Table S1: The plasmids used in this study and their relevant characteristics

Plasmid	Main Characteristics	Reference
pMD™-18-T	E. coli vector, AmpR, ColE1, Lac promoter, LacZ alpha terminator	Commercial stock
pGL1	Glyceraldehyde 3 phosphate dehydrogenase (gpd) promoter expressed in pMDTM-18-T vector	This study
pGL3	Hygromycin resistant gene expressed by promoter gpd in pGL1	This study
pGL3-LUP2	Amyrin synthase LUP2(Q8RWT0) from Arabidopsis thaliana expressed by gpd promoter in pGL3	This study
pGL3-bAS	Beta amyrin synthase bAS(O82140) from Panax ginseng expressed by gpd promoter in pGL3	This study
pGL3-CYP716A12	Beta amyrin monooxygenase CYP716A15(F6H9N6) fromVitis vinifera expressed by gpd promoter in pGL3	This study
pGL3-LUP2-CYP716A15	Amyrin synthase-Beta amyrin monooxygenase expressed by gpd promoter in Pgl3	This study
pGL3- bAS-CYP716A15	Beta amyrin synthase-Beta amyrin monooxygenase expressed by gpd promoter in pGL3	This study
pGL3- LUP2-bAS-CYP716A15	Amyrin synthase-Beta amyrin synthase-Beta amyrin monooxygenase expressed by gpd promoter in pGL3	This study
pGL3- LaeA-LUP2-bAS-CYP716A15	Transcription factor LaeA- Amyrin synthase-Beta amyrin synthase-Beta amyrin monooxygenase expressed by gpd promoter in pGL3	This study
pGL3- VeA-LaeA-LUP2-bAS-CYP716A15	Velvet protein VeA-Transcription factor LaeA- Amyrin synthase-Beta amyrin synthase-Beta amyrin monooxygenase expressed by gpd promoter in pGL3	This study
pGL3- VeA-VelB-LaeA-LUP2-bAS-CYP716A15	Velvet protein VeA-VelB-Transcription factor LaeA- Amyrin synthase-Beta amyrin synthase-Beta amyrin monooxygenase expressed by gpd promoter in pGL3	This study
pGL1-AsCpf1	CRISPR-AsCpf1 sequence expressed by gpd promoter in pGL1	This study
pGL1-LS sgRNA	CRISPR-AsCpf1 sgRNA sequence expressed by gpd promoter in pGL1	This study
pGL1-LS sgRNA-AsCpf1	CRISPR-sgRNA-AsCpf1 sequence expressed by gpd promoter in pGL1	This study
pGL1-Hgr-LS sgRNA-AsCpf1	Hygromycin resistant gene and CRISPR-LS-sgRNA-AsCpf1 sequence expressed by gpd promoter in pGL1	This study

