Supplementary Material

Synergistic effect in core microbiota associated with sulfur metabolism in spontaneous Chinese liquor fermentation

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Running Title: Interaction between *Saccharomyces* and *Lactobacillus*

FIGURES

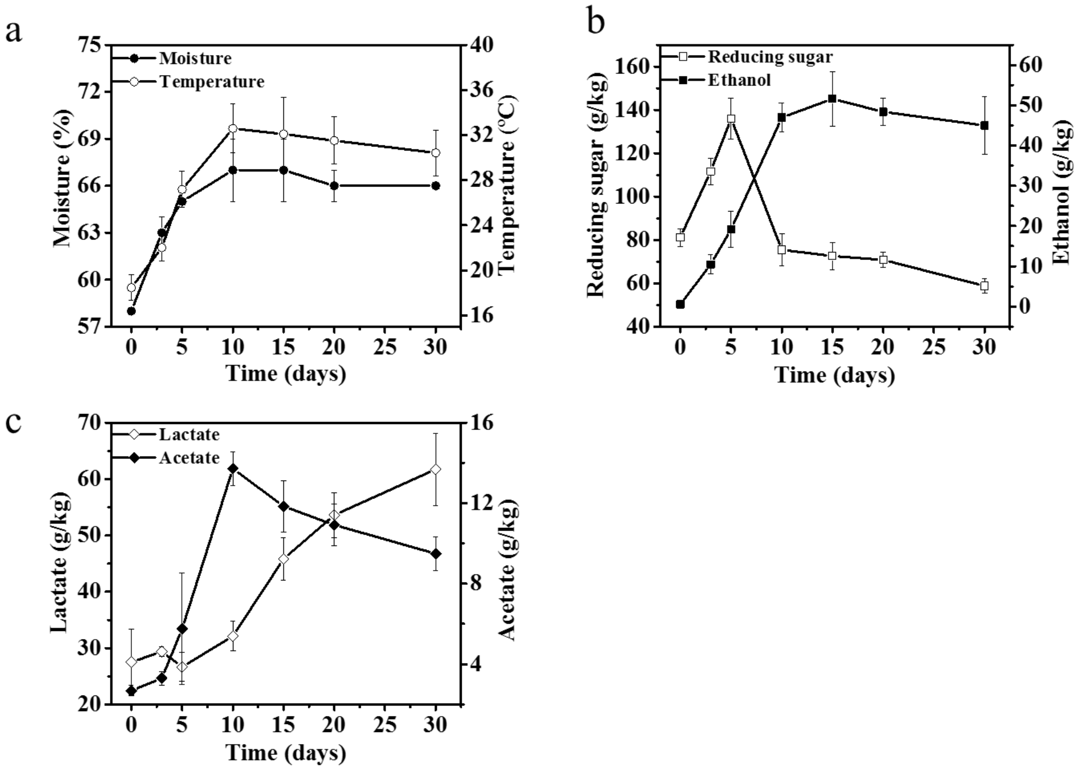

FIG S1. Dynamics of physicochemical parameters during the fermentation processes. (a) Moisture and temperature. (b) Reducing sugar and ethanol. (c) lactate and acetate. Data are shown as mean ± SD (n = 4).

