Supporting Information

Heterologous Expression of Highly Reducing Polyketide Synthase

Involved in Betaenone Biosynthesis

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General.

All reagents commercially supplied were used as received. Optical rotations were recorded on JASCO P-2200 digital polarimeter. ¹H- and ¹³C-NMR spectra were recorded on Bruker AMX-500 spectrometer, Bruker DRX-500. NMR spectra were recorded in CDCl₃ (99.8 atom % enriched, Kanto) or C₆D₆ (99.5 atom % enriched, Kanto). ¹H chemical shifts were reported in δ value based on internal TMS (0 ppm), CDCl₃ (7.26 ppm), or benzene (7.15 ppm) as references. ¹³C chemical shifts were reported in δ value based on chloroform (77.0 ppm) or benzene (128.0 ppm) as references. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant (Hz), and integration. Mass spectra were obtained with a JEOL JMS-T100LP (ESI and APCI mode). Column chromatography was carried out on 60N silica gel (Kanto Chemicals).

Strain and Culture Conditions.

Phoma betae Fr. PS-13 was obtained from the Hokkaido National Agricultural Experimental Station, Sapporo, and cultured at 30 °C in potato glucose medium. The host strain used in this study was *A. oryzae* NSAR1, a quadruple auxotrophic mutant (*niaD*⁻, *sC*⁻, $\Delta argB$, *adeA*⁻) for fungal expression.

General technique for DNA manipulation.

Genomic DNA of *Phoma betae* Fr. PS-13 was extracted according to the literature procedure. PCR reactions were performed with the KOD-Plus-Neo (TOYOBO). The primers used to amplify the genes were purchased from Hokkaido System Science Co., Ltd.

Accession number.

The betaenone biosynthetic gene cluster sequence has been deposited in the DNA Data Bank of Japan (DDBJ) with the accession number LC011911.

Construction of expression vector.

The pAdeA2, an expression vector carrying *Aspergillus nidulans adeA* gene as selectable marker, was prepared as follows: The *adeA* gene was amplified from genomic DNA of *A. nidulans* with primer set as shown in Supplemental Table S3 and inserted into the *SphI/XbaI*-digested pUSA2 using Gibson assembly cloning kit to give pAdeA2 expression vector.

Preparation of expression plasmids.

The *bet1* fragments (Fr1-Fr3) were amplified from genomic DNA of *P. betae* with primer set as shown in Supplemental Table S3. Fr1 was inserted into the *ClaI*-digested pUARA2 using In-Fusion method to obtain pUARA2-*bet1*-Fr1. Fr2 and Fr3 was inserted into the resultant plasmid in a stepwise manner to afford pUARA2-*bet1*. The strategy is summarized in Figure S9. The *bet2, bet3* and *bet4* were also amplified from genomic DNA of *P. betae* with primer set as shown in Supplemental Table S3. Each gene was inserted into the *ClaI*-digested pUSA2 or *NheI*-digested pAdeA2 using In-Fusion method to obtain pAdeA2-*bet2*, pUSA2-*bet3* and pAdeA2-*bet4*.

The bet1 mutant genes were prepared by fusion PCR; two flanking sequences were initially

amplified from pUARA2-*bet1* plasmid with primer set as shown in Supplemental Table S3. Then, amplification using nested primers fuses the two fragments into a single molecule (Fr3-S2705A, Fr3-Y2737F, and Fr3-K2741A). Resultant fragment was inserted into the pUARA2-*bet1*-Fr1, 2 to afford pUARA2-*bet1-mutant*.

Transformation of Aspergillus oryzae.

