Supplemental Information for

Construction of synthetic microbiota for reproducible flavor metabolism in Chinese light aroma type liquor produced by solid-state fermentation

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Running headline: Synthetic microbiota in food fermentation

This supplementary files include:

Supplementary figures: Fig. S1 - S4;

Supplementary tables: Table S1 - S9;

Supplementary datasets: Dataset S1 - S3.

Supplementary figures:

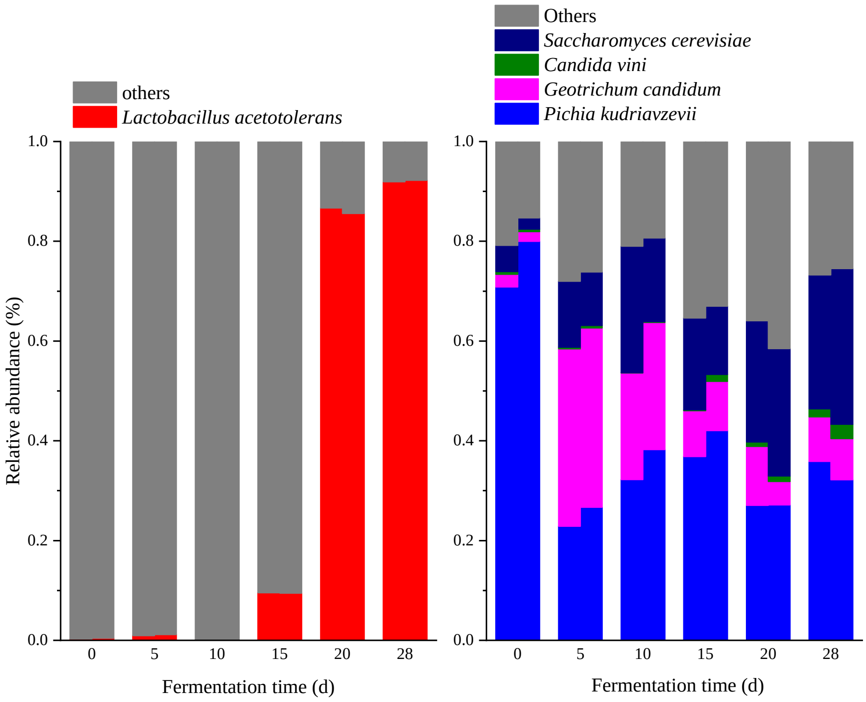

Fig. S1. The five species relative abundance of core microbiota during the fermentation in the *in situ* System.

