

Supplementary Information for:

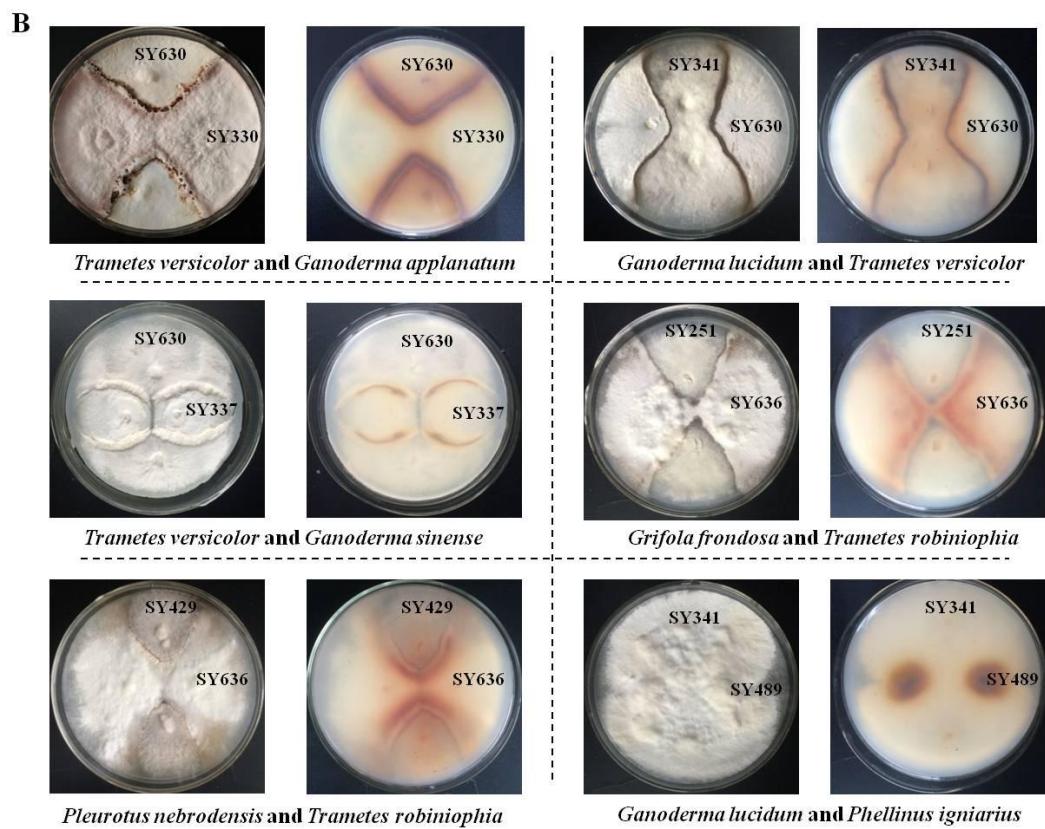
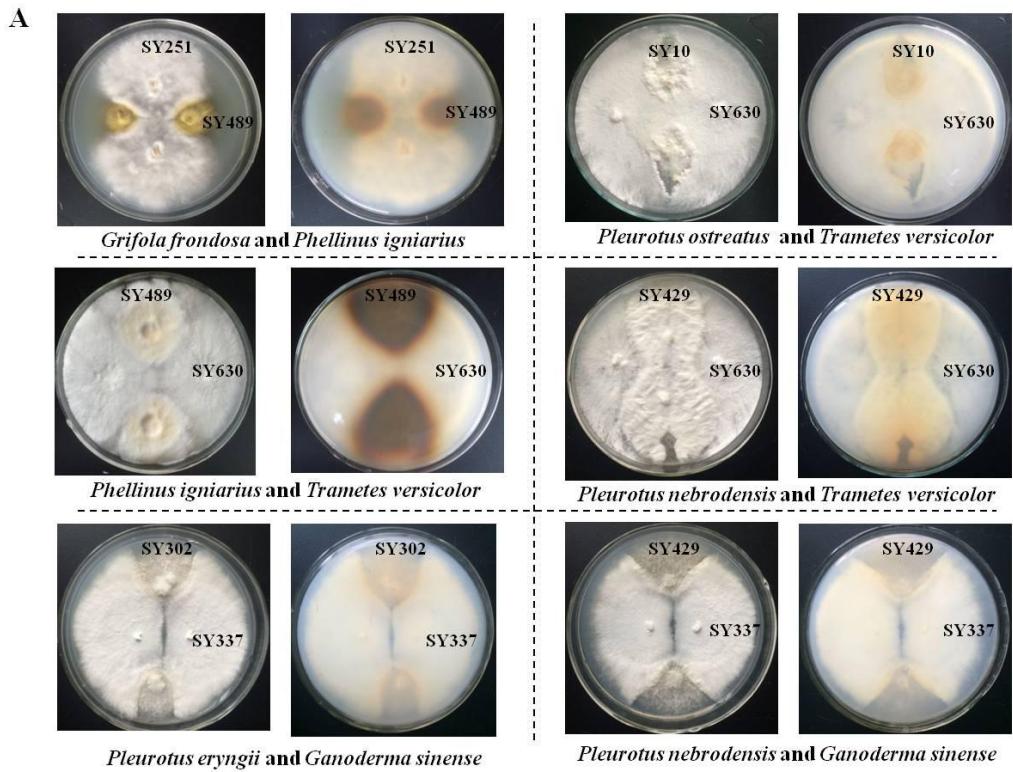
Discovery of novel xylosides in co-culture of basidiomycetes *Trametes versicolor* and

Ganoderma applanatum by integrated metabolomics and bioinformatics

Lu Yao¹, Li-Ping Zhu¹, Xiao-Yan Xu¹, Ling-Ling Tan¹, Martin Sadilek², Huan

Fan³, Bo Hu⁴, Xiao-Ting Shen¹, Jie Yang⁵, Bin Qiao⁶, Song Yang^{1,7,*}

1. School of Life Science, Qingdao Agricultural University, Shandong Province Key
Laboratory of Applied Mycology, and Qingdao International Center on Microbes
Utilizing Biogas, Qingdao 266109, Shandong Province, China
2. Department of Chemistry, University of Washington, Seattle, Washington 98195,
United States of America
3. Tianjin Academy of Agricultural Sciences, Tianjin 300192, China
4. Industrial Product Division, Intrexon Corporation, South San Francisco, CA
94080, United States of America
5. Department of Biochemistry and Molecular Biology, School of Basic Medical
Sciences, Tianjin Medical University, Tianjin 300070, China
6. School of Chemical Engineering and Technology, Tianjin University, Tianjin
300070, China
7. Key Laboratory of Systems Bioengineering, Ministry of Education, Tianjin
University, Tianjin 300072, China



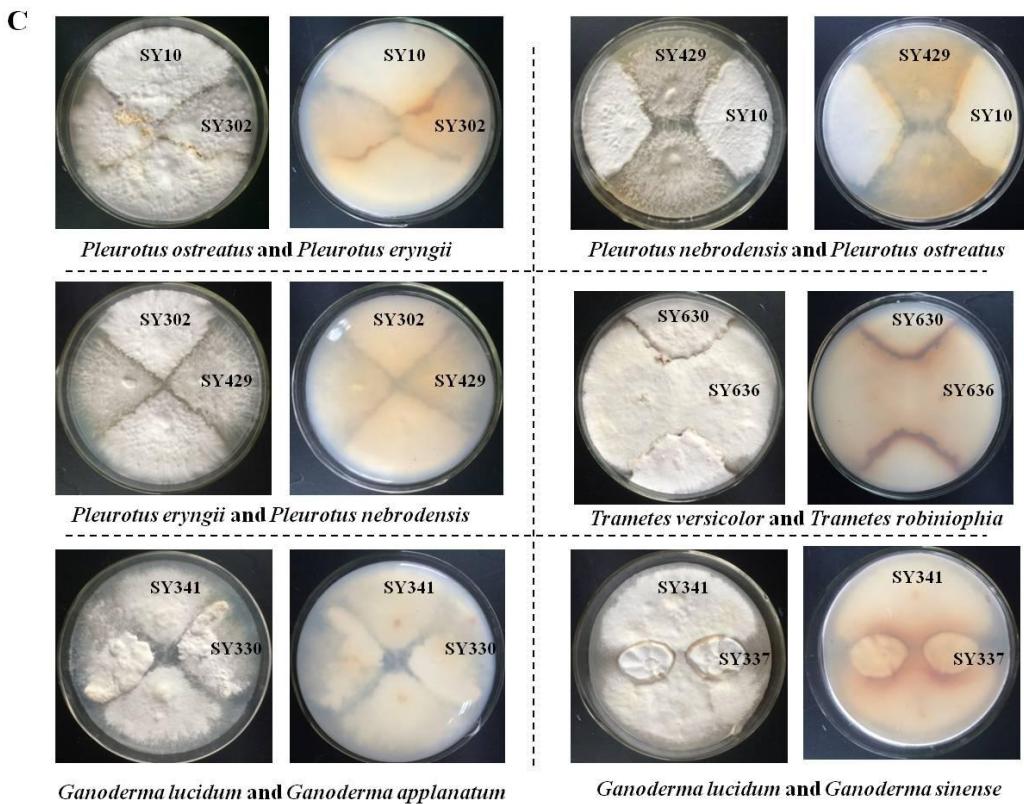


Figure S1. Colony morphologies of the representative co-cultures of basidiomycetes on agar plates. (A) Co-cultures (different order); (B) Co-cultures (different family); (C) Co-cultures (different species). For each pair, the left photo was taken from the top of plate and the right photo was taken from the bottom of plate.

