

Supplementary Material

Engineering and adaptive laboratory evolution of *Escherichia coli* for improving methanol utilization based on a hybrid methanol assimilation pathway

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1 Supplementary Figures

Fig S1. Adaptive laboratory evolution process of strain X5. Percentage of HDA in final medium was presented by the black lines.

2 Supplementary Tables

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Table S1. Comparison of ATP efficiency between different methanol assimilation pathways

Pathway	Representative microorganisms	Pathway stoichiometry	Key enzyme
RuMP	<i>Bacillus methanolicus</i>	3Methanol+4NAD ⁺ +ADP→ Pyruvate+4NADH+ATP (TA)	Mdh, Hps, Phi
XuMP	<i>Pichia pastoris</i>	3Methanol+3O ₂ +NAD ⁺ +ATP→ Pyruvate+3H ₂ O ₂ +NADH+ADP	AOX, Das
Serine cycle	<i>Methylobacterium extorquens</i>	2Methanol+ CO ₂ +2PQQ+FP +2NADH+ 2ATP→ Pyruvate+2PQQH ₂ +FPH ₂ +2NAD ⁺ +2ADP	SHMT
rGly	Engineered <i>E. coli</i>	2Methanol+CO ₂ +3NAD ⁺ +2NADPH+2ATP→ Pyruvate+3NADH+2NADP ⁺ + 2ADP	Mdh, FtfI, Fch,MdtA
Hybrid XuMP pathway	Engineered <i>E. coli</i>	3Methanol+4NAD ⁺ +ADP→ Pyruvate+4NADH+ATP	Mdh, Das, FSA
		3Methanol+4NAD ⁺ +ATP→ Pyruvate+4NADH+ADP	Mdh, Das, FBA

Table S2. Primers used in this study

Primer name	Sequence (5'– 3')
pTrc99a-mdh-das construction	
mdh-das-Frag.F	gcggataacaatttcacacaggaacagaccatggaattcaagaaggagatatacatggcggttcagaac
mdh-das-Frag.R	Agcttgcatgcctgcaggtcgactctagaggatccccgggttaaagtttattcactttgtcggtgttggt
pTrc99a-mdh-das-antigapA construction	
backbone-F	gagaatccacccggggatcctctagagtcg
backbone-R	Cattatacagaccggatgattaattgtcaattaaagtttattcactttgtcgctggtttggc
Fragment-F	Ttgacaattaatcatccggctcgataatgaaaaccgtgatacctactttgatagtcacgtcccgcaagga tgcg
Fragment-R	gactctagaggatccccgggtggattctaccaataaaaaacgccc
frmAB_KO_fragment construction	
frmAB_KO-F	Acgccagcaccagcgcgcccagcccggccatagaatgaccagagatagacgtgtaggctggagctgc ttc
frmAB_KO-R	cgtgaccaccgggtattggcgcggtacacaacacagctaaagtcagccagctgatcagtgataagctgtc aaacatgag
pfkA_KO_fragment construction	
pfkA_KO-F	Caatggccgcagccaacgtcagatctccacaataacggcccatcacttccgtgtaggctggagctgcttc

pfkA_KO-R	agacttcggcaacagatttcattttgattccaaagttcagaggtagtcctgatcagtgataagctgtcaaa catgag
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pfkB_KO_fragment construction

pfkB_KO-F	actttccgctgattcgggtgccagactgaaatcagcctataggaggaaatgctgatcagtgataagctgtcaa acatgag
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pfkB_KO-R	Ccaactcgatgttaccaattgccagtgctgcacttaacgcttcgccagaagtgtaggctggagctgcttc
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sucA_KO_fragment construction

sucA_KO-F	ggtagtatccacggcgaagtaagcataaaaaagatgcttaagggatcacgctgatcagtgataagctgtca aacatgag
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sucA_KO-R	Ccaacttcataaccacgcgctttcgacatgttcagggttcctgaaccacgtgtaggctggagctgcttc
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Table S3. Synthesized genes and their optimized sequences used in this study

Gene name	Sequence (5'– 3')
<i>mdh</i>	ATGGCGTTCAAGAACCTGGCGGATCAGACTAATGGTTTCTACATCCCGTG CGTCAGCTTGTTTCGGTCCTGGGTGTGCAAAAGAAGTGGGGGCCAAAGCAC AAAACCTTGGGGCTAAAAAAGCGTTGATCGTCACCGATGCAGGGCTTTTT AAATTCGGAGTGGCAGACATCATCGTAGGGTACCTTAAAGATGCGGGAG TCGACAGTCATGTGTTTCCGGGGGCAGAACCCAATCCCACGGACATTAAT GTATTGAATGGGGTTCAGGCCTATAATGATAATGGATGCGATTTTATTGTT TCTCTGGGTGGAGGCAGCTCACACGACTGCGCTAAGGGCATTGGGCTGGT GACGGCTGGTGGGGGTAACATTCGCGATTATGAGGGTATTGACAAATCGT CAGTCCCCATGACACCCTTAATTGCGATCAATACGACCGCTGGAACAGCC TCGGAAATGACCCGTTTTTGTATTATCACAAATACAGATACCCACGTAA GATGGCAATCGTGGATTGGCGCTGTACTCCTTTGGTCGCTATTGACGACC CGAAATTGATGATTGCTAAACCGGCTGCCCTGACTGCAGCTACTGGCATG GATGCGCTTACACATGCGGTAGAAGCGTACGTGTCGACTGCAGCCAACCC CATTACAGATGCATGCGCTGAAAAGGCGATCAGCATGATTTCTGAGTGGT TGAGTTCAGCAGTAGCAAACGGGGAAAACATCGAAGCGCGCGACGCTAT GGCTTACGCTCAGTATCTTGCGGGTATGGCGTTTAATAATGCGTCGTTAG GATATGTGCACGCCATGGCCCACCAGCTGGGTGGGTTTTACAATTTACCT CACGGTGTATGTAACGCCATCCTGCTGCCACACGTATGCGAGTTCAATCT TATTGCGTGCCCCGATCGTTTTGCGAAGATTGCACAGCTGATGGGCGTAG ATACAACAGGTATGACGGTAACTGAGGCGGGTTACGAGGCTATTGCAGC GATTCGCGAGCTTTCAGCCAGTATTGGGATTCCGAGCGGGCTGACCGAAC TGGGGGTAAAAGCCGCAGACCATGCTGTAATGACGTCTAACGCGCAAAA AGATGCTTG CATGCTGACAAATCCTCGCAAAGCGACTGACGCGCAGGTGA TTGCTATTTTTCGAAGCAGCTATGTAA
<i>das</i>	ATGTCCATGCGCATTCCCAAAGCGGCGAGCGTAAACGACGAGCAGCATC AACGCATTATCAAATATGGCCGCGCCTTGGTCCTGGACATTGTTGAACAG TACGGGGGAGGGCATCCCGGATCGGCCATGGGCGCGATGGCAATCGGCA TTGCATTATGGAAGTATACACTTAAGTACGCTCCTAATGATCCAAATTACT TCAATCGTGATCGCTTCGTTTTGAGTAATGGTCACGTTTGTCTTTTTTCAGT ACATCTTCCAGCACTTGTATGGGTAAAGTCCATGACGATGGCGCAGTTA

