

Genomic and metabolomic analysis of the endophytic fungus *Fusarium sp.* VM-40 isolated from the medicinal plant *Vinca minor*

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Table S1. List of *Fusarium* species from JGI used for comparative analysis and phylogenetic analysis.

Table S2. List of *Fusarium* isolates belonging to the *F. tricinctum* species complex (FTSC) from NCBI.

Table S3. Quality metrics for isolated genomic DNA.

Table S4. The QUAST statistics of *Fusarium sp.* VM-40.

Table S5. Gene distribution of *Fusarium sp.* VM-40 based on the six major modules of CAZymes.

Table S6. Biosynthetic gene clusters of *Fusarium sp.* VM-40 predicted by antiSMASH.

Figure S1. Contigs of *Fusarium sp.* VM-40 visualized by Bandage.

Figure S2. Taxonomic tree generated by comparing 3613 single copy orthologous genes from the genomes of 22 *Fusarium* species using OrthoFinder

Figure S3. Maximum-Likelihood (ML) phylogram of FTSC species based on the comparison of the *tef1* gene

Figure S4. Chemical structures of the secondary metabolites predicted by antiSMASH.

Figure S5. Proposed fragmentations of compound 8.

Figure S6. Proposed fragmentations of compound 10.

Table S1. List of *Fusarium* species from JGI used for comparative analysis and phylogenetic analysis.

Species	Strain	Reference
<i>Fusarium avenaceum</i>	MPI-SDFR-AT-0044	[1]
<i>Fusarium bataticola</i>	FSSC 23	[2]
<i>Fusarium commune</i>	MPI-SDFR-AT-0072	[1]
<i>Fusarium cucurbiticola</i>	FSSC 10	[2]
<i>Fusarium culmorum</i>	UK99	[3]
<i>Fusarium fujikuroi</i>	IMI 58289	[4]
<i>Fusarium graminearum</i>	Z3639	[2]
<i>Fusarium oxysporum</i>	Fo47	[5]
<i>Fusarium poae</i>	2516	[6]
<i>Fusarium proliferatum</i>	ET1	[7]
<i>Fusarium pseudograminearum</i>	CS3096	[8]
<i>Fusarium redolens</i>	MPI-CAGE-AT-0023	[1]
<i>Fusarium solani</i> f. <i>mor</i>	NRRL 22230	[9]
<i>Fusarium tenuicristatum</i>	NRRL 22470	[10-11]
<i>Fusarium tricinctum</i>	F194	[1]
<i>Fusarium vanettenii</i>	T33	[9]
<i>Fusarium venenatum</i>	MPI-CAGE-CH-0201	[1]
<i>Fusarium verticillioides</i>	7600	[12]
<i>Neonectria ditissima</i>	R0905	[13]

Table S2. List of *Fusarium* isolates belonging to the *F. tricinctum* species complex (FTSC) from NCBI.

Species	Strain	TEF1
<i>Fusarium alpinum</i>	LC6043	MW620149.1
<i>Fusarium alpinum</i>	LC6034	MW620147.1
<i>Fusarium avenaceum</i>	LC6387	MW620132.1
<i>Fusarium avenaceum</i>	LC13803	MW620118.1
<i>Fusarium acuminatum</i>	NRRL 36147	OL772880.1
<i>Fusarium arthrosporioides</i>	BCCM/IHEM 14084	KJ126123.1
<i>Fusarium paeoniae</i>	LC5166	MW620143.1
<i>Fusarium paeoniae</i>	LC7358	MW620144.1
<i>Fusarium paeoniae</i>	LC13815	MW620140.1
<i>Fusarium chongqingense</i>	LC13813	MW620136.1
<i>Fusarium tricinctum</i>	CBS 253.50	KR071775.1
<i>Fusarium tricinctum</i>	NRRL 36381	OL772830.1
<i>Fusarium</i> sp. FTSC_5	NRRL 52727	OL772885.1
<i>Fusarium</i> sp. FTSC_5	NRRL 52726	OL772884.1
<i>Fusarium</i> sp. FTSC_8	NRRL 52722	OL772890.1
<i>Fusarium</i> sp. FTSC_11	P2289a	OL964855.1
<i>Fusarium</i> sp. FTSC_12	P2285a	OL964770.1
<i>Fusarium</i> sp. FTSC_13	F164	OL964757.1
<i>Fusarium</i> sp. FTSC_14	R972	OL964801.1
<i>Fusarium</i> sp. FTSC_15	P78c	OL964788.1
<i>Fusarium</i> sp. FTSC_17	NRRL 53952	OL772853.1
<i>Fusarium</i> sp. FTSC_18	NRRL 36565	OL772876.1
<i>Fusarium</i> sp. FTSC_19	FRC R3178	OL772777.1
<i>Fusarium</i> sp. FTSC_19	FRC R3120	OL772776.1
<i>Fusarium</i> sp. FTSC_10	NRRL 52720	OL772893.1
<i>Fusarium</i> sp. FTSC_20	FRC R7848	OL772774.1
<i>Fusarium</i> sp. FTSC_21	NRRL 39843	OL772788.1
<i>Fusarium</i> sp. FTSC_22	FRC R6739	OL772764.1
<i>Fusarium</i> sp. FTSC_23	FRC R7739	OL772835.1
<i>Fusarium</i> sp. FTSC_24	FRC R7356	OL772894.1
<i>Fusarium</i> sp. FTSC_25	FRC R4587	OL772866.1
<i>Fusarium iranicum</i>	NRRL 54147	OL772891.1
<i>Fusarium flocciferum</i>	NRRL 45999	OL772882.1
<i>Fusarium petersiae</i>	CBS 143231	MG386159.1
<i>Fusarium torulosum</i>	NRRL 22748	OL772877.1
<i>Neonectria ditissima</i>	R0905	-

