

Supplementary information to

Methanol-based acetoin production by genetically engineered
Bacillus methanolicus

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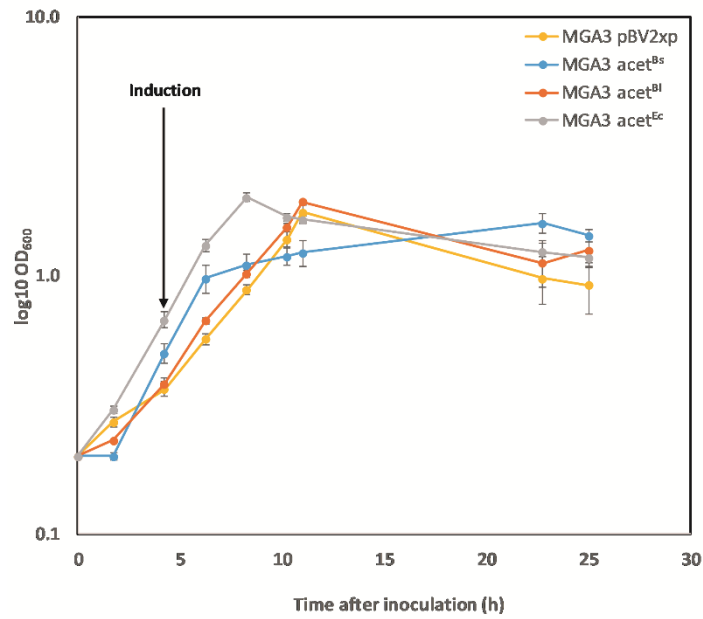


Figure S1 Biomass formation by MGA3 acetoin production strains. The strains were grown in minimal medium with 200 mM methanol and induced with 10 g/L of xylose added to the growth medium 4 hours after inoculation. The OD₆₀₀ was measured every two hours for the first 9 hours, and 25 h after inoculation. The means of triplicates with standard deviations are shown.

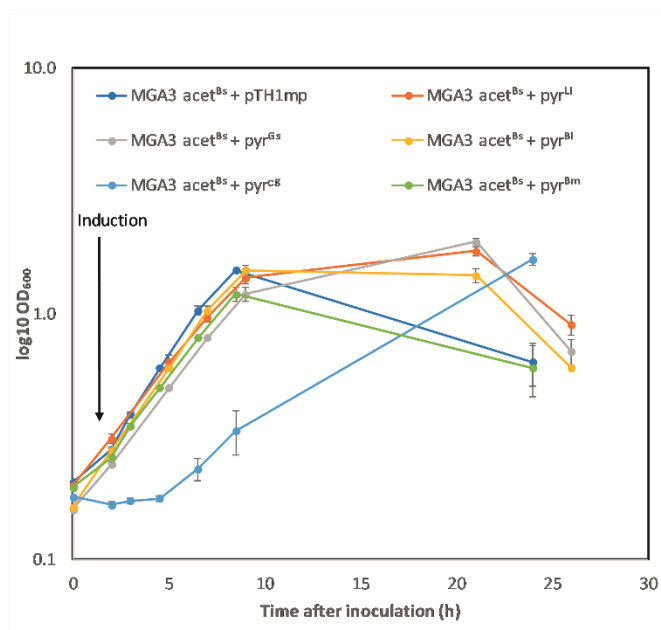


Figure S2 Biomass formation by *B. methanolicus* MGA3 acet^{Bs} strains with a second plasmid overexpressing pyruvate replenishing genes. The strains were grown in minimal medium with 200 mM methanol and induced with 10 g/L of xylose added to the growth medium two hours after inoculation. The OD₆₀₀ was measured every two hours for the first 10 hours, and 25 h after inoculation. The means of triplicates with standard deviations are shown.

