*Article*

# Identification of Three Type II Toxin-Antitoxin Systems in Model Bacterial Plant Pathogen *Dickeya dadantii* 3937

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## Supplementary Data

**Table S1.** Generation time (min) of the *D. dadantii* 3937 overexpressing the putative toxins or toxin-antitoxin complexes.

| | pBAD-toxin | pBAD-antitoxin-toxin |
|---|---|---|
| *ccdAB*$_{2Dda}$ | 63.5 +/- 2.4 | 62.4 +/-7.9 |
| *phd-doc*$_{2Dda}$ | 83.2 +/- 3.9 | 56.5 +/-2.8 |
| *dhiTA* | 78.9 +/-2.9 | 65.9 +/-1.5 |

**Table S2.** Bacterial strains and plasmids used in this study.

| Strain or plasmid | Relevant characteristics | Source or reference |
|---|---|---|
| **Strains** | | |
| ***Dickeya dadantii* 3937** | Genomic DNA and total RNA source, host for in vivo experiments with selective expression vectors | Wild-type strain isolated from *Saintpaulia ionantha* [63] |
| ***Escherichia coli*** | | |
| DH5$\alpha$ | Cloning host for recombinant vectors | [64] |
| MG1655 | Host for promoter activity assays and for in vivo experiments with selective expression vectors | [65] |
| MG1655$\Delta$*lacZ* | Host for plasmid stabilization assay | Lab stock |
| **Plasmids** | | |
| pBAD24 | Selective expression vector; amp$^R$ | [66] |
| pBAD24-ccdAB$_{2Dda}$ | pBAD24 derivative containing *ccdAB*$_{2Dda}$ genes under control of arabinose-inducible $P_{BAD}$ promoter | This study |
| pBAD24-ccdA$_{2Dda}$ | pBAD24 derivative containing *ccdA*$_{2Dda}$ gene under control of arabinose-inducible $P_{BAD}$ promoter | This study |
| pBAD24-ccdB$_{2Dda}$ | pBAD24 derivative containing *ccdB*$_{2Dda}$ gene under control of arabinose-inducible $P_{BAD}$ promoter | This study |
| pBAD24-phd-doc$_{Dda}$ | pBAD24 derivative containing *phd-doc*$_{Dda}$ genes under control of arabinose-inducible $P_{BAD}$ promoter | This study |
| pBAD24-phd$_{Dda}$ | pBAD24 derivative containing *phd*$_{Dda}$ gene under control of arabinose-inducible $P_{BAD}$ promoter | This study |

| Strain or plasmid | Relevant characteristics | Source or reference |
|---|---|---|
| pBAD24-doc$_{Dda}$ | pBAD24 derivative containing *doc$_{Dda}$* gene under control of arabinose-inducible $P_{BAD}$ promoter | This study |
| p24dhiTA | pBAD24 derivative containing *dhiTA* genes under control of arabinose-inducible $P_{BAD}$ promoter | This study |
| p24dhiA | pBAD24 derivative containing *dhiA* gene under control of arabinose-inducible $P_{BAD}$ promoter | This study |
| p24dhiT | pBAD24 derivative containing *dhiT* gene under control of arabinose-inducible $P_{BAD}$ promoter | This study |
| pBBRlux | Vector for generating transcriptional fusion to *lux*, Cm$^r$ | [55] |
| pLuxccdAB$_{2Dda}$ | pBBRlux derivative containing the 100bp fragment upstream *ccdA$_{2Dda}$* gene, cloned upstream promoter-less *luxCDABE operon* | This study |
| pLuxphd-doc$_{2Dda}$ | pBBRlux derivative containing the 100bp fragment upstream *phd$_{Dda}$* gene, cloned upstream promoter-less *luxCDABE operon* | This study |
| pLuxdhiTA | pBBRlux derivative containing the 100bp fragment upstream *dhiT* gene, cloned upstream promoter-less *luxCDABE operon* | This study |
| pRC7 | Unstable, low-copy-number, mini-F derivative of pFZY1, used for plasmid stabilization assays | [35] |
| pRC7-ccdAB$_{2Dda}$ | pRC7 derivative containing *ccdAB$_{2Dda}$* toxin-antitoxin operon including promoter region, cloned within ApaI site | This study |
| pRC7-phd-doc$_{Dda}$ | pRC7 derivative containing *phd-doc$_{Dda}$* toxin-antitoxin operon including promoter region, cloned within ApaI site | This study |
| pRC7-dhiTA | pRC7 derivative containing *dhiTA* toxin-antitoxin operon including promoter region, cloned within ApaI site | This study |