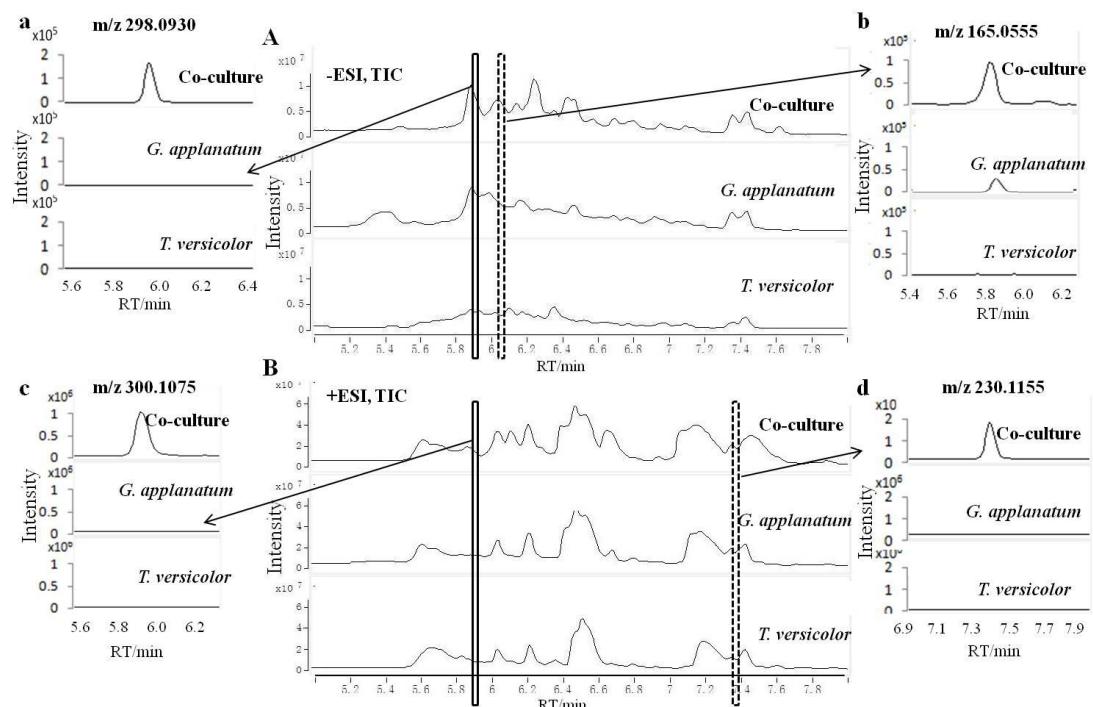


Figure S2. Comparison of total ion chromatograms (TICs) between co-culture of *T.versicolor* with *G. applanatum* and their corresponding mono-cultures on the day 23.

(A) TICs of co-culture and mono-cultures were acquired by LC-MS in the negative mode. (a) and (b) was extracted ion chromatograms (EICs) of m/z 298.0930 and 165.0555; (B) TICs of co-culture and mono-cultures were acquired by LC-MS in the positive mode. (c) and (d) was EICs of m/z 300.1075 and 230.1155.

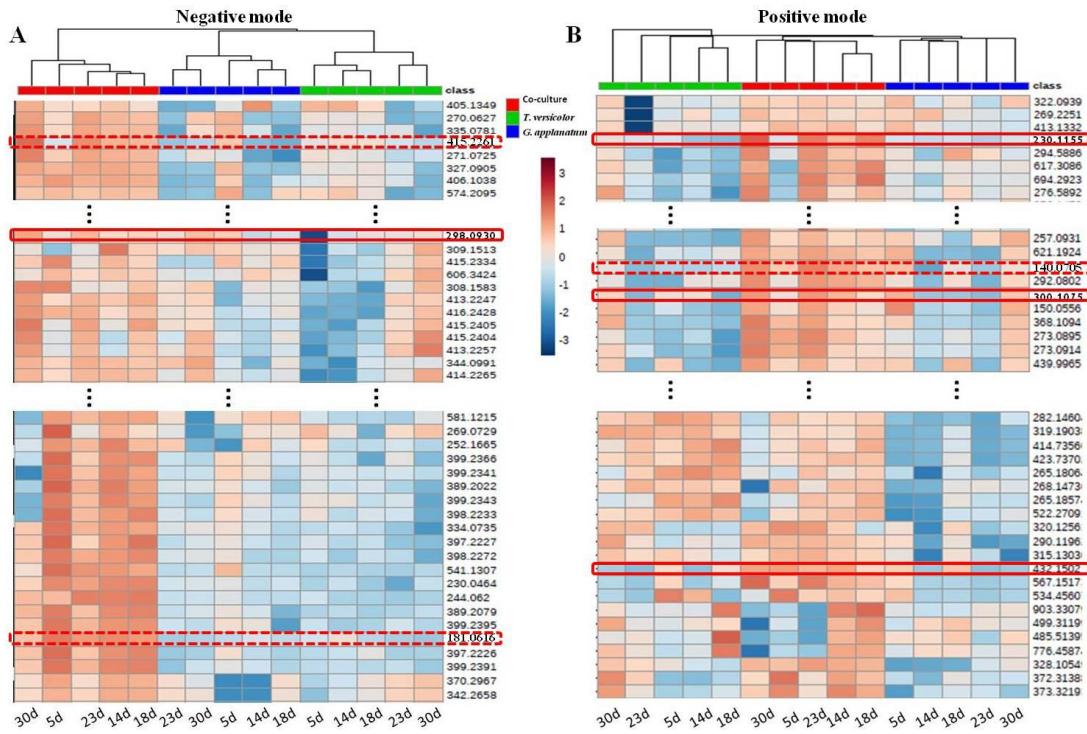


Figure S3. Heatmap analysis of co-culture of *T. versicolor* and *G. applanatum* and their corresponding mono-cultures at different time points (5, 14, 18, 23 and 30 days).

(A) Heatmap of the features analyzed by LC-MS in the negative mode; (B) Heatmap of the features analyzed by LC-MS in the positive mode. The red squares were the representative features with significant difference between co-culture and mono-cultures, and dotted line squares were features only detected by heatmap analysis. Data was acquired from three independent biological replicates and two analytical replicates.

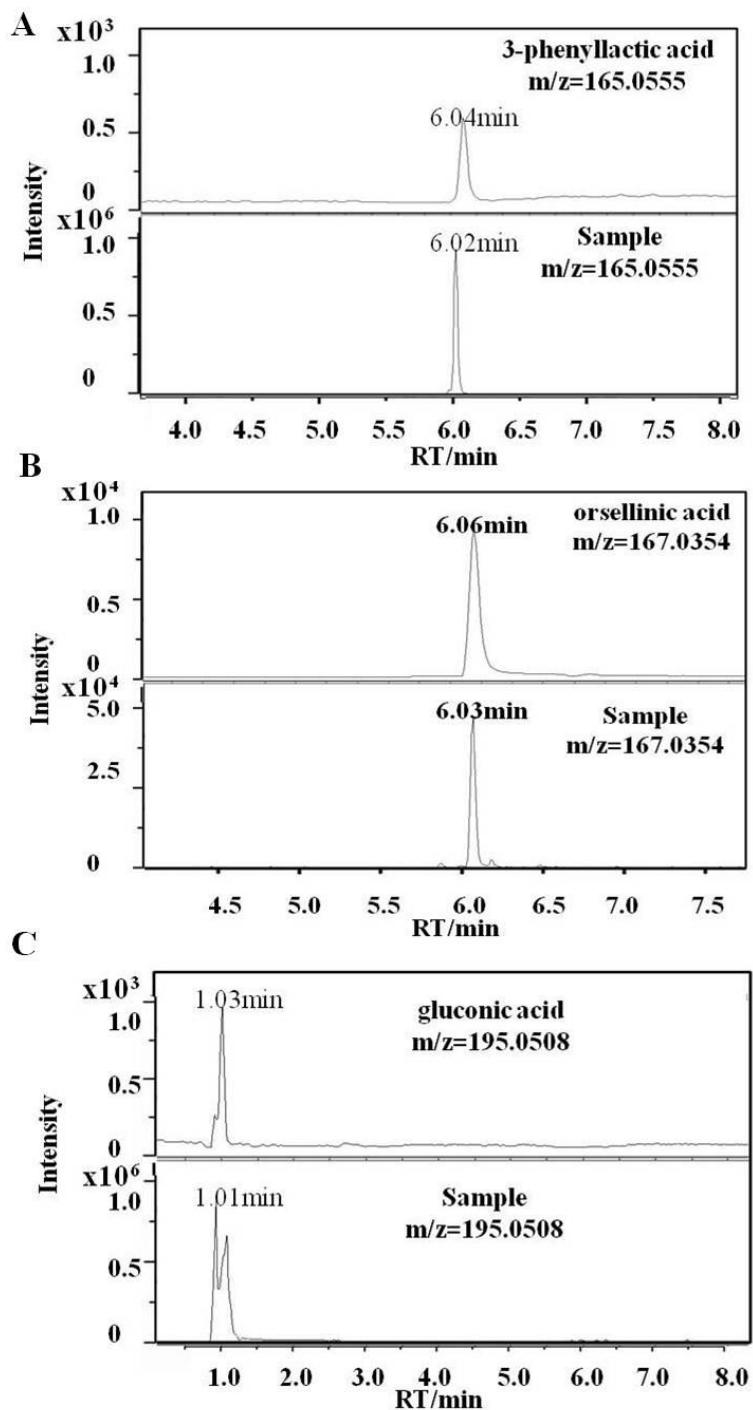


Figure S4. A comparison of retention time of three carboxylic acids between the experimental data and standards data. The extracted ion chromatograms was acquired by LC-MS in the negative mode. (A) 3-Phenyllactic acid; (B) orsellinic acid and (C) gluconic acid. Up panel was the standards and down panel was the compounds extracted from the co-culture of *T. versicolor* and *G. applanatum* on the day 9.

