Table S1 Differentially intracellular metabolites in BW25113-Δ*ast*E when compared to that those in BW25113 after exposing to 5 g/L butanol

Name	Log2fold	qvalue	Up/down	Mzmed	Rtmed
L-leucine	14.5	0.017584	UP	117.1	35.34208
11-deoxycorticosterone	-13.5	0.014418	DOWN	314.2	35.35
2-oxoglutarate	-12	0.010417	DOWN	130	38.552
γ-butyrobetaine	11.6	0.019996	UP	130.1	38.541
9-mercaptodethiobiotin	-9.7	0.029138	DOWN	245.1	32.4445
2,3-dihydroxybenzoate	-8	0.046539	DOWN	117	19.082
1-myristoyl-2-palmitoleoyl phosphatidate	7.9	0.057484	UP	308.2	33.844
Pyrazine-2-carboxylate	-7.7	0.045552	DOWN	87	19.08
6-deoxy-6-sulfo-D-fructose 1-phosphate	-7.4	0.031078	DOWN	163	8.713
2-oxo-4-methylthiobutanoate	-7.1	0.014468	DOWN	132	38.8645
S-sulfanylglutathione	-6.1	0.017348	DOWN	358.1	30.294
α-ribazole 5'-phosphate	-6.1	0.016339	DOWN	359.1	30.294
Glyoxylate	-5.9	0.041394	DOWN	119	18.31433
Malate	-5.9	0.053315	DOWN	118	19.0755
5-amino-1-(5-phospho-β-D-ribosyl)imidazole	-5.3	0.042877	DOWN	314.1	18.3
Sulfate	-5.3	0.009955	DOWN	116	38.552
5-amino-6-(D-ribitylamino)uracil	-5	0.021238	DOWN	259.1	24.4715
6,7-dimethyl-8-(1-D-ribityl)lumazine	-4.9	0.019453	DOWN	307.1	19.824
N2-succinyl-L-ornithine	-4.6	0.00941	DOWN	214.1	29.373
Ethylene glycol	-4.5	0.04192	DOWN	101	19.162
3-keto-L-gulonate 6-phosphate	-4.4	0.016669	DOWN	149	43.749
(1R,6R)-6-hydroxy-2-succinylcyclohexa-2,4-diene-1-carboxylate	-4.3	0.008353	DOWN	258.1	24.46

Oxaloacetate	-3.4	0.050957	DOWN	131	29.539
Betaine aldehyde hydrate	-3.2	0.012421	DOWN	165.1	34.9585
6-carboxy-5,6,7,8-tetrahydropterin	3	0.008353	UP	229.1	22.217
L-phenylalanine	3	0.04148	UP	225.1	30.29642
Oxalosuccinate	-2.9	0.045327	DOWN	213	18.265
7,8-dihydromonapterin	2.9	0.039561	UP	256.1	30.295
N,N-dimethyl-p-phenylenediamine	-2.9	0.010324	DOWN	181.1	34.961
2'-deoxycytidine	2.8	0.008353	UP	228.1	22.222
4-(γ-glutamylamino)butanal	-2.6	0.013737	DOWN	201.1	31.50392
Propanoate	-2.5	0.029839	DOWN	113	19.8575
N-acetylmuramate	-2.2	0.051255	DOWN	316.1	18.26433
Thymidine	2.2	0.010074	UP	243.1	22.217
Succinate semialdehyde	-2.1	0.05022	DOWN	137	18.2685
4-(γ-L-glutamylamino)butanoate	-1.8	0.010305	DOWN	256.1	19.8605
L-Valine	1.8	0.03812	UP	132.1	38.547

Table S2 Comparison of intracellular amino acids between BW25113 and BW25113-ΔastE under butanol stress

Animo acids	BW25113		ΔastE	
	Supernate (mg/100ml)	Intracellular (mg/100ml)	Supernate (mg/100ml)	Intracellular (mg/100ml)
Ala	0.25±0.03	0.37±0.04	0.73±0.10	0.21±0.15
Gly	0.13±0.01	0.54±0.14	0.21±0.01	0.20±0.09
Val	0.33±0.07	0.32±0.09	0.01±0.02	0.33±0.07
Met	0.15±0.02	0.17±0.06	0.48±0.16	0.21±0.05
Ile	0.05±0.06	0.19±0.05	0.69±0.10	0.12±0.08
Phe	0.50±0.14	0.17±0.06	0.28±0.07	0.19±0.13
NH3	17.2±0.08	0.40±0.02	23.0±0.16	0.36±0.07
His	0.00±0.00	0.10±0.07	0.00±0.00	0.12±0.12
Asp	0.17±0.03	0.10±0.005	0.19±0.03	0.03±0.01
Thr	0.23±0.01	0.25±0.05	0.00±0.00	0.28±0.16
Ser	0.05±0.01	0.22±0.03	0.07±0.02	0.13±0.09
Pro	0.00±0.00	0.12±0.07	0.00±0.00	0.05±0.03
Glu	0.36±0.03	0.79±0.22	2.84±0.34	0.30±0.21
Cys	0.04±0.04	0.00±0.00	0.27±0.01	0.69±0.10
Leu	0.32±0.11	0.18±0.13	0.59±0.02	0.34±0.09
Tyr	0.00±0.00	0.00±0.00	0.00±0.00	0.36±0.02
Lys	0.06±0.01	0.00±0.00	0.22±0.03	0.04±0.01
Arg	0.05±0.00	0.00±0.00	0.41±0.03	0.00±0.00

