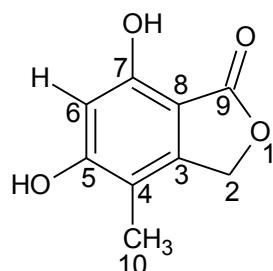
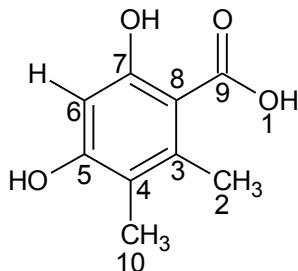


Supplementary Table S1. Top BLAST hits for *P. brevicompactum* MPA gene cluster in *Phaeosphaeria nodorum* and *Talaromyces stipitatus*.

Query (<i>P. brevicompactum</i>)	<i>P. nodorum</i> SN15 (taxid:321614)				<i>T. stipitatus</i> ATCC 10500 (taxid:441959)			
	Protein ID (top BLAST hit)	BLASTP E-value	BLASTP Identity	Identity (ClustalW)	Protein ID (top BLAST hit)	BLASTP E-value	BLASTP Identity	Identity (ClustalW)
MpaA	SNOG_05304	7.00E-17	30%	17.38%	gi 242821326	1.00E-16	20%	17.12%
MpaB	SNOG_14971	2.00E-89	39%	33.72%	gi 242779340	2.00E-120	50%	42.06%
MpaC (PKS)	SNOG_06682	0	39%	36.90%	gi 242760313	0.00E+00	49%	42.50%
MpaD	SNOG_06679	2.00E-109	47%	31.22%	gi 242760308	1.00E-113	55%	53.13%
MpaE	SNOG_06681	1.00E-81	50%	45.90%	gi 242760293	3.00E-87	50%	47.89%
MpaF (IMPDH-B)	SNOG_00421	0.00E+00	73%	70.45%	gi 242786825	0.00E+00	79%	76.64%
MpaG	SNOG_08626	2.00E-60	31%	30.35%	gi 242781136	0.00E+00	63%	61.85%
MpaH	SNOG_15010	5.00E-50	37%	26.05%	gi 242768952	2.00E-84	39%	36.59%

1. MpaD and MpaE (shown in green) were together identified as a single fusion protein in the current study. BLAST search for the fusion protein MpaDE did not find any good hits. 2. BLASTP search was performed on the NCBI BLAST server (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>, 16th March 2012) against the two genomes of interest and with the default settings. 4. For MpaC/D/E, amino acid sequence based on the coding region was used for the search. For the rest, protein sequences available in the NCBI gene bank were used.

Supplementary Table S2: ^1H and ^{13}C NMR data (extracted from HMBC experiment) for 5-methylorsellinic acid and 5,7-dihydroxy-4-methylphthalide^a



	5-methylorsellinic acid		5,7-dihydroxy-4-methylphthalide	
Position	δ_{C} (ppm)	δ_{H} mult	δ_{C} (ppm)	δ_{H} mult
2	18.6	2.28 (3H, s)	67.5	5.11 (3H, s)
3	138.9		149.0	
4	115.1		108.5	
5	159.2		162.1	
6	100.2	6.23 (1H, s)	102.0	6.41 (1H, s)
7	nd		155.6	
8	108.9		nd	
9	172.0		168.6	
10	12.1	1.93 (3H, s)	10.1	1.91 (3H, s)
5-OH		9.81 (1H, s)		10.32 (1H, s)
7-OH		10.92 (1H, bs)		10.18 (1H, s)

