Table S1. All primers for gene amplification used in this study.

Primer	Sequence(5'→3')	Genes
*Rp*matB-F	CGGGATCCGATGAACGCCAACCTGTTC	*Rp*matB
*Rp*matB-R	CCCAAGCTTTTACTTGTAGATGTCCTTG	
*Bj*matB-F	CGGGATCCGATGAACCAAGCTGCCAAC	*Bj*matB
*Bj*matB-R	CCCAAGCTTCTACTTCTTCGCGTAAATATC	
*At*matB-F	CGGGATCCGATGACCGCTACGACAACATTAAAG	*At*matB
*At*matB-R	CCCAAGCTTTTATTCTTGATTTTCCAGAG	
matC-F	CGGGATCCATGGGCATTGAAATTTTAGC	matC
matC-R	CCCAAGCTTTTACACTAAGCCCGGAAC	
mdcF-F	CGGGATCCATGACCTATGTGATTATTC	mdcF
mdcF-R	CCCAAGCTTCAGGCCGCTGGTTAAAG	
matPQM-F	CGGGATCCATGAGCAGCTTTCGTCGC	matPQM
matPQM-R	CCCAAGCTTCTGCACGGTACCCAGCATC	
dctPQM-F	CGGGATCCATGCTGACCCGTCGTATTC	dctPQM
dctPQM-R	CCCAAGCTTCATGCCCAGCAGATTCGGCAG	
matC-F1	CTCTAGAGTCGACCTGCAGATGCACCACCACCACCACCACG GCATTGAAATTTTAGCAA	matC
matC-R1	CCAAGCTTGCATGCCTGCAGTTACACTAAGCCCGGAACAA	
mdcF-F1	CTCTAGAGTCGACCTGCAGATGCACCACCACCACCACCACA CCTATGTGATTATTCATG	mdcF
mdcF-R1	CCAAGCTTGCATGCCTGCAGCAGGCCGCTGGTTAAAGAAA	
matPQM-F1	CTCTAGAGTCGACCTGCAGATGCACCACCACCACCACCACA GCAGCTTTCGTCGCAAA	matPQM
matPQM-R1	CCAAGCTTGCATGCCTGCAGCTGCACGGTACCCAGCATCT	
dctPQM-F1	CTCTAGAGTCGACCTGCAGATGCACCACCACCACCACCACC TGACCCGTCGTATTCTGG	dctPQM
dctPQM-R1	CCAAGCTTGCATGCCTGCAGCAAAAAACCCCTCAAGACC	
matC-R2	ACGCGTCGACTTACACTAAGCCCGGAAC	matC
mdcF-R2	ACGCGTCGACCAGGCCGCTGGTTAAAG	mdcF
matPQM-R2	ACGCGTCGACCTGCACGGTACCCAGCATC	matPQM
dctPQM-R2	ACGCGTCGACCATGCCCAGCAGATTCGGCAG	dctPQM
mcr-F1	GGAAGATCTCATGAGCGGAACAGGACGACT	mcr

mcr-R1	CCGCTCGAGTTACACGGTAATCGCCCGTCC	
*Rp*matB-F1	CATCACCACAGCCAGGATCCGATGAACGCCAACCTGTTCGCC	*Rp*matB
*Rp*matB-R1	CCGAGCTCGAATTCGGATCCTTACTTGTAGATGTCCTTGT	
mcr-F2	CGCGGATCCGATGAGCGGAACAGGACGACT	mcr
mcr-R2	ACGCGTCGACTTACACGGTAATCGCCCGTCC	
*Rp*matB-F2	GATATACATATGGCAGATCTCATGAACGCCAACCTGTTCGCC	*Rp*matB
*Rp*matB-R2	CCGATATCCAATTGAGATCTTTACTTGTAGATGTCCTTGT	
pntAB-F	GCCTGCAGGTCGACAAGCTTTAATACGACTCACTATAGGG	pntAB
pntAB-R	CATTATGCGGCCGCAAGCTTCAAAAAACCCCTCAAGACCC	
yfjB-F	TGTTGCAGTCAACTCTGCAGTTGCTCACATCTCACTTTAA	yfjB
yfjB-R	CGAGAAACAGCGTACCCTGCAGTTAGAATAATTTTTTTGACCAGCC	

Table S2. Strains and plasmids used in this study.