Table S3 Plasmids and strains used in this study

Strains/plasmids	Relevant characteristics	Reference
Plasmids		
pKD46	Red recombinant plasmid, Temperature-sensitive, L-arabinose induction, Amp^r	Lab. collected
pKD3	Harboring the gene of chloramphenicol resistance, Amp^r, Cm^r	Lab. collected
pCP20	Harboring the gene of FLP recombinase, Temperature-sensitive, Amp^r, Cm^r	Lab. collected
Strains		
E. coli BW25113	Host for homologous recombination	Lab. collected
BW25443-Δ*ast*E	Knocking-out of *ast*E gene in Bw25113, Cm^r	This study

Amp^r, Cm^r are ampicillin, chloramphenicol resistance, respectively

Table S4 Primers used for knocking-out and identifying in this study

Primers	**Sequences (5’-3’)**	Application
astE-cms	TTAGCTTATTTTTTCGAGCATTAATCCCGCGCGTAATGTCTTGAGCGATTGTGTAGG	Knocking-out *astE*
astE-cmas	ATGGATAATTTTCTTGCTCTGACCTTAACGGGTAAACTTAACGGCTGACATGGGAA	
astE-yans	CCAATTACTGAATCTCGGTT	Identification of Δ*ast*E
astE-yanas	AGAAAGCAACTTAATACCCG	

*astEcm represents the primers for plate pKD3; astEyan represents the identifying primers. Underline represents the homology sequences to the aim gene.

Table S5 Quantitative PCR primers of selected genes

Gene name	Sense Primer	Sequence (5'to3')	Anti-sense Primer	Sequence (5'to3')
astE	astE-qF	ATATGGCAAGTGACACGCTGAAT	astE-qR	CCAACGCTACCAACGGATTAGG
prpB	prpB-qF	TTGGCACCATCAACGCTAATCAT	prpB-qR	GTCGGTCAGCACATCATCAAGG
prpD	prpD-qF	AGGTGAATCGTTCCGCTTCCA	prpD-qR	GCTGCTTCAACTGCCGTCTG
ybfA	ybfA-qF	ACAGAGAATATCCTGCATGGCTTAT	ybfA-qR	TCGGCCTGATCCATCCACAC
ymgA	ymgA-qF	CAGGCGGTGCTCCAGATACT	mgA-qR	GCTGTCGCTGTTCTTCGGTTT
ymgC	ymgC-qF	ATGACGCATGGGTATGTTGATAGT	mgC-qR	AGAGAGCACGGATTCCCTGTC
iraD	iraD-qF	TTAATTCTCCTCATTCGGCATTACC	iraD-qR	GAGTGTGGCAGTACGCTTCTT
psuK	psuK-qF	TGAAGAGGCACTGGCATGGATT	psuK-qR	TGGCTTGAGAGTGTGGATCTGATT
gadC	gadC-qF	ACTGGTTGTTAGTTTCCTGGTGGTA	gadC-qR	TGGTGCGTTCTGACTGTTGATTG
YbaL	baL-qF	CTGATATTCCGCTGGTGGTGATTG	baL-qR	TTCTTCGTTCGCCGCATTGC
rpoA	rpoA-qF	ATTCGTCGTGCGGCAACCA	rpoA-qR	ACAGTCAATTCCAGATCGTCAACAG
astE	astE-qF	ATATGGCAAGTGACACGCTGAAT	astE-qR	CCAACGCTACCAACGGATTAGG
prpC	prpC-qF	ATCTCGACTGGTTCTCTGCTGTT	prpC-qR	CATAATTGGCGGAAGGACGGATAA
prpE	prpE-qF	CTCGTCGCTGGAAGTGCTCTA	prpE-qR	CGGATTCGGTCTGCCAGTAGT
ycgZ	ycgZ-qF	GCGGGAGCAATCACTCGTTAC	ycgZ-qR	GCGTGTTCCAGTCGGCAAAG