Table S3. Quality metrics for isolated genomic DNA.

Qubit conc. (ng/μl)	95.0
NanoDrop conc. (ng/μl)	123.9
Output (μg)	5.7
A260/280 ratio	1.80
A260/230 ratio	2.04
NanoDrop/Qubit conc. ratio	1.3
Qubit conc. after Circulomics (ng/μl)	83.4
Input for library preparation (μg)	1
Output after DNA repair and end-prep (μg)	0.978
Output after adapter ligation and clean-up (μg)	0.756

Table S4. QUAST statistics of the *Fusarium* sp. VM-40 whole genome assembly.

Statistics without reference	assembly
# contigs (>= 0 bp)	19
# contigs (>= 1000 bp)	19
# contigs (>= 5000 bp)	18
# contigs (>= 10000 bp)	18
# contigs (>= 25000 bp)	16
# contigs (>= 50000 bp)	15
Total length (>= 0 bp)	42057343
Total length (>= 1000 bp)	42057343
Total length (>= 5000 bp)	42054254
Total length (>= 10000 bp)	42054254
Total length (>= 25000 bp)	42021767
Total length (>= 50000 bp)	41979816
# contigs	19
Largest contig	5654227
Total length	42057343
GC (%)	47.72
N50	4299955
N90	1527832
L50	5
L90	11

Table S5. Gene distribution of *Fusarium* sp. VM-40 based on the six major modules of CAZymes.

Module family	Module	Numbers
Glycoside hydrolases (GH)	GH1	2
	GH10	2
	GH105	4
	GH106	1
	GH109	2
	GH11	1
	GH114	3
	GH115	2
	GH12	3
	GH125	2
	GH13	4
	GH131	1
	GH132	1
	GH135	1
	GH142	2
	GH15	1
	GH152	1
	GH154	2
	GH16	15
	GH17	5
	GH18	11
	GH2	10
	GH20	3
	GH27	2
	GH28	8
	GH29	1
	GH3	29
	GH31	8
	GH32	6
	GH33	1
	GH35	4
	GH36	4
	GH37	2
	GH38	1
	GH39	2
	GH43	27
	GH45	1

	GH47	9
	GH49	1
	GH5	23
	GH51	2
	GH53	1
	GH54	1
	GH55	3
	GH6	1
	GH62	2
	GH63	1
	GH64	1
	GH65	1
	GH67	1
	GH7	1
	GH71	1
	GH72	2
	GH74	1
	GH75	3
	GH76	7
	GH78	7
	GH81	1
	GH88	3
	GH92	1
	GH93	5
	GH95	1
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Auxiliary activities (AAs)	AA1	11
	AA11	4
	AA12	2
	AA13	1
	AA16	1
	AA2	5
	AA3	25
	AA4	3
	AA5	5
	AA6	1
	AA7	22
	AA8	4
	AA9	12
	<hr/>	
Glycosyltransferases (GTs)	GT1	16
	GT109	3

	GT15	4
	GT17	1
	GT2	17
	GT20	3
	GT21	1
	GT22	4
	GT24	1
	GT3	1
	GT31	5
	GT32	6
	GT33	1
	GT34	3
	GT35	1
	GT39	3
	GT4	6
	GT41	1
	GT48	1
	GT50	1
	GT57	2
	GT58	1
	GT59	1
	GT62	3
	GT64	2
	GT66	1
	GT69	3
	GT71	3
	GT76	1
	GT8	6
	GT90	6
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Carbohydrate esterases (CEs)	CE1	4
	CE12	3
	CE16	6
	CE18	1
	CE2	1
	CE3	10
	CE4	9
	CE5	11
	CE8	3
	CE9	1
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Carbohydrate-binding modules	CBM1	2

