Table S17: list of bacterial strains and plasmid used in this study.

|  |  |  |
| --- | --- | --- |
| **Strain/plasmid** | **Details** | **Reference/source** |
| ***E. coli*** | |  |
| DH5α | Standard laboratory cloning strain; genotype: F- *endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80dlacZΔM15 Δ(lacZYA-argF)U169, hsdR17(rK- mK+), λ–* | (1) |
| S17-1 (λpir) | Standard laboratory conjugative strain; genotype: *recA pro hsdR RP4-2-Tc::Mu-Km::Tn7* | (2) |
| C2925 | Dam and Dcm methylation deficient strain; genotype: *ara-14 leuB6 fhuA31 lacY1 tsx78 glnV44 galK2 galT22 mcrA dcm-6 hisG4 rfbD1 R*(*zgb210::Tn10*) TetS *endA1 rspL136* (StrR) *dam13::Tn9* (CamR) *xylA-5 mtl-1 thi-1 mcrB1 hsdR2* | New England Biolabs, Inc. |
| ***C. necator*** | |  |
| H16 | *C. necator* H16 wild type (DSM 428) | Leibniz Institute DSMZ-German Collection of Micro-organisms and Cell Cultures, Braunschweig, Germany |
| ΔA0792 | Mutant strain carrying the in-frame deletion of the *H16\_A0792* (*pheA*) gene, encoding a prephenate dehydratase | This study |
| ΔA3038 | Mutant strain carrying the in-frame deletion of the *H16\_A3038* (*nadA*) gene, encoding a quinolinate synthase | This study |
| ΔA3084 | Mutant strain carrying the in-frame deletion of the *H16\_A3084* (*panB*) gene, encoding a 3-methyl-2-oxobutanoate hydroxymethyltransferase | This study |
| ΔA3165 | Mutant strain carrying the in-frame deletion of the *H16\_A3165* (*ubiC*) gene, encoding a chorismate pyruvate-lyase | This study |
| ΔA3408 | Mutant strain carrying the in-frame deletion of the *H16\_A3408* (*hisE*) gene, encoding a phosphoribosyl-ATP pyrophosphatase | This study |
| ΔA3434 | Mutant strain carrying the in-frame deletion of the *H16\_A3434* (*aroB*) gene, encoding a 3-dehydroquinate synthase | This study |
| **Plasmids** |  |  |
| pMTL70115 | Suicide transposon delivery vector carrying the *tnpA* transposase-encoding gene and the miniTn5*::tetA* transposon | [This](#_ENREF_30) study |
| pMTL70621-SacB | Suicide modular vector used for deletion of genes in *C. necator*; carries the antibiotic resistance gene *tetA* and the *sacB* gene as a counter-selection marker | (3) |
| pMTL70621-SacB::ΔA0792 | pMTL70621-SacB derivative for deletion of *H16\_A0792* (*pheA*) | This study |
| pMTL70621-SacB::ΔA3038 | pMTL70621-SacB derivative for deletion of *H16\_A3038* (nad*A*) | This study |
| pMTL70621-SacB::ΔA3084 | pMTL70621-SacB derivative for deletion of *H16\_A3084* (*panB*) | This study |
| pMTL70621-SacB::ΔA3165 | pMTL70621-SacB derivative for deletion of *H16\_A3165* (*ubiC*) | This study |
| pMTL70621-SacB::ΔA3408 | pMTL70621-SacB derivative for deletion of *H16\_A3408* (*hisE*) | This study |
| pMTL70621-SacB::ΔA3434 | pMTL70621-SacB derivative for deletion of *H16\_A3434* (*aroB*) | This study |

Table S18: list of oligonucleotide primers used in this study.

