Supplementary Materials

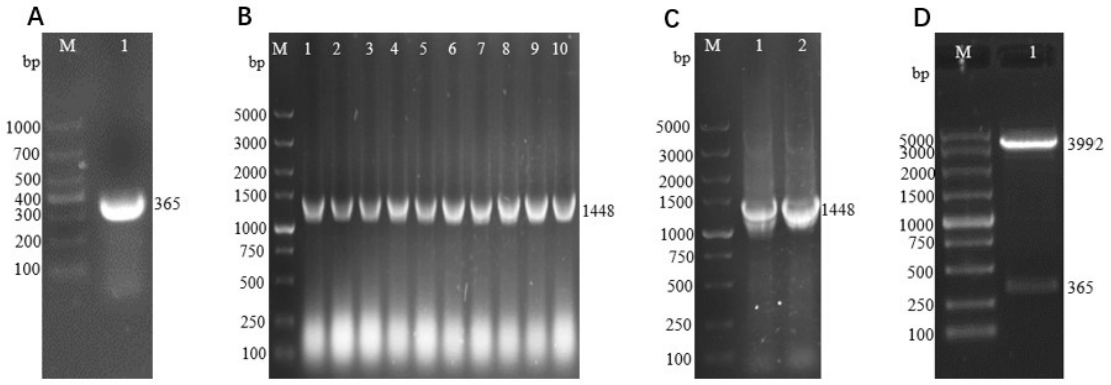

Fig. S1. Construction of pUC18+HLarm (*fadA*)+*cm* vector. (A) Amplification of HLarm (M: DL1000 Marker, line 1: HLarm); (B) Validation of colony PCR performed with primers *fadA*-HLarmF/*cm*R (M: DL5000 Marker, line 1-10: positive transformants) ; (C) Validation of Plasmid PCR performed with primers *fadA*-HLarmF/*cm*R (M: DL5000 Marker, line 1-2: positive plasmids); (D) Double digestion was verified using *Eco*RI/*Kpn*I enzymes (M: DL5000 Marker, line 1: Double enzymes digestion fragments) .

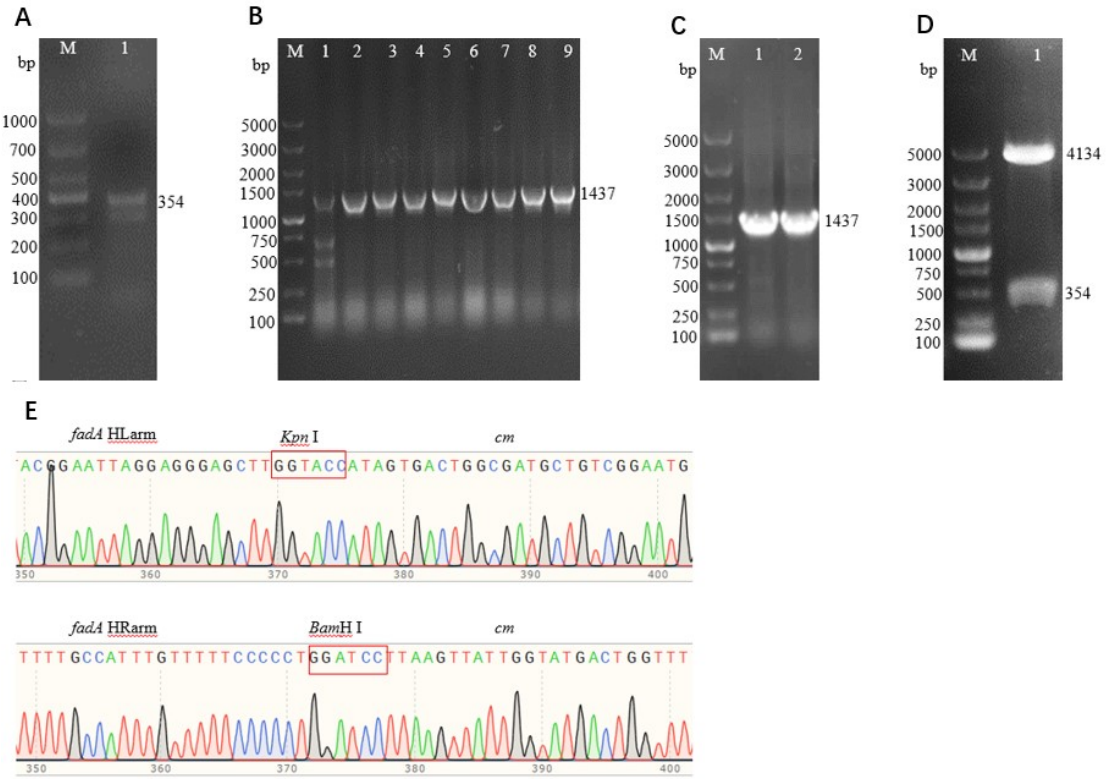

Fig. S2. Construction of pUC18+HLarm(*fadA*)+*cm*+HRarm(*fadA*) vector. (A) Amplification of HRarm (M: DL1000 Marker, line 1: HRarm); (B) Validation of colony PCR performed with primers *cm*F/*bkdB*-HRarmR (M: DL5000 Marker, line 1-19: positive transformants); (C) Validation of Plasmid PCR performed with primers *cm*F/*fadA*-HRarmR (M: DL5000 Marker, line 1-2: Primers *cm*F/*fadA*-HRarmR plasmid PCR validation); (D) Double digestion was verified using *Bam*HI/*Sal*I enzymes (M: DL5000 Marker, line 1: Double enzymes digestion fragments); (E) Sequencing of pUC18+HLarm(*fadA*)+cm+HRarm(*fadA*) plasmid.

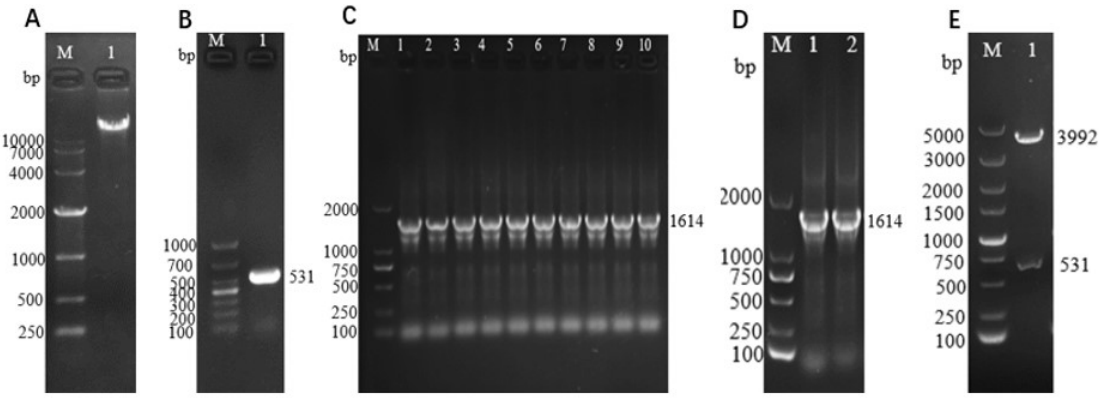

Fig. S3. Construction of pUC18+HLarm (*bkdB*)+*cm* vector. (A) BJ3-2 Genome Extraction (M: DL10000 Marker, line 1: BJ3-2 Genome); (B) Amplification of HLarm (M: DL1000 Marker, line 1: HLarm); (C) Validation of colony PCR performed with primers *bkdB*-HLarmF/*cm*R (M: DL2000 Marker, line 1-10: positive transformants); (D) Validation of Plasmid PCR performed with primers *bkdB*-HLarmF/*cm*R (M: DL2000 Marker, line 1-2: positive plasmids); (E) Double digestion was verified using *Eco*RI/*Kpn*I enzymes (M: DL5000 Marker, line 1: Double enzymes digestion fragments).