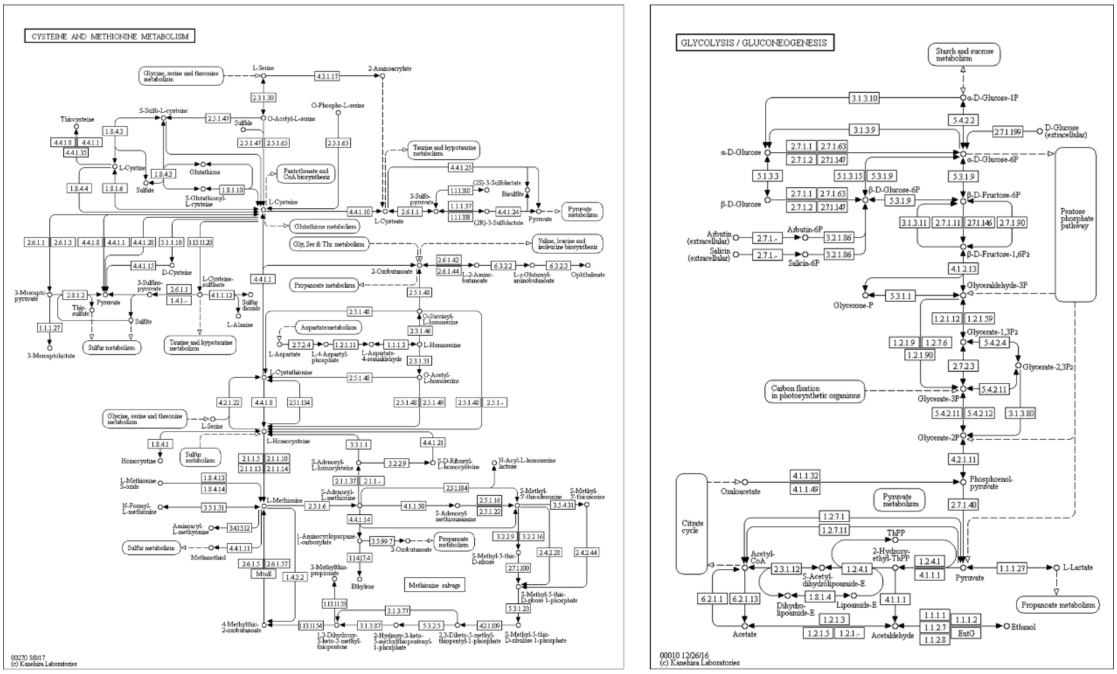

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FIG S2 The KEGG pathway associated with the synthesis of methionine, conversion of sulfur sources and generation of 3-(methylthio)-1-propanol and dimethyl disulfide.

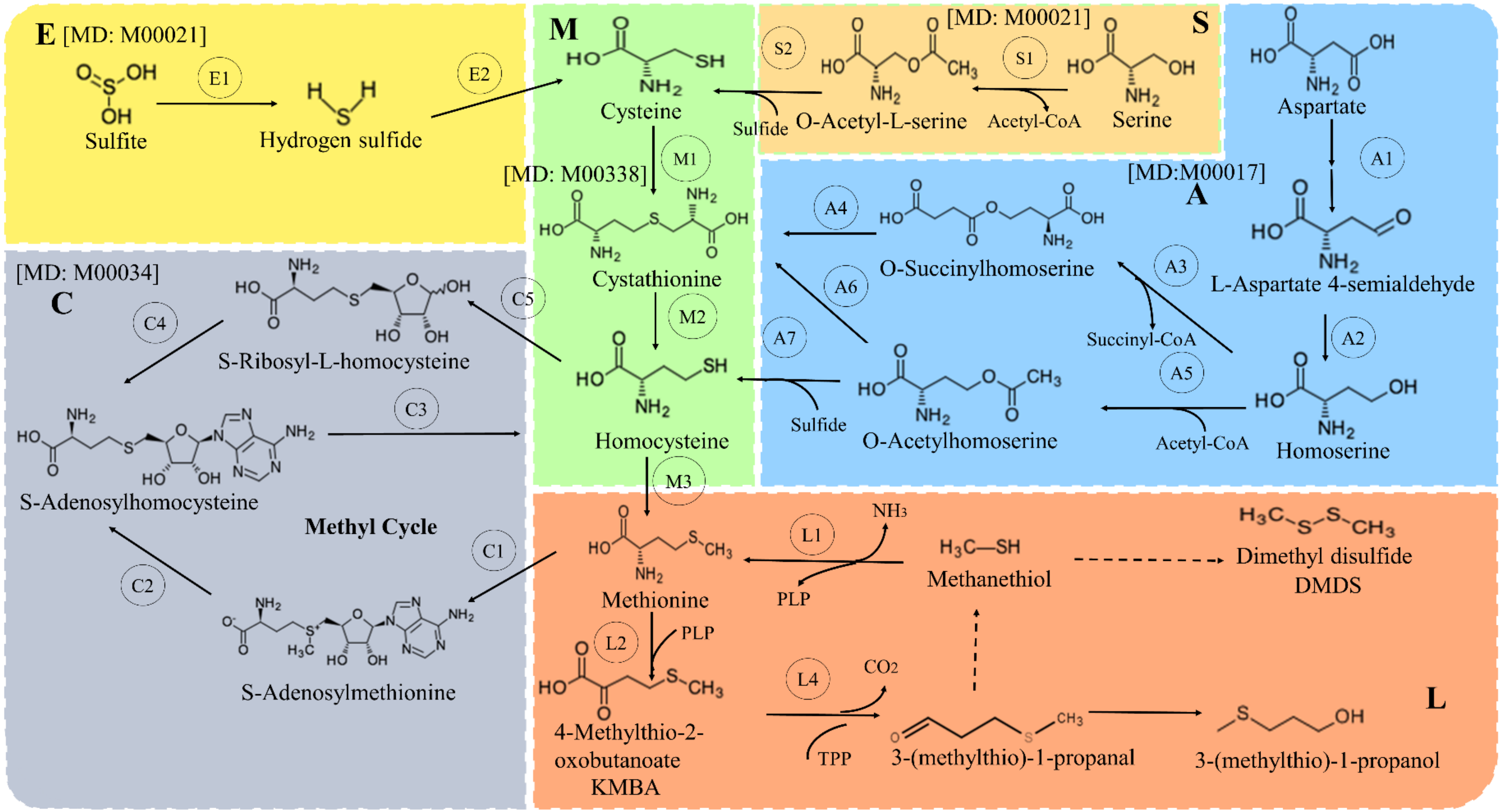


FIG S3 The predicted sulfur metabolic network for generation of 3-(methylthio)-1-propanol and dimethyl disulfide, synthesis of methionine, conversion of sulfur source.