(CBMs)		
	CBM13	1
	CBM18	14
	CBM20	1
	CBM21	1
	CBM24	2
	CBM35	3
	CBM42	2
	CBM43	2
	CBM48	2
	CBM50	8
	CBM6	1
	CBM63	3
	CBM67	4
	CBM87	1
	CBM91	7
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Polysaccharide lyases (PLs)	PL1	11
	PL11	1
	PL26	1
	PL3	9
	PL4	3
	PL42	2
	PL9	2
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Table S6. Biosynthetic gene clusters of *Fusarium sp.* VM-40 predicted by antiSMASH.

Cluster ID	Region	Contig	Type	From	To	Most similar known cluster	Genbank	Type	Similarity
1	1.1	14	NRPS-like	295355	353717				
2	1.2	14	Terpene	763502	794776	α -acorenol	HF679032.1	Terpene	1
3	1.3	14	NRPS	120598	127224				
4	2.1	5	NRPS, NRPS-like, T1PKS	164599	297127	Fusatrixin/fusapentaxin/fusaoctaxin A	CM000576.1	NRP	0.87
5	2.2	5	NRPS, T1PKS	415050	486937	ACT-Toxin II	AB516321.1	Polyketide	1
6	2.3	5	Fungal-RiPP-like	614114	705241				
7	3.1	21	T3PKS, NRPS	43028	157388	Peramine/intermediate 1/intermediate 2	MH938357.1	NRP	1
9	3.2	21	NRPS	216154	289015				
10	3.3	21	Indole	761991	793241				
11	3.4	21	Terpene, NRPS	127827	140762	Culmorin/(+)-juniperol(longiborneol)/ 15-acetyldeoxynivalenol	CM000574.1	Terpene	1
12	3.5	21	Fungal-RiPP-like	160092	169460				
13	3.6	21	T1PKS	169702	176564	Gibepyrone-A	HF679033.1	Polyketide	0.4
14	5.1	24	T1PKS	761308	828456	Oxyjavanicin	HE613440.1	Polyketide	0.87
15	5.2	24	Terpene	139953	143209	Squalestatin S1	LDZW01000177.1	Terpene	0.4

15	5.3	24	NRP-metallophore, NRPS	2598020	2681533				
16	6.1	18	Terpene	35601	68710	Gibberellin	KT216505.1	Terpene	0.85
17	6.2	18	NRPS	149800	232668				
18	7.1	15	T1PKS	13788	82708				
19	7.2	15	Fungal-RiPP-like	233298	324179				
20	7.3	15	Terpene	418888	450465				
21	7.4	15	T1PKS	495048	576701	Lijiquinone	KT874412.1	Polyketide	0.62
22	8.1	22	T1PKS	306667	371773				
23	8.2	22	Phosphonate	407924	434676	Fosfonochlorin	CM000596.1	Other	0.76
24	8.3	22	T1PKS, NRPS	438153	567620	Illicicolin H/ilicicolin J/8-epi-ilicicolin H	RQWH01000002.1	NRP + Polyketide	0.83
25	8.4	22	NRPS	899797	963512				
26	8.5	22	T3PKS	1080248	1141718				
27	8.6	22	T1PKS	2405290	2472764				
28	9.1	9	NRPS	983271	1052669	Beauvericin	EU886196.1	NRP	0.2
29	10.1	4	NRPS-like	1307934	1368649	Bassianolide	FJ439897.1	NRP	0.26
30	10.2	4	Terpene	2028925	2060184				
31	10.3	4	NRPS	2106488	2170172	Chrysogine	NC_026476.1	NRP	1