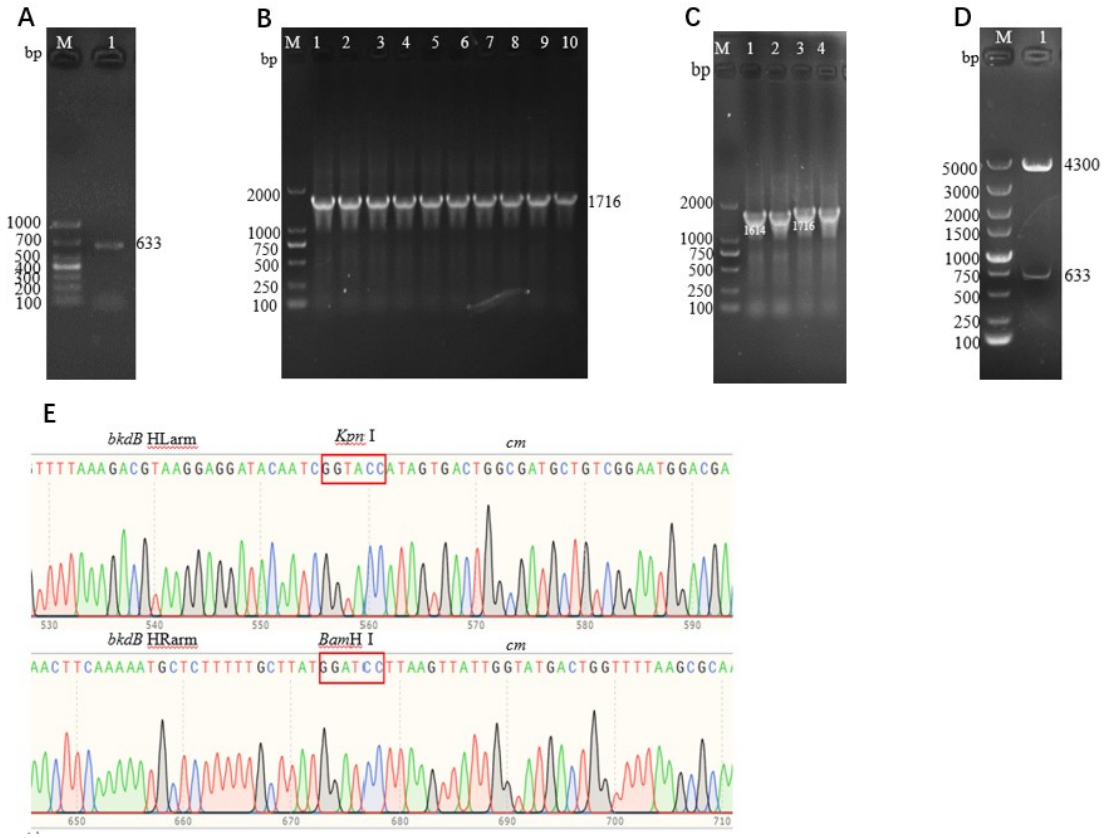

Fig. S4. Construction of pUC18+HLarm(*bkdB*)+*cm*+HRarm(*bkdB*) vector. (A) Amplification of HRarm (M: DL1000 Marker, line 1: HRarm); (B) Validation of colony PCR performed with primers *cm*F/*bkdB*-HRarmR (M: DL2000 Marker, line 1-10: positive transformants); (C) Validation of Plasmid PCR performed with primers *bkdB*-HLarmF/*cm*R and *cm*F/*bkdB*-HRarmR (M: DL2000 Marker, line 1-2: Primers *bkdB*-HLarmF/*cm*R plasmid PCR validation, line 3-4: Primers *cm*F/*bkdB*-HRarmR plasmid PCR validation); (D) Double digestion was verified using *Bam*HI/*Sal*I enzymes (M: DL5000 Marker, line 1: Double enzymes digestion fragments); (E) Sequencing of pUC18+HLarm(*bkdB*)+*cm*+HRarm(*bkdB*) plasmid.

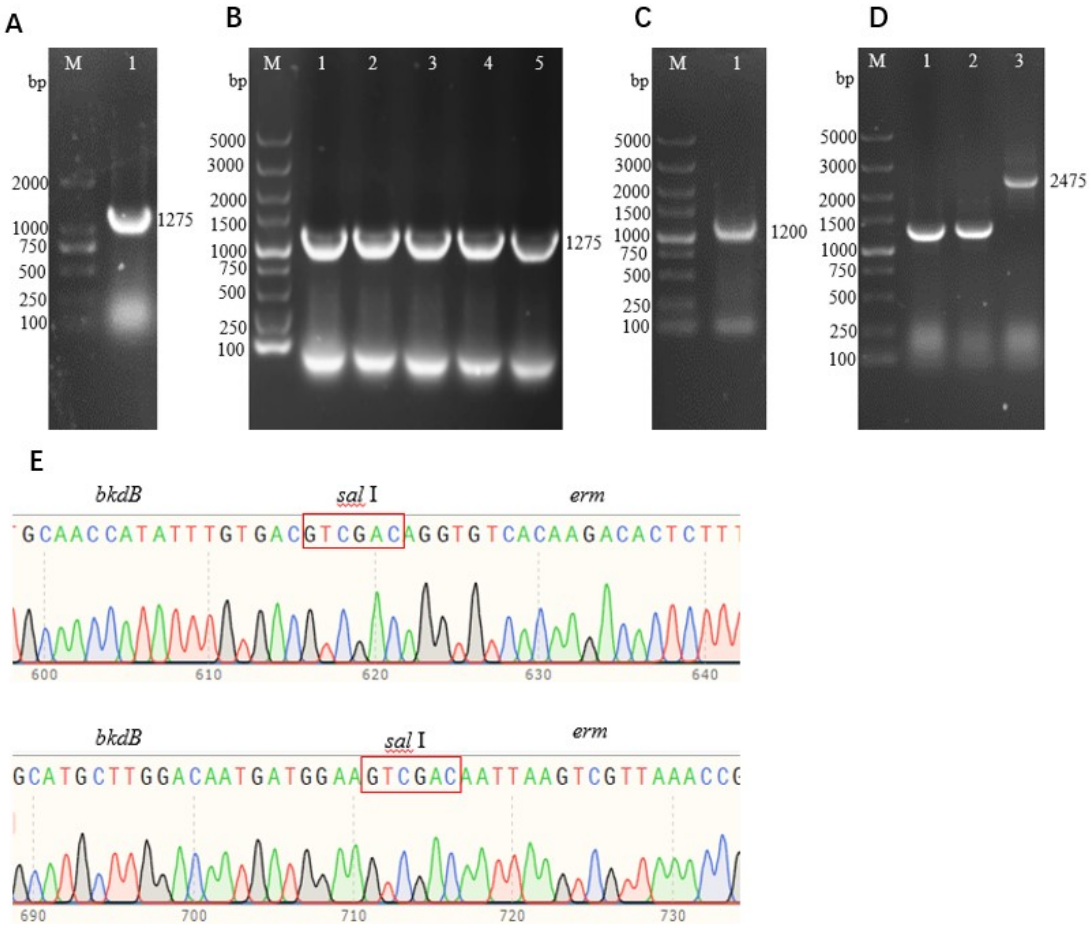

Fig. S5. Construction of pUC18-*bkdB*::*erm* vector.

A Amplification of *bkdB* (M: DL2000 Marker, line 1: *bkdB*) B PCR validation of pUC18-*bkdB* colony (M: DL5000 Marker, line 1-5: positive transformants) C Amplification of *erm* (M: DL5000 Marker, line 1: *erm*) D PCR validation of pUC18-*bkdB*::*erm* colony (M: DL5000 Marker, line 1-2: negative transformants, line 3: positive transformants) E Validation of pUC18-*bkdB*::*erm* recombinant plasmid sequencing

