

SUPPLEMENTARY MATERIALS

Exploring the role of active functional microbiota in flavor generation by integrated metatranscriptomics and metabolomics during Niulanshan baijiu fermentation

Yuanyuan Pan ^{1,†}, Ying Wang ^{2,†}, Wenjun Hao ², Sen Zhou ², Chengbao Duan ^{1,3}, Qiushi Li ^{1,3}, Jinwang Wei ^{2,*} and Gang Liu ^{1,3,*}

¹ State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China; panyy@im.ac.cn (Y.P.); 18813111804@163.com (C.D.); liqiushi192@mails.ucas.ac.cn (Q.L.)

² Niulanshan Distillery, Beijing Shunxin Agriculture Company Limited, Beijing 101301, China; wangying.2006h@163.com (Y.W.); hwj1983hwj@126.com (W.H.); ak19861118ka@126.com (S.Z.)

³ University of Chinese Academy of Sciences, Beijing 100049, China

* Correspondence: weijinwang1971@163.com (J.W.); liug@im.ac.cn (G.L.)

† These authors contributed equally to this work.

Supplementary Tables

Table S1. Numbers of genera and species in fermented grains based on the metatranscriptomic sequencing.

	Genera					Species				
	D0	D6	D8	D16	D27	D0	D6	D8	D16	D27
> 0.1%	39	25	21	29	26	59	41	37	50	45
> 1%	14	9	10	8	9	19	12	13	11	12

Table S2. 395 polar compounds identified by LC-MS during NLS fermentation.

No	compounds	No	compounds
	Amino acids	201	Mannitol 1-phosphate
1	beta-Alanine	202	Arbutin
2	2-Aminoacrylic acid	203	N-Acetylmuramate
3	4-Aminobutyraldehyde	204	N-Acetyl-alpha-D-glucosamine 1-phosphate
4	Sarcosine	205	Ribose 1,5-bisphosphate
5	1-Aminocyclopropanecarboxylic acid	206	Sucrose
6	L-Serine	207	Cellobiose
7	gamma-Aminobutyric acid	208	D-Maltose
8	2-Phenylethanol	209	Trehalose
9	p-Cresol	210	Digalacturonate
10	Pyrrole-2-carboxylic acid	211	Trehalose 6-phosphate
11	Imidazole-4-acetaldehyde	212	Stachyose
12	Histamine	213	R-Methylmalonyl-CoA
13	Creatinine		Cofactors and vitamins
14	(S)-1-Pyrroline-5-carboxylate	214	Niacinamide
15	1-Pyrroline-2-carboxylic acid	215	Nicotinic acid
16	L-Proline	216	Gamma-delta-Dioxovaleric acid
17	5-Aminopentanoic acid	217	p-Aminobenzoic acid
18	Guanidoacetic acid	218	6-Hydroxynicotinic acid
19	Methylmalonic acid	219	5-(2-Hydroxyethyl)-4-methylthiazole
20	Indole	220	Methylitaconate
21	Betaine	221	Maleic acid homopolymer
22	L-Valine	222	2-Dehydropantoate
23	Phenylacetaldehyde	223	Pimelic acid
24	L-Allothreonine	224	Quinolinic acid
25	L-Threonine	225	Homogentisic acid
26	Phenylethylamine	226	Pyridoxamine
27	Picolinic acid	227	Pyridoxine
28	Ciliatine	228	Ascorbate
29	(S)-2,3,4,5-tetrahydropyridine-2-carboxylate	229	L-Gulonolactone
30	Creatine	230	4-Pyridoxic acid
31	Pyrrolidonecarboxylic acid	231	8-Amino-7-oxononanoate
32	Pyroglutamic acid	232	Diketogulonic acid
33	L-Pipecolic acid	233	Galactaric acid
34	L-Glutamic gamma-semialdehyde	234	Dethiobiotin
35	5-Amino-2-oxopentanoic acid	235	Pantothenic acid
36	Glutaric acid	236	Pyrimidodiazepine
37	Ureidopropionic acid	237	4-Amino-4-deoxychorismate
38	L-Leucine	238	Porphobilinogen

