

Supplementary Materials: Enhanced Lycopene Production in *Escherichia coli* by Expression of Two MEP Pathway Enzymes from *Vibrio* sp. Dhg

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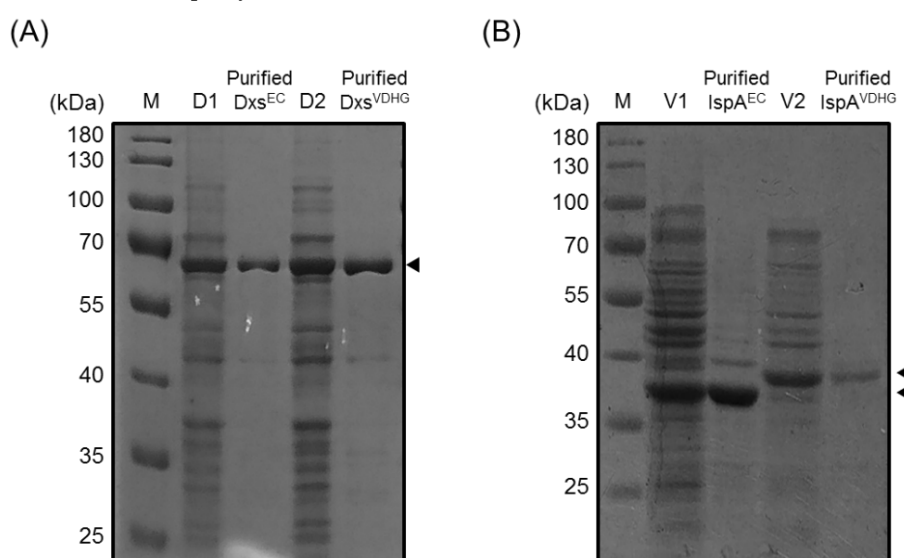


Figure S1. SDS-PAGE of total cell lysates and purified enzymes. Dxs from D1 and D2 strains (A). IspA from V1 and V2 strains (B). The marked bands indicate the purified enzymes. Dxs^{EC}, 68.4 kDa; Dxs^{VDHG}, 68.4 kDa; IspA^{EC}, 33.0 kDa; IspA^{VDHG}, 33.3 kDa (the expected protein size based on amino acid sequence).

Table S1. Synthetic 5'-UTR sequences for gene expression.

Gene	5'-UTR sequence (5'-3')	Predicted expression level ^a (a.u.)
<i>crtE</i>	gcggataacaattaaggaggtaaac	2,630,169.02
<i>crtB</i>	gcggataacaattaaggaggtgatc	2,941,466.04
<i>crtI</i>	gcggataacaattaaggaggcctc	3,110,669.92
<i>dxs^{EC}</i>	gcggataacaattaaggaggtgat	10,353,587.83
<i>dxs^{VDHG}</i>	gcggataacaattaaggaggagaa	3,110,669.02
<i>ispA^{EC}</i>	gcggataacaattaaggaggaaaat	1,236,139.37
<i>ispA^{VDHG}</i>	gcggataacaattaaggagaaatat	3,110,669.92
<i>RAM1</i>	gataacaatttaaggaggaaatact	733,702.64

^aArbitrary unit of the expression [1].

Table S2. Oligonucleotides used in this study.

Name	Sequence (5'–3')
pACYC_F	ccatgaattggatatcgccccaccgtgagcaataacta
pACYC_R	cattatacgagccgatgattaattgtcaaggccgcaagctgtcgacctgc
Tac_crtE_F	gcaggctcgacaagcttgcggccttgacaattaatcatcggctcgataatgtgtggaattgtgagcggataacaatta
crtE_R	aggaggtaaacatggtatctggctcaaaggctg
crtB_F	cggtaggaggagccacacccttacgcaattttcatcaccgg
Tac_crtB_F	gggtgtggctcctccatccgttgacaattaatcatcggctcgataatgtgtggaattgtgagcggataacaattaagg
crtB_R	aggatgatcatgtcccaaccccccttg
crtI_F	gggctacgacgacggcaccgttacaccggcgctgccaca
Tac_crtI_F	cggtgccgtcgtcgtagcccttgacaattaatcatcggctcgataatgtgtggaattgtgagcggataacaattaagg
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crtEBI_R	ttgacaattaatcatcggctcgataatg
dxsE_F	gcaggctgacaagcttgcggcc
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dxsV_F	ctgcgaaatcgctggctacttgacaattaatcatcggctcgataatgtgtggaattgtgagcggataacaattaag
dxsV_R	gaggttgatatgagtttgatattgccaaatacc
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PFT_his_R	ggaggaaaatatggactttccgcagcaactcg
dxsE_his_F	gtagccacgcgatttcgcagactagtttatttattacgctggatgatgtagtccg
dxsE_his_R	gccccatgaattggatatcgcccttgacaattaatcatcggctcgataatgtgtggaattgtgagcggataacaattaag
ispAE_his_F	ggagaaatataatgaacagacattgacttctttcc
ispAE_his_R	gtagccacgcgatttcgcagttatttttgcgctcgatgacatac
dxsV_his_F	ttgacaattaatcatcggctcgataatgtgtggaattgtgagcggataacaattaaggaggaaataactatgcgcc
dxsV_his_R	gctgttggctcgtagc
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pCDF_HR	atcaccacagtttgatattgcaaataccgac
	tgactagtctatgaaaaaaaaaccccgccgaagcgggggttttttttgagctcttaatggtggtggtgatg
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	catcaccacactcttgatattcaagtaacca
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	attgacttctttc
	tgactagtctatgaaaaaaaaaccccgccgaagcgggggttttttttgagctcttaatggtggtggtgatg
	gggtttttttcatagactagtaaacctcaggcatttgagaagc
	cattatacgagccgatgattaattgtcaacataaggagagcgtcgagatc

Table S3. The coding sequence of *dxs*^{VDHG}, *ispA*^{VDHG}, and *RAM1*.