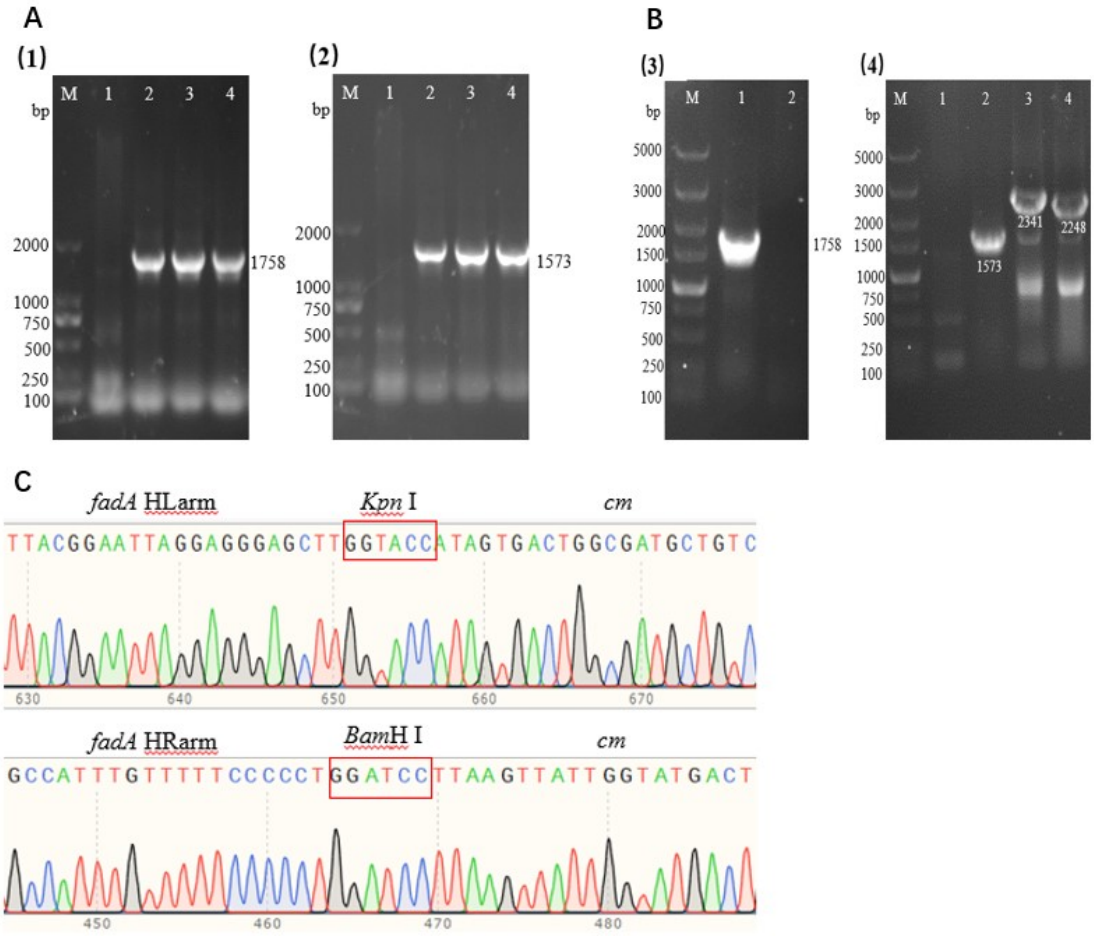

Fig. S6. BJ3-2Δ*fadA* colony PCR, genome PCR and sequencing validation. (A) BJ3-2Δ*fadA* colony PCR validation. (1) Validation of colony PCR performed with primers *fadA*-DEDPF/*cm*R (M: DL2000 Marker, line 1: BJ3-2 , line 2-4: positive transformants); (2) Validation of colony PCR performed with primers *cm*F/*fadA*-DEDPR (M: DL2000 Marker, line 1: BJ3-2, line 2-4: positive transformants). (B) BJ3-2Δ*fadA* genome PCR. (1) Validation of genome PCR performed with primers *fadA*-DEDPF/*cm*R (M: DL5000 Marker, line 1: BJ3-2, line 2: BJ3-2Δ*fadA*); (2) Validation of genome PCR performed with primers *cm*F/*fadA*-DEDPR and *fadA*-DEDPF /*fadA*-DEDPR (M: DL5000 Marker, line 1,3: BJ3-2 , line 2,4: BJ3-2Δ*fadA*). (C) Sequencing of BJ3-2Δ*fadA*.

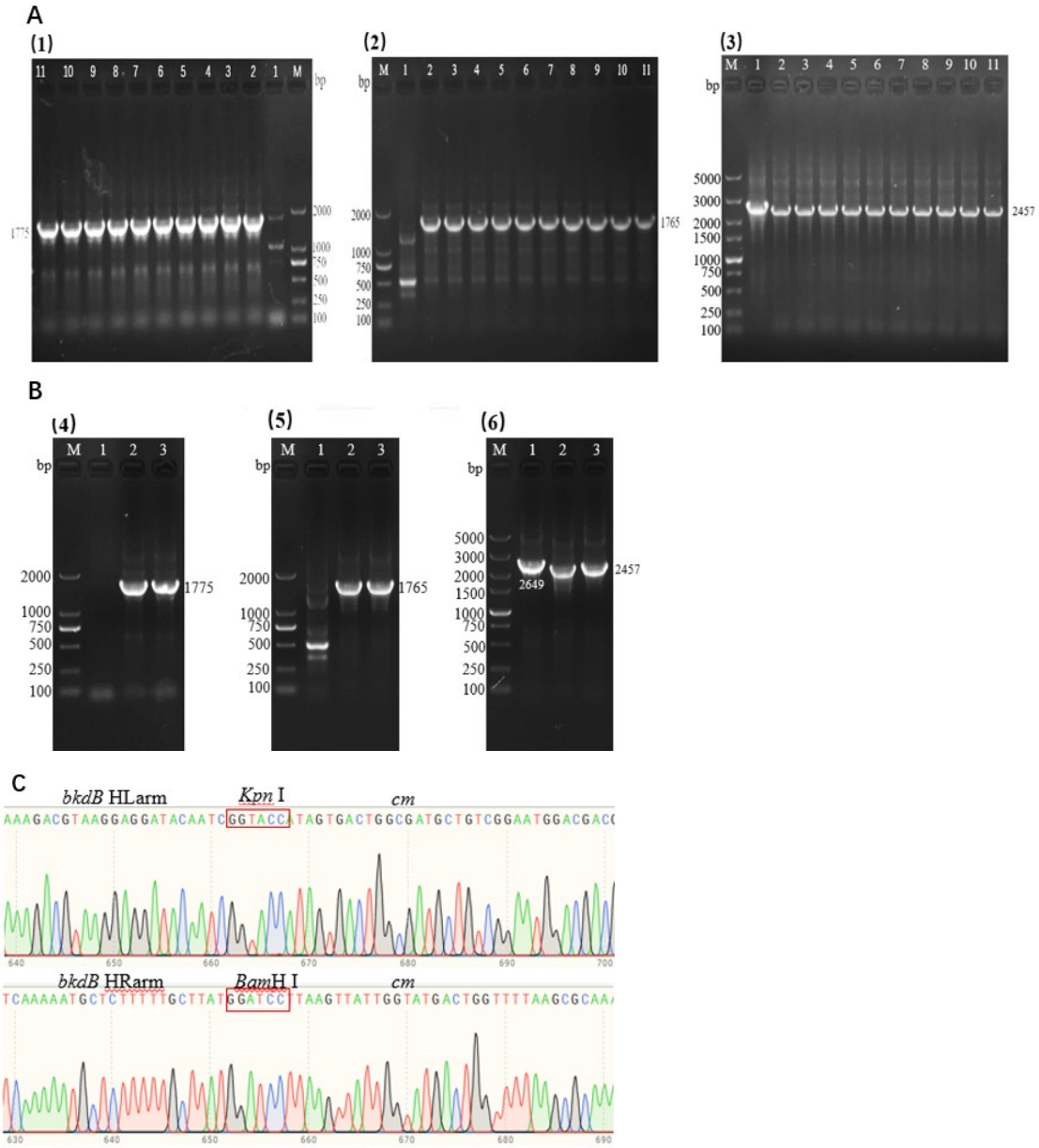

Fig. S7. BJ3-2Δ*bkdB* colony PCR, genome PCR and sequencing validation. (A) BJ3-2Δ*bkdB* colony PCR validation. (1) Validation of colony PCR performed with primers *bkdB*-DEDPF/*cm*R (M: DL2000 Marker, line 1: BJ3-2 , line 2-11: positive transformants) ; (2) Validation of colony PCR performed with primers *cm*F/*bkdB*-DEDPR (M: DL2000 Marker, line 1: BJ3-2 , line 2-11: positive transformants); (3) Validation of colony PCR performed with primers *bkdB*-DEDPF/*bkdB*-DEDPR (M: DL5000 Marker, line 1: BJ3-2 , line 2-11: positive transformants). (B) (4) Validation of genome PCR performed with primers *bkdB*-DEDPF/*cm*R (M: DL2000 Marker, line 1: BJ3-2 , line 2-3: BJ3-2Δ*bkdB*); (5) Validation of genome PCR performed with primers *cm*F/*bkdB*-DEDPR (M: DL2000 Marker, line 1: BJ3-2 , line 2-3: BJ3-2Δ*bkdB*); (6) Validation of genome PCR performed with primers *bkdB*-DEDPF/*bkdB*-DEDPR (M: DL5000 Marker, line 1: BJ3-2 , line 2-3: BJ3-2Δ*bkdB*) C Sequencing of BJ3-2Δ*bkdB.*

