# Characterization of Two-Component System CitB Family in *Salmonella* Pullorum

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## Supplementary Figures

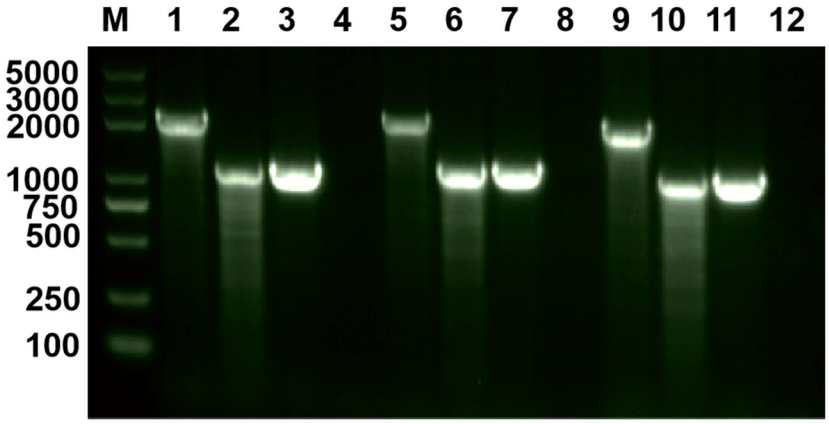


**Figure S1**. Identification of mutants (Δ*cit*, Δ*dcu*, Δ*dpi* and Δ3) by PCR. M: DL 5,000; 4,8,12: blank control; 1,5,9: WT; 2: Δ*cit;* 6: Δ*dcu*; 10: Δ*dpi*; 3,7,11: Δ3. 1-4 used primers which identified whether *cit* was deleted. 5-8 used primers which identified whether *dcu* was deleted. 9-12 used primers which identified whether *dpi* was deleted.

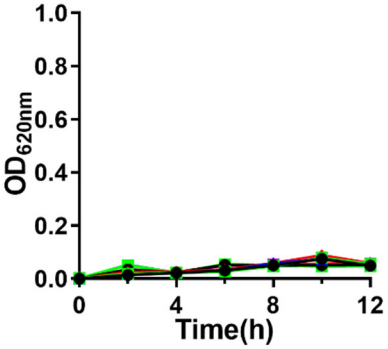


**Figure S2**. The growth curves of five strains in static M9 minimal medium under aerobic and anaerobic conditions, of which glucose was replaced with an equal concentration of citrate (citrate is the only carbon source).

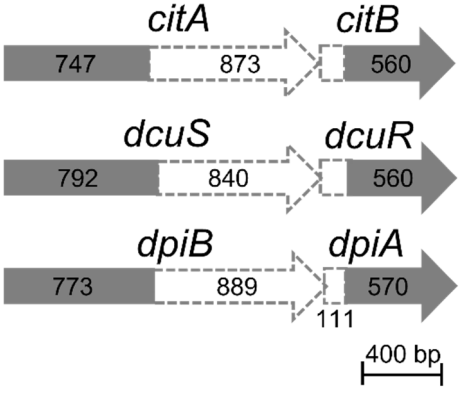


**Figure S3.** The deleted regions for three pairs of two component systems in wild-type strain R51. The dashed unfilled boxes indicate the deleted parts.

## Supplementary Tables

**Table S1.** Bacterial strains and plasmids used in this study

| Strains or plasmids | Genotype or relevant phenotype | Source or reference |
|---|---|---|
| *S.* Pullorum WT | Wild-type R51 | Clinical isolate |
| Δ*cit* | WT derivative; *citAB*-knockout mutant | This study |
| Δ*dcu* | WT derivative; *dcuSR*-knockout mutant | This study |
| Δ*dpi* | WT derivative; *dpiBA*-knockout mutant | This study |
| Δ3 | WT derivative; Δ*citAB*Δ*dcuSR*Δ*dpiBA* -knockout mutant; | This study |
| *Escherichia coli* DH5α | $F^-$ *endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG* ϕ80d*lacZ*ΔM15 Δ(*lacZYA-argF*) *U169 hsdR17* ($r_K^-$ $m_K^+$) $\lambda^-$ | This study |
| *Escherichia coli* TG1 | [F′ *traD36 proAB lacIqZ*ΔM15] *supE thi-1* Δ(*lac-proAB*) Δ(*mcrB-hsdSM*)5($r_K^-$*mk*$^-$) | This study |
| *Escherichia coli* 25922 | CLSI control strain for antimicrobial susceptibility testing | Lab collection |
| *Pseudomonas aeruginosa* 27853 | CLSI control strain for antimicrobial susceptibility testing | Lab collection |
| Rosetta (DE3) | BL21 derivatives; F-*ompT hsdSB* (*rB*$^-$*mB*$^-$ *gal dcm* (DE3) pRARE (*argU, argW, ilex, glyT, leuW, proL*) ($CHL^R$) | This study |
| pTarget F | Constitutive expression of sgRNA without donor editing template DNA; $SPEC^R$ | [28] |
| pCas | Temperature-sensitive vector; Constitutive expression of cas9 and inducible expression of λ-Red and sgR; $KAN^R$ | [28] |
| pET30a | Prokaryotic expression vector; $KAN^R$ | Lab collection |

