SUPPLEMENTARY INFORMATION

Identification of Viridicatumtoxin and Griseofulvin Gene Clusters from *Penicillium aethiopicum*

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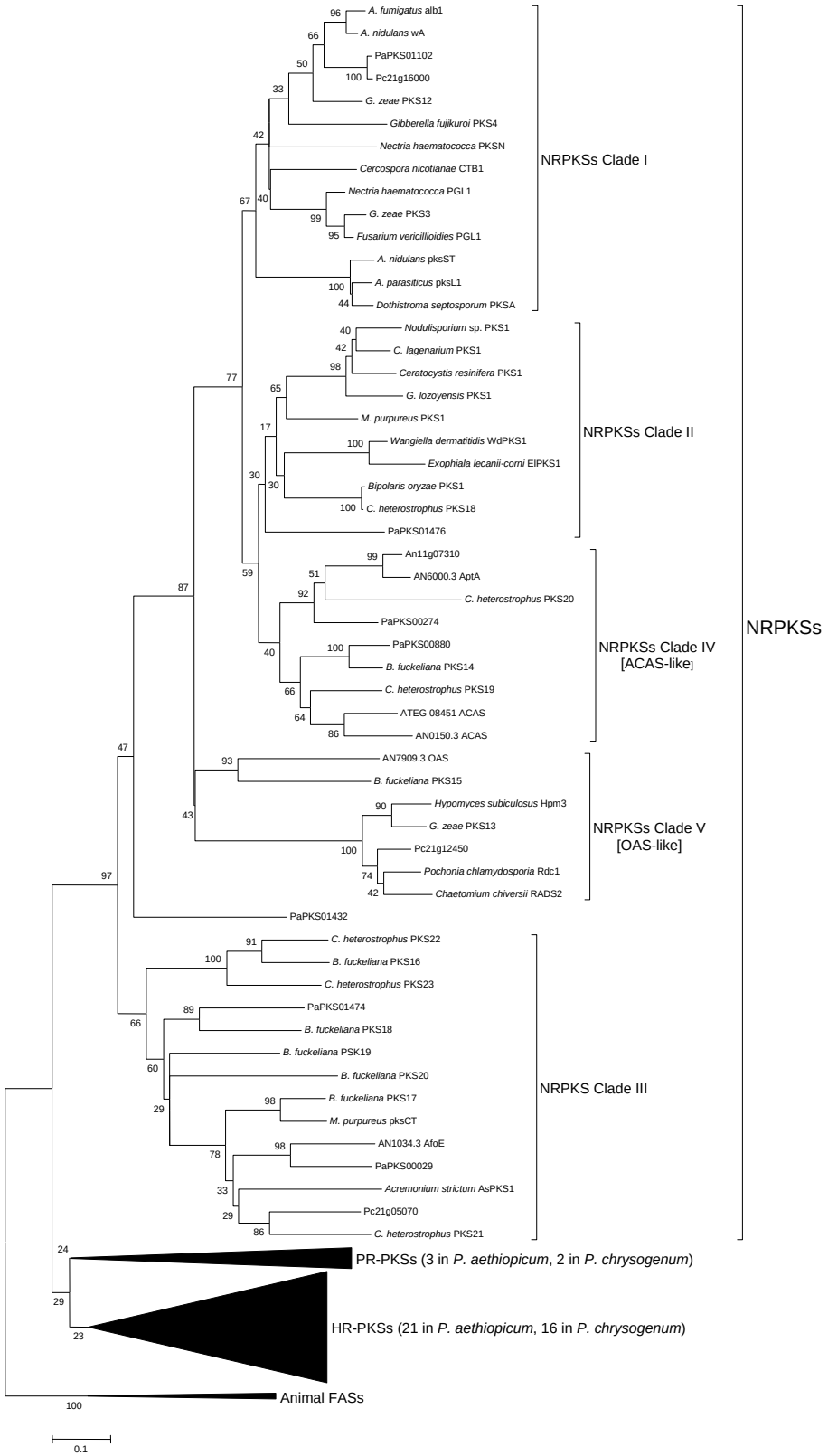


Figure S1. The KS domain phylogeny inferred using the Minimum Evolution method (bootstrap replicates = 500). The clades for PR-PKSs, HR-PKSs and animal FASs were compressed (not shown). Assignment of NR-PKSs clade I, II and III is based on Kroken et al. (2003). Two NRPKS clades (previously grouped as NRPKSs basal to clade I and II) were assigned as Clade IV (ACAS-like) and V (OAS-like), based on their recently characterized function.