Table S2: Primers for plasmids construction used in this study

Primers	Sequence (5'-3')	For plasmid
gpd -F	gtccggcgtagcctaggatcgaCatcgatGtTGTGAGAAAGAGACGAGGTG	pGL1
gpd -R	tatagtcgactcctcctttcgctagccGCGGCGTTCTAAGTAGCCTCAAAC	pGL1
HgR-F	GTTTGGCCCCCTTCTCACAGGTCAAGcctgaactcaccgcgacgtctg	pGL3
HgR-R	ACAGTCCCCAAACGCACATtctcGGTACCctattcctttgccctcggacg	pGL3
LUP2-F	GTTTGGCCCCCTTCTCACAGGTCAATGGAAACTTAAAATCGGAGAAGGCA	pGL3-LUP2
LUP2-R	GTCCCCAAACGCACATtctcGGTACCTTATTACAGGTCCTGGTGCGTGGCAAAT	pGL3-LUP3
bAS-F	AtaaccgcagGtaaccgcagTGGAAACTTAAAATTGCCGAGGG	pGL3-bAS
bAS-R	GCACATtctcGGTACCTTATTAATTGTTCTCAAGAGGGAGAAGGA	pGL4-bAS
CYP716A15-F	CAGGTCAAGtaaccgcagGAACCTAACTTCTACCTTTCTCTGC	pGL3-CYP716A15
CYP716A15-R	CAAACGCACATtctcGGTACCTTAAGCTTTGTGGGGGTACAG	pGL3-CYP716A15
LUP2-CYP716A15-F	GtaaccgcagGAACCTAACTTCTACCTTTCTCTGCTGCTGCTGTTTGTTA	pGL3-LUP2-CYP716A15
LUP2-CYP716A15-R	AAGACCTCCCTATTCGTCTGTACCCCCACAAAGCTTAAGGTACCgagaAT	pGL3-LUP2-CYP716A15
bAS-CYP716A15-R	AtaaccgcagGtaaccgcagTGGAAACTTAAAATTGCCGAGGGTCAAAAC	pGL3- bAS-CYP716A15
bAS-CYP716A15-R	GTCCCCAAACGCACATtctcGGTACCTTATTAATTGTTCTCAAGAGGGAG	pGL3- bAS-CYP716A15
LUP2-bAS-CYP716A15-F	CCCTTCTCACAGGTCAATGGAAACTTAAAATCGGAGAAGGCAACGGCGA	pGL3- LUP2-bAS-CYP716A15
LUP2-bAS-CYP716A15-R	CCAGGACCTGTAATAAGGTACCgagaATGTGCGTTTGGGGACTGTTCGTAG	pGL3- LUP2-bAS-CYP716A15
LaeA-LUP2-bAS-CYP716A15-F	CTTGGCCCCCTTCTCACAGGTCAAGTCTCGTGTGGATCCCGTCCAATTCA	pGL3- LaeA-LUP2-bAS-CYP716A15
LaeA-LUP2-bAS-CYP716A15-R	AGTCCCCAAACGCACATtctcGGTACCTTATAATGCTTTTCTTCGTGCGT	pGL3- LaeA-LUP2-bAS-CYP716A15
VeA-LaeA-LUP2-bAS-CYP716A15-F	AGTCCCCAAACGCACATtctcGGTACCTTATCCGACAACCTCATAGAGAG	pGL3- VeA-LaeA-LUP2-bAS-CYP716A15
VeA-LaeA-LUP2-bAS-CYP716A15-R	TCATCGTTTGGCCCCCTTCTCACAGGTCAAGTTTCTTATCCTCTGCTTTA	pGL3- VeA-LaeA-LUP2-bAS-CYP716A15
VeA-VelB-LaeA-LUP2-bAS-CYP716A15-F	CAAACGCACATtctcGGTACCTTAATCGTAATCATCACCGTCATCATCAT	pGL3- VeA-VelB-LaeA-LUP2-bAS-CYP716A15
VeA-VelB-LaeA-LUP2-bAS-CYP716A15-R	GTTTGGCCCCCTTCTCACAGGTCAAATGTATGCTATCGAGGAGCGAGCTC	pGL3- VeA-VelB-LaeA-LUP2-bAS-CYP716A15
AsCpf1-F	TTTGGCCCCCTTCTCACAGGTCAAGacacagttcgagggctttac	pGL1-AsCpf1
AsCpf1-R	GCACATtctcGGTACCctctcagatttacttttttcttttttgcct	pGL1-AsCpf1
LS sgRNA-F	CCCCCTTCTCACAGGTCAAGtaatttctactcttgtagatAAGGCACGCGCATGCCTCCA	pGL1-LS sgRNA
LS sgRNA-R	taatttctactcttgtagatAAGGCACGCGCATGCCTCCAatctgagagGGTACCgagaATG	pGL1-LS sgRNA
LS sgRNA-AsCpf1-F	gtatcaggtgagcaagacactgcggtt	pGL1-LS sgRNA-AsCpf1
LS sgRNA-AsCpf1-R	acaaaaggccggcggccacgaaaaaggccggcc	pGL1-LS sgRNA-AsCpf1
Hgr-LS sgRNA-AsCpf1-F	TTTGGCCCCCTTCTCACAGGTCAAGcctgaactcaccgcgacgtctgtcg	pGL1-Hgr-LS sgRNA-AsCpf1
Hgr-LS sgRNA-AsCpf1-R	actcgtccgagggcaaaggaatagGGTACCgagaATGTGCGTTTGGGGA	pGL1-Hgr- sgRNA-AsCpf1