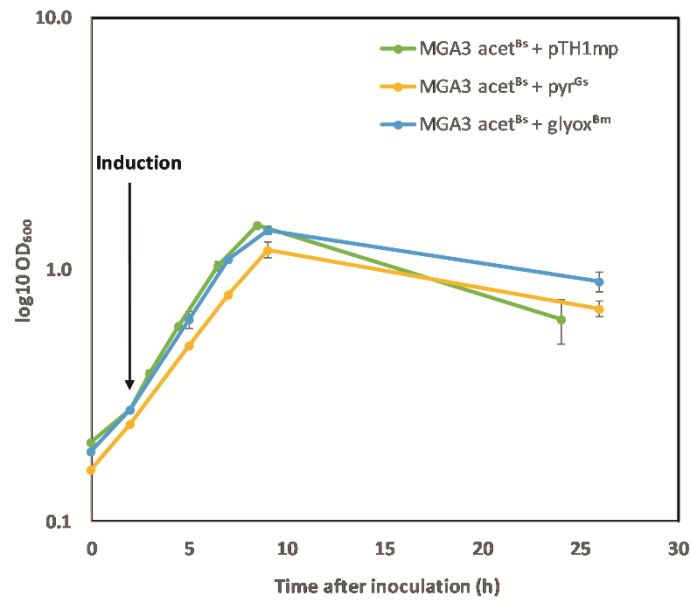


Figure S3 Biomass formation by MGA3 acet^{Bs} strain with overexpressed genes coding for the glyoxylate shunt pathway compared to control strains. The strains were grown in minimal medium with 200 mM methanol and induced with 10 g/L of xylose added to the growth medium two hours after inoculation. The OD₆₀₀ was measured every two hours for the first 10 hours, and 25 h after inoculation. The means of triplicates with standard deviations are shown.

Table S1. Primers used in this study.