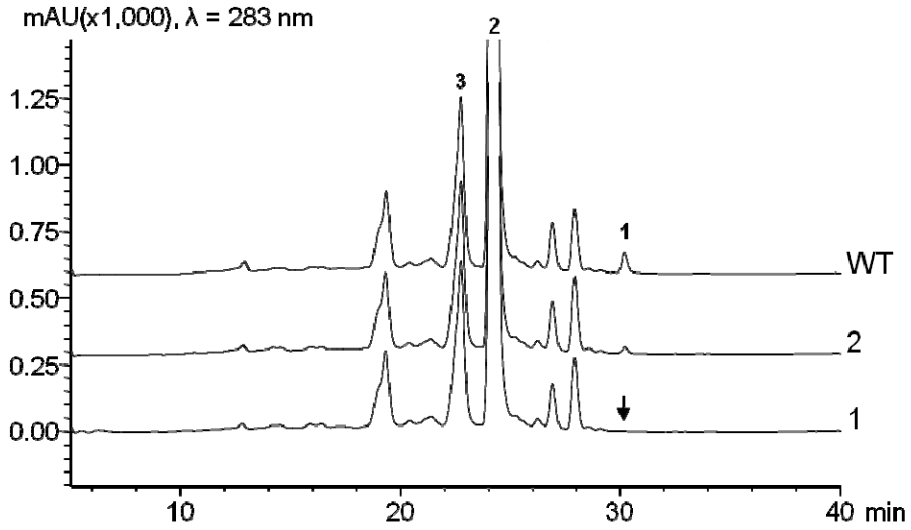


Figure S2. RNA-silencing of *vrtA* (PaPKS0274). Transformants PKS274si-2 showed reduced production of **1,** while production of **1** is completely abolished in PKS274si-1.

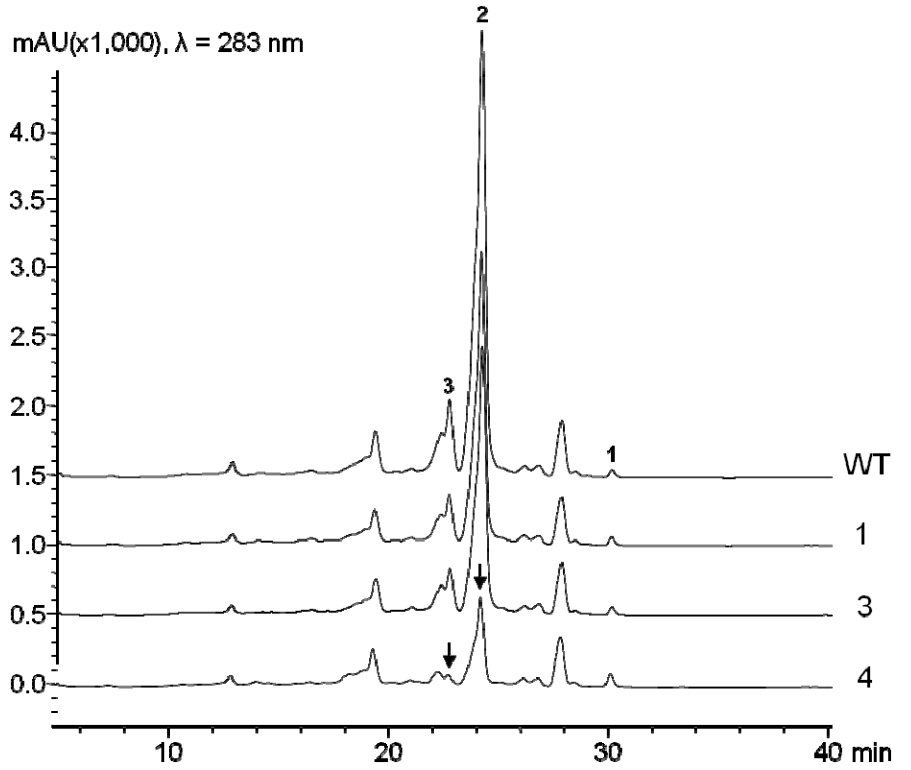


Figure S3. RNA-silencing of *gsfA* (PaPKS0880). Transformants PKS880si-1 and -3 have lower **2** and **3** levels than *WT*, while PKS880si-4 has the lowest level of **2** and **3** among all the RNA-silencing transformants.

