Supporting Information

An overlooked cyclase plays a central role in the biosynthesis of indole diterpenes

*Rosannah C. Cameron, Daniel Berry, Alistair T. Richardson, Luke J. Stevenson, Yonathan Lukito, Kelly A. Styles, Natasha S. L. Nipper, Rose M. McLellan, and Emily J. Parker**

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1.1 General methods:

1.1.1 Bacterial protocols

Routine growth of *Escherichia coli* was performed at 37 °C in Luria Broth (LB). Chemically competent *E. coli* HST08 Stellar cells (Clontech Laboratories, Inc., 636763) and in-house DH5α cells (original stock - NEB® 5-alpha, C2988J) were used for transformation and maintenance of plasmids.

Bacterial transformations were performed using standard protocols, 1 µl of plasmid was added to 25 µl of competent cells and incubated on ice for 30 min. Heat shock at 42 °C for 45 seconds was followed by immediate transfer to ice for 2 min. Then, 400 µl of SOC medium was added, and the cells were incubated at 37 °C for 1 hour with shaking. Afterward, 50-100 µl of the transformation mixture was spread onto LB agar plates containing appropriate selection agents and incubated overnight at 37 °C. Individual colonies were selected using a sterile pipette tip. Colonies were transferred to a 14 ml sterile tube containing 4 ml of LB (and appropriate selection agents) using a sterile pipette tip. The cultures were then incubated overnight at 37 °C with shaking (200 rpm).

1.1.2 Media and reagents used for bacterial protocols

Luria Broth:

Luria Broth was prepared by adding 25 g of Miller's LB base (Invitrogen, 12795-084) to 1 l of deionised water and autoclaving. LB agar (LBA) was prepared by dissolving 25 g of Miller's

LB base (Invitrogen, 12795-084) and 20 g of select agar (SigmaAldrich, A5054-1KG) in 1 l of deionised water and autoclaving.

SOC medium:

Super Optimal Broth with Catabolite Repression (SOC) medium was prepared as follows: 20g of Tryptone (ThermoFisher, LP0042B), 5 g of yeast extract (Oxoid, LP0021), 2 ml of 5 M sodium chloride (Sigma, S9888-10KG), and 1.25 ml of 2 M potassium chloride (Sigma Aldrich, P9333) were added to 1 l of deionised water and autoclaved. After the medium cooled, 10 ml of a 2 M filter-sterilized glucose solution (20 mM working concentration) was added, along with 5 ml each of filter-sterilized 2 M magnesium chloride (10 nM working concentration) (Sigma, M8266) and 2 M $MgSO_4.7H_2O$ (10 mM working concentration) (Aaron Chemicals, AR0002CV).

Selection

MIDAS level 1 agar plates: LBA was supplemented with Isopropyl β-d-1-thiogalactopyranoside (IPTG) (PureScience, N1038215) at a working concentration 0.26 µM of and 5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside (X-Gal) (PureScience, N1016210-1), at a working concentration of 53.3 µg/ml.

MIDAS level 2 agar plates: LBA was supplemented with 4-chloro-dl-phenylalanine (4CP) at a working concentration of 0.25 g/l (Sigma, C6506-5G) and kanamycin (ThermoFisher, 11815032) at a working concentration of 50 µg/ml.

MIDAS level 3 agar plates: LBA was supplemented with IPTG at a working concentration of 0.26 mM and X-Gal at a working concentration of 53.3 µg/ml. Either spectinomycin (Sigma, S4014-5G) or carbenicillin (Aaron Chemicals, AR00I9G0) were also added at working concentrations of 120 µg/ml and 100 µg/ml respectively.