AAGTCTTACCACTCGAATGACTTTCATTTCATTATGTCCTGGCCACCCGGAG
ATCGAACACGATGCGGTTGAGGTCCTACCGGACCGCTGGGGCAGGGAA
TCTCTAACTCGGTTGGTTTGGCTATCGCCACCAAGAACCTTGCCGCGACCT
ACAACAAACCAGGCTTCGATATCATTACTAATAAAAGTTTACTGTATGGTT
GGAGATGCATGTTTACAAGAAGGGCCTGCTCTGGAAGTATCAGTTTGGC
TGGACACATGGGGTTGGATAATTTAATTGTCCTTTACGACAACAATCAAG
TATGCTGCGATGGCTCGGTCGACATCGCAAATACCGAGGATATCTCGGCT
AAATTTAAGGCGTGCAATTGGAATGTCATCGAGGTTGAAAATGCCAGTGA
GGACGTTGCAACCATCGTCAAGGCGCTGGAATATGCACAGGCTGAGAAG
CACCGCCCGACTTTAATTAATTGTCGTAAGTGTATCGGTAGTGGGGCAGC
ATTTGAGAACCACTGTGCAGCTCATGGGAACGCTTTAGGCGAAGACGGG
GTACGCGAGTTAAAAATTAAATACGGAATGAACCCGGCGCAAAAATTTT
ATATTCCGCAGGATGTATATGACTTTTTCAAAGAGAAGCCAGCCGAAGGT
GACAAGCTGGTTGCCGAGTGGAAGTCTTGTGGCCAAGTACGTGAAGG
CATACCCGGAAGAGGGTCAAGAATTCCTTGCCCGTATGCGCGGCGAATTG
CCCAAAAACCTGGAAGTCGTTTCTTCCCAACAAGAGTTTACAGGAGATGC
TCCCACACGCGCCGCTGCCCGTGAGTTGGTTCGCGCCCTTGGTCAAAATT
GTAAGTCTGTAATCGCTGGATGTGCCGACTTGTCCGTTTCGGTGAATTTAC
AGTGGCCTGGCGTTAAGTACTTCATGGATCCCTCATTATCCACGCAGTGC
GGGCTTTCCGGAGATTATTCGGGTCGTTATATCGAGTACGGTATTCGCGA
ACATGCGATGTGTGCGATCGCTAACGGATTGGCAGCCTATAATAAAGGGA
CGTTTCTGCCATCACGTCGACCTTCTTCATGTTCTATTTGTACGCTGCGC
CTGCCATTCTGATGGCAGGCCTTCAAGAGTTAAAGGCTATCCATATCGGG
ACGCACGACTCAATTAACGAGGGTGAAAATGGGCCCACACATCAACCCG
TTGAGTCACCAGCGCTGTTCCGCGCTATGCCAAATATTTACTACATGCGCC
CGGTTGACTCCGCAGAAGTGTTCCGGTCTTTTTTCAGAAGGCAGTCGAACTT
CCATTCTCCTCCATCCTGTCGCTTTCACGTAATGAGGTATTGCAATATCCA
GGGAAGTCCTCCGCAGAGAAAGCGCAACGCGGTGGGTACATCCTGGAAG
ACGCGGAGAATGCCGAAGTGCAAATCATTGGAGTCGGGGCCGAAATGGA
ATTTGCTTACAAGGCTGCGAAAATCCTTGGTCGCAAGTTTCGCACTCGCG
TTTTATCAATCCCTTGCACTCGTTTATTTGATGAGCAATCGATTGGATACC
GTCGTAGCGTGCTTCGTAAGGATGGCCGCCAGGTACCCACTGTAGTAGTG
GATGGCCACGTCGATTTCGGCTGGGAACGTTACGCAACCGCAAGCTATTG
TATGAATACTTATGGAAGTCATTACCTCCCGAGGTAATTTATGAATACTT
TGGGTATAATCCGGCGACTATCGCGAAGAAGGTGGAGGCTTACGTACGC

GCTTGCCAGCGCGACCCCTTATTATTACATGATTTCTTAGACTTAAAAGAG
AAGCCAAACCACGACAAAGTGAATAAACTTTAA