39	cis-4-Hydroxy-D-proline	239	Biotin
40	3-Methylindole	240	Lumichrome
41	N-Carbamoylputrescine	241	Nicotinate D-ribonucleoside
42	L-Isoleucine	242	4-(beta-D-Glucosyloxy)benzoate
43	L-Asparagine	243	D-4-Phosphopantothenate
44	Ornithine	244	2,5-Diamino-6-(5-phospho-D- ribosylamino)pyrimidin-4(3H)-one
45	L-Aspartic acid	245	Riboflavin
46	Phenylacetic acid	246	S-Adenosylmethionine
47	3-Hydroxyanthranilic acid	247	Folic acid
48	4-Hydroxyphenylacetaldehyde	248	13(1)-Oxo-magnesium-protoporphyrin IX 13-monomethyl ester
49	2-Phenylacetamide		Energy
50	2-Aminobenzoic acid	249	Dimethyl sulfone
51	Tyramine	250	Hydrogen phosphate
52	Gentisate aldehyde	251	3-Methylthiopropionic acid
53	Urocanic acid	252	N-methyl-L-glutamic Acid
54	Ectoine	253	Pyrophosphate
55	Spermidine	254	Fructose 1,6-bisphosphate
56	2-Keto-glutaramic acid		Lipid
57	4-Oxoglutaramate	255	Acetoacetic acid
58	2-Keto-6-aminocaproate	256	Butyric acid
59	4-Guanidinobutanoic acid	257	Glycerol
60	(2R,5S)-2,5-Diaminohexanoate	258	Malonate
61	L-Glutamine	259	2-Hydroxybutyric acid
62	L-Lysine	260	Maltol
63	(3S)-3,6-Diaminohexanoate	261	O-Phosphoethanolamine
64	trans-Cinnamate	262	Caprylic acid
65	L-Glutamic acid	263	Acetylcholine
66	O-Acetylserine	264	L-2-Hydroxyglutaric acid
67	1-Phenyl-1,2-propanedione	265	2-Oxo-4-methylthiobutanoic acid
68	L-Methionine	266	(+)-(S)-Carvone
69	3,4-Dihydroxyphenylglycol	267	Perillyl aldehyde
70	3,4-Dihydroxyphenylacetaldehyde	268	rac-Glycerol 3-phosphoate
71	Ortho-Hydroxyphenylacetic acid	269	Limonene-1,2-diol
72	Vanillin	270	Phosphorylcholine
73	L-Histidine	271	Dodecanoic acid
74	Gentisic acid	272	(+)-7-Isojasmonic acid
75	3-Indoleacetonitrile	273	Jasmonic acid
76	Isopropylmaleate	274	Capsidiol
77	5-Acetamidovalerate	275	2-trans,6-trans-Farnesal
78	Indoleacetaldehyde	276	Methyl jasmonate
79	Oxoadipic acid	277	Traumatic Acid
80	Tryptophanol	278	Myristic acid

81	D-4-Hydroxy-2-oxoglutarate	279	1-Hexadecanol
82	Phenylpyruvic acid	280	Xanthoxin
83	m-Coumaric acid	281	Abscisic alcohol
84	Formylanthranilic acid	282	Palmitic acid
85	L-Methionine S-oxide	283	Glycerophosphocholine
86	D-Phenyllactic acid	284	(S)-Abscisic acid
87	L-Phenylalanine	285	Xanthoxic acid
88	3,4-Dihydroxybenzeneacetic acid	286	Stearic acid
89	1-Methylhistidine	287	Stearidonic acid
90	Norepinephrine	288	Alpha-Linolenic acid
91	Tetrahydrodipicolinate	289	Linoleic acid
92	3-Dehydroshikimate	290	Oleic acid
93	N-Acetyl-L-glutamate 5-semialdehyde	291	Sphingosine
94	1H-Indole-3-acetamide	292	9(S)-HPOT
95	N-Acetylmethionine	293	12-OPDA
96	L-Arginine	294	13(S)-HpOTrE
97	N-Formyl-L-methionine	295	3-Dehydrosphinganine
98	Citrulline	296	Sphinganine
99	Hippuric acid	297	19(S)-HETE
100	4-Hydroxyphenylpyruvic acid	298	Arachidonic acid
101	L-Homophenylalanine	299	2,3-Dinor-8-iso prostaglandin F2alpha
102	3,4-Dihydroxyhydrocinnamic acid	300	Phytosphingosine
103	(R)-3-(4-Hydroxyphenyl)lactate	301	5-KETE
104	Beta-Tyrosine	302	12-Keto-tetrahydro-leukotriene B4
105	L-Tyrosine	303	12-KETE
106	Homovanillic acid	304	Prostaglandin E2
107	Se-Methylselenocysteine	305	Prostaglandin-c2
108	Epinephrine	306	8,9-DiHETrE
109	3,4-Dihydroxymandelic acid	307	6-Ketoprostaglandin E1
110	Normetanephrine	308	Prostaglandin G2
111	Phosphohydroxypyruvic acid	309	Lithocholic acid
112	Phosphoserine	310	Cholesterol
113	Vanylglycol	311	Deoxycholic acid
114	Kynurenic acid	312	3-Keto-4-methylzymosterol
115	2-Keto-6-acetamidocaproate	313	Lanosterin
116	N6-Acetyl-L-lysine	314	beta-Carotene
117	N-Acetylglutamic acid		Nucleotide
118	Diaminopimelic acid	315	Cytosine
119	5-Hydroxyindoleacetic acid	316	Uracil
120	Selenomethionine	317	Dihydrouracil
121	L-Dopa	318	Thymine
122	gamma-Glutamyl-beta-aminopropionitrile	319	(R)-5,6-Dihydrothymine
123	4-Fumarylacetoacetate	320	Oxalureate