A spore suspension of A. oryzae NSAR1 or dehydroprobetaenone I-producing transformant (1.0 x 10⁸ cells) were inoculated into DPY (dextrin-polypeptone-yeast extract: 2% dextrin, 1% polypeptone, 0.5% yeast extract, 100 mL) medium supplemented with appropriate nutrients. After 3 days incubation at 30 °C, mycelia was collected by filtration and washed with water. Protoplasting was performed using Yatalase (Takara; 5 mg mL⁻¹) in Solution 1 (0.8 mM of NaCl, 10 mM of NaH₂PO₄, pH 6.0) at 30 °C for 2 h. Protoplasts were centrifuged at 2,000 rpm (Beckman JLA10.500) for 5 min and washed with 0.8 M of NaCl solution. Then, protoplasts were adjusted to 2.0 x 10⁸ cells/mL by adding Solution 2 (0.8 M of NaCl, 10 mM of CaCl₂, 10 mM of Tris-HCl, pH 8.0) and Solution 3 (40% (w/v) of PEG4000, 50 mM of CaCl₂, 50 mM of Tris-HCl, pH 8.0) in 4/1 volume ratio. Plasmids (pUARA2-bet1 and pUSA2-bet3 for the first transformation of wild type strain. pAdeA2-bet2 or pAdeA2-bet4 for the second transformation of dehydroprobetaenone I-producing strain. 12 µg each) added to the protoplast solution (200 µL). The aliquot was incubated on ice for 20 min and then Solution 3 (1 mL) added to the aliquot. After 20 min incubation at room temperature, Solution 2 (10 mL) added to the mixtures and the mixture was centrifuged at 2,000 rpm (Beckman JLA10.500) for 5 min. The transformation mixture was poured onto the Czapek-Dox (3.5%) agar plate supplemented with 0.8 M of NaCl and appropriate nutrients and then overlaid with the soft-top agar (1.2 M of sorbitol, 3.5% of Czapek-Dox, 0.6% of agar). The plates were incubated at 30 °C for 3-7 days.

Production and HPLC analysis of the metabolites.

Mycelia of *A. oryzae* transformants were inoculated into a solid medium containing polished rice (100 g) and adenine (10 mg) in 500 mL Erlenmeyer flasks. Each culture was incubated at 30 °C for 2 weeks. After extraction with ethyl acetate, the extract was then concentrated in vacuo and the residues were extracted with ethyl acetate (100 mL x 2). The combined organic layers were washed with brine, dried over Na₂SO₄ and concentrated in vacuo. The crude extracts were directly analyzed by LC-MS equipped with ZORBAX XDB-C18 column (50 mm x 2.1 mm) at the following conditions:

LC conditions to analyze dehydroprobetaenone I and probetaenone I;

A linear gradient from 50% to 100% acetonitrile for 15 min and 100% acetonitrile for 15 min at a flow rate of 0.2 mL/min.

LC conditions to analyze betaenone B;

A linear gradient from 30% to 85% acetonitrile for 15 min and 100% acetonitrile for 15 min at a flow rate of 0.2 mL/min.

Dehydroprobetaenone I (5).

HR-ESIMS analysis of **5**; calcd. for $C_{21}H_{35}O_2$ [M+H]⁺: 319.2632, found: 319.2682. [α]_D²³ -19.3 (c 0.3 CHCl₃). ¹H-NMR (500 MHz, C₆D₆) δ 15.5 (s, 1H), 7.39 (s, 1H), 5.41 (d, *J* = 4.9 Hz, 1H), 5.18 (s, 1H),

1.96 (t, J = 9.5), 1.85 (m, 1H), 1.71 (m, 1H), 1.67 (s, 3H), 1.66-1.60 (m, 2H), 1.59 (s, 1H), 1.53 (m, 1H), 1.45 (m, 1H), 1.20 (m, 1H), 1.00 (d, J = 7.1 Hz, 3H), 0.99 (s, 3H), 1.05-0.97 (m, 2H), 0.96 (s, 1H), 0.92 (t, J = 7.2, 3H), 0.86 (d, J = 6.5, 3H), 0.85 (d, J = 7.0, 3H). ¹³C-NMR (125 MHz, C₆D₆) δ 207.7, 173.5, 131.2, 127.4, 100.1, 58.8, 50.7, 46.8, 44.1, 42.7, 42.5, 38.1, 35.3, 34.0, 27.1, 25.9, 22.4, 22.3, 22.0, 18.7, 13.4.