Table S3: RT-qPCR primers used in this study

Enzyme	Type	Sequence	Start	Length	Tm	GC Percent
HMGR	HMGR-Forward Primer	GAGGTTCGTCGTATCAAGAAGG	1180	22	62.103	50
	HMGR-Reverse Primer	GAGACAAGACTCCGCGAATAG	1288	21	61.856	52.381
MVK	MVK-Forward Primer	CCGTCCCTATGTATGGCTATTC	527	22	60.56	50
	MVK-Reverse Primer	ATTGTCGGAGTATGGTGTGGA	863	21	61.78	50
SQS	SQS-Forward Primer	CAAGGAAGTGGAGGGTGATTTA	201	22	61.968	45.455
	SQS-Reverse Primer	TGTGGAATTGTCGGAGTATGG	331	21	61.908	47.619
SE	SE-Forward Primer	GCTATTCCCGTCCCTATGTATG	1350	22	61.703	50
	SE-Reverse Primer	CAGTGAAGAAGTGTCGGAAGA	1461	21	61.665	47.619
LS	LS-Forward Primer	GCGCTCATGTACGCGAAATA	1960	20	62.866	50
	LS-Reverse Primer	CTTGTTGAACACGCCCTCTATC	2082	22	62.903	50
18S rRNARrna	18S Rrna-Forward Primer	CCTCGACGAAGTGCCATTTA	426	20	62.06	50
	18S Rrna-Reverse Primer	GCCAGGGTTTCCCATAGTTTA	523	21	62.039	47.619

Table S4: Molecular characteristics and origin of major genes used in this study

Gene/Origin	Amino acids (base pairs)	Protein homologue in Ganoderma spp.	Similarity/Identity (%)	Proposed function in the engineered mushroom
LUP2/Arabidopsis thaliana	763(2683)	*G. lucidum* LSS	46/52	Converts 2,3-oxidosqualene to lupeol, beta-amyrin and alpha-amyrin
bAS/Panax ginseng	763(2298)	*G. lucidum* LSS	53/58	Converts 2,3-oxidosqualene into beta-amyrin
CYP716A15/Vitis vinifera	480(1443)	*G. leucocontextum* Cytochrome P450	27/28	Catalyzes the carboxylation of beta-amyrin, alpha-amyrin and lupeol to form oleanolic acid ursolic acid and betulinic acid respectively.
VeA/Ganoderma leucocontextum	196(588)	*G. leucocontextum* VeA	100/100	Forms a heterodimer with VelB and stimulates spore development and fruiting body formation
VelB/Ganoderma leucocontextum	531 (1593）	*G. leucocontextum* VelB	85.38/85	Forms a heterodimer with VeA and stimulate the formation of fruiting bodies
LaeA/Ganoderma lucidum	378(1137)	*G. lucidum* LaeA	96.83/97	Regulator secondary metabolism and the development of the mushroom

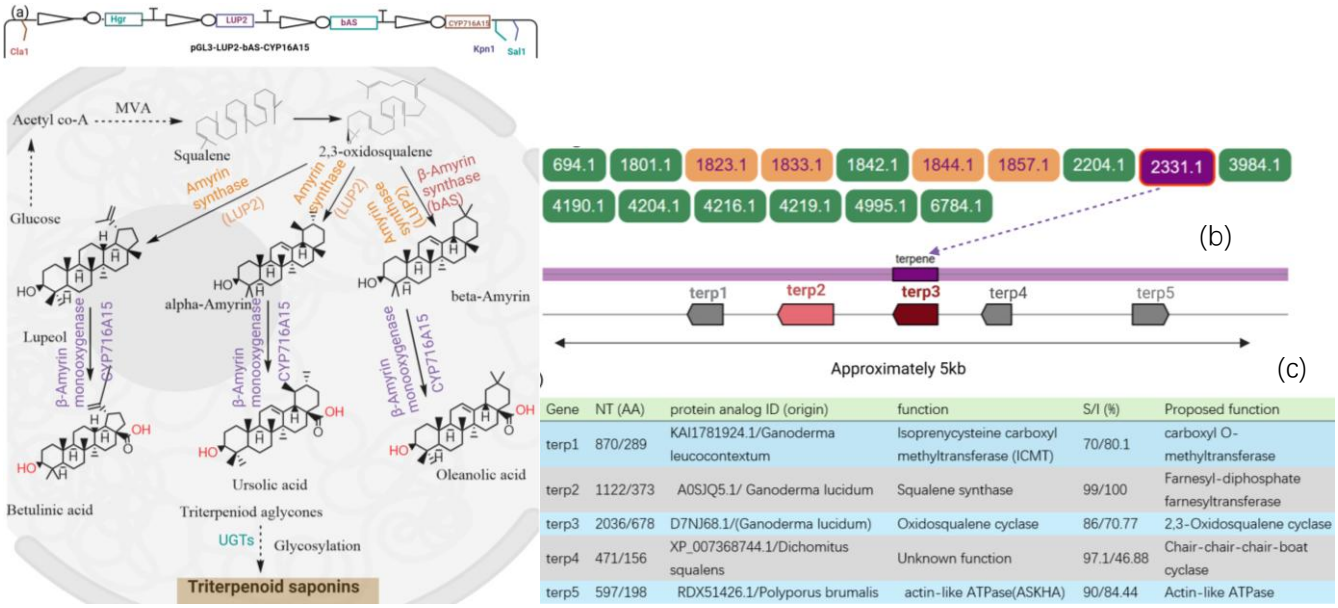
Table S5: Ganoderic acid profile produced by the GLE029 strain