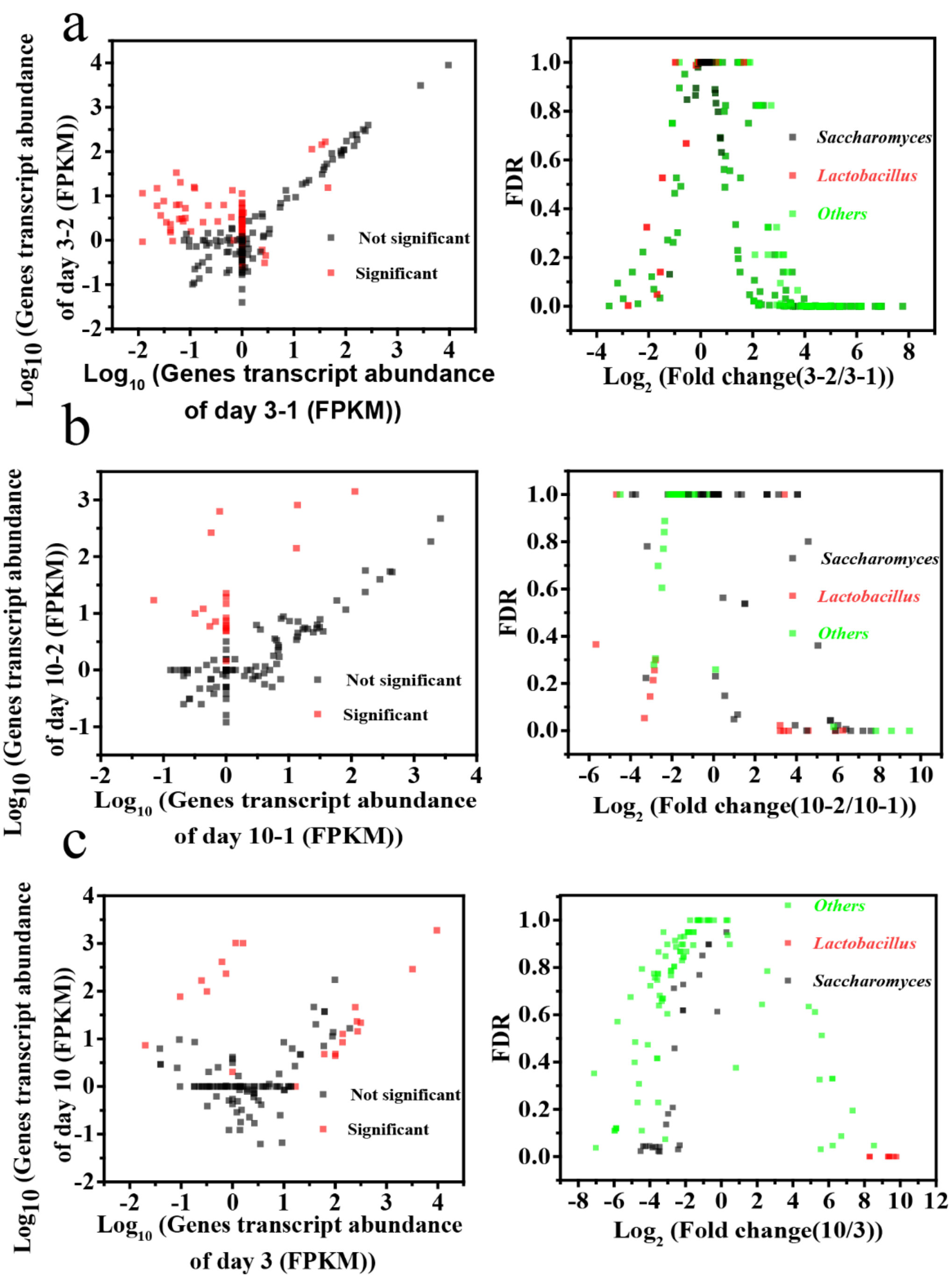
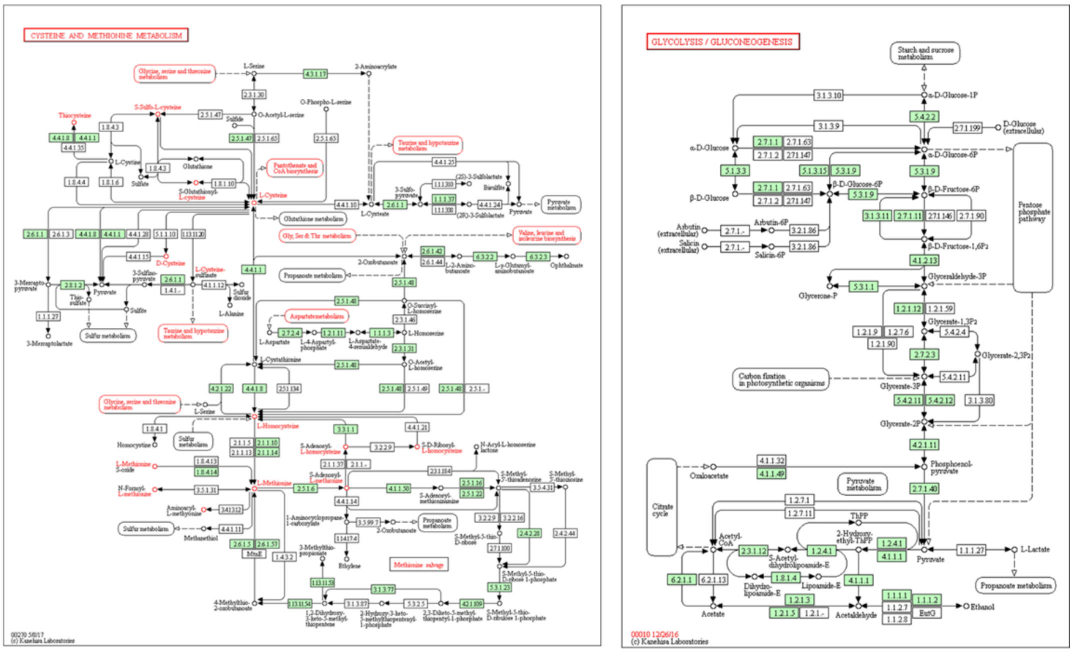