Betaenone B (2).

HR-APCIMS analysis of **2**; calcd. for $C_{21}H_{35}O_5$ [M+H]⁺: 369.2636, found: 369.2599. [α]_D²⁴ 24.1 (c 0.3 CHCl₃).



Reduction of dehydroprobetaenone I (5)

To a solution of dehydroprobetaenone I (28.6 mg, 89.9 μ mol) in methanol (2.0 mL) was added NaBH₄ (1.3 mg, 30 μ mol) in methanol (0.5 mL) and the mixture was stirred for 50 min at room temperature. After concentration, the crude residue was dissolved in ethyl acetate. The solution was washed with water, dried over anhydrous Na₂SO₄, and concentrated in vacuo. The crude residue was purified by silica gel chromatography (hexane/EtOAc 10:1) to give probetaenone I (28.6 mg, quant.).

Conformational search of dehydroprobetaenone I (5)

The lowest energy conformer of **5** was determined by a conformational search. Calculations were performed using Maestro v9.8 (Force field: OPLS_2005; Solvent: None; Conformational search: MonteCarlo Multiple Minimum (MCMM) method; Minimization method: Powell-Reeves conjugate gradient (PRCG); Steps: 10000). 6 conformers were found within a range up to 8 kJ/mol above the global minimum. The conformers are summarized in Supplementary Figure S6.

Figure S1. Gene integration analysis of (A) *bet1/3* gene in double transformants, (B) *bet4* in *bet1/3/4* triple transformants, and (C) *bet2* in *bet1/2/3* triple transformants.

(A) *bet1/3* double transformant *bet1*-Fr1 bp~2278 bp bet3 Μ 1-1 1-2 1-3 1-4 1-5 1-1 1-2 1-3 1-4 1-5 Μ (B) *bet1/3/4* triple transformant bet4 2-1 2-2 2-3 2-4 2-5 2-6 Μ (C) *bet1/2/3* triple transformant bet2 Μ 3-1 3-2 3-3 3-4 3-5

HO (A) 319 0= н dehydroprobetaenone I (5) *m/z* 319 [M+H]⁺ 303 (B) HO 321 0= н (C) probetaenone I (4) *m/z* 321 [M+H]⁺ 351 369 HO 333 0: н (D)] 369 351 HO H H OF betaenone B (2) ÔН 333 m/z 369 [M+H]+ 300 360 380 320 340 m/z

Figure S2. MS spectra of metabolites. (A) **5** from *bet1/3* transformant, (B) **4** from *bet1/3/4* transformant, (C) **2** from *bet1/2/3* transformant, and (D) authentic **2** from *P. betae*.

Figure S3. Schematic representation of the fungal expression vector, pAdeA2. P_{amyB} , amyB promoter; T_{amyA} , amyA terminator; T_{amyB} , amyB terminator; adeA, phosphoribosylaminoimidazolesuccino-carboxamide synthase.



Figure S4. Multiple sequence alignment of R domains. The sequences referred to are as follows; AfoE (XM_653546) from asperfuranone biosynthetic gene cluster and AzaA (AGN71604) from azaphilone biosynthetic gene ccluster.