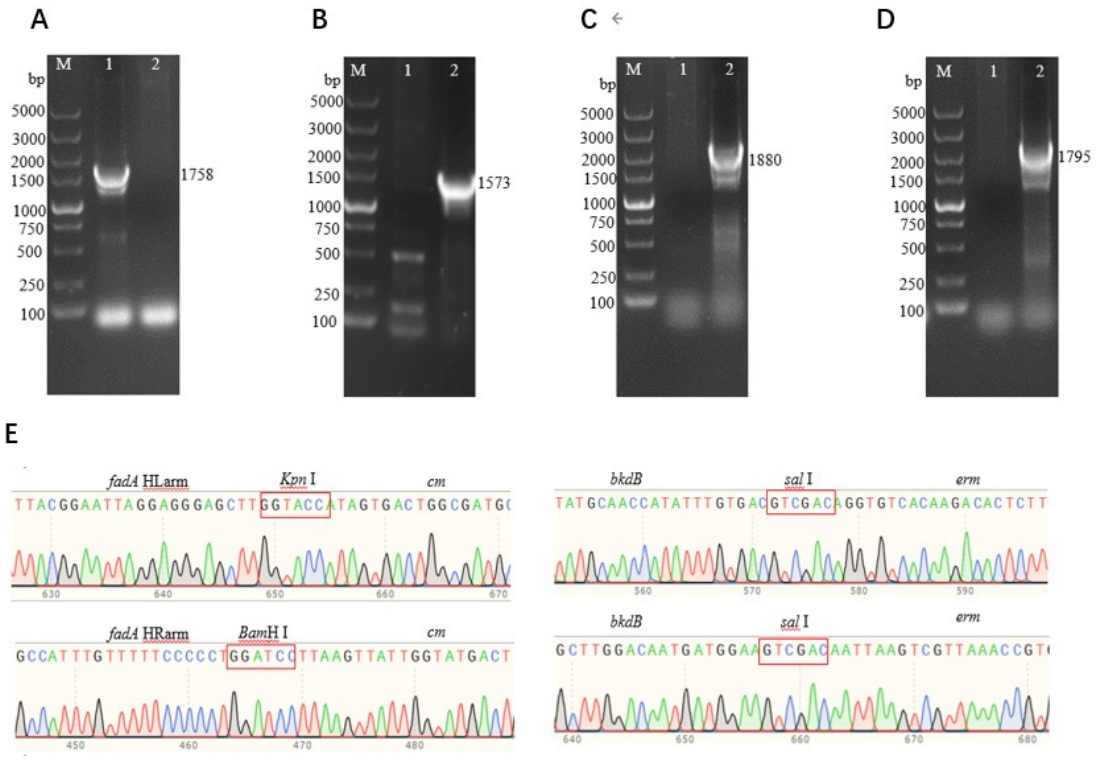

Fig. S8. Validation of BJ3-2Δ*fadA*Δ*bkdB* genome PCR and sequencing. (A) *fadA*-DEDPF/*cm*R Genome PCR Validation (M: DL5000 Marker, line 1: BJ3-2Δ*fadA*Δ*bkdB*, line 2: BJ3-2); (B) *cm*F/*fadA*-DEDPR Genome PCR Validation (M: DL5000 Marker, line 1: BJ3-2, line 2: BJ3-2Δ*fadA*Δ*bkdB*); (C) *bkdB*F/*erm*R Genome PCR Validation (M: DL5000 Marker, line 1: BJ3-2, line 2: BJ3-2Δ*fadA*Δ*bkdB*); (D) *erm*F/*bkdB*R Genome PCR Validation (M: DL5000 Marker, line 1: BJ3-2, line 2: BJ3-2Δ*fadA*Δ*bkdB*); (E) Validation of BJ3-2Δ*fadA*Δ*bkdB* sequencing.

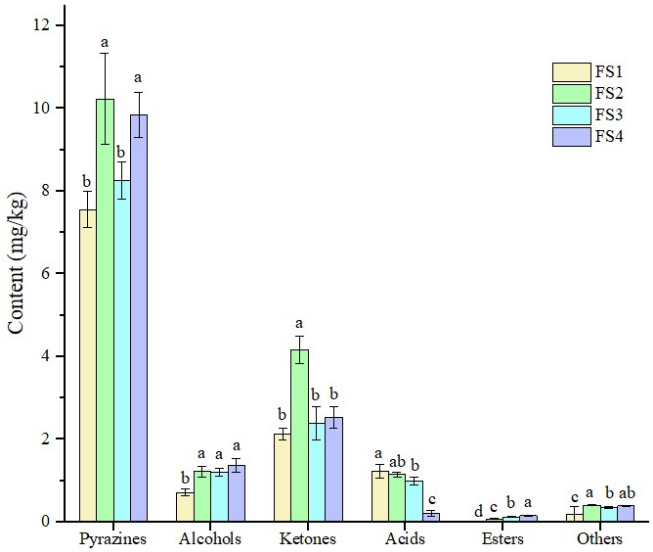

Fig. S9. Determination of volatile content in fermented soybeans.

Table S1. The strains and plasmids used for this study

Strain or plasmid	Comment [a]	Source
Strains		
BJ3-2	Wild type (WT)	Laboratory storage
BJ3-2Δ*fadA*	BJ3-2 mutant obtained by deletion of *fadA*	This work
BJ3-2Δ*bkdB*	BJ3-2 mutant obtained by deletion of *bkdB*	This work
BJ3-2Δ*fadA*Δ*bkdB*	BJ3-2 mutant obtained by deletion of *fadA* and *bkdB*	This work
E. coli DH5α	Competent cells for transformation	Purchased from TaKaRa
Plasmids		
pUC18	Cloning vector	Purchased from TaKaRa
pMarA	Amplification of *erm* gene	Generous donation from China Agricultural University
pUC18-HLarm(*serA*)-*cm*-HRarm(*serA*)	pUC18-derived plasmid, *E. coli* (Amp^r), *B. subtilis*（Cm^r）, for *serA* gene knockout	Laboratory storage
pUC18-HLarm(*fadA*)-*cm*-HRarm(*fadA*)	pUC18-derived plasmid, *E. coli* (Amp^r), *B. subtilis* (Cm^r), for *fadA* gene knockout	This work
pUC18-HLarm(*bkdB*)-*cm*-HRarm(*bkdB*)	pUC18-derived plasmid, *E. coli* (Amp^r), *B. subtilis* (Cm^r), for *bkdB* gene knockout	This work
pUC18-*bkdB*::*erm*	pUC18-derived plasmid, *E. coli* (Amp^r), *B. subtilis* (Cm^r and Ery^r) for *fadA* and *bkdB* genes knockout	This work

[a]Amp^r: ampicillin resistant; Cm^r, chloramphenicol resistant; Ery^r, erythromycin resistant.

Table S2. Primers and PCR details used in this study

Primer	Sequence (5'→3') [a]	Comment
16S rRNA	F: ACTCCTACGGGAGGCAGCAG	RT-qPCR internal reference gene.
	R: ATTACCGCGGCTGCTGG	
q-*fadA*	F: CGAGCAGGTTGCGAGAAAATAC	Determination of gene expression
	R: CGCCTTCATCCTGAGAAAACAC	
q-*bkdB*	F: TGTCACAGGCGTAAGAAA	Determination of gene
	R: ATGCTATTCATTTGCGGG	expression
fadA-HLarm	F: GC**GAATTC**GAATCAAGACCACCTGC	Amplification of the upstream regions of *fadA*
	R: CGG**GGTACC**AAGCTCCCTCCTAATTC	

Primer	Sequence (5'→3') [a]	Comment
fadA-HRarm	F: CGC**GGATCC**AGGGGGAAAAACAAATG	Amplification of the downstream regions of *fadA*
	R: ACGC**GTCGAC**CGCCATAGGAAAGAGAA	
fadA-DEDP	F: AAATACAGTGCGAAACCG	Double exchange detection primers to validate *fadA* knockout
	R: GCATCAGACCCAGAGCC	
bkdB-HLarm	F: GC**GAATTC**AAAGCCGCAGTTCGTG	Amplification of the upstream regions of *bkdB*
	R: CGG**GGTACC**GATTGTATCCTCCTTA	
bkdB-HRarm	F: CGC**GGATCC**ATAAGCAAAAAGAGCATT	Amplification of the downstream regions of *bkdB*
	R: ACGC**GTCGAC**GCGGCAAATCAGGGAGAA	
bkdB-DEDP	F: GCAACAATGACTGGAGC	Double exchange detection primers to validate *bkdB* knockout
	R: CACAGGACTTAGAGATGG	
bkdB	F: CGG**GGTACC**ATGGCAATTGAACAAATG	Amplification of *bkdB* for construction of pUC18-*bkdB*::*erm*
	R: CCC**AAGCTT**TTAGTAAACAGATGTCT	
erm	F: TGGACAATGATGGAA**GTCGAC**AATTAAG	Amplification of erm for construction of pUC18-*bkdB*::*erm*
	TCGTTAAACCGTGTGC	
	R: AACCATATTTGTGAC**GTCGAC**AGGTGTCA	
	CAAGACACTCTTTT	
cm	F: CGG**GGTACC**ATAGTGACTGGCGATGCTG	PCR validation
	R: CGC**GGATCC**TTAAGTTATTGGTATGACTGG	
	TT	

[a] Restriction sites used in this study are shown in bold.

