

Supporting Information

Engineering Fungal Nonreducing Polyketide Synthase by Heterologous Expression and Domain Swapping

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Supplemental Methods

Phylogenetic analysis

We performed phylogenetic analyses of DtbA and other NR-PKSs that are found in the genome of *Aspergillus nidulans*. Amino acid sequences of NR-PKSs were obtained from the National Center for Biotechnology Information (NCBI) or the Broad Institute *Aspergillus* Database (http://www.broadinstitute.org/annotation/genome/aspergillus_group/MultiHome.html)

Identification of KS, AT, ACP, CMeT, TE, and R domains was performed using the NCBI Conserved Domain Database. (<http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>). Multiple sequence alignments were constructed using ClustalX (<http://www.clustal.org>) with default settings, and phylogenetic analyses were conducted using MEGA5 (<http://www.megasoftware.net>). The evolutionary history was inferred using the Minimum Evolution (ME) method.¹ The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The ME tree was searched using the Close-Neighbor-Interchange (CNI) algorithm at a search level of 0. The Neighbor-joining algorithm was used to generate the initial tree.²

Generation of fusion PCR fragments, *A. nidulans* protoplasting and transformation

We generated fusion PCR fragments for the heterologous expression of *A. niger* NR-PKS (DtbA) in *A. nidulans*. Construction of fusion PCR products, protoplast production and transformation were carried out as described.³ In brief, a PCR fragment from the start site of e_gw1_19.204 to the stop site after est_GWPlus_C_190476 was generated. This fragment was then fused to a selectable marker and inducible *alcA* promoter cassette and transformed to *A. nidulans* as a heterologous host. The transformants with the correct insertion were further verified with diagnostic PCR. The primers for fusion PCR and diagnostic PCR are listed in Table S1. In each case at least two transformants carrying the correct insertion were used for further study (Table S2).

cDNA generation and sequencing analysis

Total RNA of *A. nidulans* mutant strains was extracted using the Qiagen RNeasy Plant Mini Kit according to the manufacture's instructions. First-strand cDNA was synthesized using gene specific reverse primer and TaqMan Reverse Transcription Reagents (Applied Biosystems) following the supplied protocols. The cDNA was then used as template for PCR amplification and underwent sequencing analysis.

Metabolite profile determination using fermentation and HPLC-DAD-MS analysis

For fermentation, 3.0×10^7 spores of *A. nidulans* were grown in 30 ml liquid LMM medium (15 g/l lactose, 6 g/l NaNO₃, 0.52 g/l KCl, 0.52 g/l MgSO₄·7H₂O, 1.52 g/l KH₂PO₄, and 1 ml/l trace elements)⁴ in 125-ml flasks at 37°C with shaking at 180 rpm and supplemented with uracil (1 g/l), uridine (10 mM) or pyridoxine (0.5 mg/l) when necessary. For *alcA* promoter induction, cyclopentanone at a final concentration of 10 mM was added to the medium after 18 hr of incubation. Culture medium was collected 48 hr after cyclopentanone induction by filtration and extracted with the same volume of ethyl acetate (EtOAc) twice. The combined EtOAc layers were evaporated *in vacuo*, re-dissolved in 0.5 ml of 1:4 DMSO/MeOH, and 10 µl was injected for HPLC-DAD-MS analysis. Conditions for MS included a capillary voltage 5.0 kV, a sheath gas flow rate at 60 arbitrary units, an auxiliary gas flow rate at 10 arbitrary units, and the ion transfer capillary temperature at 350°C. HPLC-DAD-MS analysis was carried out using a ThermoFinnigan LCQ Advantage ion trap mass spectrometer with an reverse phase C₁₈ column (Alltech Prevail C₁₈; particle size, 3 µm; column, 2.1 by 100 mm) at a flow rate of 125 µl/min.

Product fractionation and isolation by HPLC

For scaling up compounds **1** and **2**, CW2261 were cultivated in 17 of 30 ml liquid LMM medium in 125-ml flasks. After 2 days of induction, the medium was collected by filtration and then extracted with equal amounts of EtOAc twice. The combined EtOAc extracts were evaporated *in vacuo*. The crude extract in EtOAc layer was coated on C₁₈ reverse phase gel (Cosmosil 75C₁₈-OPN, Nacalai USA), which was then suspended in 10% of MeOH/ddH₂O and applied to a C₁₈ reverse phase column (10 × 19 mm). This column was then eluted with MeOH/ddH₂O mixtures of decreasing polarity (fraction A, 10% MeOH, 20 ml; fraction B, 30% MeOH, 20 ml; fraction C, 70% MeOH, 20 ml; and fraction D, 100% MeOH, 20 ml). All fractions were analyzed by HPLC-DAD-MS. Fraction which contained the product was subjected to semi-preparative reverse phase HPLC (Phenomenex Luna 5 µm C₁₈, 250 × 10 mm) with a flow rate of 5.0 ml/min and measured by a UV detector at 280 nm. The solvent gradient for HPLC was 100% MeCN (solvent B) in 5% MeCN/ddH₂O (solvent A), both containing 0.05% trifluoroacetic acid (TFA), as follows: 20% B from 0 to 20 min, 60 to 100% B from 20 to 24 min, maintained at 100% B from 24 to 27 min, 100 to 20% B from 27 to 28 min, and re-equilibration with 20% B from 28 to 32 min. Compounds **1** (5.51 mg) and **2** (4.19 mg) were eluted at 16.7 and 19.5 min, respectively. For scaling up compounds **3** and **4**, CW2297 were cultivated in 17 of 30 ml liquid LMM medium in 125-ml flasks. After 2 days of induction, the medium was collected by filtration, acidified with concentrated HCl to pH of 2.0, and then extracted with equal amounts of EtOAc twice. The combined EtOAc extracts were evaporated *in vacuo*., the crude extract in EtOAc layer was collected and coated on C₁₈ reverse phase gel (Cosmosil 75C₁₈-OPN, Nacalai USA), which was then suspended in 10% of MeOH/ddH₂O and applied to a C₁₈ reverse phase column (10 × 29 mm). This column was then eluted with MeOH/ddH₂O mixtures of decreasing polarity (fraction A, 10% MeOH, 25 ml; fraction B, 30%

MeOH, 25 ml; fraction C, 70% MeOH, 25 ml; and fraction D, 100% MeOH, 25 ml). All fractions were analyzed by HPLC-DAD-MS. Fraction which contained the product was subjected to semi-preparative reverse phase HPLC (Phenomenex Luna 5 μ m C₁₈, 250 \times 10 mm) with a flow rate of 5.0 ml/min and measured by a UV detector at 254 nm. The solvent gradient for HPLC was 100% MeCN (solvent B) in 5% MeCN/ddH₂O (solvent A), both containing 0.05% TFA, as follows: 20% B from 0 to 18 min, 44 to 100% B from 18 to 20 min, maintained at 100% B from 20 to 23 min, 100 to 20% B from 23 to 24 min, and re-equilibration with 20% B from 24 to 28 min. Compounds **3** (1.55 mg) and **4** (1.13 mg) were eluted at 13.8 and 17.0 min, respectively.

Compound structure identification with NMR analysis

For compound structure elucidation, ¹H and ¹³C spectra were collected on a nuclear magnetic resonance (NMR) Varian Mercury Plus 400 and Varian VNMRS-600 spectrometer. High resolution electrospray ionization mass spectrum (HRESIMS) was obtained with an Agilent Technologies 1200 series high-resolution mass spectrometer.

Structural Characterizations

Compound **1** had a molecular formula of C₁₀H₁₂O₃ on the basis of its HRESIMS, ¹H, and ¹³C NMR spectroscopic data. The ¹H and ¹³C NMR spectroscopic data of compound **1** were similar to those of compound **2**.⁵ Comparison of the NMR spectra between **1** and **2** indicated three methyl groups [δ_{H} 2.08, 2.14, and 2.48 (each 3H, s) and δ_{C} 7.8 (q), 11.7 (q), and 13.8 (q)] were attached on the benzene of compound **1**. Therefore, compound **1** was 2,4-dihydroxy-3,5,6-trimethylbenzaldehyde.