**Table S3.** Primers used in this study.

| Primer | Sequence (5′-3′) | Relevant characteristics |
|---|---|---|
| 1 | GCTAGCAGGAGGAATTCACCATGCCGACTACAAAAGCATACGA | Primer (forward) for *ccdA(B)$_{2Dda}$* amplification with primer 2 or 4, fragment cloned into pBAD24 digested with SmaI |
| 2 | GTCGACTCTAGAGGATCCCCTTAAAACGTCCTGAATCAT | Primer (reverse) for *ccdA$_{2Dda}$* amplification with primer 1, fragment cloned into pBAD24 digested with SmaI |
| 3 | GCTAGCAGGAGGAATTCACCATGCAGTACATGGTGTACG | Primer (forward) for *ccdB$_{2Dda}$* amplification with primer 4, fragment cloned into pBAD24 digested with SmaI |
| 4 | GTCGACTCTAGAGGATCCCCGGGTCAAAACCCGTCTAGCAAAA | Primer (reverse) for *ccdA(B)$_{2Dda}$* amplification with primer 1 or 3, fragment cloned into pBAD24 digested with SmaI |
| 5 | GCTAGCAGGAGGAATTCACCATGAAGACTTATACC | Primer (forward) for *phd(doc)$_{Dda}$* amplification with primer 6 or 8, fragment cloned into pBAD24 digested with SmaI |
| 6 | GTCGACTCTAGAGATCCCCTCATCTGTCGGCGAG | Primer (reverse) for *phd$_{Dda}$* amplification with primer 5, fragment cloned into pBAD24 digested with SmaI |
| 7 | GCTAGCAGGAGGAATTCACCATGAAATGGGTGAGTGCGC | Primer (forward) for *doc$_{Dda}$* amplification with primer 8, fragment cloned into pBAD24 digested with SmaI |