1.1.3 Fungal protocols – *Penicillium paxilli* protoplast preparation

The preparation of fungal protoplasts for transformation followed the protocol outlined by Yelton et al.[1] with modifications. Protoplasts were generated by combining 1 x 10^7 spores with 50 ml of CYDE medium in 250 ml Erlenmeyer flasks and incubating at 28 °C with shaking (200 rpm) for 31 hours. The resulting mycelia were collected and filtered through a sterile blue cloth, followed by three rinses with sterile water and one rinse with OM buffer (10 mM Na_2HPO_4 (SigmaAldrich, S9390-1KG) and 1.2 M $MgSO_4.7H_2O$ (Aaron Chemicals, AR0002CV), adjusted to pH 5.8 with 100 mM $NaH_2PO_4.2H_2O$ (Vetec, V800376-500G)). The mycelia were then resuspended in 50 ml of filter-sterilized Lysing Enzyme solution (prepared by resuspending VinoTaste® Pro (Novozymes) 1 g in 50 ml OM buffer) and incubated for 18 hours at 30 °C, 80 rpm. Protoplasts were filtered through a sterile blue cloth into a 250 ml

Erlenmeyer flask and further filtered through a sterile 40 µM cell strainer to remove any residual mycelia. The protoplasts were divided between two sterile 50 ml centrifuge tubes and overlaid with 10 ml of ST buffer (0.6 M sorbitol (Sigma Aldrich, S1876-1KG) and 0.1 M Tris-HCl (Invitrogen, 1550420) at pH 8.0) to create a gradient, followed by centrifugation at 4 °C, 4300 rpm for 15 min. Protoplasts were then washed with STC buffer (1 M sorbitol (Sigma Aldrich, S1876-1KG), 50 mM Tris-HCl at pH 8.0, and 50 mM $CaCl_2$ (Sigma, C3306-500G) and pelleted by centrifugation. After pooling into a single tube, the protoplasts were resuspended in STC buffer and their concentration was estimated using a hemocytometer. The protoplast stock was diluted to a final concentration of 1.25 x 10^8 protoplasts per ml of STC buffer. Aliquots of protoplasts (100 µl) were either used immediately or preserved in 8% PEG solution (80 µl of protoplasts added to 20 µl of 40% w/v PEG 4000 (Sigma, 81240) in STC buffer) in 1.7 ml micro-centrifuge tubes. Protoplasts were slowly cooled to -80 °C at 1 °C/min using a Mr. Frosty freezing container (Nalgene, C1562-1).

1.1.4 Fungal protocols – transformation of *P. paxilli*

P. paxilli protoplasts were transformed following a method modified from Vollmer and Yanofsky and Oliver et al.[2],[3] Transformations were conducted in 1.7 ml micro-centrifuge tubes using 100 µl (1.25×10^7) of *P. paxilli* protoplasts. In a separate 1.7 ml micro-centrifuge tube, 2 µl of spermidine (50 mM in H_2O) (Sigma, S2626) and 5 µl heparin (Sigma, H3393-100KU) (5 mg/mL in STC buffer) were combined with 5-15 µg of plasmid, for random integration, or 100-500 ng of plasmid for CRISPR-Cas9 transformations, with the amount of plasmid adjusted according to plasmid size. The contents of the 1.7 ml micro-centrifuge tube were then combined with the 100 µl of protoplasts and incubated on ice for 30 min. Subsequently, 900 µl of 40% PEG solution (40% w/v PEG 4000 in STC buffer) was added, and protoplasts were further incubated for 20 min. Protoplasts were then transferred to 20 ml of 0.8% RGA medium (prewarmed to 50 °C) in sterile 50 ml tubes, mixed by inversion, and 3.5 ml aliquots were dispensed onto 5 x 1.5% RGA plates. Following overnight incubation at 28 °C, 5 ml of 0.8% RGA supplemented with either nourseothricin, G418, or hygromycin was overlaid onto each plate, with selection agent concentrations adjusted to account for the agar volume of the entire plate. Plates were further incubated for 4 days at 28 °C, and spores were picked from individual colonies and streaked onto CDYE agar plates supplemented with the appropriate selection. Streaked plates were incubated at 28 °C for an additional 4 days. Spores from individual colonies were suspended in 50 µl of 0.01% v/v triton X-100 (Sigma, T8787-100ML) and spread onto 35 mm cell culture dishes containing 3 ml of RGA (containing appropriate selection). Spore plates were then incubated at 28 °C for 3-4 days, and spore stocks were prepared as follows: the top layer, containing the mycelia and spores, was removed from the 35 mm dishes and added to 3 ml of 0.01% v/v triton X-

100 in glass vials. Spores were separated from the mycelia by shaking, and 800 µl of the spore suspension was mixed with 200 µl of 50% w/v glycerol in a 1.7 ml micro-centrifuge tube. Spore concentration was estimated by measuring the optical density at 600 nm of a 1/10 dilution, and spore stocks were adjusted to a concentration of 1 x 10^8 spores/ml. Spore stocks were either used immediately for growth in liquid culture or stored at -80 °C

1.1.5 Fungal protocols – Growth of *P. paxilli* in liquid culture (small scale)

Fungal liquid cultures were grown in 125 ml Erlenmeyer flasks sealed with cotton wool, each filled with 25 ml of CDYE medium. The flasks were inoculated with 25 µl (2.5x10^6 spores) of spore stock and incubated at 28°C with shaking at 200 rpm for 7 days.