32	10.4	4	T1PKS, NRPS	222093 6	237515 3	Lucilactaene	LC515193.1	Polyketide	0.61
33	11.1	27	T1PKS	1	62583				
34	11.2	27	Fungal-RiPP-like	450256	541207				
35	13.1	11	T1PKS	54592	124703				
36	13.2	11	NRPS	452458	520206				
37	13.3	11	Terpene	144236 8	147349 5				
38	14.1	19	NRPS, Indole	32215	97591	Hexadehydroastech rome/terezine- D/astechrome	CM000171.1	NRP	1
39	14.2	19	T1PKS	170110	230638	Aurofusarin	CM000574.1	Polyketide	0.9
40	14.3	19	NRPS, T1PKS	289487	372153	Fusaristatin A	CM000575.1	NRP + Polyketide	1
41	14.4	19	Terpene	285286 2	288398 8				
42	15.1	3	T1PKS	605930	683015	Orcinol/orsellinic acid	CM000575.1	Polyketide	0.55
43	15.2	3	Fungal-RiPP-like	224519 7	233826 2				
44	15.3	3	NRPS-like	407744 6	414078 7				
45	15.4	3	Indole	420380 4	423507 8				
46	17.1	7	T1PKS, T3PKS	28890	94430				
47	17.2	7	Terpene	158798	189551	PR-toxin	HG792016.1	Terpene	0.5
48	17.3	7	NRPS, T1PKS	390515	462676	Fusaridione A/ (3R,5S)-5-(4-	AY700570.3	NRP + Polyketide	0.18

						hydroxybenzyl)-3-methyl-3-((2E,4E,6E,8E,10E)-4,8,10-trimethyldodeca-2,4,6,8,10-pentaenoyl)pyrrolidine-2,4-dione			
49	17.4	7	NRPS-like	646679	710753				
50	17.5	7	NRPS-like	794850	858701	Choline	CH236925.1	NRP	1
51	17.6	7	Betalactone	120736	124971				
				0	7				
52	19.1	8	NRPS-like	447125	509494	Equisetin	KC439347.1	NRP + Polyketide	0.18
53	19.2	8	NRPS	514479	591098				
54	19.3	8	Fungal-RiPP-like	612755	703644				
55	19.4	8	Terpene	823883	853011	Koraiol	HF679030.1	Terpene	1
56	19.5	8	Terpene	120916	124177				
				5					

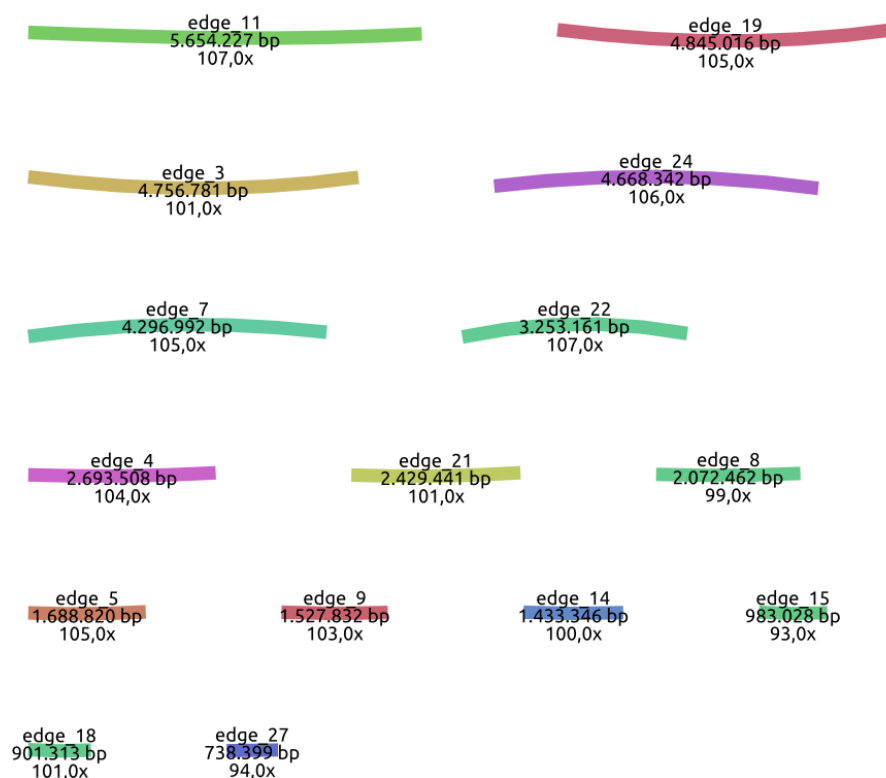


Figure S1. Contigs of *Fusarium sp.* VM-40 visualized by Bandage.