Primer name	Sequence 5'→3'	Characteristics
alsSD B. subtilis FW	CTTAAGGGGAAATGGCAAATGACAAAAGCAACAAAGAACAAAAATC	Amplification of <i>alsSD</i> from <i>B. subtilis</i>
alsSD B. subtilis RV	ACGGCCAGTGAATTCGAGCTTTATTCAGGGCTTCCTTCAGTTGT	Amplification of <i>alsSD</i> from <i>B. subtilis</i>
alsSD B. licheniformis FW	CTTAAGGGGAAATGGCAAATGAATAATGTAGCCGCTAAAAATGAAAC	Amplification of <i>alsSD</i> from <i>B. licheniformis</i>
alsSD B. licheniformis RV	ACGGCCAGTGAATTCGAGCTTTACTCGGGATTGCCTTCGG	Amplification of <i>alsSD</i> from <i>B. licheniformis</i>
budAB E. cloacae FW	CTTAAGGGGAAATGGCAAATGATGCACTCATCTGCCTGCCA	Amplification of <i>budAB</i> from <i>E. cloacae</i>
budAB E. cloacae RV	ACGGCCAGTGAATTCGAGCTTCACAAAATCTGGCTGAGATGGA	Amplification of <i>budAB</i> from <i>E. cloacae</i>
AC01	TAAACAATTACATAAATAGGAGGTAGTACATATGCGTTTTGGACGAATTGCC ACCCGAGATGG	Amplification of <i>odx</i> from <i>C. glutamicum</i>
AC02	TAGACCTATGGCGGGTACCATATGTTAGGCGTCCACAACCTGGGTGCCAGCT T	Amplification of <i>odx</i> from <i>C. glutamicum</i>
AC03	TAAACAATTACATAAATAGGAGGTAGTACATATGGTTGATTTTAATAAAGTT TTAGATTTACAC	Amplification of <i>citM</i> from <i>Lactococcus lactis</i> IL1403
AC04	TAGACCTATGGCGGGTACCATATGTTATTTTGCTGAATCTTTACCGCTTTT	Amplification of <i>citM</i> from <i>Lactococcus lactis</i> IL1403
AC05	TAAACAATTACATAAATAGGAGGTAGTACATATGAACAACATGAGAAGAAG AAGGAAGGACATTT	Amplification of <i>mae</i> from <i>B. licheniformis</i>
AC06	TAGACCTATGGCGGGTACCATATGTTAGTCTTGTTTTGCAACAA	Amplification of <i>mae</i> from <i>B. licheniformis</i>
AC07	TAAACAATTACATAAATAGGAGGTAGTACATATGAATTCTGTTACGATTTCA AGCGAGTTG	Amplification of <i>pckA</i> from <i>B. methanolicus</i>
AC08	TTATGCGATAGGACCGCCTTTT	Amplification of <i>pckA</i> from <i>B. methanolicus</i>
AC09	AGGCGGTCTATCGCATAAGTAAACAATTACATAAATAGGAGGTAGTAAGAA TGCGCAGAACAAAAATCG	Amplification of <i>pyk</i> from <i>B. methanolicus</i>
AC10	TAGACCTATGGCGGGTACCATATGTTATAATACGCTCGCATGAC	Amplification of <i>pyk</i> from <i>B. methanolicus</i>
AC11	TAAACAATTACATAAATAGGAGGTAGTACATATGGCATTACCAGCGGGGCA GCCATGAACATTA	Amplification of <i>mae</i> from <i>G. stearothermophilus</i>
AC12	TAGACCTATGGCGGGTACCATATGTTACTGCCCTGTATATCCGCCAACCGGGA TATTG	Amplification of <i>mae</i> from <i>G. stearothermophilus</i>
AC13	CGCCATAGGTCTAGAGCTTGTAACAATTACATAAATAGGAGGTAGTAAGAA TGACAGATTCTAGAGTACAACAATTA	Amplification of <i>aceA</i> from <i>B. methanolicus</i>
AC14	TGTAAAACGACGGCCAGTGAATTCTACTTTTCTTTTAAAAACGTTTTTGA	Amplification of <i>aceA</i> from <i>B. methanolicus</i>
MI09	GATACCAAATACTGTCCTTCTAGTGTAGCCG	Creation of pMI2mp vector
MI10	CGGCTACACTAGAAGGACAGTATTTGGTATC	Creation of pMI2mp vector
aceB_pBx_fw	TGATGGATAAACTTGTTCAAGAGGTAGTACATATGTCCACTCAAACGAC AGGC	Amplification of <i>aceBA</i> from <i>B. methanolicus</i> for pBV2xp
aceA_pBx_rv	GTACGGATCCCATTTCCCTTAAGTTATTTTGGCGGATAAATTGTTCTACT TCCG	Amplification of <i>aceBA</i> from <i>B. methanolicus</i> for pBV2xp
aceB_pEC_fw	ATTGAGCTCGGTACCCGGGAAAGGAGGCCCTTCAGATGTCCACTCAAACGA CAGGC	Amplification of <i>aceBA</i> from <i>B. methanolicus</i> for pEC-XT99A

aceA_pEC_rv	CCTGCAGGTCGACTCTAGAGTTATTTTGGGGCGATAAATTGTTCTACTTCCG	Amplification of <i>aceBA</i> from <i>B. methanolicus</i> for pEC-XT99A
xp-pBV2_fw	CTGCCCCGGGGACGTCGACTCTAACTTATAGGGGTAACTTAAAAAGAAT CAATAACGA	Creation of pBV2xp vector
xp-pBV2_rv	TGAATTCGAGCTCATGGTACGGATCCCATTTCCCCCTTAAGTGAACAAGTTTA TCCATCAACTATCTTAATTGAGTTAGT	Creation of pBV2xp vector

Table S2. Plasmid backbones, promoters and gene sequences used in this study.