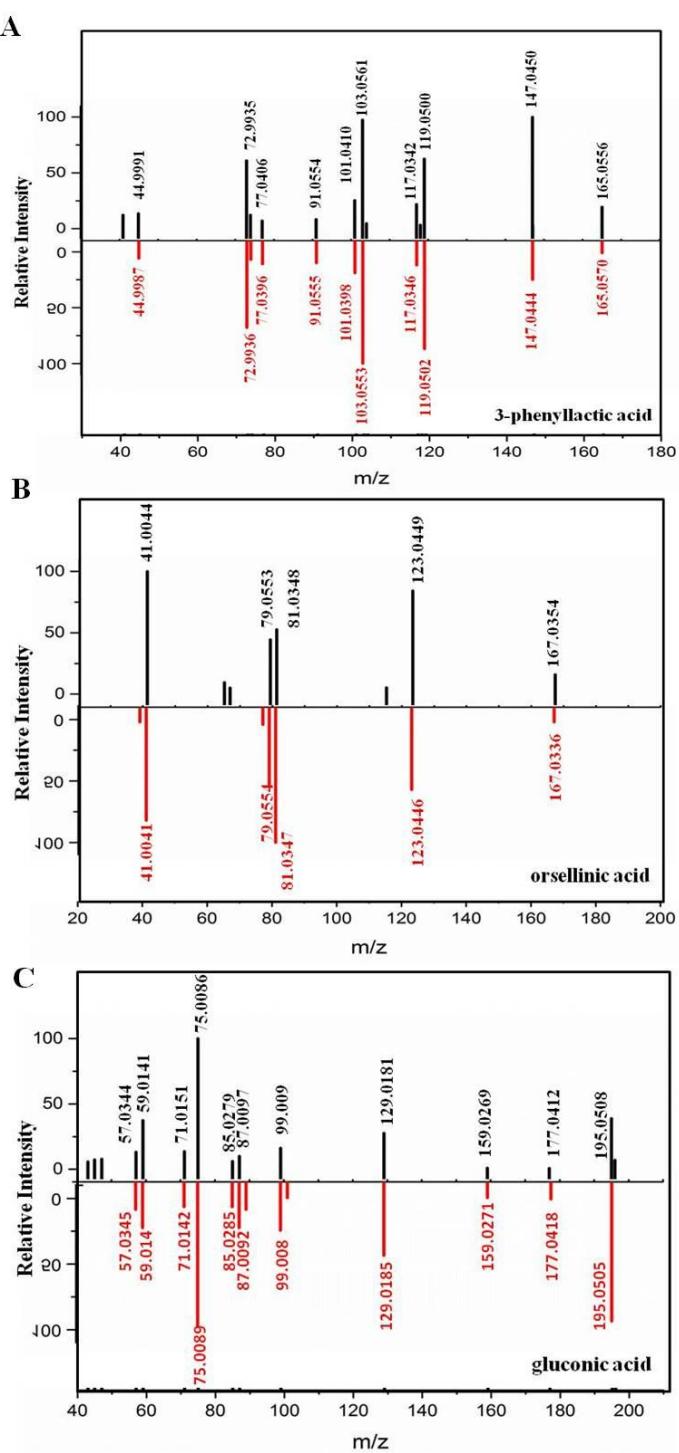
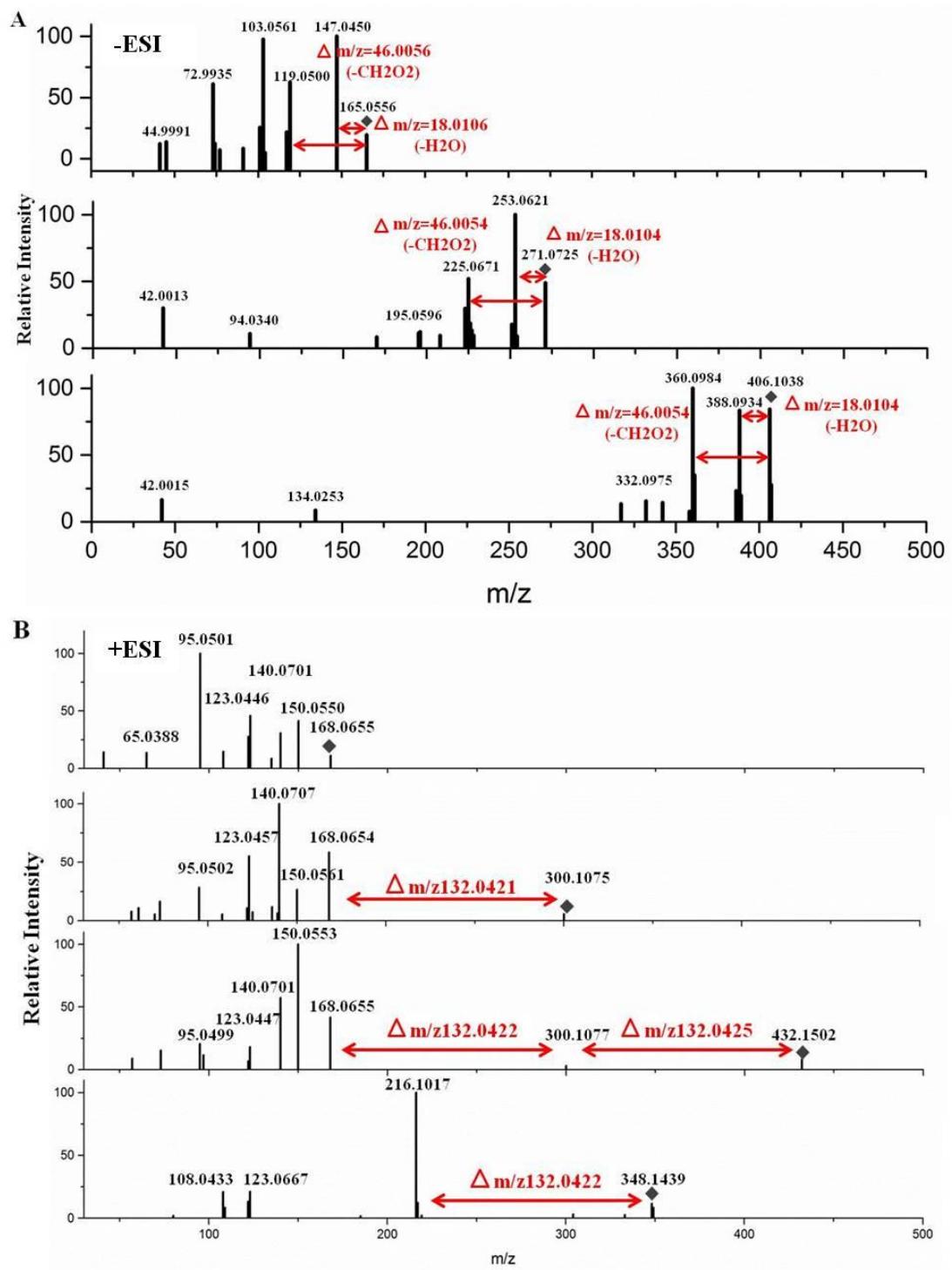


Figure S5. A comparison of MS/MS pattern of three carboxylic acids between the experimental data (black) and METLIN data (red). The mass spectral data was acquired by LC-MS/MS in the negative mode. (A) m/z 165.0555 matched with 3-phenyllactic acid; (B) m/z 167.0354 matched with orsellinic acid; (C) m/z 195.0508 matched with gluconic acid.



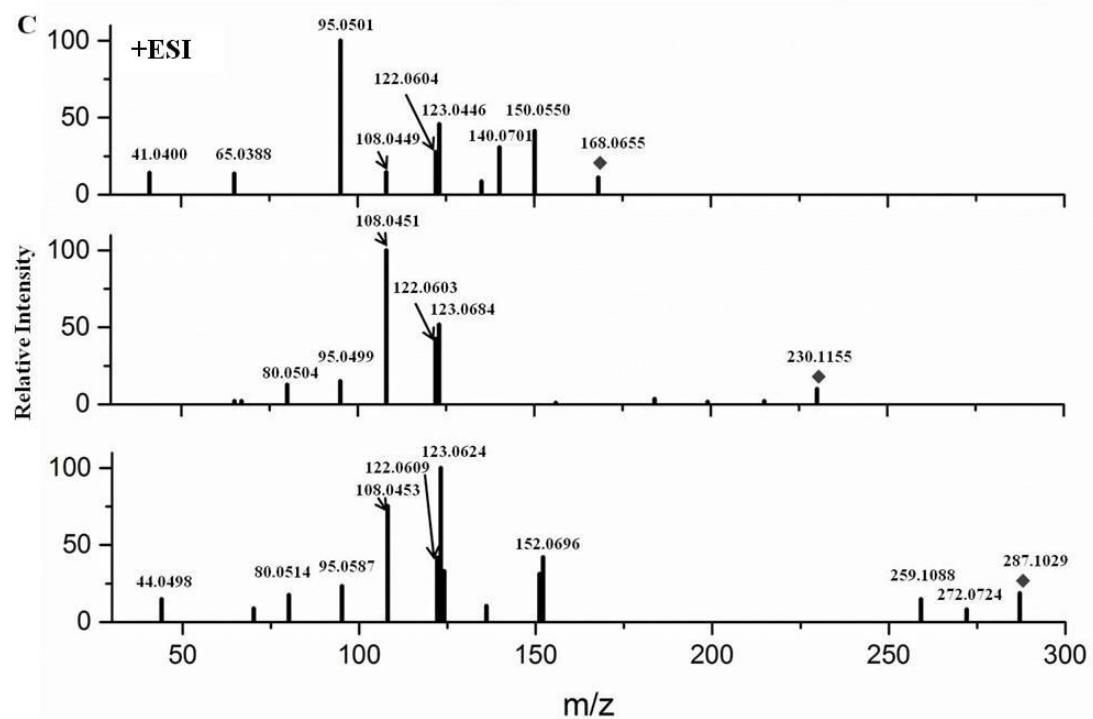


Figure S6. A comparison of MS/MS pattern of the features revealed by molecular network analysis. The mass spectral data was acquired by LC-MS/MS. (A) MS/MS spectra of m/z 165.0555, 271.0725, 406.1038 in the negative mode; (B) MS/MS spectra of xylosides (m/z 300.1075, 432.1502 and 348.1439) and m/z 168.0655 in the positive mode; (C) MS/MS spectra of m/z 168.0655 and its clusters (m/z 230.1155 and 287.1029) in the positive mode, and black arrow points out the same fragment ions generated from different parent ions.

