

SUPPLEMENTARY FILE S1

(Sayari et al - *Ceratocystidaceae Polyketide biosynthesis gene clusters*)

To confirm the order of genes within the different PKS clusters identified, a PCR-based approach was used. For each cluster type, primers were designed that allow amplification of individual genes, as well as the regions between them. Correlation between predicted and observed fragment sizes were used as evidence that the specific cluster was correctly assembled.

For these PCRs, DNA was extracted from the five representative isolates: *Ceratocystis manginecans* (CMW17570), *Thielaviopsis punctulata* (CMW1032), *Endoconidiophora laricicola* (CMW20928), *Huntiaella moniliformis* (CMW10134) and *Davidsoniella virescens* (CMW17339). This was done using 10-day-old fungal cultures grown at room temperature on malt-extract-Agar (MEA; Merck) medium and the DNeasy Plant Mini Kit (Qiagen, Carlsbad, CA, USA).

The table below lists the primer sequences and expected amplicon sizes for all of the PCRs conducted in this study. Each PCR mixture contained 2.5Mmol MgCl₂, 150μM of each dNTP, 0.1μmol of each primer, 1U of *Taq* polymerase and 10X reaction buffer (Roche Applied Science, Mannheim, Germany) in a total volume of 25μl. Amplification was carried out with the GeneAmp PCR system 9700 (Applied biosystems) with initial denaturation 94°C for 4min, followed by 30 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min, and a final extension step at 72°C for 5 min. The sizes of the resulting PCR products were then estimated by making use of 1% (w/v) agarose (whiteheads scientific, South Africa) gel electrophoresis, GelRed™ (Biotium, Inc., Fremont, California) nucleic acid staining and an UV-transilluminator.

Table 1. Sequences, annealing temperatures, GC content and expected amplicon size for the primers used in this study.

A. Primers for individual genes in the R-PKS-I A group

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)	Amplicon size
HK-1 (F)	CATCTCTACGACGACTGCT	59	55	2099
HK-1 (R)	AGTAGAGTCACCCGTCTCCT	59	55	
HK-2 (F)	AACATACTGTCCTGCGTTGC	59	50	2654
HK-2 (R)	CATAACATCGTCGCACCCAG	59	55	
MF (F)	GGTCTTCGTTGCGGATTGT	59	50	1754
MF (R)	CACCCCTGCCAGTTCTATCA	59	55	
R-PKS-I-1 (F)	TGTGAATGCGGGCGAATTAG	59	50	2692
R-PKS-I-1 (R)	CCCCTGATCTTGCCTCATG	59	55	
Myosin-1 (F)	AGTTTAATTGCCGATCCGCC	59	50	2967
Myosin-1 (R)	AATCGCAGCTCACGTCAATC	59	50	
Myosin-2 (F)	GATTGACGTGAGCTGCGATT	59	50	3016
Myosin-2 (R)	GGCCGATCAATGGTTCAGTC	59	55	
Hypothetical-1 (F)	GCAAAGTCGGGCTAAGATGG	59	55	509
Hypothetical-1 (R)	CTATCATGCGCTGTCCAACC	59	55	
Hypothetical-2 (F)	GCGGTTACCCAAATTCTCC	59	55	722
Hypothetical-2 (R)	ACCCCTAGACTCATCCACCT	59	55	
Hydrolase (F)	ACTACCTTGCTTTGCTGCCT	59	50	1784
Hydrolase (R)	TATGCTGCCTCTACCTCTGC	59	55	

