Supporting information

Sortase A-mediated enzyme assembly on multimeric protein for improving mevalonate production

Materials and Methods

Plasmid construction

The plasmids used in this study are shown in Table S1, and the primers used are listed in Table S2. Polymerase chain reactions were performed using KOD Plus or KOD One Master Mix. Vectors and inserts were linked using NEBuilder, according to the manufacturer's protocol. Using synthetic gene of the mutant of sortase A as templates [1], primers 1 (GAGGAGAAAGGTACCATGCAGGCAAAACCGCAGATTCCGAAAGAT) and 2 (TTCGATATCAAGCTTTTATTTATCATCATCATCTTTATAATCTTT) were used to amplify the *srtAm7* gene. Using synthetic gene of the plasmid pZA23LMCS as templates [2], primers 3 (AAGCTTGATATCGAATTCCTGCAGCCCGGG) and 4 (GGTACCTTTCTCCTCTTTAATGAATTCTGT) were used to amplify the pZAMCS lacI gene. Two gene fragments were conjugated and the resulting plasmid was named pZAsrtA.

Table S1. Strains and plasmids used in this study

Strains or plasmids	Characteristics	Source of reference
Strain		
E. coli DH5α	*F-, Φ80dlacZΔM15, Δ(lacZYA-argF)U169, deoR, recA1, endA1, hsdR17(rK-, mK+), phoA, supE44, λ-, thi-1, gyrA96, relA1*	Novagen
E. coli BW25113	$lacI^q$ $rrnB_{T14}$ $\Delta lacZ_{WJ16}$ *hsdR514* $\Delta araBAD_{AH33}$ $\Delta rhaBAD_{LD78}$	Novagen
Plasmid		
pTrcHisB	pBR322 ori; Amp^R; P_{trc}::MCS::T_{rrnBTT}	Thermo Fisher Scientific Inc.
pMev	pBR322 ori; Amp^R; P_{trc}::*atoB mvaS mvaE*::T_{rrnBTT}	(8)
pMevlp	pBR322 ori; Amp^R; P_{trc}::*atoB mvaS-lp mvaE-lp*::T_{rrnBTT}	This study
pZA23MCS	p15A ori; Kan^R; $P_{A1lacO-1}$::MCS::T_{rrnBTT}	EXPRESSYS
pZAsrtA	pZA23MCS but P_{laci}^q::*lacI*; $P_{A1lacO-1}$::*srtAm7*::T_{rrnBTT}	This study
pZAsrtA_s	p15A ori; Kan^R; P_{laci}^q::*lacI*; $P_{A1lacO-1}$::*srtAm7g-SA*::T_{rrnBTT}	This study
pZAsrtA_c	pZA23MCS harboring P_{laci}^q::*lacI*; $P_{A1lacO-1}$::*srtAm7 g-cutA*::T_{rrnBTT}	This study

Table S2. Primers used in this study

No.	Primer name	Sequence
1	mvaElp_fwd	taattaaagaggtatatattatgTGAAAACCGTGGTGATTATTG
2	mvaElp_rev	TgtaccagctgcagatctcgagctcggatcttagccgccggtcgccggcagCTGTTTACG
3	mvaSlp_fwd	tctgcagctggtacaaaattaaagaggtatatattaatgACCATTGGCAT
4	mvaSlp_rev	ttcgaattcccatatggtacttagccgccggtcgccggcagATTACGATAGCTACGC
5	pMev_fwd	GTACCATATGGGAATTCG
6	pMev_rev	TAATATATACCTCTTTAATTAATTCAACC
7	gSA_fwd	gaattcctgcagcccttaaagagGTATATATTAATG
8	gSA_rev	ACCATGGGATCCCCCTTAggaggcggcggacgg
9	cutA_fwd	taatgggcggcggcggctcgATGCTTGATGAAAAAAGTTC
10	cutA_rev	cgcgtaccatgggatcccccTCAGCGTAAAGATGCGTTGA
11	pMevlp_fwd	tcaacgcatctttacgctgaGGGGGATCCCATGGTACGCG
12	pMev_rev	gaacttttttcatcaagcatCGAGCCGCCGCCGCCCATTA

Table S3. The gene sequence of synthetic gene flagment

Sortase A mutant (srtAm7)

CAGGCAAAACCGCAGATTCCGAAAGATAAAAGCAAAGTGGCAGGCTATATTGAAATTCC
GGATGCCGATATTAAAGAACCGGTTTATCCGGGTCCGGCAACCAGCGAACAGCTGAATC
GTGGTGTTAGCTTTGCAAAAGAAAATCAGAGCCTGGATGATCAGAATATTAGCATTGCA
GGCCATACCTTTATTGATCGTCCGAATTATCAGTTTACCAACCTGAAAGCAGCAAAAAAA
GGTAGCATGGTGTATTTCAAAGTGGGTAATGAAACCCGCAAATACAAAATGACCAGCAT
TCGTAATGTTAAACCGACCGCAGTTGAAGTTCTGGATGAACAAAAAGGCAAAGATAAAC
AGCTGACCCTGATTACCTGTGATGATTATAACGAAAAAACCGGTGTTTGGGAAACGCGC
AAAATCTTTGTTGCAACCGAAGTGAAA