ariR	ariR-qF	GTTAGAAGAAGAATCAGCAGTGTTAGG	ariR-qR	TGTTGTTTCCAAGGAGTGTATCAGA
spy	spy-qF	ATGCCGCAGACACCACTACC	spy-qR	CGTCGGTCAGGTTCAGGTCTT
psuG	psuG-qF	TTACTGGGTCGTGAAGGGCATAA	psuG-qR	CGCCGCAATAATCATCGTTGAAG
csrB	csrB-qF	GGAGTCAGACAACGAAGTGAACATC	csrB-qR	GCGTCCTGCGTCATCCTCTT
adiA	adiA-qF	CGCTGTCACAGGCTTCTTATATTCA	adiA-qR	TCAACCGCTTCGTCAATCACTTC
gadC	gadC-qF	ACTGGTTGTTAGTTTCCTGGTGGTA	gadC-qR	TGGTGCGTTCTGACTGTTGATTG
YbaT	baT-qF	TGGCGGTATTGTGGCGATGT	baT-qR	CCAGCGTCAACAGGTACAGTAAC
yidE	yidE-qF	CCGCTGATTACTGTTGGCATTCT	yidE-qR	GCACCGCTGGTTGGATGAAG
cysB	cysB-qF	ATATCTTCAGCCACAGTACAACCAA	cysB-qR	CAGCCGCATCAACGACATCA

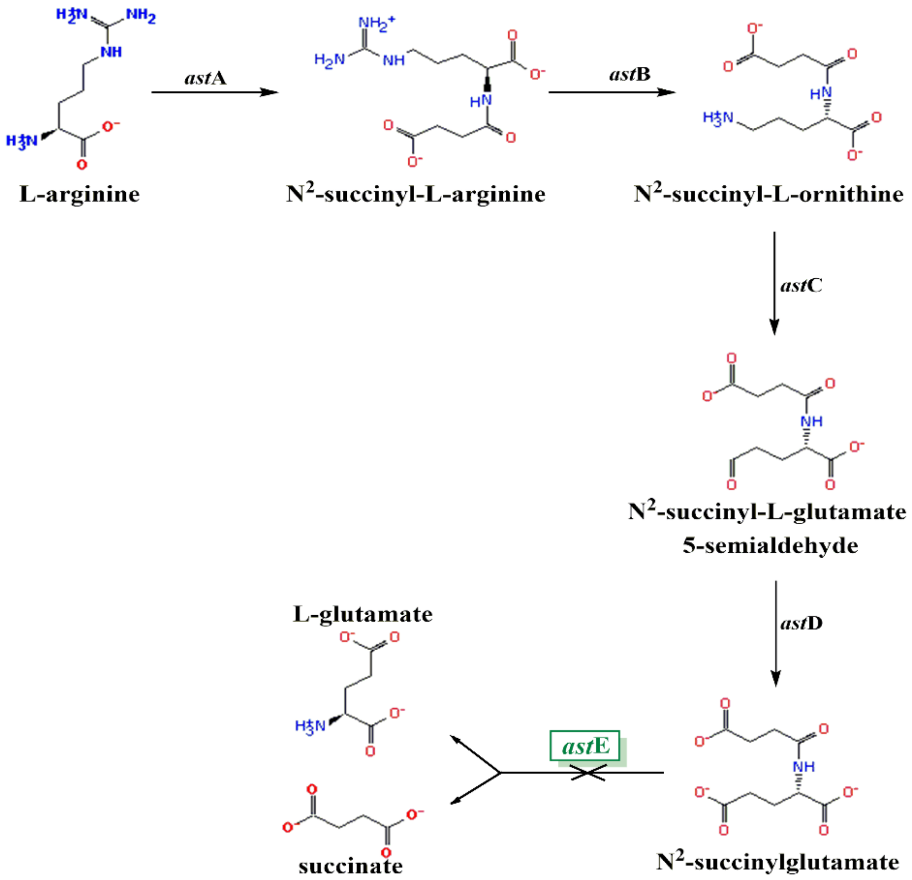


Fig. S1 The catabolism pathway of L-arginine in *E. coli*. *ast*A, Arginine N-succinyltransferase; *ast*B, N-succinylarginine dihydrolase; *ast*C, Succinylornithine transaminase; *ast*D, Succinylglutamate-semialdehyde dehydrogenase; *ast*E, Succinylglutamate desuccinylase.