124	Xanthurenic acid	321	Adenine
125	L-Tryptophan	322	Hypoxanthine
126	4-(2-Aminophenyl)-2,4-dioxobutanoic acid	323	5-Methylbarbiturate
127	N-Acetyl-L-phenylalanine	324	Guanine
128	L-Kynurenine	325	Xanthine
129	N-Acetyldemethylphosphinothricin	326	4,5-Dihydroorotic acid
130	2-Hydroxy-6-ketonoatrienedioate	327	Uric acid
131	beta-Alanyl-L-lysine	328	Deoxyuridine
132	gamma-Glutamylalanine	329	Cytidine
133	D-Lysopine	330	Thymidine
134	L-Cystine	331	Pseudouridine
135	5-(Methylthio)-2,3-dioxopentyl phosphate	332	Uridine
136	beta-Alanyl-L-arginine	333	2-Deoxyadenosine
137	D-Octopine	334	Inosine
138	Linatine	335	Adenosine
139	5-Methylthioribulose 1-phosphate	336	Deoxyguanosine
140	Subaphylline	337	Guanosine
141	(2R,3R)-3-Methylglutamyl-5-semialdehyde-N6-lysine	338	Xanthosine
142	N2-Succinyl-L-arginine	339	dGMP
143	4-Hydroxycinnamoylagmatine	340	3-AMP
144	Saccharopine	341	AMP
145	N-Succinyl-2-amino-6-ketopimelate		Peptide
146	Glutathione	342	Carnosine
147	S-Adenosylhomocysteine		Unknown
	Carbohydrate	343	2-Methyl-3-oxopropanoic acid
148	3-Butyrate	344	5-Aminopentanal
149	Pyruvic acid	345	D-1-Piperidine-2-carboxylic acid
150	Diacetyl	346	3-Amino-4-hydroxybenzoate
151	L-Lactic acid	347	L-4-Hydroxyphenylglycine
152	Maleic acid	348	Choline sulfate
153	Succinic acid semialdehyde	349	D-Arabinose 5-phosphate
154	2-Ketobutyric acid	350	3-Deoxy-D-manno-octulosonate
155	(R)-3-Hydroxybutyric acid		Xenobiotics
156	Glyceric acid	351	Cyclopropanecarboxylate
157	Fumaric acid	352	m-Cresol
158	Succinic acid	353	Phenol
159	Deoxyribose	354	Aniline
160	Itaconic acid	355	Sulfate
161	Mesaconate	356	Benzaldehyde
162	Citraconic acid	357	Catechol
163	L-Malic acid	358	Hydroquinone
164	Acetylphosphate	359	2-Aminophenol

165	L-Fucose	360	4-Aminophenol
166	Oxoglutaric acid	361	Epsilon-caprolactam
167	D-Xylonate	362	epsilon-Caprolactone
168	D-Ribose	363	3-Hydroxybenzoic acid
169	D-Xylose	364	4-Methylbenzaldehyde
170	D-Lyxose	365	Benzoate
171	Tartaric acid	366	3-Hydroxybenzyl alcohol glucoside
172	L-Arabinose	367	4-Methylcatechol
173	L-Arabitol	368	2-Hydroxybenzaldehyde
174	D-Xylitol	369	3-Hydroxybenzaldehyde
175	Ribitol	370	Erythritol
176	2-Hydroxy-3-oxoadipate	371	4-Aminocatechol
177	Alpha-D-Glucose	372	Aminohydroquinone
178	L-Rhamnono-1,4-lactone	373	1,2,3-Trihydroxybenzene
179	D-Fructose	374	1,3,5-Trihydroxybenzene
180	L-Rhamnofuranose	375	Adipate semialdehyde
181	Gluconolactone	376	Aminocaproic acid
182	Galactonic acid	377	m-toluate
183	Glucosamine	378	(R)-mandelic Acid
184	Allose	380	4-Hydroxybenzoic acid
185	D-Mannose	381	Salicylic acid
186	L-Sorbose	382	2-Methylbenzoic acid
187	myo-Inositol	383	Cuminaldehyde
188	Galactitol	384	3-Hydroxyphenylacetic acid
189	Sorbitol	385	2-Pyrocatechuic acid
190	Mannitol	386	Protocatechuic acid
191	Citric acid	387	3-Oxoadipic acid
192	Isocitric acid	388	cis-1,2-Dihydronaphthalene-1,2-diol
193	D-Glucuronic Acid	389	4-Hydroxycinnamic acid
194	Gluconic acid	390	Gallic acid
195	N-Acetyl-D-glucosamine	391	(S)-4-Hydroxymandelate
196	Inositol phosphate	392	1-Hydroxy-2-naphthoate
197	D-Mannose 1-phosphate	393	2,4-Dinitrotoluene
198	Glucose 6-phosphate	394	Syringic acid
199	Fructose 6-phosphate	395	5-Carboxyvanillic acid
200	Beta-D-Glucose 6-phosphate		

Table S3. 83 compounds identified by derivative GC-MS during NLS fermentation.