Name	mz	HPLC-MS/MS fragments	Actual mass	Retention time (RT)	Formular	Quantification (g/L)	
						pGL3	GLE029
Ganoderic acid B*	516.691	501.11025, 483.1627	516.7	2.1002	C30H44O7	0.001	0.1212
Ganoderenic acid D	535.2648691	511.26307, 494.8652	512.6	2.2355	C30H40O7	0.004	0.1032
Ganoderic acid alpha	595.2994325	497.95117, 480.67152	574.7	2.5987	C32H46O9	0	0.07211
Ganoderenic acid B	497.2915517	513.08641, 495.18923	514.6	2.8195	C30H42O7	0	0.03
Ganoderic acid F*	612.3203967	511.0825, 495.16723	570.7	3.0615	C32H42O9	0	0.005
Ganoderic acid I*	553.2723296	515.6728, 495.16723	532.7	3.5889	C30H44O8	0.07	0.208
Lucidenic acid E2	499.2700904	480.7825	516.6	3.8167	C29H40O8	0.008	0.1036
Lucidenic acid K	493.2140903	475.2711	472.6	3.8738	C27H36O7	0	0.06
Lucidenic acid L	509.223562	501.83623	474.6	3.9595	C27H38O7	0	0.01
Lucidenic acid J*	1025.513618	513.17298, 452.8923	490.6	4.0023	C27H38O8	0.04	0.05
Lucidenic acid E*	1031.517413	516.17263, 485.16728	515.6	4.3444	C29H40O8	0.01	0.04
Ganoderic acid G*	1082.594045	531.02671, 513.02671	532.7	4.9426	C30H44O8	0	0.06
Ganoderic acid theta	553.276101	551.26712, 497.51728	530.6	5.3417	C30H42O8	0	0.007
Ganoderiol D	453.3361445	411.36728, 267.81627	488.7	5.89725	C30H48O5	0.006	0.005
Ganoderic acid A*	516.309	515.36722, 497.10836	516.7	7.25	C30H44O7	0.008	0.06
Lanosterin*	427.3890524	427.67318	426.7	8.6332	C30H50O	0.03	0.06

*Means compound verified with the authentic standard

Table S6: Quantification of the meroterpenoids produced by the GLE029 strain

Name	mz	HPLC-MS/MS mz fragments	Actual mass	Retention time (RT)	Formular	Quantification (g/L)	
						pGL3	GLE029
10'-Hydroxy-GHQ	261.1496	290.11136, 291.12360	262.34	12.093	C16H22O3	0	1.57
(+)-chizhine A	289.10809	265.14789	290.31	7.785	C16O18O5	0	2.36
(+)-chizhine C	305.10275	306.10617, 307.10818	306.31	6.486	C16H18O6	0.38	6.79



Gene	NT (AA)	protein analog ID (origin)	function	S/I (%)	Proposed function
terp1	870/289	KAI1781924.1/Ganoderma leucocontextum	Isoprenycysteine carboxyl methyltransferase (ICMT)	70/80.1	carboxyl O-methyltransferase
terp2	1122/373	A0SJQ5.1/ Ganoderma lucidum	Squalene synthase	99/100	Farnesyl-diphosphate farnesyltransferase
terp3	2036/678	D7NJ68.1/(Ganoderma lucidum)	Oxidosqualene cyclase	86/70.77	2,3-Oxidosqualene cyclase
terp4	471/156	XP_007368744.1/Dichomitus squalens	Unknown function	97.1/46.88	Chair-chair-chair-boat cyclase
terp5	597/198	RDX51426.1/Polyporus brumalis	actin-like ATPase(ASKHA)	90/84.44	Actin-like ATPase

Fig. S1: (a) The illustrative metabolic map of the co-expressed -LUP2- bAS -CYP716A15 pathway. (b) The biosynthetic gene clusters in the mushroom mycelium. The clusters in green colour are the 11 nonribosomal peptide synthases (NRPS); the clusters in brown colour are the 4 polyketide synthases (PKS), and the purple cluster is the terpene gene clusters. (c) The reconstructed terpenoid gene cluster in the mushrroom.