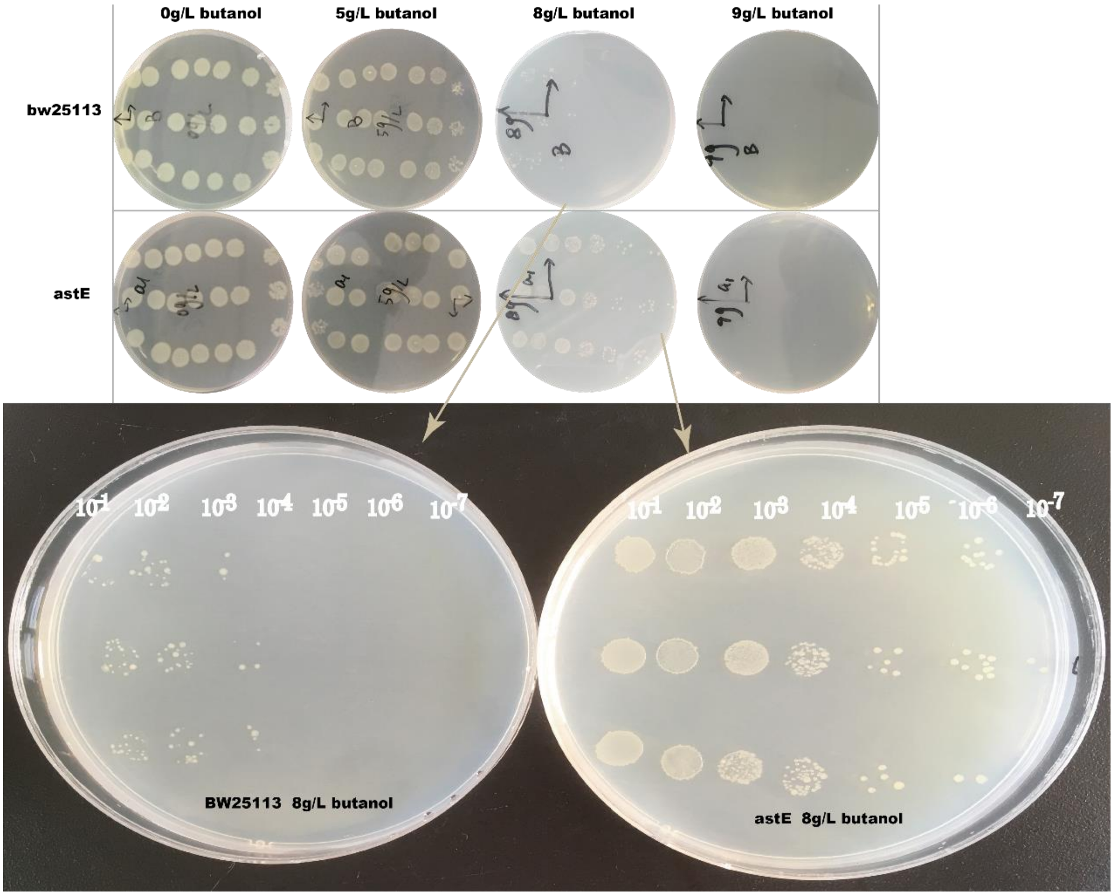


Fig. S2 Cell growth against 8 g/L butanol stress: Application of a spotting assay