Compound **4** had a molecular formula of C₁₁H₁₄O₄ on the basis of its HRESIMS, ¹H, and ¹³C NMR spectroscopic data. The ¹H and ¹³C NMR spectroscopic data of compound **4** were similar to those of compound **3**.⁵ Comparison of the NMR spectra between **4** and **3** indicated an ethyl group [δ_{H} 3.03 (2H, q, $J = 7.2$ Hz, H₂-8) and 1.15 (3H, t, $J = 7.2$ Hz, H₃-9); and δ_{C} 25.1 (t, C-8) and 15.2 (q, C-9)] at C-7 in compound **4**. Therefore, compound **4** was 6-ethyl-2,4-dihydroxy-3,5-dimethylbenzoic acid.

2,4-dihydroxy-3,5,6-trimethylbenzaldehyde (**1**)

¹H NMR (acetone-*d*₆, 400 MHz) δ 2.08 (3H, s), 2.14 (3H, s), 2.48 (3H, s), 10.21 (1H, s), 12.97 (1H, s); ¹³C NMR (acetone-*d*₆, 400 MHz) δ 7.8 (q), 11.7 (q), 13.8 (q), 108.8 (s), 113.8 (s), 116.5 (s), 139.9 (s), 162.1 (s), 162.9 (s), 195.2 (s); UV-Vis and ESIMS spectra, see Figure S5; HRESIMS, [M – H][–] m/z found 179.0715 calc. for C₁₀H₁₁O₃:179.0714.

6-ethyl-2,4-dihydroxy-3,5-dimethylbenzoic acid (**4**)

¹H NMR (acetone-*d*₆, 600 MHz) δ 1.16 (3H, t, $J = 7.2$ Hz), 2.10 (3H, s), 2.18 (3H, s), 3.04 (2H, q, $J = 7.2$ Hz); ¹³C NMR (acetone-*d*₆, 600 MHz) δ 8.8 (q), 11.7 (q), 15.2 (q), 25.1 (t), 105.2 (s), 109.2 (s), 115.8 (s), 144.6 (s), 159.3 (s), 161.7 (s), 174.9 (s); UV-Vis and ESIMS spectra, see Figure S5; HRESIMS, [M – H][–] m/z found 209.0821 calc. for C₁₁H₁₃O₄:209.0819.

Table S1. Primers used in this study

Primer	Sequence (5'→3')
yA_P1	TTCTTCCAGCTTCTGCTGCGT
yA_P2	CGACAACCAAGGGAAGTCAA
yA_P3	CGCATTCTAGAGAGAGTGTG
yA_P4	ATTGCGTCCCATCAAATGGG
yA_P5	CAAACCTCCTTGACACCGT
yA_P6	GAGTCTGCAGCAAAGGCATTGA
Aspnil_194381_Af	CCAATCCTATCACCTCGCCTCAAATGGAACAGCCAGCACTGGT
Aspnil_194381_Ar1	GAATAGCCACCATCCTTC
Aspnil_194381_Ar2	GTTCCACAAGACAGAGGATG
Aspnil_194381_Bf1	GCAGTGTTTCTCTTGGAAGG
Aspnil_194381_Bf2	GATATCAGGATTGGAAGAGAG
Aspnil_194381+R_S1_Re	CCCATTTGATGGGACGCCTATCACCCCAGAAAGCCAGT
Aspnil_194381_de_R_Re	AGTTAGAAGCACAACTGACC
ANID_08383_TE_Fw	GGTCAGTTGTGCTTCTAACTACTGTGCAGGAGCAAACGGT
ANID_08383_Re	CGAAGAGGGTGAAGAGCATTGCAGTAGAAGACAGAGCCAG
ANID_07825_TE_Fw	GGTCAGTTGTGCTTCTAACTAGATCCTGTTTCATGCTCC
ANID_07825_Re	CGAAGAGGGTGAAGAGCATTGCTACTCAGCACTGTCCAC
ANID_08209_TE_Fw	GGTCAGTTGTGCTTCTAACTAAGACCCTCTTTCTCTTCCC
ANID_08209_Re	CGAAGAGGGTGAAGAGCATTGCAGGGAGTGCTGAAACTG
ANID_07909_TE_Fw	GGTCAGTTGTGCTTCTAACTGATGCCGCCGTCTCGCGTG
ANID_07909_Re	CGAAGAGGGTGAAGAGCATTGGTGCTTTGACACGCCAAG
ANID_06448_TE_FwL	GGTCAGTTGTGCTTCTAACTGGGTTCCAGCATGTTTCGTG
ANID_06448_Re	CGAAGAGGGTGAAGAGCATTGCAACATCCACCGATAAG
PyroA_F3	GATCAAGGAGATCATGGCTG
AfpyroA_NR	CAATTACCATCCTCTCTTGGC
AN8383_TE-S123A_Fw	CAATGTCGTTGCCGTAGGCTGGGCAACTGGTGGTCACCTAGCTATG
AN8383_TE-S123_Re	CCAGCCTACGGCAACGACATTG

AN8383_TE-H317F_Fw GTATCGTGCGTGATGGGCTATTCTTGTTTGACCTAGAGGCCA

AN8383_TE-H317F_Re TAGCCCATCACGCACGATAC

AN8383_TE-D285N_Fw CTTGATTCATGGTACTCTGGATAACTTGATTCCCGTACAACAG

AN8383_TE-D285N_Re CAGAGTACCATGAATCAAG

AN8383_TE-S152A_Fw GAGGCAATTCTCTCCTTCTACGCTCCTACGGATTACACTGACCCGTTTC

AN8383_TE-S152A_Re GTAGAAGGAGAGAATTGCCTC

AN8383_TE-S162A_Fw GATTACACTGACCCGTTCTGGGCTAAACCGAACTTCCCGTATAGAG

AN8383_TE-S162A_Re CCAGAACGGGTCAGTGTAAT

Table S2. *A. nidulans* strains used in this study

Strain	Secondary metabolite mutations	Genotype
LO2026	<i>stcJ</i> Δ	<i>pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB</i>
CW2261 to CW2265	<i>stcJ</i> Δ, <i>alcA</i> (p)- <i>dtbA</i>	<i>pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;</i> <i>yA::AfpG-alcA</i> (p)- <i>dtbA</i>
CW2296 to CW2300	<i>stcJ</i> Δ, <i>alcA</i> (p)- <i>dtbA</i> Δ <i>R</i> + <i>TE</i> _(<i>ausA</i>)	<i>pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;</i> <i>yA::AfpG-alcA</i> (p)- <i>dtbA</i> , <i>dtbA::dtbA</i> Δ <i>R</i> + <i>TE</i> _(<i>ausA</i>) - <i>AfpyroA</i>
CW2321 to CW2325	<i>stcJ</i> Δ, <i>alcA</i> (p)- <i>dtbA</i> Δ <i>R</i> + <i>TE</i> / <i>CLC</i> _(<i>stcA</i>)	<i>pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;</i> <i>yA::AfpG-alcA</i> (p)- <i>dtbA</i> , <i>dtbA::dtbA</i> Δ <i>R</i> + <i>TE</i> / <i>CLC</i> _(<i>stcA</i>) - <i>AfpyroA</i>
CW2326 to CW2330	<i>stcJ</i> Δ, <i>alcA</i> (p)- <i>dtbA</i> Δ <i>R</i> + <i>TE</i> _(<i>orsA</i>)	<i>pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;</i> <i>yA::AfpG-alcA</i> (p)- <i>dtbA</i> , <i>dtbA::dtbA</i> Δ <i>R</i> + <i>TE</i> _(<i>orsA</i>) - <i>AfpyroA</i>
CW2331 to CW2335	<i>stcJ</i> Δ, <i>alcA</i> (p)- <i>dtbA</i> Δ <i>R</i> + <i>TE</i> / <i>CLC</i> _(<i>wA</i>)	<i>pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;</i> <i>yA::AfpG-alcA</i> (p)- <i>dtbA</i> , <i>dtbA::dtbA</i> Δ <i>R</i> + <i>TE</i> / <i>CLC</i> _(<i>wA</i>) - <i>AfpyroA</i>
CW2456 to CW2460	<i>stcJ</i> Δ, <i>alcA</i> (p)- <i>dtbA</i> Δ <i>R</i> + <i>TE</i> _(<i>ausA</i>) ^{<i>S152A</i>}	<i>pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;</i> <i>yA::AfpG-alcA</i> (p)- <i>dtbA</i> , <i>dtbA::dtbA</i> Δ <i>R</i> + <i>TE</i> _(<i>ausA</i>) ^{<i>S152A</i>} - <i>AfpyroA</i>
CW2461 to CW2465	<i>stcJ</i> Δ, <i>alcA</i> (p)- <i>dtbA</i> Δ <i>R</i> + <i>TE</i> _(<i>ausA</i>) ^{<i>S162A</i>}	<i>pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;</i> <i>yA::AfpG-alcA</i> (p)- <i>dtbA</i> , <i>dtbA::dtbA</i> Δ <i>R</i> + <i>TE</i> _(<i>ausA</i>) ^{<i>S162A</i>} - <i>AfpyroA</i>
CW2466 to CW2470	<i>stcJ</i> Δ, <i>alcA</i> (p)- <i>dtbA</i> Δ <i>R</i> + <i>TE</i> _(<i>ANID_06448</i>)	<i>pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;</i>