B. Primers for intergenic regions in the R-PKS-I A group

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)	Amplicon size
HK-MFS (F)	GTCAAGCCGTTCCGTATTCC	59	55	1520
HK-MFS (R)	TGCTCTCGTTATTTGCGTGG	59	50	
MFS-PKS-1 (F)	CCGGGCTTATTGTTTCAAGG	59	55	3458
MFS-PKS-1 (R)	AGGCCTGTGGTTTCTTAGAC	59	55	
MFS-PKS-2 (F)	TCCATTTCCCTTCCCTCCCC	59	55	3425
MFS-PKS-2 (R)	CCATTCCAACAACCACAGCA	59	50	
PKS-Myosin (F)	ACGTACACATGGGATTTGCG	59	50	3877
PKS-Myosin (R)	CCGGACATGGAGGCTAAGAT	59	55	
Myosin-Hypo-1 (F)	GACTGAACCATTTGATCGGCC	59	55	2905
Myosin-Hypo-1 (R)	CCATCTTAGCCGACTTTGC	59	55	
Hypo-1-Hypo-2 (F)	AGAAGGGAAGCAGCGAGTAG	59	55	1512
Hypo-1-Hypo-2 (R)	TTGAGACGTACCATGCTCCA	59	50	
Hypo-2-Hydrolase (F)	AGGTGGATGAGTCTAGGGGT	59	55	3401
Hypo-2-Hydrolase (R)	AGGCAGCAAGACAAGGTAGT	59	54	

C. Primers for individual genes in the *Huntiaella* R-PKS-I A cluster

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)	Amplicon size
FA desaturase (F)	CCCGCGAGATAGACTTCTA	59	55	1154
FA desaturase (R)	CGTTGAAGCAGTGTCCGGTTA	58	50	
SSR (F)	AAAGCGCCGTTGAAGTTTCT	59	45	1721
SSR (R)	TGCTGGTGTGGTTATTTGGC	59	50	
Transporter (F)	CCCTCCGCCTCTATCGAATT	59	55	526
Transporter (R)	AAGGGTTGACAGCAGGACAT	59	50	
MPV17 (F)	TCACTGCTCTCACTACCCC	59	55	294
MPV17 (R)	TTGTGATGCGGTGATTGGTG	59	50	
MFS (F)	AACAGCCACCAAGTACTCGA	59	50	2024

MFS (R)	CAACACCACCACACTGATG	59	55	
R-PKS-1 (F)	TTGCCGTCTCCCTTCTACAG	59	55	3731
R-PKS-1 (R)	TGTCACCTTTCTCGTCTCCC	59	55	
R-PKS-2 (F)	GGGAGACGAGAAAAGGTGACA	59	55	2387
R-PKS-2 (R)	CTCCGCTCAGTGTGATACT	59	55	
RNA polymerase (F)	TGGGTGAAATCTTCGGTGGA	59	50	3078
RNA polymerase (R)	TGTTTCATCGGTTTGAGCTGC	59	50	
Panthonate kinase (F)	AATCGCCGCTTCCATCTTTC	59	50	605
Panthonate kinase (R)	CATTGAGGTCGCGAGGATG	59	55	
ATP synthase (F)	GGCGAGTTGTCCAATGTTGT	59	50	990
ATP synthase (R)	GTCTACCAAATCACACGGCC	59	55	
NAC (F)	TGGCAGCAGTCTTCTCATCA	59	50	1860
NAC (R)	CGCTACTGAGGAGACCAAGA	59	55	
60 S (F)	TGAGACGGAGGACCTTGAAG	59	55	718
60 S (R)	TAACGAACCCAGCTGGC	60	61.11	