GxxGxxG motif

AfoE	VTGATGSLGSHVVGYLSRLPNVHTVVCLNRRSTVPATIRQEEALKVRGISLDDNSRSKLV	60
AzaA	VTGATGSLG ⁵ HLVSHFAQLNDVTSVVCINRVSREDPTRRQQQSMESKGISLNNTALSKLI	60
BetFA	LTGCSGLLGHHLLNTLIAQPSICKIICLAVRRLSSRLESGDLPAPSERIC	50
	:* <mark>**.:* **</mark> *::. : .: .::*: * :: .: ::	
AfoE	VLEVETAKPLLGLPVETYQKLVNTATHIVHSAWPMSLTRPIRGYESQFKVMQNLINLARE	120
AzaA	$\tt ILETDTSKPMLGLPAEQYQHLVNNVTHVLHNAWPMSGKRPVKGFELQFQVMKNLITLAWD$	120
BetF	YYEGDLTSTYFGLDTTTWTSIFHETDAVIHNGSDTSHLKYYSALKQANVESTK	103
	* : : :** . : :.: . ::* * . :::: : .	
	SYK motif	
AfoE	VAAWRPVPFKFSFQFISSIGVVGYYPLRYG-EIIAPEETMTADSVLPVGYAEAKLVCERM	179
AzaA	ISCRRGPDFKVRFQLISSISVVGYYPLRTG-NRHVPEERVCIEDLLPNCYSDAKYVCELM	179
BetF	QLVSTCLQRMIPLHYISSAGVALFAGLAAFPPISCTQTGKTPPADGSHQYMCQKWVCEKM	163
	· :: *** ·*· : * · ·: · · * ·* *** *	
A foE		226
AzaA		230
BetF		230
200		225
AfoE	SWCPVDDVSATLGELLISNTTPYSIYHIENPSROOWRKMVKTLAOSLDIPRDGIIPFDOW	296
AzaA	SWTPVNDVAGTLADLLLADNTPYPIYHIDNPVROPWREMIPILADGLDIPKGNVIPFPEW	296
BetF	DLVSVETCCEDVVRELPNRGREGITYVNNVGDVVIPMAOMADVGLSKVEKRYSVLPMEEW	283
	*: . : * * : : : : .::*: :*	
AfoE	IERVRNSSASIN-DNPARQLLEFFDQHFIRMSCGNLILDTTKTREHSATLRERGPVGPGL	355
AzaA	VQRVRRFPGSVELDNPAAKLIDFLDDNFLRMSCGGLLLDTSKSREHSPTLADVVPVSAEA	356
BetF	TKIVVNAGMHPAVAALIETFDEPGVEKYPALLRSEDA	320
	: *	
TO A A F		
ZaAAI oFBet	VEKYISAWKTMGFLD 370	
F	VHKYIQAWKEMGFLH 371	
1		

Figure S5. LC-MS profiles monitored at *m/z* 319 of the metabolites from R domain mutant strains. (A) *A. oryzae* NSAR1. (B) *bet1* transformant. (C) *bet1/3* transformant. (D) *bet1*-S2705A/*bet3*. (E) *bet1*-Y2737F/*bet3*. (F) *bet1*-K2741A/ *bet3*.



Figure S6. (A) calculated conformations of 5. (B) Detailed analysis of conformer B.









Structure	Potential energy (kJ/mol)	Relative potential energy (kJ/mol)	Boltzmann populations (%)
А	202.835	0	54.6
В	205.025	2.19	22.6
С	207.247	4.412	9.2
D	207.928	5.093	7
Е	209.848	7.013	3.2
F	210.763	7.928	2.2

(B)



Figure S7. A phylogenetic tree of trans-ER and cis-ER domain of HR-PKS and PKS-NRPS in biosynthetic gene cluster of various polyketide metabolites. * shows inactive cis-ERs because of the point mutation on NADPH binding motif.



Figure S8. Multiple sequence alignment of ER domains. The sequences referred to are summarized in Table S2.