| Primer | Sequence (5′-3′) | Relevant characteristics |
|---|---|---|
| 8 | GTCGACTCTAGAGGATCCCCTTATTTTTCCACCCGTTG | Primer (reverse) for *phd(doc)*$_{Dda}$ amplification with primer 5 or 7, fragment cloned into pBAD24 digested with SmaI |
| 9 | GCTAGCAGGAGGAATTCACCATGCCTGAAATTGAC | Primer (forward) for *dhiT*(*A*) amplification with primer 10 or 12, fragment cloned into pBAD24 digested with SmaI |
| 10 | GTCGACTCTAGAGGATCCCCTCACAATTTTCCCGGATG | Primer (reverse) for *dhiT* amplification with primer 10, fragment cloned into pBAD24 digested with SmaI |
| 11 | GCTAGCAGGAGGAATTCACCATGCATAAAATCATC | Primer (forward) for *dhiA* amplification with primer 12, fragment cloned into pBAD24 digested with SmaI |
| 12 | GTCGACTCTAGAGGATCCCCCTACTTCTCTTCCAG | Primer (reverse) for *dhiT(A)* amplification with primer 10 or 11, fragment cloned into pBAD24 digested with SmaI |
| 13 | GGTGGCGGCCGCTCTAGAACTAGTGCCTGAGCGGGTATCGCGGCG | Primer (forward) for putative promoter region of *ccdAB*$_{2Dda}$ amplification with primer 14, fragment cloned into pBBRlux digested with BamHI |
| 14 | ATCCATTTTGCGGCCGCAACTAGAGGTTGCCCTCCATTTACACGT | Primer (reverse) for putative promoter region of *ccdAB*$_{2Dda}$ amplification with primer 13, fragment cloned into pBBRlux digested with BamHI |
| 15 | GGTGGCGGCCGCTCTAGAACTAGTGGGTTGGGGGCTTTTTTTATA | Primer (forward) for putative promoter region of *phd-doc*$_{Dda}$ amplification with primer 16, fragment cloned into pBBRlux digested with BamHI |
| 16 | ATCCATTTTGCGGCCGCAACTAGAGGGCTCCCCCTTGAAATGTAC | Primer (reverse) for putative promoter region of *phd-doc*$_{Dda}$ amplification with primer 15, fragment cloned into pBBRlux digested with BamHI |
| 17 | GGTGGCGGCCGCTCTAGAACTAGTGGTGGAATCAACATGAAACCT | Primer (forward) for putative promoter region of *dhiTA* amplification with primer 18, fragment cloned into pBBRlux digested with BamHI |
| 18 | ATCCATTTTGCGGCCGCAACTAGAGTGGCACTCCTTGTCAATGAG | Primer (reverse) for putative promoter region of *dhiTA* amplification with primer 17, fragment cloned into pBBRlux digested with BamHI |
| 19 | TCACCAGCAAATCGCGCTGTTAGCGTGAGCGGGTATCGCGGCGTA | Primer (reverse) for *ccdAB*$_{2Dda}$ amplification, including putative promoter region, used with primer 20, fragment cloned into pRC7 digested with ApaI |
| 20 | AGACGCGCCGAGACAGAACTTAATGTCAAAACCCGTCTAGCAAAA | Primer (forward) for *ccdAB*$_{2Dda}$ amplification, including putative promoter region, used with primer 19, fragment cloned into pRC7 digested with ApaI |
| 21 | TCACCAGCAAATCGCGCTGTTAGCGTTGGGGGCTTTTTTTATAAC | Primer (reverse) for *phd-doc*$_{Dda}$ amplification, including putative promoter region, used with primer 22, fragment cloned into pRC7 digested with ApaI |

| Primer | Sequence (5′-3′) | Relevant characteristics |
|---|---|---|
| 22 | AGACGCGCCGAGACAGAACTTAATGTTATTTTTCCACCCGTTGTC | Primer (forward) for *phd-doc*$_{Dda}$ amplification, including putative promoter region, used with primer 21, fragment cloned into pRC7 digested with ApaI |
| 23 | TCACCAGCAAATCGCGCTGTTAGCGTGGAATCAACATGAAACCTT | Primer (forward) for *dhiTA* amplification, including putative promoter region, used with primer 24, fragment cloned into pRC7 digested with ApaI |
| 24 | AGACGCGCCGAGACAGAACTTAATGCTACTTCTCTTCCAGTTGTA | Primer (reverse) for *dhiTA* amplification, including putative promoter region, used with primer 23, fragment cloned into pRC7 digested with ApaI |
| 25 | ACTACAAGCATACGACAC | Primer (forward) for RT-PCR analysis of *ccdAB*$_{2Dda}$ module transcript |
| 26 | TCATCACCAGATAATCAC | Primer (reverse) for RT-PCR analysis of *ccdAB*$_{2Dda}$ module transcript |
| 27 | TATACCATTACTGAAGCC | Primer (forward) for RT-PCR analysis of *phd-doc*$_{Dda}$ module transcript |
| 28 | CTTAATGGTGAGTTCTTC | Primer (reverse) for RT-PCR analysis of *phd-doc*$_{Dda}$ module transcript |
| 29 | GATGATGAGTTGATCATC | Primer (forward) for RT-PCR analysis of *dhiTA* module transcript |
| 30 | CTCTTCCAGTTGTAACAC | Primer (reverse) for RT-PCR analysis of *dhiTA* module transcript |