Name	Description	Reference
Strains		
E. coli BL21 (DE3)	F$^-$ *ompT gal dcm lon hsdSB* (rB$^-$ mB$^-$) λ(DE3)	Invitrogen
E. coli C43 (DE3)	F$^-$ *ompT hsdSB* (rB$^-$ mB$^-$) *gal dcm* (DE3)	Invitrogen
SGN01	*E. coli* BL21 (DE3)/pSGN-01	This study
SGN15	*E. coli* BL21 (DE3)/pSGN15	This study
SGN16	*E. coli* BL21 (DE3)/pSGN16	This study
SGN10	*E. coli* C43 (DE3)/pSGN10	This study
SGN18	*E. coli* C43 (DE3)/pSGN18	This study
SGN20	*E. coli* C43 (DE3)/pSGN20	This study
SGN21	*E. coli* C43 (DE3)/pSGN21	This study
SGN22	*E. coli* C43 (DE3)/pSGN22	This study
SGN25	*E. coli* C43 (DE3)/pSGN25	This study
SGN27	*E. coli* C43 (DE3)/pSGN27	This study
SGN28	*E. coli* C43 (DE3)/pSGN28	This study

SGN69	*E. coli* C43 (DE3)/pSGN69	This study
SGN70	*E. coli* C43 (DE3)/pSGN70	This study
SGN68	*E. coli* C43 (DE3)/pSGN68	This study
SGN72	*E. coli* C43 (DE3)/pSGN72	This study
SGN34	*E. coli* C43 (DE3)::yfjB	This study
SGN47	*E. coli* C43 (DE3)/pSGN36	This study
SGN73	*E. coli* C43 (DE3)/pSGN68;pSGN-36	This study
SGN74	*coli* C43 (DE3)/pSGN68;pSGN-41	This study
SGN75	*E. coli* C43 (DE3)/pSGN68;pSGN-40	This study
SGN78	*E. coli* C43 (DE3)::yfjB/pSGN68;pSGN-42	This study
plasmids		
pET28a	lacI; expression vector; T7 promoter; Kan^r	Novagen
pBAD-24	expression vector; arabinose Bad promoter; Amp^r	Novagen
pMAL-c2x	expression vector; tac promoter; Amp^r	Novagen
pRSFDuet-1	lacI; expression vector; T7 promoter; two sets of MCS; MCS I, His6-N; MCS II,S-tag-N; Kan^r	Novagen
pACYCDuet-1	lacI; expression vector; T7 promoter; two sets of MCS; MCS I, His6-N; MCS II,S-tag-N; Cm^r	Novagen
pSGN-01	pACYCDuet-1 carrying *Rp*matB from *R. palustris*; Cm^r	This study
pSGN-15	pACYCDuet-1 carrying *Bj*matB from *B. japonicum*; Cm^r	This study
pSGN-16	pACYCDuet-1 carrying *At*matB from *A. thaliana*; Cm^r	This study
pSGN-10	pET28a carrying matC from *R. leguminosarium bv trifolii*; Kan^r	This study
pSGN-18	pET28a carrying mdcF from *K. pneumoniae* ; Kan^r	This study
pSGN-20	pET28a carrying matPQM from *S. meliloti*; Kan^r	This study
pSGN-21	pET28a carrying dctPQM from *R. capsulatus*; Kan^r	This study
pSGN-22	pMAL-c2x carrying matC from *R. leguminosarium bv trifolii*; Amp^r	This study
pSGN-25	pMAL-c2x carrying mdcF from *K. pneumoniae* ; Amp^r	This study
pSGN-27	pMAL-c2x carrying matPQM from *S. meliloti*; Amp^r	This study
pSGN-28	pMAL-c2x carrying dctPQM from *R. capsulatus*; Amp^r	This study
pSGN-69	pBAD-24 carrying matC from *R. leguminosarium bv trifolii*; Amp^r	This study
pSGN-70	pBAD-24 carrying mdcF from *K. pneumoniae*; Amp^r	This study

pSGN-68	pBAD-24 carrying matPQM from *S. meliloti*; Amp^r	This study
pSGN-72	pBAD-24 carrying dctPQM from *R. capsulatus*; Amp^r	This study
pSGN-35	pRSFDuet-1 carrying mcr(linker) from *C. aurantiacus*; Kan^r	This study
pSGN-36	pRSFDuet-1 carrying *mcr*(linker; MCSI) from *C. aurantiacus*; *Rp*matB(MCSII) from *R. palustris*	This study
pSGN-40	pRSFDuet-1 carrying *Rp*matB(MCSI) from *R. palustris*; *mcr*(MCSII) from *C. aurantiacus*	This study
pSGN-41	pRSFDuet-1 carrying *Rp*matB(MCSI) from *R. palustris*; *mcr*(linker; MCSII) from *C. aurantiacus*	This study
pSGN-42	pRSFDuet-1 carrying *Rp*matB(MCSI) from *R. palustris* and PntAB from *E. coli* DH5α; *mcr*(linker; MCSII) from *C. aurantiacus*	This study