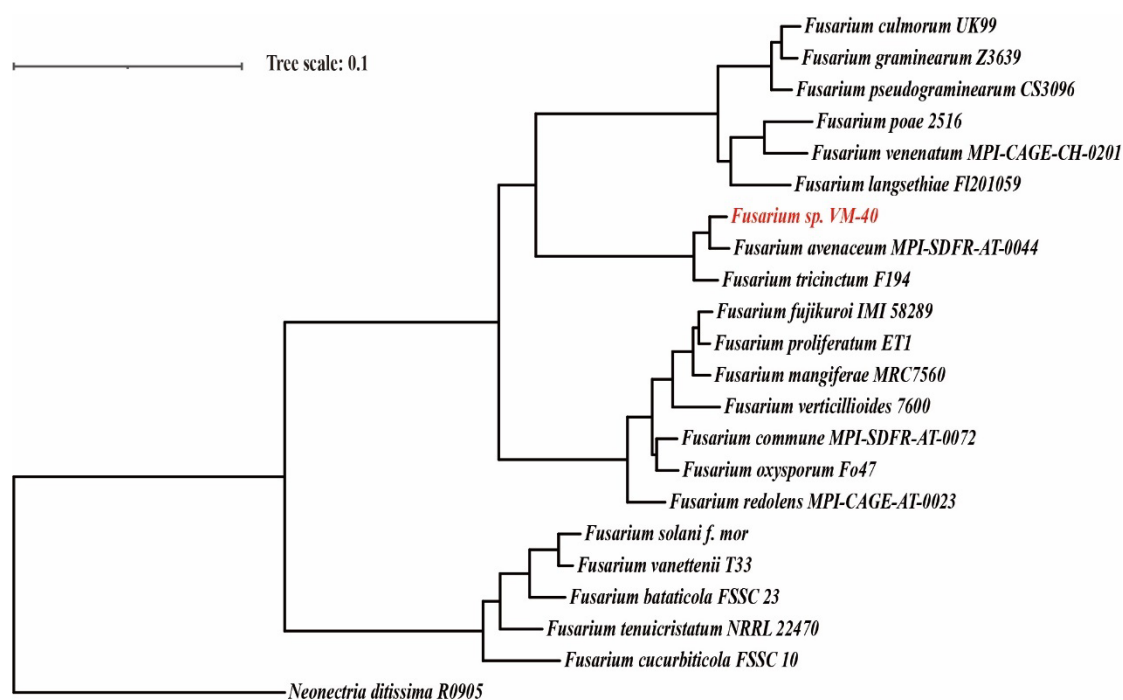


Figure S2. Taxonomic tree generated by comparing 3613 single copy orthologous genes from the genomes of 22 *Fusarium* species using OrthoFinder. Phylogenetic tree was conducted using FastTree with local bootstrap values of 1000 replicates.