D. Primers for intergenic regions in the *Huntella* R-PKS-I A cluster

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)	Amplicon size
FA-SSR (F)	GCGGATTGTCTGGTCCCTAT	59	55	448
FA-SSR (R)	AGAACTTCAACGGCGCTTT	59	45	
SSR-Transporter (F)	TGCGTTGCCAAATAACCACA	59	45	936
SSR-Transporter (R)	GGATGATGAAGAGGAGGCGA	59	55	
Transporter-MPV17 (F)	ATGTCCTGTGTCAACCTT	59	50	1112
Transporter-MPV17 (R)	GGGTGTAGTGAGAGCAGTGA	59	55	
MPV-17-MFS (F)	ATGACCCATCCACACCAAT	59	50	1562
MPV-17-MFS (R)	TCGAGTACTTGGTGGCTGTT	59	50	
MFS-PKS-1 (F)	GTGTTGCAATCGTGGAAGT	59	50	3236
MFS-PKS-1 (R)	TGGAAAGCAGCAAGGAAAGG	59	50	
MFS-PKS-2 (F)	TGTTCTCGGTTGCCTAGTGT	59	50	3872
MFS-PKS-2 (R)	TGCCAATAACAACAGGTGCC	59	50	
PKS-RNA polymerase (F)	AGTATCGACTGAGCGGAG	59	55	3292
PKS-RNA polymerase (R)	TCCACCGAAGATTTACCCCA	59	50	
RNA polymerase-P kinase (F)	CGATGAACAATTCGCGCATG	59	50	1611
RNA polymerase-P kinase (R)	GAAAGATGGAAGCGCGATT	59	50	
P-kinase-ATP synthase (F)	TTTGCCCGGAGAACCCTTAT	59	50	409
P-kinase-ATP synthase (R)	ACAACATTGGACAACCTCGCC	59	50	
ATP synthase-NAC (F)	GGCCGTGTGATTTGGTAGAC	59	55	3897
ATP synthase-NAC (R)	TGATGAGAAGACTGCTGCCA	59	50	
NAC-60S (F)	TCTTGGTCTCCTCAGTAGCG	59	55	1206
NAC-60S (R)	TCCTCCGTCTCAAGAAGCAG	59	55	

E. Primers for the individual genes in the R-PKS-I B group

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)	Amplicon size
Dehydrogenase (F)	AGGTCCGTGAGTACATCGAC	59	55	2402
Dehydrogenase (R)	ACTCACTGGCCCACTTAGAC	59	55	
Transporter (F)	CCCCTTTCCCATCCTCACTT	59	55	193
Transporter (R)	TGACCCTAAAGCCACACTCG	59	55	
Hydrolase (F)	GGATTTTCATGACCACGGCC	59	58	469
Hydrolase (R)	CTACGAAGCTCCTCTCTCCG	59	60	
PKS-1 (F)	GCGATTGACTTTGAGGGGTG	59	55	3908
PKS-1 (R)	GTCCGGTGATTAGTTGTGCC	59	55	
C-450 (F)	CAAGCTCATCGGGTCCTTTG	59	55	2085
C-450 (R)	TCAGGTCCATCGTCATGTCC	59	55	
Hypo (F)	AAGAGTCCGTCCCGAGAAAG	59	55	909
Hypo (R)	TGGAACTCATAACCATGAGC	59	55	

F. Primers for intergenic regions in the R-PKS-I B group

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)	Amplicon size
Dehydrogenase-Transporter (F)	GTCTAAGTGGGCCAGTGAGT	59	55	1791
Dehydrogenase-Transporter (F)	AAGTGAGGATGGGAAAGGGG	59	55	
Transporter-Hydrolase (F)	CGAGTGTGGCTTTAGGGTCA	59	55	1317
Transporter-Hydrolase (R)	GGCCGTGGTCATGAAATCC	59	58	
Hydrolase-PKS	CGGAGAGAGGAGCTTCGTAG	59	60	2916

(F)				
Hydrolase-PKS (R)	CACCCCTCAAAGTCAATCGC	59	55	
PKS-C-450 (F)	GGCACAACATAATCACCGGAC	59	55	1738
PKS-C-450 (R)	CAAAGGACCCGATGAGCTTG	59	55	
C-450-Hypo (F)	CACGTGGTTTTGAAAGTCGCT	59	50	3180
C-450-Hypo (R)	GTGAAGAACGCTGGGTACG	59	58	