CW2476 to CW2480	<i>stcJ</i> Δ, <i>alcA</i> (p)- <i>dtbA</i> ΔR+ <i>TE</i> _(<i>ausA</i>) ^{S123A}	<i>yA</i> :: <i>Afp</i> <i>pyrG</i> - <i>alcA</i> (p)- <i>dtbA</i> , <i>dtbA</i> :: <i>dtbA</i> ΔR+ <i>TE</i> _(ANID_06448) - <i>Afp</i> <i>pyroA</i> <i>pyrG89</i> ; <i>pyroA4</i> , <i>nkuA</i> :: <i>argB</i> ; <i>riboB2</i> , <i>stcJ</i> :: <i>AfriboB</i> ;
CW2481 to CW2485	<i>stcJ</i> Δ, <i>alcA</i> (p)- <i>dtbA</i> ΔR+ <i>TE</i> _(<i>ausA</i>) ^{H317F}	<i>yA</i> :: <i>Afp</i> <i>pyrG</i> - <i>alcA</i> (p)- <i>dtbA</i> , <i>dtbA</i> :: <i>dtbA</i> ΔR+ <i>TE</i> _(<i>ausA</i>) ^{S123A} - <i>Afp</i> <i>pyroA</i> <i>pyrG89</i> ; <i>pyroA4</i> , <i>nkuA</i> :: <i>argB</i> ; <i>riboB2</i> , <i>stcJ</i> :: <i>AfriboB</i> ;
CW2486 to CW2490	<i>stcJ</i> Δ, <i>alcA</i> (p)- <i>dtbA</i> ΔR+ <i>TE</i> _(<i>ausA</i>) ^{D285N}	<i>yA</i> :: <i>Afp</i> <i>pyrG</i> - <i>alcA</i> (p)- <i>dtbA</i> , <i>dtbA</i> :: <i>dtbA</i> ΔR+ <i>TE</i> _(<i>ausA</i>) ^{H317F} - <i>Afp</i> <i>pyroA</i> <i>pyrG89</i> ; <i>pyroA4</i> , <i>nkuA</i> :: <i>argB</i> ; <i>riboB2</i> , <i>stcJ</i> :: <i>AfriboB</i> ;
		<i>yA</i> :: <i>Afp</i> <i>pyrG</i> - <i>alcA</i> (p)- <i>dtbA</i> , <i>dtbA</i> :: <i>dtbA</i> ΔR+ <i>TE</i> _(<i>ausA</i>) ^{D285N} - <i>Afp</i> <i>pyroA</i>

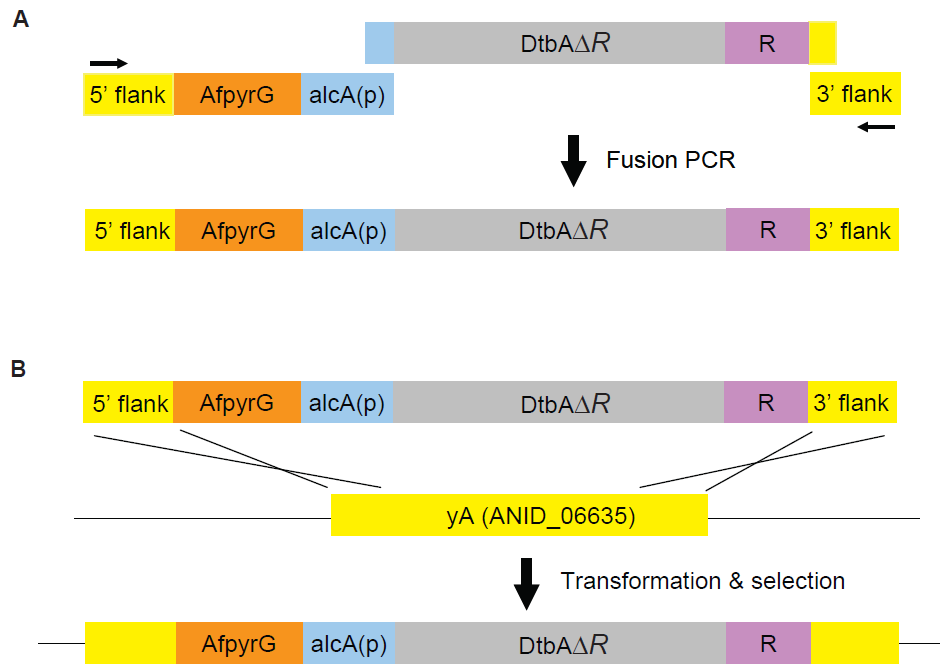


Figure S1. Heterologous expression of DtbA in *A. nidulans* using fusion PCR (A) and transformation methods (B).

(A)

5461
dtbA gDNA | ATTCCTGTGG ATGAGATTGG CAATGACAGC ACTCTTGAAG CACTTGGAAAT AGATTCTCTT ATGAGAAAAG
cDNA-putative | ATTCCTG
cDNA-revised | ATTCCTGTGG ATGAGATTGG CAATGACAGC ACTCTTGAAG CACTTGGAAAT AGATTCTCTT ATGAGAAAAG

5531
dtbA gDNA | AGGTCCAATC TGAGCTCCGG AAGAATCTGG GCCGTGCAGA TATTCCAGAA AATCTTCATG AATACTCTGT
cDNA-putative | AGGTCCAATC TGAGCTCCGG AAGAATCTGG GCCGTGCAGA TATTCCAGAA AATCTTCATG AATACTCTGT
cDNA-revised | AGGTCCAATC TGAGCTCCGG AAGAATCTGG GCCGTGCAGA TATTCCAGAA AATCTTCATG AATACTCTGT

5601
dtbA gDNA | CACATCACTT GCCGCATATC TCGCCGACCA GGATTGTGGT AAAAGGAATG ACCTTTCGTG CGGTACGCCG
cDNA-putative | CACATCACTT GCCGCATATC TCGCCGACCA GGATTGTGGT AAAAGGAATG ACCTTTCGTG CGGTACGCCG
cDNA-revised | CACATCACTT GCCGCATATC TCGCCGACCA GGATTGTGGT AAAAGGAATG ACCTTTCGTG CGGTACGCCG

5671
dtbA gDNA | CCGGGCAGTG TTTCTCTTGG AAGGCATTGC GATATTAAAG CGCTGGGTTC TCATACGCAA TTGATGGCCC
cDNA-putative | CCGGGCAGTG TTTCTCTTGG AAGGCATTGC GATATTAAAG CGCTGGGTTC TCATACGCAA TTGATGGCCC
cDNA-revised | CCGGGCAGTG TTTCTCTTGG AAGGCATTGC GATATTAAAG CGCTGGGTTC TCATACGCAA TTGATGGCCC

5741
dtbA gDNA | CAGAAATGAT ATCAGGATTG GAAGAGAGGC AGGAACTCGT GAAAAGAAAT GATAGAAGCC AGCTATTTCA
cDNA-putative | CAGAAATGAT ATCAGGATTG GAAGAGAGGC AGGAACTCGT GAAAAGAAAT GATAGAAGCC AGCTATTTCA
cDNA-revised | CAGAAATGAT ATCAGGATTG GAAGAGAGGC AGGAACTCGT GAAAAGAAAT GATAGAAGCC AGCTATTTCA

5811
dtbA gDNA | GCTATCGAGT CCACCGAAAC CTCTTTGTTC CAGCACCGTT GGGGGATTCA CACTAAAGGA CCTTGCTGCC
cDNA-putative | GCTATCGAGT CCACCGAAAC CTCTTTGTTC CAGCACCGTT GGGGGATTCA CACTAAAGGA A CCTTGCTGCC
cDNA-revised | GCTATCGAGT CCACCGAAAC CTCTTTGTTC CAGCACCGTT GGGGGATTCA CACTAAAGGA CCTTGCTGCC

