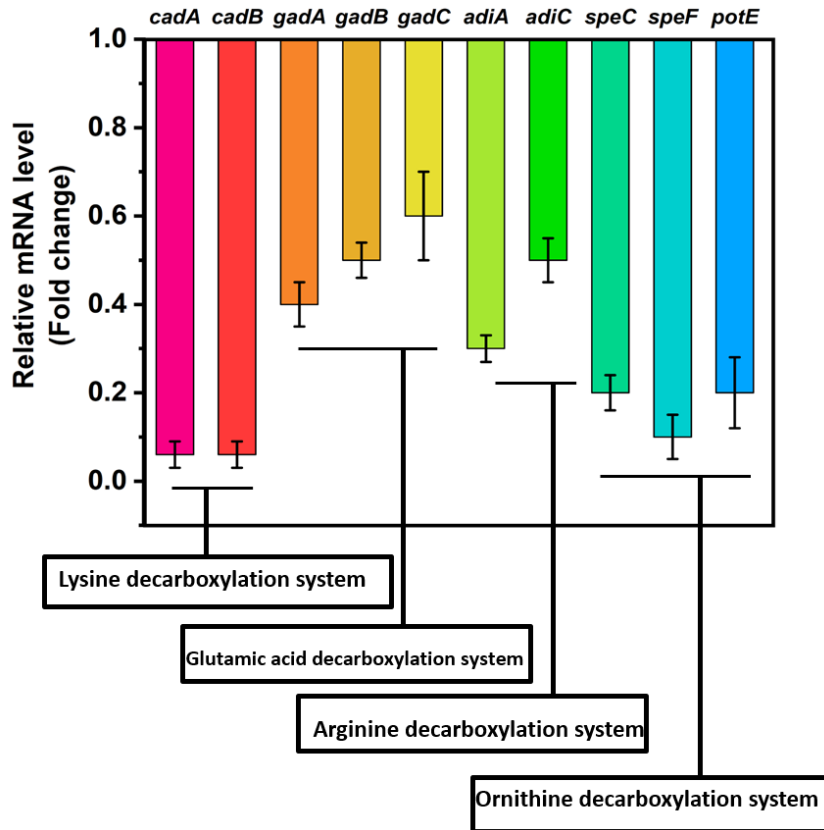


1 **Molecular mechanism of proteolytic cleavage-dependent activation of**
2 **CadC-mediated response to acid in *E. coli***
3 **Supplementary data**



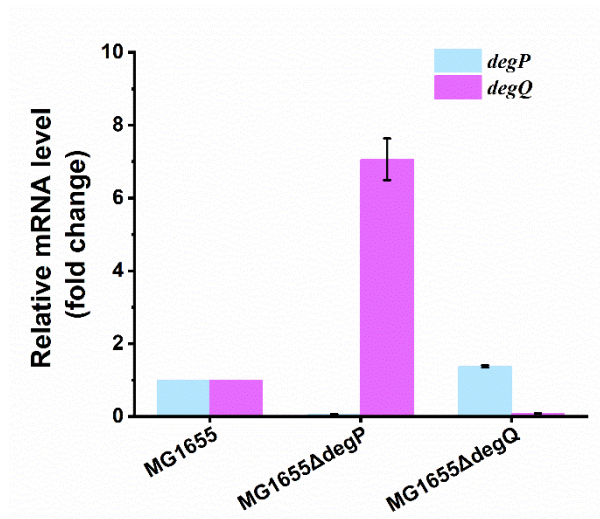
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5 **Supplementary Figure S1. N-terminal sequencing results of CadC protein.** The
6 sequences of the six N-terminal aa were determined consecutively as NH₂-Leu-Pro-
7 Met-Ser-Lys-Ser.



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9 **Supplementary Figure S2. Expression of other amino acid decarboxylation**
 10 **system-related genes in $\Delta cadC$.** Red is the expression of genes related to the lysine
 11 decarboxylation system, yellow is the expression of genes related to the glutamic acid
 12 decarboxylation system, green is the expression of genes related to the arginine
 13 decarboxylation system, and blue is the expression of genes related to the ornithine
 14 decarboxylation system.

