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| **Table S1. Strains, plasmids, and oligonucleotides used in this study.** |  |
|   | Relevant genotype/comments | Source or reference |
| **Strains** |  |  |
| ***S. venezuelae*** |  |  |
| NRRL B-65442 | Wild type | (NCBI Reference Sequence: NZ\_CP018074.1) |
| SVNT20 | *cdgC::apr*; AprR | This study |
| SVNT21 | *cdgD::apr*; AprR | This study |
| SVNT22 | *cdgE::apr*; AprR | This study |
| SVNT23 | *cdgB::apr*; AprR | This study |
| SVNT24 | *cdgA::apr*; AprR | This study |
| SVNT25 | *cdgF::apr*; AprR | This study |
| SVNT26 | *rmdB::apr*; AprR | This study |
| SVNT27 | *rmdA::apr*; AprR | This study |
| SVNT28 | *hdgA::apr*; AprR | This study |
| SVNT29 | *hdgB::apr*; AprR | This study |
| SVSN1 | *cdgC* (G658A/G659L/D660L) | This study |
| SVSN3 | *cdgC::apr* *attB*ΦBT1::pMS82-*cdgC*; AprR, HygR | This study |
| SVSN55 | *cdgC* (G658A/G659L/D660L) *attB*ΦBT1::pMS82-*cdgC*; AprR, HygR | This study |
| SVSN56 | *cdgC* (G658A/G659L/D660L) *attB*ΦBT1::pMS82; AprR, HygR | This study |
| SVSN57 | *cdgC::apr* *attB*ΦBT1::pMS82; AprR, HygR | This study |
| SVABK-4 | *cdgC::apr*  *attB*ΦBT1::pIJ10257-*cdgB*; AprR, HygR | This study |
| SVNT30 | *cdgA::apr; attB*ΦBT1*::*p3xFLAG*-cdgA*; AprR, HygR | This study |
| SVNT31 | *rmdA::apr; attB*ΦBT1*::*p3xFLAG*-rmdA*; AprR, HygR | This study |
| SVNT34 | *hdgA::apr; attB*ΦBT1*::*p3xFLAG*-hdgA*; AprR, HygR | This study |
| SVNT35 | *hdgB::apr; attB*ΦBT1*::*p3xFLAG*-hdgB*; AprR, HygR | This study |
| SVNT36 | *cdgF::apr; attB*ΦBT1*::*p3xFLAG*-cdgF*; AprR, HygR | This study |
| SVNT37 | *cdgE::apr; attB*ΦBT1*::*p3xFLAG*-cdgE*; AprR, HygR | This study |
| SVNT38 | *cdgD::apr; attB*ΦBT1*::*p3xFLAG*-cdgD*; AprR, HygR | This study |
| SVNT39 | *rmdB::apr; attB*ΦBT1*::*p3xFLAG*-rmdB*; AprR, HygR | This study |
| SVSL1 | *cdgB::apr; attB*ΦBT1*::*p3xFLAG*-cdgB*; AprR, HygR | This study |
| SVSN6 | *cdgC::apr; attB*ΦBT1*::*p3xFLAG*-cdgC*; AprR, HygR | This study |
| ***E. coli*** |  |  |
| ET12567/pUZ8002 | *dam*, *dcm*, *hsd*, KanR, CmR | (Paget et al., 1999) |
| BW25113/pIJ790 | (Δ(*araD-araB*)*567*, Δ*lacZ4787*(::*rrnB-4*), *lacIp-4000*(lacIQ), λ-, *rpoS369*(Am), *rph-1*, Δ(*rhaD-rhaB*)568, *hsdR514*; CmR | (Datsenko and Wanner, 2000) |
| HME68 | W3110 *galKtyr145UAG* Δ*lacU169* [λ *c*I*857* Δ*(crobioA)*]*mutS*<>*cat;* defective methyl-directed mismatch repair system | (Thomason et al., 2014) |