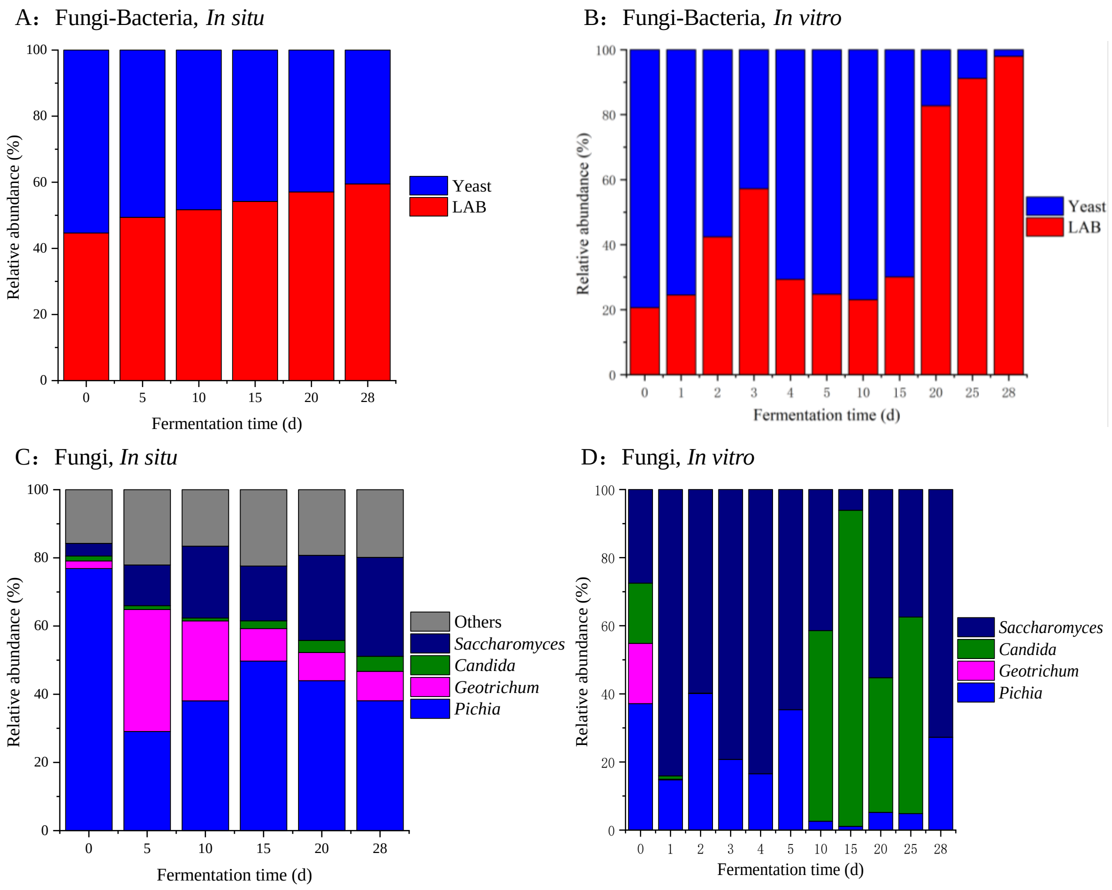

Fig. S2. Comparison of microbial composition in two systems. (A) The percent of yeasts and LAB in the *in situ* system. (B) The percent of yeasts and LAB in the *in vitro* system. (C) The percent of yeasts in the *in situ* system. (D) The percent of yeasts in the *in vitro* system.

