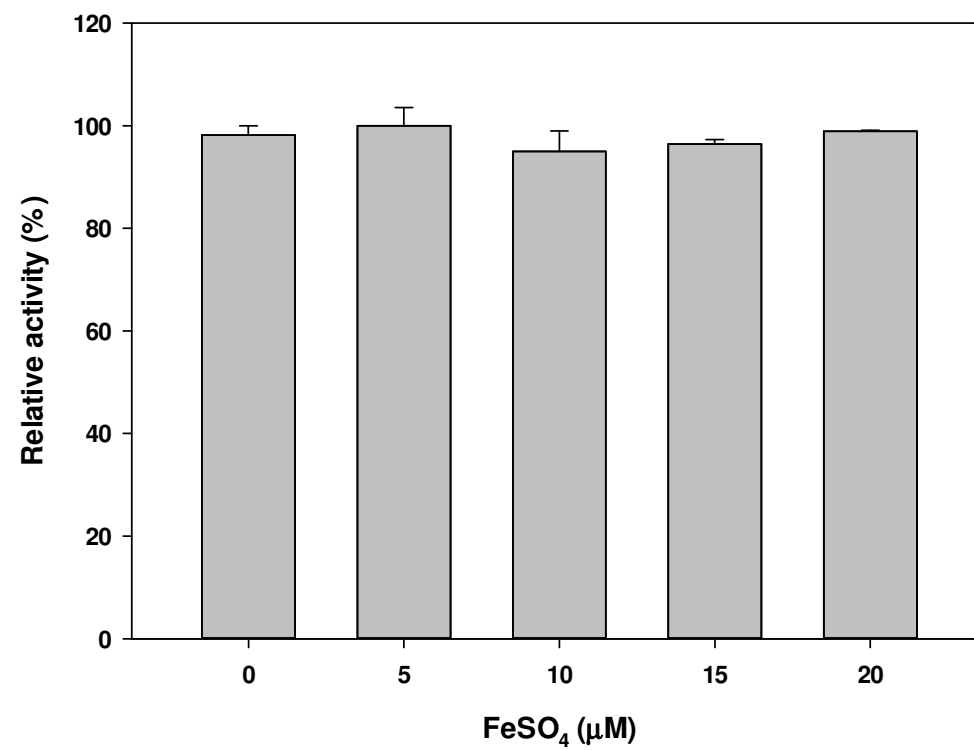
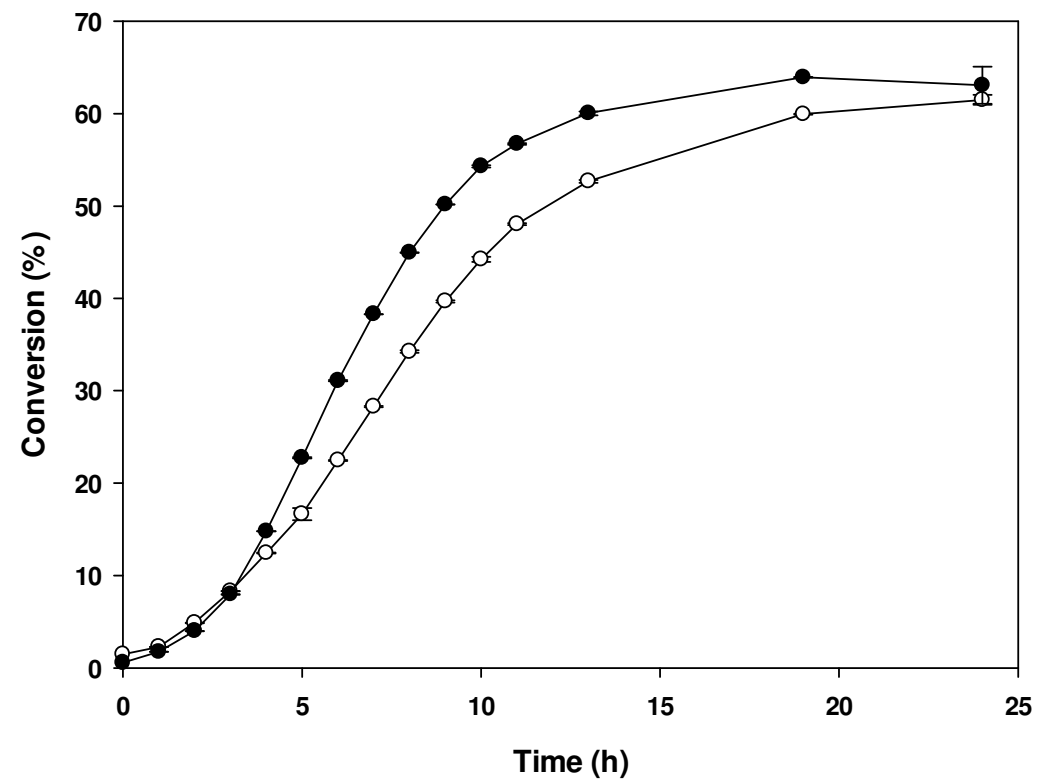