NADPH binding motif

	(FSL5	FRSLDVPGYPEAPATERIPVLVYGGSTATGTLAIOLLK-LSGLIPITTCSPHN
	EaxC	FKSLGLPGNPLSPATEKLPVLVYGGSSATGTAAIOLVR-LAGFAPITTCSPRN
	CcsC	FRTLELPGTPEEPAOKPLTVLVHGGSSSVGTMAMOLLR-LVGHIPITTCSPRN
	LovC	LLGLPLPSPSADOPPTHSKPVYVLVYGGSTATATVTMOMLR-LSGYIPIATCSPHN
trans-ER <	CheB	YOEMRLKCTPSAPAKKGFPVLVSGAGTATGALATOILT-LSGLOPIVTSSPGS
	ApdC	YHCLRLPMKPEOAGKSPY-VLVYGGSTATGTLAIOILT-RSGYAAITTCSPHN
	DmbC	YLHISMPSLLEAMSRSIAAPSASHDHDGASDGDANVFILVYGGGTSTGAIAIQILK-AAGFTPITCCSSES
	Bet3	PTALNLPGTPSKPDARSLPVLVYGGATATGIIAIQMLK-RSGYIPIAVCSAQS
	LovF	VARILPGETVLIHAGAGGVGQAAIILAQ-LTGAEVFTTAGSET
	AzaB	TARLORGEIGAEIYATASTPA
	CazF	VARLRHGQVARLRHGQSVLIHAAAGGVGQAAVMLAQDYLGAEVYATVGSQE
	AfoG	AARLSQGQAARDSQCAAVILAKEYLGAEVFATVGSQE
active	Rdc5	LAKLRPGQFATVGTDT
	Hpm8	IAKLRKGQVLIHAAAGGVGQAAIQLAK-HLGLITYVTVGTED
cis-ER ^{<}	PKSN	AGGIVAGEAGAIMIAQ-AKGAEIFATVGADT
	EasB	KGSLSKGEHIGAEVFATVGSEA
	Fum1P	VGGLRPGQVGGLEIYTTVGNEE
	Sol1	VTQLKDQEAGAINATAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
	Acrts2	TARLTKNE = = = = = = = = = = = = = = = = = = =
	PKSF	YCGTNSGDVILVHDAASVLG <u>D</u> AIIKIAAIHGYTKIFATVSSAG
	ApdA	CNRLPASNHFSFVSADKAS
	CcsA	lrdvpygsqialqpnsllapalreaaqdkgvtlhlwstqasd
	Actts3	LRCISPNSVALABAIRHHAHENTVDVFPATSNREK
	FSL1	MYMLPPTGORTALIST MULTER MALE AND
inactive cis-ER	Pyr2	FDQLPPFGVIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII
	LovB	ISTAKCLGOTDSILVLNPPSICGQMLLHAGEEIGLQVHLATTSGNR
	Bet1	FCDSLFTG
	EqxS	LERLAKGEVALANA LERLAKGETLVVHDTPPHVRVALDKLAKTASVAIFYTSSDEAM
l	CheA	IVQSHQTARANDADDLLARAISQTIEGRLHFSASNAVVHQD
	CDmbS	RRHGQRQRSALIYGADEELAELTSKRCAVRESKIYFASSHSAA