No	compounds	No	compounds
	amine	43	Quinic acid
1	Ethanolamine	44	Ribonic acid
2	Histamine	45	Shikimic acid
	Amino acids	46	Succinic acid
3	2-Aminobutyric acid	47	Threonic acid
4	4-Aminobutyric acid	48	Trans-Ferulic acid
5	Alanine	49	2,4-Dihydroxybutanoic acid
6	Asparagine	50	4-Hydroxy-3-Methoxybenzoic acid
7	Aspartic acid	51	4-Hydroxybutanoic acid
8	Beta-Alanine	52	DL-Beta-Hydroxybutyric acid
9	Glutamic acid		Others
10	Glutamine	53	Adenosine
11	Glycine	54	Threonic acid-1,4-Lactone
12	Isoleucine	55	Uracil
13	Leucine	56	Urea
14	Lysine	57	Uridine
15	Methionine	58	Hypoxanthine
16	Phenylalanine	59	Thymine
17	Proline		Phosphoric acid
18	Pyroglutamic acid	60	Glucose-6-Phosphate
19	Serine	61	Glycerol-3-Phosphate
20	Threonine	62	Monomethylphosphate
21	Tyrosine	63	Myo-Inositol-1-Phosphate
22	Valine	64	Phosphoric acid
23	Ornithine	65	Fructose-6-Phosphate
	Fatty acid	66	Sorbitol-6-Phosphate
24	Heptanoic acid		Polyol
25	Hexadecanoic acid	67	Erythritol
26	Octadecanoic acid	68	Glycerol
27	9-(Z)-Octadecenoic acid	69	Mannitol
28	9,12-(Z,Z)-Octadecadienoic acid	70	Myo-Inositol
	Organic acid	71	Sorbitol
29	2-Hydroxyglutaric acid	72	1-Monohexadecanoylglycerol
30	2-Keto-L-Gluconic acid	73	Ribitol
31	Citric acid	74	Threitol
32	Erythronic acid		Sugar
33	Fumaric acid	75	Fructose
34	Galactaric acid	76	Galactose
35	Glyceric acid	77	Glucose
36	Glycolic acid	78	Maltose
37	Lactic acid	79	Ribose

38	Malic acid	80	Sucrose
39	Nicotinic acid	81	Trehalose
40	Oxalic acid	82	Fucose
41	Parabanic acid	83	Gentiobiose
42	Pyruvic acid		

Table S4. 181 VOCs identified by HS-SPME-GC-MS during NLS fermentation.

No	compounds	No	compounds
	Esters	93	1-Octen-3-ol
1	Nonoic acid, 2,4,6-trimethyl-, methyl ester, (2R,4S,6R)-(-)-	94	1,1,3,3,5,5,7,7-Octamethyl-7-(2-methylpropoxy)tetrakisloxan-1-ol
2	Ethyl formate	95	2-Furanmethanol
3	Arsenous acid tris(trimethylsilyl) ester	96	3-(Methylthio)-1-propanol
4	Acetic ester	97	Benzyl alcohol
5	2-Methylpropyl acetate	98	Phenylethyl alcohol
6	Ethyl butyrate	99	4aH-Cycloprop[e]azulen-4a-ol, decahydro-1,1,4,7-tetramethyl-
7	Ethyl 2-methylbutyrate	100	(-)-Epiglobulol
8	Ethyl isovalerate	101	noethylene glycol
9	Isoamyl acetate	102	Prenol
10	Ethyl hexanoate		Ketones
11	Sulfurous acid, butyl nonyl ester	103	2,3-Butanedione
12	Pentyl formate	104	2,3-Pentanedione
13	Ethyl 5-methylhexanoate	105	4-Heptanone
14	Isoamyl isovalerate	106	2-Heptanone
15	Ethyl heptanoate	107	3-Octanone
16	Docosanoic acid, ethyl ester	108	2-Nonone
17	Ethyl lactate		Acids
18	ethyl (2S)-lactate	109	Acetate
19	4-Heptenoic acid, ethyl ester, (E)-	110	Propanoic acid
20	Methyl caprylate	111	Orsellite
21	Ethyl octanoate	112	3-Methylbutanoic acid
22	3-Methylbutyl hexanoate	113	Hexanoic acid
23	Ethyl 4Z-octenoate	114	Octanoic acid
24	Ethyl nonoate	115	Octanoic acid, silver(1+) salt
25	Ethyl dl-2-hydroxycaproate	116	Lactic acid
26	n-Caprylic acid isobutyl ester	117	Linoleate
27	Octyl formate		Alkene
28	Ethyl 3-(methylthio)propanoate	118	cis-2-Octene
29	2-Methylpropanoate	119	trans-3-Octene
30	Methoxyacetic acid, 3-methylbutyl ester	120	Styrene
31	Phosphonessigsaeure-tri-(trimethylsilylester)	121	prehnitene
32	gamma-Butyrolactone	122	Cyperene
33	4-Decenoic acid, methyl ester	123	1-Epi-bicyclosquiphellandrene
34	Nonoic acid, 2,6-dimethyl-, methyl ester	124	Aristolene
35	Ethyl decanoate	125	Berkheyaradulene
36	Ethyl 4-decenoate	126	(-)-alpha-Gurjunene
37	Diethyl succite	127	beta-Caryophyllene