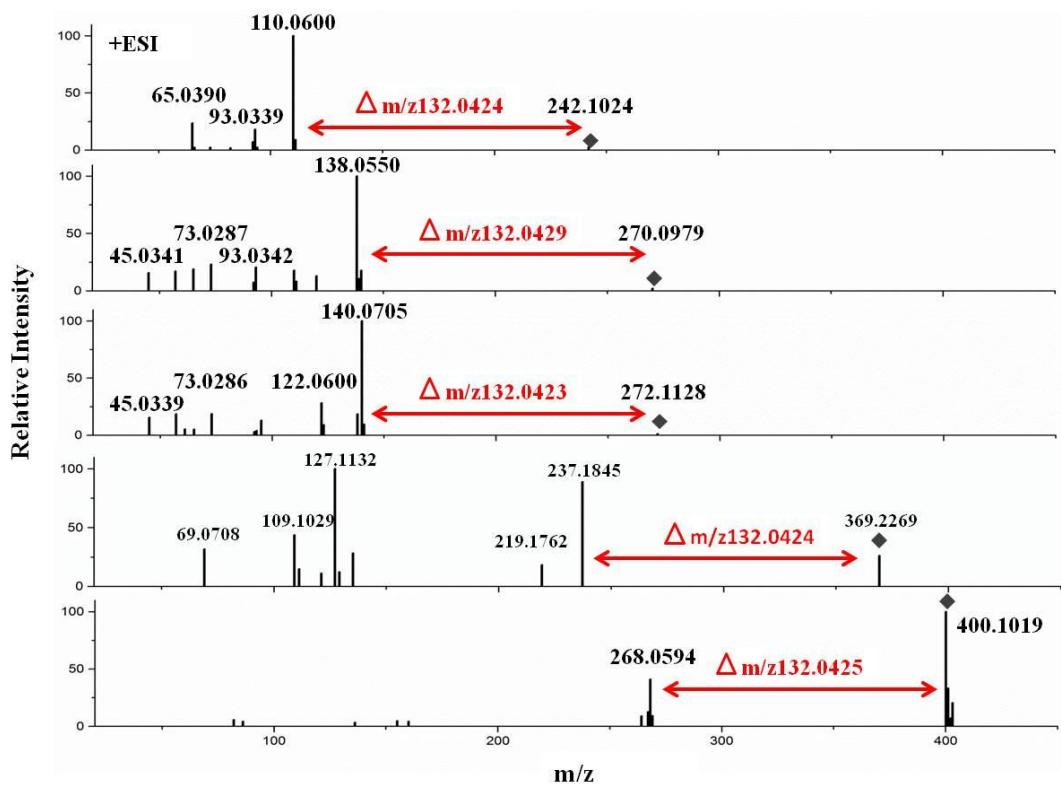
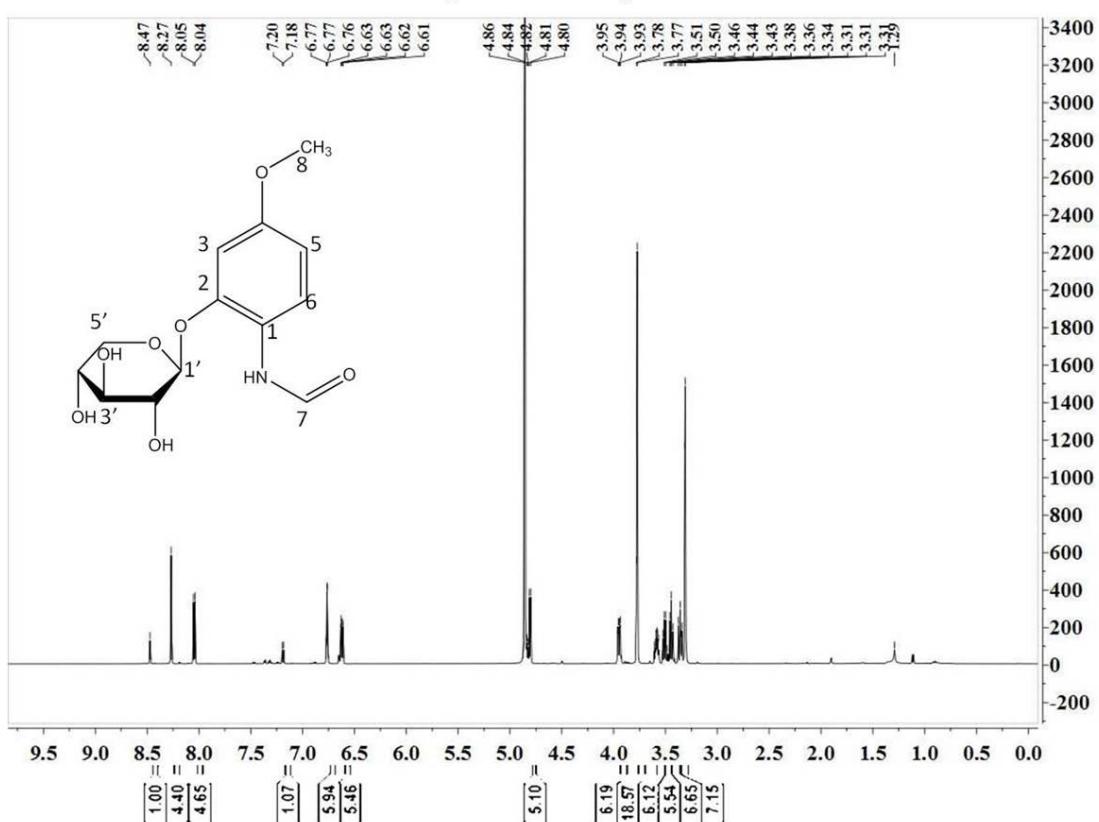
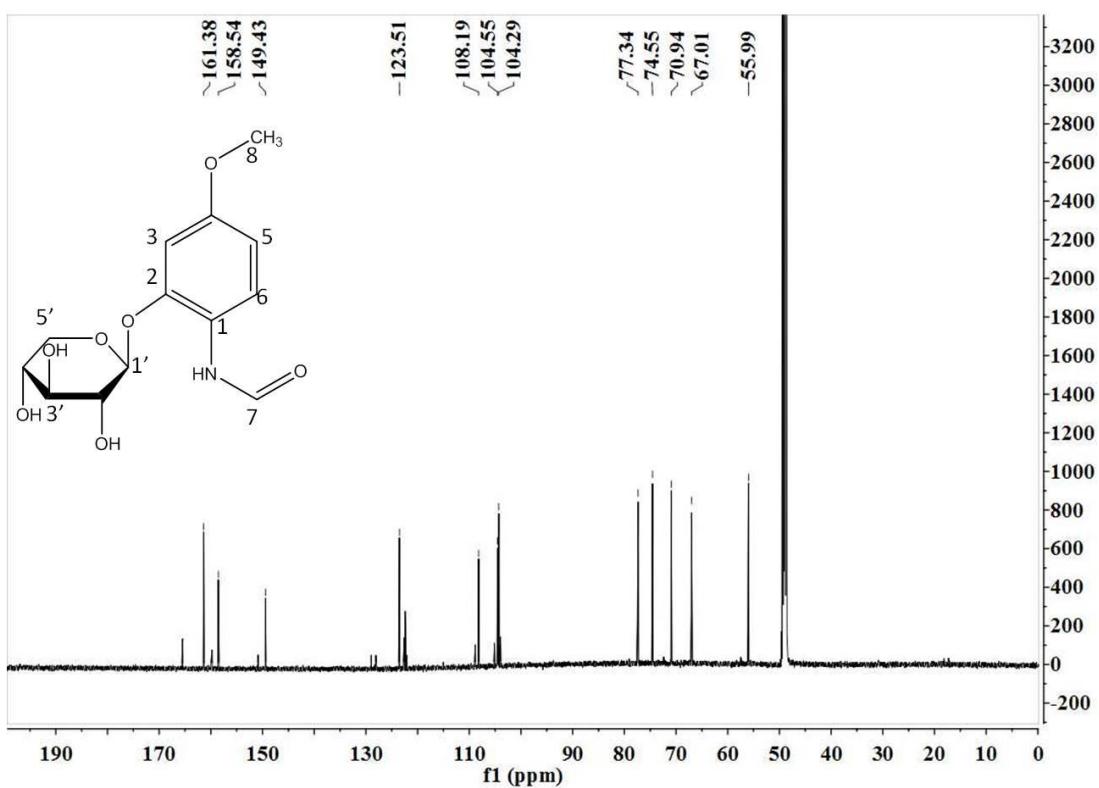


Figure S7. The xylosides were revealed by neutral loss scanning for a loss of 132 Dalton by LC-QQQ-MS. The figure shows that their MS/MS spectra were confirmed by high resolution LC-TOFMS in the positive mode.

