

Supplementary Table 1. Promoter and terminator sequences used in this study

Promoter/terminator	Sequence
<i>Lac</i> promoter	GCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAG GCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAG CGGATAACAATTTACACAGGAAACA
<i>PJ5</i> promoter	AGCGGATATAAAAACCGTTATTGACACAGGTGGAAATTTAGAATAT ACTGTTAGTAAACCTAATGGATCGACCTTCCCATGTGAGGCAACTG ATATGATCATTACATCAGTTAGTACAGATGAGGGTCTACTGGCCCGA TATGGTTTAGCAGAAATAAATGTAACAGTGAGGTATCAGCCTCCAC GTAGGTCACTTCGCAGATAAGTTACAGATTAGGAGAAAATAATTA
<i>rrnB</i> terminator	GCTGTTTTGGCGGATGAGAGAAGATTTTCAGCCTGATACAGATTAA ATCAGAACGCAGAAGCGGTCTGATAAACAGAATTTGCCTGGCGG CAGTAGCGCGGTGGTCCCACCTGACCCCATGCCGAACCTCAGAAGT GAAACGCCGTAGCGCCGATGGTAGTGTGGGGTCTCCCCATGCGAG AGTAGGGAACCTGCCAGGCATCAAATAAACGAAAGGCTCAGTCG AAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTTCGGTGAACGCTC TCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGTTGCGA AGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAACT GCCAGGCATCAAATTAAGCAGAAGGCCATCCTGACGGATGGCCTT TTGCGTTTCTACAAACTCTT

Supplementary Table 2. Primers used in this study

Name	Sequence (5' → 3')	Restriction sites
5'-OL lacp PacI	GATTAATTAAGCGCAACGCAATTAATGT	<i>PacI</i>
3'-OL lacp NdeI	GCCATATGTGTTTCCTGTGTGAAATTGTTATCCG	<i>NdeI</i>
5'-OL ispS NdeI	CACATATGCGCCTGCTCCGT	<i>NdeI</i>
3'-OL ispS XhoI	ATCTCGAGTACATGTACGTATCAGCGCTCAAACGGG	<i>XhoI</i>
5'-OL mvaK1 XhoI	TACTCGAGATGACGAAGAAGGTGGGC	<i>XhoI</i>
3'-OL mvaK1 ClaI	ATATCGATTCACAGGCTCTCAATCCAG	<i>ClaI</i>
5'-OL mvaD ClaI	GAATCGATTCTAGCCAAGAAGGAGATATAAAAAATGG ACCGCGAACCG	<i>ClaI</i>
3'-OL mvaD fusionP	CTCCTTCTTAAATCTACAGGATGCCAATTCATTACAGC AGCAGTCGTCCTG	
5'-OL mvaK2 fusionP	TGGCATCCTGTAGATTTAAGAAGGAGATATAAAAAAT GATCGCGGTGAAGACC	
3'-OL mvaK2 MluI	TCACGCGTTTACGACTTGTCGTCATGGC	<i>MluI</i>
5'-OL idi EcoRI	GTGAATTCATGCAGACCGAGCACG	<i>EcoRI</i>
3'-OL idi XmaI	GCCCCGGGTCACTTGAGCTGGGTGAACG	<i>XmaI</i>
5'-OL mvaE infusion	ATGCACTAGAGTCGAAAGAAGGAGATATAAAAAATG AAGACCGTGGTCATCA	
3'-OL mvaE infusion	AGCAGAATAATTTCTGCTGAACTGTTTTTCTAGATT ACTGCTTGCGCAGATCA	
5'-OL mvaS-rrnBt infusion1	TTTCAGGCCACGAAAGAAGGAGATATAAAAAATGAC CATCGGGATCGAC	
3'-OL mvaS-rrnBt infusion2	CAAAACAGCGATGATTCAGTTGCGGTAGGAGCG	
5'-OL mvaS-rrnBt infusion3	CGCAACTGAATCATCGCTGTTTTGGCGGATGAGA	
3'-OL mvaS-rrnBt infusion4	TGGAGCTCCACCGCGGGATGATATTTAAATAAGAGT TTGTAGAAACGCAAAAAGG	
5'-OL PJ5p PacI	GATTAATTAAGCGGATATAAAAAACCGTTATTGAC	
3'-OL PJ5p NdeI	GCCATATGTTAATTATTTTCTCCTAATCTGTAACCTAT CTGCG	
5'-OL lacp-mvaK1 infusion1	CGTACATGTACTCGAGGCGCAACGCAATTAATGTG	
3'-OL lacp-mvaK1 infusion2	CACCTTCTTCGTCATTGTTTCCTGTGTGAAATTGTTA TCCG	
5'-OL lacp-mvaK1 infusion3	TTCACACAGGAAACAATGACGAAGAAGGTGGGC	
3'-OL lacp-mvaK1 infusion4	TTGGCTAGAATCGATTCACAGGCTCTCAATCCAGG	
5'-OL lacp-idi infusion1	GCGTGATCGTGAATTCGCGCAACGCAATTAATGTG	

