Supplemental material

Supplemental Tables

Table S1. Overview of the metaproteomics results across samples

Samples	Total Spectrum	Peptide-Spectrum Matches	Peptide sequences	Proteins	Protein groups
Jiuqu & FG00 & FG03	499, 255	97, 198	15, 228	5, 311	2, 373

Table S2. Identified non-redundant protein groups across samples as an Excel file: Table_S2.xls in data sheet 1.

Table S3. Distribution of the identified non-redundant protein groups into Clusters of Orthologous Groups by the metaproteomics analysis.

Type	Functional categories	Number of proteins
Information storage and processing	[A] RNA processing and modification	1

Information storage and processing	[B] Chromatin structure and dynamics	4
Information storage and processing	[J] Translation, ribosomal structure and biogenesis	235
Information storage and processing	[K] Transcription	22
Information storage and processing	[L] Replication, recombination and repair	16
Cellular processes and signaling	[D] Cell cycle control, cell division, chromosome partitioning	9
Cellular processes and signaling	[M] Cell wall/membrane/envelope biogenesis	28
Cellular processes and signaling	[O] Posttranslational modification, protein turnover, chaperones	234
Cellular processes and signaling	[T] Signal transduction mechanisms	25
Cellular processes and signaling	[U] Intracellular trafficking, secretion, and vesicular transport	8
Cellular processes and signaling	[Z] Cytoskeleton	36
Metabolism	[C] Energy production and conversion	329
Metabolism	[E] Amino acid transport and metabolism	149
Metabolism	[F] Nucleotide transport and metabolism	42

Metabolism	[G] Carbohydrate transport and metabolism	338
Metabolism	[H] Coenzyme transport and metabolism	69
Metabolism	[I] Lipid transport and metabolism	69
Metabolism	[P] Inorganic ion transport and metabolism	54
Metabolism	[Q] Secondary metabolites biosynthesis, transport and catabolism	23
Poorly characterized	[R] General function prediction only	121
Poorly characterized	[S] Function unknown	7

Table S4. Distribution of carbohydrate hydrolases across samples as an Excel file: Table_S4.xls in data sheet 2.

Table S5. Bacterial and fungal microbiota diversity index based on 16S rRNA and ITS amplicons sequencing across samples

ID	Sample	Clean_reads	OTUs	Goods' coverage	Chao1 richness	Shannon diversity index
Bacteria	*Jiuqu*_01	31802	229	0.9944	319.91	3.50

*Jiuqu*_02	32917	241	0.9940	302.23	3.35
*Jiuqu*_03	36227	239	0.9941	313.87	3.84
FG00_01	28263	248	0.9938	306.50	4.09
FG00_02	21263	226	0.9944	302.81	4.05
FG00_03	39094	233	0.9946	289.98	4.10
FG03_01	37735	155	0.9942	243.56	2.90
FG03_02	14624	94	0.9963	212.75	2.93
FG03_03	25433	141	0.9962	183.01	3.15
FG05_01	16004	134	0.9963	176.55	2.03
FG05_02	19666	135	0.9959	190.73	2.09
FG05_03	18722	131	0.9955	254.44	2.19
FG10_01	18556	108	0.9961	171.77	1.69
FG10_02	19834	110	0.9966	152.83	1.80

	FG10_03	21504	134	0.9959	189.34	1.57
	FG15_01	23107	230	0.9931	323.07	2.92
	FG15_02	20310	116	0.9960	259.29	2.23
	FG15_03	25347	219	0.9926	355.72	2.40
	*Jiuqu*_01	44094	50	0.9992	66.07	2.12
	*Jiuqu*_02	41630	65	0.9989	96.47	2.25
	*Jiuqu*_03	39092	55	0.9990	78.29	2.29
	FG00_01	26798	88	0.9988	96.09	2.80
Fungi	FG00_02	29079	74	0.9991	79.92	2.99
	FG00_03	32663	74	0.9987	102.73	2.46
	FG03_01	29963	42	0.9993	52.29	1.59
	FG03_02	25953	51	0.9988	81.18	1.46
	FG03_03	33619	45	0.9991	78.93	1.48

FG05_01	28584	34	0.9995	40.24	0.64
FG05_02	20186	36	0.9995	38.54	0.76
FG05_03	42702	27	0.9995	33.11	0.60
FG10_01	28168	43	0.9993	63.26	2.16
FG10_02	22844	73	0.9992	78.72	2.20
FG10_03	23843	47	0.9992	58.41	2.39
FG15_01	18753	36	0.9992	57.45	0.73
FG15_02	19821	39	0.9992	53.34	0.80
FG15_03	27512	36	0.9992	47.10	0.58