**Production of itaconate by whole-cell bioconversion
of citrate mediated by expression of multiple cis-
aconitate decarboxylase (*cadA*) genes in *Escherichia
coli***

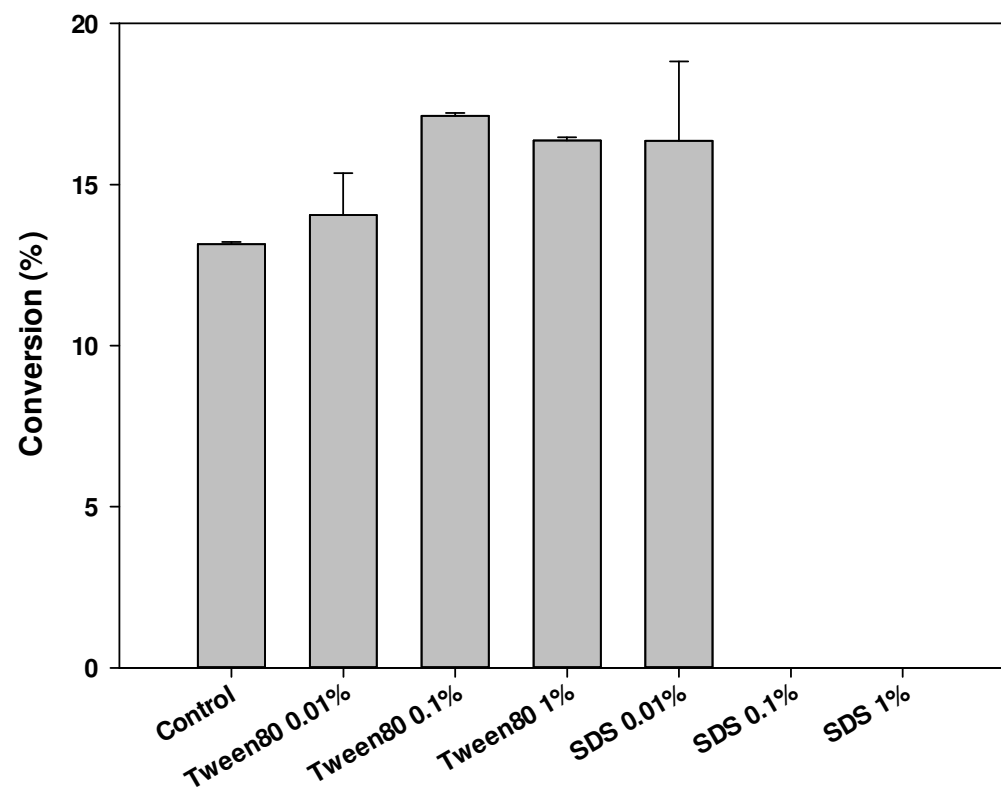
Junyoung Kim¹⁾, Hyung-Min Seo¹⁾, Shashi Kant Bhatia²⁾, Hun-Seok Song¹⁾,
Jung-Ho Kim¹⁾, Jong-Min Jeon¹⁾, Kwon Young Choi³⁾, Wooseong Kim⁴⁾,
Jeong-Jun Yoon⁵⁾, Yun-Gon Kim⁶⁾, and Yung-Hun Yang^{1)*}