^a recorded in DMSO-*d*₆ at 25 °C, referenced to 2.50 ppm

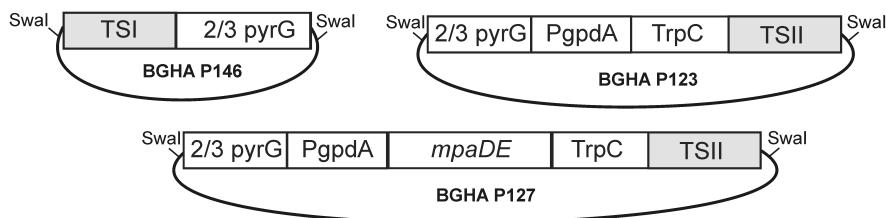
δ_{C} : carbon chemical shift values

δ_{H} : proton chemical shift values

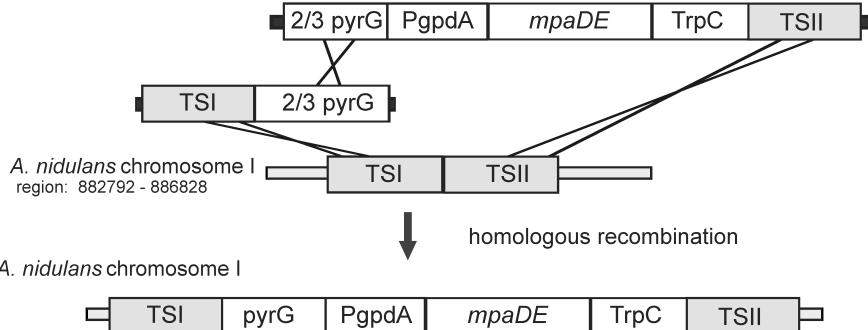
Mult: multiplicity of proton NMR peaks (s: singlet, bs: broad singlet)

HMBC: Heteronuclear Multiple Bond Correlation (nd: not detected via HMBC experiment)

A



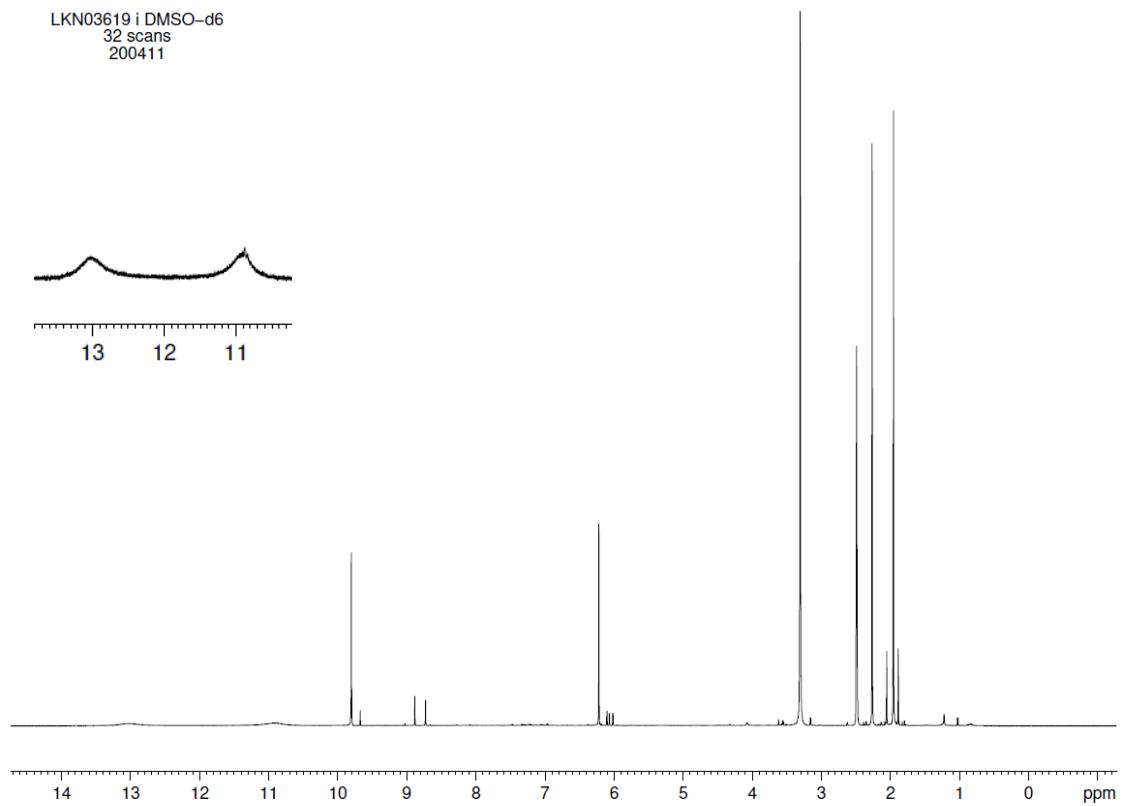
B



Supplementary Figure S1.

