Supporting Information

D-2-Hydroxyglutarate dehydrogenase plays a dual role in L-serine biosynthesis and D-malate utilization in the bacterium *Pseudomonas stutzeri*

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Running title: Dual role of D-2-hydroxyglutarate dehydrogenase

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TABLE S1.

Strains and plasmids used in this study.

Strain/plasmid	genotype or relevant characteristics
Strain	
E. coli DH5α	F^-, φ80 *lacZ*Δ*M15*, Δ(*lacZYA-argF*) *U*169, *recA*1, *endA*1, *hsdR*17, *phoA*, *supE*44λ^-, *thi*$^{-1}$, *gyrA*96, *relA*1
E. coli BL21 (DE3)	F^- *ompT gal dcm lon hsdS*$_B$(rB^- mB^-)λ(DE3), used for protein expression
P. stutzeri A1501-WT	wild strain of *P. stutzeri* A1501
P. stutzeri A1501-pk18-*d2hgdh′*	*P. stutzeri* A1501 strain harboring chromosomal integration with pk18-*d2hgdh′* plasmid; Km^r *
P. stutzeri A1501-pk18-*etf′*	*P. stutzeri* A1501 strain harboring chromosomal integration with pk18-*etf′* plasmid; Km^r *
P. stutzeri A1501-Δ*d2hgdh*	*P. stutzeri* A1501 strain mutant obtained by exchanging the *d2hgdh* gene with the *d2hgdh′*
P. stutzeri A1501-Δ*etf*	*P. stutzeri* A1501 strain mutant obtained by exchanging the *etf* gene with the *etf′*
P. stutzeri A1501-Δ*d2hgdh-d2hgdh*$^+$	A1501-Δ*d2hgdh* strain harboring the plasmid pBBR1MCS-5-*d2hgdh*; Gm^r *
P. stutzeri A1501-Δ*etf-etf*$^+$	A1501-Δ*etf* strain harboring the plasmid pBBR1MCS-5-*etf*; Gm^r *
P. stutzeri A1501-Δ*d2hgdh-dmlA*$^+$	A1501-Δ*d2hgdh* strain harboring the plasmid pBBR1MCS-5-*dmlA*; Gm^r *
P. stutzeri A1501-pME6522-P_{d2hgdh}	*P. stutzeri* A1501 strain harboring the transcriptional fusions plasmid pME6522-P_{d2hgdh}
BL-D2HGDH	*E. coli* BL21(DE3) strain harboring the expression plasmid pET-*d2hgdh*
BL-ETF	*E. coli* BL21(DE3) strain harboring the expression plasmid pET-*etfAB*
BL-SerA	*E. coli* BL21(DE3) strain harboring the expression plasmid PET-*serA*
BL-DmlA	*E. coli* BL21(DE3) strain harboring the expression plasmid PET-*dmlA*
E. coli K-12 MG1655-WT	wild strain of *E. coli* MG1655
E. coli K-12 MG1655-Δ*dmlA*	*E. coli* MG1655 strain mutant obtained by exchanging the *dmlA* gene with the *Kana*
E. coli K-12 MG1655-Δ*dmlA-dmlA*$^+$	MG1655-Δ*dmlA* strain harboring the plasmid pMMB66EH-*dmlA*; Ap^r *
E. coli K-12 MG1655-Δ*dmlA-d2hgdh*$^+$	MG1655-Δ*dmlA* strain harboring the plasmid pMMB66EH-*d2hgdh*; Ap^r *
Plasmid	
pk18*mobsacB*	Allelic exchange suicide vector mobilized by *E. coli* S17-1λ*pir*; for selecting double crossover; Km^rSuc^s *
pk18-*d2hgdh′*	pK18*mobsacB* carrying *d2hgdh′*
pk18-*etf′*	pK18*mobsacB* carrying *etfAB′*
pET28a(+)	vector for protein expression, Km^r *

pET-*dmlA*	pET28a(+) carrying *dmlA* gene
pBBR1MCS-5	broad host range cloning vector; Gm^r *
pBBR-*dmlA*	pBBR1MCS-5 carrying *dmlA* gene
pMMB66EH	broad host range *tac*P expression vector; *lac*I^q ; Ap^r *
pMM-*d2hgdh*	pMMB66EH carrying *d2hgdh* gene
pMM-*dmlA*	pMMB66EH carrying *dmlA* gene
pME6522	*E. coli–Pseudomonas* shuttle vector for transcriptional *lacZ* fusions and promoter probing, Tc^r *
pME6522-P_{d2hgdh}	A 199 bp fragment upstream translation initiation site of *d2hgdh* was ligated into the pME6522

*Km^r, Gm^r, Tc^r and Ap^r indicate resistance to kanamycin, gentamicin sulphate, tetracycline and ampicillin, respectively. Suc^s indicates sensitive to sucrose.

TABLE S2.

Primers used in this study.

Primer	Sequence
PD1	5'- TATCCATGGGCATGATGAAAACGATGCGT - 3' †
PD2	5'- GACGGATCCACGCAAAATAATTTTGCAAATCG - 3' †
PBA1	5'- AATTGGATCCATGATGAAAACGATGCG - 3' †
PBA2	5'- AATTAAGCTTTTAACGCAAAATAATTT - 3' †
PMD1	5'- AATTGGATCCATGACCGACCCCGCCCTGA - 3' †
PMD2	5'- AATTAAGCTTTCAGGCCGCGAAGATCTTGC - 3' †
PMA1	5'- AATTGGATCCATGATGAAAACGATGCG - 3' †
PMA2	5'- AAGGAAGCTTTTAACGCAAAATAATTT - 3' †
6522D1	5'- AATTGAATTCCTGCATTAAAACCTTAAGGCC - 3' †
6522D2	5'- AATTCTGCAGCACGGGAACTCTCATACGAT - 3' †

† *Nco*I, *Bam*HI, *Hin*dIII, *Eco*RI, and *Pst*I restriction sites introduced in the primers are underlined.