"Fingerprint" region for active ER

	FSL5	TPDEIRKFTRN-SLKYVLDCISEPETMQFCYKCIGRTGGKYTALEPFPQFLH-TRPTIQPDWV	-LGPTLLGKPIGWGPP
	EqxC	CISDIKKHTKN-NIRYALDCISTTQSMQFCYQAIGRAGGKYTALEPYSEAVARTRKMVKPDWI	-MGPQMLGKEIRWPEP
trans-ER <	CcsC	CGQKIKAYTRN-TLRYVLDPFTDAKSIALCCGAMGRAGGRYACLEMYPDYLV-EKRTLRVGFV	-MGPALLGHRLELDYG
	LovC	LAQTIRTYTKN-NLRYALDCITNVESTTFCFAAIGRAGGHYVSLNPFPEHAA-TRKMVTTDWT	-LGPTIFGEGSTWPAP
	CheB	CGSDIREYTAN-ELGCALDCHADSGSMTICYNAIGSDGGRYVALNPFPLRVH-RRRSVQPRWV	-FMFTQFDQTIPWKRP
	ApdC	CGRQIRDFSSG-NLWYALDCITDTRSMAVCYEAIGPSGGRYLSLDPFPIRGH-TRRSVKPNWV	-LSVTMYNQPIPWKRP
	DmbC	CGRDIREYTND-SLALAIDCLSESASMAICYEAIGSAGGRYVSLDPFPIRGC-VRRSIVPDWI	-CSFTQFGHSINWAPP
	Bet3	CVQDIKALANGQSIKHALDCITDPESTTVCLASLARIGGRYACLEAVSDACI-TRRSVAVKVV	-MGFEGQNFDVDLGHP
	LovF	FVDGIKTRTRGKGVDVVLNSLAGPLL-QKSFDCLARFG-RFVEIGKKDLEQNSR	-LDMSTFVRNVSFSSV
	AzaB	FATDVHLRTDGRGVDVVLNSLAGRLL-QESFNCLAEFG-RMVEIGKRDLEQHSG	-LDMYPFTRNVSFSSV
active cis-ER	CazF	FAPAVLAATGGRGVDAVLNSLGGSLL-QASFEVLAPFG-NFVEIGKRDLEQNSL	-LEMATFTRAVSFTSL
	AfoG	FAPAALAATAGRGVDCLIEVLAPFG-HFVEIGKRDLEQNSL	-LEMATFTRAVSFTSL
	Rdc5	FVKGIERATNGRGVDCVLNSLSGELLRLSWG-CLATFG-TFVEIGLRDITDNMR	-LDMRPFAKSTTFSFI
	Hpm8	FVKGVQRVTNGRGVDCVLNSLSGELLRASWG-CLATFG-HFIEIGLRDITNNMR	-LDMRPFRKSTSFTFI
	PKSN	FVKGVLRATDGQGVDLVLNSLAGEALRLSWTDCLAKFG-RFLEIGKADLFANTG	-LDMKPLLDNKSYIGV
	EasB	FYGEIMKSTGGYGVDVVLNSLSGEMFRESCN-LMASFG-RFVEIGRKDLMDDAL	-MPMEFLLRNITFSYV
	Fum1F	FLYDVRAATQGRGVDLVLNSLSGDLLHASWQ-CVAPYG-KMLEIGKRDFIGKAR	-LEMDLFEANRSFIGI
	Sol1	DFAKLMEATDGKGFNVILSTSSGELLYDSIK-MLAPMG-RIIDVGRIDVQNSTS	-LALELFKRNATFTSF
	Acrts2	FRDSLLCKTNNRGVDVVVNSLSGELLTETWA-VIAAFG-RFVEIGKKDAFLNNS	-LPMRPFNNNVTLSAI
	PKSF	FQHGIRHLTNGEGVAVVVGSEDHLQDSWPCIAPFG-RFVGVGEKDVFHSTANG	SKEIVLPPTTKNIAFV
	ApdA	DFDALIDCTGE	-LPENLIACLSRNCRV
	CcsA	NVTCFFDMGGDESIATKILAC	-LPDHTQAKKEASITA
	Actts3	NTGCFINLSFK	-PPGALSRALLQQTII
inactive	FSL1	GVTFFIDCGQAEDVIHEGSHGKDHGLGLRLHNSLSRTC	-VKRTLQDLTSRTASV
	Pyr2	DVSAVAVLDRRGQGIYDR	-MLSLLPDNATRIQID
cis-ER	LovB	GVQALVDLSADQSCEGLTQRMMKVLMPG	CAHYRAADLFTDTVST
	Bet1	DIVCFVDFSASIQAENVAMITS	CLPSYCRKENVNTIFS
	EqxS	SASKFIDLSQDSDKNETSKVIS	-MCLPWDYETIDTAHL
	CheA	DIAMLLDCSEQSQSNE	-VLVQCIPATCRIAGL
l	↓ DmbS	GVQVFIDCLGGTESFDACR	-TLQSCLPTTCTVHRL

Figure S9. Construction of expression plasmid, pUARA2-bet1.



 Table S1. Proposed function for betaenone biosynthetic genes from Phoma betae.