Table S3. All primers used in transcription analysis of transporter genes

Primer	Sequence (5'→3')
matC-F	TGTGCTGGGTAGTATGACCA
matC-R	TGCAAACAGATAGGTCACG
mdcF-F	ATTGTTGCCCAGTCTCCGTT
mdcF-R	ACGGTTAAGGTCAGAACTGCT
matPQM-F	GATCAGTGGAATGCAGTGAC
matPQM-R	TGGTATACAGATCACTAC
dctPQM-F	GAAGAACTGGAAGCACTGCAG
dctPQM-R	ATCCGAATAGCCAGCTGTAT

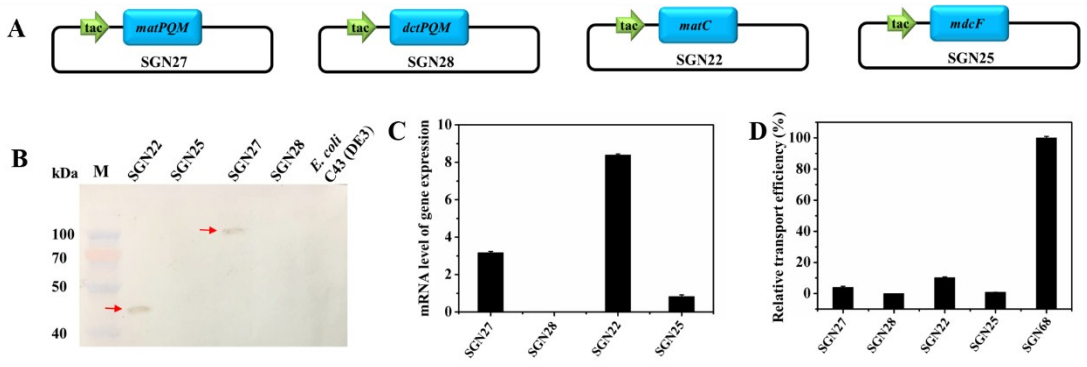

Fig.S1 Identification of malonate transporters expression and function under tac promoter

in *E. coli.* (A) Construction of recombinant strains carrying malonate transport genes. (B) Western Blot analysis of expressed malonate transport proteins. (C) Transcription analysis of transporter genes. (D) Relative malonate transport efficiency of tested transporters.

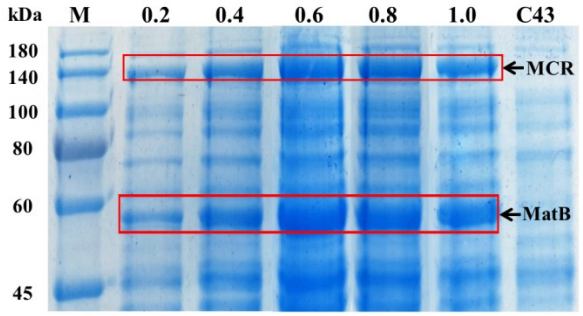

Fig.S2 SDS-PAGE analysis of the expression levels of the enzymes by varying the concentration of the inducer IPTG.

