## Supplemental material

Table S1. The oligonucleotides used in this study.

| Primer | Sequence | Added restriction site |
|---|---|---|
| **RT-qPCR** | | |
| ro01338 | | |
| Forward primer | CACCGTTCCAGGACTTCATC | |
| Reverse primer | TGTGCATGTCGAGTTCGTG | |
| Probe | CGTGACCAGATGTCGCCCCA | |
| ro02797 | | |
| Forward primer | ATCGCTCCGTTCAACTTCC | |
| Reverse primer | AGAATCACTGAGTTGCCCAG | |
| Probe | ATCCTCTCGATCCGCGCAGTG | |
| ro02845 | | |
| Forward primer | GTACGCCGTTGTCCTTCG | |
| Reverse primer | GAAAACTGTTGCACGGACTG | |
| Probe | ACGCTGAGGACCGAACGCTT | |
| ro02986 | | |
| Forward primer | CACTGCCATTGGGTGAATTC | |
| Reverse primer | CCACATCAACGACCAGACC | |
| Probe | AATCGCGTGCCGTTGCCG | |
| ro04165 | | |
| Forward primer | GAGCCGATCCTGTTCTGG | |
| Reverse primer | CCGTAGGTGAAACCGTGATAG | |
| Probe | CCGCTACCCGCTGTCCGAA | |
| DNA polymerase IV | | |
| Forward primer | TGAGCAAGTCCGTGTTCG | |
| Reverse primer | CGCAGTCCCTCGCAATAG | |
| Probe | ACGCCTCGTCCATCGACAGTTG | |
| **Cloning** | | |
| ro02797 Forward | TATATATCATATGAGCCTGCTCGACATCGACTGG | *NdeI* |
| ro02797 Reverse | TATATAAGCTTCAGAACGGGTACTGCTGAATCGGC | *HindIII* |
| ro02845 Forward | CCTCCTCATATGAGCATGTTCACTGTCACTGTCA | *NdeI* |
| ro02845 Reverse | CCTCTGGATCCTCAGGGAAGTTCGACGACGAG | *BamHI* |
| ro02986 Forward | GGGGGCATATGACTGACCTTCTCGGCGGC | *NdeI* |
| ro02986 Reverse | CCGCAAGCTTCAGAACGGGAACTGTTGGGCCA | *HindIII* |
| ro04163 Forward | CCTTCCTCATATGCCTATCCCACCCGAACC | *NdeI* |
| ro04163 Reverse | CCTCTGGATCCTCACAGTTCCAGCGTCAGAC | *BamHI* |

| | | |
|---|---|---|
| ro04165 Forward | TTTCTCTCATATGTCCACGATCGCCCGCAACC | *NdeI* |
| ro04165 Reverse | TTCTTAAGCTTCAGCCCACCGCCGCGGTGACG | *HindIII* |
| **Gene knockout** | | |
| "Up" fragment of *vdh* | | |
| 2986_F1 | TTTTAAGCTTGGTGCTGAGCCGCAGCCCCC | *HindIII* |
| 2986_F2 | TTTTTCTAGAGGTGACCCAGTTGCCGTCG | *XbaI* |
| "Down" fragment of *vdh* | | |
| 2986_R1 | GGTGTCTAGATTCGGAACGCAGAGCAGC | *XbaI* |
| 2986_R2 | TTTTGGATCCGAGCACGAGTACCGGGTTC | *BamHI* |
| "Up" fragment of *vanA* | | |
| 4165_F1 | CTCTTTCTAGACCGCCCTGGACCAACTCGCC | *XbaI* |
| 4165_F2 | CTCTTGTTAACGGATCGGCTCCTCGCAGATGG | *HpaI* |
| "Down" fragment of *vanA* | | |
| 4165_R1 | CTCCTGTTAACACCAGGAGCTGTCGATCAACATC | *HpaI* |
| 4165_R2 | CTCCTAAGCTTCCGCAGCAGTAGACGAGGGTG | *HindIII* |
| **Screening knockout mutant** | | |
| 2986M1 | CGGAGGGAGTACTGCACGTAC | |
| 2986M2 | GGTGTTCGTTGATGAGGGGG | |
| 4165M1 | GGATTCCCCCGACTACACCGTGG | |
| 4165M2 | GAGGAACGTCGTGACGGCGTCG | |