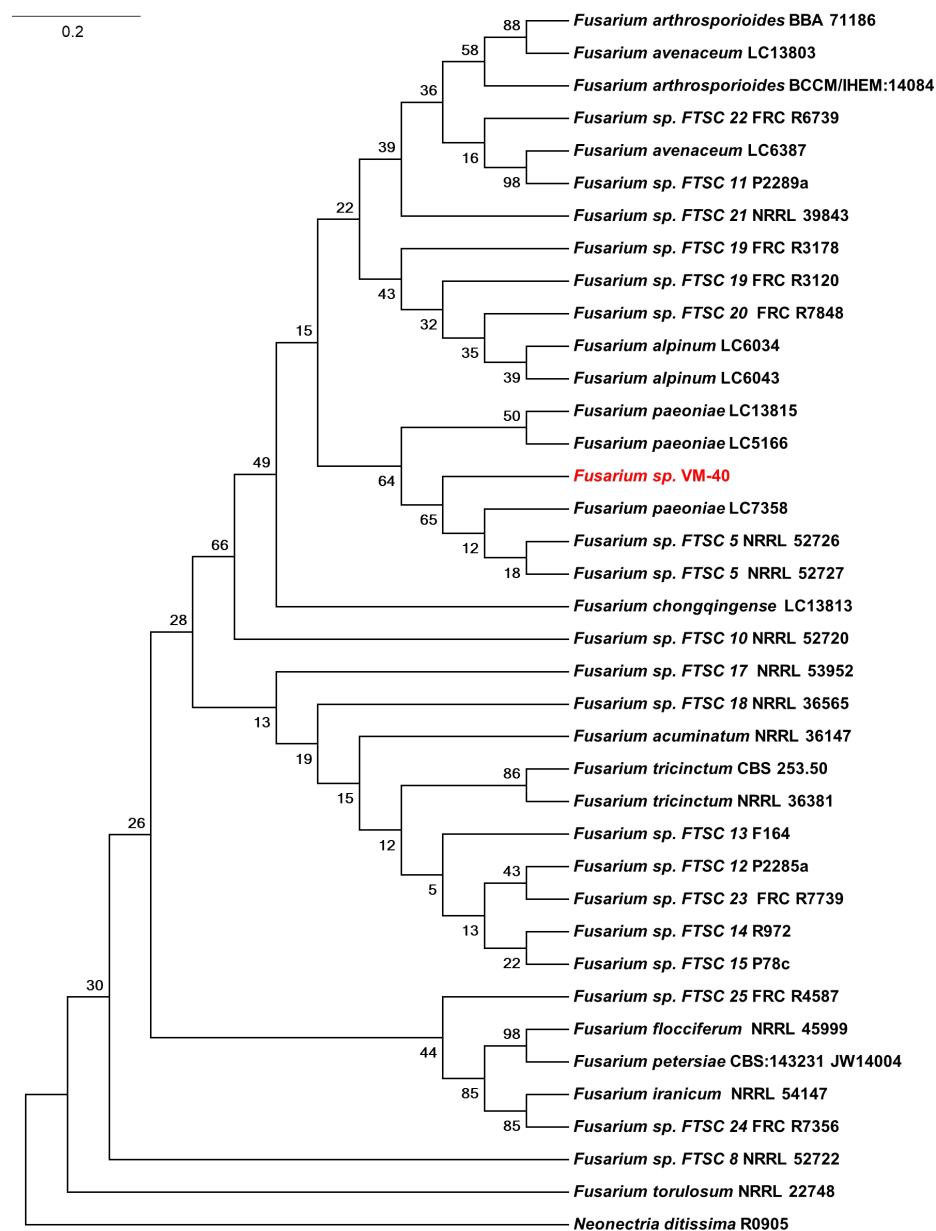
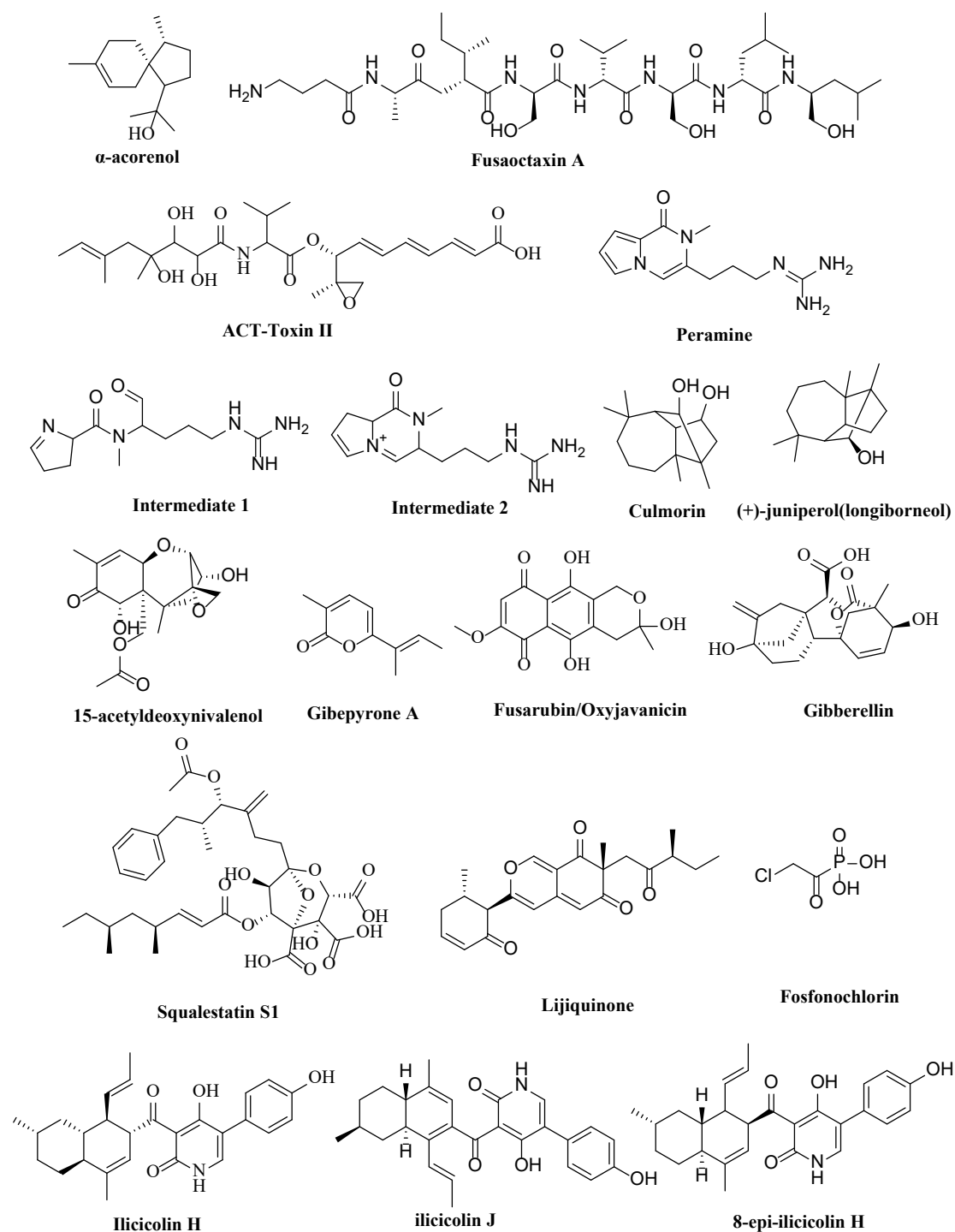