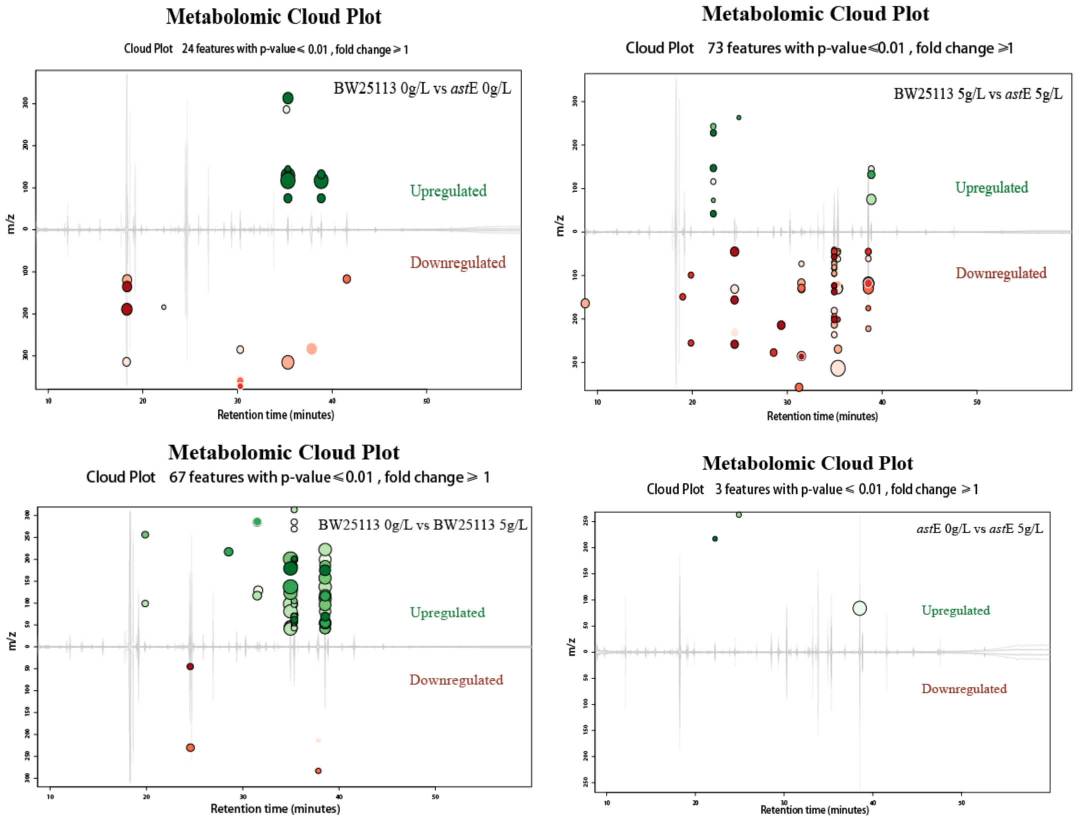


Fig. S3 Metabolomic cloud plot of strains in the presence and absence of butanol(Green, up/down-regulated metabolites; red, up/down-regulated metabolites; $p<0.01$; FC≥1).

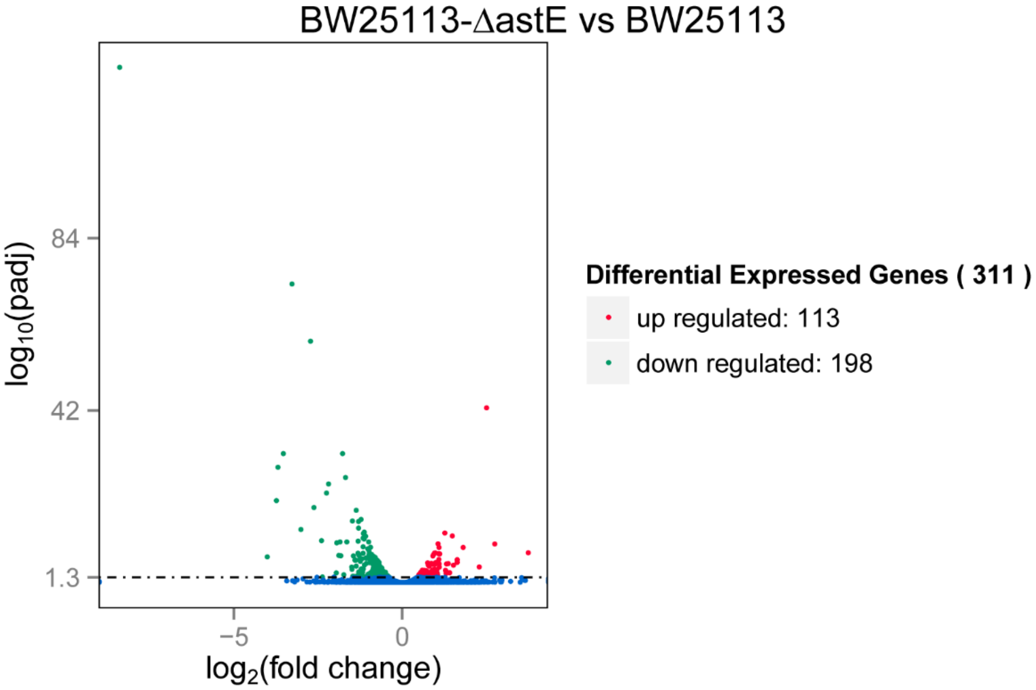


Fig. S4 Overview of the differentially expressed genes between BW25113 and BW25113-Δ*ast*E against 5 g/L butanol stress (Green, downregulated genes; red, upregulated genes; $p<0.05$; FC≥2).