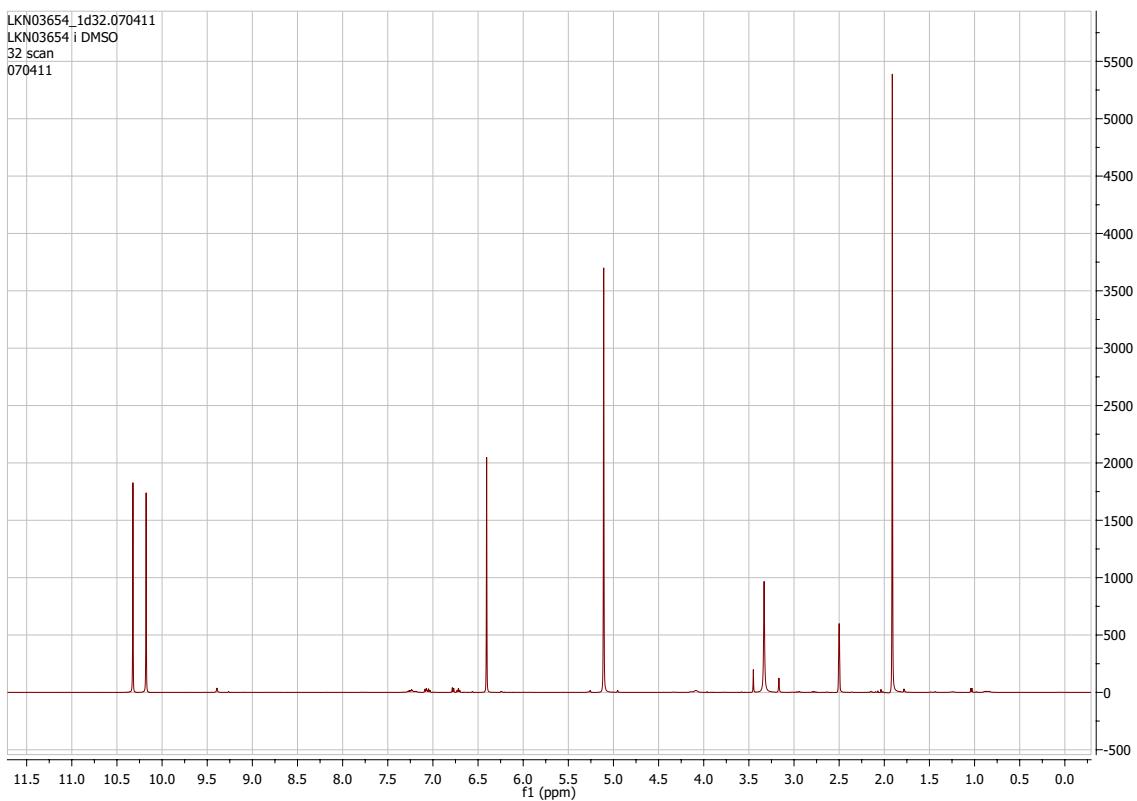
Integration of expression cassette into integration site II on *A. nidulans* chromosome I by homologous recombination using bipartite transformation. A) Schematic drawing of BGHA P127, BGHA P146 and BGHA P123. TSI and TSII are the sequences for homologous integration in IS2. The selection marker is *pyrG* from *A. fumigatus* shown in bipartite gene targeting configuration. PgpdA and TrpC define promoter and terminator of native *gpdA* and *trpC*, respectively, controlling and enabling constitutive *mpaDE* expression. Transformation substrates are generated by SwaI digest of the constructs. B) Integration of the *mpaDE* expression cassette into *A. nidulans* by homologous recombination. Control strain is generated by bipartite transformation with SwaI digested BGHA P146 and BGHA P123. Drawing is not to scale.

LKN03619 i DMSO-d₆
32 scans
200411



Supplementary Figure S2.

¹H NMR spectrum of 5-methylorsellinic acid (5-MOA) measured in DMSO-*d*₆ (¹H at 500 MHz, ¹³C at 125 MHz) and referenced to solvent residual signals and solvent signals at 2.50 ppm (¹H NMR).

