**Table S1. Bacterial strains and plasmids.**

| Bacterial strain or plasmid | Relevant characteristics* | Reference or source |
|---|---|---|
| *C. botulinum* strains | | |
| ATCC 3502 | Isolated in 1940, producing type A1 neurotoxin | Sebaihia *et al*. (2007) |
| *codY* mutant | ATCC 3502 derivative, *codY* inserted with the Ll.LtrB-derived intron at the antisense site of 526 bp | This study |
| WT-pMTL82151 | *C. botulinum* ATCC 3502 strain carrying plasmid pMTL82151 | This study |
| *codY*-pMTL82151 | *codY* mutant carrying plasmid pMTL82151 | This study |
| WT-pMTL82151::*codY* | *C. botulinum* ATCC 3502 strain carrying plasmid pMTL82151::*codY* | This study |
| *codY*-pMTL82151::*codY* | *codY* mutant carrying plasmid pMTL82151::*codY* | This study |
| *E. coli strains* | | |
| CA434 | *E. coli* HB101 carrying the Incβ conjugative plasmid R702 | Purdy *et al*. (2002) |
| CA434-pMTL82151 | *E. coli* CA434 carrying plasmid pMTL82151 | This study |
| CA434-pMTL82151::*codY* | *E. coli* CA434 carrying plasmid pMTL82151::*codY* | This study |
| NEB 5-alpha strain | cloning strain, DH5α™ derivative | NEB |
| NEB 5-alpha-pBAD30::*codY* | NEB 5-alpha carrying plasmid pBAD30::*codY* | This study |
| LMG 194 | F$^-$Δ*lacX*74 *galE galK thi rpsL*Δ*phoA* (*Pvu*II) Δ*ara*714 *leu::*Tn*10* | Life Technologies |
| LMG 194-pBAD30::*codY* | LMG 194 carrying plasmid pBAD30::*codY* | This study |
| Plasmid | | |
| pMTL007C-E2 | Inducible clostridial expression vector for expression of ClosTron, containing Erm RAM, ColE1, pCB102, Cm$^R$ | Heap *et al*. (2010) |
| pMTL007C-E2::*codY* | pMTL007C-E2 containing intron retargeted to *codY* (antisense insertion at 526 bp) | This study; DNA 2.0 |
| pMTL82151 | *Clostridium-E.coli* shuttle plasmid; pBP1 replicon; Cm$^R$ | Heap *et al*. (2009) |

| | | |
|---|---|---|
| pMTL82151::*codY* | pMTL82151 containing the DNA fragment amplified by PCR using primers *codY*-82151-F and *codY*-82151-R | This study |
| pBAD30 | expression vector; *araBAD* promoter; $Amp^R$ | Gift from Bruno Dupuy |
| pBAD30::*codY* | pBAD30 containing the DNA fragment amplified by PCR using primers *codY*-30-F and *codY*-30-R | This study |

* $Amp^R$, ampicillin resistance; $Cm^R$, chloramphenicol/ thiamphenicol resistance

**References**


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Purdy, D., O'Keeffe, T.A., Elmore, M., Herbert, M., McLeod, A., Bokori-Brown, M., *et al*. (2002) Conjugative transfer of clostridial shuttle vectors from *Escherichia coli* to *Clostridium difficile* through circumvention of the restriction barrier. *Mol Microbiol* **46**: 439-452.

Sebaihia, M., Peck, M.W., Minton, N.P., Thomson, N.R., Holden, M.T., Mitchell, W.J., *et al*. (2007) Genome sequence of a proteolytic (Group I) *Clostridium botulinum* strain Hall A and comparative analysis of the clostridial genomes. *Genome Res* **17**: 1082-1092.

**Table S2. Oligonucleotides.**

| Primer | Sequence(5'-3')* |
|---|---|
| **PCR screening** | |
| *codY*-F | TTGAATGTGACGAAGTAAGG |
| *codY*-R | TTCTGTGCCATCTAACTCAT |
| **Complementation and overexpression** | |
| *codY*-82151-F | NNNNNNGCGGCCGCGTGAAGAATGTGGAAGTTAC |
| *codY*-82151-R | NNNNNNGCTAGCCTCCTTAGGTAGTATATCACAC |
| **qRT-PCR** | |
| *botA*-F | CGCGAAATGGTTATGGCTCT |
| *botA*-R | GCCTGCACCTAAAAGAGGATTT |
| 16S*rrn*-F | AGCGGTGAAATGCGTAGAGA |
| 16S*rrn*-R | GGCACAGGGGGAGTTGATAC |
| **Protein expression** | |
| *codY*-30-F | NNNNNNGAATTCAGGAGGAATTAACCATGGGGGGTTCT**CATCATCATCATCATCAT**GGTAT GAGTTCATTACTAGATAA |
| *codY*-30-R | NNNNNNGCATGCCTATTTTATCTTCTTTAATTCTTCTAAA |
| **EMSA** | |
| P*ntnh-botA*-F | IRD700-GGCTTTAGAGAGATTAGAACCCATA |
| P*ntnh-botA*-R | IRD700-CATTTTTATTATCTACCGGGGAA |

*, Restriction enzyme site is indicated by underline. Sequence encoding the 6×His tag is marked in bold.

**Fig. S1. Growth curves of *Clostridium botulinum* ATCC 3502 wild-type strain and *codY* mutant.** Growth of WT and the *codY* mutant in tryptose-peptone-glucose-yeast extract medium. Error bars indicate standard deviations of three biological replicates.

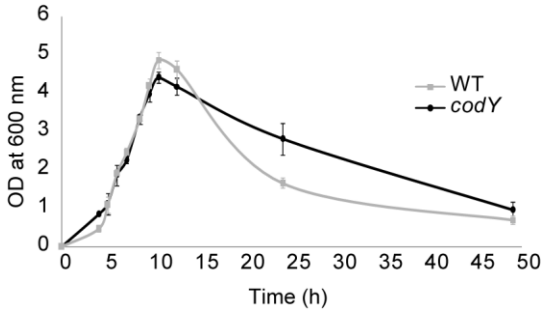


**Fig. S2. Growth curves of the complementation and overexpression strains.** Growth of the complementation strain *codY*-pMTL82151::*codY*, overexpression strain WT-pMTL82151::*codY*, and vector control strains WT-pMTL82151 and *codY*-pMTL82151 in tryptose-peptone-glucose-yeast extract medium. Error bars indicate standard deviations of three biological replicates.