|  |  |  |
| --- | --- | --- |
| **Primer** | **5’ to 3’ sequence** | **Details** |
| MCSTn5\_FOR | GCTTCCCGGGGATCAT | Primers used to verify insertion of the miniTn5*::tetA* transposon in the *C. necator* H16 genome |
| Tn5\_NCOseq 3\_REV | GTGGCGGGACCAGTGA |
| A0792\_U\_Fwd | tttatcaggaaacagctatgaccgcggccgcGTTCCAGGACATTCCCGATATC | Primers used to amplify the *H16\_A0792* upstream region. Sequences in capital letters anneal on the *C. necator* H16 genome. Primer A0792\_U\_Fwd was also used to screen for H16 *ΔH16\_A0792* mutants, in combination with A0792\_D\_Rev |
| A0792\_U\_Rev | ggctgccttacttTGTCATTTTGTATGAATCCGG |
| A0792\_D\_Fwd | atacaaaatgacaAAGTAAGGCAGCCAGGCAAG | Primers used to amplify the *H16\_A0792* downstream region. Sequences in capital letters anneal on the *C. necator* H16 genome. Primer A0792\_D\_Rev was also used to screen for H16 *ΔH16\_A0792* mutants, in combination with A0792\_U\_Fwd |
| A0792\_D\_Rev | tgccaagcttgcatgtctgcaggcctcgagACACCATCAGGTTCGGGTAG |
| A3038\_U\_Fwd | tttatcaggaaacagctatgaccgcggccgCTGATCATTGGTGGCGAAGAG | Primers used to amplify the *H16\_A3038* upstream region. Sequences in capital letters anneal on the *C. necator* H16 genome. Primer A3038\_U\_Fwd was also used to screen for H16 *ΔH16\_A3038* mutants, in combination with A3038\_D\_Rev |
| A3038\_U\_rev | ttcacgctcatgcGGTCATCTCCGGTACTCCTC |
| A3038\_D\_Fwd | accggagatgaccGCATGAGCGTGAATTCGATTTTC | Primers used to amplify the *H16\_A3038* downstream region. Sequences in capital letters anneal on the *C. necator* H16 genome. Primer A3038\_D\_Rev was also used to screen for H16 *ΔH16\_A3038* mutants, in combination with A3038\_U\_Fwd |
| A3038\_D\_Rev | tgccaagcttgcatgtctgcaggcctcgagATTGATCCTGACCGCATCCTG |
| A3084\_U\_Fwd | tttatcaggaaacagctatgaccgcggccGCCATGCTCGATCACCTGCG | Primers used to amplify the *H16\_A3084* upstream region. Sequences in capital letters anneal on the *C. necator* H16 genome. Primer A3084\_U\_Fwd was also used to screen for H16 *ΔH16\_A3084* mutants, in combination with A3084\_D\_Rev |
| A3084\_U\_Rev | caccggctcaggcGCCCATATAGGACGGCTTTGC |
| A3084\_D\_Fwd | gtcctatatgggcGCCTGAGCCGGTGGAGATCG | Primers used to amplify the *H16\_A3084* downstream region. Sequences in capital letters anneal on the *C. necator* H16 genome. Primer A3084\_D\_Rev was also used to screen for H16 *ΔH16\_A3084* mutants, in combination with A3084\_U\_Fwd |
| A3084\_D\_Rev | tgccaagcttgcatgtctgcaggcctcgaGTGGTCATGGGCAACTGAGG |
| A3165\_U\_Fwd | tttatcaggaaacagctatgaccgcggccgCGACCACGTGCTGGTGATGAGC | Primers used to amplify the *H16\_A3165* upstream region. Sequences in capital letters anneal on the *C. necator* H16 genome. Primer A3165\_U\_Fwd was also used to screen for H16 *ΔH16\_A3165* mutants, in combination with A3165\_D\_Rev |
| A3165\_U\_Rev | tgcagtttcatctGCTCATGCCGGGCCTGTGCC |