3'-OL lacp-idi infusion2	GTGCTCGGTCTGCATTGTTTCCTGTGTGAAATTGTTA TCCG
5'-OL lacp-idi infusion3	TTCACACAGGAAACAATGCAGACCGAGCACG
3'-OL lacp-idi infusion4	TAGTGCATGCCCCGGGTCACTTGAGCTGGGTGAACG
5'-OL PJ5p-mvaK1 infusion1	CGTACATGTACTCGAGAGCGGATATAAAAACCGTTA TTGACA
3'-OL PJ5p-mvaK1 infusion2	CACCTTCTTCGTCATTTAATTATTTTCTCCTAATCTGT AACTTATCTGCGA
5'-OL PJ5p-mvaK1 infusion3	GGAGAAAATAATTAAATGACGAAGAAGGTGGGC
3'-OL PJ5p-mvaK1 infusion4	CTTCTTGGCTAGAATCGATTCACAGGCTCTCAATCC AGG
5'-OL PJ5p-idi infusion1	GCGTGATCGTGAATTCAGCGGATATAAAAACCGTTA TTGACA
3'-OL PJ5p-idi infusion2	GTGCTCGGTCTGCATTTAATTATTTTCTCCTAATCTGT AACTTATCTGCGA
5'-OL PJ5p-idi infusion3	GGAGAAAATAATTAAATGCAGACCGAGCACG
3'-OL PJ5p-idi infusion4	TAGTGCATGCCCCGGGTCACTTGAGCTGGGTGAACG

Supplementary Table 3. Codon usage table of mevalonate pathway genes in *R. eutropha*