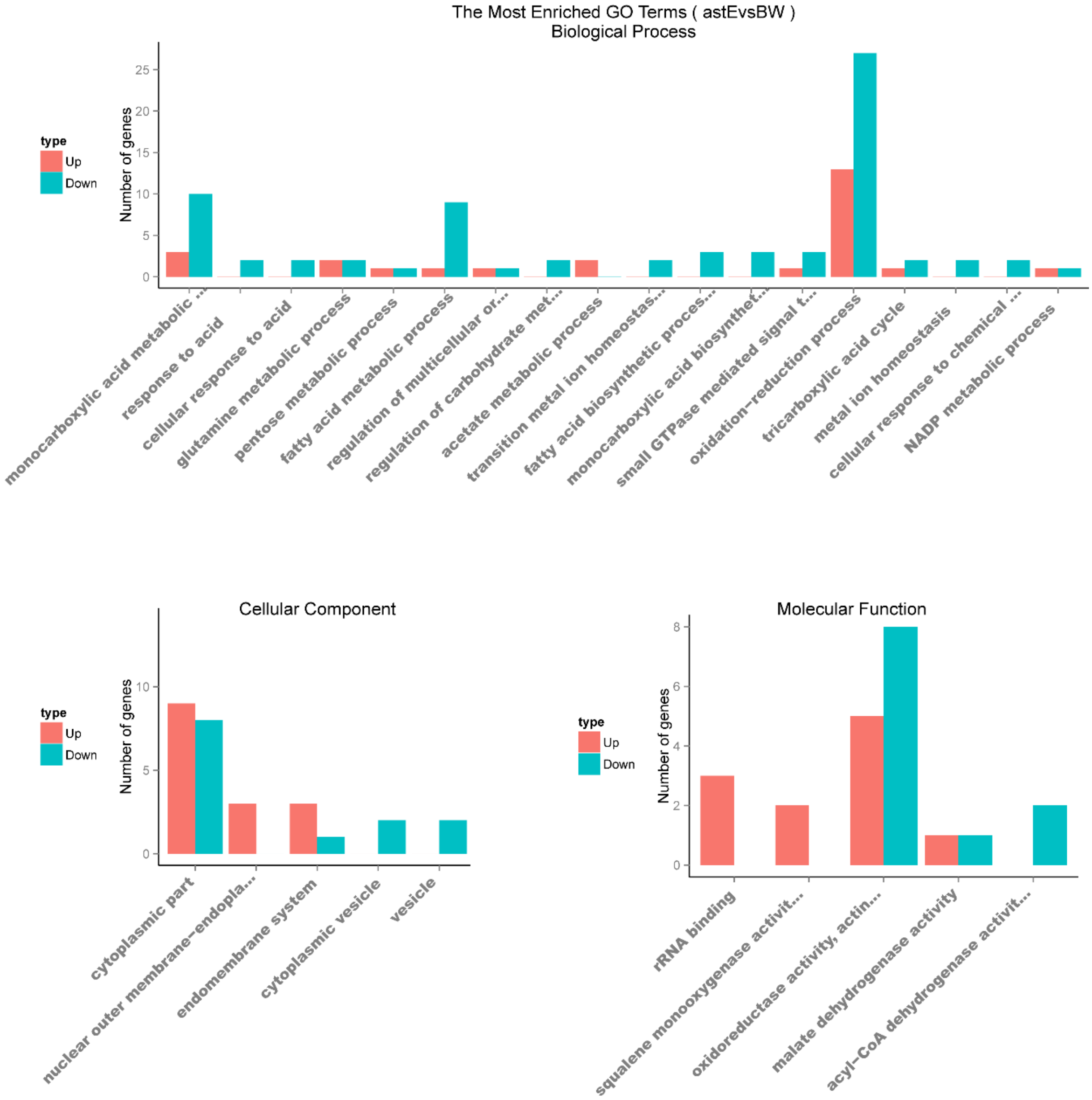


Fig. S5 GO enrichment analysis of differentially expressed genes between BW25113 and BW25113-ΔastE after exposure to 5 g/L butanol stress.