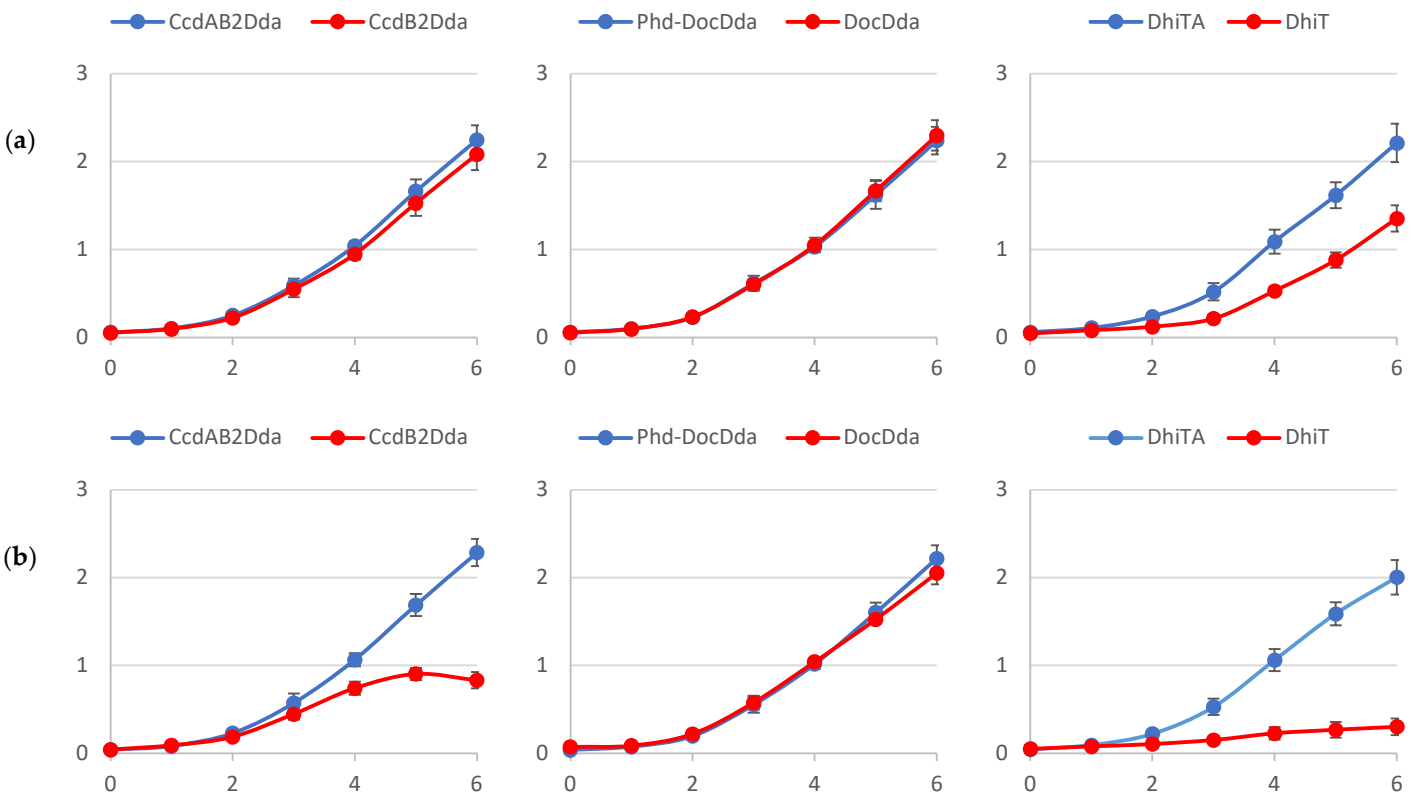
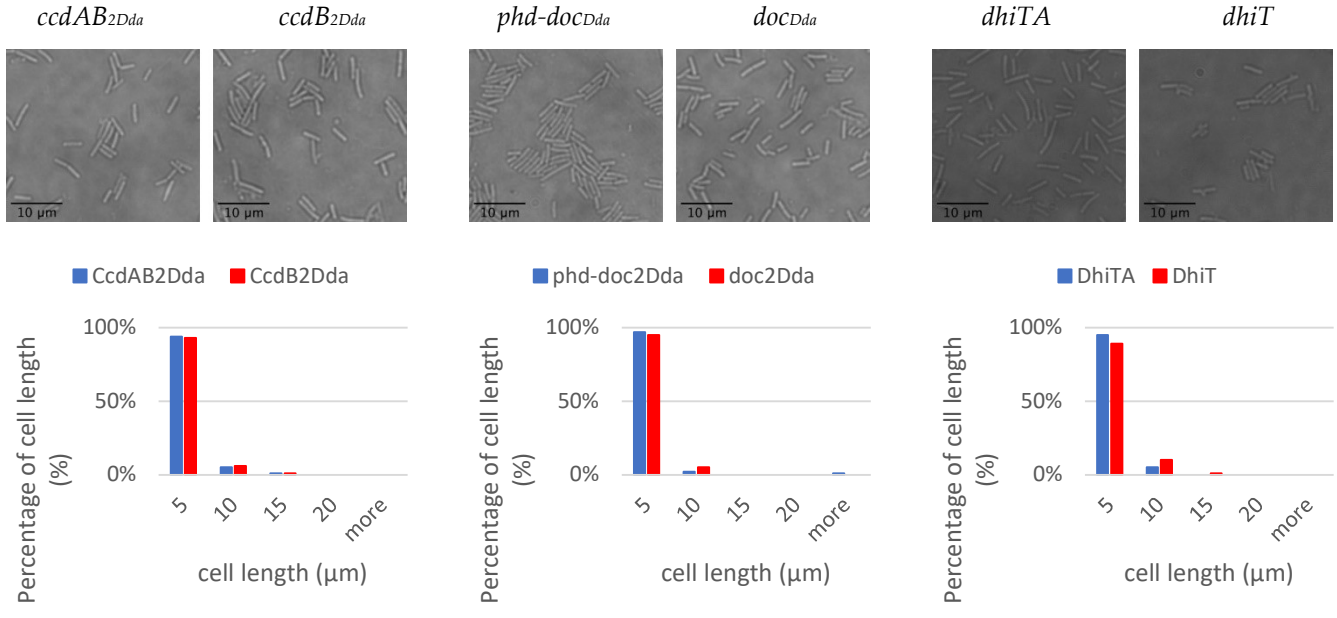