Table S2. Strains and plasmids used in this study

| Strain / plasmid | Relevant genotype/comments | Source |
|---|---|---|
| Plasmids | | |
| pET28a+ | pT7 promoter, *lacI*, $Kan^R$ | Novagen |
| pETvanABC | pET28a+ with *vanABC* cloned into *Nde*I and *BamH*I sites | This study |
| pETvdh | pET28a+ with *vdh* cloned into *Nde*I and *Hind*III sites | This study |
| pETvanA | pET28a+ with *vanA* cloned into *Nde*I and *Hind*III sites | This study |
| pETvanB | pET28a+ with *vanB* cloned into *Nde*I and *BamH*I sites | This study |
| pET2797 | pET28a+ with ro02797, cloned into *Nde*I and *Hind*III sites | This study |
| pET2845 | pET28a+ with ro02845 cloned into *Nde*I and *BamH*I sites | This study |
| pK18*mobsacB* | 5.7-kb mobilizable suicide vector used for triple ligation; *sacB alphII* | (5) |
| pK18vanA | pK18*mobsacB* containing 2.0-kb fusion PCR fragment flanking Δ*vanA*; used to make strain RHA046 | This study |
| pK18vdh | pK18*mobsacB* containing 2.0-kb fusion PCR fragment flanking Δ*vdh*; used to make strain RHA045 | This study |
| pTipII | Rhodococcal expression vector; *tipA* promoter | (2) |
| pTipvanA | *vanA* cloned into pTipII; used for complementation test | This study |
| pTipvdh | *vdh* cloned into pTipII; used for complementation test | This study |
| Strains | | |
| *E. coli* | | |
| DH5α | $F^-$ λ$^-$ *endA1 hsdR17 hsdM*$^+$ *supE44 thi-1 recA1 gyrA96 relA1* Δ(*argF lacZYA*)*U169* φ*80d* Δ(*lacZ*)*M15*; Used to clone and propagate DNA. | Bethesda Research |
| S17-1 | *recA1 pro thi*; has the *tra* genes from plasmid RP4 integrated in the chromosome; Donor strain for conjugation | (4) |
| BL21(DE3) | *hadS gal*(λ*cIts857 indI Sam7 nin5 lac*UV5-T7 *gene1*) | (1) |
| *Rhodococcus jostii* | | |
| RHA1 | Wild-type, $Cm^R$ | |
| RHA005 | Mutant derivative of RHA1 with *pcaL* replaced with $Apra^R$ | (3) |
| RHA045 | Mutant derivative of RHA1 with *vdh* disrupted. | This study |

| | | |
|---|---|---|
| RHA046 | Mutant derivative of RHA1 with *vanA* disrupted. | This study |

*Gene cloning and protein expression* –DNA fragments containing each of *vdh*, ro02797, ro02845, *vanACB, vanA*, and *vanB* were amplified by PCR according to standard procedures using RHA1 genomic DNA as a template and the respective primers indicated in Table 1. The amplicons were cloned into pET28a+ vector using the endonucleases indicated in Table 2, and their nucleotide sequences were verified. The resulting plasmids, listed in Table 2, were freshly transformed into *E. coli* BL21 to facilitate the production of recombinant protein. Individual colonies were used to inoculate 5-mL of LB medium containing 50 μg/mL kanamycin. Cultures were grown overnight at 37°C and then used to inoculate 200 ml of LB medium containing 50 μg/mL kanamycin. These cultures were incubated at 37°C until they reached an $OD_{600}$ of ~0.6, at which point the temperature was lowered to 16°C. After 30 min, expression was induced by the addition of 100 mg/L IPTG and the cultures were incubated overnight. The cells were harvested by centrifugation at 4000 *x g* for 10 min and were then stored at -20°C.

Protein production was verified using 12% SDS-PAGE analysis of whole cells. Polypeptides corresponding to Vdh, Ro02797, Ro02845, VanA and VanB were detected in cells containing pETvdh, pET2797, pET2845, pETvanA and pETvanB, respectively. In cells containing pETvanABC, only polypeptide corresponding to VanA was detected.

## References for supplemental material


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3. **Patrauchan, M.A., C. Florizone, M. Dosanjh, W.W. Mohn, J. Davies, and L.D. Eltis**. 2005. Catabolism of benzoate and phthalate in *Rhodococcus* sp. strain RHA1: redundancies and convergence. J. Bacteriol. **187:**4050-4063.
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5. **Schäfer, A., Tauch, A., Jäger, W., Kalinowski, J., Thierbach, G. and Pühler, A**. 1994. Small mobilizable multi-purpose cloning vectors derived from the *Escherichia coli* plasmids pK18 and pK19: selection of defined deletions in the chromosome of *Corynebacterium glutamicum*. Gene **145**: 69-73.