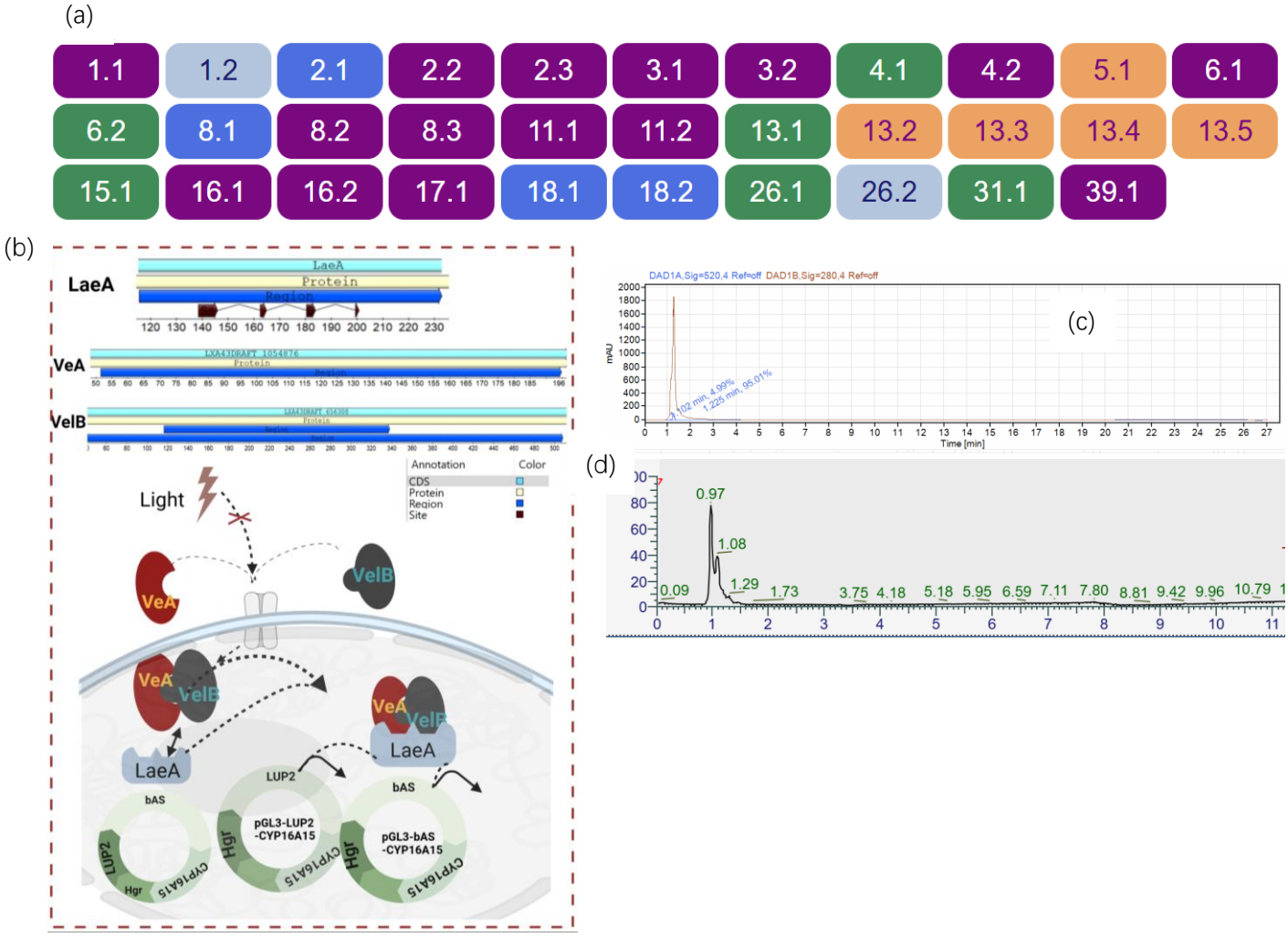


Fig. S2: The biosynthetic gene clusters from the sequenced genome of the fruity body of the mushroom. The clusters in green colour are the six (6) nonribosomal peptide synthases (NRPS); the clusters in brown colour are the five (5) polyketide synthases (PKS), and the purple cluster is the fifteen (15) terpene gene clusters; the blue colours are the four (4) RiPP, and the light blue are the two (2) hybrid gene clusters. (b) The UGENE-based structural analysis of the VeA – VelB- LaeA proteins and their proposed reaction mechanism. (c-d) The HPLC and LC-MS/MS of the meroterpenoids.