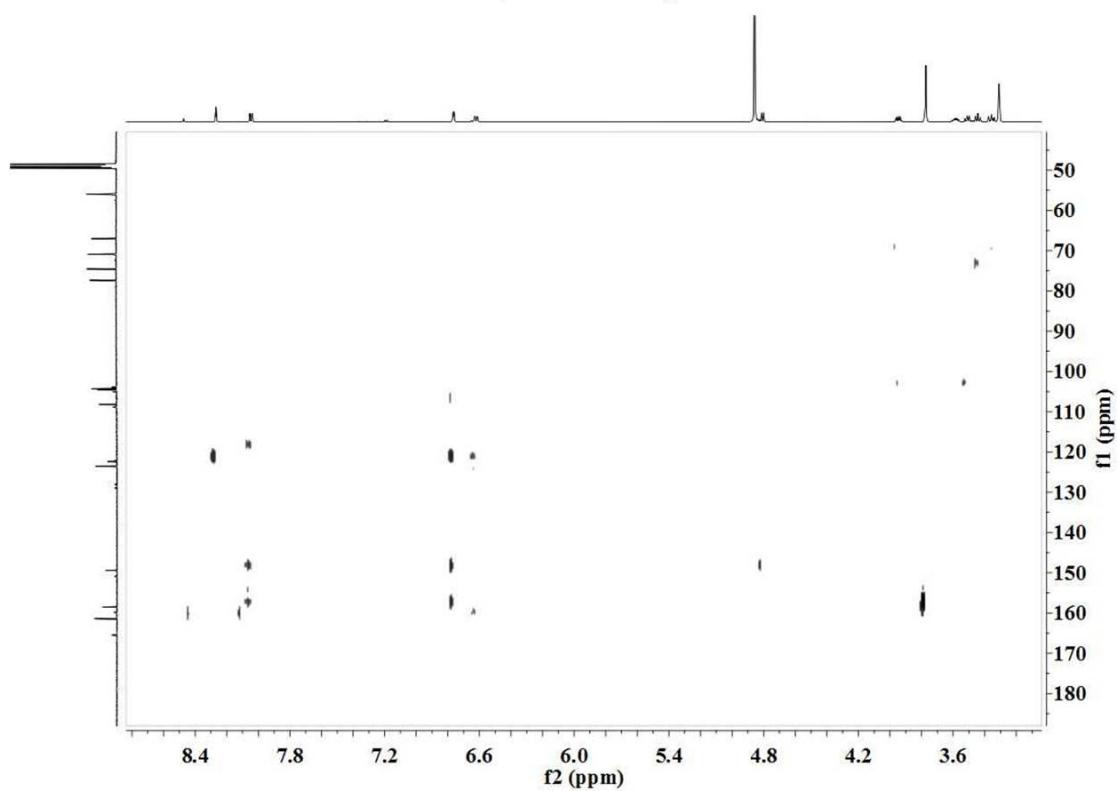
¹H spectrum of compound 2



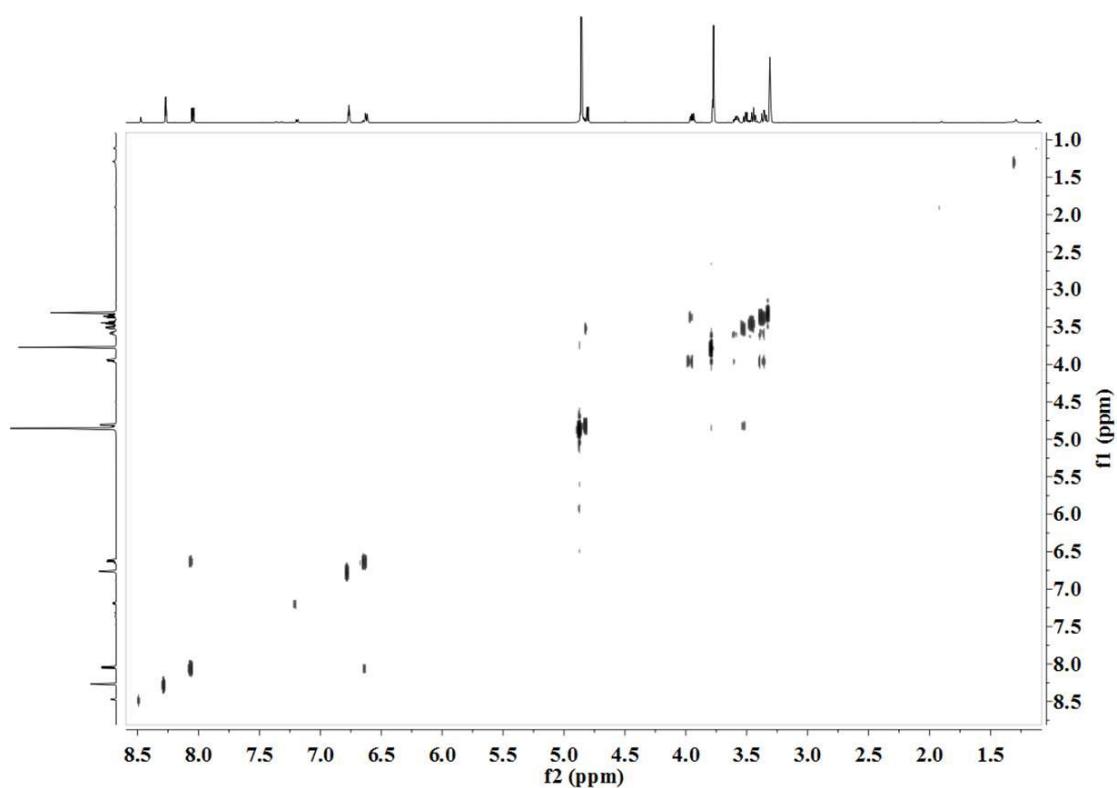
¹³C spectrum of compound 2



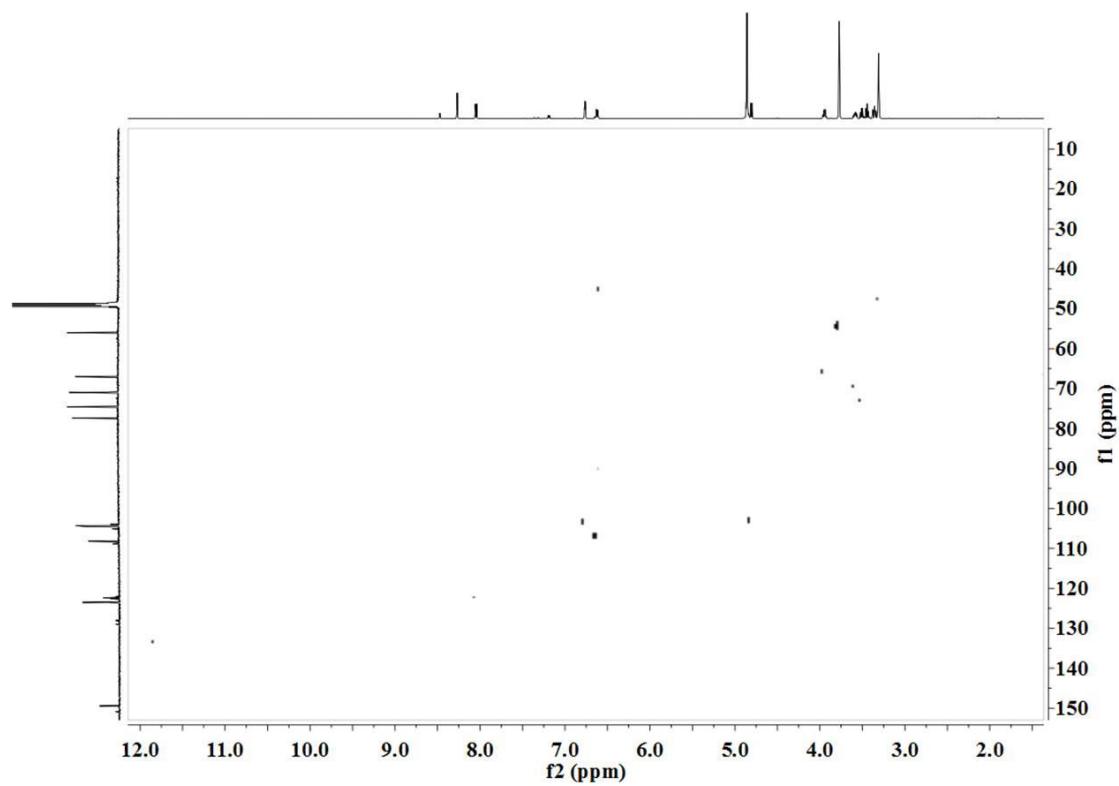
HMBC spectrum of compound **2**



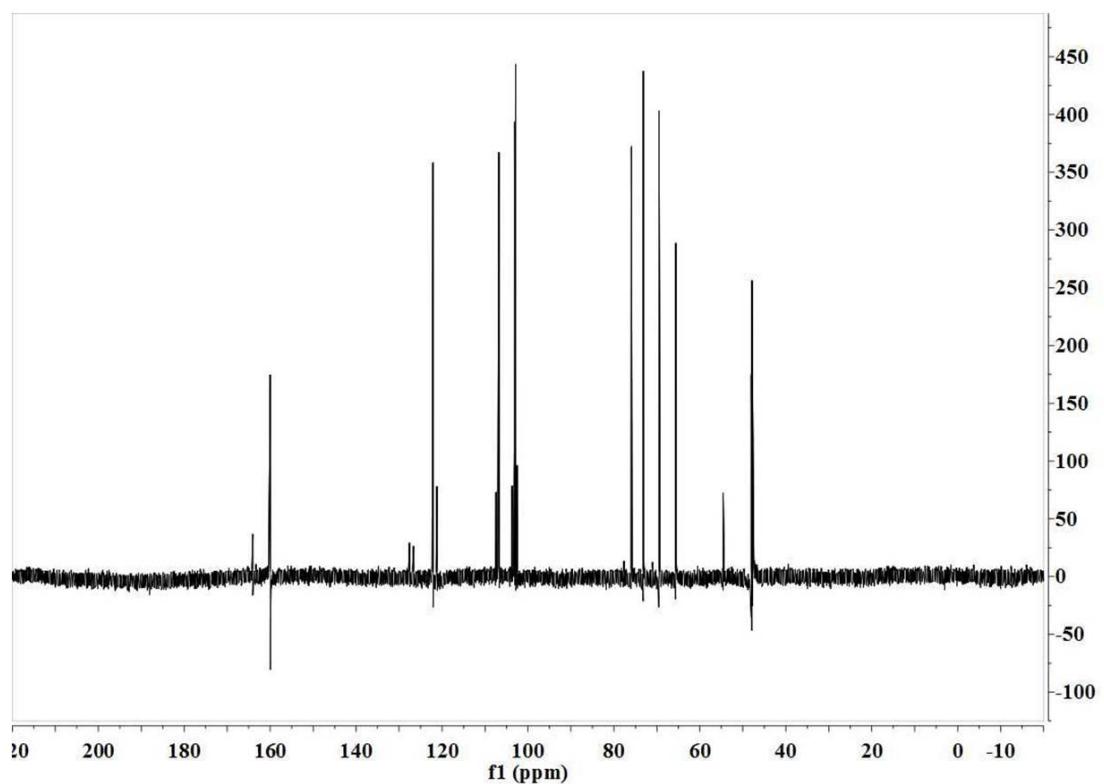
COSY spectrum of compound **2**



HSQC spectrum of compound **2**



DEPT90° spectrum of compound **2**



DEPT135° spectrum of compound **2**

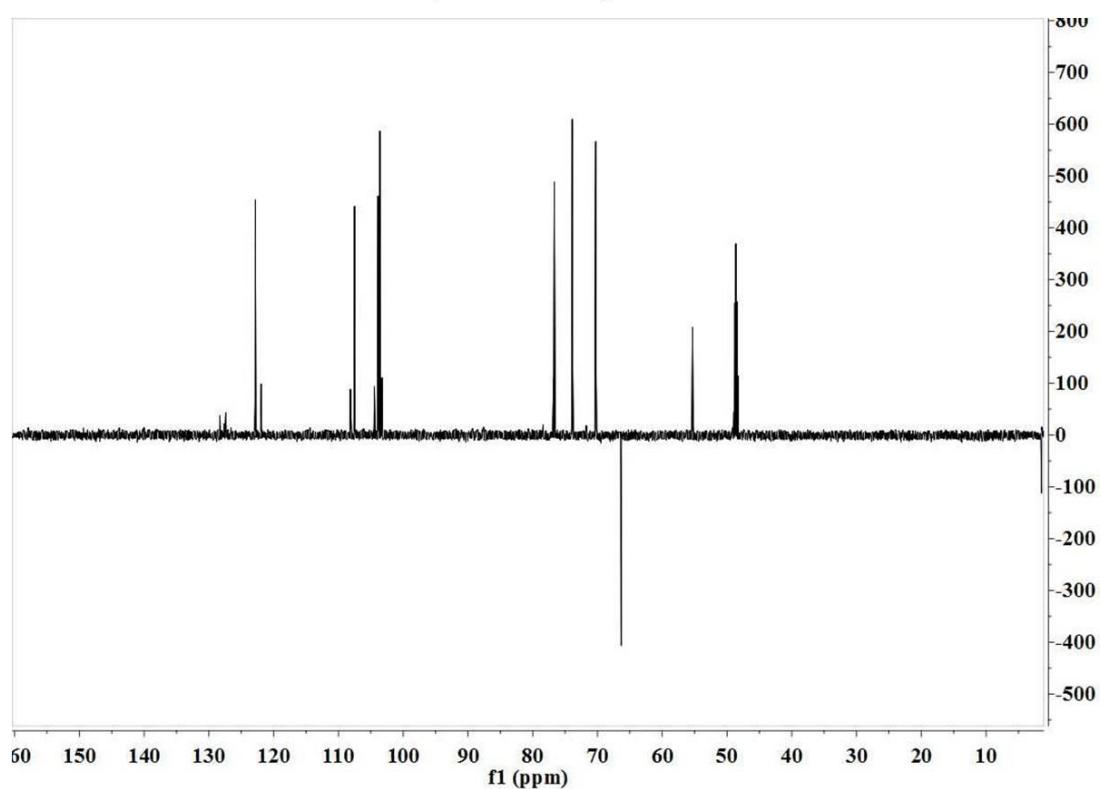
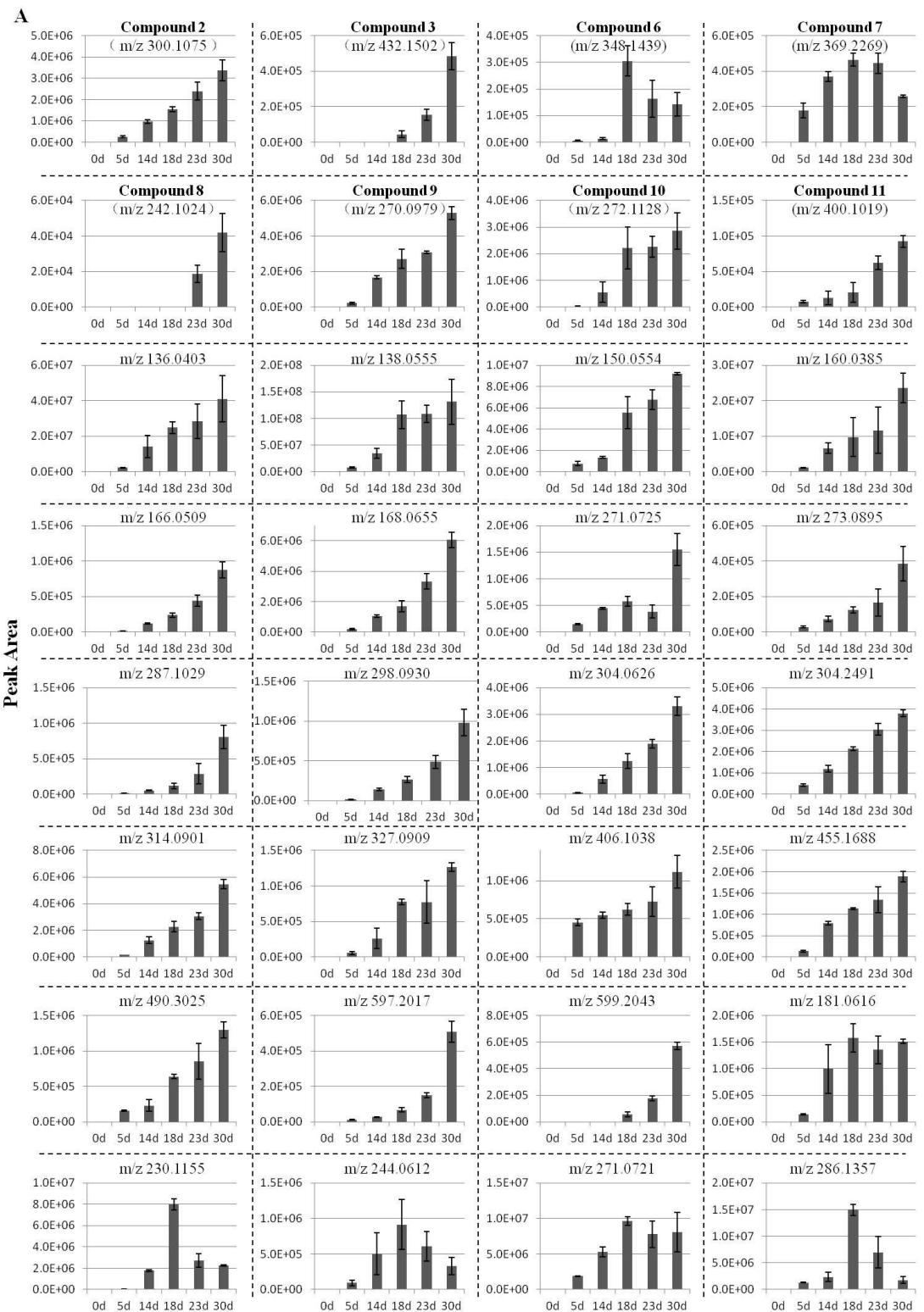


Figure S8. NMR spectra of compound **2**.