Figure S3. Maximum-Likelihood (ML) phylogram of FTSC species based on the comparison of the *tef1* gene. Phylogenetic tree was conducted using IQ-Tree with local bootstrap values of 1000 replicates.



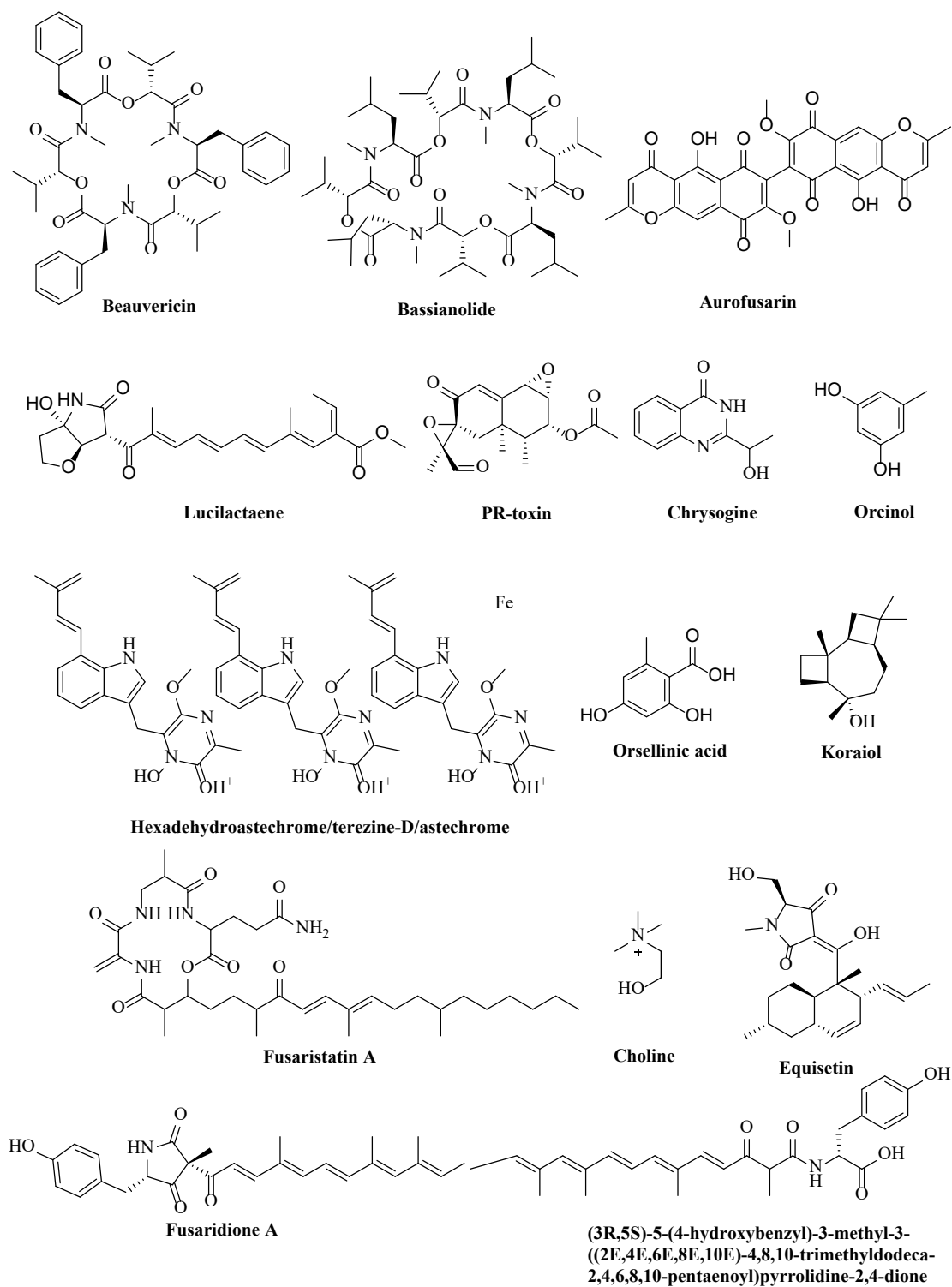


Figure S4. Chemical structures of the types of secondary metabolites that *Fusarium* sp. VM-40 may be able to produce based on the presence of known BGCs in its genome predicted by antiSMASH.