Gene	Size (bp)	Homolog		Identity (%)/ Similality (%)
orf2	1839	dehydrogenase	XP_007587319	67%/79%
orf1	1761	FAD binding domain protein	XP_008031658	71%/82%
bet4	1041	short-chain dehydrogenase	XP_008031659	83%/90%
bet3	1095	enoyl reductase (trans-ER)	XP_003306797	82%/88%
bet2	1566	cytochrome P450	EMD93172	87%/91%
bet1	8715	polyketide synthase [KS-AT-DH-MT-ERº-KR-ACP-R]	AFN68297	84%/90%
orf3	405	hypothetical protein	XP_007688029	49%/52%
orf4	3369	hypothetical protein	XP_003303789	39%/54%

Table S2. Summary of functionally characterized HR-PKSs.

Environ. Microbiol. 2012, 14, 1159.	unknown	EYB31404	FSL5	ESU17181	FSL1 [KS-MAT-DH-MT-ERº-KR-ACP]	fusarielin F
Science 2009, 326, 589.	LovG catalyzing hydrolysis	AAD34554	LovC	AAD39830	LovB [KS-MAT-DH-MT-ER°-KR-ACP-CON]	lovastatin
Science 2009, 326, 589.	LovD catalyzing acyl transfer	I	I	AAD34559	LovF [KS-MAT-DH-MT-ER-KR-ACP]	lovastatin
FEMS Microbiol. Lett. 2005, 248, 257.	unknown	ļ	I	AAD43562	Fum1p [KS-MAT-DH-MT-ER-KR-ACP]	fumonisin B1
Mol Plant Microbe Interact. 2010, 23, 406.	unknown	I	I	BAJ14522	Actts3 [KS-MAT-DH-MT-ER°-KR-ACP]	ACT-toxin
Mol Plant Microbe Interact. 2012, 25, 1419.	unknown	I	I	BAN19720	Acrts2 [KS-MAT-DH-MT-ER°-KR-ACP]	ACR-toxin
Chem. Biol. 2008, 15, 527.	unknown	I	I	XP_660151	EasB [KS-MAT-MT-DH-ER-KR-ACP]	emericellamide
J. Am. Chem. Soc. 2012, 134, 17900.	transfer to NR-PKS	ļ	I	XP_001216280	CazF [KS-MAT-DH-MT-ER-KR-ACP]	chaetomugilin A
Chem. Biol. 2012, 19, 1049.	transfer to NR-PKS	I	I	EHA28244	AzaB [KS-MAT-DH-MT-ER-KR-ACP]	azanigerone A
J. Biol. Chem. 2010, 285, 41412.	transfer to NR-PKS	I	I	ACD39774	Rdc5 [KS-MAT-DH-MT°-ER-KR-ACP]	radicicol
J. Am. Chem. Soc. 2009, 131, 2965.	transfer to NR-PKS	ļ	I	XP_658640	AfoG [KS-MAT-DH-MT-ER-KR-ACP]	asperfuranone
J. Am. Chem. Soc. 2013, 135, 1735.	transfer to NR-PKS	I	I	ACD39767	Hpm8 [KS-MAT-DH-MT°-ER-KR-ACP]	hypothemycin
Nat. Chem. 2010, 2, 858.	spontaneous cleavage	I	I	XP_751268	Pyr2 [KS-MAT-DHº-MTº-ERº-KRº-ACP]	pyripyropene
ChemBioChem 2006, 7, 920.	spontaneous cleavage	I	I	BAE80697	PKSF [KS-MAT-DH-ER-KR-ACP]	aslanipyrone
Chem. Biol. 2005, 12, 1301.	spontaneous cleavage	I	I	BAD83684	PKSN [KS-MAT-DH-MT-ER-KR-ACP]	alternapyrone
ChemBioChem 2010, 11, 1245.	spontaneous cleavage	I	I	BAJ09789	Sol1 [KS-MAT-DH-MT-ER-KR-ACP]	solanapyrone
Reference	Chain release mechanism	Accession No.	trans-ER	Accession No.	HR-PKS	Compound

 Table S3. Oligonucleotides used for construction of expression plasmids.