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Supplementary Figure S3. The expression of the *degP* and *degQ* gene varies in the MG1655Δ*degP* strain and MG1655Δ*degQ* strain. In the Δ*degP* strains, *degQ* gene was up-regulated . In the Δ*degQ* strains , *degP* gene expression was stable .

Supplementary Table S1 His-CadC cross-linked mass spectrometry results

Accession	Gene	Description	Coverage	Peptides
P23890	<i>cadC</i>	Transcriptional activator CadC	65	12
A0A140NCH9	<i>hns</i>	DNA-binding protein	45	5
P0ABT2	<i>dps</i>	DNA protection during starvation protein	38	5
P69776	<i>lpp</i>	Major outer membrane lipoprotein Lpp	33	2
A0A6M6NZT1	HO397_07230	Lipoprotein	25	1
A0A140NFE9	<i>skp</i>	Chaperone protein Skp	17	2
A0A6M6P8N7	<i>degP</i>	Serine endoprotease DegP	17	5
A0A6M6P4Y3	<i>degQ</i>	Serine endoprotease DegQ	12	6
A0A6M6NZL6	<i>fur</i>	Ferric iron uptake transcriptional regulator	8	1
A0A6M6PIE0	<i>rsd</i>	Sigma D regulator	7	1
A0A6M6PGX3	<i>trpS</i>	Tryptophan--tRNA ligase	6	1

Supplementary Table S1 His-CadC cross-linked mass spectrometry results.

Accession	Gene	Description	Coverage	Peptides
A0A6M6P1V3	<i>ispC</i>	1-deoxy-D-xylulose-5-phosphate reductoisomerase	6	1
A0A140NDE3	<i>ompA</i>	Outer membrane protein A	4	1
A0A6M6P5G2	<i>comA</i>	Carboxy-S-adenosyl-L- methionine synthase CmoA	2	1

Supplementary Table S2. Bacterial strain used in this study.

E.coli strain	Relevant characteristics	Source
DH5α	A host for plasmid cloning	Our lab
MG1655	Strain K-12, F-λ- rph-1	Our lab
MG1655 ΔcadC	<i>cadC</i> deletion mutant of MG1655	This study
MG1655 ΔdegP	<i>degP</i> deletion mutant of MG1655	This study
MG1655 ΔdegQ	<i>degQ</i> deletion mutant of MG1655	This study
MG1655 ΔdegP degQ	<i>degP degQ</i> deletion mutant of MG1655	This study
MG1655 ΔdsbC	<i>dsbC</i> deletion mutant of MG1655	This study
MG1655 ΔdsbG	<i>dsbG</i> deletion mutant of MG1655	This study
MG1655 ΔlysP	<i>lysP</i> deletion mutant of MG1655	This study
BL21(DE3)	F' <i>ompT hsdSB (rB⁻ mB⁻) gal dcm met</i> (DE3)	This study

Supplementary Table S3. Oligonucleotides used in this study for gene knockout.

The letters with underline represented homologous sequences for recombination.

Oligonucleotides	Sequence
cadC-genta-PF	TAAACCCATCTATAGATGGTAAAAATAGGTTGTGGCAATTATCATTGCATCATTC
	TTTTCGAATGAGTTTCTATT AGCTGAATTACATTCCCAACCG
cadC-genta-PR	CACGATGCGGCAAAATTTTCTGGAGCGTCTCCTGCATTGTTGTGGCCACGGTTGGT
	TTAACGCTTTTGATAAACTC CAACTTAAATGTGAAAGTGGGTC
degP-genta-PF	TTACCTTTTGCAGAACTTTAGTTCGGAAGTTTCTAGGCTATAAAACGAATCTGAAGAA
	CACAGCAATTTGCGTTATCTGTAAATCGAGACTGAAATAC AGCTGAATTACATTCCCAACCG
degP-genta-PR	AAATGCCTAAAGGATGATGAAGATGTATGGAGTTGTGGTGAAGTTCACAGATTGTAA
	GGAGAACCCCTTCCCGTTTTTCAGGAAGGGGTTGAGGGAGA CAACTTAAATGTGAAAGTGGGTC