Gene	Sequence
<i>dxs</i> from <i>Vibrio</i> sp. dhg	atgactcttgatatttcaaagtaccaacactggcgcttagcaaatacacccgaagagttgcgtctct tcctaaagaacattacctgctctgtgtgatgagcttcgaacgtatctgctaaattcagtgcgcaat caagcggacacttagcgtctggcttaggcacagtagagctcacagttgcctgcattatgtatataa cacaccagtagaccagttaatctgggatgttggtcatcaggcttaccacataaaatctgaccgga cgtcgtgacaaaatgccgaccatccgtcagaaaggtgggcttaccattcccttggcgctcagag agcgaatacgacacgctgtcgggttggtcactcttcacttcaatcagtgtggaacttggcatggccat cagtgcacagaaagaaggtaaaggcgtaaggctgtgagtggttattggtgacggcgccattacc gcgggtatggcattcgaagcaatgaaccacgctggcgatgttcatccagatatgtggtgattctg aacgataacgaaatgtcgatttcagaaaacgttgggtgcgttaaacaaccacttagcgaattactgt ctggtagcctgtatacgtcgatttcggaaggcggtaaaaagtgttcttggcgcttccgccgatcaa agagctgggtcgtctacagaagaacacctcaaaggcatggtcgtccctggcacactattcgaaga gttcggatttaactacatcgcccaattgatgggcatgatgtcaatgaactgggtcagacttgaag aacatgcgtgagttgaaaggccctcaattctgcacatcatgacaaaaaggcaagggttatgag ccagcagagaaagacctatcggttaccatgtctgtacaaaagttcgcaccatcaaataacagcttgc caaagagcagcgggtgtaaaccaacgttctcaaagatcttgggtgacttctatgcatatggccgc gcaagatcctaagctgatggcgattaccagcaatgcgtgaagggttctggcatggtgcgtttctc gaaagagtcccagaacaatattcgatgttgctatcgctgagcagcatgcagtgcgctcgctacc ggatggcgattgtcggcaataaccaatcgttgccatttactcgaccttctacaacgcgggttatgat cagctcatccacgacatcgcaattatggatttgcagtcattgtcgctatcgaccgagcaggttgggt tggtgctgatggtcagactaccaagggtgcgtttgacttgagctttatgcgctgattccaaacatgg tgatcatggcaccagcgacgagaatgaatgtcgtcaaatgctatacactgggtcacaagcacacg gggtccgagcgcagttcgttacctcgtggaaacgggtatgggaaccgacatcgaaagtgaattacc gcacttgagatcggtaaaggccgaattgtacgtcaaggcgaaaaagtcgcatcctgagcttgggt accttctggggaaatgcgttagaagccgtgaaaaccttaatgcaacgggtgtgacatgcgcttgg ttaagccactagatgaaacgttgattcgtcagctagcgagcgaacacgatgtactcgtgacactgg aagagaatgcgattgtcggcggtgccggtgcaggtgttctgaattcatgatgaaagaaaaatc atcaagccagtactcaaccttggcttacctgataagttgttcatcaaggcactcaggtgagctgca tgaagagcttggctggtatgcgaaaggaattgaacaatccatcaacgattatctggcgaagtaa
<i>ispA</i> from <i>Vibrio</i> sp. dhg	atgcaacagacattgacttcttccaacaaagaacaatcaacaattaaacttggctagaacagc ttccttatcaagaactccattgattgatgcaatgaaatatggccttttctagggcggaacgcgttc gcccttttctgtttacatcacaggccaaatgttcgggtgtaaacctgaagatctggacacaccagcc gcagcgattgaatgcattcacgcttactcacttattcatgatgacctgcagcgatggatgacgatg agttacgccgtggtaaccgacttgcataattaagttcgatgaagcgaccgcaatactgactgggtga cgctctacaaacgctcgcttactatttggcagatgggtctttaaataccagaagctgaaagccagc gtatcaatatgataaaagctctggctcattcgtctggcttaacggcatgtgtgtcggaagaagcactg gatttaagtgcagagaatcgtaaaatttctcgaagaaatggaagaaatccatcgtaaaaagacc ggagccctaattgactgtgcggttaaattaggcgcttggccgctgggtgataagggtatcgagttt tacctcatttagagcgctattcgaaagcaattgggttggcggttcagggttaagacgatattcttgata tcattagtgcacagaaacattgggtgaagcctcaggggttgatcaagaactcaataagagcactta cccttcctgctgggttttagagggagcgatagagaaagctcactctctgttacaggaagcactca

gcattggaagctatccatacaatactcagttactgaagagttcgcccgatatgtcatcgagcgca
aaaattaa

Codon-optimized
RAM1
from *S. cerevisiae*

atgcgccagcgtgttggtcgtagcattgcgcgcgaaatttataacaccgccttgctgggcccga
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