38	ethyl 9-decenoate	128	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydrophthalene
39	Succinic acid, ethyl 2-methylphenyl ester	129	Cedrene-V6
40	Ethyl phenylacetate	130	valencene
41		131	(1R,4aS,8aS)-7-methyl-4-methylidene-1-propan-2-yl-2,3,4a,5,6,8a-hexahydro-1H-phthalene
	Succinic acid, ethyl 3-heptyl ester	132	alpha-muurolene
42	4-Ethylbenzoic acid, dodec-9-ynyl ester	133	.gamma.-Elemene
43	Phenethyl acetate	134	delta-Cadinene
44	Methyl 3-phenylpropanoate	135	Caryophyllene epoxide
45	Lauric Acid ethyl ester	136	Longifolene
46	Ethyl 3-phenylpropanoate	137	
47	1-O-ethyl 4-O-(3-methylbutyl) butanedioate		gamma-gurjunene
48	methyl 2-methyltetradecanoate		Alkanes
49	Tetradecanoic acid, ethyl ester	138	2,4-dimethylheptane
50	Ethyl 13-methyl-tetradecanoate	139	Octamethyltrisiloxane
51	Octadecanoic acid, 9-methyl-, ethyl ester	140	Methylene chloride
52	Ethyl cinmate	141	2,2,4,6,6-pentamethylheptane
53	Pentadecanoic acid, ethyl ester	142	N-Decane
54		143	trisiloxane, 1,1,1,5,5,5-hexamethyl-3-[(trimethylsilyl)oxy]-
	(Z)-Ethyl pentadec-9-enoate	144	Decane, 3,7-dimethyl-
55	Palmitic Acid ethyl ester	145	Undecane
56	Methyl hexadec-9-enoate	146	Decamethyl-Tetrasiloxane
57	Ethyl 9-hexadecenoate	147	3,8-Dimethyldecane
58	Oxalic acid, pentyl 2-phenylethyl ester	148	3-methyl-Undecane
59	methyl 17-methyloctadecanoate	149	Decamethylcyclopentasiloxane
60	Dihydroactinidiolide	150	Dodecane, 1-iodo-
61	Heptadecanoic acid, ethyl ester	151	Dodecane
62	(Z)-Ethyl heptadec-9-enoate	152	heptacosane
63	Ethyl hydrogen succite	153	Eicosane, 1-iodo-
64	Stearic Acid ethyl ester	154	Tridecane
65	Oleic Acid ethyl ester	155	Heptadecane
66	cis-10-Pentadecenoic acid, propyl ester	156	tetradecane
67	octadeca-9,12-dienoic acid ethyl ester	157	
68	Phthalic acid, isobutyl 4-methylpent-2-yl ester		dodecamethylpentasiloxane
69		158	3-(1,3-Dihydroxyisopropyl)-1,5,8,11-tetraoxacyclotridecane
	Linolenic Acid ethyl ester	159	1,4,7,10,13,16-Hexaoxanodecane, 18-(2-propenyl)-
70			Others
	Ethyl 9.cis.,11.trans.-octadecadienoate	160	Ether
71	Linoleic Acid ethyl ester	161	Benzene
72	7,10-Hexadecadienoic acid, methyl ester		
73	n-Propyl 9,12-octadecadienoate		