(B)

6951
dtbA gDNA | AATAAGCGTG AAGATGGATC ATCAATACGA CCTTCCAAG TGGGAACTG GTAGCGAGAA GTGCACAGGG
cDNA-putative | AATAAGCG
cDNA-revised | AATAAGCGTG AAGATGGATC ATCAATACGA CCTTCCAAG TGGGAACTG GTAGCGAGAA GTGCACAGGG

7021
dtbA gDNA | GACAATTTTG AAATAGTCAC TTCACGTCAA CCATTGTCAG TATATTCAAC ACCTGATCTA CTTAAGGAAC
cDNA-putative | GACAATTTTG AAATAGTCAC TTCACGTCAA CCATTGTCAG TATATTCAAC ACCTGATCTA CTTAAGGAAC
cDNA-revised | GACAATTTTG AAATAGTCAC TTCACGTCAA CCATTGTCAG TATATTCAAC ACCTGATCTA CTTAAGGAAC

7091
dtbA gDNA | CTGGGCTTAG TGGGTCAGTT GTGCTTCTAA CTGGCGGCAC AGGTAATCTT GGCACCCACG TCCTCCACCA
cDNA-putative | CTGGGCTTAG TGGGTCAGTT GTGCTTCTAA CTGGCGGCAC AGGTAATCTT GCGGCAC AGGTAATCTT GGCACCCACG TCCTCCACCA
cDNA-revised | CTGGGCTTAG TGGGTCAGTT GTGCTTCTAA CTGGCGGCAC AGGTAATCTT GGCACCCACG TCCTCCACCA

7161
dtbA gDNA | GCTGATTAAT CGAGCAGACG TCCGCCGGGT GATATGCTTG AATCGATTGA CAACAAATGA TGATCCAATT
cDNA-putative | GCTGATTAAT CGAGCAGACG TCCGCCGGGT GATATGCTTG AATCGATTGA CAACAAATGA TGATCCAATT
cDNA-revised | GCTGATTAAT CGAGCAGACG TCCGCCGGGT GATATGCTTG AATCGATTGA CAACAAATGA TGATCCAATT

7231
dtbA gDNA | CAACGGCAAC GACGTGCTCT CAGAGATAAA GGTATTGATC TTAAAGAACG ACAGTGGGAA AAAATTGAAG
cDNA-putative | CAACGGCAAC GACGTGCTCT CAGAGATAAA GGTATTGATC TTAAAGAACG ACAGTGGGAA AAAATTGAAG
cDNA-revised | CAACGGCAAC GACGTGCTCT CAGAGATAAA GGTATTGATC TTAAAGAACG ACAGTGGGAA AAAATTGAAG

7301
 dtbA gDNA TTCTCGAAGC AAAATCAAGC CACACGGCCC TCGGTTTGCA AACCGAACAA TACCAGCGAT TACGAGACCA
 cDNA-putative TTCTCGAAGC AAAATCAAGC CACACGGCCC TCGGTTTGCA AACCGAACAA TACCAGCGAT TACGAGACCA
 cDNA-revised TTCTCGAAGC AAAATCAAGC CACACGGCCC TCGGTTTGCA AACCGAACAA TACCAGCGAT TACGAGACCA

7371
 dtbA gDNA AGTAACCCAT ATTGTCCACA ACGCGTGGCC AATGAGCTTC AAGCGGTCCC TGCACACATT TGAACCTCAA
 cDNA-putative AGTAACCCAT ATTGTCCACA ACGCGTGGCC AATGAGCTTC AAGCGGTCCC TGCACACATT TGAACCTCAA
 cDNA-revised AGTAACCCAT ATTGTCCACA ACGCGTGGCC AATGAGCTTC AAGCGGTCCC TGCACACATT TGAACCTCAA

7441
 dtbA gDNA TTAAAAACAC TGCAAAACCT GTTGAAGCTC TGCCACGAAG CCAAGTACGG TGCAAGACTC TTATTTCATCT
 cDNA-putative TTAAAAACAC TGCAAAACCT GTTGAAGCTC TGCCACGAAG CCAAGTACGG TGCAAGACTC TTATTTCATCT
 cDNA-revised TTAAAAACAC TGCAAAACCT GTTGAAGCTC TGCCACGAAG CCAAGTACGG TGCAAGACTC TTATTTCATCT

7511
 dtbA gDNA CCTCGATAGG CGTAGTTGGC CGCCACCCCA ATACATTTGC GAATAAGCCA GTTCCGGAGG ATCCGGTGAG
 cDNA-putative CCTCGATAGG CGTAGTTGGC CGCCACCCCA ATACATTTGC GAATAAGCCA GTTCCGGAGG ATCCGGTGAG
 cDNA-revised CCTCGATAGG CGTAGTTGGC CGCCACCCCA ATACATTTGC GAATAAGCCA GTTCCGGAGG ATCCGGTGAG

7581
 dtbA gDNA GGACTGTCAA AGTTCACTCG GATTCGGATA CAGCCAGGCG AAATATCACT GCGAGCAGAT CATAAATCGG
 cDNA-putative GGACTGTCAA AGTTCACTCG GATTCGGATA CAGCCAGGCG AAATATCACT GCGAGCAGAT CATAAATCGG
 cDNA-revised GGACTGTCAA AGTTCACTCG GATTCGGATA CAGCCAGGCG AAATATCACT GCGAGCAGAT CATAAATCGG

7651
 dtbA gDNA GCGCTAGAGC AAGATACCCG CCTCGAAGCT TCCTACGTCC GAATTGGACA GATAACGGGT TCTCAACACT
 cDNA-putative GCGCTAGAGC AAGATACCCG CCTCGAAGCT TCCTACGTCC GAATTGGACA GATAACGGGT TCTCAACACT
 cDNA-revised GCGCTAGAGC AAGATACCCG CCTCGAAGCT TCCTACGTCC GAATTGGACA GATAACGGGT TCTCAACACT

7721
 dtbA gDNA TCGGTTTGTG GAATACAGAG GAGCACGTTT CCGCTTTACT GAGAACATCG CAAACAATAG GCGCTCTGCC
 cDNA-putative TCGGTTTGTG GAATACAGAG GAGCACGTTT CCGCTTTACT GAGAACATCG CAAACAATAG GCGCTCTGCC
 cDNA-revised TCGGTTTGTG GAATACAGAG GAGCACGTTT CCGCTTTACT GAGAACATCG CAAACAATAG GCGCTCTGCC

7791
 dtbA gDNA CCACCTTGAT GGAGTAAGTT CTTGGCATAG TTTGAATTGG CATTTGTGAA CTGCTACTTA CGATGACACA
 cDNA-putative CCACCTTGAT GGA
 cDNA-revised CCACCTTGAT GGA

7861
 dtbA gDNA GCTTGCCTCC TGGCTCCCAA TCGATATTGC CGCTGCCACC GTCAGTGAGC TCCTATTGGA TACGGCTCAT
 cDNA-putative CTTGCCTCC TGGCTCCCAA TCGATATTGC CGCTGCCACC GTCAGTGAGC TCCTATTGGA TACGGCTCAT
 cDNA-revised CTTGCCTCC TGGCTCCCAA TCGATATTGC CGCTGCCACC GTCAGTGAGC TCCTATTGGA TACGGCTCAT

7931
 dtbA gDNA CTTCGGATGG TGTATCATGT CGAAAACCCCT GTTCGTCAGC CGTGGTGTGA GCTGCTTGGT TATCTCTCTG
 cDNA-putative CTTCGGATGG TGTATCATGT CGAAAACCCCT GTTCGTCAGC CGTGGTGTGA GCTGCTTGGT TATCTCTCTG
 cDNA-revised CTTCGGATGG TGTATCATGT CGAAAACCCCT GTTCGTCAGC CGTGGTGTGA GCTGCTTGGT TATCTCTCTG

8001
 dtbA gDNA CGCATTTGAG GCTGCCTATC ATTCCGTATA AGGAATGGCT GAGTCGAATG GAGACCAATG GAGACTCCGT
 cDNA-putative CGCATTTGAG GCTGCCTATC ATTCCGTATA AGGAATGGCT GAGTCGAATG GAGACCAATG GAGACTCCGT
 cDNA-revised CGCATTTGAG GCTGCCTATC ATTCCGTATA AGGAATGGCT GAGTCGAATG GAGACCAATG GAGACTCCGT