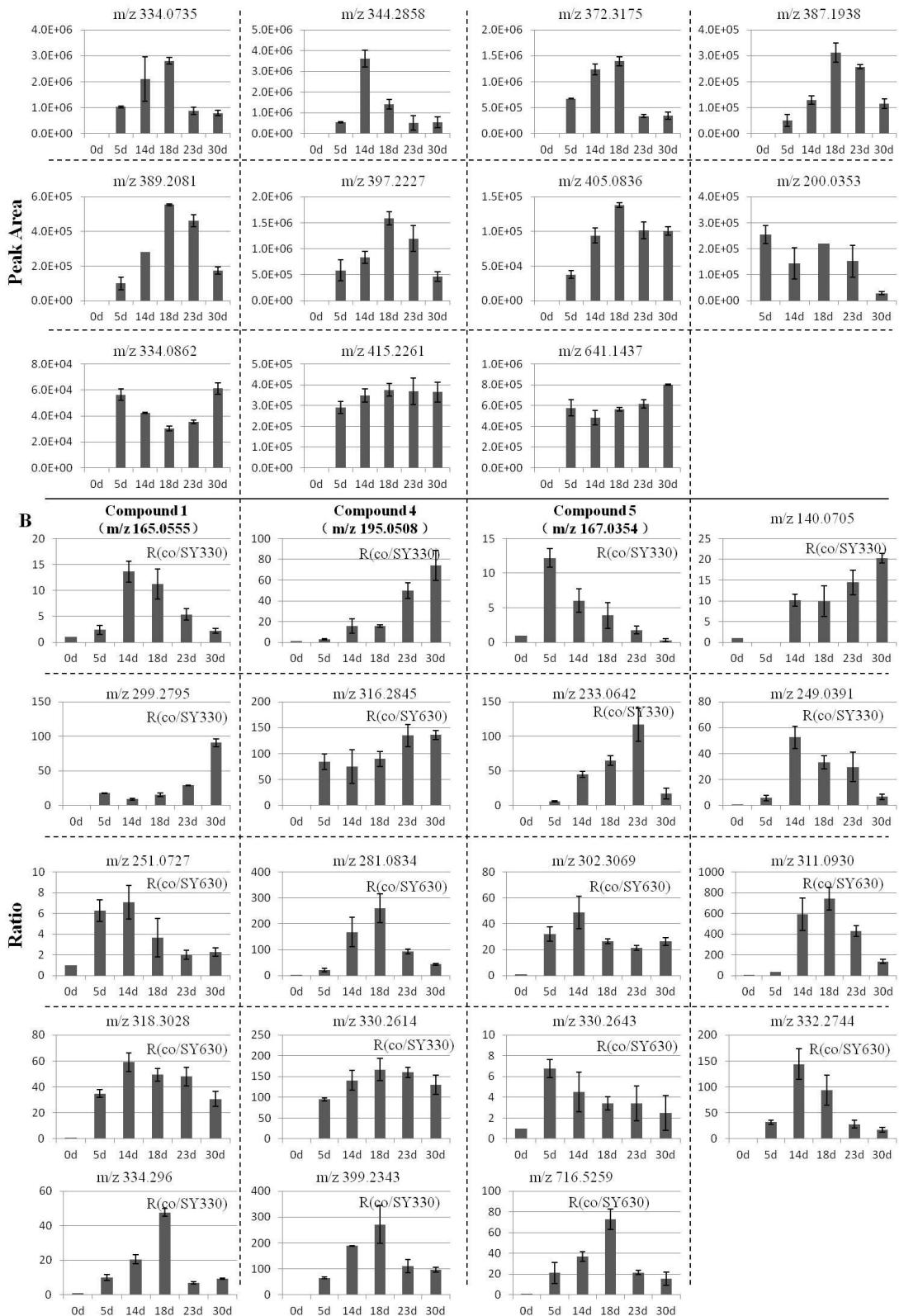


Figure S9. Dynamics of induced features accumulation in fungal co-culture from the day 0

to day 30. (A) Induced newly features; (B) Increased features in the co-culture.

R(co/SY330) and R(co/SY630) means the ratio of the abundance in the co-culture to the mono-culture of *G. applanatum* SY330 and the ratio of the abundance in the co-

culture to the mono-culture of *T. versicolor* SY630, respectively. Data show the mean with error bars indicating standard deviation calculated from three independent biological replicates.