Table S6. The relative abundance of bacterial and fungal genera across samples

ID	Genus	Relative abundance (%, mean ± sd)					
		Jiuqu	FG00	FG03	FG05	FG10	FG15

	Acetobacter	0.95±0.45	1.28±0.36	0.24±0.11	0.11±0.08	0.05±0.02	0.07±0.05
	Acinetobacter	0.97±0.58	1.12±0.27	1.69±2.51	0.28±0.06	0.14±0.10	0.26±0.10
	Bacillus	0.47±0.2	0.64±0.10	0.12±0.14	0.01±0.00	0.02±0.01	0.11±0.11
	Klebsiella	1.20±0.07	5.52±2.06	7.88±2.55	1.05±1.15	0.91±0.34	0.40±0.14
Bacteria	*Lactobacillus*	27.33±3.00	21.58±1.33	61.24±18.10	86.31±1.2	93.94±1.42	90.63±3.46
	Pantoea	0.23±0.03	0.86±0.16	2.62±3.49	0.17±0.21	0.12±0.07	0.05±0.03
	Pediococcus	20.83±2.83	11.46±2.66	8.30±7.84	1.46±0.26	1.13±0.73	0.74±0.16
	Weissella	31.83±8.76	24.34±6.78	10.18±11.14	3.80±1.39	0.51±0.11	2.10±0.62
	Others	16.19±5.08	33.2±5.55	7.74±3.90	6.81±1.25	3.18±0.63	5.64±2.47
	Aspergillus	8.31±2.71	6.38±1.18	0.44±0.04	0.15±0.15	9.18±4.02	0.14±0.03
	Candida	5.89±1.37	5.49±0.68	1.41±0.19	0.15±0.08	0.62±0.33	0.18±0.04
Fungi	*Millerozyma*	0.30±0.33	0.16±0.10	0.01±0.00	0.02±0.01	0.37±0.36	0.07±0.02
	Pichia	2.73±0.87	2.34±0.61	0.55±0.09	2.68±1.19	3.48±2.80	3.04±0.99

	Rhizopus	17.38±1.58	8.87±3.87	4.04±1.63	0.72±0.61	11.01±4.07	0.62±0.20
	Saccharomyces	2.61±0.67	31.72±7.54	58.73±4.17	89.98±0.89	52.92±6.40	89.17±2.74
	Saccharomycopsis	58.13±3.30	35.27±4.11	33.09±5.29	5.74±2.22	18.32±7.65	6.23±2.87
	Others	4.66±0.68	9.77±5.64	1.73±0.11	0.56±0.08	4.10±0.57	0.55±0.16

Table S7. The concentration of metabolites in the baijiu fermentation

Category	Compounds	Content (mg/g DW)				
		FG00	FG03	FG05	FG10	FG15
alcohols	ethanol	51.81±4.84	211.1±8.25	186.5±6.82	157.1±6.19	144.3±5.88

Table S8. The ethanol formation catalyzed by various mixtures of key carbohydrate hydrolases

Run	Content (mg/g starch)		Ethanol (mg/L)
	GH13	GH15	

1	0.10	0.38	415.4±7.83
2	0.06	0.44	478.5±7.98
3	0.12	0.22	405.5±8.10
4	0.06	0.22	388.7±6.99
5	0.06	0.22	389.4±8.55
6	0.02	0.08	218.0±8.71
7	0.06	0.22	387.4±8.82
8	0.10	0.08	321.8±8.10
9	0.06	0.22	374.3±9.81
10	0.00	0.22	373.4±8.36
11	0.06	0.02	343.0±7.37
12	0.02	0.38	397.0±8.82
13	0.06	0.22	380.5±7.79

Table S9. The ethanol formation catalyzed by a mixture of GH13 and GH15 (in a ratio of 1:6, w/w)

Time (h)	Content (mg/L)
24	158.7±4.99
48	305.3±7.90
72	435.8±6.80

Supplemental Figure Legends

Fig. S1 Profiles of Chinese Chixiang-style *Jiuqu* and baijiu production. In *Jiuqu* preparations, raw materials (rice, soybean) are stewed for 1 h under 0.1-0.2 MPa. Cooked materials are cooled to 30 °C, and mixed with caky balls (inoculated with strains). Mixed materials are shaped to bricks, and transferred into a making-house. The bricks are fermented for 7 days under 30-40 °C. Matured *Jiuqu* bricks are stored for two weeks, and smashed to powder for baijiu fermentation. In baijiu fermentation, raw materials (rice) are stewed for 30 min under 0.1-0.2 MPa, and cooled to 30 °C. Cooked rice are mixed with *Jiuqu* powder and other materials (water). Mixed materials are transferred into a 50 m^3 silo, and fermented for 15 days under 30-35 °C. Then the mash is distilled to make liquors.