8071
 dtbA gDNA GACAGGGTCA CCTAATCCGG CGAAGAACTT AAGGGATTTC TTCAAAAATG ATTTTCTTCA TATGTCTGT
 cDNA-putative GACAGGGTCA CCTAATCCGG CGAAGAACTT AAGGGATTTC TTCAAAAATG ATTTTCTTCA TATGTCTGT
 cDNA-revised GACAGGGTCA CCTAATCCGG CGAAGAACTT AAGGGATTTC TTCAAAAATG ATTTTCTTCA TATGTCTGT

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      8141
      |
dtbA gDNA      GGGTCAGTTG TGATGAGTAC GACCAGTACA GCGTCTGTCT CTGCGGCTCT ACGATCAGCC GGTCCCGTGA
cDNA-putative GGGTCAGTTG TGATGAGTAC GACCAGTACA GCGTCTGTCT CTGCGGCTCT ACGATCAGCC GGTCCCGTGA
cDNA-revised  GGGTCAGTTG TGATGAGTAC GACCAGTACA GCGTCTGTCT CTGCGGCTCT ACGATCAGCC GGTCCCGTGA

      8211
      |
dtbA gDNA      GTCCAGGAAC TCTGTTATTG TATATTGAGC AGTGGAGGAG GACTGGCTTT CTGGGGTGA
cDNA-putative GTCCAGGAAC TCTGTTATTG TATATTGAGC AGTGGAGG
cDNA-revised  GTCCAGGAAC TCTGTTATTG TATATTGAGC AGTGGAGGAG GACTGGCTTT CTGGGGTGA

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Figure S2. Comparison of the nucleotide sequence between *dtbA* genomic DNA (gDNA), the putative *dtbA* coding sequence from the Broad Institute (cDNA-putative) and *dtbA* cDNA from RT-PCR of mRNA obtained in this study (cDNA-revised). Position numbering is shown starting from start codon of *dtbA* genomic DNA. Highlighted in gray are regions predicted to be introns in the database. (A) Gene region from 5461 bp to 5880 bp. The revised cDNA sequence (cDNA-revised) revealed a new ACP domain located between the first ACP and the CMeT domain of DtbA. (B) Gene region from 6951 bp to 8269 bp. The stop codons are in red.

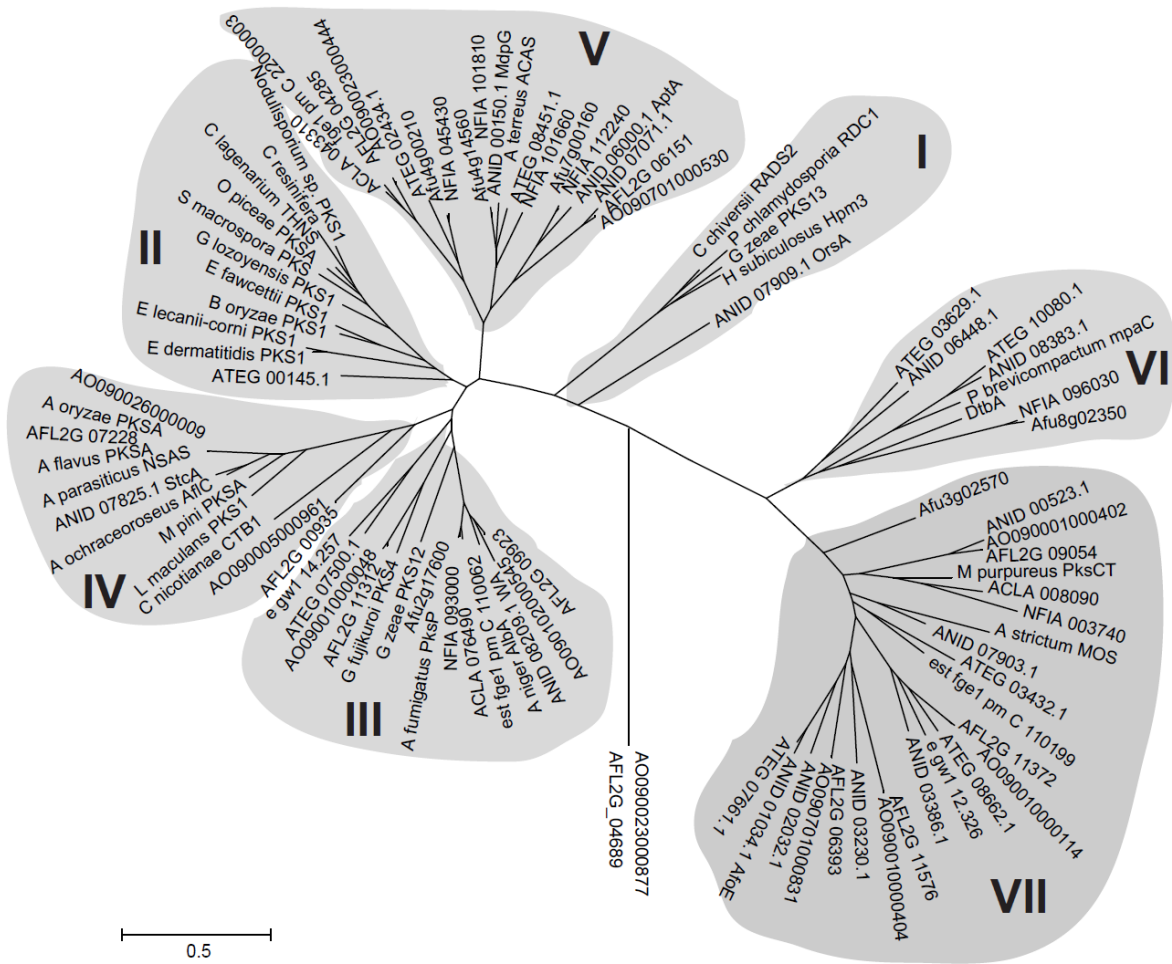


Figure S3. Phylogenetic analysis of the reannotated DtbA and NR-PKSs obtained from the Broad Institute Aspergillus Comparative Database (http://www.broadinstitute.org/annotation/genome/aspergillus_group/MultiHome.html).

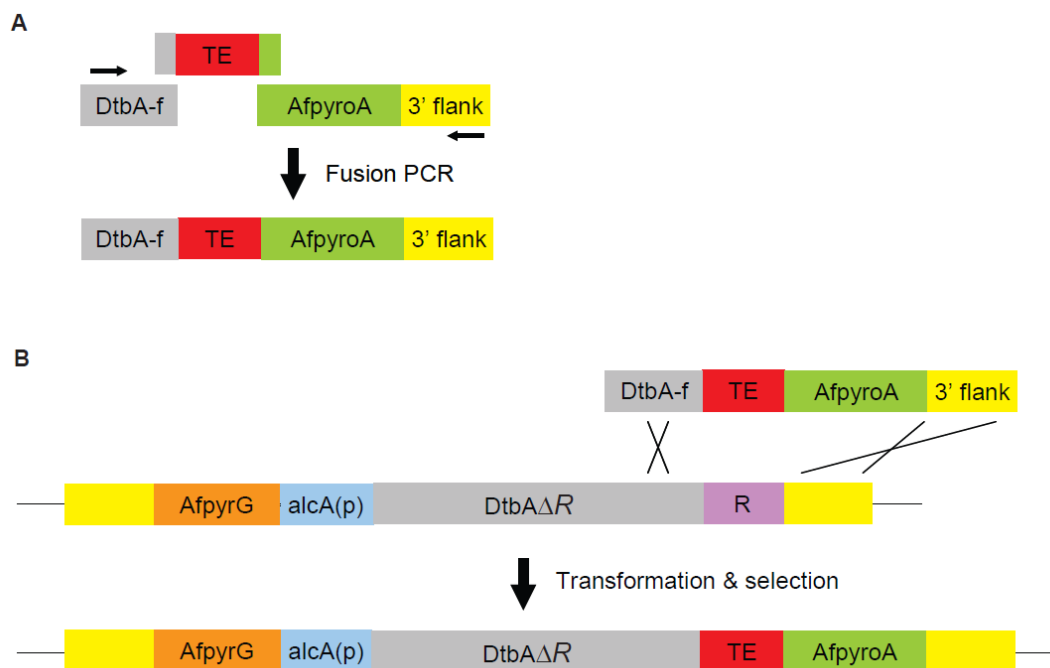


Figure S4. Generation of chimeric modules in which the SAT-KS-AT-PT-ACP-CMeT fragment from DtbA (DtbA Δ R) was fused to a TE or TE/CLC domain from other NR-PKSs of *A. nidulans* using fusion PCR approaches (A) and followed by transformation methods (B).