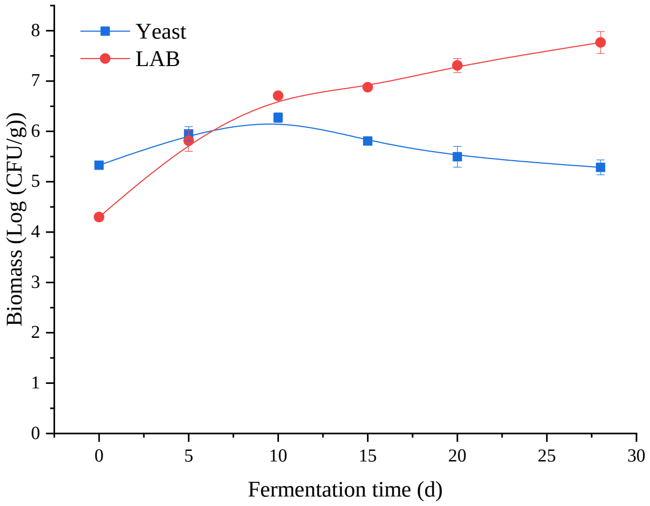

Fig. S3. The biomass changes of yeast and LAB in the *in situ* system

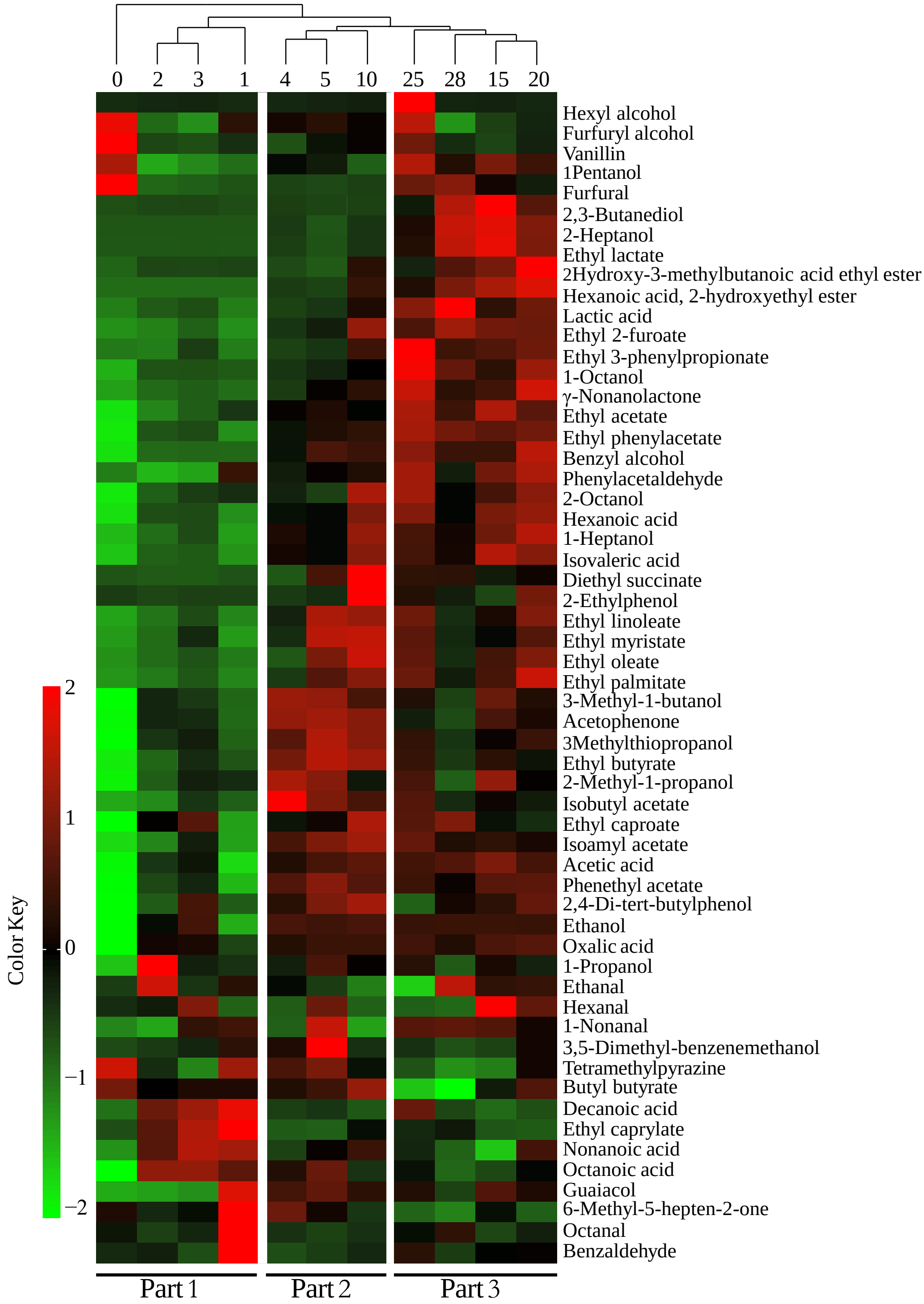

Fig. S4. Heatmap of flavor metabolites and hierarchical clustering in the *in vitro* fermentation process. Favor compounds were transformed by z-score. Clustering analysis was performed using Pearson correlation coefficient and Euclidean distance based on the flavor compound contents during the fermentation process.

Supplementary tables:

Table S1. Bacterial and fungal statistical information about Illumina sequencing results during the fermentation process.