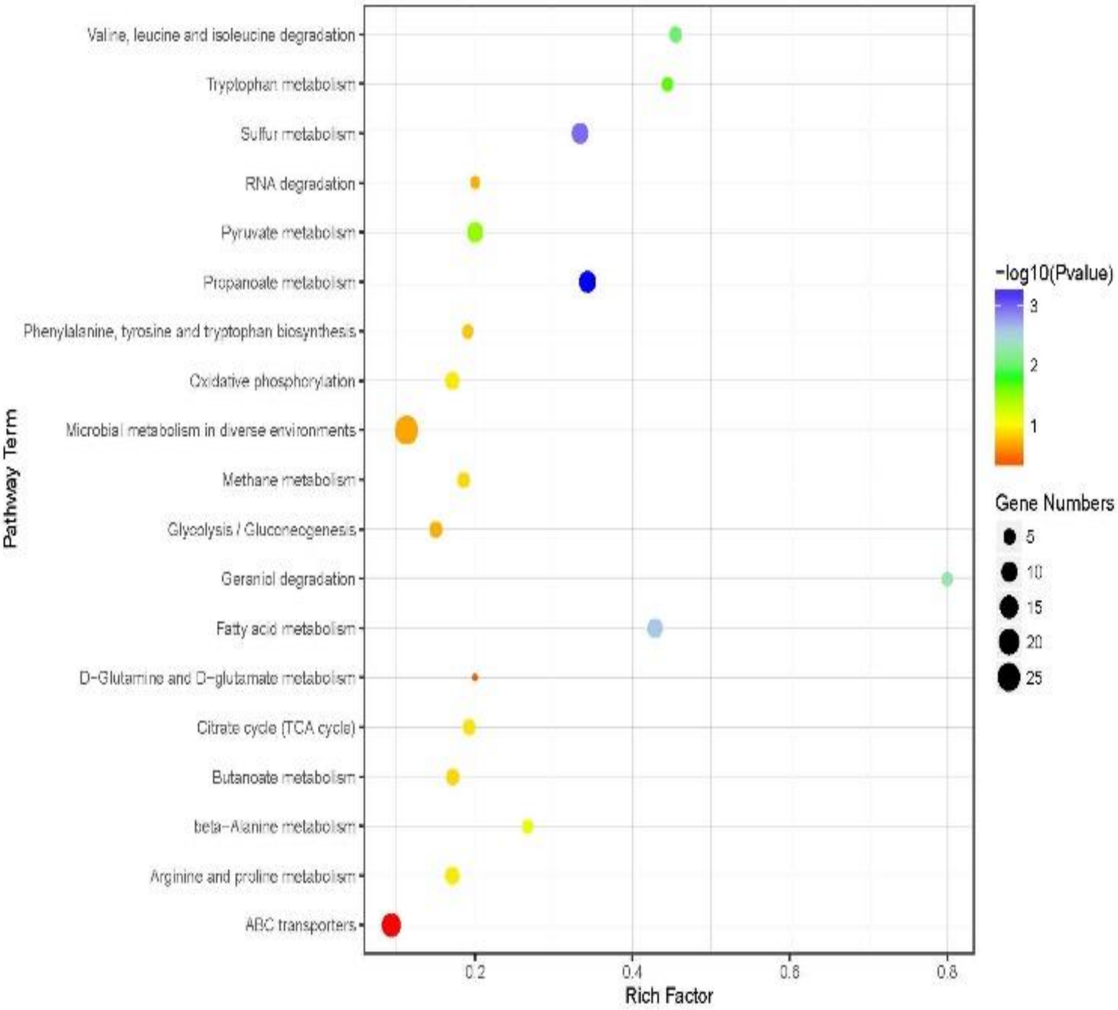
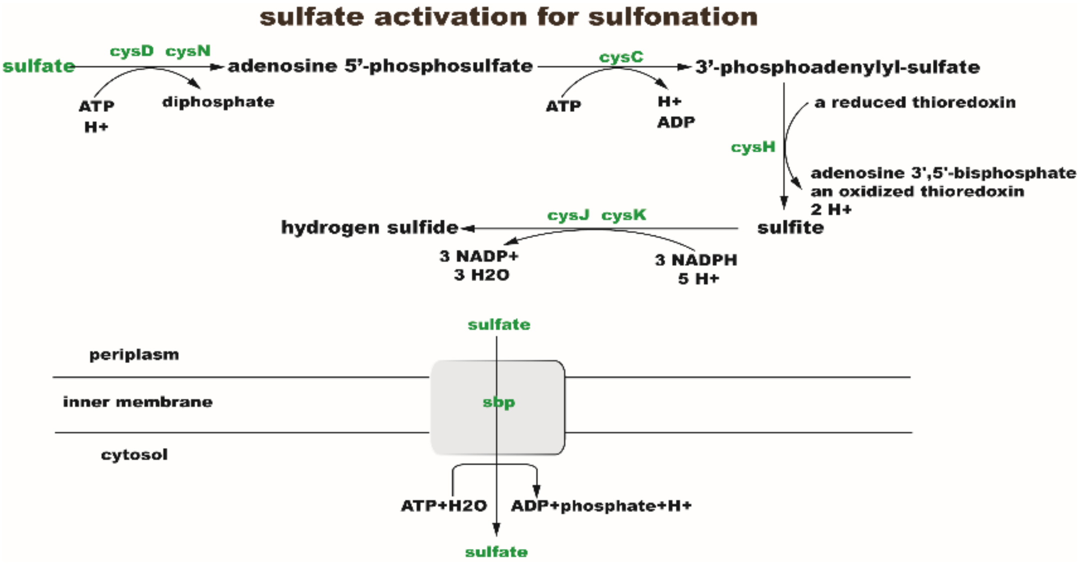
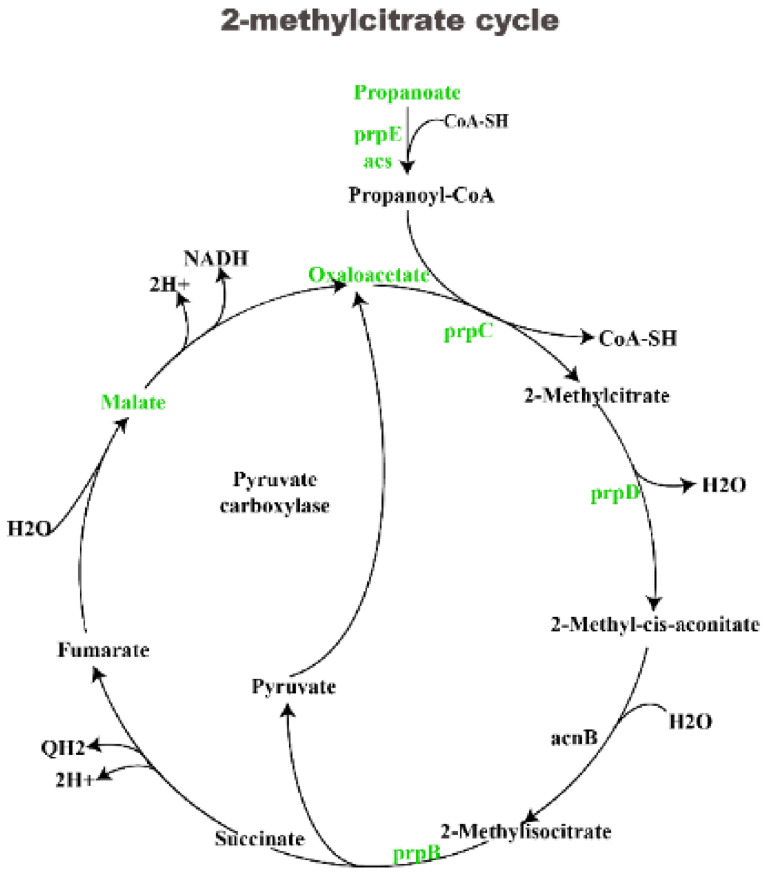