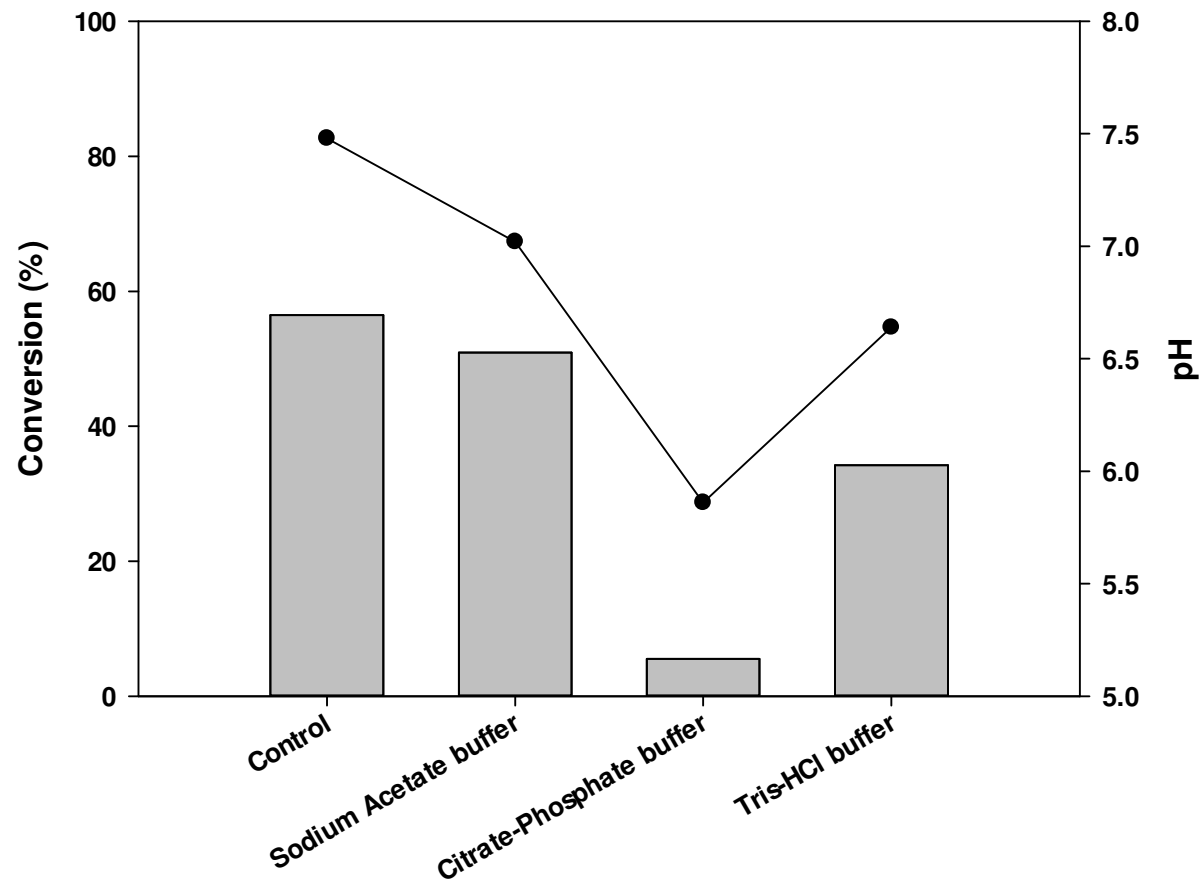
Supplement Figure 1. Effect of FeSO₄. No effect was detected.



Supplement Figure 2. *citT*, a citrate carrier protein, had no effect on conversion. JY001 (●) and JY002 (○).



Supplement Figure 3. Surfactant screening. Tween 80 and SDS were tested. The reaction was conducted at pH 7 and 35°C.



Supplement Figure 4. Effect of Buffers. Sodium acetate buffer (pH 5.5, 300mM), Citrate-Phosphate buffer(pH 5.5, 300mM), Tris-HCl buffer(pH 6.8, 300mM) was tested. However, none of these showed positive effect.

Supplementary table 1. Activity of each enzymes

Strain	<i>acn</i>	<i>cadA</i>	Activity (U)	Relative activity
pCDF (<i>cadA</i>), pRSF (<i>cadAx2</i>)	X	OOO	0.38	1
pCDF (<i>acn,cadA</i>), pRSF (<i>cadAx2</i>)	O	OOO	1.46	3.5

Strain	<i>acn</i>	<i>cadA</i>	Activity (U)	Relative activity
pCDF (<i>acn</i>)	O	X	0	-
pCDF (<i>acn,cadA</i>)	O	O	0.002	1
pCDF (<i>acn,cadA</i>), pRSF (<i>cadA</i>)	O	OO	0.151	75.5
pCDF (<i>acn,cadA</i>), pRSF (<i>cadAx2</i>)	O	OOO	1.46	730

Unit (U) = Amount of whole cell catalyst converting 1μmol substrate to 1μmol itaconate per minute.