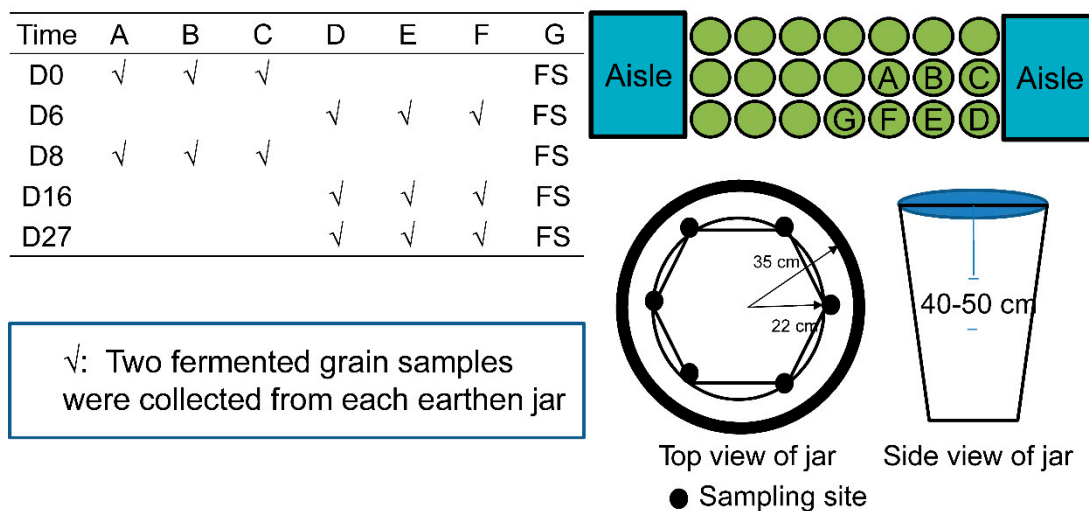


Figure S1. Fermentation time points and methods of sampling for untargeted metabolomics. D0 represents the samples isolated from the fermentation start time. Samples from A, B and C earthen jars were named as D0A, D0B and D0C, respectively. D6 represents the samples isolated from the 6 day of fermentation. Samples from D, E and F earthen jars were named as D6A, D6B and D6C, respectively. The following samples were named in the same way. Both fermented grain samples were collected from each earthen jar at the same time. Samples located between 40 and 50 cm in the earthen jars were collected and mixed well immediately for use, and then supply the same amount of feedstock from earthen jar G to the sampling spaces. FS, Feedstock supply earthen jar; cm, centimeter.

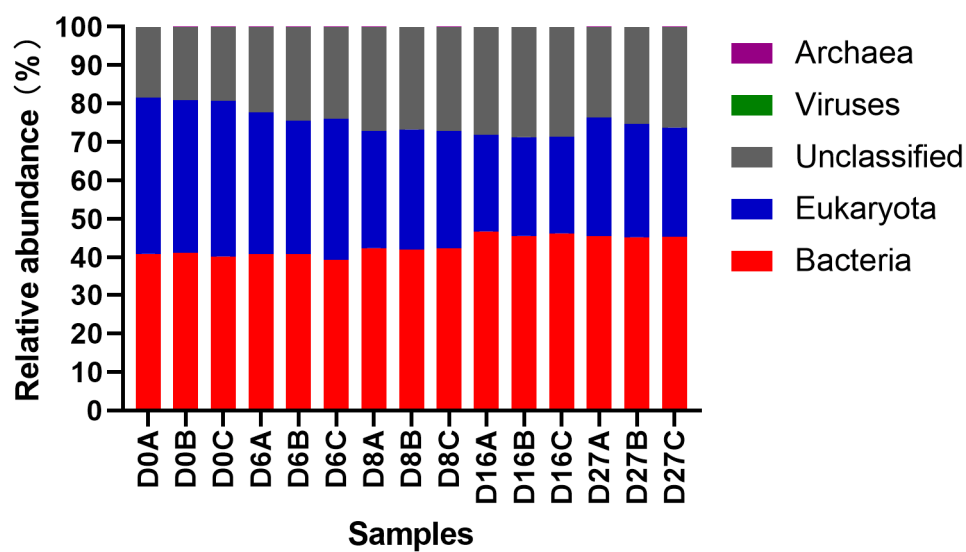


Figure S2. Distribution of microorganisms at the superkingdom level.