FIG S4 The statistical testing results of genes transcriptions in sulfur metabolic pattern, and each dot represents one coding sequences. The statistical testing analysis of the two biological replicates in dya 3 (3-1, 3-2) (a) and in day 10 (10-1, 10-2). (b) The statistical testing results of genes transcription profile between day 3 and day 10 (c).

a



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b

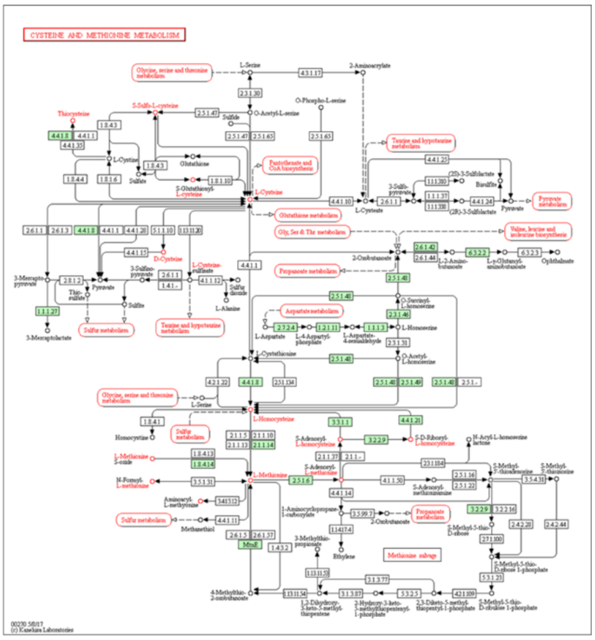

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FIG S5 The KEGG pathway associated with 3-(methylthio)-1-propanol and dimethyl disulfide of *S. cerevisiae* (a). and methyl cycle pathway of *L. buchneri* (b).

TABLES

Table S1 Summary of the metatranscriptomic sequencing assembly information.

Data	3-1	3-2	10-1	10-2
Insert size(bp)	250	250	250	250
Read length(bp)	150	150	150	150
Raw reads	164177322	155669670	138719046	127993572
Raw bases (bp)	24626598300	23350450500	20807856900	19199035800
Clean reads	161014964	152831948	136080680	125546508
Clean bases (bp)	22996584167	21646209707	19713007177	18089122586
Percent in raw	98.1	98.2	98.1	98.1
Non-rRNA	67718588	46970100	53736208	75340206
Percent in raw	41.2	30.2	38.7	58.9
Sequences	11862	34573	10887	8264
Sequences bases	16167654	33828138	16167654	9014791
N50 (bp)	2665	1541	2398	1776
N90 (bp)	470	392	450	403
Max (bp)	23527	21206	76654	48348
Min (bp)	300	300	300	300
ORFs	9150	24859	9321	6758
Total length (bp)	9445638	19247766	7975278	5024652
Average length	1032	774	856	744
Max (bp)	14706	14706	18177	12702
Min (bp)	123	123	123	123

Table S2 Details information of the 31 enzymes associated with generation of sulfur compounds, synthesis of methionine and conversion of sulfur sources.

Enzymes	Reactions	EC number of enzymes	Entry	Pathway	Enzyme name
1	A1	[EC:2.7.2.4]	K00928	ko00270	aspartate kinase
2	A1	[EC:1.2.1.11]	K00133	ko00270	aspartate-semialdehyde dehydrogenase
3	A2	[EC:1.1.1.3]	K00003	ko00270	homoserine dehydrogenase
4	A3	[EC:2.3.1.46]	K00651	ko00270	homoserine O-succinyltransferase
5	A4, A6	[EC:2.5.1.48]	K01739	ko00270	cystathionine gamma-synthase
6	A5	[EC:2.3.1.31]	K00641	ko00270	homoserine O-acetyltransferase
7	A7	[EC:2.5.1.49]	K01740	ko00270	O-acetylhomoserine (thiol)-lyase
8	C1	[EC:2.5.1.6]	K00789	ko00270	S-adenosylmethionine synthetase
9	C2	[EC:2.1.1.37]	K00558	ko00270	DNA (cytosine-5-)-methyltransferase
10	C3	[EC:3.3.1.1]	K01251	ko00270	adenosylhomocysteinase
11	C4	[EC:3.2.2.9]	K01243	ko00270	S-adenosylhomocysteine/5'-methylthioadenosine