[illegible]

		CAAACGGCATGTTTGTGTCATCTGCCAACGCGGTGCGGAACATGTGGTATCATGAAAAGCGGGTGCAGGTTCTTCTGCCGAAAATGCACGACCTGCAAGACGTATCCGTTCAAGTGGCGATGCAGCTGTGTAAGAGCTGCCATTAAAGACGGCGTGGCCCAAGTTGACGCTGAAGATGTGCAAAAGCGCTGCGCGAAGCCATGTGGCGTCCGAGTATAAAAACAATTGTTGCAAAAACAGACTAA	
<i>odx</i> ^{Cs}	pTH1mp- <i>odx</i> ^{Cs}	ATGCGTTTGGACGAATTGCCACCCAGATGGAATGTGTTTTTGTCTAATTGAAGGCGAAGCGGATGATGAGCTAACCTCACCGCCGTGAGATCGAGGGTACCCCTTCACTGAACCAAAGTTACCGGTGCGGAATGGCCACTTAAAGATGACGCTGCTGGCACCATGTCTGCCAAGC AAAGTCGTCGCGATTGGCGGTAACACCGCGATCAGCTTGCTGAGGTGTTTAAAGAATCCGCAGAATCACTGCCACCAACCTGTTCTGAAGCCACCAACAGCTGTCACCGGACACAGAGTCCCAATCCGAATCCCTTCTTTGCCACCAAGGTGGAATTGGAAGGTGAGCTCGCAGTAGTTA TGGCAAGCCCTGCAAGAACGTCAAGGCTGATGACTGGAAGTCTGTGTTTTGGGCTTCAACTCATCAACGAGCTCTCTCCGCTGACCTTCAGTTCGCTGACGGCCAGTGGGACCGGGCTAAGGGCAATTGACACCTTGGGGCCATCGGACCATGGATTGAACTGACATCAACTCCATCGA CTTGGACAACCTGCCATCAAGGCAGCCTCACCCACGACGGCGAAACCAATTGAAGCAGGACTCCAACCCAACAGATGATCATGAAGATGGGCGAAATTATCGAGTTATCATCACGCTCCATGACCTGCTCCGACGGCAGCTTATTGCAACCGGTTCTCCAGCAGGCCGGAAGCAATG GTTGACGGGACTACATCGAAATCGAAATTCAGGCATCGGCAAGCTGGCAACCCAGTTGTGGACGCTTAA	Oxaloacetate decarboxylase gene (<i>C. glutamicum</i>): <i>odx</i> (fwd)
<i>pckA-pyk</i> ^{Bm}	pTH1mp- <i>pckA-pck</i> ^{Bm}	ATGAATTCGTGTTACGATTTCAAGCGAGTTGAGTGATTGTTTAAAGGAAATAATGTTCAAAATCAATTGTCGCTCCCTCAATTAGTTGAGAAGTTTTATACCGTAAAGAAGGGAAGTTGACTTCAACCGGAGCAGTCCGCGCCACAACCGGGAATATACCGGACGTTGCGCTAAAGATAA ATATATTGTTGAAGAAGCTTCTACGAAAGATAAAATCGAATTGGGGCACTGTTAATCAGCCAATTTCCGAAGAAGCATTTACTAAACTATATAATAAAGTTATCAACTATTGTAAGGAAAAAGATGAAATATTGTATTCAAAGGATTTCGGGGTGCTGACAAGAAATACCGCATGCCAATT CAAGTAATAAATGAATATGCATGGCATAACCTGTTGGCCATCAATTATTATCCGCCCTACAGAAGAAGAAATGTTGGAGCATCAAGCGGAATTCACCGTGATTTCGCGACCAAAATTTAAAGCAGATCCTGCAGTTGATGGTACTAGATCGGAAGCTTTATCAITATTCTTTTGAACGT CGCACCTGTTAATTGGCGGTACAGAAATGACAGGAGAAATGAAAAATCTATTCTCTGTCATGAACATATATGCTTCAGAAAAACGGGATCCTATCTATGCATTGTTCCGAAATGAGGCAAGTGAAGGAGATGTGCTTTATTCTTCGGCCTTTCAGGTACAGGAAAAACAATTTGTC