Sample ID	Bacteria					fungi				
	Reads	OTUs	Good's coverage	Chao1	Shannon	Reads	OTUs	Good's coverage	Chao1	Shannon
0-1	25104	81	99.95%	79.33	2.62	74017	101	99.91%	97.30	2.05
0-2	37867	94	99.96%	89.88	4.45	50235	98	99.92%	96.35	1.50
5-1	51207	52	99.95%	37.55	1.48	67964	108	99.91%	100.21	3.09
5-2	16623	42	99.93%	45.50	1.49	63428	105	99.89%	111.07	3.01
10-1	29386	34	99.93%	46.33	0.90	22411	73	99.93%	82.39	2.99
10-2	32275	55	99.90%	65.00	1.47	39621	113	99.90%	114.48	2.88
15-1	37811	59	99.93%	52.88	2.39	71038	123	99.89%	120.83	3.26
15-2	50874	65	99.88%	78.50	2.31	33937	108	99.91%	112.80	3.18
20-1	26684	29	99.97%	28.50	0.83	44646	122	99.89%	125.97	3.44
20-2	25854	35	99.93%	37.50	0.88	38933	117	99.90%	117.87	3.33
28-1	38417	34	99.91%	51.00	0.52	30936	118	99.87%	148.81	3.37
28-2	41758	29	99.95%	26.00	0.49	47662	112	99.91%	109.51	3.22

Table S2. The constitution of ubiquitously distributed microbiota. The number represent the presence of corresponding microbes in 14 samples.

Microbes	Number of samples
Lactobacillus	14
Pediococcus	13
Pichia	14
Geotrichum	14
Saccharomyces	14
Saccharomycopsis	14
Rhizopus	11
Aspergillus	13
Candida	11
Thermoascus	8

Table S3. The constitution of flavor-producing microbiota. The number represent kinds of flavor compounds which correlated with microbes.

Microbes	Number of flavor compounds	Microbes	Number of flavor compounds
Lactobacillus	26	*Kroppenstedtia*	1
Saccharomyces	26	*Pediococcus*	1
Clavispora	16	*Pichia*	1
Candida	14	*Staphylococcus*	1
Aspergillus	2	*Streptomyces*	1
Bacillus	1	*Thermomyces*	1
Geotrichum	1	*Trichosporon*	1
Herbaspirillum	1	*Weissella*	1

Table S4. The constitution of co-occurring microbiota. The number represent the kinds of microbes which co-occurrence with the corresponding microbes. The rate represent the ratio of co-occurrences' number in the total co-occurrences' number.

Microbes	Number of co-occurrences	co-occurrence rate（%）	Microbes	Number of co-occurrences	co-occurrence rate（%）
Flavobacterium	24	8.05%	*Clavispora*	10	3.36%
Lactobacillus	22	7.38%	*Geotrichum*	10	3.36%
Brevibacterium	20	6.71%	*Lichtheimia*	10	3.36%
Herbaspirillum	20	6.71%	*Thermoascus*	10	3.36%
Pichia	20	6.71%	*Rhizopus*	8	2.68%
Staphylococcus	20	6.71%	*Achromobacter*	6	2.01%
Bacillus	18	6.04%	*Pseudomonas*	6	2.01%
Weissella	18	6.04%	*Stenotrophomonas*	6	2.01%
Kroppenstedtia	16	5.37%	*Candida*	4	1.34%
Leuconostoc	14	4.70%	*Saccharomycopsis*	4	1.34%
Saccharomyces	14	4.70%	*Streptomyces*	4	1.34%
Aspergillus	10	3.36%			

Table S5**.** The changing of environmental factors in the *in situ* fermentation process.