**Figure S1.** Effect of induction of expression of the putative toxins on growth of the *E.* coli K-12 MG1655. *E. coli* cells, harbouring plasmids encoding the toxin (red ♦) or antitoxin-toxin (blue •) genes under control of the $P_{BAD}$ promoter, were grown to $OD_{600}$ of 0.05–0.08. Each culture was then supplemented with either 0.2 % D-glucose (**a**) or 0.2 % L-arabinose (**b**). Culture growth was monitored by measuring $OD_{600}$ every hour. The results are an averages of at least 3 independent experiments with SD indicated.




(**a**) (**b**) (**c**)

**Figure S2.** The effect of overexpression of the toxins on the *D. dadantii* 3937 cell morphology. pBAD24 derivatives harbouring genes encoding (**a**) $CcdAB_{2Dda}$ system or toxin $CcdB_{2Dda}$, (**b**) Phd-$Doc_{Dda}$ system or $Doc_{Dda}$, toxin (**c**) DhiTA system or DhiT toxin were introduced into *D. dadantii* 3937 cells and were induced with 0.2 % arabinose. *D. dadantii* 3937 cells cultured in the same conditions were used as a cell length control. Light microscope morphology of *D. dadantii* 3937 cells (×100), cell length was measured for 100 bacteria for each strain. Examples of relevant microphotographs are above the graphs.