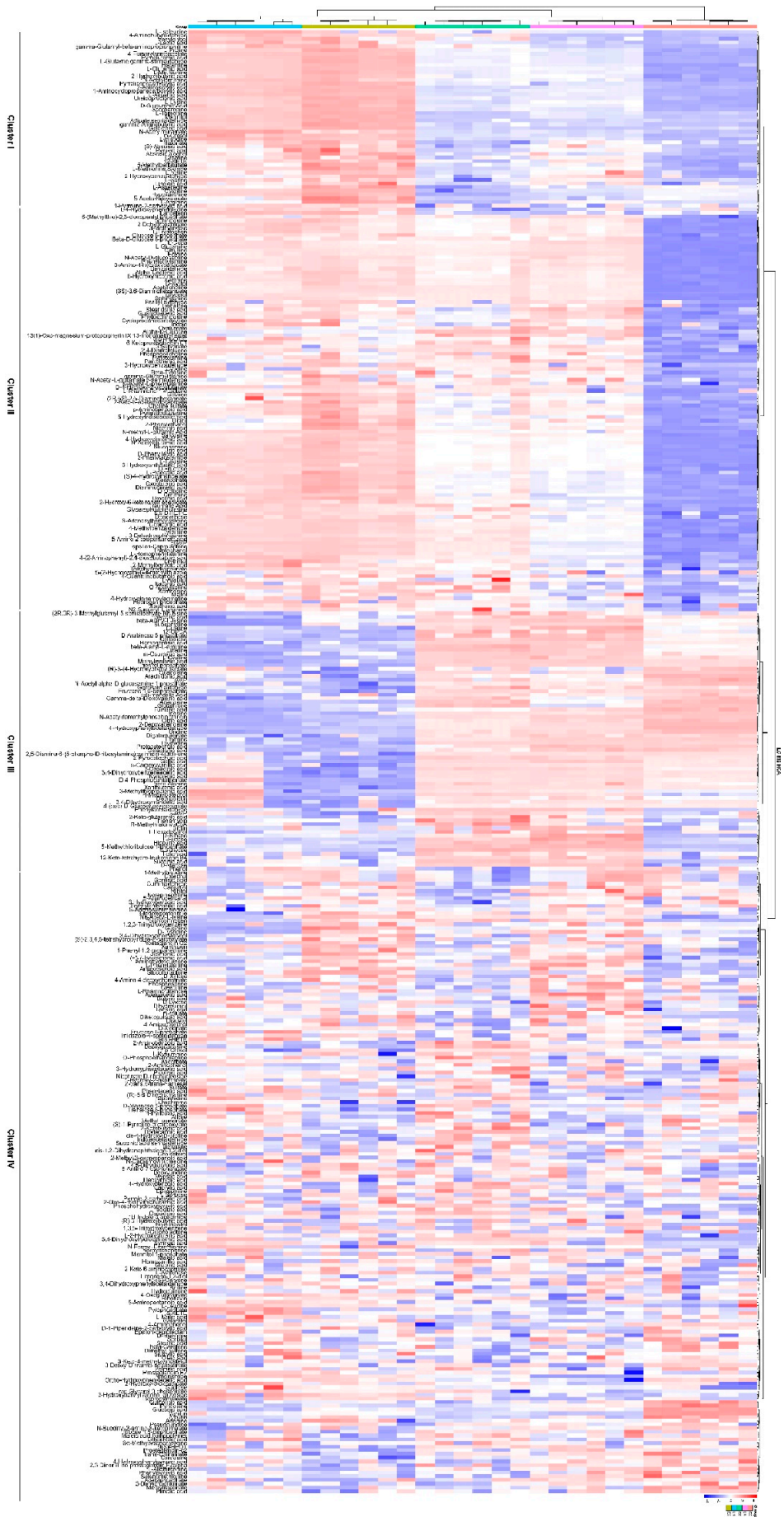


Figure S3. Hierarchical cluster analysis of compounds during NLS fermentation based on the LC-MS detection. Rows represent the compounds and columns from different batches. Color key indicates the relative abundance of different compounds, green indicates the lowest and red indicates the highest. D0, D6, D8, D16 and D27 represent the fermentation sample of 0, 6, 8, 16 and 27 days, respectively.

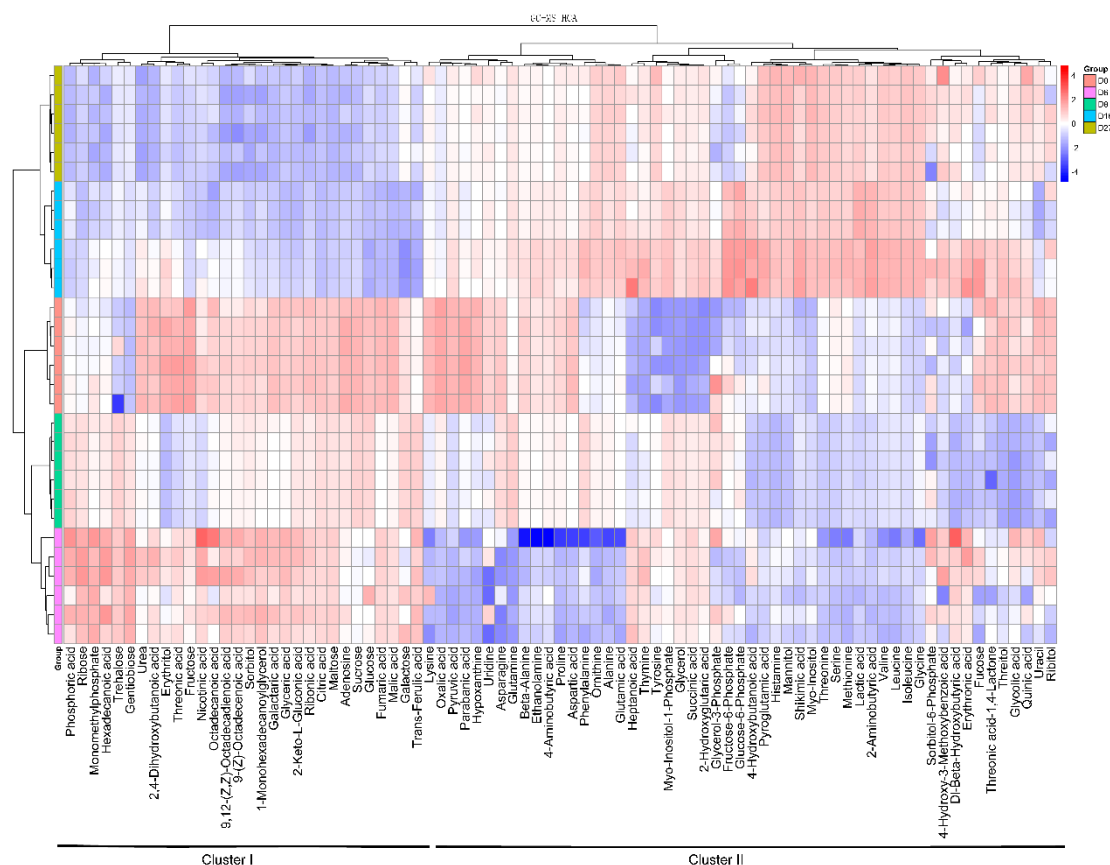


Figure S4. Hierarchical cluster analysis of compounds during NLS fermentation based on the GC-MS detection. Rows represent the compounds and columns represent different batches. Color key indicates the relative abundance of differential compounds, green indicates the lowest and red indicates the highest. D0, D6, D8, D16 and D27 represent the fermentation sample collected on 0, 6, 8, 16 and 27 day, respectively.