| A3165\_D\_Fwd | gcccggcatgagcAGATGAAACTGCAGGGTCGG | Primers used to amplify the *H16\_A3165* downstream region. Sequences in capital letters anneal on the *C. necator* H16 genome. Primer A3165\_D\_Rev was also used to screen for H16 *ΔH16\_A3165* mutants, in combination with A3165\_U\_Fwd |
| A3165\_D\_Rev | tgccaagcttgcatgtctgcaggcctcgaGCAGTTCAGAGCGACATGCC |
| A3408\_U\_Fwd | tttatcaggaaacagctatgaccgcggccgCTGGATGCGGTCGAATGGGC | Primers used to amplify the *H16\_A3408* upstream region. Sequences in capital letters anneal on the *C. necator* H16 genome. Primer A3408\_U\_Fwd was also used to screen for H16 *ΔH16\_A3408* mutants, in combination with A3408\_D\_Rev |
| A3408\_U\_Rev | ccagctagtccttGTCGCTCATGGCTTGGTGTAG |
| A3408\_D\_Fwd | agccatgagcgacAAGGACTAGCTGGCGCAGGC | Primers used to amplify the *H16\_A3408* downstream region. Sequences in capital letters anneal on the *C. necator* H16 genome. Primer A3408\_D\_Rev was also used to screen for H16 *ΔH16\_A3408* mutants, in combination with A3408\_U\_Fwd |
| A3408\_D\_Rev | tgccaagcttgcatgtctgcaggcctcgagCTAGCAAGCAGCTCCCCTTC |
| A3434\_U\_Fwd | tttatcaggaaacagctatgaccgcggccgcGAAGATGATGCAGTTCCC | Primers used to amplify the *H16\_A3434* upstream region. Sequences in capital letters anneal on the *C. necator* H16 genome. Primer A3434\_U\_Fwd was also used to screen for H16 *ΔH16\_A3434* mutants, in combination with A3434\_D\_Rev |
| A3434\_U\_Rev | cgatcggtcaggcAATCATGGATTGGGGTCC |
| A3434\_D\_Fwd | ccaatccatgattGCCTGACCGATCGGTTATG | Primers used to amplify the *H16\_A3434* downstream region. Sequences in capital letters anneal on the *C. necator* H16 genome. Primer A3434\_D\_Rev was also used to screen for H16 *ΔH16\_A3434* mutants, in combination with A3434\_U\_Fwd |
| A3434\_D\_Rev | tgccaagcttgcatgtctgcaggcctcGAGCTGCTCCAGCGTCAG |
| A0792\_int\_FOR | GAGGTCGGTGAGGTCAAGAA | Primers annealing within the *H16\_A0792* gene, used to confirm H16 *Δ H16\_A0792* mutants |
| A0792\_int\_REV | AGGTACCGACAGGATCATCG |
| A3038\_int\_FOR | ATCGATCAAGACCGTCGAGT | Primers annealing within the *H16\_A3038* gene, used to confirm H16 *Δ H16\_A3038* mutants |
| A3038\_int\_REV | TGGATATAGCTGCCCAGGTG |
| A3084\_int\_FOR | CCATGAGCTACCTCCTCGAT | Primers annealing within the *H16\_A3084* gene, used to confirm H16 *Δ H16\_A3084* mutants |
| A3084\_int\_REV | GTCAGCGATTGCGTGATCT |
| A3165\_int\_FOR | ACCTGGCCTTTGATGCAG | Primers annealing within the *H16\_A3165* gene, used to confirm H16 *Δ H16\_A3165* mutants |
| A3165\_int\_REV | TAGAGGGGCTTCGGTCTGTA |
| A3408\_int\_FOR | GACAACCAACTCAGCAGCAA | Primers annealing within the *H16\_A3408* gene, used to confirm H16 *Δ H16\_A3408* mutants |
| A3408\_int\_REV | AAGTTGGCCAGCAATACCAT |
| A3434\_int\_FOR | GCAGCTACCCCATCCATATC | Primers annealing within the *H16\_A3434* gene, used to confirm H16 *Δ H16\_A3434* mutants |
| A3434\_int\_REV | GTGTGGCCGAAATTGAGAAT |

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