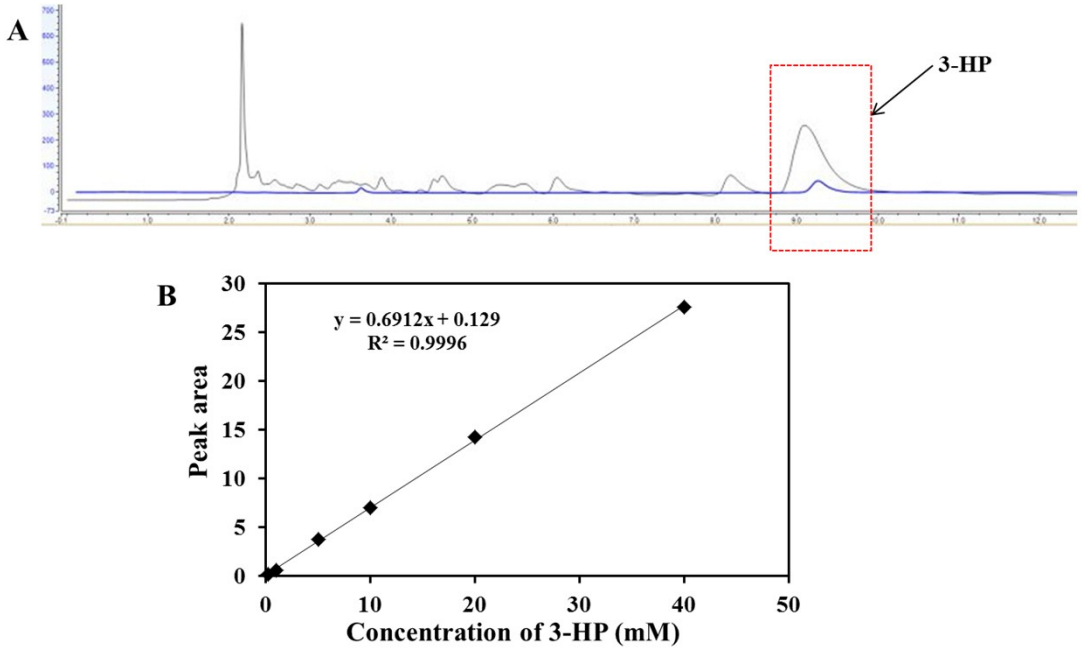

Fig.S3 The HPLC chromatogram and calibration curve of 3-HP. (A) The HPLC chromatogram of 3-HP Standards (blue line) and engineered strain constructed in this study (black line). The retention time of 3-HP was 9.15 min. (B) The calibration curve of 3-HP.

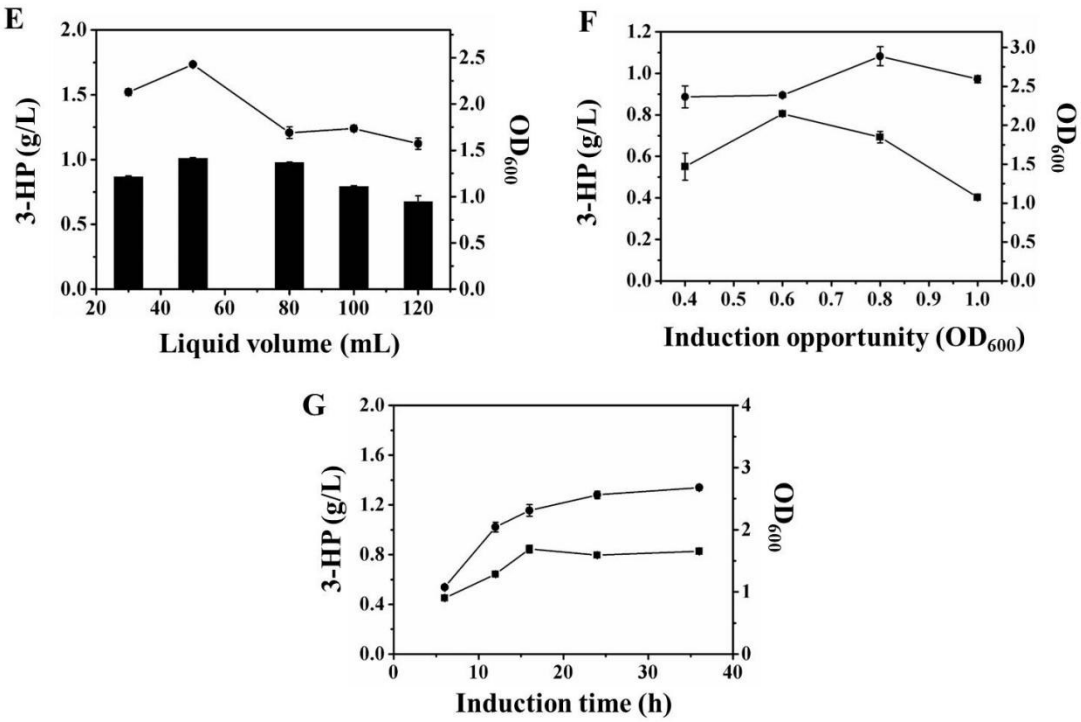

Fig.S4 Effects of fermentation conditions on 3-HP production by SGN74. (E): Effect of volume of fermentation medium on 3-HP production; (F): Effect of induction opportunity on 3-HP production; (G): Effect of induction time on 3-HP production. All the experiments were performed in triplicates. Black line of filled circle, OD_{600} value of strain SGN74. Black columns and black line of filled square, 3-HP production under different fermentation conditions.