Table S3. Transcriptome sequencing results

Gene	CT53-1	CT53-2	CT53-3	AT37-1	AT37-2	AT37-3	log_2FC(53℃/37℃)
fadA	873.35	892.68	793.92	4891.91	3263.88	3945.77	-2.24
bkdB	478.54	515.21	410.53	1414.23	2456.91	2108.30	-2.09

Table S4. RT-qPCR results

Gene	$2^{-\Delta\Delta CT}$ (53℃)	$2^{-\Delta\Delta CT}$ (37℃)	Log_2FC(53℃/37℃)	Mean log_2FC(53℃/37℃)
fadA	0.29	0.81	-1.49	-1.22
	0.37	1.11	-1.58	

Gene	$2^{-\Delta\Delta CT}$ (53℃)	$2^{-\Delta\Delta CT}$ (37℃)	Log_2FC(53℃/37℃)	Mean log_2FC(53℃/37℃)
	0.74	1.12	-0.59	
bkdB	0.24	1.11	-2.21	-2.01
	0.16	1.29	-3.00	
	0.39	0.70	-0.83	

Table S5. Results of variance analysis of OD600 values at each period in the growth curves of the four strains

Time (h)	OD600 values (Mean ±SD)			
	BJ3-2	BJ3-2Δ*fadA*	BJ3-2Δ*bkdB*	BJ3-2Δ*fadA*Δ*bkdB*
0	0.009±0.001 [a]	0.008±0.001 [a]	0.009±0.000 [a]	0.01±0.002 [a]
2	0.124±0.003 [a]	0.100±0.010 [b]	0.124±0.002 [a]	0.09±0.005 [b]
4	0.526±0.022 [a]	0.395±0.023 [b]	0.366±0.010 [b]	0.249±0.031 [c]
6	0.752±0.032 [a]	0.666±0.035 [b]	0.692±0.012 [ab]	0.472±0.013 [c]
8	0.832±0.015 [a]	0.714±0.078 [a]	0.764±0.035 [a]	0.693±0.012 [a]
10	1.061±0.035 [a]	0.901±0.078 [ab]	0.934±0.039 [ab]	0.836±0.053 [b]
12	1.202±0.048 [a]	1.103±0.081 [a]	1.168±0.065 [a]	1.020±0.044 [a]
14	1.379±0.049 [a]	1.278±0.060 [a]	1.328±0.038 [a]	1.247±0.046 [a]
16	1.540±0.063 [a]	1.424±0.048 [a]	1.509±0.022 [a]	1.423±0.005 [a]
18	1.711±0.052 [a]	1.566±0.056 [b]	1.644±0.023 [ab]	1.591±0.056 [ab]
20	1.813±0.071 [a]	1.732±0.030 [a]	1.767±0.027 [a]	1.624±0.118 [a]
22	1.996±0.066 [a]	1.833±0.047 [ab]	1.866±0.029 [ab]	1.742±0.091 [b]
24	2.103±0.089 [a]	1.901±0.072 [b]	1.955±0.024 [ab]	1.854±0.075 [b]
26	2.185±0.058 [a]	2.007±0.088 [ab]	2.063±0.026 [ab]	1.953±0.080 [b]
28	2.301±0.03 [a]	2.126±0.072 [b]	2.164±0.031 [ab]	2.044±0.075 [b]
30	2.295±0.03 [a]	2.237±0.055 [ab]	2.261±0.016 [ab]	2.158±0.041 [b]
32	2.277±0.014 [a]	2.266±0.005 [a]	2.278±0.003 [a]	2.185±0.007 [b]
34	2.252±0.017 [a]	2.241±0.023 [a]	2.265±0.001 [a]	2.179±0.015 [b]

[a-c] Indicates statistical significance ($p < 0.05$).

TableS6. Identification and quantification of volatile compounds in different fermented soybeans

NO.	Aroma Compounds	CAS	RIc[a]	RIr[b]	Compound Content (μg /kg)				Identification Methods[c]
					FS1	FS2	FS3	FS4	
	Pyrazines								
1	Pyrazine	290-37-9	1208.91	1212	8.48±0.36	13.03±0.66	10.17±1.24	14.83±1.36	MS, RI
2	2-Methylpyrazine	109-08-0	1263.52	1264	38.18±3.93	82.05±6.63	54.78±6.31	88.52±7.27	MS, RI
3	2,5-Dimethylpyrazine	123-32-0	1333.13	1323	1868.70±41.22	2459.33±108.38	1927.81±323.07	5781.23±268.62	MS, RI, O, S
4	2-Ethyl-5-methylpyrazine	13360-64-0	1391.25	1392	28.55±6.70	45.85±17.46	31.90±2.80	34.54±12.40	MS, RI
5	2,3,5-Trimethylpyrazine	14667-55-1	1408.95	1416	4655.50±368.80	4942.39±1343.98	5160.84±154.00	3799.16±277.16	MS, RI, O, S
6	3-Ethyl-2,5-dimethylpyrazine	13360-65-1	1444.29	1447	63.55±22.79	113.41±23.50	64.53±9.25	48.96±7.51	MS, RI, O, S
7	2,6-Diethylpyrazine	13067-27-1	1450.45	1436	36.44±13.52	37.33±10.61	31.93±11.24	ND	MS, RI
8	2,3,5,6-Tetramethylpyrazine	1124-11-4	1479.99	1478	806.05±73.03	1678.76±180.24	927.60±171.08	31.79±13.69	MS, RI, O
9	2,3,5-Trimethyl-6-ethylpyrazine	17398-16-2	1517.99	1491	47.23±7.11	103.15±20.36	37.77±3.89	ND	MS, RI
10	2,6-Dimethylpyrazine	108-50-9	1326.40	1334	ND	754.79±55.14	ND	ND	MS, RI
11	3,5-Diethyl-2-methyl-pyrazine	18138-05-1	1553.08	1509	ND	ND	10.20±0.22	25.68±1.31	MS, RI
12	2-Ethyl-6-methylpyrazine	13925-03-6	1388.24	1385	ND	ND	ND	17.87±4.15	MS, RI
	Alcohols								
13	Methanethiol	74-93-1	-	-	0.71±0.05	1.49±0.25	1.20±0.09	0.90±0.08	MS

NO.	Aroma Compounds	CAS	RIc[a]	RIr[b]	Compound Content (μg /kg)				Identification
14	Glycidol	556-52-5	-	-	82.46±11.36	199.04±16.09	92.02±23.58	8.64±2.82	MS
15	2-Pentanol	6032-29-7	1090.35	1114	1.87±0.50	ND	ND	16.22±1.76	MS, RI
16	3-Methyl-2-pentanol	565-60-6	1191.25	1202	1.07±0.16	ND	ND	ND	MS, RI
17	2-Methyl-1-butanol	137-32-6	1198.94	1210	2.37±1.26	2.80±0.11	2.37±0.20	6.46±0.80	MS, RI
18	3-Methyl-3-buten-1-ol	763-32-6	1241.32	1245	4.33±1.27	ND	3.95±0.76	5.46±0.61	MS, RI
19	2-Heptanol	543-49-7	1310.15	1312	11.99±3.94	ND	ND	ND	MS, RI
20	Prenol	556-82-1	1311.37	1317	4.66±3.41	13.96±5.80	9.64±1.06	13.79±1.37	MS, RI
21	1-Hexanol	111-27-3	1343.23	1345	106.94±7.54	155.52±6.78	123.65±11.62	138.34±10.74	MS, RI, O
22	3-Octanol	589-98-0	1384.59	1385	12.18±2.01	0.55±0.26	21.22±1.65	33.83±2.42	MS, RI, O
23	1-Octen-3-ol	3391-86-4	1437.04	1435	283.77±71.33	425.08±112.61	543.22±18.78	606.03±56.61	MS, RI, O, S
24	2,3-Butanediol	513-85-9	1527.95	1539	89.13±10.51	200.42±16.12	212.07±29.31	108.95±44.99	MS, RI
25	(R, R)-2,3-Butanediol	24347-58-8	1563.36	1544	18.64±7.74	59.97±4.10	23.17±4.91	ND	MS, RI
26	3-Furanmethanol	4412-91-3	1646.19	1679	67.71±4.95	111.91±14.10	117.50±13.06	89.79±1.92	MS, RI, O
27	Benzyl alcohol	100-51-6	1869.26	1872	6.24±0.47	10.95±1.88	11.02±0.80	9.11±1.28	MS, RI
28	Phenethyl alcohol	60-12-8	1909.08	1910	19.79±5.25	29.48±12.12	26.18±2.44	ND	MS, RI
29	2-Methyl-1-propanol	78-83-1	1088.36	1113	ND	3.38±0.63	ND	ND	MS, RI
30	1-Penten-3-ol	616-25-1	1152.62	1152	ND	4.57±0.05	5.61±0.53	10.40±1.14	MS, RI