G. Primers for individual genes in the *Davidsoniella* R-PKS-IC cluster

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)	Amplicon size
TF (F)	GGGGTCCATGACATCCTCA	59	55	1045
TF (R)	TATGAGTCTTGGGGCAGTGG	59	55	
MFS (F)	CAACGGGAGGGAATTGTGTG	59	55	2631
MFS (R)	CTCTTCCGTTTCCAGCAG	59	55	
Hydroxylase (F)	CGCGGTGTCAGTGTATTACG	59	55	591
Hydroxylase (R)	CCCAAGCTTCATCCTCAACG	59	55	
PKS-I-1 (F)	AACCGTTCTGTCAATGCCC	59	50	3837
PKS-I-1 (R)	TGTTCTGTGAGATTGGCCCT	59	50	
PKS-I-2 (F)	AGGGCCAATCTCACAGAACA	59	50	2484
PKS-I-2 (R)	CAAGAAGTTCGACAGCAGCA	59	50	
GTP (F)	GTCTTGGTGCGAGTCTCAAC	59	55	1786
GTP (R)	TTGCCACAGAGATCTCACGT	59	50	
Dehydrogenase (F)	CTGGACCGGACAAGTAGTT	59	55	763
Dehydrogenase (R)	TTGCATCACCCAGTCCATCT	59	50	
60S (F)	CACGCCTAGTCTCTATCG	59	60	418
60S (R)	GGCTCGCTCTGCTTATTTT	59	50	
P-kinase (F)	TCAAACACAGACACAGCAGC	59	50	2945
P-kinase (R)	AGTAGACGCAGAATACGCCA	59	50	
P-450 (F)	GAAGCTGAATGAGGGCAAGG	59	55	163
P-450 (R)	TGTCACGGAACAGCTGGTAA	59	50	

H. Primers for intergenic regions in the *Davidsoniella* R-PKS-IC cluster

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)	Amplicon size
TF-MFS (F)	CCACTGCCCAAGACTCATA	59	55	2224
TF-MFS (R)	CACACAATCCCTCCCGTTG	59	55	
MFS-Hydroxylase (F)	CAGGGTTTGGATGGTGAAGC	59	55	3450
MFS-Hydroxylase (R)	CGCGGTGTCAGTGTATTACG	59	55	
Hydroxylase-PKS (F)	CGTTGAGGATGAAGCTTGGG	59	55	3114
Hydroxylase-PKS (R)	CACGGCCGTATTCTTCCAG	59	55	
PKS-GTP-1 (F)	TGAACATGCTGCTGTCGAAC	59	50	3206
PKS-GTP-1 (R)	CGTGTCGTCGATTGCAGATT	59	50	
PKS-GTP-2 (F)	AATCTGCAATCGACGACACG	59	50	3583
PKS-GTP-2 (R)	GTTGAGACTCGACCAAGAC	59	55	
GTP-Dehydrogenase (F)	AGAAGGCACGGATACGTG	59	50	1356
GTP-Dehydrogenase (R)	AACTACTGTCCCGTCCAG	59	55	
Dehydrogenase-60S (F)	AGATGGACTGGGTGATGCAA	59	50	2102
Dehydrogenase-60S (R)	GCCCACAACGAGCTTCAC	59	61.11	
60S-P-kinase (F)	AAAATCAAGCAGAGCGAGCC	59	50	3066
60S-P-kinase (R)	GCTGCTGTGTCTGTGTTGA	59	50	
P-kinase-P450 (F)	GCGTATTCTGCGTCTACTGC	59	55	1720
P-kinase-P450 (R)	CCTTGCCCTCATTACAGCTTC	59	55	

I. Primers for individual genes in the *Thielaviopsis* R-PKS-IC cluster

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)	Amplicon size
Hydrolase (F)	GAGGAAAAGTGCGACCCAAG	59	55	602
Hydrolase (R)	TGGTTTTCGTCTTGGTGCT	58	45	
PKS (F)	TATCTTCTCTCGCGCTCAG	59	55	3420
PKS (R)	GTTCTCTCCACCAGTTGCG	59	55	
Hyp (F)	GGCTGCTTCATCAACACCAA	59	50	589
Hyp (R)	TGGAAGTACGCGGAGATGAC	59	55	
ATP (F)	GCCTTCTCTGACTCCGA	59	55	2460
ATP (R)	AATGAGTTGGGCGAGTCTGA	59	50	
Oxygenase (F)	CAAACCCCTACTCGCGTCTG	59	55	936
Oxygenase (R)	CTTCCTTCGGTGCCTCTAT	60	55	