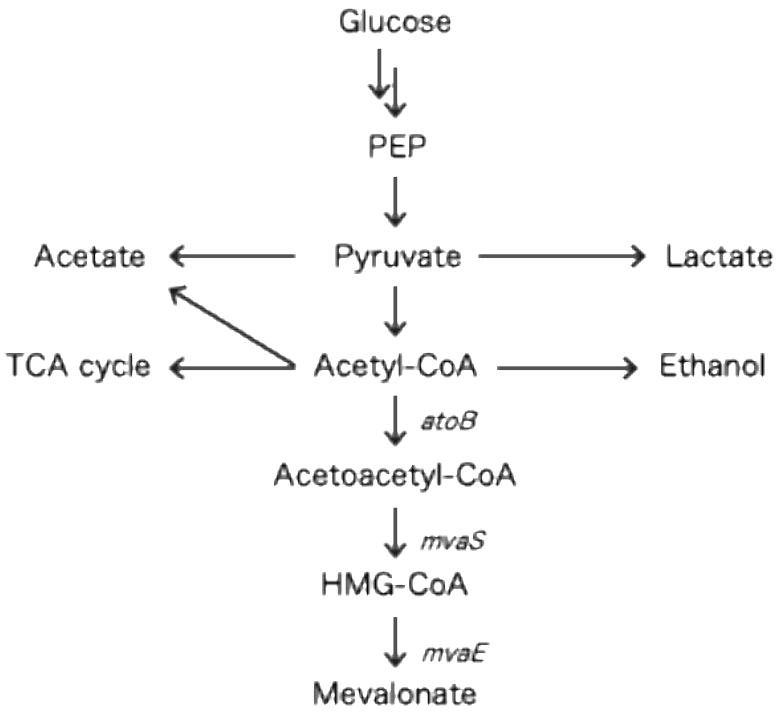

Figure S1 Overview of the mevalonate biosynthetic pathway

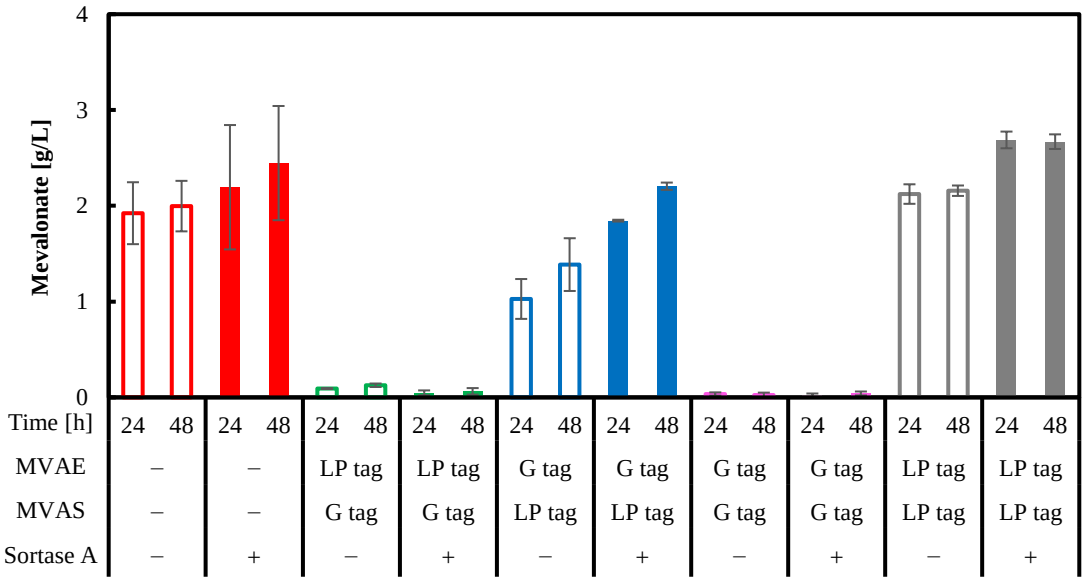

Figure S2 Comparison of mevalonate production using LP- or G-tagged mvaE and mvaS. Data are shown as the mean of three independent experiments, each starting from separately isolated colonies, and error bars represent standard deviations.

Reference

[1] Witte MD, Wu T, Guimaraes CP, Theile CS, Blom AEM, Ingram JR, Li Z, Kundrat L, Goldberg SD, Ploegh HL. Site-specific protein modification using immobilized sortase in batch and continuous-flow systems. *Nat Protoc* **2015**, *10*, 508-516.

[2] Matsumoto T, Furuta K, Tanaka T, Kondo A. Sortase A-Mediated Metabolic Enzyme Ligation in Escherichia coli. *ACS Synth Biol* **2016**, *5*, 1284-1289.