| Rosetta (DE3) | contains pRARE to supply tRNAs for rare codons; CmR  | (Novagen) |
| **Plasmids** |  |  |
| pMS82 | Vector for conjugal transfer of DNA from *E. coli* to *Streptomyces*; integrates site-specifically at the ΦBT1 attachment site; HygR | (Gregory et al., 2003) |
| pIJ773 | Plasmid template for amplification of the *apr-oriT* cassette for ‘Redirect’ PCR-targeting | (Gust et al., 2003) |
| pIJ790 | Modified l RED recombination plasmid [*oriR101*] [*repA101*(ts)] *araBp-gam-be-exo*, CmR | (Gust et al., 2003) |
| pUZ8002 | RP4 derivative with defective oriT, KanR | (Paget et al., 1999) |
| pIJ10257 | Plasmid integrating at the ϕBT1 *attB* attachment sitecontaining the constitutive *ermEp*\* promoter, HygR | (Hong et al., 2005) |
| pIJ10350 | pIJ10257 carrying *cdgB* from *S. coelicolor* | (Tran et al., 2011) |
| pSVOJ1 | pMS82-*cdgC* | This study |
| pIJ10770 | pMS82 with an extended Multiple Cloning Site (MCS) lacking an intrinsic apramycin promoter upstream of the extended MCS | (Schlimpert et al., 2017) |
| p3xFLAG | pIJ10770 containing triple Flag Tag downstream of the MCS in the XhoI/KpnI sites | This study |
| pSVSL-4 | pSS170 *cdgA*-FLAG | This study |
| pSVSL-11 | pSS170 *cdgB*-FLAG | This study |
| pSVSN-23 | pSS170 *cdgC*-FLAG | This study |
| pSVSL-3 | pSS170 *cdgD*-FLAG | This study |
| pSVSL-5 | pSS170 *cdgE*-FLAG | This study |
| pSVSL-6 | pSS170 *cdgF*-FLAG | This study |
| pSVJH01 | pSS170 *rmdA*-FLAG | This study |
| pSVJH02 | pSS170 *rmdB*-FLAG | This study |
| pSVNT-11 | pSS170 *hdgA*-FLAG | This study |
| pSVNT-12 | pSS170 *hdgB*-FLAG | This study |
| pSVSN-2 | pET15b-∆TM-*cdgC* | This study |

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| **Oligonucleotides used for amplification of the *oriT-apr* cassette with gene-specific extensions**  |
| Oligonucleotide | 5′-3′ Sequence |
| *cdgC*\_H1-P1-fw | GCGGCCGGGTGCGGTGGCGCGGCTCCGGAGGGACCGGTGATTCCGGGGATCCGTCGACC |
| *cdgC*\_H2-P2-rev | CATGGCACTGACCCTCCCTCTCCGCCCTCACCACTGTCATGTAGGCTGGAGCTGCTTC |
| *cdgD*\_H1-P1-fw | CGTCGTCCGCGACATGCAGAAGGGCCGGTAGCGGCCATGATTCCGGGGATCCGTCGACC |
| *cdgD*\_H2-P2-rev | CGGAAGTTTGGCGACCGAGGCCGCTCCGGGGGCCGCTCATGTAGGCTGGAGCTGCTTC |
| *cdgE*\_H1-P1-fw | AAGGGAAAACGGATCAAACCTCCCTAAGCTGGAGGAATGATTCCGGGGATCCGTCGACC |
| *cdgE*\_H2-P2-rev | ACGCCCCCCCGAGGGGAAGCCCGCGCCCGCGGCTCCTCATGTAGGCTGGAGCTGCTTC |
| *cdgB*\_H1-P1-fw | CTGGAGTTGCTGTCGAGGTCTCGGGGAGAGCGAGCGATGATTCCGGGGATCCGTCGACC |