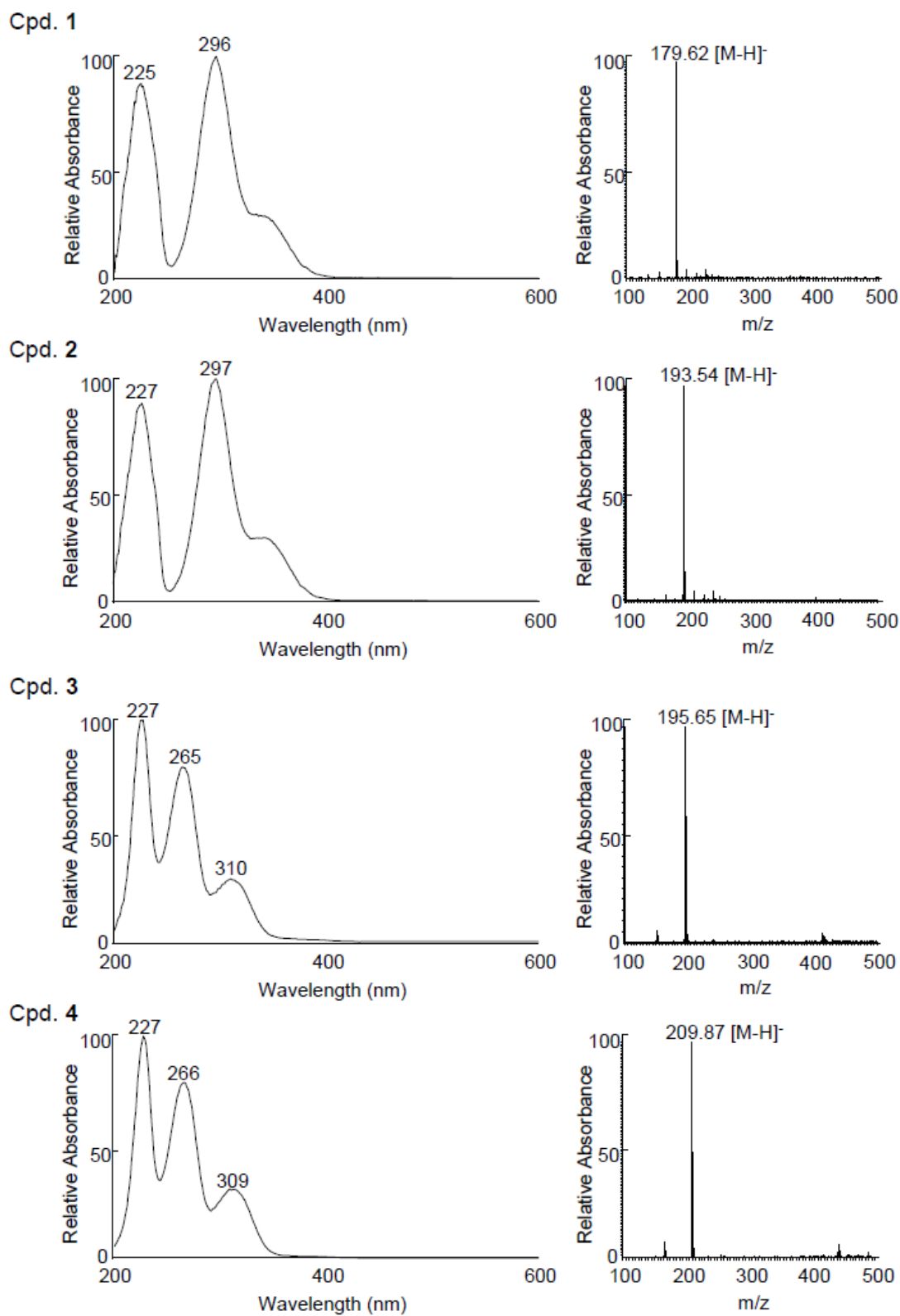


Figure S5. UV-vis and ESIMS spectra in negative mode of compounds **1-4**.

CW2261_1
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Sample id : s_20111006_001
Pulse Sequence: s2pu1
Solvent: acetone
Temp: 50.0 C / 323.1 K
Operator: walkup
File: Proton_01
Mercury-400BB "MVX"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.595 sec
Width 6402.0 Hz
32 repetitions
OBSERVE H1, 400.1025402 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 40 sec

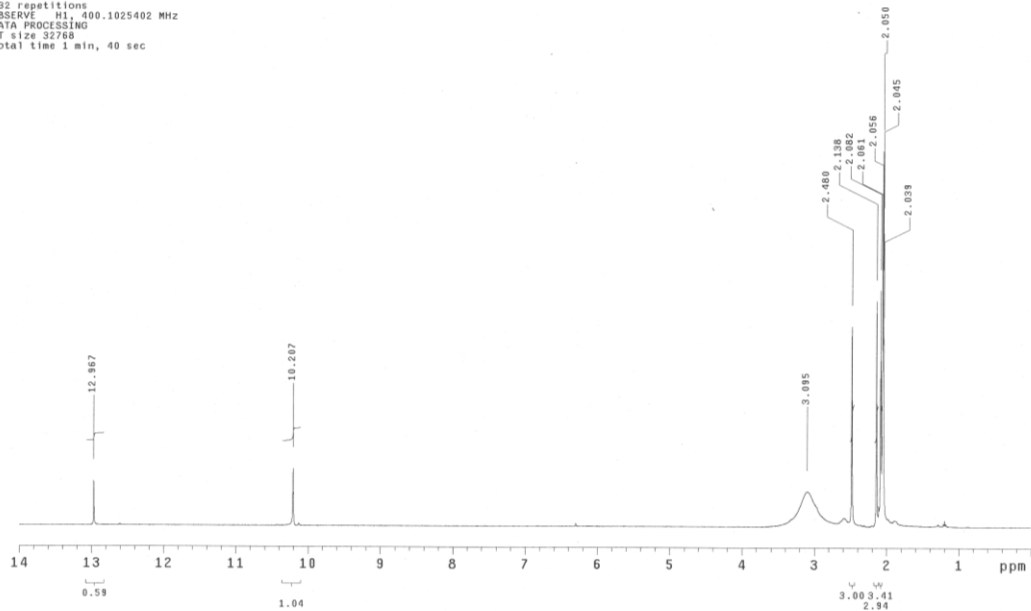


Figure S6. ^1H NMR spectrum of compound **1** in acetone- d_6 .

CW2261_1
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Sample id : s_20111006_002
Pulse Sequence: s2pu1
Solvent: acetone
Temp: 50.0 C / 323.1 K
Operator: walkup
File: Carbon_01
Mercury-400BB "MVX"
Relax. delay 1.000 sec
Pulse 29.5 degrees
Acq. time 1.300 sec
Width 24150.8 Hz
15000 repetitions
OBSERVE C13, 100.6057659 MHz
DECOUPLE H1, 400.1045743 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65586
Total time 13 hr, 48 min, 27 sec