12	C5	[EC:4.4.1.21]	K07173	ko00270	S-ribosylhomocysteine lyase
13	E1	[EC:1.8.1.2]	K00381	ko00270	sulfite reductase (NADPH) hemoprotein
14	E1	[EC:1.8.1.2]	K00380	ko00270	sulfite reductase (NADPH) flavoprotein
15	E2, S2	[EC:2.5.1.47]	K01738	ko00270	cysteine synthase
16	L1	[EC:4.4.1.11]	K01761	ko00270	methionine-gamma-lyase
17	L2	[EC:2.6.1.58 2.6.1.28]	K05821	ko00270	aromatic amino acid aminotransferase
18	L2	[EC:2.6.1.57 2.6.1.27 2.6.1.5]	K00838	ko00270	aromatic amino acid aminotransferase
19	L3	[EC:4.1.1.1]	K01568	ko00010	pyruvate decarboxylase
20	L3	[EC:4.1.1.-]	K12732	ko00360	phenylpyruvate decarboxylase
21	L4	[EC:1.1.2.8]	K00114	ko00010	alcohol dehydrogenase (cytochrome c)
22	L4	[EC:1.1.1.2]	K00002	ko00010	alcohol dehydrogenase (NADP+)
23	L4	[EC:1.1.1.1]	K00001	ko00010	alcohol dehydrogenase
24	L4	[EC:1.1.1.1]	K13953	ko00010	alcohol dehydrogenase, propanol-preferring
25	M1	[EC:4.4.1.1]	K01758	ko00270	cystathionine gamma-lyase

26	M2	[EC:4.4.1.8]	K01760	ko00270	cystathione beta-lyase
27	M2	[EC:4.2.1.22]	K01697	ko00270	cystathionine beta-synthase
28	M2	[EC:2.1.1.13]	K00548	ko00270	5-methyltetrahydrofolate--homocysteine
29	M3	[EC:2.1.1.10]	K00547	ko00270	homocysteine S-methyltransferase
30	M3	[EC:2.1.1.14]	K00549	ko00270	5-methyltetrahydropteroyltriglutamate--
31	S1	[EC:2.3.1.30]	K00640	ko00270	serine O-acetyltransferase

Table S3. The complete list of the transcription level of enzymes in sulfur metabolic pattern of microbiota in Chinese liquor fermentation

Genera	Transcript abundance (FPKM)				Reactions	Enzymes
	3-1	3-2	10-1	10-2		
Kazachstania	0.0	0.0	0.0	6.3	A1	Aspartate kinase [EC:2.7.2.4]
Lactobacillus	0.3	0.4	166.5	31.9	A1	Aspartate kinase [EC:2.7.2.4]
Oceanobacillus	0.0	2.4	0.0	0.0	A1	Aspartate kinase [EC:2.7.2.4]
Ogataea	0.3	2.7	0.4	0.3	A1	Aspartate kinase [EC:2.7.2.4]
Penicillium	0.1	1.5	0.0	0.0	A1	Aspartate kinase [EC:2.7.2.4]
Saccharomyces	37.5	39.2	81.4	11.6	A1	Aspartate kinase [EC:2.7.2.4]
Sporosarcina	0.0	13.9	0.0	0.0	A1	Aspartate kinase [EC:2.7.2.4]
Virgibacillus	0.0	4.1	0.0	0.0	A1	Aspartate kinase [EC:2.7.2.4]
Kazachstania	1.0	2.9	0.9	0.8	A1	Aspartate-semialdehyde dehydrogenase [EC:1.2.1.11]

Lactobacillus	0.3	0.2	286.2	49.5	A1	Aspartate-semialdehyde dehydrogenase [EC:1.2.1.11]
Oceanobacillus	0.1	33.6	0.0	0.0	A1	Aspartate-semialdehyde dehydrogenase [EC:1.2.1.11]
Saccharomyces	55.8	66.7	6.8	2.8	A1	Aspartate-semialdehyde dehydrogenase [EC:1.2.1.11]
Sporosarcina	57.2	141.0	293.9	54.1	A1	Aspartate-semialdehyde dehydrogenase [EC:1.2.1.11]
Virgibacillus	0.1	3.2	0.0	0.0	A1	Aspartate-semialdehyde dehydrogenase [EC:1.2.1.11]
Ogataea	0.6	1.8	0.3	0.3	A2	Homoserine dehydrogenase [EC:1.1.1.3]
Sporosarcina	0.0	2.8	0.0	0.0	A2	Homoserine dehydrogenase [EC:1.1.1.3]
Saccharomyces	92.6	110.2	6.4	3.3	A2	Homoserine dehydrogenase [EC:1.1.1.3]
Sporosarcina	0.0	1.4	0.0	0.0	A3	Homoserine O-succinyltransferase [EC:2.3.1.46]
Naumovozyma	0.0	0.1	0.0	5.8	A4	Cystathionine gamma-synthase [EC:2.5.1.48]
Ogataea	0.0	0.4	0.0	0.0	A4	Cystathionine gamma-synthase [EC:2.5.1.48]
Brettanomyces	1.1	4.2	0.0	1.4	A4	Cystathionine gamma-synthase [EC:2.5.1.48]