NO.	Aroma Compounds	CAS	RIc[a]	RIr[b]	Compound Content (μg /kg)				Identification
31	3-Methyl-2-butanol	598-75-4	1086.50	1118	ND	ND	1.72±0.37	ND	MS, RI
32	2-Ethyl-1-hexanol	104-76-7	1477.59	1470	ND	ND	4.01±1.89	ND	MS, RI
33	(R)-(-)-2-Pentanol	31087-44-2	1119.94	1112	ND	ND	ND	16.22±1.76	MS, RI
34	2-Hexanol	626-93-7	1214.23	1211	ND	ND	ND	6.86±0.69	MS, RI
35	6-Methyl-2-heptanol	4730-22-7	1381.12	1365	ND	ND	ND	6.01±1.04	MS, RI
36	1-Octanol	111-87-5	1578.56	1573	ND	ND	ND	1.70±0.32	MS, RI
37	2-Tridecanol	1653-31-2	1878.36	1898	ND	ND	ND	251.18±63.76	MS, RI
38	2-Tetradecanol	4706-81-4	2000.86	2005	ND	ND	ND	27.58±2.41	MS, RI
39	2-Pentadecanol	1653-34-5	2099.63	2128	ND	ND	ND	30.00±2.54	MS, RI
	Others								
40	Carbon disulfide	75-15-0	699.05	710	3.25±0.65	4.48±0.13	4.88±0.77	8.80±1.77	MS, RI
41	Dimethyl sulfide	75-18-3	719.02	720	0.24±0.04	0.31±0.01	0.34±0.05	0.61±0.07	MS, RI
42	2-Methylfuran	534-22-5	857.51	866	0.41±0.04	0.82±0.28	0.71±0.14	0.50±0.04	MS, RI
43	3-Methylfuran	930-27-8	880.87	851	0.34±0.08	0.34±0.02	0.55±0.07	1.19±0.16	MS, RI
44	2-Ethylfuran	3208-16-0	942.78	944	4.33±2.47	8.91±0.44	10.09±2.30	18.43±4.09	MS, RI, O, S
45	Toluene	108-88-3	1044.20	1040	1.18±0.05	1.79±0.27	1.35±0.28	1.19±0.72	MS, RI
46	Dimethyl disulfide	624-92-0	1078.44	1061	2.76±1.09	1.35±0.09	ND	6.19±0.65	MS, RI

NO.	Aroma Compounds	CAS	RIc[a]	RIr[b]	Compound Content (μg /kg)				Identification
47	N-Methylpyrrole	96-54-8	1137.74	1137	1.01±0.16	1.99±0.38	1.70±0.38	3.54±0.11	MS, RI
48	Pyridine	110-86-1	1186.05	1191	7.83±0.63	8.87±1.54	6.63±1.82	10.10±4.17	MS, RI
49	2,4,5-Trimethyloxazole	20662-84-4	1197.31	1197	71.21±9.84	135.81±6.81	69.95±6.54	33.66±3.07	MS, RI, O, S
50	2-Pentylfuran	3777-69-3	1226.42	1229	7.86±2.16	21.37±3.67	16.40±3.94	24.64±8.77	MS, RI, O, S
51	Benzaldehyde	100-52-7	1525.32	1529	15.29±3.30	10.19±1.81	9.50±1.37	133.00±13.53	MS, RI
52	Guaiacol	90-05-1	1853.74	1859	49.01±0.72	117.12±24.32	128.70±7.26	55.16±0.70	MS, RI, O, S
53	2-Acetopyrrole	1072-83-9	1968.96	1970	1.32±0.07	23.26±21.14	2.73±1.12	1.57±0.05	MS, RI
54	Phenol	108-95-2	1987.59	1993	29.26±1.63	53.64±8.07	62.00±4.65	37.32±2.06	MS, RI
55	1-Ethoxy-2-methylpropane	627-02-1	1336.22	1364	ND	10.23±0.43	ND	ND	MS, RI
56	3-Furaldehyde	498-60-2	1453.96	1455	ND	ND	5.71±1.42	ND	MS, RI
57	Maltol	118-71-8	2002.60	2004	ND	ND	36.36±6.38	ND	MS, RI
58	Trimethylamine	75-50-3	-	-	ND	ND	ND	0.99±0.24	MS
59	3-Methylbutyraldehyde	590-86-3	903.78	902	ND	ND	ND	32.26±4.58	MS, RI
60	2-n-Butylfuran	4466-24-4	1134.72	1123	ND	ND	ND	0.99±0.45	MS, RI
61	Thiazole	288-47-1	1245.56	1240	ND	ND	ND	1.38±0.27	MS, RI
62	Benzeneacetaldehyde	122-78-1	1656.33	1656	ND	ND	ND	11.68±2.14	MS, RI
	Acids								

NO.	Aroma Compounds	CAS	RIc[a]	RIr[b]	Compound Content (μg /kg)				Identification
63	Acetic acid	64-19-7	1432.87	1433	103.29±7.41	145.47±12.12	10.18±4.12	ND	MS, RI, O
64	Isobutyric acid	79-31-2	1554.35	1561	216.54±74.52	275.04±32.67	303.57±57.18	35.43±6.56	MS, RI
65	2-Methylbutyric acid	116-53-0	1653.67	1670	906.28±88.38	722.88±14.39	665.02±27.36	163.29±76.6	MS, RI, O, S
66	4-Methylpentanoic acid	646-07-1	1803.18	1809	ND	ND	9.97±1.99	ND	MS, RI
	Ketones								
67	Acetone	67-64-1	807.95	809	657.05±36.96	701.48±88.21	594.43±78.73	787.24±70.66	MS, RI
68	2-Butanone	78-93-3	888.90	888	225.52±32.75	259.96±9.44	238.02±33.96	489.36±42.58	MS, RI
69	3-Methyl-2-butanone	563-80-4	950.36	929	11.88±4.19	ND	9.57±3.51	ND	MS, RI
70	2,3-Butanedione	431-03-8	959.45	954	263.20±27.18	665.64±55.86	291.21±69.68	149.24±15.31	MS, RI, O, S
71	2-Pentanone	107-87-9	991.99	970	44.84±15.49	56.2±6.00	52.19±9.15	264.21±43.56	MS, RI
72	3-Methyl-2-pentanone	565-61-7	1025.76	1016	26.61±2.54	2.96±0.15	26.34±2.88	ND	MS, RI
73	2-Hexanone	591-78-6	1085.39	1098	5.72±1.86	7.11±1.40	7.15±1.57	46.46±2.86	MS, RI
74	5-Methyl-2-hexanone	110-12-3	1142.71	1146	73.83±3.79	15.21±2.25	118.18±12.04	107.38±7.37	MS, RI
75	2-Heptanone	110-43-0	1181.68	1184	134.73±19.13	67.43±5.74	172.20±5.34	156.27±6.40	MS, RI, O, S
76	3-Hydroxy-3-methyl-2-butanone	115-22-0	1236.66	1243	92.80±16.86	213.09±7.13	76.82±30.99	ND	MS, RI
77	3-Octanone	106-68-3	1251.78	1253	77.46±15.95	94.37±10.74	89.61±12.43	114.59±21.53	MS, RI, O
78	Acetoin	513-86-0	1278.58	1286	490.60±89.67	1963.38±188.10	652.11±174.58	40.63±4.75	MS, RI, O, S