Table S1. Domain architectures of putative PKSs in *P.aethiopicum* and comparison to the closest homologs in *P. chrysogenum* and other fungi. Highlighted rows indicate *P. aethiopicum* PKS genes that have an ortholog in *P. chrysogenum.*

	Contig No.	PKS Gene ID	length (a.a.)	PKS domains	PKS clades	Closest homolog in BlastP
1	00274	PaPKS0274	1824	SAT-KS-AT-PT-ACP	NR-PKS	NFIA_112240, 63%
2	01102	PaPKS1102	2138	SAT-KS-AT-PT-ACP-TE	NR-PKS	Pc21g16000, 96%
3	00880	PaPKS0880	1797	SAT-KS-AT-PT-ACP	NR-PKS	BC1G_08227, 61%
4	01476	PaPKS1476	2146	SAT-KS-AT-PT-ACP-TE	NR-PKS	PODANSg8506, 56%
5	01474	PaPKS1474	2521	SAT-KS-AT-PT-ACP-ACP-CM-TE	NR-PKS	AN6448.2, 40%;
6	00029	PaPKS0029	2684	SAT-KS-AT-PT-ACP-CM-R	NR-PKS	ATEG_07661, 58%;
7	01432	PaPKS1432	2108	SAT-KS-AT-DH-ACP-TE	NR-PKS	AFLA_112840, 87%
8	00990	PaPKS0990	2575	A-ACP-KS-AT-DH-KR-ACP-R	PR-PKS/NRPS	ChPKS24, 54%
9	00370	PaPKS0370	3181	A-ACP-KS-AT-DH-KR-ACP-R	PR-PKS/NRPS	ChPKS24, 57%
10	00002	PaPKS0002	2469	KS-AT-DH-CM-ER-KR-ACP	HR-PKS	PMAA_001080, 42%
11	00148	PaPKS0148	1737	KS-AT-DH-KR-ACP	PR-PKS	Pc22g08170, 93%
12	00861	PaPKS0861	3106	KS-AT-DH-CM-ER-KR-ACP	HR-PKS	Pc13g08690, 88%
13	01385	PaPKS1385	2181	KS-AT-DH-CM-KR	HR-PKS	AN1784, 50%
14	00181	PaPKS0181	2980	KS-AT-DH-CM-KR-ACP-C	HR-PKS	ACLA_055680, 67%
15	00107	PaPKS0107	2349	KS-AT-DH-ER-KR-ACP	HR-PKS	Pc16g03800, 95%
16	00876	PaPKS0876	2793	KS-AT-DH-CM-ER-KR-ACP	HR-PKS	ATEG_06056, 71%
17	00275	PaPKS0275	2474	KS-AT-DH-CM-ER-KR-ACP	HR-PKS	Pc16g11480, 83%
18	00891	PaPKS0891	2432	KS-AT-DH-CM-ER-KR-ACP	HR-PKS	FG01790, 42%
19	00927	PaPKS0927	2347	KS-AT-DH-CM-ER-KR-ACP	HR-PKS	Pc12g11530, 86%
20	00403	PaPKS0403	2582	KS-AT-DH-CM-ER-KR-ACP	HR-PKS	Pc13g04470, 88%
21	00150	PaPKS0150	2510	KS-AT-DH-CM-ER-KR-ACP	HR-PKS	PODANSg7686, 62%
22	00817	PaPKS0817	2665	KS-AT-DH-ER-KR-ACP	HR-PKS	AN2035, 62%
23	00430	PaPKS0430	3014	KS-AT-DH-CM-ER-KR-ACP-□TE	HR-PKS	Pc22g23750, 92%
24	01084	PaPKS1084	2442	KS-AT-DH-CM-ER-KR-ACP	HR-PKS	NFIA_062550,47%
25	00591	PaPKS0591	2457	KS-AT-DH-CM-KR-ACP	HR-PKS	Pc12g05590, 87%
26	00681	PaPKS0681	2724	KS-AT-DH-CM-ER-KR-ACP	HR-PKS	ACLA_002670, 66%
27	00337	PaPKS0337a	2527	KS-AT-DH-ER-KR-ACP	HR-PKS	SQTKS, 44%
28	00337	PaPKS0337b	4077	KS-AT-DH-CM-KR-ACP-C-A-T-R	HR-PKS/NRPS	ACLA_078660, 43%
29	00993	PaPKS0993	3974	KS-AT-DH-CM-KR-ACP-C-A-T-R	HR-PKS/NRPS	Pc14g00080, 87%
30	00310	PaPKS0310	3927	KS-AT-DH-CM-KR-ACP-C-A-T-R	HR-PKS/NRPS	ACLA_077660, 39%
31	00033	PaPKS0033	157	KS-AT(truncated)	HR-PKS/NRPS	CheA, 88%

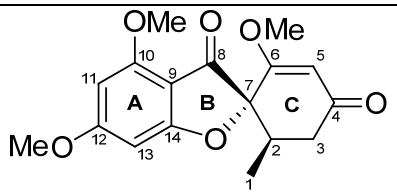
Abbreviations for functional domains: SAT – starter unit acyltransferase, KS – ketosynthase, AT – acyltransferase, PT – product template, ACP – acyl carrier protein, TE – thioesterase, R – reductase, CM – *C*-methyltransferase, DH – dehydratase, ER – enoylreductase, KR, ketoreductase, C – condensation, A – adenylation, T – thiolation.