Saccharomyces	63.1	60.2	63.2	11.7	A4	Cystathionine gamma-synthase [EC:2.5.1.48]
Kazachstania	0.0	0.0	0.0	8.3	A5	Homoserine O-acetyltransferase [EC:2.3.1.31]
Sporosarcina	0.0	0.7	0.0	0.0	A5	Homoserine O-acetyltransferase [EC:2.3.1.31]
Naumovozyma	1.1	0.0	0.2	4.8	A5	Homoserine O-acetyltransferase [EC:2.3.1.31]
Ogataea	0.9	2.0	0.4	0.0	A5	Homoserine O-acetyltransferase [EC:2.3.1.31]
Penicillium	0.1	5.2	0.3	0.0	A5	Homoserine O-acetyltransferase [EC:2.3.1.31]
Saccharomyces	38.7	45.1	35.5	4.8	A5	Homoserine O-acetyltransferase [EC:2.3.1.31]
Naumovozyma	0.0	0.1	0.0	5.8	A6	Cystathionine gamma-synthase [EC:2.5.1.48]
Ogataea	0.0	0.4	0.0	0.0	A6	Cystathionine gamma-synthase [EC:2.5.1.48]
Brettanomyces	1.1	4.2	0.0	1.4	A6	Cystathionine gamma-synthase [EC:2.5.1.48]
Saccharomyces	63.1	60.2	63.2	11.7	A6	Cystathionine gamma-synthase [EC:2.5.1.48]
Naumovozyma	0.0	0.1	0.0	5.8	A7	O-acetylhomoserine (thiol)-lyase [EC:2.5.1.49]

Kazachstania	0.0	0.2	0.0	19.3	A7	O-acetylhomoserine (thiol)-lyase [EC:2.5.1.49]
Ogataea	0.0	0.4	0.0	0.0	A7	O-acetylhomoserine (thiol)-lyase [EC:2.5.1.49]
Sporosarcina	0.0	8.0	0.0	0.0	A7	O-acetylhomoserine (thiol)-lyase [EC:2.5.1.49]
Virgibacillus	0.0	2.5	0.0	0.0	A7	O-acetylhomoserine (thiol)-lyase [EC:2.5.1.49]
Brettanomyces	1.1	4.2	0.0	1.4	A7	O-acetylhomoserine (thiol)-lyase [EC:2.5.1.49]
Saccharomyces	198.0	294.9	77.0	15.6	A7	O-acetylhomoserine (thiol)-lyase [EC:2.5.1.49]
Bacillus	0.0	0.2	0.0	0.0	C1	S-adenosylmethionine synthetase [EC:2.5.1.6]
Lactobacillus	1.0	0.5	413.2	54.7	C1	S-adenosylmethionine synthetase [EC:2.5.1.6]
Naumovozyma	0.7	0.8	0.0	2.1	C1	S-adenosylmethionine synthetase [EC:2.5.1.6]
Saccharomyces	260.2	370.7	35.3	8.5	C1	S-adenosylmethionine synthetase [EC:2.5.1.6]
Sporosarcina	0.0	16.7	0.0	0.0	C1	S-adenosylmethionine synthetase [EC:2.5.1.6]
Lactobacillus	0.8	0.5	13.7	813.6	C2	DNA (cytosine-5-)-methyltransferase [EC:2.1.1.37]

Bacillus	0.0	2.3	0.0	0.0	C2	DNA (cytosine-5-)-methyltransferase [EC:2.1.1.37]
Naumovozyma	0.5	0.2	0.0	0.0	C3	Adenosylhomocysteinase [EC:3.3.1.1]
Ogataea	0.2	0.8	1.2	0.0	C3	Adenosylhomocysteinase [EC:3.3.1.1]
Saccharomyces	237.3	294.5	18.0	28.2	C3	Adenosylhomocysteinase [EC:3.3.1.1]
Lactobacillus	2.9	0.3	561.6	1466.9	C4	S-adenosylhomocysteine/5'-methylthioadenosine nucleosidase [EC:3.2.2.9]
Oceanobacillus	0.0	2.8	0.2	0.0	C4	S-adenosylhomocysteine/5'-methylthioadenosine nucleosidase [EC:3.2.2.9]
Sporosarcina	0.0	2.9	0.0	0.0	C4	S-adenosylhomocysteine/5'-methylthioadenosine nucleosidase [EC:3.2.2.9]
Sporosarcina	0.1	2.7	0.0	0.0	C5	S-ribosylhomocysteine lyase [EC:4.4.1.21]
Virgibacillus	0.1	3.1	0.0	0.0	C5	S-ribosylhomocysteine lyase [EC:4.4.1.21]