AGCTGATCCAAACCGCGGACTTATTGGTGATGATGAGCATGGCTGCTCCTCAATGGGCTATTTAATATTGAGGGTGGCTGATGCGAAATGCATCAACTTCTCCGTGAAAAAGAACCGCAAATTTTGTATGCCATCCGTTTCGGTTCAGTTTATAGAAATGTGTGATTAATTTAGAAA CACGCGTTCGCGACTATGATGACAGCACATTAAACAGAAAAATACACGTGCAGCTTATCGAATCAAGCGATTGACAATATCGTTGATCCAAGCGTTCGCGGCCATCCGAACACAATTTATTTCTTAACCTGCTGATGCTTCGAGGTGCTCCACCGGATAGCAAACTAACCAAGCAACAGGC TGTACCACCTTCTTAAGTGGCTATACATCCAAGCTTGGCGGAACAGAGCGCGGAATTACTTCTCCGCAAGCAACATTTCAACTTGCTTTGGATCCTGCTTCCCTCAACTCTCTGCAACGGCTATCGCGGAAATGCTTGGAGAAAAATCGATGAGCATAACGCAACGTAATTTCTAGTCAATAAC AGGCTGGACCGCGGGAATACGGCACAGGCACGCGCATGAAGCTCGCTTATACAGTGGGATGGTCAAGCTGCTTGAAGGAGAACTGAACAACACGGAACCGTTAAAGATAAATCTTTGGACTAGAAATTCCTTTACATGTTCCCGGGGTTCTCTGATGAAGTCTTCAGCCAAACAA AACATGGGAATGCCAGAAGCATATGAAGCAAAAGCAAAAGAACTGCAGAAAAATTCGTGAGAAGTTCAAAAAAGTTTAAAAATGTTCCTGCTGAAATTTGAAGAAAAAGGCGGCTCATCGCTAACTAAACAAATACATAAATAGGAGTAGTAAGAATGGCGAGAACAAAAATCGTTTT GTACGATTGGTCCGGCAAGTGAAAGTGTGGAGAAGCTTACGCAATTAATAGAAGCGGGAATGAATGTTGCACGTCTGAATTTTTCACATGGGAACCAAGAAACATGCGCAGCGGATTAAAAACATTCGGGAAGCTGCAGAAAGAACCGGCAAAAAATTTGGGATCTCTTTGACACGAAA GGTCTGAAATACGCACGATGATGGAAGCGGTCTATCGAATTAAAGCCGGACAGGAAATTTATTTCTATGAAGAAGTCTTGGGAACGCTGAAAAATTTTCAGTTACATATACGGGTTTAAATGATGATGTTCAAAAAGGCTCAAAAAATCTCTGATAGATGACGGATTATCGA ACTTGAAGTAACCAAAATCGACAAGCTAATAATGAAATTCATACAAAAATCTTAAACAGCGGCATCTTAAAAATAGAAAGGTGCAATGTTCCGGGAGTATCTGTCAAACCTTCGGGAATAACAGAAAAAGATGAAATGATATTATTTTGGGATTGAGCATGGCATAGATTTTATA GCCGCTCATTTGTGAGAAGAGCAAAAGATGTGCTTGAGATCCGCCAATTGTTAGAAGAAGCAAAATGCGACTAATATTCATATTATCCCTAAGATTGAAATCAAGAAGGGTCGACAATATTGATGAAATTTTAGAAGTATCAGACGGTTAATGTTAGCAAGGGGTGACCTCGGAGTAGA ACTTCTGCTGAAGAAGTTCTCTTGTTGCAGAAAAAATGATCAAAAAATGCAATGGCGTTGGAAAAACCGGTAATTTACTGCTACGCAAAATGCTTGATTCCATGCAAGAAATCCACGGCCGACACGTGCGGAAGCAAGTGACGTAGCAAAATGGATCTTTGACGGGAACAGACGCAATTTATGC