J. Primers for intergenic regions in the *Thielaviopsis* R-PKS-IC cluster

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)	Amplicon size
PKS-Hyp (F)	GAGGCGCTGCAGAAGAATG	59	58	3700
PKS-Hyp (R)	TTTGCCCCAATGTAGAGTGT	57	45	
Hyp-ATP (F)	GTCATCTCCCCTCAGTTCCA	59	55	3535
Hyp-ATP (R)	TAGCGCCAAGACTCCTTCAA	59	50	
ATP-oxy (F)	TCAGACTCGCCCAACTCATT	59	50	777
ATP-oxy (R)	CCAACATGTTCAAGCGCAAC	59	50	
Oxy-hyp (F)	GGAGGGAGAGGGTCTGTAGA	59	63	2050
Oxy-hyp (R)	CCCAAGCGTGTATGATGAGC	60	55	

K. Primers for individual genes in the R-PKS-ID

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)	Amplicon size
CoA (F)	TGCTATTTCTCTGACCGCT	59	50	1452
CoA (R)	TAGTATCGCAACGCAAAGCC	59	50	
Dehydrogenase (F)	CGACCTTCTCTGCTTGATG	58	55	1641
Dehydrogenase (R)	CAAACACTCTGCAGCACCTT	59	50	
Oxidoreductase (F)	CGCGGGAATGAACGGTTTAG	60	55	456
Oxidoreductase (R)	ACGACGACTTTATGCAGTGC	60	50	
NUP-54 (F)	ACAACAACAATTCCAGCCCG	59	50	818
NUP-54 (R)	ATCTGCTCCACAACCTCTCC	59	55	
PKS-I-1 (F)	GTCGCTCCTCATCAACAAGC	59	55	4028
PKS-I-1 (R)	CCAGATAAGGCTCGGCAAAC	59	55	
PKS-I-2 (F)	GTTTGCCGAGCCTTATCTGG	59	55	3791
PKS-I-2 (R)	TGCTCACCATCGAATCCAGT	59	50	

L. Primers for intergenic regions in the R-PKS-ID

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)	Amplicon size
CoA-Dehydrogenase (F)	GGCTTTGCGTTGCGATACTA	59	50	559
CoA-Dehydrogenase (R)	CTTTGTGAAGCGCCTACTGG	59	55	
Dehydrogenae-Oxidoreductase (F)	AAGGTGCTGCAGAGTGTTTG	59	50	2452
Dehydrogenae-Oxidoreductase (R)	CTAAACCGTTTCATTCGCCG	60	55	
Oxidoreductase-NUP (F)	GCACTGCATAAAGTCGTCGT	60	50	1259
Oxidoreductase-NUP (R)	CGGGCTGGAATTGTTGTTGT	59	50	
NUP-PKS (F)	GGAGGAGTTGTGGAGCAGAT	59	55	3979
NUP-PKS (R)	GCTTGTTGATGAGGAGCGAC	59	55	

M. Primers for individual genes in the NR-PKS-I cluster

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)	Amplicon size
BZZ-1 (F)	GCCATGATACGGTTCCTCCG	59	55	2243
BZZ-1 (R)	GACGATTCTGTCTTTTGG	59	50	
Helicase (F)	CCTCCCCAGAACCTCATTGC	59	55	2599
Helicase (R)	GTCAGTATCAGCTTTGT	59	55	
CEFM (F)	GAGCCTGATCCGGAGACTTA	58	55	3927
CEFM (R)	GGGAAGGGCGAAGTATACGA	59	55	
PKS-I-1 (F)	TCAACAAGACTGTTGAGGG	59	50	3002
PKS-I-1 (R)	GTGGTAGTTGATACGACC	59	50	
PKS-I-2 (F)	GGTCGTATCAACTACCAC	59	55	2757
PKS-I-2 (R)	TCTCCAGTGTACTGCAGC	59	55	
THN (F)	CTTGGATCCCCTGACAAAAG	59	55	286
THN (R)	ATATCAGCAAGCCGAGGAA	59	50	
TF (F)	CAGCGGCCATAATACCATG	59	55	2277
TF (R)	GAGTCCCCTTCCATCTTGA	59	55	