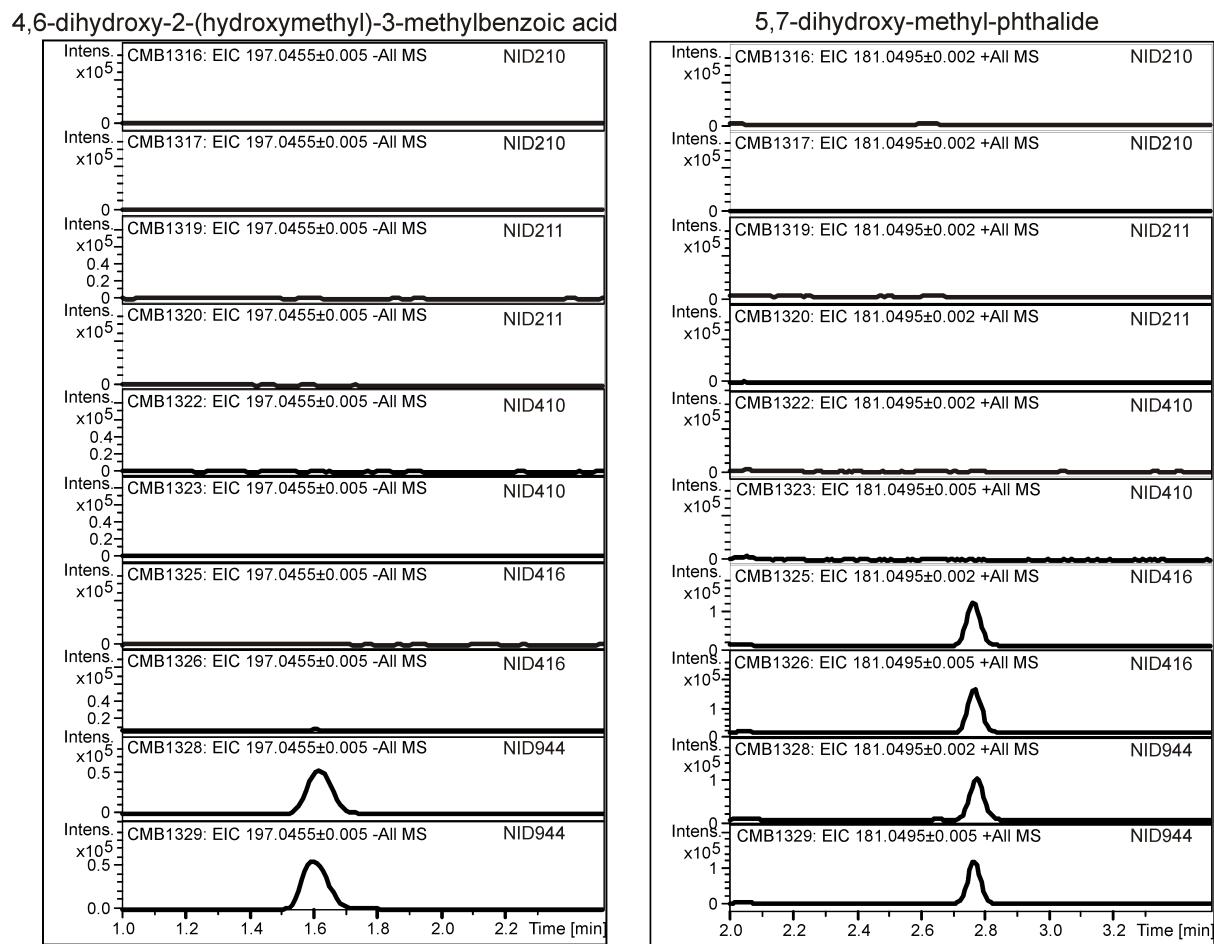


Supplementary Figure S3.

¹H NMR spectrum of 5,7-dihydroxy-4-methylphthalide (DHMP) measured in DMSO-*d*₆ (¹H at 500 MHz, ¹³C at 125 MHz) and referenced to solvent residual signals and solvent signals at 2.50 ppm (¹H NMR).