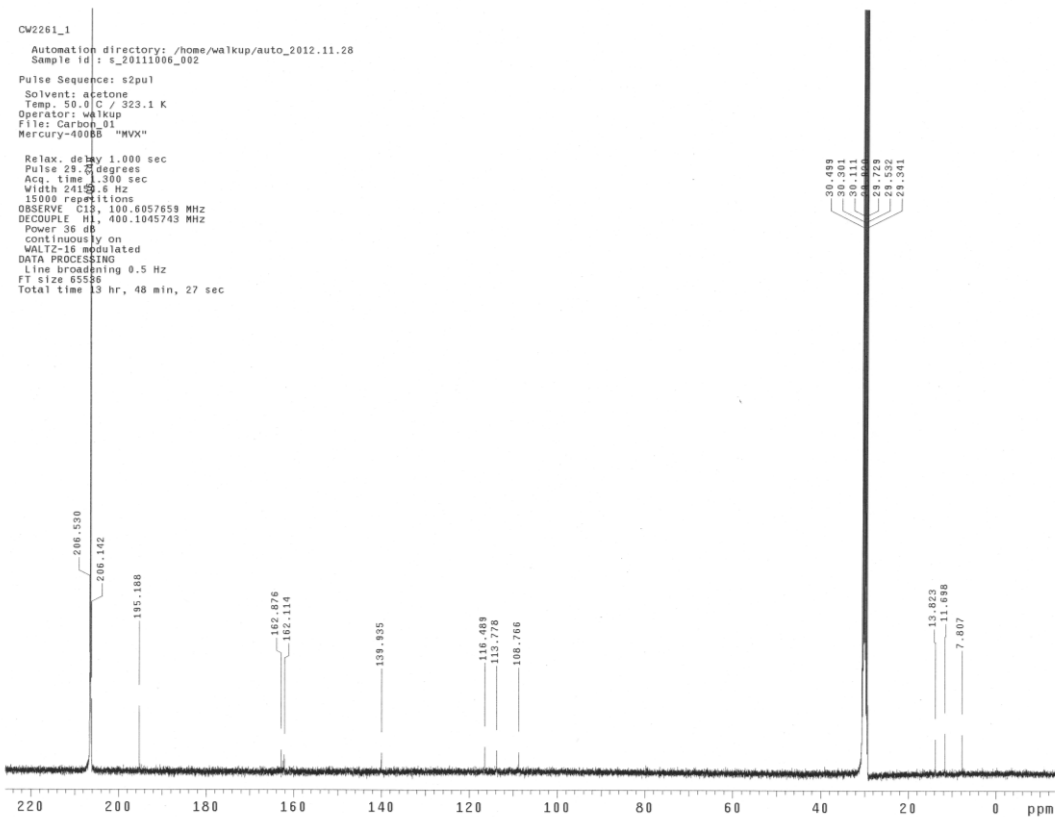


Figure S7. ^{13}C NMR spectrum of compound **1** in acetone- d_6 .

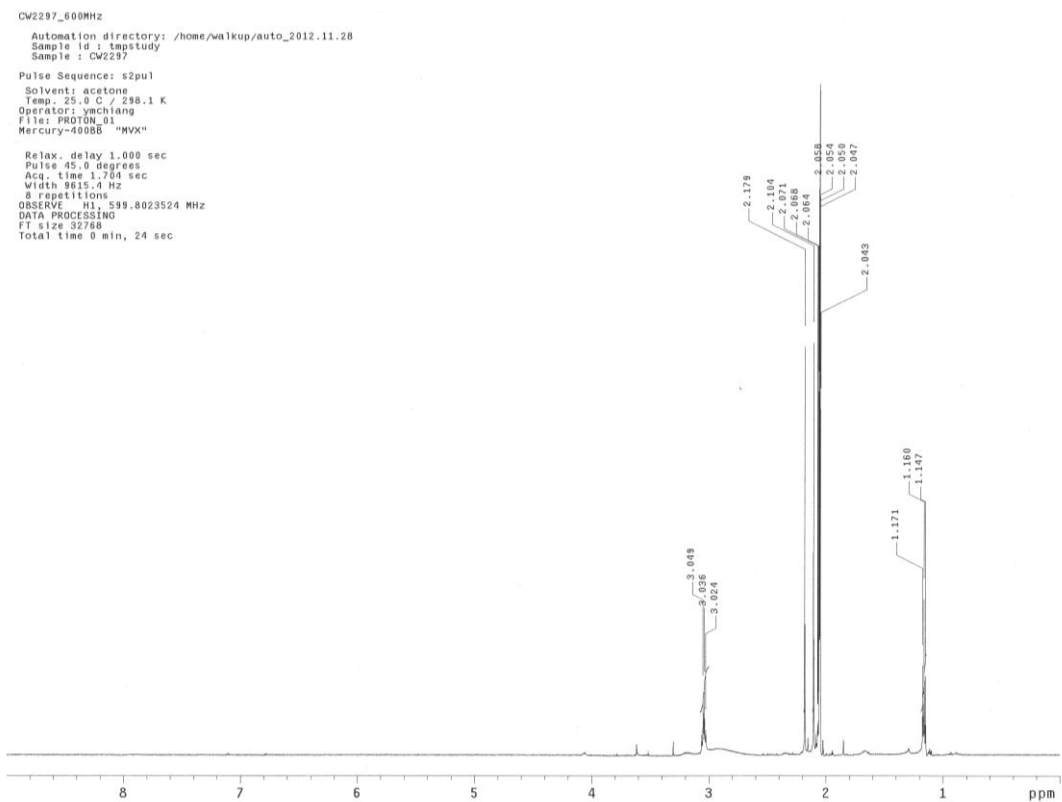


Figure S8. ^1H NMR spectrum of compound **4** in acetone- d_6 .

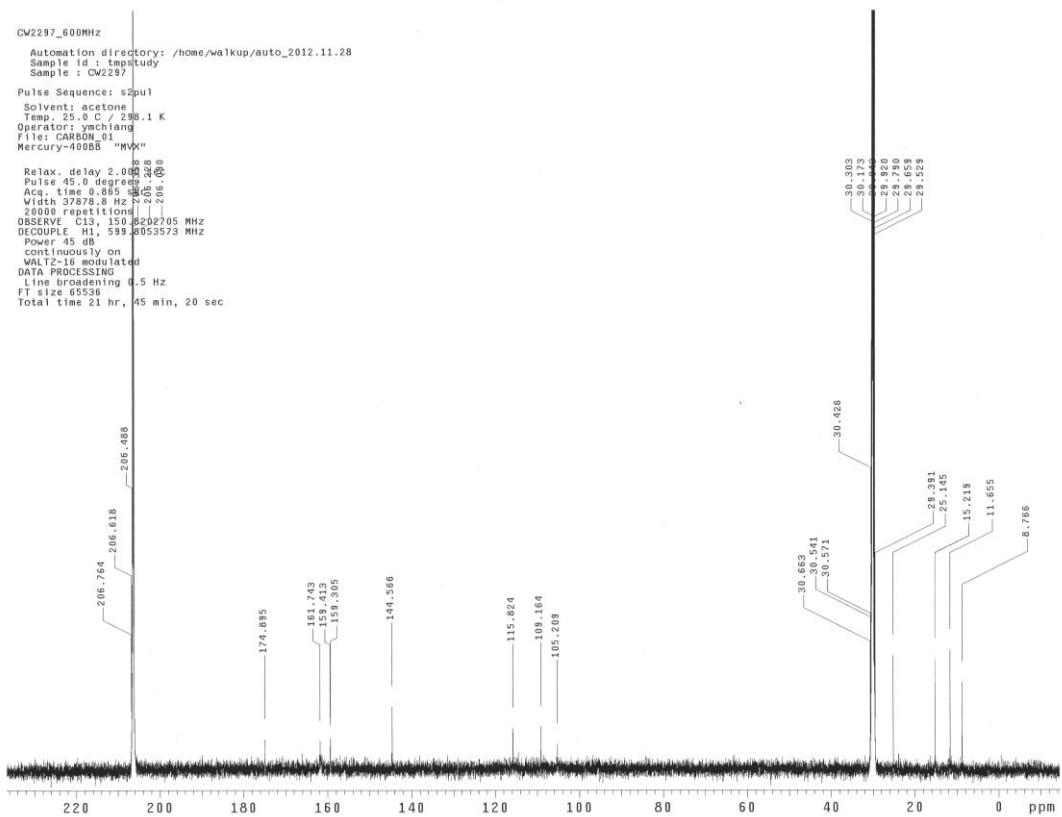


Figure S9. ^{13}C NMR spectrum of compound **4** in acetone- d_6 .