Table S2. List of primers used in this study.

Primer Name	Sequence	Application
274PKS-KO-P1	tcgagcaatgaagaaagctc	fusion PCR of *vrtA* deletion cassette, Δ*vrtA* mutant PCR screening
274PKS-KO-P2	ctgcctgttacgttatggct	fusion PCR of *vrtA* deletion cassette
274PKSbar-KO-P3	tgcccgtcaccgagatttagg-atggcctcgtatgcagtcac	fusion PCR of *vrtA* deletion cassette
274PKSbar-KO-P4	tcaatatcatcttctgtcgac-caacgagcaggtgtacagac	fusion PCR of *vrtA* deletion cassette
274PKS-KO-P5	cacgtgctgtgacccgttag	fusion PCR of *vrtA* deletion cassette
274PKS-KO-P6	agcaaggtctatggagtaga	fusion PCR of *vrtA* deletion cassette, Δ*vrtA* mutant PCR screening
880PKS-KO-P1	ctctcgcgatccgagaggct	fusion PCR of *gsfA* deletion cassette, Δ*gsfA* mutant PCR screening
880PKS-KO-P2	ttagcggtctatgtacatga	fusion PCR of *gsfA* deletion cassette
880PKSbar-KO-P3	tgcccgtcaccgagatttagg-ggatctacatgtgtttcgac	fusion PCR of *gsfA* deletion cassette
880PKSbar-KO-P4	tcaatatcatcttctgtcgac-gaggcagaacttcccgctgt	fusion PCR of *gsfA* deletion cassette
880PKS-KO-P5	acgtcatgagatagaatgtc	fusion PCR of *gsfA* deletion cassette
880PKS-KO-P6	tgcatcgtctcccattactg	fusion PCR of *gsfA* deletion cassette, Δ*gsfA* mutant PCR screening
bar-F	agtaaccatgagcccagaacgac	internal primers for PCR screening of double homologous recombination
bar-R	agaaacccacgtcatgccagttc	internal primers for PCR screening of double homologous recombination
PtrpC-strt-F	gtcgacagaagatgatattga	amplification of PtrpC:*bar* resistance cassette from pBARKS1 plasmid
bar-stp-R	cctaaatctcggtgacgggca	amplification of PtrpC:*bar* resistance cassette from pBARKS1 plasmid
VrtB-F	atggctccgtatctacaacat	Δ*vrtA* mutant PCR screening, amplification of template for DIG-labeled probe (Δ*vrtA* Southern blot)
VrtB-R	tcaattctgcgttgcccact	amplification of template for DIG-labeled probe (Δ*vrtA* Southern blot)
GsfI-F	atggcgattcctcaatcttgt	Δ*gsfA* mutant PCR screening, amplification template for DIG-labeled probe (Δ*gsfA* Southern blot)
GsfI-R	ctacatttggagatcccctgca	amplification of template for DIG-labeled probe (Δ*gsfA* Southern blot)

Note: Primers with name ending with P1 – P6 followed the designation for fusion PCR as described previously (Szewczyk, E., Nayak, T., Oakley, C.E., Edgerton, H., Xiong, Y., Taheri-Talesh, N., Osmani, S.A., and Oakley, B.R. (2007). Fusion PCR and gene targeting in Aspergillus nidulans. Nat Protocols *1*, 3111-3120.)

Table S3. ^{13}C and ^{1}H NMR for **3**[a].



No.	^{13}C δ (ppm)	^{1}H δ (ppm) (m, area, J_{HH} (Hz))
1	14.6	0.95 (d, 3H, 6.7)
2	36.9	2.74 (dqd, 1H, 4.7, 6.7, 13.4)
3	40.4	2.38 (dd, 1H, 4.7, 16.7)
		3.04 (dd, 1H, 13.4, 16.7)
4	197.6	-
5	105.1	5.52 (s, 1H)
6	170.7	-
7	90.2	-
8	192.8	-
9	104.6	-
10	159.4	-
11	88.9	6.22 (d, 1H, 1.7)
12	176.0	
13	93.7	6.03 (d, 1H, 1.7)
14	171.6	-
MeO	56.4	3.89 (s, 3H)
MeO	56.4	3.89 (s, 3H)
MeO	56.9	3.61 (s, 3H)

[a] Spectra were obtained at 400 MHz for proton and 100 MHz for carbon and were recorded in chloroform-*d*.