TTTCCGGTGAACAGCTGCTGGACAATATCTCTGATAGAGGCTGTCAGACAATGCATAATATTGCATCAGCAGGTGAATCTGCATTAATACCAATGAGATATTATCGATACGAAGCAAGACAATGAACACAATAATACAGATGCAATTTGGCAATCGGTTGCCATACAGCATTAATCTG GATGTAATGGGATTATAACGCTACAGAAAGCGGTCTACAGCAAGGATGATTTGAAATATCGGCCAAAGTGCGGATTGTGCGAGTTACATCAAAATGACTTTTCCGCGCGGCTCTGGCATTAGTTTGGGAGTATATCCATTCTTGAAAAAGAGCTACCAACAACAGCGAAATGCT TGAGATTGCTGTTCAGGAAAGTCTTAATAGTGAATTTGTTACCAATGGCGATTTAGTAGTTATAACTGCAGCGCTCCAGTTGGAGAGAGCGGAACGACAACCTTATGAAATTCATGTAGTAGGTGATTCTATTATCAAGGGACAGGGATCGGAAGAAAAATCTCGCTACGGAAGAGTC GTGATCGCCGAGATGCAAAAGAAGCTTTAAGCAAAGTGAAGAAGGCCATATTTAGTGACATCGGTTCCGACCCGGAATGATACTGCTATTGAGAAATGACGGGCCCTTATTACCGAGGAAGGCGGATTGACAAGCCATGGAGCAGTTGTGGCTTAAACATTGGCATTCTGTTAT TGTGGTGCTGAAAAATGCAACAAAAATCTTAAAGATGGGCAAGAAATCATGTGGATGCAGCACGTGGAATTTTACAACGGTCTATGCGAGCGTATTATAA	Phosphoenolpyruvate carboxykinase gene (<i>B. methanolicus</i>): <i>pckA</i> (fwd) Pyruvate kinase gene (<i>B. methanolicus</i>): <i>pyk</i> (fwd)
<i>mae</i> ^{Gs-ace} ^{Bm}	pTH1mp- <i>mae</i> ^{Gs-ace} ^{Bm}	ATGGCATTACAGCGGGGACGGCATGAACATTACGATTGCTGCCAGTTGCAAAAAAGATATCGTCTCATTACGGATATCGCCCGCGGATTGGCAAGCGGGCGGGACATTGTGGGATTGACGTCAATTTCGTCAAGCAAAGTTACACGGTGGCGCATATTACCGTCAGCGCCTCGAT ACGAAGCAGTGGCACTTGATCAATTGAGGCGCTGAAAAAATTCGGCGGCTCAAAATTTGGAAGCTTTCCGACCGCAGCTTTTGTATGCACATCGGGGGGAAAAATTGAAACAAATTCAAAATCCCAAGTGAACACCGCGACGACTTGTGCGGGGTATACACGCGGGCGTGGCGCGCTCTCG ACGGCGATTGCCGAAGATCCGCGAAGGGCGTATTTCGTGACGATTAAACGGAATACGGTGCCTGCTTGTCTGGACGGCACGGCGGCTGTTGGCTTGGCGACATCGTCCGTACGCGCGCATGCCAGTCATGGAAGGAAAGCGATGCTGTTAAGGAATTTCCGCGAGTGACGCGTCCCG ATTTGTTTGGATACGAAGACACGGAAGAAATCATTCAAATTTGAAAGCGATCGCCCGGCTTTGGCGGCATTAACTGGAGGACATTTCGCGCCACGTTGTTTGAATTTGAAAAGCGGCTGAAAGAAGAGCTGGATATCCCGTGTTTCATGATGACCAGCATGGCACGGCGGTGCTG CTCTTGGCGGACTGCTTAACGCGCTGAAAAATCGTTGACAAAAAGCTCGAAGACATTAAGTCGTCTTAACCGGCATCGGTGCGGCGGCATCGCGTGCAGGAAAAATTTGCTTGGCGGCGGCTGGCAACATTATCGGGGTGACCGCCATGGCGCCATCCACGCGATGAAGCGTACGAAA