Supplementary table 2. DNA and amino acid sequences of synthetic *cadA*

DNA sequence of synthetic <i>cadA</i>
ATGACCAAGCAGAGCGCAGATAGCAACGCAAAGAGCGGTGTCACG AGCGAAATCTGCCATTGGGCAAGCAACCTGGCAACGGATGATATCC CAAGCGACGTACTGGAACGTGCAAAGTACCTGATCCTGGACGGTAT CGCATGCGCTTGGGTAGGTGCACGTGTCCCTTGGTCTGAAAAGTACG TCCAGGCAACTATGTCCTTCGAGCCGCCTGGTGCTTGTCTGTAATCG GTTACGGCCAGAACTGGGTCCTGTGGCAGCCGCCATGACTAACAG CGCATTCATCCAGGCAACTGAGCTGGACGACTACCATAGCGAGGCT CCACTGCATAGCGCTAGCATCGTCCTGCCAGCTGTTTTCGCTGCTTCT GAGGTACTGGCTGAGCAAGGTAAAACCATCTCCGGTATCGACGTCAT CCTGGCTGCTATCGTGGGTTTCGAGAGCGGTCCGCGTATCGGTAAAG CTATCTACGGCAGCGACCTGCTGAACAACGGTTGGCATTGCGGTGCT GTGTACGGTGCTCCGGCGGGTGCACTGGCGACTGGTAAACTGCTGG GTCTGACTCCGGATTCTATGGAAGATGCCCTGGGTATTGCCTGTACTC AAGCCTGTGGTCTGATGTCTGCGCAATATGGTGGTATGGTTAAACGT GTGCAGCACGGTTTCGCGGCGCGTAATGGTCTGCTGGGTGGTCTGCT GGCGCACGGCGGCTATGAAGCGATGAAAGGCGTTCTGGAACGTTCT TATGGCGGCTTCTGAAAATGTTACCAAAGGCAATGGCCGTGAAC CGCCGTATAAAGAAGAGGAGGTGGTGGCGGGCCTGGGCTCTTTCTG GCACACGTTACCATTCGTATTAAACTGTACGCGTGCTGTGGCCTGG TTCACGGCCCCGGTAGAAGCGATTGAAAACCTGCAGGGCCGTTACCC GGAAGTCTGAATCGTGCCAACCTGTCTAACATCCGTACGTTACG TTCAGCTGTCTACCGCTCTAACTCTCACTGTGGCTGGATTCCGGAA GAACGTCCGATCTCTTCCATCGCGGGCCAGATGTCTGTTGCGTACATC CTGGCCGTACAGCTGGTTGATCAGCAGTGCCTGCTGTCTCAGTTTTC TGAATTTGACGACAACTGGAACGCCCGGAAGTGTGGGACCTGGCG CGTAAAGTGACCTCTCCAGTCCGAAGAATTTGACCAGGACGGCA ACTGCTGTCCGCGGGCCGCGTACGCATTGAATTTAACGACGGCTCC TCCATTACCGAATCCGTTGAAAAACCGCTGGGCGTTAAAGAACCGAT GCCGAACGAACGCATCCTGCACAAATATCGCACCTGGCGGGCTCC GTTACCGATGAATCCCGGTTAAAGAAATTGAAGATCTGGTGCTGG GCCTGGATCGCCTGACCGATATTTCCCGCTGCTGGAGCTGCTGAAC TGCCCGGTTAAATCCCGCTGGTT

Amino acid sequence of synthetic <i>cadA</i>
MTKQSADSNKSGVTSEICHWASNLATDDIPSDVLERAKYLILDGIA CAWVGARVPWSEKYVQATMSFEPPGACRVIGYGQKLGPVAAAMT NSAFIQATELDDYHSEAPLHSASIVLPAVFAASEVLAEQGKTISGIDVIL AAIVGFESGPRIGKAIYGSDLLNNGWHCGAVYGAPAGALATGKLLGL TPDSMEDALGIACTQACGLMSAQYGGMVKRVQHGFAARNGLLGGL LAHGGYEAMKGVLSYGGFLKMFMTKGNGREPPYKEEEVVAGLGSF WHTFTIRIKLYACCGLVHGPVEAIENLQGRYPELLNRLNLSNIRHVHV QLSTASNSHCGWIPEERPISSIAGQMSVAYILAVQLVDQQCLLSQFSE FDDNLERPEVWDLARKVTSSQSEFDQDGNCLSAGRVRIEFNDGSSI TESVEKPLGVKEPMPNERILHKYRTLGSVTDSESRVKEIEDLVGLDRL TDISPLLELLNCPVKSPV