Primers

Incort	Restriction	Section 25' 2'	Size
Insert	site	Sequence 5 -5	Vector
hatl Er1	Cla	F: CAAGCTCCGAATTCGAATCGTTATGTCATCATCAGC	2.3 kb
Dell-FII	Ciai	R: CGCTAGCTCAAATCGATGGCATCTTCGTCC	pUARA2
h - 41 E-2	Chal	F: GGGGACGAAGATGCCATCGATGAGATCGCGGC	3.2 kb
Dell-Ff2	Clai	R: CGCTAGCTCAAATCGATCCGCGGGACTAGCTCTCG	pUARA2-bet1 Fr1
h - 41 E-2	CII	F: GGACGAGAGCTAGTCCCGCGGTTGTTTACC	3.4 kb
Dell-FI3	Sacii	R: TAGCTCAAATCGATCCTCACGCAGCCTCCGACC	pUARA2-bet1 Fr1, 2
12		F: CTCCGAATTCGAATCGATATGCCCCCAACGAACG	1.2 kb
bet3	Clai	R: GCGGCCGCTAGCTCAAATCGATCTATGAAGATATATTGACC	pUSA2
1.0	177.1	F: AATTCGAATCGATTTGAGCTAGCATGGATCTATTCAAGACCG	1.8 kb
bet2	Nhel	R: TAGTGCGGCCGCTAGCTACTGCGCTTCC	pAdeA2
1.4	N71 - X	F: TTCGAATCGATTTGAGCTAGCATGACGCCGGCGAAAGC	1.2 kb
bet4	Nhel	R: GTCACTAGTGCGGCCGCTAGCCTACAGGAAGGCTTTG	pAdeA2
<i>C</i> 1	<i>V</i> I	F: TCCGGAATTCGAGCTCGGTACCATGAAATCATTCGC	1.9 kb
orj1	Kpn1	R: ACTACAGATCCCCGGGTACCCTAAACCCTACAAAGC	pAdeA2-bet2
(2)	N/L -1	F: TCGAATCGATTTGAGCTAGCATGGTTGAACATATCGG	1.9 kb
Orj2	Innei	R: TAGTGCGGCCGCTAGCTCAATTCGCCCAGTC	pUNA2
adal		F: GTGCCAAGCTTGCATGTCGCAGATAAACATGTTGTGGC	2.7 kb
uueA		R: CAGAACGGCAGATCCTCGTGCGGAACTCCGAATCCTC	pUSA2
bet1-Fr3-		F: GGACGAGAGCTAGTCCCGCGGTTGTTTACCATCAAGGC	
S2705A-1		R: GAGGGCTACACCGGC <u>TGC</u> GGAGATGTAGTGCAG	
bet1-Fr3-		F: CTGCACTACATCTCC <u>GCA</u> GCCGGTGTAGCCCTC	
S2705A-2		R: TAGCTCAAATCGATCCGCGGTCACGCATCCTCCGACC	
bet1-Fr3-		F: GGACGAGAGCTAGTCCCGCGGTTGTTTACCATCAAGGC	
Y2737F-1	_	R: CCATTTTCCACACAT <u>GAA</u> CCCGTGCGAGCCGTCG	
bet1-Fr3-		F: CGACGGCTCGCACGGG <u>TTC</u> ATGTGTGGAAAATGG	
Y2737F-2	_	R: TAGCTCAAATCGATCCGCGGTCACGCATCCTCCGACC	_
bet1-Fr3-		F: GGACGAGAGCTAGTCCCGCGGTTGTTTACCATCAAGGC	
K2741A-1		R: GCATCTTCTCGCACACCCA <u>CGC</u> TCCACACATATACCC	
bet1-Fr3-		F: GGGTATATGTGTGGAGAGAGAGATGC	
K2741A-2		R: TAGCTCAAATCGATCCGCGGTCACGCATCCTCCGACC	













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