Kazachstania	0.0	0.0	0.0	14.7	E1	Sulfite reductase (NADPH) flavoprotein alpha-component [EC:1.8.1.2]
Sporosarcina	0.0	1.0	0.0	0.0	E1	Sulfite reductase (NADPH) flavoprotein alpha-component [EC:1.8.1.2]
Penicillium	0.0	1.7	0.2	0.0	E1	Sulfite reductase (NADPH) flavoprotein alpha-component [EC:1.8.1.2]
Ogataea	0.6	1.9	0.2	0.8	E1	Sulfite reductase (NADPH) flavoprotein alpha-component [EC:1.8.1.2]
Naumovozyma	3.4	0.9	0.2	0.3	E1	Sulfite reductase (NADPH) flavoprotein alpha-component [EC:1.8.1.2]
Saccharomyces	80.6	102.0	17.0	10.7	E1	Sulfite reductase (NADPH) flavoprotein alpha-component [EC:1.8.1.2]
Kazachstania	0.0	0.0	0.0	4.1	E1	Sulfite reductase (NADPH) hemoprotein beta-component [EC:1.8.1.2]
Naumovozyma	0.0	0.0	0.0	7.6	E1	Sulfite reductase (NADPH) hemoprotein beta-component [EC:1.8.1.2]
Bacillus	0.0	0.6	0.0	0.0	E1	Sulfite reductase (NADPH) hemoprotein beta-component [EC:1.8.1.2]
Sporosarcina	0.0	1.2	0.0	0.0	E1	Sulfite reductase (NADPH) hemoprotein beta-component [EC:1.8.1.2]
Penicillium	0.2	5.0	1.0	0.2	E1	Sulfite reductase (NADPH) hemoprotein beta-component [EC:1.8.1.2]
Ogataea	1.8	5.5	0.3	0.6	E1	Sulfite reductase (NADPH) hemoprotein beta-component [EC:1.8.1.2]

Saccharomyces	53.7	66.7	6.4	7.8	E1	Sulfite reductase (NADPH) hemoprotein beta-component [EC:1.8.1.2]
Kuraishia	0.0	0.5	0.0	0.0	E2	Cysteine synthase A [EC:2.5.1.47]
Ogataea	7.0	13.4	0.7	1.0	E2	Cysteine synthase A [EC:2.5.1.47]
Saccharomyces	19.5	22.6	6.9	2.5	E2	Cysteine synthase A [EC:2.5.1.47]
Sporosarcina	0.2	24.5	0.0	0.0	E2	Cysteine synthase A [EC:2.5.1.47]
Virgibacillus	0.2	27.2	0.0	0.0	E2	Cysteine synthase A [EC:2.5.1.47]
Sporosarcina	0.0	10.0	0.0	0.0	L1	Methionine-gamma-lyase [EC:4.4.1.11]
Naumovozyma	0.0	0.2	0.0	4.9	L2	Aromatic amino acid aminotransferase I [EC:2.6.1.57 2.6.1.27 2.6.1.5]
Brettanomyces	0.6	1.1	0.6	0.4	L2	Aromatic amino acid aminotransferase I [EC:2.6.1.57 2.6.1.27 2.6.1.5]
Naumovozyma	1.3	0.7	0.0	0.0	L2	Aromatic amino acid aminotransferase I [EC:2.6.1.57 2.6.1.27 2.6.1.5]
Saccharomyces	91.4	109.9	7.1	1.7	L2	Aromatic amino acid aminotransferase I [EC:2.6.1.57 2.6.1.27 2.6.1.5]
Brettanomyces	0.0	0.0	0.2	0.9	L2	Aromatic amino acid aminotransferase II [EC:2.6.1.58 2.6.1.28]

Kazachstania	0.0	0.0	0.0	0.9	L2	Aromatic amino acid aminotransferase II [EC:2.6.1.58 2.6.1.28]
Saccharomyces	24.6	4.7	5.6	6.9	L2	Aromatic amino acid aminotransferase II [EC:2.6.1.58 2.6.1.28]
Brettanomyces	0.8	0.0	0.0	0.0	L3	Pyruvate decarboxylase [EC:4.1.1.1]
Kazachstania	0.0	5.4	0.0	0.0	L3	Pyruvate decarboxylase [EC:4.1.1.1]
Kuraishia	75.0	309.1	17.9	15.4	L3	Pyruvate decarboxylase [EC:4.1.1.1]
Naumovozyma	2.9	14.0	0.0	0.5	L3	Pyruvate decarboxylase [EC:4.1.1.1]
Ogataea	1.5	1.4	0.0	0.0	L3	Pyruvate decarboxylase [EC:4.1.1.1]
Penicillium	1.4	1.2	2.6	0.7	L3	Pyruvate decarboxylase [EC:4.1.1.1]
Saccharomyces	9784.1	9352.4	2680.9	1105.5	L3	Pyruvate decarboxylase [EC:4.1.1.1]
Saccharomyces	2.4	3.9	0.5	0.5	L3	Phenylpyruvate decarboxylase [EC:4.1.1.-]
Lactobacillus	1.2	1.1	1851.9	184.3	L4	Alcohol dehydrogenase, propanol-preferring [EC:1.1.1.1]
Saccharomyces	3020.2	3377.0	239.4	341.0	L4	Alcohol dehydrogenase, propanol-preferring [EC:1.1.1.1]