ispS				mvaK1			
aa	codon	number	frequency	aa	codon	number	frequency
END	TGA	1	0.67	END	TGA	1	0.67
END	TAA	0	0.17	END	TAA	0	0.17
END	TAG	0	0.16	END	TAG	0	0.16
ALA	GCC	22	0.47	ALA	GCC	16	0.47
ALA	GCG	18	0.39	ALA	GCG	13	0.39
ALA	GCA	4	0.09	ALA	GCA	3	0.09
ALA	GCT	2	0.05	ALA	GCT	2	0.05
CYS	TGC	6	0.91	CYS	TGC	5	0.91
CYS	TGT	1	0.09	CYS	TGT	0	0.09
ASP	GAC	27	0.74	ASP	GAC	9	0.74
ASP	GAT	9	0.26	ASP	GAT	3	0.26
GLU	GAG	33	0.60	GLU	GAG	16	0.60
GLU	GAA	22	0.40	GLU	GAA	10	0.40
PHE	TTC	23	0.82	PHE	TTC	4	0.82
PHE	TTT	5	0.18	PHE	TTT	1	0.18
GLY	GGC	15	0.76	GLY	GGC	17	0.76
GLY	GGG	3	0.13	GLY	GGG	3	0.13
GLY	GGT	1	0.07	GLY	GGT	2	0.07
GLY	GGA	1	0.04	GLY	GGA	1	0.04
HIS	CAC	8	0.64	HIS	CAC	6	0.64
HIS	CAT	4	0.36	HIS	CAT	4	0.36
ILE	ATC	27	0.86	ILE	ATC	20	0.86
ILE	ATT	4	0.12	ILE	ATT	3	0.12
ILE	ATA	1	0.02	ILE	ATA	0	0.02
LYS	AAG	31	0.90	LYS	AAG	12	0.90
LYS	AAA	3	0.10	LYS	AAA	1	0.10
LEU	CTG	47	0.74	LEU	CTG	24	0.74
LEU	CTC	9	0.14	LEU	CTC	5	0.14
LEU	TTG	4	0.06	LEU	TTG	2	0.06
LEU	CTT	3	0.05	LEU	CTT	2	0.05
LEU	CTA	1	0.01	LEU	CTA	0	0.01
LEU	TTA	0	0.00	LEU	TTA	0	0.00
MET	ATG	7	1.00	MET	ATG	7	1.00
ASN	AAC	23	0.76	ASN	AAC	5	0.76
ASN	AAT	7	0.24	ASN	AAT	1	0.24
PRO	CCG	7	0.61	PRO	CCG	6	0.61
PRO	CCC	3	0.27	PRO	CCC	3	0.27
PRO	CCA	1	0.06	PRO	CCA	1	0.06
PRO	CCT	1	0.06	PRO	CCT	1	0.06
GLN	CAG	13	0.86	GLN	CAG	10	0.86
GLN	CAA	2	0.14	GLN	CAA	2	0.14
ARG	CGC	22	0.68	ARG	CGC	5	0.68
ARG	CGG	5	0.17	ARG	CGG	1	0.17
ARG	CGT	3	0.08	ARG	CGT	1	0.08
ARG	AGG	1	0.04	ARG	AGG	0	0.04
ARG	CGA	1	0.03	ARG	CGA	0	0.03
ARG	AGA	0	0.01	ARG	AGA	0	0.01

SER	TCG	16	0.37
SER	AGC	15	0.35
SER	TCC	8	0.18
SER	TCA	2	0.04
SER	AGT	1	0.03
SER	TCT	1	0.03
THR	ACC	17	0.57
THR	ACG	10	0.34
THR	ACT	1	0.05
THR	ACA	1	0.04
VAL	GTG	17	0.59
VAL	GTC	9	0.32
VAL	GTT	1	0.05
VAL	GTA	1	0.04
TRP	TGG	9	1.00
TYR	TAC	16	0.70
TYR	TAT	7	0.30

SER	TCG	7	0.37
SER	AGC	7	0.35
SER	TCC	3	0.18
SER	TCA	1	0.04
SER	AGT	1	0.03
SER	TCT	1	0.03
THR	ACC	8	0.57
THR	ACG	5	0.34
THR	ACT	1	0.05
THR	ACA	1	0.04
VAL	GTG	13	0.59
VAL	GTC	7	0.32
VAL	GTT	1	0.05
VAL	GTA	1	0.04
TRP	TGG	2	1.00
TYR	TAC	6	0.70
TYR	TAT	3	0.30

mvaD & mvaK2

aa	codon	number	frequency
END	TGA	1	0.67
END	TAA	0	0.17
END	TAG	0	0.16
ALA	GCC	27	0.47
ALA	GCG	22	0.39
ALA	GCA	5	0.09
ALA	GCT	3	0.05
CYS	TGC	10	0.91
CYS	TGT	1	0.09
ASP	GAC	31	0.74
ASP	GAT	11	0.26
GLU	GAG	28	0.60
GLU	GAA	19	0.40
PHE	TTC	21	0.82
PHE	TTT	5	0.18
GLY	GGC	30	0.76
GLY	GGG	5	0.13
GLY	GGT	3	0.07
GLY	GGA	2	0.04
HIS	CAC	3	0.64
HIS	CAT	2	0.36
ILE	ATC	29	0.86
ILE	ATT	4	0.12
ILE	ATA	1	0.02
LYS	AAG	43	0.90
LYS	AAA	5	0.10
LEU	CTG	53	0.74
LEU	CTC	10	0.14
LEU	TTG	4	0.06
LEU	CTT	4	0.05