degQ-genta-PF	ATTTAATCTGGTGTCTCATTGTTAGCCGTCTGAAAATTCAATAACATCAAACCTGTTTT GAATCTCTTTTCTTATCATTACAGGTACGAGAGCAGGAATA AGCTGAATTACATTCCCAACCG
degQ-genta-PR	TAACGCATCAGCAGATAGATGCTTTCATTGCCGCGTACAATTTGCAGGGCGATGATG GCCGGTTTTGCCGCCAGCACTTTACGCATTTACAGCAATCG CAACTTAAATGTGAAAGTGGGTC
lysP-genta-PF	AACCGTGTATTTCTGCCGGAAGGATTGCCAATCGTCTGCTACAATCGCGCCTCATT TTAAGATGGATAGCATTTTTGT AGCTGAATTACATTCCCAACCG
lysP-genta-PR	ATATCCGCATGATTTGTGAGGAAATGGAATTGAAAAAGCCCTCTCGGTTGAGAGG GCTTAGCAAGGAAGGGAGGAAAC CAACTTAAATGTGAAAGTGGGTC
dsbC-genta-PF	GTCGCTACCGAGCGTCTGCCGCAACTTCATCAACAGCATCACCCGCGGGCGTGATG TCTGAAAAGAACGGGAAGATTT AGCTGAATTACATTCCCAACCG
dsbC-genta-PR	AGGGAGGCAATTCAGCGGGCAAGTCTGCCGTTTCATCGACTTCACGGCGACGAAGT TGTATCTGTTGTTTCACGCGAA CAACTTAAATGTGAAAGTGGGTC
dsbG-genta-PF	CTGATCTAAGCTCTTACCTATGACAGTGATAGGTTATGCCTTTTACTCGACTTTTGCA CTGACTGAAAAGGACAAATTA AGCTGAATTACATTCCCAACCG
dsbG-genta-PR	TAGGGCAGAGCGAGCAATTCATGCTTTTTTAGGATGGTTGAAAAGCAGGGATATAGT TCAACCATCCATGAATTACCTT CAACTTAAATGTGAAAGTGGGTC
cadC-test-PF	TGCAAAGACTAACAATCCATTAAAATAGTAAGTGCT
cadC-test-PR	TTATTCTGAAGCAAGAAATTTGTGCGAGATAAGGT
degP-test-PF	GCCTGTGCTTATCAGACTTTACTGAACT
degP-test-PR	CTGTGAAAGTACCAGCAATGCACC
degQ-test-PF	GGTGTCTCATTGTTAGCCGTCTG
degQ-test-PR	CAATTAATCCAATCGCAACGGAACGTAA
lysP-test-PF	TGCTGTGTCGATTTCTGTGA
lysP-test-PR	TCAGCCTTTTTTGATTGCG
dsbC-test-PF	CAAATTTATACGCATGTCGCTACCGAG
dsbC-test-PR	AATTCAGCGGGCAAGTCTGC
dsbG-test-PF	AAATGCAACAACGACATGGATTA
dsbG-test-PR	GTTTTTTGCGTATATATTTTT

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45 **Supplementary Table S4. Plasmids used in this study.**

Plasmids	Relevant characteristics	Source
pTKRED	Gene knockout plasmid, expression of three λ -Red recombination enzyme	Qingsheng Qi
pCM157	Resistance gene remove plasmid, expression of flippase recombination enzym	Qingsheng Qi
PR6K-fliB-genta- LoxM	Amplification the genta gene cassettes flanked by lox recognition target site	Our lab
pCL1920	Cloning vector, lac promoter, pSC101 ori, spc	Qingsheng Qi
pCL1920 <i>cadC</i>	<i>cadC</i> from MG1655 cloned into pCL1920 vector	This study
pCL1920 <i>cadCR184Q</i>	<i>cadCR184Q</i> from MG1655 cloned into pCL1920 vector	This study
pGL01	Expression vector, T7 lac promoter, N-terminal His tag PPase cleavage site between the His-tag and the expressed protein,Amp	Our lab
pGL01 <i>degQ</i>	DegP protein expression construct into pGL01 vector, N-terminal His Amp	This study
pET29b	Expression vector, T7 lac promoter, C-terminal His tag, Kan	Our lab
pET29b <i>cadC</i>	CadC protein expression construct into pET29b vector, C-his tag Kan	This study
pET29b <i>cadCR184Q</i>	<i>CadCR184Q</i> mutant protein expression construct into pET29b vector, C-his tag Kan	This study
pET21b	Expression vector, T7 lac promoter, C-terminal His tag, Kan	Our lab
pET21b <i>degP</i>	DegQ protein expression construct into pET21b vector, C-terminal His , Amp	This study
pCL1920 <i>CadCC172S</i>	<i>CadCC172S</i> mutant protein expression construct into pCL1920 vector, Spc	This study
pCL1920 <i>CadCC208S</i>	<i>CadCC172S</i> mutant protein expression construct into pCL1920 vector, Spc	This study
pCL1920 <i>CadCC272S</i>	<i>CadCC272S</i> mutant protein expression construct into pCL1920 vector, Spc	This study