Sample ID	Lactic acid (mg/g)	Ethanol (mg/g)	Acetic acid (mg/g)	Moisture (%)	pH
0-1	3.94	3.62	0.00	55.43%	4.98
0-2	3.86	4.56	0.00	55.33%	4.96
5-1	9.19	28.86	0.43	61.92%	4.00
5-2	9.45	34.19	0.38	67.03 %	5.86
10-1	12.96	45.34	0.53	66.77%	3.85
10-2	12.92	43.30	0.76	65.90%	3.98
15-1	17.64	56.64	0.97	66.80%	3.81
15-2	15.71	60.81	1.25	63.36%	3.72
20-1	14.82	78.16	1.52	65.32%	3.67
20-2	15.84	78.19	1.13	66.72%	3.73
28-1	17.11	72.79	2.03	67.64%	3.51
28-2	14.30	75.29	3.11	65.49%	3.41

Table S6**.** Explanations of variation partitioning analysis of the core microbiota with environmental factors in the *in situ* and *in vitro* systems.

Fermentation system	Constrained	Unconstrained
In situ	73.03%	26.97%
In vitro	53.65%	46.35%

Table S7**.** Monte Carlo permutation test of the core microbiota with environmental factors in the *in situ* and *in vitro* systems.

Monte Carlo permutation test	*In situ*		*In vitro*	
	r^2	*P*	r^2	*P*
pH	0.4947*[a]	0.017	0.7980***	0.001
Moisture (%)	0.7118**	0.002	0.7588***	0.001
Ethanol (mg/g)	0.8217***	0.001	0.7228***	0.001
Acetic acid (mg/g)	0.8197***	0.001	0.5180***	0.001
Lactic acid (mg/g)	0.6323**	0.005	0.6620***	0.001

[a] Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

Table S8. The species of core microbiota. Similarity were based on comparison results of NCBI Blast. Maximum relative abundance were calculated based on 14 samples in the related genus.

Core microbiota	Dominant species	Similarity (%)	Maximum relative abundance (%)
Lactobacillus	*Lactobacillus acetotolerans*	100%	93.50%
Pichia	*Pichia kudriavzevii*	100%	98.28%
Geotrichum	*Geotrichum candidum*	100%	100.00%
Candida	*Candida vini*	100%	53.54%
Saccharomyces	*Saccharomyces cerevisiae*	100%	98.88%

Table S9. The changing of environmental factors in the *in vitro* fermentation process.

Fermentation time (d)	Lactic acid (mg/g)	Ethanol (mg/g)	Acetic acid (mg/g)	Moisture (%)	pH
0	n. d[a]	0.82 ± 0.01	n. d	57.85 ±0.52	6.39 ±0.00
1	n. d	14.10 ± 0.34	0.06 ± 0.01	59.23 ±1.06	5.44 ±0.02
2	2.08 ± 0.06[b]	31.41 ± 0.05	0.42 ± 0.01	63.02 ±0.04	4.70 ±0.01
3	2.80 ± 0.17	38.96 ± 0.34	0.51 ± 0.06	63.99 ±0.69	4.56 ±0.04
4	3.63 ± 0.37	39.61 ± 0.96	0.61 ± 0.04	64.07 ±0.90	4.53 ±0.03
5	4.29 ± 0.34	38.48 ± 0.46	0.70 ± 0.03	64.80 ±0.12	4.44 ±0.04
10	8.60 ± 0.40	39.61 ± 0.33	0.76 ± 0.03	64.94 ±0.51	3.95 ±0.08
15	9.82 ± 0.59	38.08 ± 0.28	0.83 ± 0.01	64.18 ±0.24	3.94 ±0.01
20	13.71 ± 0.37	37.55 ± 0.14	0.70 ± 0.08	63.72 ±0.26	3.80 ±0.07
25	15.02 ± 1.75	37.56 ± 0.32	0.69 ± 0.00	64.58 ±0.28	3.63 ±0.14
28	21.59 ± 0.3	38.08 ± 0.33	0.73 ± 0.18	65.12 ±0.78	3.62 ±0.14

[a] "n. d" indicates not detected.

[b] Values are shown as the mean ± SD (n = 3) (mg/g wet weight).