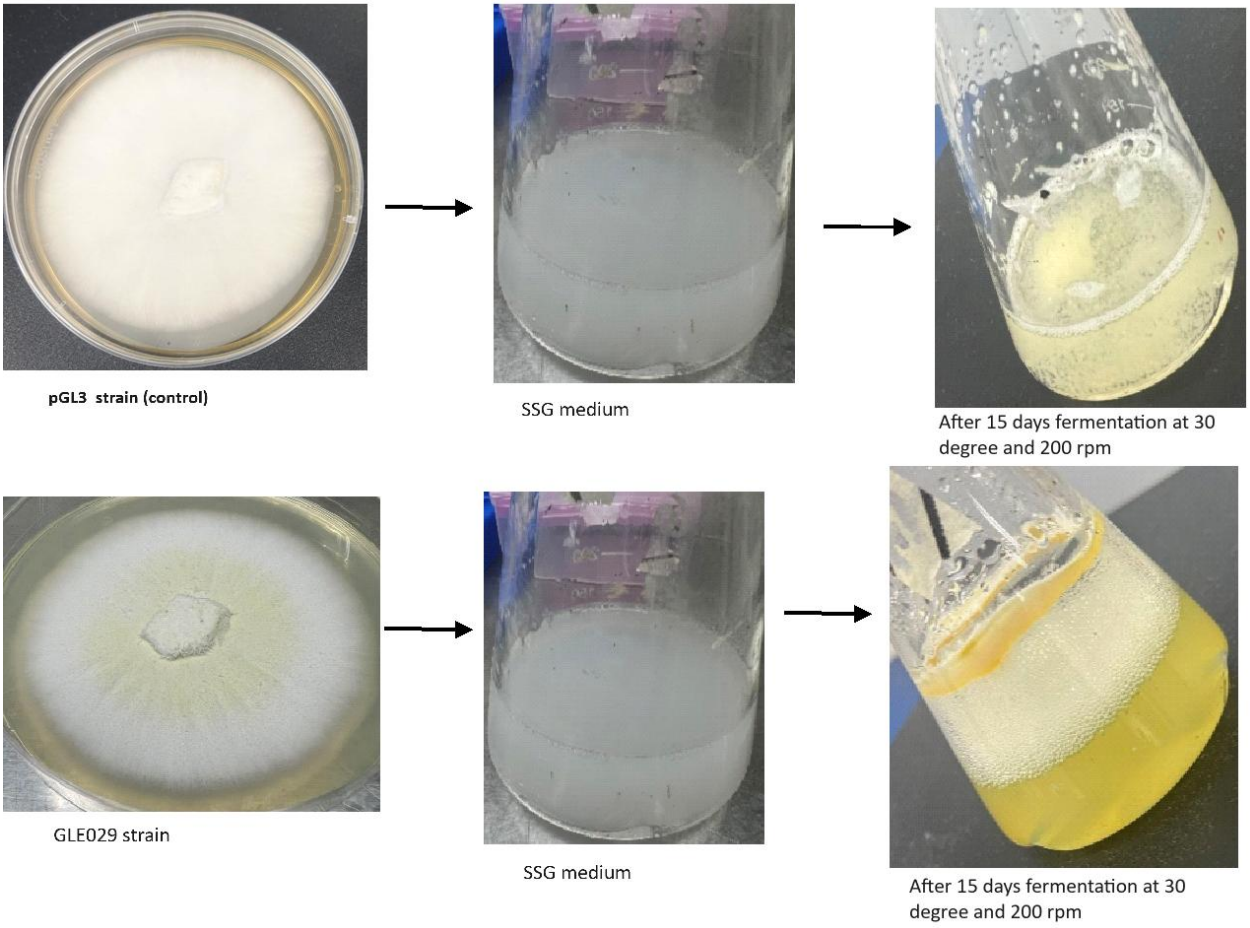


Fig. S3:The morphological appearance of the GLE029 and pGL3 strain (control) and its fermentation products