NO.	Aroma Compounds	CAS	RIc[a]	RIr[b]	Compound Content (μg /kg)				Identification
79	2-Hydroxy-3-pentanone	5704-20-1	1351.51	1361	8.18±1.58	32.60±2.21	19.83±5.56	ND	MS, RI
80	Acetophenone	98-86-2	1672.53	1669	1.40±0.16	ND	ND	3.47±0.30	MS, RI
81	2,3-Pentanedione	600-14-6	1055.12	1056	ND	1.68±0.07	1.58±0.25	ND	MS, RI
82	Acetoin acetate	4906-24-5	1369.14	1361	ND	81.21±30.50	ND	ND	MS, RI
83	3-Methyl-1,2-cyclopentanedione	80-71-7	1827.16	1837	ND	6.27±0.81	4.73±0.32	ND	MS, RI
84	6-Methyl-2-heptanone	928-68-7	1234.16	1237	ND	ND	34.89±4.15	8.12±0.25	MS, RI
85	2,3-Heptanedione	96-04-8	1147.57	1153	ND	ND	ND	15.43±1.12	MS, RI
86	2-Octanone	111-13-7	1291.71	1283	ND	ND	ND	10.75±0.92	MS, RI
87	2-Undecanone	112-12-9	1585.63	1586	ND	ND	ND	12.18±4.65	MS, RI
88	2-Dodecanone	6175-49-1	1687.45	1692	ND	ND	ND	21.01±8.55	MS, RI
89	2-Tridecanone	593-08-8	1779.18	1817	ND	ND	ND	304.91±175.27	MS, RI
	Esters								
90	Ethyl acetate	141-78-6	874.82	889	2.64±0.49	8.69±1.17	3.62±1.13	1.14±0.06	MS, RI
91	Methyl 2-methylbutyrate	868-57-5	1017.87	1004	7.00±1.97	6.12±0.87	10.58±1.37	25.72±0.20	MS, RI, O, S
92	4-Hexanolide	695-06-7	1717.36	1715	6.34±0.38	ND	10.18±3.04	ND	MS, RI
93	Acetic acid methyl ester	79-20-9	820.57	839	ND	54.61±2.78	89.29±6.62	87.81±15.36	MS, RI
94	Methyl tiglate	6622-76-0	1188.95	1188	ND	1.61±0.04	ND	ND	MS, RI

NO.	Aroma Compounds	CAS	RIc[a]	RIr[b]	Compound Content (μg /kg)				Identification
95	Hexyl acetate	142-92-7	1267.22	1275	ND	2.31±0.73	ND	ND	MS, RI
96	Methyl isobutyrate	547-63-7	910.97	921	ND	ND	3.47±0.30	ND	MS, RI
97	Methylbutyrolactone	1679-47-6	1589.86	1555	ND	ND	0.38±0.06	ND	MS, RI
98	Methyl isovalerate	556-24-1	1028.11	1011	ND	ND	ND	29.48±1.21	MS, RI
99	2-Methylbutyric acid ethyl ester	7452-79-1	1057.64	1062	ND	ND	ND	3.70±0.33	MS, RI
100	Isovaleric acid ethyl ester	108-64-5	1076.52	1059	ND	ND	ND	2.12±0.28	MS, RI

a: Retention indices of the compounds calculated on a DB-WAX column (60 m x 0.25 mm x 0.25 μm) with a homologous series of n-alkanes (C7-C30).

b: Retention index obtained from a specific database (https://webbook.nist.gov/chemistry/name-ser/).

c: MS, RI, O, and S represent the identification of the product by the NIST 20 mass spectrometry database, linear retention index, olfactometry, and standards, respectively.

-: Not confirmed.

ND: Not detected.

TableS7. Intensity of aroma compounds detected by GC-O in four fermented soybeans

NO.	Aroma Compounds	GC-O (average odor intensity)			
		FS1	FS2	FS3	FS4
1	2-Ethylfuran	1.00	1.50	1.50	2.17
2	2,3-Butanedione	1.00	1.83	1.33	1.00

NO.	Aroma Compounds	GC-O (average odor intensity)			
3	2-Methylbutyric acid methyl ester	1.00	1.00	1.17	2.00
4	2-Pentylfuran	1.00	1.50	1.00	1.67
5	Acetoin	1.50	2.00	1.67	1.00
6	2,5-Dimethylpyrazine	3.00	3.17	3.33	4.00
7	2-Heptanone	1.83	1.00	2.00	1.83
8	2,4,5-Trimethyloxazole	1.67	2.00	1.50	1.00
9	3-Octanone	1.00	1.00	1.00	1.50
10	1-Octanol	1.00	1.83	1.00	1.00
11	3-Octanol	1.50	1.17	1.83	2.17
12	2,3,5-Trimethylpyrazine	2.67	3.00	3.33	2.33
13	Acetic acid	1.00	1.50	1.00	0.00
14	1-Octen-3-ol	2.00	2.33	2.50	2.83
15	2,3,5,6-Tetramethylpyrazine（Tetramethylpyrazine）	1.50	2.33	1.67	1.00
16	3-Furanmethanol	1.00	1.33	1.50	1.17
17	3-Ethyl-2,5-dimethylpyrazine	1.00	1.67	1.00	1.00
18	2-Methylbutyric acid	3.67	3.00	2.83	2.00

NO.	Aroma Compounds	GC-O (average odor intensity)			
19	Guaiacol	2.00	3.17	3.33	2.17

TableS8. ROAV values of all aroma compounds.

NO.	Aroma Compounds	Threshold [a] (μg /kg)	ROAV value			
			FS1	FS2	FS3	FS4
1	2-Ethylfuran	2.3	1.88	3.87	4.39	8.01
2	2,3-Butanedione	3	87.73	221.88	97.07	49.75
3	2-Methylbutyric acid methyl ester	0.25	28.00	24.46	42.32	102.87
4	Dimethyl disulfide	0.06	45.97	22.57	ND	103.23
5	2-Heptanone	70	1.92	0.96	2.46	2.23
6	2,4,5-Trimethyloxazole	5	14.24	27.16	13.99	6.73
7	2-Pentylfuran	4.8	1.64	4.45	3.42	5.13
8	Acetoin	40	12.27	49.08	16.30	1.02
9	2,5-Dimethylpyrazine	20	93.44	122.97	96.39	289.06
10	2,3,5-Trimethylpyrazine	71	65.57	69.61	72.69	53.51
11	1-Octen-3-ol	7	40.54	60.73	77.60	86.58
12	3-Ethyl-2,5-dimethylpyrazine	25	2.54	4.54	2.58	1.96

NO.	Aroma Compounds	Threshold [a]	ROAV value			
13	2-Methylbutyric acid	0.7	1294.69	1032.69	950.03	233.27
14	Guaiacol	0.75	65.35	156.16	171.61	73.54
15	Methyl isovalerate	0.4	ND	ND	ND	73.71
16	2-Methylbutyric acid ethyl ester	0.15	ND	ND	ND	24.66
17	Isovaleric acid ethyl ester	2	ND	ND	ND	1.06
18	Benzeneacetaldehyde	9	ND	ND	ND	1.30
19	Methanethiol	2	0.35	0.74	0.60	0.45
20	Glycidol	-	/	/	/	/
21	Carbon disulfide	-	/	/	/	/
22	Dimethyl sulfide	30	0.01	0.01	0.01	0.02
23	Acetone	100000	0.01	0.01	0.01	0.01
24	2-Methylfuran	8200000	0.00	0.00	0.00	0.00
25	Ethyl Acetate	11000	0.00	0.00	0.00	0.00
26	3-Methylfuran	-	/	/	/	/
27	2-Butanone	3000	0.08	0.09	0.08	0.16
28	3-Methyl-2-butanone	10000	0.00	ND	0.00	ND
29	2-Pentanone	300	0.15	0.19	0.17	0.88

NO.	Aroma Compounds	Threshold [a]	ROAV value			
30	3-Methyl-2-pentanone	-	/	/	/	/
31	Toluene	6000	0.00	0.00	0.00	0.00
32	2-Hexanone	90	0.06	0.08	0.08	0.52
33	2-Pentanol	85000	0.00	ND	ND	ND
34	N-Methylpyrrole	-	/	/	/	/
35	5-Methyl-2-hexanone	7000	0.01	0.00	0.02	0.02
36	Pyridine	2000	0.00	0.00	0.00	0.01
37	3-Methyl-2-pentanol	-	/	/	/	/
38	2-Methyl-1-butanol	6000	0.00	0.00	0.00	0.00
39	Pyrazine	300000	0.00	0.00	0.00	0.00
40	3-Hydroxy-3-methyl-2-butanone	400000	0.00	0.00	0.00	ND
41	3-Methyl-3-buten-1-ol	-	/	/	/	/
42	3-Octanone	1000	0.08	0.09	0.09	0.11
43	2-Methylpyrazine	280000	0.00	0.00	0.00	0.00
44	2-Heptanol	100	0.12	ND	ND	ND
45	Prenol	-	/	/	/	/
46	1-Hexanol	200	0.53	0.78	0.62	0.69