idi

aa	codon	number	frequency
END	TGA	1	0.67
END	TAA	0	0.17
END	TAG	0	0.16
ALA	GCC	9	0.47
ALA	GCG	7	0.39
ALA	GCA	2	0.09
ALA	GCT	1	0.05
CYS	TGC	4	0.91
CYS	TGT	0	0.09
ASP	GAC	7	0.74
ASP	GAT	3	0.26
GLU	GAG	7	0.60
GLU	GAA	4	0.40
PHE	TTC	5	0.82
PHE	TTT	1	0.18
GLY	GGC	7	0.76
GLY	GGG	1	0.13
GLY	GGT	1	0.07
GLY	GGA	0	0.04
HIS	CAC	3	0.64
HIS	CAT	2	0.36
ILE	ATC	6	0.86
ILE	ATT	1	0.12
ILE	ATA	0	0.02
LYS	AAG	5	0.90
LYS	AAA	1	0.10
LEU	CTG	12	0.74
LEU	CTC	2	0.14
LEU	TTG	1	0.06
LEU	CTT	1	0.05

LEU	CTA	1	0.01
LEU	TTA	0	0.00
MET	ATG	19	1.00
ASN	AAC	17	0.76
ASN	AAT	5	0.24
PRO	CCG	11	0.61
PRO	CCC	5	0.27
PRO	CCA	1	0.06
PRO	CCT	1	0.06
GLN	CAG	25	0.86
GLN	CAA	4	0.14
ARG	CGC	18	0.68
ARG	CGG	4	0.17
ARG	CGT	2	0.08
ARG	AGG	1	0.04
ARG	CGA	1	0.03
ARG	AGA	0	0.01
SER	TCG	21	0.37
SER	AGC	20	0.35
SER	TCC	10	0.18
SER	TCA	2	0.04
SER	AGT	2	0.03
SER	TCT	2	0.03
THR	ACC	17	0.57
THR	ACG	10	0.34
THR	ACT	2	0.05
THR	ACA	1	0.04
VAL	GTG	24	0.59
VAL	GTC	13	0.32
VAL	GTT	2	0.05
VAL	GTA	2	0.04
TRP	TGG	8	1.00
TYR	TAC	17	0.70
TYR	TAT	7	0.30

LEU	CTA	0	0.01
LEU	TTA	0	0.00
MET	ATG	4	1.00
ASN	AAC	5	0.76
ASN	AAT	2	0.24
PRO	CCG	6	0.61
PRO	CCC	3	0.27
PRO	CCA	1	0.06
PRO	CCT	1	0.06
GLN	CAG	7	0.86
GLN	CAA	1	0.14
ARG	CGC	8	0.68
ARG	CGG	2	0.17
ARG	CGT	1	0.08
ARG	AGG	0	0.04
ARG	CGA	0	0.03
ARG	AGA	0	0.01
SER	TCG	4	0.37
SER	AGC	4	0.35
SER	TCC	2	0.18
SER	TCA	0	0.04
SER	AGT	0	0.03
SER	TCT	0	0.03
THR	ACC	8	0.57
THR	ACG	5	0.34
THR	ACT	1	0.05
THR	ACA	1	0.04
VAL	GTG	8	0.59
VAL	GTC	4	0.32
VAL	GTT	1	0.05
VAL	GTA	1	0.04
TRP	TGG	6	1.00
TYR	TAC	4	0.70
TYR	TAT	2	0.30