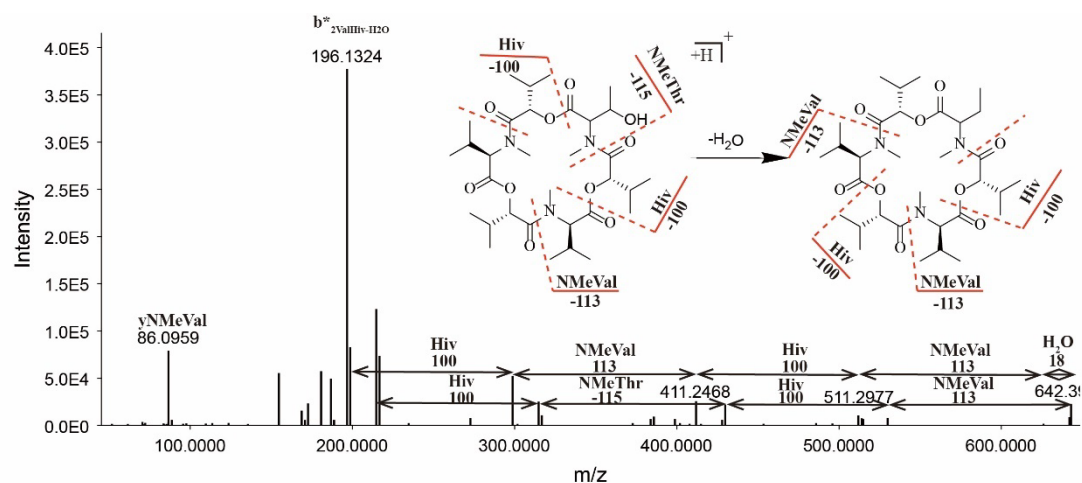


Figure S5. Proposed fragmentations of compound 8: $[M+H]^+$ - m/z 642.3979, $[M+H\text{-methyl-valine (NMeVal)}]^+$ - m/z 529.3160, $[M+H\text{-NMeVal-2-hydroxy-isovaleric acid (Hiv)}]^+$ - m/z 429.2618, $[M+H\text{-NMeVal-Hiv-N-methyl-Threonine(NMeThr)}]^+$ - m/z 314.1987, $[M+H\text{-NMeVal-Hiv-NMeThr-Hiv}]^+$ - m/z 214.1449, $[M+H\text{-H}_2\text{O}]^+$ - m/z 624.3943, $[M+H\text{-H}_2\text{O-NMeVal}]^+$ - m/z 511.3055, $[M+H\text{-H}_2\text{O-NMeVal-Hiv}]^+$ - m/z 411.2516, $[M+H\text{-H}_2\text{O-NMeVal-Hiv-NMeVal}]^+$ - m/z 298.1658, $[M+H\text{-H}_2\text{O-NMeVal-Hiv-NMeVal-Hiv}]^+$ - m/z 196.1343, y_{NMeVal} - m/z 86.0968, $b^*_{2ValHiv-H_2O}$ - m/z 196.1343

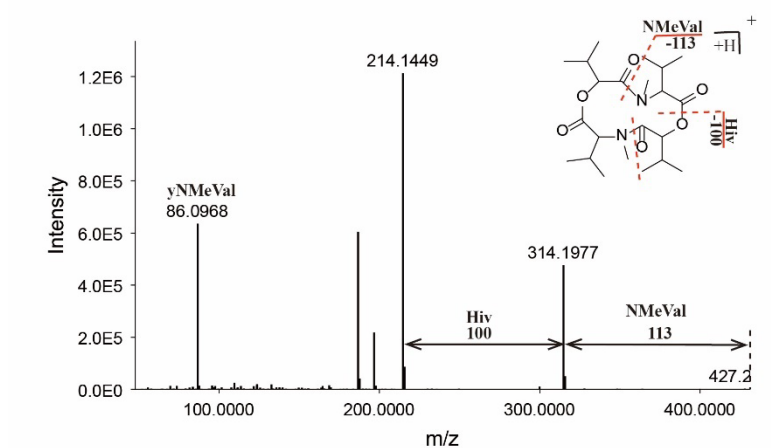


Figure S6. Proposed fragmentations of compound **10** (m/z 427.282).

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