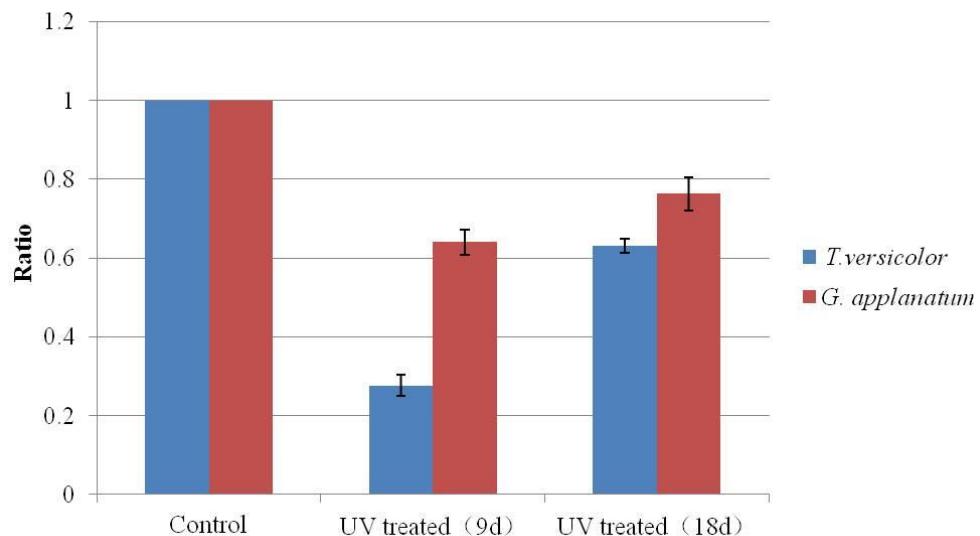


Figure S10. Comparison of mycelium wet weight between UV-treated fungi and the control on the days 9 and 18. The y axis is the ratio of the wet weight of UV-treated *G.applanatum* or *T. versicolor* to the wet weight of fungi without treatment (the control). The average value for the control was set to 1. Data show the mean with error bars indicating standard deviation calculated from three independent biological replicates.

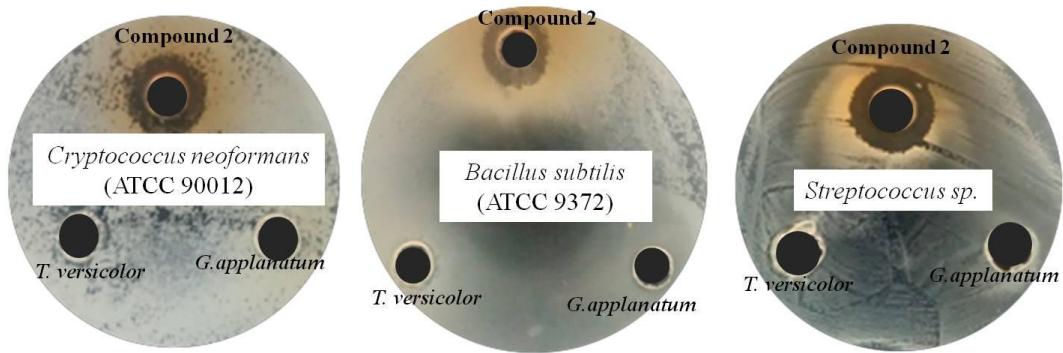


Figure S11. Antimicrobial activities of compound **2** by disc diffusion assay.

Cryptococcus neoformans, *Bacillus subtilis* and *Streptococcus* sp. were employed as antagonistic pathogenic microbe. 170 μM of compound **2** was added to sterile borer, and 20-fold concentrated supernatant from mono-cultures of *T. versicolor* and *G.applanatum* were used as the control. The incubation was 12 hours, and zone of inhibition of compound 2 was about 1.0 cm and the control was zero.

Table S1. A list of seventeen basidiomycetes in this research. They were either deposited at the Shandong Province Key Lab of Applied Mycology or stored in China General Microbiological Culture Collection Center (CGMCC).

Strains	Strain ID	Order	Family	Genus	Wood-decaying
<i>Pleurotus ostreatus</i>	SY10	Agaricales	Pleurotaceae	Pleurotus	Yes
<i>Lentinus edodes</i>	SY103	Agaricales	Marasmiaceae	Lentinula	Yes
<i>Agaricus bisporus</i>	SY153	Agaricales	Agaricaceae	Agaricus	No
<i>Flammulina velutipes</i>	SY204	Agaricales	Physalacriaceae	Flammulina	Yes
<i>Grifola frondosa</i>	SY251	Polyporales	Meripilaceae	Grifola	Yes
<i>Pleurotus eryngii</i>	SY302	Agaricales	Pleurotaceae	Pleurotus	Yes
<i>Ganoderma applanatum</i>	SY330	Polyporales	Ganodermataceae	Ganoderma	Yes
<i>Ganoderma sinense</i>	SY337	Polyporales	Ganodermataceae	Ganoderma	Yes
<i>Ganoderma lucidum</i>	SY341	Polyporales	Ganodermataceae	Ganoderma	Yes
<i>Pleurotus nebrodensis</i>	SY429	Agaricales	Pleurotaceae	Pleurotus	Yes
<i>Phellinus igniarius</i>	SY489	Hymenochaetales	Hymenochaetaceae	Phellinus	Yes
<i>Cordyceps militaris</i>	SY501	Hypocreales	Clavicipitaceae	Cordyceps	No
<i>Boletus edulis</i>	SY613	Boletales	Boletaceae	Boletus	Yes
<i>Trametes versicolor</i>	SY630	Polyporales	Polyporaceae	Trametes	Yes
<i>Trametes roboriphila</i>	SY636	Polyporales	Polyporaceae	Trametes	Yes
<i>Schizophyllum commune</i>	SY658	Agaricales	Schizophyllaceae	Schizophyllum	Yes
<i>Sclerotium xylariae nigripis</i>	SY687	Xylariales	Xylariaceae	Sclerotium	Yes

Table S2. List of induced features in the co-culture of *T. versicolor* and *G.applanatum*.

m/z (-)	RT/min	Difference	m/z (-)	RT/min	Difference	m/z (+)	RT/min	Difference
195.0508	1.09	increased	334.0735	6.34	newly synthesized	300.1075	5.86	newly synthesized
181.0616	2.13	newly synthesized	327.0909	6.35	newly synthesized	432.1502	5.87	newly synthesized
304.0626	2.13	newly synthesized	405.0836	6.3	newly synthesized	599.2043	5.88	newly synthesized
314.0901	2.24	newly synthesized	490.3025	6.37	newly synthesized	150.0554	5.99	newly synthesized
136.0403	2.57	newly synthesized	251.0727	6.68	increased	304.2491	6.01	newly synthesized
271.0721	5.48	newly synthesized	399.2343	7.61	increased	287.1029	6.05	newly synthesized
271.0725	5.71	newly synthesized	397.2227	7.61	newly synthesized	330.2643	6.08	increased
244.0612	5.36	newly synthesized	387.1938	7.61	newly synthesized	334.296	6.09	increased
406.1038	5.79	newly synthesized	389.2081	7.62	newly synthesized	332.2744	6.15	increased
166.0509	5.86	newly synthesized	m/z (+)	RT	Difference	318.3028	6.15	increased
298.093	5.87	newly synthesized	249.0391	1.1	increased	316.2845	6.17	increased
597.2017	5.88	newly synthesized	233.0642	1.18	increased	348.1439	6.22	newly synthesized
455.1688	5.86	newly synthesized	242.1024	1.42	newly synthesized	369.2269	6.24	newly synthesized
165.0555	6.02	increased	272.1128	2.09	newly synthesized	334.0862	6.26	newly synthesized
167.0354	6.05	increased	140.0705	2.19	increased	400.1019	6.35	newly synthesized
330.2614	6.13	increased	270.0979	2.27	newly synthesized	299.2795	6.43	Increased
311.0930	6.17	increased	160.0385	2.59	newly synthesized	344.2858	7.12	newly synthesized
200.0353	6.17	newly synthesized	138.0555	2.7	newly synthesized	230.1155	7.46	newly synthesized
281.0834	6.2	increased	286.1357	5.03	newly synthesized	716.5259	7.54	Increased
641.1437	6.26	newly synthesized	273.0895	5.68	newly synthesized	302.3069	7.54	Increased
415.2261	6.27	newly synthesized	168.0655	5.87	newly synthesized	372.3175	8.16	newly synthesized

Table S3. ^1H -NMR and ^{13}C -NMR data for compound **2**, δ in ppm

	δ_{H}	δ_{C}
1		122.4
2		149.0
3	6.76 (1H, d)	104.5
4		159.0
5	6.62 (1H, q)	108.4
6	8.04 (1H, d)	123.9
7	8.27 (1H, brs)	161.4
8	3.77 (3H, brs)	56.9
1'	4.81 (1H, d)	104.4
2'	3.50 (1H, d)	74.9
3'	3.44 (1H, s)	77.5
4'	3.58 (1H, m)	71.3
5'	3.36 (1H, s)	67.4
	3.95 (1H, m)	

Table S4. Bioinformatics analysis of the potential xylosyltransferases encoded by *T.versicolor*. Only the potential query protein with an identity of $\geq 20\%$ to the database and the query length no less than 120 bps are shown.

No	Locus tag	Protein annotation	length	NCBI Accession	query	Identity	Query	Query	objective sequences ID	objective sequence function
					length	(%)	start	stop		
1	TRAVE_DRAFT_31538	capsular associated protein	630bp	GI 636624487 ref XP_008043378.1	645	27.29	6	612	GI 927760026 gb KPA43099.1	beta-xylosyltransferase 1 [Fusarium langsethiae]
					641	28.71	30	628	GI 647399448 emb CDR44236.1	RHTO0S09e01508g1_1 [Rhodotorula toruloides]
					604	33.61	65	623	GI 647397492 emb CDR40532.1	RHTO0S05e04610g1_1 [Rhodotorula toruloides]
					600	26.17	59	626	GI 751532302 gb KIL90024.1	beta-xylosyltransferase 1 [Fusarium avenaceum]
					600	23.17	65	626	GI 949392729 dbj GAP84327.1	putative glycosyltransferase family 90 protein [Rosellinia necatrix]
					599	50.58	60	626	GI 924113091 emb CUA78292.1	Beta-1,2-xylosyltransferase 1 [Rhizoctonia solani]
					599	28.05	60	628	GI 816192268 emb CRG85785.1	Beta-1,2-xylosyltransferase 1 [Talaromyces islandicus]
					598	27.26	60	626	GI 358386117 gb EHK23713.1	glycosyltransferase family 90 protein [Trichoderma virens Gv29-8]
					598	27.26	60	626	GI 927424180 ref XP_013957921.1	glycosyltransferase family 90 protein [Trichoderma virens Gv29-8]
					598	26.76	64	626	GI 927760719 gb KPA43756.1	xylosyltransferase 1 [Fusarium langsethiae]
					597	28.81	58	626	GI 358398656 gb EHK48007.1	glycosyltransferase family 90 protein [Trichoderma atroviride IMI 206040]
					597	28.81	58	626	GI 927400707 ref XP_013946199.1	glycosyltransferase family 90 protein [Trichoderma atroviride IMI 206040]
					592	29.39	65	625	GI 647397714 emb CDR40950.1	RHTO0S05e09538g1_1 [Rhodotorula toruloides]
					590	27.8	71	626	GI 358377868 gb EHK15551.1	glycosyltransferase family 90 protein [Trichoderma virens Gv29-8]
					590	27.8	71	626	GI 927406821 ref XP_013949749.1	glycosyltransferase family 90 protein [Trichoderma virens Gv29-8]
					589	49.41	57	615	GI 751836596 emb CEL53282.1	Beta-1,2-xylosyltransferase 1 OS=Cryptococcus neoformans var. neoformans serotype D (strain JEC21 / ATCC MYA-565) GN=CXT1 PE=1 SV=1 [Rhizoctonia solani AG-1 IB]
					586	27.13	71	628	GI 358386962 gb EHK24557.1	glycosyltransferase family 90 protein [Trichoderma virens Gv29-8]
					586	27.13	71	628	GI 927425858 ref XP_013958760.1	glycosyltransferase family 90 protein [Trichoderma virens Gv29-8]
					585	30.09	65	626	GI 647402717 emb CDR48932.1	Weakly similar to uniprot Q5K8R6 Cryptococcus
					583	29.67	63	629	GI 647397729 emb CDR40965.1	RHTO0S05e09890g1_1 [Rhodotorula toruloides]
					582	28.35	65	629	GI 74682361 sp Q5K8R6.1 CXT1_CRYN	RecName: Full=Beta-1,2-xylosyltransferase 1; Short=Cxt1p
					582	28.35	65	629	GI 58261216 ref XP_568018.1	cryptococcal xylosyltransferase 1 [Cryptococcus neoformans var. neoformans JEC21]
					582	28.35	65	629	GI 57230100 gb AAW46501.1	cryptococcal xylosyltransferase 1 [Cryptococcus neoformans var.