20 I 40 I 60 I 80 I 100 I
 MpaD_MpaE ----- MKSLSLTWIT AVAVVLYLVQ RYVRSYWRLK DIPGPV-LAK LTDLQRVWWV -KTGRAHEFH RDMHAMYGP VRFGPN--M VSVDSPRVIP 85
 2j2 ----- MTIK ----- EMPQPKTFFGE LKLNPLL--- .NTDKPVQAL MKIADELGEI FKFEPAPGRVT RYLSQSRLIK 60
 3kv ----- MASR APKEVPLCPL MTDGETRNTV DLPGPTNWPL LGSLLEIFWK GGLKKHQDTL AEYHKKYQGI FRMKLGSFDS VHLSGSPSLLE 84
 2f9q ----- MAKK TSSKGKL -PPGPLPLPG LGNLLHV-- DFQNTPYCF DQLRRRGFDV FSQQLAWTPV VVLNGLAAVR 66
 TSTA_060710 MDSMNIFPGY RQADSIVFIA VGSILGLSLL HLLRTWRLR YIPGPFLAR FTNLQRVYWV -KTTRAHEIH QQMHEKYGNY VRFGPN--M VSLDGPALIP 95
 SNOG_06679 MDRNVLFVDF GMAGGVAVVA VVLLIVAFVTA HTARTWWRLLR HIPGPFLAS ITDLYRMNNWV -KTTRAHLLK QOCHERYGKL VRIGPN--T VSFSDPVAILP 95
 3dha -----
 2r2d -----
 1smI -----
 TST_060680 -----
 SNOG_06681 -----
 120 I 140 I 160 I 180 I 200 I
 MpaD_MpaE TIYPSRPGF KGDFYRTQKP YTRNKGAMPA VFNT-QDELD HKQLRSPIAS LYSMT--NV RLEPLVDETL TVLSKQLDER FVGTNDKPFD LGDWLQYFAF 182
 2j2 EACDESFRDK NLSQALKFVR ---DFAGDG LFSTSWTHEKN WKKAHNILLP SFSSQO--AMK GHYAMMVDA QVQVKWERL NADEH--IE VPEDMTRLTL 151
 3kv ALYRTESAHQ QRLEIKPWKA YRDHRHNEAY LMIL-E-GOE WQRVRSAFQK KLMKP-VEIM KLDDKNEVIL ADFLERMDL CDERGRI-PD LYSELNKWSF 180
 2f9q EALVTHGED ADRPPVITQ ILFGFGRPSQG VFLLA-RYGPRA WREGRPSVSL TLRNLLGLGKX SLEQWVTEA ACLCAAFAHNS GRGP---FR PNGLDDKAYS 161
 TSTA_060710 SLYPYRGPFF KSDFYRSLMP YTRKGGSLPA VFNT-RDENL HKV1KTP1AP LFSLS--NL PLEVFNVRV LVLFOQLDTR FVSSGDT-FD LADWLQYFAF 191
 SNOG_06679 EVPPMRAGMP KSDFYVTLR YT-SGQALHA VFNT-TEESI LKQ1KPP1AP LFNIS--SAA TLEPLVDEVL ECIRGRFDER FVGTQI-FD MGQWLQFFAF 190
 3dha -----
 2r2d -----
 1smI -----
 TST_060680 -----
 SNOG_06681 -----
 220 I 240 I 260 I 280 I 300 I
 MpaD_MpaE DSMGTLTFSR RYGFLEQGR --DHMGHLQ EIWNFMTTRVA VMGQ1PWFDE IWKNKSFTL FKRTGFGVL KVVDNF---- ISOR VSSRENDEKA 268
 2j2 DT1GLCGFNY RFNSFVRDOP -HPF1TSMWVRL ALDEAMMNLQ RANDPDPOYD ENKROFQEDI ---K--- VWMNDL-VDK IADRKASGE 229
 3kv E5ICLVLVYK RFGF---LLQKE TEEEAFTIIT AIIKTMFTRP KMMVTPVELH KRLN1KWWQA HTLAWD---TIFKS-VKP CIDNRRLQRY 261
 2f9q NVIASLTLGCR RFE-YDQPR -FLRLLDLAQ EGLKEESGFL REVNLNAVPK RH1PALAKVY -.KAFLTQLDE LLTEHMRMTWD PA---O 244
 TSTA_060710 DVMGTLTFSK RYGFLEHGR --DVNNMIL TIWTYMSNC A PMTQ1PWFDV VWNKNAF1L FRRAS---SG LS1LGH-VGE LIADRRQRNN TPSAVSDEKA 282
 SNOG_06679 DVMGTMFTSK RYGFLEHGR --DVGGMLG A1VDFMRTAA PMTQSP1LLD LLLRNKVNVDT LROQYIRPTAS LT1LSF-VQQ A1KEKKEKME SKTSKSE- 282
 3dha -----
 2r2d -----
 1smI -----
 TST_060680 -----
 SNOG_06681 -----
 320 I 340 I 360 I 380 I 400 I
 MpaD_MpaE DEKDMLSQFL DIQASNPH --SIMPWAPR AWTFSNVYMG SDSTANVMRT MMYNLLVDRD TLRSRLRAELL --EAENSNGL SRSLPSWQGV RSLPYLDACV 362
 2j2 QSDDLTHML NGKDPETG --EPLDDEINR YQ1ITFLI AG HETTSQGLSF ALYFLVKNPH VLQKAAEEA- ARVLV D-PVPSYKVQ KQLYYGMVL 319
 3kv SQQPGADFLC DIY- ----- ESSFDNENLR IVVADFLSAG MYTTSTTLAW GLLLMIHLPD VQRRVQE----- DDVIG QVRPPEMDQ AHMPYTTAVI 338