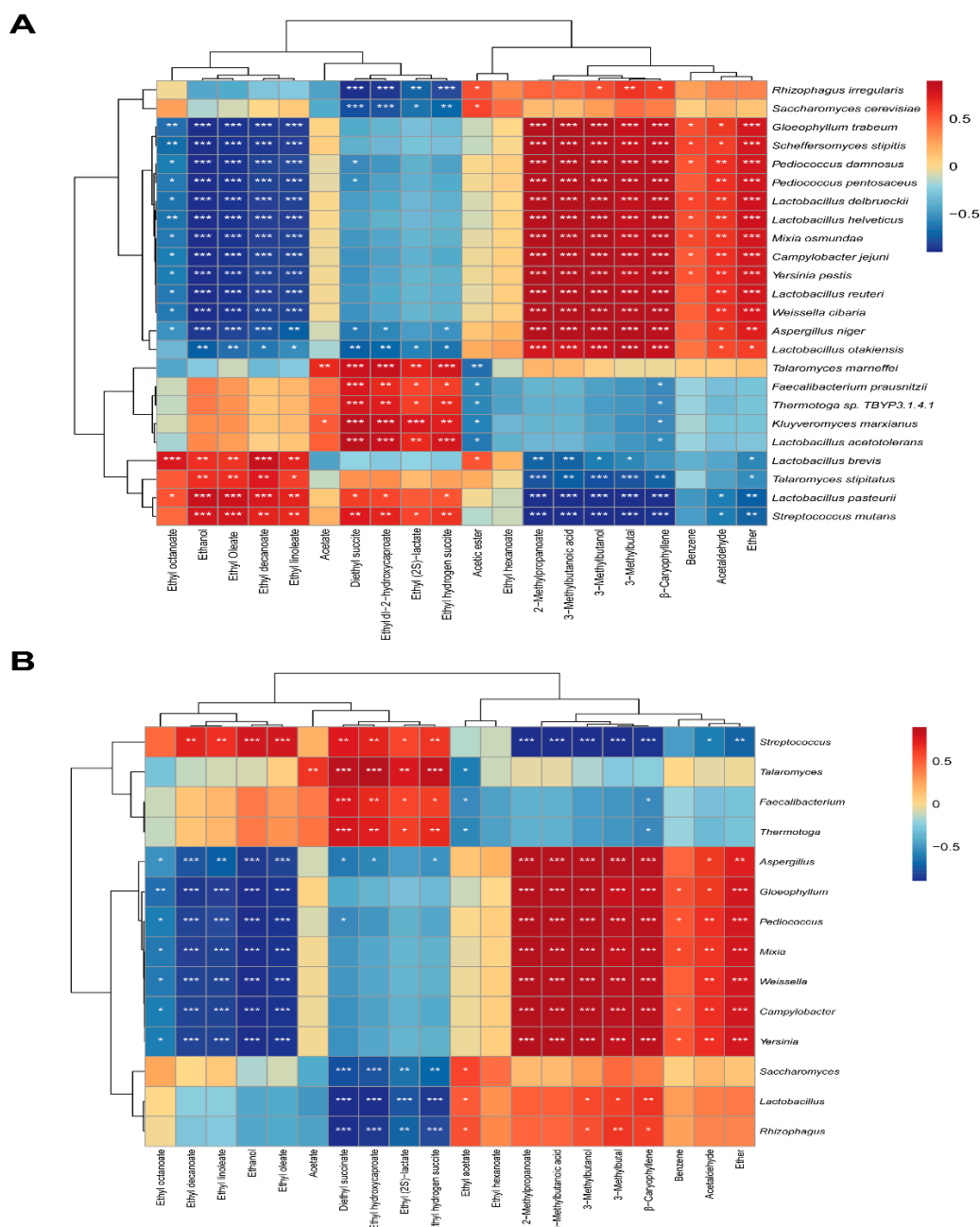


Figure S6. Correlation analysis between microbiota and volatile compounds by O2PLSDA model. (A) Statistical pearson's correlations between important genera (VIP > 1) and top 20 volatile compounds. (B) Statistical pearson's correlations between important species (VIP > 1) and top 20 volatile compounds. The correlated relationship with p-value < 0.05, 0.01, 0.001 was displayed by one, two and three asterisks, respectively.