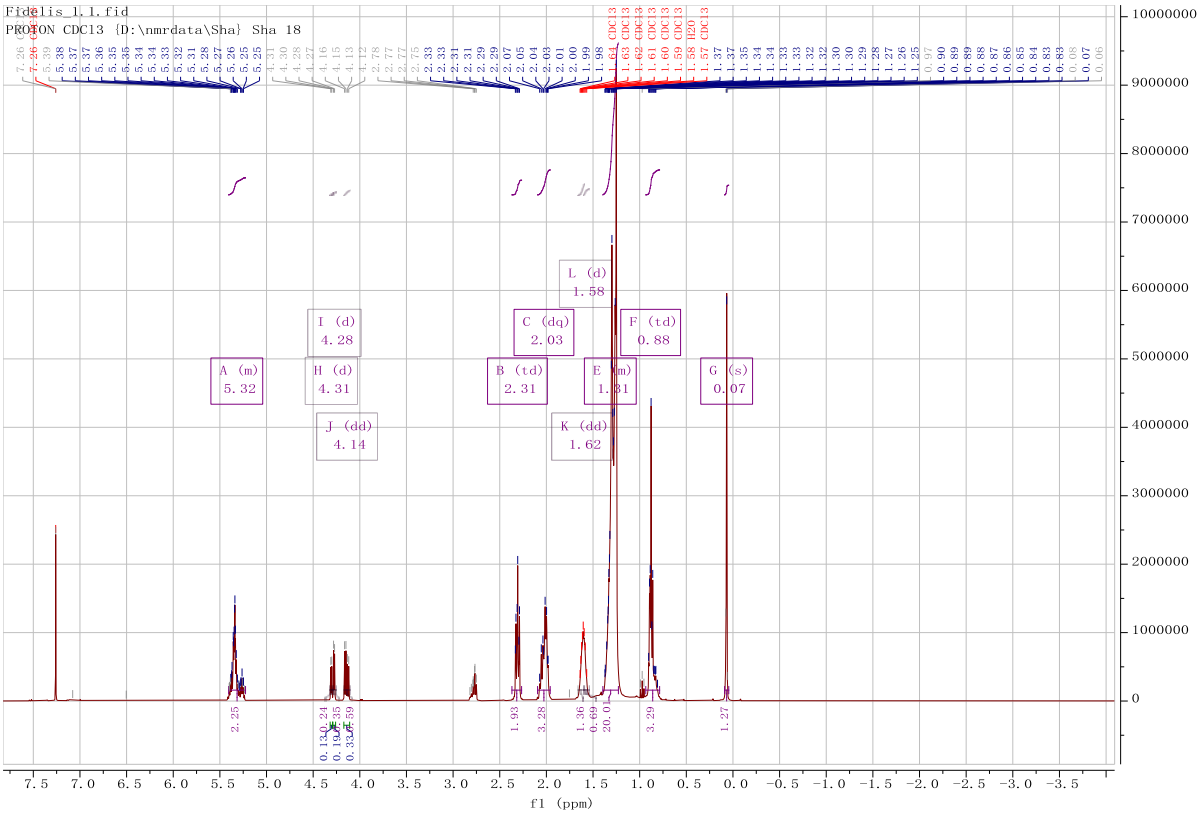


Fig. S4: The ^{1}H NMR Spectrum of (**1**) (^{1}H NMR (400 MHz, $CDCl_3$)

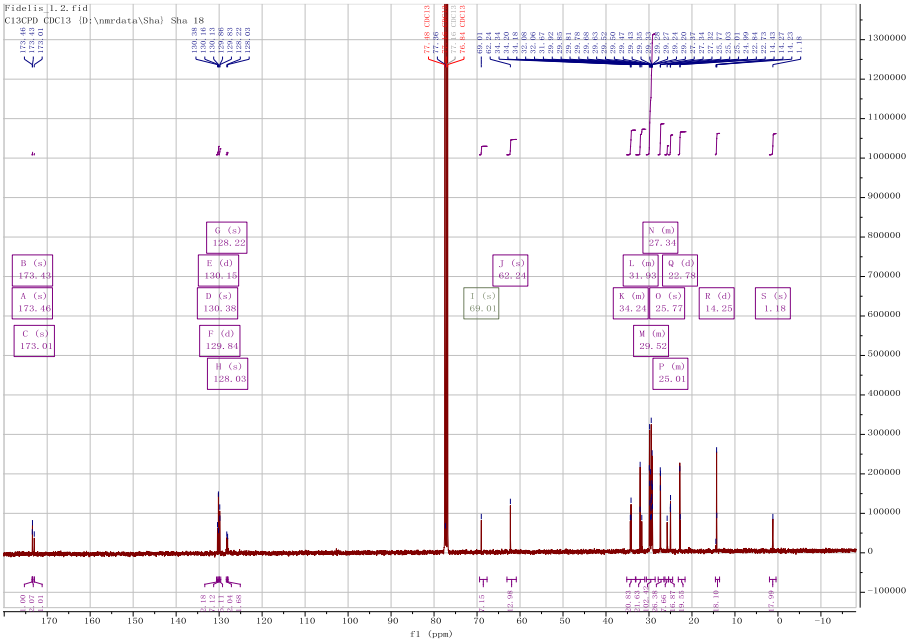


Fig.S5: The ^{13}C NMR spectrum of (**1**). (^{13}C NMR (101 MHz, $CDCl_3$)