| *cdgB*\_H2-P2-rev | CGAGCCCCCGAAGCGGCCCCGGGCGTGTCAGGAGCCTCATGTAGGCTGGAGCTGCTTC |
| *cdgA*\_H1-P1-fw | ACATGTGACCGGCCCGACTGCACTACGGGAGCGAGCGTGATTCCGGGGATCCGTCGACC |
| *cdgA*\_H2-P2-rev | TGCCTACCGCTCAGACCCGTACCGGCTCCGTACCGTTCATGTAGGCTGGAGCTGCTTC |
| *cdgF*\_H1-P1-fw | CCCGACTCCGTCGCCGAGGGGACCCGGCTCCTGCCCGTGATTCCGGGGATCCGTCGACC |
| *cdgF*\_H2-P2-rev | GGGGCGGCGCCGGCGGGCGCGGCCCGGTCCCGTGCGTCATGTAGGCTGGAGCTGCTTC |
| *rmdB*\_H1-P1-fw | ACACCGACGGCACCGCCGCCTGTGAGAGGGACGGAAATGATTCCGGGGATCCGTCGACC |
| *rmdB*\_H2-P2-rev | GGTTTGCCCGGGGTCGTCGCGCCGGGGTGACACGGCCTATGTAGGCTGGAGCTGCTTC |
| *rmdA*\_H1-P1-fw | CGCCTTCTCCGGTTTCGCAGGGGTGGGGCGCAAGGCGTGATTCCGGGGATCCGTCGACC |
| *rmdA*\_H2-P2-rev | CTTCGCGCGGGGAGGCCGGTTTCGTGCGTCCGGGTCTCATGTAGGCTGGAGCTGCTTC |
| *hdgA*\_H1-P1-fw | GCGGCACGCCCGCGACGGCCTCCCGCGTATATCAGCTTGATTCCGGGGATCCGTCGACC |
| *hdgA*\_H2-P2-rev | TCATGCGCGTTCCCTGGGCCGGGGCGGCAGCGGCGCGCCTGTAGGCTGGAGCTGCTTC |
| *hdgB*\_H1-P1-fw | CCCGGCGCCCTCACCGTCGGCGCCGTGCACGGGGCCGCCATTCCGGGGATCCGTCGACC |
| *hdgB*\_H2-P2-rev | CCATGCCCGGTCGGACGCGCGCTCATTACCCATGGGTTATGTAGGCTGGAGCTGCTTC |
| **Oligonucleotides used for verification of deletion mutants** |
| *cdgC*-HindIII-MS82-fw | GGTGGTAAGCTTGTCACGGAGAGTACTGCCAC |
| *cdgC*-KpnI-MS82-rev | AGTAGTGGTACCCATCTCTTCTCGAAGCAGAG |
| *cdgD*-test-fw | GACACCACGATCAACAAGTAG |
| *cdgD*-KpnI-MS82-rev | AGTAGTGGTACCGTAGCGAAGCCGTCGCAGTC |
| *cdgE*-test-fw | CCTTCAACGAGAAGCGGAAG |
| *cdgE*-test-rev | CAGCACCCTACGGATTGAAG |
| *cdgB*-HindIII-MS82-fw | GGTGGTAAGCTTCACACCCGCACGCTAGTC |
| *cdgB-*KpnI-MS82-rev | AGTAGTGGTACCGGTTCCTCTCCATGCTCGC |
| *cdgA*-HindIII-MS82-fw | GGTGGTAAGCTTCACGTGGACCTCCGGTTCG |
| *cdgA*-KpnI-MS82-rev | AGTAGTGGTACCCGCTGAGTCCCGCATGAAC |
| *cdgF*-test-fw | CTGCCACACCGACGAGCTGAC |
| *cdgF*-test-rev | GCATCAGCGGATAGAGGTTG |
| *rmdB*-HindIII-MS82-fw | GGTGGTAAGCTTGAAGCTCACCCGTTCGGCG |
| *rmdB*-KpnI-MS82-rev | AGTAGTGGTACCGGTGAGCTTGATGGTGTGG |
| *rmdA*-test-fw | TCGTCCTCACCCACGCCTC |
| *rmdA*-test-rev | TGAGTGTTACCGTCCTACCG |
| *hdgA*-test-fw | GCAGCGACGACGTCCCCAC |
| *hdgA*-test-rev | TTCGCCGACGGCGGGTGC |
| *hdgB*-test-fw | CTTCGACCCCCGGATGGT |
| *hdgB*-test-rev | TCCTCGTACCGTCCGTCC |
| **Oligonucleotides used for cloning of *cdgC* into pMS82 and for amplification of *cdgC* (G658A/G659L/D660L) PAS-PAC-GGDEF region for sequencing** |
| 5187-HindIII-pMS82-f  | CAGAAGCTTGAGCCTGTGAACGTCCTC  |