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50 **Supplementary Table S5. Oligonucleotides used in this study for plasmid**
51 **construction. The letters with underline represented homologous sequences for**
52 **recombine**

Oligonucleotides	Sequence
CadC-pET29bPF	GAAGGAGATATACAT ATGCAACAACCTGTAGTTCGCGTTGG
CadC-pET29bPR	GTGGTGCTCGAGTTCTGAAGCAAGAAATTTGTCGAGAT
PGLO1 15b-F	CTCGAGTCTGGTAAAGAAAC
PGLO1 15b-R	GGATCCGGGCCCCCTGGAA
pET21b 21b-F	CTCGAGCACCACCACCAC
pET21b 21b-R	CATATGTATATCTCCTTCTTA
pET29b-F	CTCGAGCACCACCACCACCACCCTGAGATCCGGCTGCTAACAAAGC
pET29b-R	CATATGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTCTAGAGGGGAATT
DegP-pGLO1PF	AGGGGCCCCGGATCC ATGGAGACTTCTTCAGCAACGACAG
DegP-PGLO1PR	TTCTTTACCAGACTCGAG TTAAGTGCATTAACAGGTAGATGGTG
DegP-pET21bPF	GAAGGAGATATACATATG ATGGAGACTTCTTCAGCAACGACAG
DegP-PET21bPR	TGGTGGTGCTCGAGCTGCATTAACAGGTAGATGGTG
DegQ-pGLO1PF	AGGGGCCCCGGATCC ATGTCGATTCCAGGCCAGGT
DegQ-pGLO1PR	TTCTTTACCAGACTCGAG TTAACGCATCAGCAGATAGATGCTTTCA
DegQ-pET21bPF	GAAGGAGATATACATATG ATGTCGATTCCAGGCCAGGT
DegQ-pET21bPR	TGGTGGTGCTCGAGACGCATCAGCAGATAGATGCTTTCA
DegPS 210APF	AGACCGATGCAGCGATCAACCGTGGTAACGCCGGTGGTGC
DegPS 210APR	CGCCGTTTCAGGTTAACCAGCGCACCACCGGCGTTACCACG
DegPS 210APF	CAGACAGATGCTTCCATTAACCGCGGTAACGCCGGC GGTG
DegPS 210APR	ACCGTTAAGGTTTAATAGTGCACCGCGGCGTTACCGCGG
pCL1920-Np-F	TAAGCCAGCCCCGACACC
pCL1920-Np-R	ACGCGAATTCCCGACAGTAAGA
CadC-pCL1920PF	ATTTACACAGGAAACAGCTATGCAACAACCTGTAGTTCGCGTTGG
CadC-pCL1920PR	CGGGTGTCGGGGCTGGCTTATTCTGAAGCAAGAAATTTGTCGAGAT

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59 **Supplementary Table S6 Primers used in real-time quantitative PCR**

Gene	Forward sequence (5'-3')	Reverse sequence (5'-3')
<i>gapA</i>	AACTGAATGGCAAAGTACTGGTA	TTTCATTTTCGCCTTCAGCAGC
<i>cadA</i>	GCACGCTACCATTGCTAAGC	TCGGTGTTGTACAGCAGACC
<i>cadB</i>	ACTGGATTGGTAACCTGGCG	AGCAATACAGGCGATACCCG
<i>cadC</i>	GCGTTTGTTAATCAGCGCCA	GTGGCCACGGTTGGTTTAAC

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