ATCCGTACTGGCAAGATATGCCAACTCAGGAACCCGGATAATCTGAAAGGAAGCTTGCCGATGTCATCGCGCGGCAGATGATTATCGCGGCTTTCGGCTCCGGCAATTTAAAGTGAAGATGTGAAAAAATGGCGCGCATCCGATCGTGTTTGGATGGCCAAACCGGATCCGG AAATGATCCGGAGCTGGCGAACCGTACGTGCGCTCATGGCGACCGGGCTTCGCACTATCCGAACCAATCAACAAGTCCTTTGCTCCCGGGCATTTTCGCGGGGCGCTGACTCGCGGCGGAGAGAAATTAACGAGGAAATGAAGCTCGCTCGCGGAAGGGGATTTGCTCTTGCTG GACGGAGGATGAATTGAACGAAACATACATATCCGAGGCTCTTCAACGCAAGTGTGCGAACGCTGCAGACAAGCGGTGCTGCAAGCGGCTTACCGCACTGGGTGGCGGGAAGGACAATAATCCCGTTGGCGGATATACAGGCGAGTAAATATGGTACCGGCCATAGGCTAGATCC ATATGTTACCCGCCATAGGCTAGAGCTTGAACAATTACATAAATAGGAGGTAGTAAGAATGACAGATTCTAGAGTACAACAATTACAGGAAGCTGGGGAAGGATTTCGGCTGGAAAGGAATTACCGGCTCTTATTACGACAGAGGTGTAATCAAAATACGTGGGTCGAATTGACATT GAGTATACATTGGCACGCGCGGTGGGAAAAAGCTTTGGAAGCTTGAATGAAGAAGATTTTGTAAATGCCCTTGGTGCACTACAGGCAATCAGGCTGTTGACGAAGTAAAGCCGAGTGAAGACTATTACTTAAGCGGCTGGCAAGTTGCAGCTGATGCAAACTGGCAGGTGATAT GTACCTTGACCAAAGTTTATATCCGCAAAATAGTTTCGGCAGTAGTAAAAAGTATTAATCAAGCGTTGCAGCGTGTGACCAAAATCCATCATGCTGAAGGGGACCAATCAATTGATTGGTTTGTCTCGGATCGTAGCTGATGCAGAGCAGGCTTGGCGGACAGCTGAACGTGTTTGAATT GATGAAAGCGATGATTGAAGCGGGGCTGAGGGGTTCACTCGAAGATCAGCTTCTTCAGAAAAAGAAATGCGGACACCTTGGAGGAAAAAGTATTACTTCCAAACACAACCTGAGTGAAAAAATCGATTGCTGCAGCTTATGACAGCTGATGAATGGGTGTACCAACAGTGTAAATGTCTG GGACAGATGCAAAACGACGGGATATGTTAACAAGTGATGTGGATCCATACGATGCTCCGTTCAATTACTGGAGAGCGTACAGAAGAAGGATTTCCGCGCAAAAAGCTGGTTAGATCAAGCGATTGCTCGCGGATTGGCATATGCACCGTATGCTGATATGATCTGGTGAACACTGAG CCAAACTTAGAGGAAGCAAGACGATTTCGGAAGCTATTATGAGAAGTTCCCTGGCAAGCTGTAGCTTACAACCTGCTCACCATCATTTAATCGGAAGAAAAAGCTTGATGATGAAGACGATTGCAATTTCCAATTTGAGCTCGGAAAAATGGGCTACAAGTTCCAATTCGTTACACTAGC AGGATTCCATACATTGAACCACAGCATGTTGGAATAGCACGTCAATACAAGACCGGGAATGGCGCTTATTCTGAATTGCAGCAAGCTGAATTTGCAAGTGAAGAATACGGCTACACAGTACAAGACACACGCGTGAAGTTGGCACAGGCTACTTTGATGAAGTTGCCATGGTGTAGC GTGGTGAAGTCTTCAACTACTGCATTAAAGGCTCAACGGAAGTAGAACAATTTATCGCGCAAAAATAA	Malic enzyme gene (<i>G. stearothermophilus</i>): <i>mae</i> ^{Gs} (fwd) Isocitrate lyase gene (<i>B. methanolicus</i>): <i>aceA</i> (fwd)