Fig. S2 Sample collections in Chinese Chixiang-style baijiu production. In *Jiuqu* samples collection, we tracked 3 *Jiuqu* making-houses produced in 7, 8 and 9 July, 2017. Each house was an independent batch. We selected three biological samples of *Jiuqu* powder, after storage and ready for baijiu fermentation. In baijiu fermentation, we tracked three silos that used corresponding *Jiuqu* samples. Fermented samples were collected from three points (1, 2 and 3) at the upper, middle and bottom layer (“a”, “b” and “c”) in every fermentation silo. We selected fermented samples at 0, 3, 5, 10 and 15 days.

Fig. S3 Phylogenetic tree of identified GH13, GH15 in baijiu fermentation and the commercial ones for Central Composite analysis.

Fig. S4 Dynamic of isolated strains, enzymatic activity, and physicochemical indexes by the culture-dependent method. Growth curve of *Rhizopus microsporus* (a), *Lactobacillus fermentum* and *Lactobacillus helveticus* (c). Dynamic of saccharifying activity (b) and pH (d).

Fig. S5 Dominant microbial compositions at species across *Jiuqu* and fermented samples.

Fig. S1

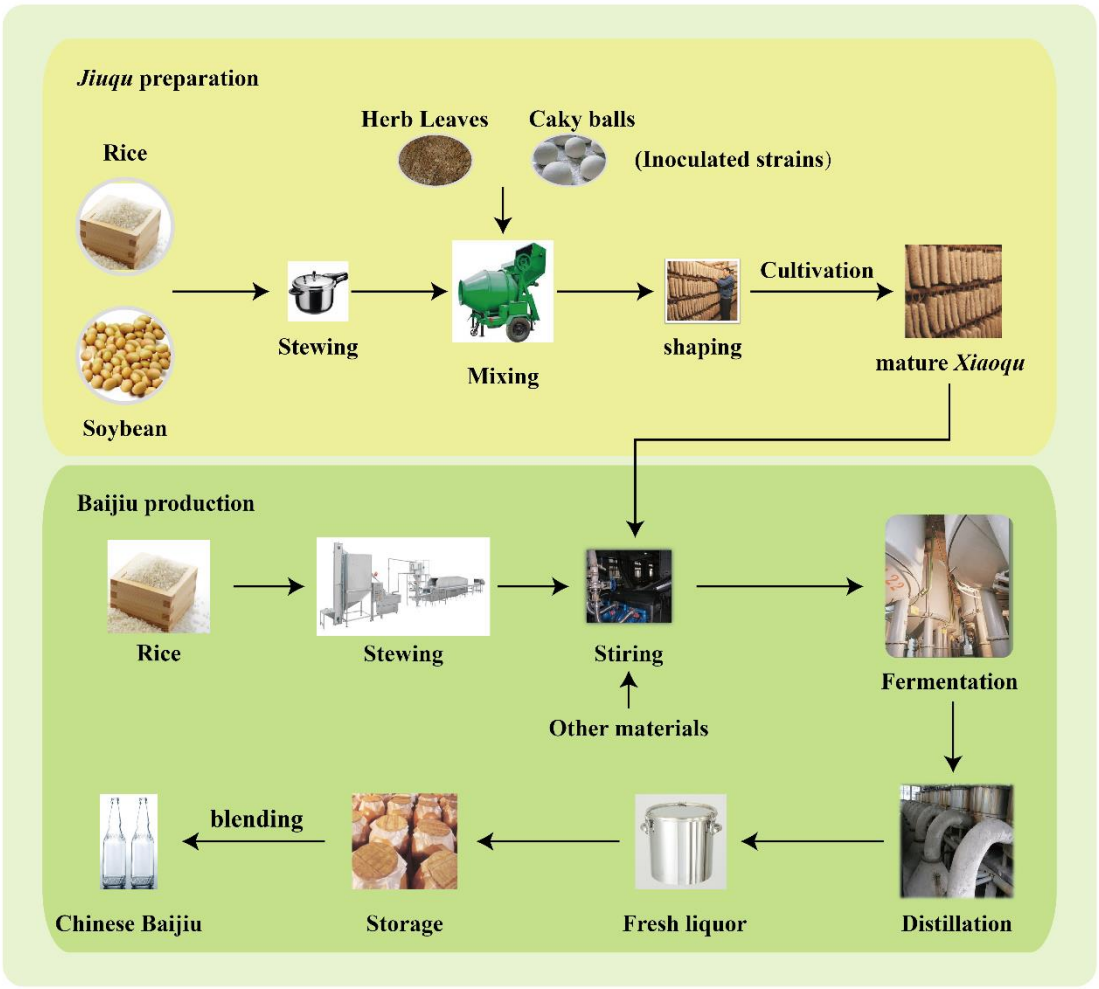

Jiuqu preparation
Herb Leaves
Caky balls
Rice
(Inoculated strains)
Stewing
Mixing
shaping
Cultivation
mature *Xiaoqu*
Soybean
Baijiu production
Rice
Stewing
Stiring
Fermentation
Other materials
blending
Chinese Baijiu
Storage
Fresh liquor
Distillation

Fig. S2

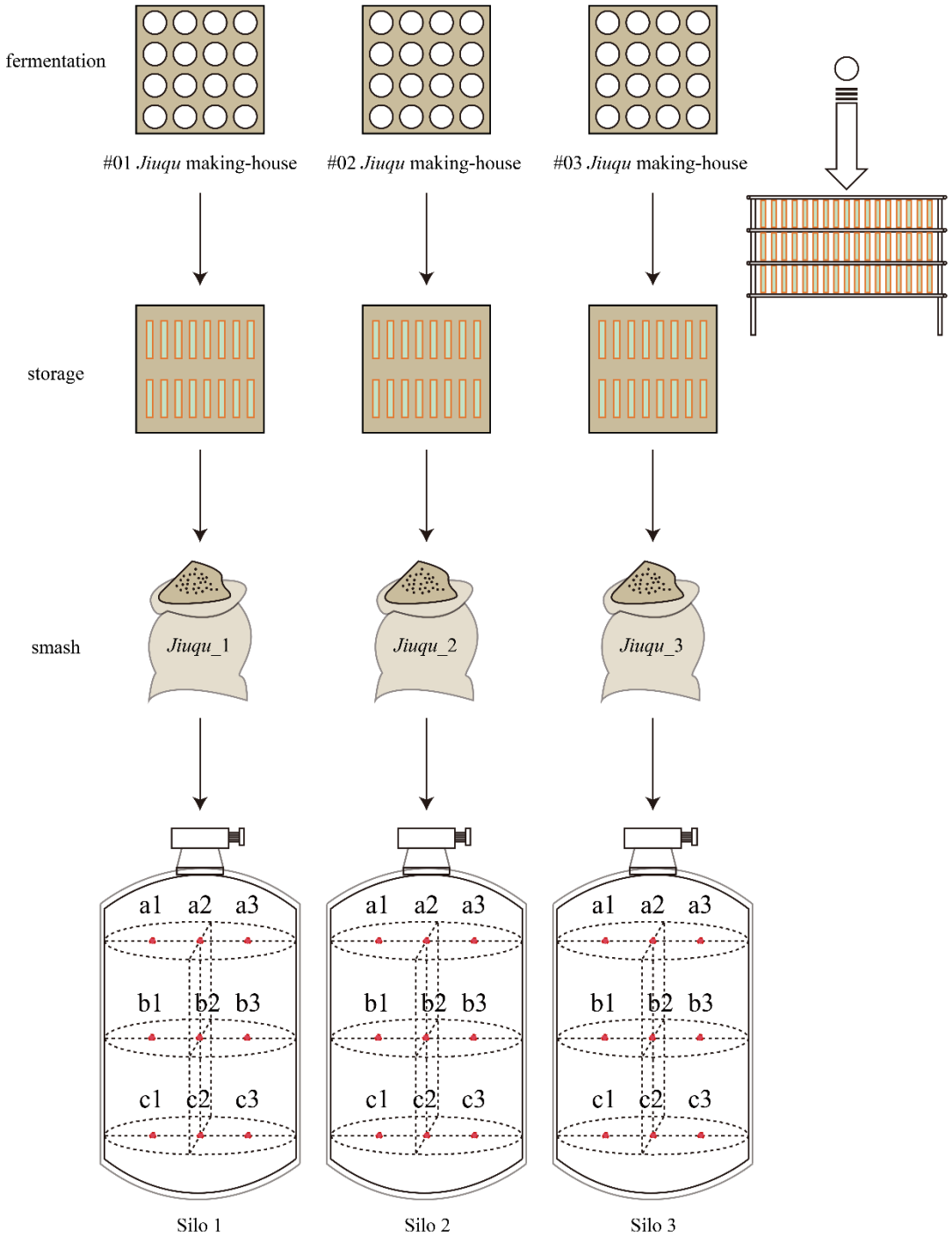
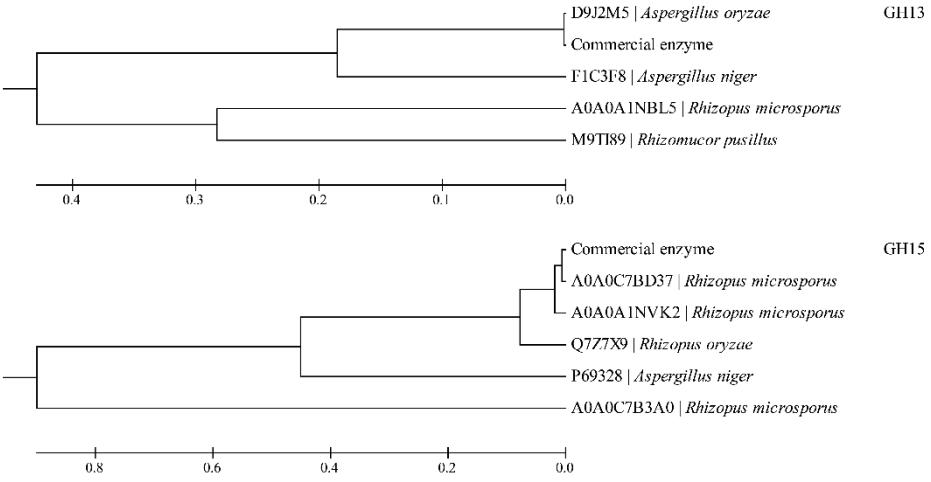