Fig. S6 KEGG enrichment analysis of differentially expressed genes between BW25113 and BW25113-ΔastE after exposure to 5 g/L butanol.



(a)



(b)

Fig. S7 KEGG enrichment analysis of significantly downregulated genes

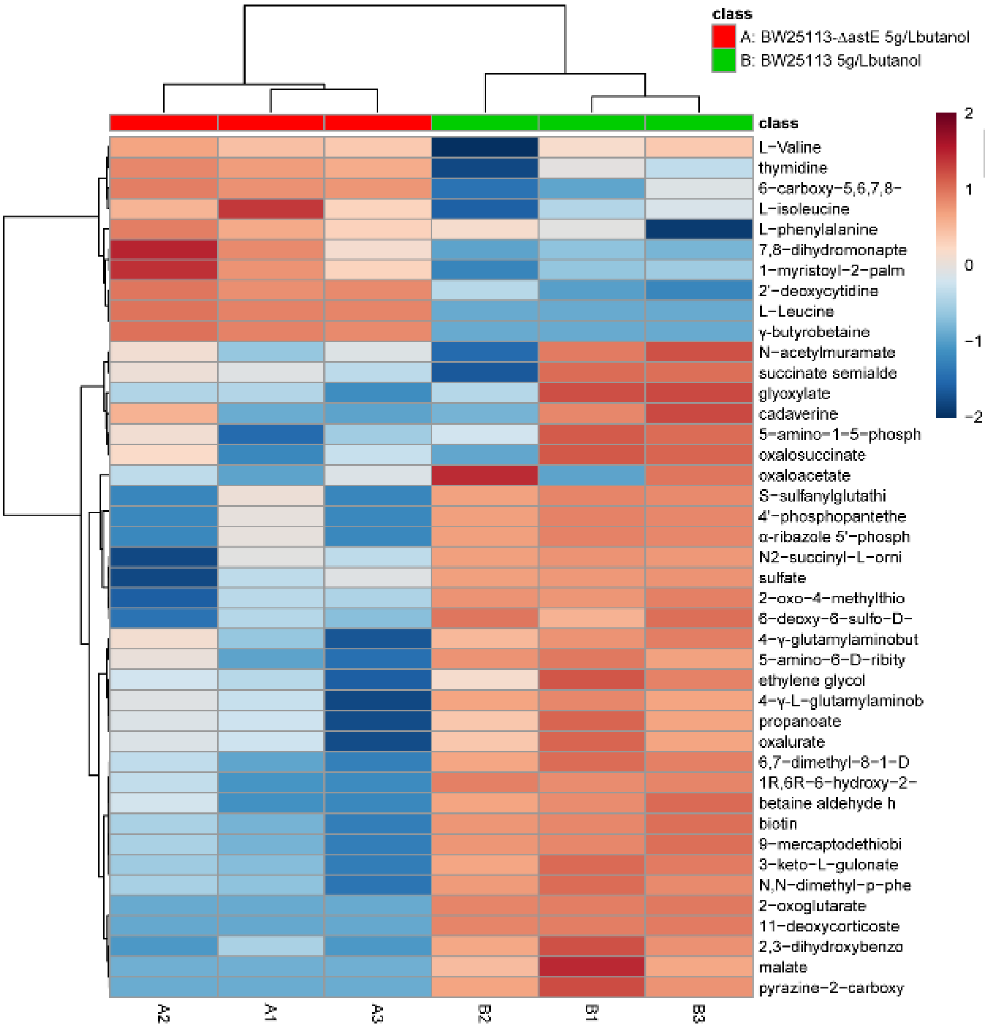


Fig. S8 Different metabolites: Clustering heat map. analysis related to the different metabolites of BW25113-ΔastE and BW25113 after exposure to 5 g/L butanol and metabolic pathways (red, upregulated; blue and green, downregulated)