NO.	Aroma Compounds	Threshold [a]	ROAV value			
47	2-Hydroxy-3-pentanone	-	/	/	/	/
48	3-Octanol	100	0.12	0.01	0.21	0.34
49	2-Ethyl-5-methylpyrazine	1000	0.03	0.05	0.03	0.03
50	Acetic acid	1200	0.09	0.12	0.01	ND
51	2,6-Diethylpyrazine	-	/	/	/	/
52	2,3,5,6-Tetramethylpyrazine	100000	0.01	0.02	0.01	0.00
53	2,3,5-Trimethyl-6-ethylpyrazine	-	/	/	/	/
54	Benzaldehyde	1500	0.01	0.01	0.01	0.09
55	2,3-Butanediol	1000000	0.00	0.00	0.00	0.00
56	Isobutyric acid	1000	0.22	0.28	0.30	0.04
57	(R,R)-2,3-Butanediol	400000	0.00	0.00	0.00	ND
58	3-Furanmethanol	-	/	/	/	/
59	Acetophenone	3000	0.00	ND	ND	0.00
60	4-Hexanolide	13000	0.00	ND	0.00	ND
61	Benzyl alcohol	5500	0.00	0.00	0.00	0.00
62	Phenylethyl Alcohol	41100	0.00	0.00	0.00	ND
63	2-Acetopyrrole	100000	0.00	0.00	0.00	0.00

NO.	Aroma Compounds	Threshold [a]	ROAV value			
64	Phenol	60000	0.00	0.00	0.00	0.00
65	Acetic acid methyl ester	550000	ND	0.00	0.00	0.00
66	2,3-Pentanedione	5	ND	0.34	0.32	ND
67	2-Methyl-1-propanol	5250	ND	0.00	ND	ND
68	1-Penten-3-ol	3000	ND	0.00	0.00	0.00
69	Methyl tiglate	-	/	/	/	/
70	Hexyl acetate	40	ND	0.06	ND	ND
71	2,6-Dimethylpyrazine	10000	ND	0.08	ND	ND
72	1-Ethoxy-2-methylpropane	-	/	/	/	/
73	Acetoin acetate	-	/	/	/	/
74	3-Methyl-1,2-cyclopentanedione	2000	ND	0.00	0.00	ND
75	Methyl isobutyrate	-	/	/	/	/
76	3-Methyl-2-butanol	-	/	/	/	/
77	6-Methyl-2-heptanone	-	/	/	/	/
78	3-Furaldehyde	-	/	/	/	/
79	2-Ethyl-1-hexanol	-	/	/	/	/
80	3,5-Diethyl-2-methyl-pyrazine	-	/	/	/	/

NO.	Aroma Compounds	Threshold [a]	ROAV value			
81	Methylbutyrolactone	-	/	/	/	/
82	4-Methylpentanoic acid	-	/	/	/	/
83	Maltol	20000	ND	ND	0.00	ND
84	Trimethylamine	600	ND	ND	ND	0.00
85	3-Methylbutyraldehyde	200	ND	ND	ND	0.16
86	2-Pentanol	85000	ND	ND	ND	0.00
87	2-n-Butylfuran	-	/	/	/	/
88	Acetyl valeryl	-	/	/	/	/
89	2-Hexanol	6700	ND	ND	ND	0.00
90	Thiazole	23000	ND	ND	ND	0.00
91	2-Octanone	40	ND	ND	ND	0.27
92	6-Methyl-2-heptanol	-	/	/	/	/
93	2-Ethyl-6-methylpyrazine	860000	ND	ND	ND	0.00
94	1-Octanol	90	ND	ND	ND	0.02
95	2-Undecanone	30	ND	ND	ND	0.41
96	2-Dodecanone	460000	ND	ND	ND	0.00
97	2-Tridecanone	500000	ND	ND	ND	0.00

NO.	Aroma Compounds	Threshold [a]	ROAV value			
98	2-Tridecanol	-	/	/	/	/
99	2-Tetradecanol	-	/	/	/	/
100	2-Pentadecanol	-	/	/	/	/

a: The odor threshold in water, derived from previously reported literature. [1-4]

-: Not available

/: Not calculable

ND: Not detected.

Table S9. Screening of key aroma skeleton components and auxiliary flavor components in combination with previous findings.

NO.	SGR1 [a]	SGR2 [b]	GR1 [c]	GR2 [d]	Key aroma skeleton compounds [e]	Auxiliary compounds [f]
1	2-Ethylfuran	3-Methyl-2-butanone	2-Ethylfuran	2-Ethylfuran	2-ethylfuran	Acetoin
2	2,3-Butanedione	2-Ethylfuran	2,3-Butanedione	2,3-Butanedione	2,3-butanedione	3-Octanone
3	Methyl 2-methylbutyrate	2,3-Butanedione	Methyl 2-methylbutyrate	Methyl 2-methylbutyrate	methyl 2-methylbutyrate	1-Octanol
4	2-Pentylfuran	Methyl 2-methylbutyrate	2-Heptanone	2-Heptanone	2-heptanone	3-Octanol
5	Acetoin	2-Heptanone	2,4,5-Trimethyloxazole	2,4,5-Trimethyloxazole	2,4,5-trimethyloxazole	Acetic acid
6	2,5-Dimethylpyrazine	2,4,5-Trimethyloxazole	2-Pentylfuran	2-Pentylfuran	2-pentylfuran	3-Furanmethanol
7	2-Heptanone	2-Pentylfuran	Acetoin	3-Octanone	2,5-dimethylpyrazine	Dimethyl disulfide
8	2,4,5-Trimethyloxazole	3-Octanone	2,5-Dimethylpyrazine	2,5-Dimethylpyrazine	2,3,5-trimethylpyrazine	Methyl isovalerate

NO.	SGR1 [a]	SGR2 [b]	GR1 [c]	GR2 [d]	Key aroma skeleton compounds [e]	Auxiliary compounds [f]
9	3-Octanone	2-Octanone	2,3,5-Trimethylpyrazine	2,3,5-Trimethylpyrazine	1-octen-3-ol	2-Methylbutyric acid ethyl ester
10	1-Octanol	2,5-Dimethylpyrazine	1-Octen-3-ol	1-Octen-3-ol	3-ethyl-2,5-dimethylpyrazine	Isovaleric acid ethyl ester
11	3-Octanol	2-Nonanone	3-Ethyl-2,5-diMethylpyrazine	3-Ethyl-2,5-diMethylpyrazine	2-methylbutyric acid	Benzeneacetaldehyde
12	2,3,5-Trimethylpyrazine	1-Hexanol	2-Methylbutyric acid	2-Methylbutyric acid	Guaiacol	3-Methyl-2-butanone
13	Acetic acid	2-Ethyl-5-methylpyrazine	Guaiacol	Guaiacol		2-Octanone
14	1-Octen-3-ol	2,3,5-Trimethylpyrazine				2-Nonanone
15	2,3,5,6-Tetramethylpyrazine	1-Octen-3-ol				1-Hexanol
16	3-Furanmethanol	3-Ethyl-2,5-dimethylpyrazine				2-Ethyl-5-methylpyrazine
17	3-Ethyl-2,5-diMethylpyrazine	2,3,5,6-Tetramethylpyrazine				2,3,5,6-Tetramethylpyrazine
18	2-Methylbutyric acid	3-Octanol				
19	Guaiacol	2-Methylbutyric acid				
20	Dimethyl disulfide	Guaiacol				
21	Methyl isovalerate					
22	2-Methylbutyric acid ethyl					

NO.	SGR1 [a]	SGR2 [b]	GR1 [c]	GR2 [d]	Key aroma skeleton compounds [e]	Auxiliary compounds [f]
	ester					
23	Isovaleric acid ethyl ester					
24	Benzeneacetaldehyde					

[a] Compounds identified in this study using GC-O with ROAV>1; [b] Compounds identified in previous studies using GC-O with ROAV>1; [c] Compounds identified in this study using GC-O combined with ROAV>1; [d] Compounds identified in previous studies using GC-O combined with ROAV>1; [e] Aroma compounds common to GR1 and GR2; [f] The remaining aroma components of GR1 and GR2 except for the common compounds.

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