 2f9q PPRDLTEAFL AEMEKAIGNP ESSFDNENLR IVVADFLSAG MYTTSTTLAW GLLLMIHLPD VQRRVQE----- DDVIG QVRPPEMDQ AHMPYTTAVI 338
 TSTA_060710 SDRDMLSRFF ELQEKDSK ---I PRWAVT AWAFLSVI AG SDSTAVTMR TWFGLLSHPE TLKTLRKEEL DODSKLEG1 TRFPFAWKO CNLAYLDACV 377
 SNOG_06679 GO1DFLTRYI ELQKHNPNS ---I PPWAPT AWTFSNV1AG SDSVGTCMRT MLYHLLAYPN TFDRLYKEL ---LAAKT SRPFPSYSEV RELPYLEACV 371
 3dha -----
 2r2d -----
 1smI -----
 TST_060680 -----
 SNOG_06681 -----
 420 I 440 I 460 I 480 I 500 I
 MpaD_MpaE LEARLRLHPP CLPFERVVP E GGTVC-E-TY LPAGTVVGIS PYLANRDKQT FGDDADKWRP SRWLDSLRED RVKLENSRL FGAGRRTCLG KNAIAILEIKK 461
 2j2 NEARLRWPTA P-AFSLYAKE -DTVLGGEY LEKGDELMLV1 IPQLHDKTI WGDDVEEFP ERFENPSA- -IOPQHAFK FNGNQRAC1G QQFALHEATL 413
 3kv KEVQRFGDIV PLGMTHMTRSR -DIEVQG-FR IPKGTTLITN LSSVLLKDEAV W-EKPFHFP EHFLDAQG- HFVKEPAFL FSGARRALC EPLARAMEFL 433
 2f9q NEARLRLHPP CLPFERVVP GGLTIGG-TY PAGTVGPM PWVNVNHRP1 FGDAAEWSRP ERVMVPEE-H RRKLEQSVLT FGAGRRVCLG KHIAMLEIKK 475
 TSTA_060710 SNOG_06679 QEARIHVHPP ALPLERVVPD GGMVTLG-YF LPAGTVGIGS PYVVNDRDTSM FGEDAAEWRP ERWLGDDAA KRSLEASMLT FGAGRRICLG RHVGILEIKK 470
 3dha -----
 2r2d -----
 1smI -----
 TST_060680 -----
 SNOG_06681 -----
 520 I 540 I 560 I 580 I 600 I
 MpaD_MpaE LFPMILLNLY IEIVNPENY TTNAWFFRQW GLOAVIRKQL APERDDTIEQ KAS1PPALN PPSSSTV EVR IIDSGTLDDL RPDLFWTPDL PGLLK- 556
 2j2 VLCMMLKHF DEDHTNVEYL IKTETLTKPE CFVVKAKSKK1 IPLGG1PSPS TEQSAKKV- -----
 3kv ALCWIQKDY IIVATDNEPVE MLHQL1LVP5 RELPIAFLPRP -----
 2f9q FFTSLLQHFS FSVPCTCOPR SHHGVFALVY SPSPYELCAV PR-----
 TSTA_060710 SNOG_06679 LTAALALNYY FDLDDLPKRFK VENGWFFRQV GMVDVKARAK VWEEDKKQ- -----
 3dha -----
 2r2d -----
 1smI -----
 TST_060680 SNOG_06681 -----
 620 I 640 I 660 I 680 I 700 I
 MpaD_MpaE -VTAPTYCYFL ISNGTRHVLF DLAVRQDWEN LPPSIVAMIK SQTV1QEPRN I-----SDV LDDESS-LG VRSKD1EAI1 WSHAHFDHIG DPSTFPNSTE 648
 2j2 -----
 3kv -----
 2f9q -----
 TSTA_060710 SNOG_06679 -----
 3dha -----
 2r2d -----
 1smI -----
 TST_060680 SNOG_06681 -----
 720 I 740 I 760 I 780 I 800 I
 MpaD_MpaE LVVGPGRD1 HWPGPTNPD A1NLNTD1QG RNVRE1SFEK TQKGATK1GS FDAOVDYFGD SFY1-LDAAG HSVGHIG-AL ARVTT- -SPVSPFVFM 739
 2j2 -----
 3kv -----
 2f9q -----
 TSTA_060710 SNOG_06679 -----
 3dha -----
 2r2d -----
 1smI -----
 TST_060680 SNOG_06681 -----
 820 I 840 I 860 I 880 I 900 I
 MpaD_MpaE GGD---SCHH AGVLRPTKYL P----- CPLDS GDTSLPCK- --SDSVF- -TLSP-ALPT DYTAALRTVE NIKELDACE 803
 2j2 -----
 3kv -----
 2f9q -----
 TSTA_060710 SNOG_06679 -----
 3dha -----
 2r2d -----
 1smI -----
 TST_060680 SNOG_06681 -----
 920 I 940 I 960 I 980 I 1000 I
 MpaD_MpaE VFVFLAHDAT LKGKVFDFYPS KINDWKKEY GKKTWKWLYK DIENSIEGQK 853
 2j2 -----
 3kv -----
 2f9q -----
 TSTA_060710 SNOG_06679 -----
 3dha -----
 2r2d -----
 1smI -----
 TST_060680 SNOG_06681 -----
 1020 I 1040 I 1060 I 1080 I 1100 I