| 5187-KpnI-pMS82-rev  | CAGGGTACCGCATCTCTTCTCGAAGCAG  |
| 5187-NdeI-pET15b-GGDEF-fw | CAGCATATGATCATGATCGCCGCTCCC |
| 5187\_pET15b-XhoI-PPG-rev | GACTCTCGAGTTAGACCACCTCGGCCTG |
| **ssOligonucleotides used for generation of G658A/G659L/D660L mutations in *cdgC* on the PI1\_C11 cosmid** |
| ssRec5187 ALLEF | GGCCGGGGACACGGCCGCCCGGCTCGCGCTCCTCGAGTTCGCCGCCCTCATCCTGGGCGACGGCGGACGC |
| **Oligonucleotides used for specific amplification of *cdgC* (G658A/G659L/D660L) for selection of clones containing the desired mutation** |
| 5187-NdeI-pET15b-GGDEF-fw | CAGCATATGATCATGATCGCCGCTCCC |
| test\_5187-ALLEF-rev | CGGCGAACTCGTCCGCCG |
| **Oligonucleotides used for amplification of the *oriT-apr* cassette with *neo*-specific extensions for replacement of the kanR cassette on the PI1\_C11 cosmid and for verification of the exchange** |
| neo\_H1-P1-fw | AGATCTGATCAAGAGACAGGATGAGGATCGTTTCGCatgATTCCGGGGATCCGTCGACC |
| neo\_H2-P2-rev | TCGCTTGGTCGGTCATTTCGAACCCCAGAGTCCCGCtcaTGTAGGCTGGAGCTGCTTC |
| neo\_test-fw | GTTTTATGGACAGCAAGCG |
| neo\_test-rev | GAATCGAAATCTCGTGATG |
| **Oligonucleotides used for generation of the Flag constructs and for overexpression of ∆TM-*cdgC*** |
| 5058\_NdeI-f | GCTGCATATGCTCCCGGCCCCCTCG |
| 5058\_AvrII-r | CATGCCTAGGCTGGACGACCTGGCCG |
| 6683-4\_HindIII-for | GATCAAGCTTCAACGCGCGGGATTCGC |
| 6684\_XhoI-rev | GCAGCCTCGAGGACGGCGTCCGCCAG |
| sven15\_3908\_XhoI-r | GCAGCCTCGAGGTGTGTGTTGCGCCTGC |
| sven15\_3910\_NdeI-f | GCTGCATATGGAACCGCTTCCACCACGT |
| 4502\_AvrII-rev | CATGCCTAGGCCCGCCCCCGTCCC |
| 4502\_NdeI-for | GCTGCATATGGTGGAGGCGGCGGA |
| sven15\_3942\_NdeI-f | GCTGCATATGGGGCCCTCGGGGGG |
| sven15\_3942\_NruI-r | CATGTCGCGAGCCGGCCCGCCGG |
| 2547\_AvrII-r | CATGCCTAGGGACCCGCAGCGGTTCC |
| 2547\_NdeI-f | GCTGCATATGGGCGCGCGGGGTCGA |
| sven15\_0422\_AvrII\_r | CATGCCTAGGCGGCTCCGGCGTCCG |
| sven15\_0422\_NdeI\_f | GCTGCATATGGCCGAACCGAGCGGAC |
| 4769-70\_NdeI-for | GCTGCATATGGACACGGACTGCACCAAG |
| 4769-FLAG\_XhoI-rev | GCAGCCTCGAGTGCGCGTTCCCTGGGC |
| 4770-FLAG\_XhoI-rev | GCAGCCTCGAGCCCAGCGGGGGCGGA |
| 5187-HindIII-pMS82-f | CAGAAGCTTGAGCCTGTGAACGTCCTC |
| sven15\_5080\_XhoI\_r | GCAGCCTCGAGAGTGGGTGGGACAGGCG |
| 3xFLAG\_XhoI\_f | CATCTCTCGAGGACTACAAGGAC |
| 3xFLAG\_KpnI\_r | CACTGGTACCCTACTTGTCGTCATCGTC |
| 5187\_pET15b-NdeI-PPGEoTM-fw | GACCATATGATCATGCTGCTCGACAAC |
| 5187-XhoI-pET15b-EAL-rev | CAGTCTCGAGTTCAAGTGGGTGGGACA |

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