N. Primers for intergenic regions in the NR-PKS-I cluster

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)	Amplicon size
BZZ-Helicase (F)	TTGTTGCACATCTTCGAGCC	59	50	1250
BZZ-Helicase (R)	CCTCGTCATCACCAACAACC	59	55	
Helicase-CEFM (F)	TGGGATGAAACGGAGGAGAG	59	55	1362
Helicase-CEFM (R)	CTCTCCTCCGTTTCATCCCA	59	55	

CEFM-PKS (F)	TCGTATACTTCGCCCTTCCC	59	55	3375
CEFM-PKS (R)	CAACGACTTCTGGAGACCCT	59	55	
PKS-THN (F)	CCCGAGTGGCTGCTTCC	59	50	2519
PKS-THN (R)	CCCTGGCATGGACATGAC	59	55	
THN-TF (F)	GCTTTCTCGCTTGCTTGCAT	59	50	1720
THN-TF (R)	AACCATCGGAGATTCTTGG	58	55	

O. Primers for individual genes in the PKS-III cluster

Primer	Primer sequence (5'→3')	Annealing temperature (□C)	GC content (%)	Amplicon size
Ribonuclease (F)	GAGATTGTGACTGGCTTGCC	59	55	3513
Ribonuclease (R)	TGAAGCAGGGAGTTGGCATA	59	50	
Mito-carrier (F)	GTTTGTGTGTTGTTGCAGGC	59	50	540
Mito-carrier (R)	CGTCATCCAGCCTGTAGACA	59	55	
Methyltransferase (F)	GGCCAGAGTCTTTAGCAGGA	59	55	577
Mito-carrier (R)	CCACTATGTCCCAGAGCAGT	58	55	
Ankyrin (F)	TTGGTAGTAATAAGAAC	59	50	3752
Ankyrin (R)	GGATGGGAAGAAGAGGATG	59	50	
Mito 2 (F)	GAACGACGAATGGGGAAAGG	59	55	419
Mito 2 (R)	GACTTGACAACGTCCATGGG	59	55	
PKS-III (F)	CATCCTCTGGTGGTGTGTCT	59	55	852
PKS-III (R)	CTGTTGAGCGTGTCTCCTA	59	55	
TF (F)	GGCTCAAACGCAGATACCAG	59	55	2277
TF (R)	ACCCCGCTGATGTTGTTAGA	59	50	

P. Primers for intergenic regions in the PKS-III cluster

Primer	Primer sequence (5'→3')	Annealing temperature (□C)	GC content (%)	Amplicon size
Ribo-Mito (F)	GCAAACCTCTCGCTAGCCTTC	59	55	1192
Ribo-Mito (R)	GCCTGCAACAACACACAAAAC	59	50	
Mito-Methyl -1 (F)	TGCGGACCTTGATCATGTCT	59	50	4028
Mito-Methyl -1 (R)	CGAGCGACATCAACCAAGAG	59	55	
Mito-Methyl -2 (F)	CTCTTGTTGATGTCGCTCG	59	55	3242
Mito-Methyl -2 (R)	TCCTGCTAAAGACTCTGGCC	59	55	
Methyl-Ankyrin (F)	GCTTGCCTCGTCTGTTTCTT	59	50	1366
Methyl-Ankyrin (R)	GAAGACGAACAGAAGCGCAT	59	50	
Ankyrin-Mito (F)	TGGATCATCAAAGCTGGGGT	59	50	2940
Ankyrin-Mito (R)	CCTTTCCCATTCGTCGTTT	59	55	
Mito-PKS (F)	GCAATTGGTGGTACTGTCCG	59	55	2489
Mito-PKS (R)	AGACACACCACCAGAGGATG	59	55	