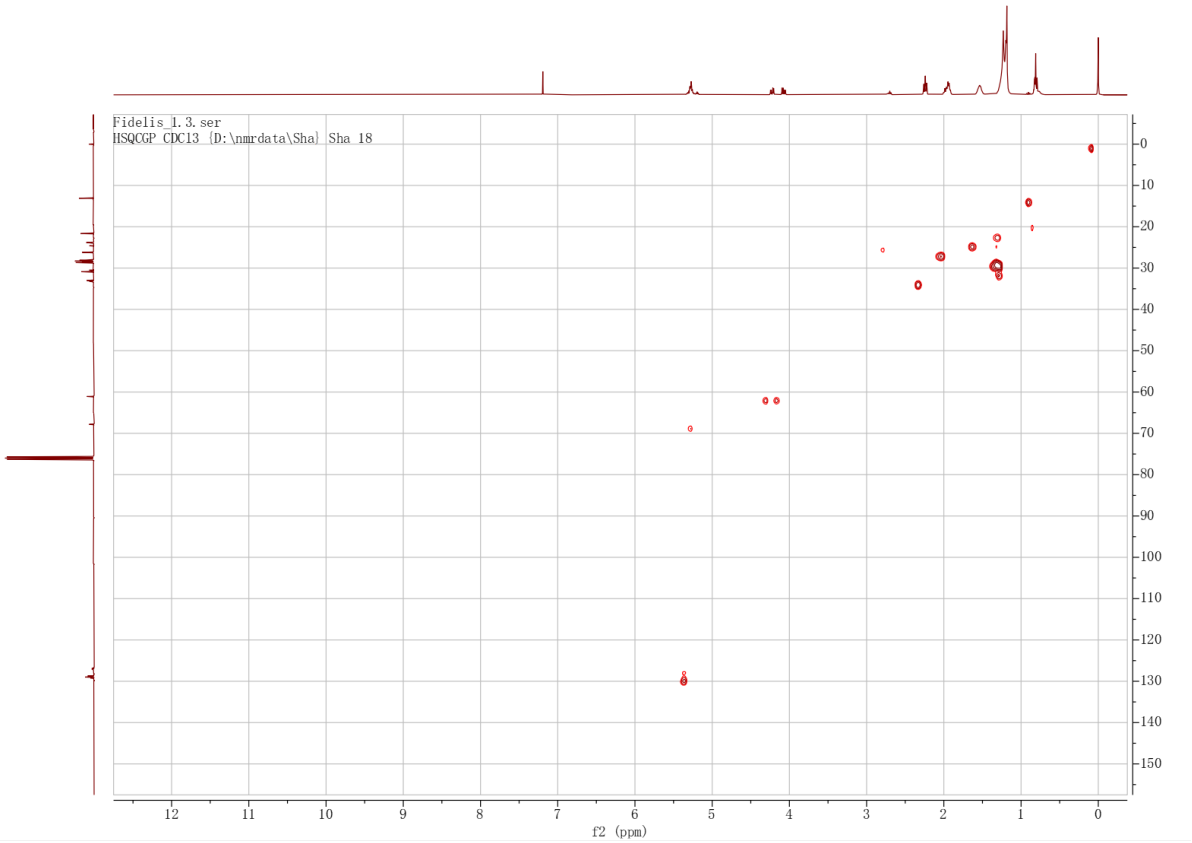


Fig. S6: The DEPT spectrum of (**1**)

Supplementary Methodology

LC-MS/MS CONDITIONS

The LC-MS/MS was run in both ESI positive and ESI negative ion modes. An Accucore™ C18 HPLC column (2.6um, 2.1x150mm) was used for both positive and negative ESI modes. The mobile phase consisted of 0.1% formic acid in water (phase A) and methanol (phase B), and separation was achieved using the following gradient: 0min 5% B; 0.5 min, 5%B; 13min, 95%B; 16.9min 95%B; 17min, 5%B; 20min, 5%B. The flow rate was 0.3mL/min, and the column temperature was 45 ℃. The injection volume was 1 μL. The mass range was from m/z 100 to 1,000. The resolution was 70,000 for the full MS scans and 17,500 for HCD MS/MS scans. The Collision energy was set at 20, 50, 80eV. The mass spectrometer operated as follows: spray voltage, 3,800 V (+) and 3,200 V (-); sheath gas flow rate, 40 arbitrary units; auxiliary gas flow rate, 8 arbitrary units; capillary temperature, 320°C; Probe Heater Temperature, 350°C; S-lens RF level, 50.