Supplementary Figure S4. Alignment of MpaDE with the CYPs; *Bacillus megaterium* P450 BM3 (2ij2), *Rattus norvegicus* CYP24A1 (PDB 3k9v) , *Homo sapiens* CYP2D6 (PDB 2f9q), *Talaromyces stipitatus* TSTA_060710 and *Phaeosphaeria nodorum* SNOG_06679, *Bacillus thuringiensis* serovarkurstaki lactone hydrolase (PDB 3dha), *Agrobacterium tumefaciens* lactone hydrolase (PDB 2r2d), *Stenotrophomonas maltophilia* lactone hydrolase (PDB 1sml), *Talaromyces stipitatus* TSTA_060680, *Phaeosphaeria nodorum* SNOG_06681. The last three amino acids in the MpaD expressed in this study are marked in green.



Supplementary Figure S5.

MpaD catalyses the conversion of 5MOA to 4,6-dihydroxy-2-(hydroxymethyl)-3-methylbenzoic acid. UHPLC-UV/VIS-HRMS analyses NID210, NID211, NID410, NID416 and NID944. For each strain, two extracts were analysed, resulting in two chromatograms for each strain for each compound. Left column: extracted ion chromatogram, m/z 197.0455 ± 0.005 corresponding to the $[M+H]^+$ ion of 4,6-dihydroxy-2-(hydroxymethyl)-3-methylbenzoic acid. To the right: extracted ion chromatogram, m/z 181.0495 ± 0.002 corresponding to the $[M+H]^+$ ion of 5,7-dihydroxy-methyl-phthalide (DHMP).