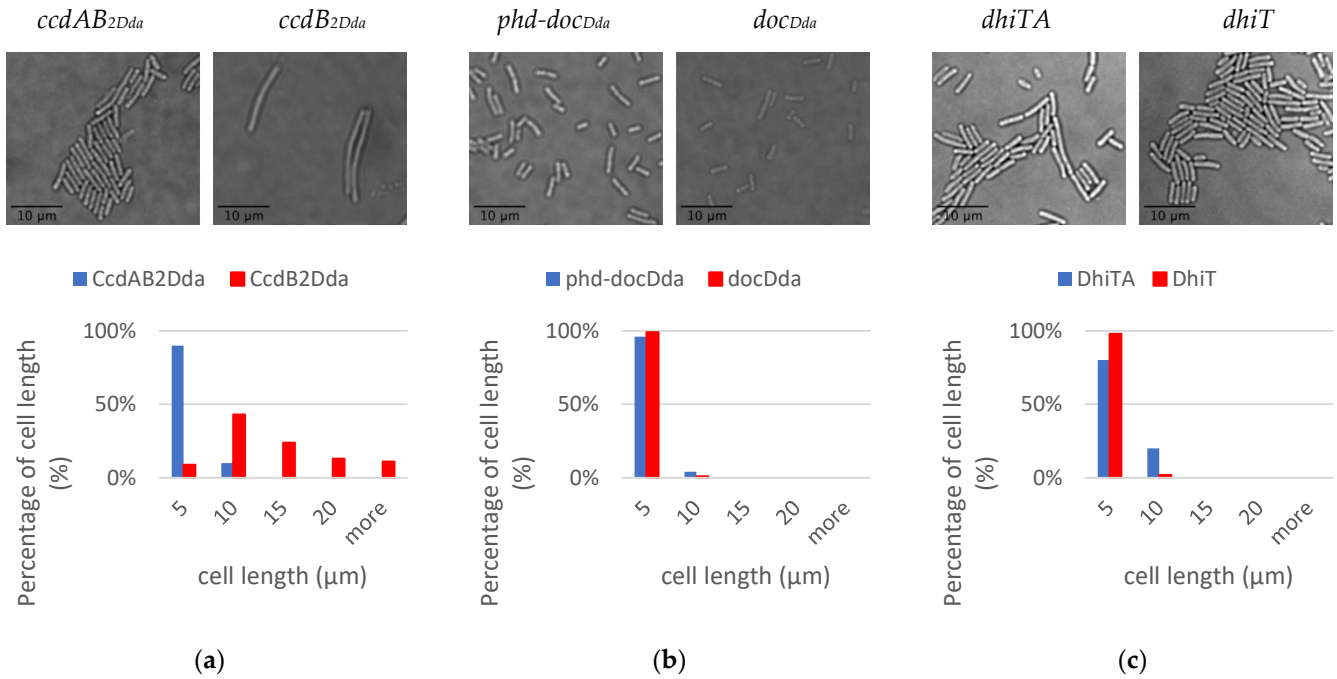



**Figure S3.** The effect of overexpression of the *D. dadantii* 3937 toxins on the *E. coli* cell morphology. pBAD24 derivatives harbouring genes encoding (**a**) CcdAB$_{2Dda}$ system or toxin CcdB$_{2Dda}$, (**b**) Phd-Doc$_{Dda}$ system or Doc$_{Dda}$ toxin (**c**) DhiTA system or DhiT toxin were introduced into *E. coli* cells and were induced with 0.2 % arabinose. *E. coli* cells cultured in the same conditions were used as a cell length control. Light microscope morphology of *E. coli* cells (×100), cell length was measured for 100 bacteria for each strain. Examples of relevant microphotographs are above the graphs.