mvaE

aa	codon	number	frequency
END	TGA	1	0.67
END	TAA	0	0.17
END	TAG	0	0.16
ALA	GCC	44	0.47
ALA	GCG	37	0.39
ALA	GCA	8	0.09
ALA	GCT	5	0.05
CYS	TGC	3	0.91
CYS	TGT	0	0.09
ASP	GAC	24	0.74
ASP	GAT	8	0.26
GLU	GAG	38	0.60
GLU	GAA	26	0.40

mvaS

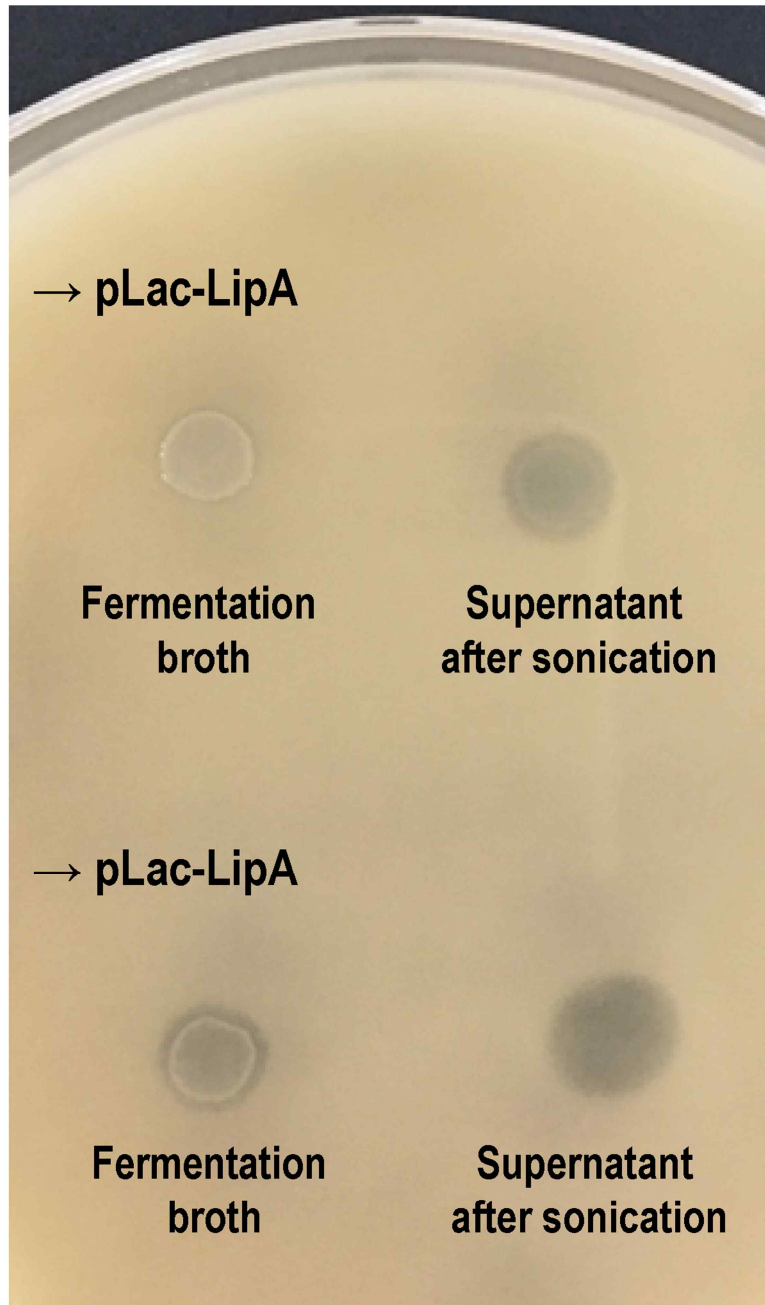
aa	codon	number	frequency
END	TGA	1	0.67
END	TAA	0	0.17
END	TAG	0	0.16
ALA	GCC	21	0.47
ALA	GCG	18	0.39
ALA	GCA	4	0.09
ALA	GCT	2	0.05
CYS	TGC	1	0.91
CYS	TGT	0	0.09
ASP	GAC	18	0.74
ASP	GAT	6	0.26
GLU	GAG	17	0.60
GLU	GAA	11	0.40