neoformans JEC21]										
			582	28.35	65	629	GI 146742896 gb ABQ42713.1	beta-1,2 xylosyltransferase 1 [<i>Cryptococcus neoformans</i> var. <i>neoformans</i>]		
			578	27.85	60	622	GI 647397408 emb CDR40393.1	RHTO0S05e02784g1_1 [<i>Rhodotorula</i> <i>toruloides</i>]		
			577	29.81	65	630	GI 647397582 emb CDR40723.1	RHTO0S05e06634g1_1 [<i>Rhodotorula</i> <i>toruloides</i>]		
			577	26.69	54	623	GI 949389023 dbj GAP88019.1	putative glycosyltransferase family 90 protein [<i>Rosellinia necatrix</i>]		
			576	27.08	65	628	GI 924115849 emb CUA75853.1	hypothetical protein RSOLAG22IBB_01853 [<i>Rhizoctonia</i> <i>solanii</i>]		
			574	28.57	65	627	GI 647397677 emb CDR40913.1	RHTO0S05e08724g1_1 [<i>Rhodotorula</i> <i>toruloides</i>]		
			574	27.18	65	628	GI 751840883 emb CEL55146.1	hypothetical protein RSOLAG1IB_01154 [<i>Rhizoctonia</i> <i>solanii</i> AG-1 IB]		
			572	28.67	63	625	GI 358386481 gb EHK24077.1	glycosyltransferase family 90 protein [Trichoderma virens Gv29-8]		
			572	28.67	63	625	GI 927424890 ref XP_01395827 6.1	glycosyltransferase family 90 protein [Trichoderma virens Gv29-8]		
			546	26.74	82	622	GI 927751359 gb KPA36041.1	beta- -xylosyltransferase 1 [<i>Fusarium</i> <i>langsethiae</i>]		
			506	24.31	62	560	GI 927750330 gb KPA35555.1	beta- -xylosyltransferase 1 [<i>Fusarium</i> <i>langsethiae</i>]		
2	TRAVE DRAFT_ 31521	hypothetical protein	558bp	GI 636624477 re f XP_0080433 73.1	556	49.82	6	558	GI 751836596 emb CEL53282.1	Beta-1,2-xylosyltransferase 1 [<i>Rhizoctonia solani</i> AG-1 IB]
3	TRAVE DRAFT_ 56171	UDP- Glycosyltrans ferase/glycogen phosphorylase	515bp	GI 636605689 re f XP_0080339 79.1	331	25.98	193	509	GI 5802783 gb AAD51778.1 AF 116858_1	zeatin O-xylosyltransferase [<i>Phaseolus</i> <i>vulgaris</i>]
					331	25.98	193	509	GI 6226511 sp P56725.1 ZOX_P HAVU	Full=Zeatin O-xylosyltransferase; AltName: Full=Zeatin O-beta-D- xylosyltransferase [<i>Phaseolus vulgaris</i>]
					293	27.3	239	503	GI 15234056 ref NP_192016.1	UDP-glycosyltransferase 72B1 [<i>Arabidopsis thaliana</i>]
					293	27.3	239	503	GI 332656577 gb AEE81977.1	UDP-glycosyltransferase 72B1 [<i>Arabidopsis thaliana</i>]
					229	25.33	263	477	GI 75311632 sp Q9LVW3.1 AX YLT_ARATH	RecName: Full=Anthocyanin 3-O- glucoside 2''-O-xylosyltransferase; Short=A3G2''XylT [<i>Arabidopsis</i> <i>thaliana</i>]
4	TRAVE DRAFT_ 73641	hypothetical protein	444bp	GI 636620747 re f XP_0080415 08.1	317	25.87	130	414	GI 829746565 ref XP_01261067 5.1	PREDICTED: xylosyltransferase 1 [<i>Microcebus murinus</i>]
					286	23.78	90	332	GI 512987849 ref XP_00485585 1.1	PREDICTED: xylosyltransferase 1 [<i>Heterocephalus glaber</i>]
					267	22.47	90	306	GI 694962633 ref XP_00942865 8.1	PREDICTED: xylosyltransferase 1 isoform X1 [<i>Pan troglodytes</i>]
					267	23.22	90	306	GI 795246872 ref XP_01191773 9.1	PREDICTED: xylosyltransferase 1 [<i>Cercocebus atys</i>]
					267	23.22	90	306	GI 967501808 ref XP_01498119 3.1	PREDICTED: xylosyltransferase 1 [<i>Macaca mulatta</i>]
					266	21.05	90	306	GI 837808540 ref XP_01278291 1.1	PREDICTED: xylosyltransferase 1 [<i>Ochotonota princeps</i>]
					266	23.68	90	306	GI 71164803 sp Q86Y38.1 XYLT 1_HUMAN	RecName: Full=Xylosyltransferase 1; AltName: Full=Peptide O- xylosyltransferase 1; AltName: Full=Xylosyltransferase 1; Short=XT-I; Short=XylT-I [<i>Homo sapiens</i>]
					266	23.68	90	306	GI 162317952 gb AAI56040.1	Xylosyltransferase I, partial [<i>synthetic</i> <i>construct</i>]
					266	23.68	90	306	GI 28172878 emb CAD62248.1	xylosyltransferase I [<i>Homo sapiens</i>]

necatrix]									
			248	28.63	502	745	GI 949391510 dbj GAP85657.1	putative copper radical oxidase [<i>Rosellinia necatrix</i>]	
			222	27.93	524	742	GI 919012629 ref XP_013390783.1	PREDICTED: xylosyltransferase oxt-like [<i>Lingula anatina</i>]	
			221	24.89	270	481	GI 949391510 dbj GAP85657.1	putative copper radical oxidase [<i>Rosellinia necatrix</i>]	
			219	22.83	381	592	GI 972142722 gb KUI68971.1	Xylosyltransferase oxt [<i>Valsa malii</i>]	
			213	26.76	532	732	GI 972142722 gb KUI68971.1	Xylosyltransferase oxt [<i>Valsa malii</i>]	
			204	26.96	542	742	GI 925201043 gb JAI52582.1	putative xylosyltransferase oxt, partial [<i>Rhodnius neglectus</i>]	
9	TRAVE DRAFT_73850	WSC-domain-containing protein	463bp	GI 636622003 ref XP_008042136.1	283	44.17	147	6	GI 949393989 dbj GAP83249.1 putative wsc domain protein [<i>Rosellinia necatrix</i>]
					176	27.84	112	8	Xylosyltransferase oxt [<i>Madurella mycetomatis</i>]
					140	27.86	93	4	GI 780631854 ref XP_011685660.1 PREDICTED: xylosyltransferase oxt [<i>Wasemannia auropunctata</i>]
					137	35.77	81	3	GI 949391510 dbj GAP85657.1 putative copper radical oxidase [<i>Rosellinia necatrix</i>]
					133	25.56	91	3	GI 908390298 gb KND90936.1 Xylosyltransferase oxt [<i>Tolyphocladium ophioglossoides</i> CBS 100239]
					132	28.79	73	7	GI 961440096 emb CUT99591.1 xylosyltransferase [<i>Echinococcus multilocularis</i>]
					130	30.77	67	7	GI 972140057 gb KUI66306.1 Xylosyltransferase oxt [<i>Valsa malii</i>]
					128	28.13	76	3	GI 949391510 dbj GAP85657.1 putative copper radical oxidase [<i>Rosellinia necatrix</i>]

Table S5. Primers used in this study.

Primer Name	Sequence (5'-3')	Tm	Length(bp)
GAPDH-F	CGGAATCAACGGTTAGGTC	57.2	221
GAPDH-R	TCTCCTGGAAGACGGTGATG	57.9	
1-GI636624487-F	TCCCGAGGCCATTACTACTACG	57.4	197
1-GI636624487-R	GAATCTATGACGCTGATACTGTGC	58.7	
2-GI636624477-F	CTGATGATTCCAACCCTTTC	58.5	189
2-GI636624477-R	ATACTGAACCGCCAACGATAC	57.0	
3-GI636605689-F	CGGGGAGAAACAACAGTCAT	57.2	193
3-GI636605689-R	ATGGCTCAGGATGAATGGG	57.6	
4-GI636620747-F	TCTGCCCTTCCCTTTTC	59.1	124
4-GI636620747-R	GCTTGATCGCTGCTTAGTTC	59.0	
5-GI636606177-F	CTGCTTGACTCGGCAATAGA	58.3	98
5-GI636606177-R	AGAAAACTGGTGAAGGGGAAA	58.1	
6-GI636616829-F	ACCATTCCCCAGCAACTACC	58.8	226
6-GI636616829-R	GGTTCAAGTGGCGTCAGGA	58.8	