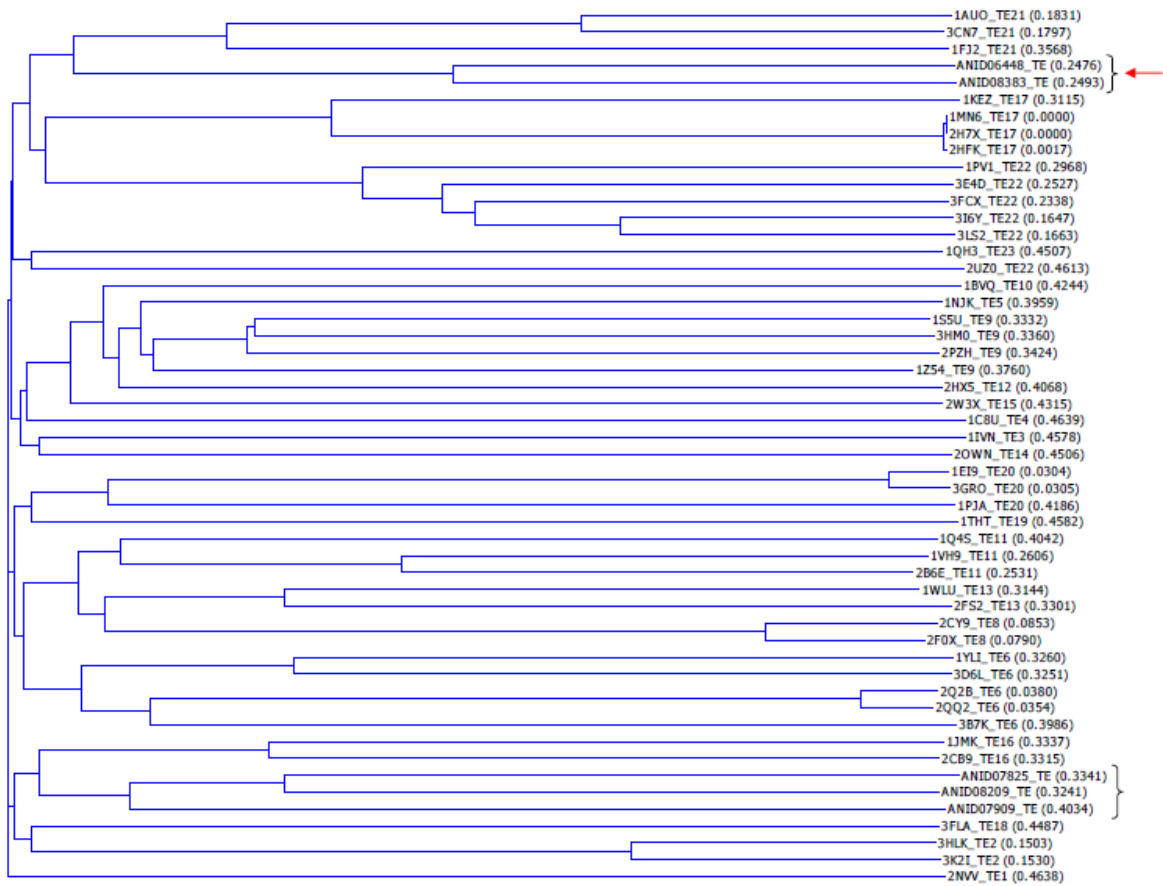


Figure S10. Phylogenetic analysis of five TE domains for which we conducted domain swapping and 47 representative members which have been identified from 23 TE families (TE1-TE23).

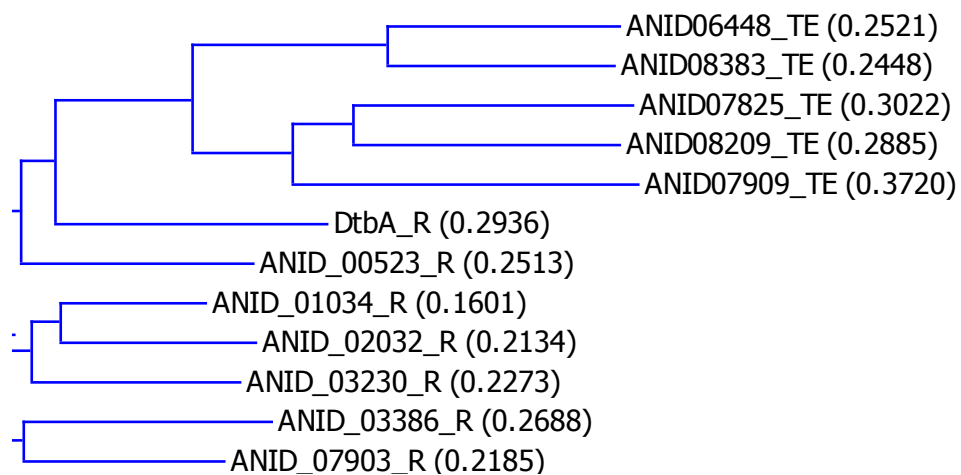


Figure S11. Phylogenetic analysis of the DtbA R domain, five TE domains for which we conducted domain swapping, and six R domains excised from the NR-PKSs of *A. nidulans*.

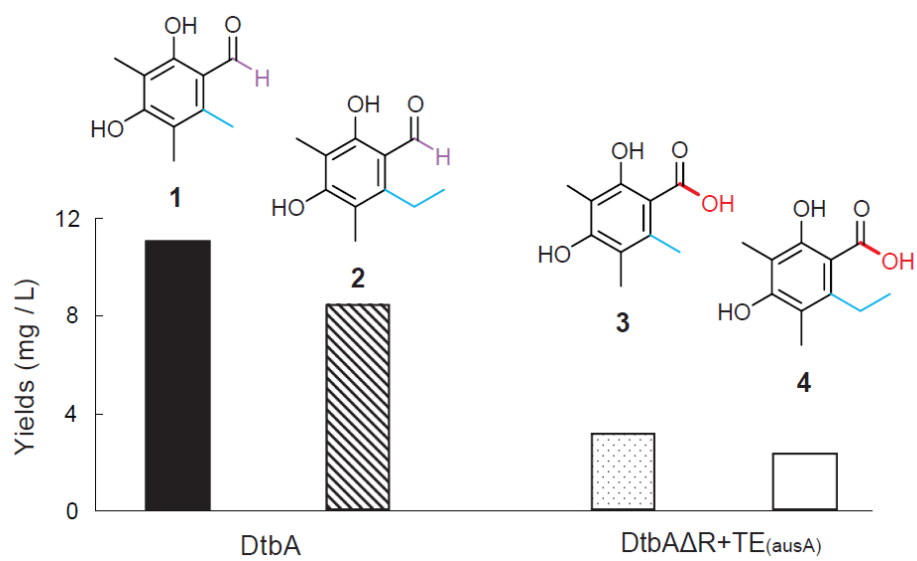


Figure S12. Production yield of compounds 1-4.



Figure S13. Ribbon diagram of the AusA TE domain. The location of the S123-H317-D285 catalytic triad is shown.

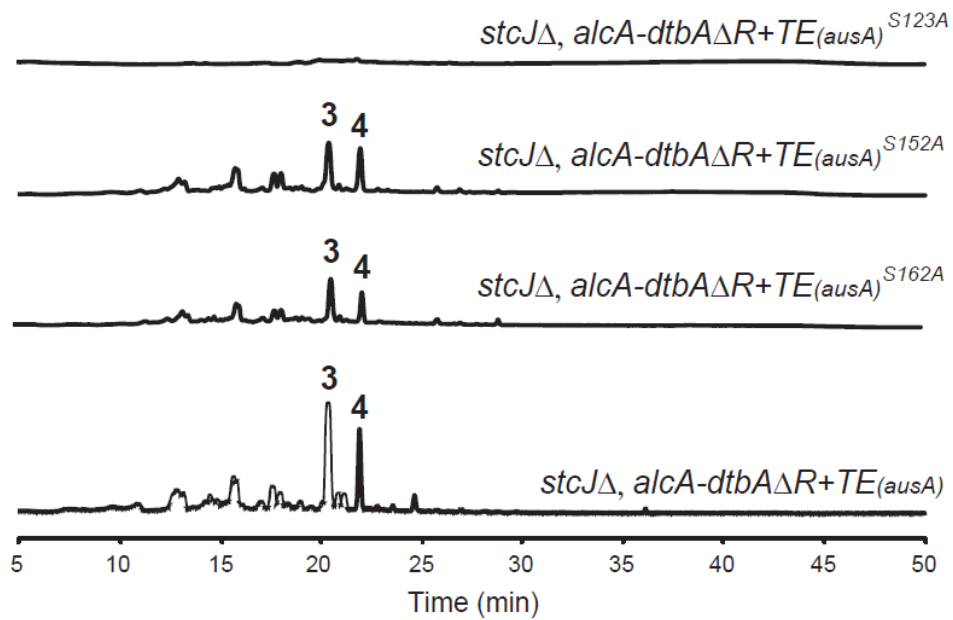


Figure S14. HPLC-DAD-MS analysis of TE mutant strains.

Supplemental References

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- (3) Szewczyk, E.; Nayak, T.; Oakley, C. E.; Edgerton, H.; Xiong, Y.; Taheri-Talesh, N.; Osmani, S. A.; Oakley, B. R., *Nat. Protoc.* **2006**, 1, 3111-3120.
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