CHL, chloramphenicol; SPEC, spectinomycin; KAN, kanamycin; CLSI, Clinical and Laboratory Standards Institute

**Table S2.** Primers used in this study

| Primer's name | Sequence (5′ to 3′) |
|---|---|
| Primers for constrcution of gene deletion mutants | |
| *cit*-sgRNA-F | GTCCTAGGTATAATACTAGTAAGCTAATAACCCAGCCCTGGTTTTAGAGCTAGAAATAGCAAGTT |
| *dcu*-sgRNA-F | GTCCTAGGTATAATACTAGTTGACGATGATCTCTTCATCGGTTTTAGAGCTAGAAATAGCAAGTT |
| *dpi*-sgRNA-F | GTCCTAGGTATAATACTAGTCTGGAAGACAATGATCCTTGGTTTTAGAGCTAGAAATAGCAAGTT |
| sgRNA-R | AAATAGTCGGTGGTGATAAACTTAT |
| sgRNA vector-F | ACTAGTATTATACCTAGGACTGAGC |
| sgRNA vector-R | ATAAGTTTATCACCACCGACTATTT |
| Identify *cit*-sgRNA-F | AAGCTAATAACCCAGCCCTG |
| Identify *dcu*-sgRNA-F | TGACGATGATCTCTTCATCG |
| Identify *dpi*-sgRNA-F | CTGGAAGACAATGATCCTTG |
| Identify sgRNA-F | CACCGCTGATGACATCAGT |
| *cit*-Donor DNA-Xf-F | TTTTGAATTCTCTAGAGTCGTTGACCCAATGCGCTCGTTTTCGGA |
| *cit*-Donor DNA-Xf-R | TTGATAGAGGCGTATTTGCTAGCCGTTTGGTTAATGGCGG |
| *cit*-Donor DNA-Xr-F | CCGCCATTAACCAAACGGCTAGCAAATACGCCTCTATCAA |
| *cit*-Donor DNA-Xr-R | AGATCTAAGCTTCTGCAGGTTTCGAGAAAGTTATTTTTCACGCCT |
| *dcu*-Donor DNA-Xf-F | TTTTGAATTCTCTAGAGTCGTTGTGGTGACCAATATGCAGGGCAT |
| *dcu*-Donor DNA-Xf-R | CTGCATATAGATATCCAGCAGGCGTCATCCTGCGTTTTTC |
| *dcu*-Donor DNA-Xr-F | GAAAAACGCAGGATGACGCCTGCTGGATATCTATATGCAG |
| *dcu*-Donor DNA-Xr-R | TAAGCTTCTGCAGGTCGGCCTGCCGGTCACGCCGTAATGA |
| *dpi*-Donor DNA-Xf-F | TTTTGAATTCTCTAGAGTCGGGGACGGATTTTGACTATGTGGTGA |
| *dpi*-Donor DNA-Xf-R | ATTCTCGCCTGCGCCAGATTGGCAGCCCCAACATTTTTCT |
| *dpi*-Donor DNA-Xr-F | AGAAAAATGTTGGGGCTGCCAATCTGGCGCAGGCGAGAAT |
| *dpi*-Donor DNA-Xr-R | AGATCTAAGCTTCTGCAGGTCGACTCGCGCAATATTCCAGGTAGC |
| Donor DNA vector-F | CGACTCTAGAGAATTCAAAAAAAGC |