PHE	TTC	16	0.82
PHE	TTT	4	0.18
GLY	GGC	48	0.76
GLY	GGG	8	0.13
GLY	GGT	4	0.07
GLY	GGA	3	0.04
HIS	CAC	8	0.64
HIS	CAT	4	0.36
ILE	ATC	46	0.86
ILE	ATT	6	0.12
ILE	ATA	1	0.02
LYS	AAG	37	0.90
LYS	AAA	4	0.10
LEU	CTG	59	0.74
LEU	CTC	11	0.14
LEU	TTG	5	0.06
LEU	CTT	4	0.05
LEU	CTA	1	0.01
LEU	TTA	0	0.00
MET	ATG	21	1.00
ASN	AAC	24	0.76
ASN	AAT	8	0.24
PRO	CCG	13	0.61
PRO	CCC	6	0.27
PRO	CCA	1	0.06
PRO	CCT	1	0.06
GLN	CAG	41	0.86
GLN	CAA	7	0.14
ARG	CGC	22	0.68
ARG	CGG	5	0.17
ARG	CGT	3	0.08
ARG	AGG	1	0.04
ARG	CGA	1	0.03
ARG	AGA	0	0.01
SER	TCG	21	0.37
SER	AGC	20	0.35
SER	TCC	10	0.18
SER	TCA	2	0.04
SER	AGT	2	0.03
SER	TCT	2	0.03
THR	ACC	25	0.57
THR	ACG	15	0.34
THR	ACT	2	0.05
THR	ACA	2	0.04
VAL	GTG	36	0.59
VAL	GTC	20	0.32
VAL	GTT	3	0.05
VAL	GTA	2	0.04
TRP	TGG	2	1.00
TYR	TAC	15	0.70

PHE	TTC	11	0.82
PHE	TTT	3	0.18
GLY	GGC	20	0.76
GLY	GGG	3	0.13
GLY	GGT	2	0.07
GLY	GGA	1	0.04
HIS	CAC	6	0.64
HIS	CAT	3	0.36
ILE	ATC	25	0.86
ILE	ATT	3	0.12
ILE	ATA	1	0.02
LYS	AAG	17	0.90
LYS	AAA	2	0.10
LEU	CTG	27	0.74
LEU	CTC	5	0.14
LEU	TTG	2	0.06
LEU	CTT	2	0.05
LEU	CTA	0	0.01
LEU	TTA	0	0.00
MET	ATG	10	1.00
ASN	AAC	11	0.76
ASN	AAT	4	0.24
PRO	CCG	8	0.61
PRO	CCC	4	0.27
PRO	CCA	1	0.06
PRO	CCT	1	0.06
GLN	CAG	13	0.86
GLN	CAA	2	0.14
ARG	CGC	9	0.68
ARG	CGG	2	0.17
ARG	CGT	1	0.08
ARG	AGG	1	0.04
ARG	CGA	0	0.03
ARG	AGA	0	0.01
SER	TCG	8	0.37
SER	AGC	7	0.35
SER	TCC	4	0.18
SER	TCA	1	0.04
SER	AGT	1	0.03
SER	TCT	1	0.03
THR	ACC	14	0.57
THR	ACG	8	0.34
THR	ACT	1	0.05
THR	ACA	1	0.04
VAL	GTG	12	0.59
VAL	GTC	7	0.32
VAL	GTT	1	0.05
VAL	GTA	1	0.04
TRP	TGG	2	1.00
TYR	TAC	13	0.70

TYR	TAT	6	0.30
-----	-----	---	------

TYR	TAT	5	0.30
-----	-----	---	------



Supplementary Figure 1. Comparison of Lac and J5 promoter for alkaline lipase (LipA) production in *R. eutropha*