fermentation
#01 *Jiuqu* making-house
#02 *Jiuqu* making-house
#03 *Jiuqu* making-house
storage
smash
*Jiuqu*_1
*Jiuqu*_2
*Jiuqu*_3
a1 a2 a3
b1 b2 b3
c1 c2 c3
Silo 1
Silo 2
Silo 3

Fig. S3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Commercial enzyme
F1C3F8 | Aspergillus niger
A0A0A1NBL5 | Rhizopus microsporus
M9TI89 | Rhizomucor pusillus
GH13
0.4
0.3
0.2
0.1
0.0
Commercial enzyme
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A0A0A1NVK2 | Rhizopus microsporus
Q7Z7X9 | Rhizopus oryzae
P69328 | Aspergillus niger
A0A0C7B3A0 | Rhizopus microsporus
GH15
0.8
0.6
0.4
0.2
0.0

Fig. S4

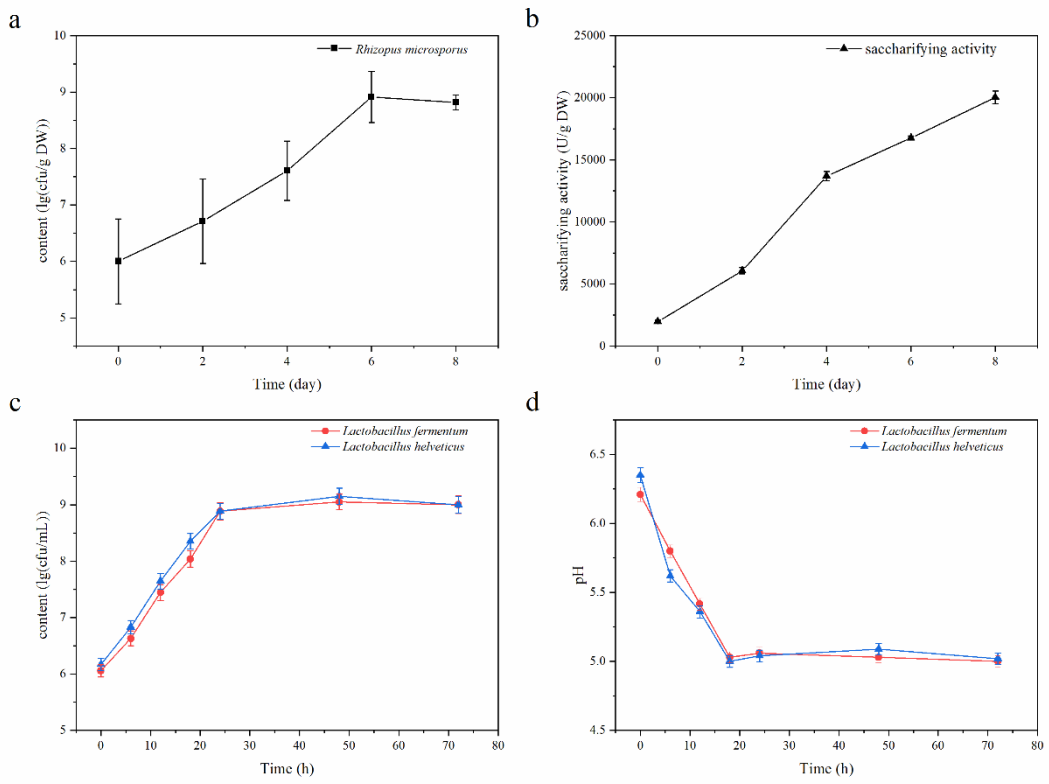
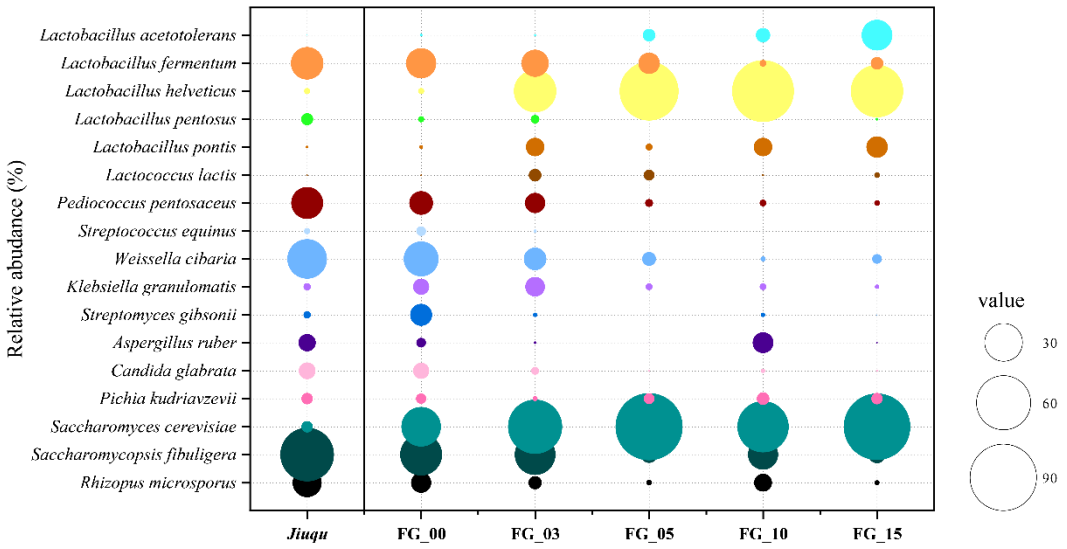

a
Rhizopus microsporus
content (lg(cfu/g DW))
Time (day)
b
saccharifying activity
saccharifying activity (U/g DW)
Time (day)
c
Lactobacillus fermentum
Lactobacillus helveticus
content (lg(cfu/mL))
Time (h)
d
Lactobacillus fermentum
Lactobacillus helveticus
pH
Time (h)

Fig. S5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Lactobacillus fermentum
Lactobacillus helveticus
Lactobacillus pentosus
Lactobacillus pontis
Lactococcus lactis
Pediococcus pentosaceus
Streptococcus equinus
Weissella cibaria
Klebsiella granulomatis
Streptomyces gibsonii
Aspergillus ruber
Candida glabrata
Pichia kudriavzevii
Saccharomyces cerevisiae
Saccharomycopsis fibuligera
Rhizopus microsporus
Relative abudance (%)
Jiuqu
FG_00
FG_03
FG_05
FG_10
FG_15
value
30
60
90