Lactobacillus	0.2	0.0	13.3	140.1	L4	Alcohol dehydrogenase [EC:1.1.1.1]
Bacillus	0.0	15.0	0.0	0.0	L4	Alcohol dehydrogenase [EC:1.1.1.1]
Saccharomyces	14.5	18.4	4.2	1.2	L4	Alcohol dehydrogenase [EC:1.1.1.1]
Saccharomyces	84.9	93.6	17.0	5.7	L4	Alcohol dehydrogenase (NADP+) [EC:1.1.1.2]
Acetobacter	0.7	0.0	3.3	0.8	L4	Alcohol dehydrogenase (cytochrome c) [EC:1.1.2.8]
Penicillium	0.0	1.5	0.0	0.0	M1	Cystathionine gamma-lyase [EC:4.4.1.1]
Naumovozyma	6.0	3.0	0.0	0.0	M1	Cystathionine gamma-lyase [EC:4.4.1.1]
Saccharomyces	103.3	173.8	8.2	8.8	M1	Cystathionine gamma-lyase [EC:4.4.1.1]
Bacillus	0.1	7.4	0.0	0.0	M2	Cystathione beta-lyase [EC:4.4.1.8]
Penicillium	0.0	0.5	0.0	0.0	M2	Cystathione beta-lyase [EC:4.4.1.8]
Saccharomyces	10.9	9.1	13.7	3.3	M2	Cystathione beta-lyase [EC:4.4.1.8]
Sporosarcina	0.0	18.4	0.1	0.0	M2	Cystathione beta-lyase [EC:4.4.1.8]

Penicillium	0.2	0.4	0.0	0.8	M2	Cystathionine beta-synthase [EC:4.2.1.22]
Naumovozyma	3.2	1.9	0.5	1.2	M2	Cystathionine beta-synthase [EC:4.2.1.22]
Saccharomyces	144.7	134.8	20.2	5.3	M2	Cystathionine beta-synthase [EC:4.2.1.22]
Kazachstania	0.0	0.4	0.1	17.1	M3	5-Methyltetrahydropteroyltriglutamate--homocysteine methyltransferase [EC:2.1.1.14]
Bacillus	0.0	1.0	0.0	0.0	M3	5-Methyltetrahydropteroyltriglutamate--homocysteine methyltransferase [EC:2.1.1.14]
Penicillium	0.2	2.6	0.5	1.2	M3	5-Methyltetrahydropteroyltriglutamate--homocysteine methyltransferase [EC:2.1.1.14]
Komagataella	2.6	1.3	0.0	1.6	M3	5-Methyltetrahydropteroyltriglutamate--homocysteine methyltransferase [EC:2.1.1.14]

Sporosarcina	0.1	5.3	0.0	0.0	M3	5-Methyltetrahydropteroyltriglutamate--homocysteine methyltransferase [EC:2.1.1.14]
Ogataea	3.5	6.9	1.8	0.5	M3	5-Methyltetrahydropteroyltriglutamate--homocysteine methyltransferase [EC:2.1.1.14]
Saccharomyces	214.4	333.3	19.8	8.9	M3	5-Methyltetrahydropteroyltriglutamate--homocysteine methyltransferase [EC:2.1.1.14]
Bacillus	0.0	1.4	0.0	0.0	M3	Homocysteine S-methyltransferase [EC:2.1.1.10]
Saccharomyces	7.4	8.9	2.9	0.8	M3	Homocysteine S-methyltransferase [EC:2.1.1.10]
Sporosarcina	0.0	7.0	0.0	0.1	M3	5-Methyltetrahydrofolate--homocysteine methyltransferase [EC:2.1.1.13]
Truepera	0.1	0.0	0.4	12.0	S1	Serine O-acetyltransferase [EC:2.3.1.30]
Virgibacillus	0.0	0.7	0.0	0.0	S1	Serine O-acetyltransferase [EC:2.3.1.30]

Kuraishia	0.0	0.5	0.0	0.0	S2	Cysteine synthase A [EC:2.5.1.47]
Ogataea	7.0	13.4	0.7	1.0	S2	Cysteine synthase A [EC:2.5.1.47]
Saccharomyces	19.5	22.6	6.9	2.5	S2	Cysteine synthase A [EC:2.5.1.47]
Sporosarcina	0.2	24.5	0.0	0.0	S2	Cysteine synthase A [EC:2.5.1.47]
Virgibacillus	0.2	27.2	0.0	0.0	S2	Cysteine synthase A [EC:2.5.1.47]