| | |
|---|---|
| Donor DNA-R | CACCGCTGATGACATCAGT |
| pCas-F | TTTGTGGAGCAGCATAAGC |
| pCas-R | CCGCTCATGTCTAGATTAAGA |
| Identify Δ*cit*-F | AAGAACGCCTGCATTATCAG |
| Identify Δ*cit*-R | ATAGAGTCTCTCCGGCCT |
| Identify Δ*dcu*-F | GGTCCATCTGATCTATTTTTCG |
| Identify Δ*dcu*-R | CAGTAAAGAGTAATGTTCCG |
| Identify Δ*dpi*-F | CATGTTCGTGATATGGCGATGAATC |
| Identify Δ*dpi*-R | CAGTAAAGAGTAATGTTCCG |
| Primers for expression of recombinant fimbrial proteins | |
| pET30a-F | ATGATGATGATGATGGTGCATATG |
| pET30a-R | GAATTCGAGCTCCGTCGACAAGC |
| *bcfA* segment-F | ACATATGCACCATCATCATCATCATGATACTACAACCGTCACTGG |
| *bcfA* segment-R | AAGCTTGTCGACGGAGCTCGAATTCTCAGGAATAAACCATGCTAA |
| *fimA* segment-F | ACATATGCACCATCATCATCATCATGCTGATCCTACTCCGGTGAGC |
| *fimA* segment-R | AAGCTTGTCGACGGAGCTCGAATTCTTATTCGTATTTCATGATAAA |
| *lpfA* segment-F | ACATATGCACCATCATCATCATCATGCTGAATCTGGTGACGGCAC |
| *lpfA* segment-R | AAGCTTGTCGACGGAGCTCGAATTCAACGTGAGCGGTTCATGATT |
| *safA* segment-F | ACATATGCACCATCATCATCATCATGGCTCGCTCGTGCCGAATAC |
| *safA* segment-R | AAGCTTGTCGACGGAGCTCGAATTCTTAAGCTTGATACTCCGCTA |
| *stdA* segment-F | ACATATGCACCATCATCATCATCATGTCGATCCACCCACAGCAGG |
| *stdA* segment-R | AAGCTTGTCGACGGAGCTCGAATTCTCACAGGTATTTCAGGGTGT |
| *sthA* segment-F | ACATATGCACCATCATCATCATCATCAGAACACGATTACGTTCAA |
| *sthA* segment-R | AAGCTTGTCGACGGAGCTCGAATTCTTACTGATACGAAACGGTAT |

***Procedures for Four Mutants of Wild-type Strain R51***

The two-plasmid-based (pTargetF and pCas) CRISPR/Cas9 mediated genome-editing system was employed to created three individual mutants and one triple-deficient strain [28]. The primers are listed in the **Table S2**. Take the *citAB* mutant for example of individual mutant, the sgRNA (20-bp region) was designed through the online web server (http://chopchop.cbu.uib.no). The vector pTarget F was used as the template. The primers *cit*-sgRNA-F/sgRNA-R and sgRNA vector-F/R were used to amplify the small fragment with sgRNA sequence and the corresponding large vector fragment, respectively. The recombinase was used to ligate the two fragments to obtain the pTargetF (sgRNA), which chemically transformed into *E.* coli TG1 competent cell and spread onto the LB plate with spectinomycin. The single colonies were picked up and identified by PCR. Then the genome of the strain was used as the template. The primers *cit*-Donor DNA-Xf-F/R and cit-Donor DNA-Xr-F/R were used to amplify the Xf and Xr fragments, respectively. The donor DNA (Xf and Xr) was obtained by overlap PCR. The vector pTarget T (containing sgRNA and Donor DNA) was obtained by the same method as above. Prepare the R51 competent cells. L-arabinose-induced plasmid pCas was chemically transformed into R51. The single colonies growing on the LB plate with kanamycin were pick up and identified by PCR. The vector pTarget T was transformed into R51 harbouring the plasmid pCas and spread onto the LB plate with kanamycin and spectinomycin. After about three days, the single colony grown on the plate was confirmed by PCR whether the target fragment was deleted. The target colony harboring both pCas and pTargetT was inoculated into LB medium containing kanamycin and IPTG (isopropyl-D-thiogalactopyranoside, 0.5 mM) to cure pTargetT. After curing the pTargetT, the colony was stored at -80 °C for construction of double deletion mutant and used to cure the plasmid pCas. Because pCas is a temperature-sensitive plasmid which cannot maintain at 42 °C, pCas was cured by growing the colony overnight at 42°C nonselectively to obtain clean and traceless single deletion strain. The Δ*dcuSR* and Δ*dpiBA* mutants were also obtained in the same protocol. The triple-deficient strain was created based on a double strain and the methods were the same as above.