1.1.6 Fungal protocols – *Epichloë festucae*

Epichloë festucae strains were grown at 22 °C on 2.4% (w/v) PDA. *E. festucae* protoplasts were prepared and transformed as previously described.[4-5] *Lolium perenne* seeds were surface sterilised and artificially inoculated using a method adapted from Latch and Christensen.[6] Seeds were soaked in 50% (v/v) H_2SO_4 for 30 min, rinsed in sterile H_2O and soaked in 50% (v/v) commercial bleach (NaOCl 21.5 g/l), rinsed thoroughly with sterile H_2O and air-dried in a laminar flow cabinet. Inoculation was performed by making a shallow 2-3 mm long incision between the mesocotyl and coleoptile regions of *L. perenne* seedlings (7 d old germinated on 1.5% PDA) and a small amount of *E. festucae* mycelia were placed into this cut. Inoculated seedlings were maintained on PDA for 7 days in darkness followed by 7 days with a 16 h photoperiod, then were transferred to root trainers containing fungicide-free organic seed mix (Daltons®) and maintained in a Conviron GEN1000 plant growth chamber with a photoperiod of 16 h of light (25% intensity) and 75% humidity.

1.1.7 Media and reagents used for fungal protocols

CDYE (Czapex-Dox/Yeast extract) + trace elements medium:

Made with deionised water and contained 3.34% (w/v) Czapex-Dox (Duchefa Biochemie, C1714), 0.5% (w/v) yeast extract (Oxoid, LP0021), and 0.5% (v/v) trace element solution. For agar plates, select agar (SigmaAldrich, A5054-1KG) was added to 1.5% (w/v). Trace element solution was made in deionised water and contained 0.004% (w/v) cobalt(II) chloride hexahydrate (ThermoFisher, 1697492), 0.005% (w/v) copper(II) sulfate pentahydrate (ThermoFisher, BSPCL942.500), 0.05% (w/v) iron(II) sulfate heptahydrate (Sigma, F7002), 0.014% (w/v) manganese(II) sulfate tetrahydrate (ThermoFisher, 1683385) and 0.05% (w/v) zinc sulfate heptahydrate (Acros Organics, 205980010). The solution was preserved with 1 drop of 12 M hydrochloric acid.

Regeneration (RG) medium:

Made with deionised water and contained 2% (w/v) malt extract (ThermoFisher, LP0039B), 2% (w/v) D(+)-glucose anhydrous (Scharlau, SCARGL01251000), 1% (w/v) mycological peptone (ThermoFisher, LP0040B), and 27.6% sucrose (BayStyle Crown Brands, BSWS3). Depending on whether the media was to be used for plates (1.5% RGA) or overlays (0.8% RGA), Select agar (SigmaAldrich, A5054-1KG) was added to 1.5% or 0.8% (w/v), respectively.

Potato dextrose medium:

Potato dextrose broth was prepared by adding 24 g of potato dextrose broth powder (Bio-strategy, SCAR02-483-500) to 1 l of deionised water. Potato dextrose agar (PDA) was prepared by dissolving 39 g of potato dextrose agar mix (Oxoid, CM0139B) in 1 l of deionised water.

Agar plate Selection:

Agar plates were supplemented with the following selection agents: Geneticin (G418) (PureScience, G-418-10G) at a working concentration of 150 µg/ml, nourseothricin (Pure Science, N-500-1) at a working concentration of 100 µg/ml and hygromycin (Invitrogen, 10687010) at a working concentration of 150 µg/ml. For negative selection, 5-Fluoro-2'-deoxyuridine (fdu) (AK Scientific, J50306) was used at a working concentration of 50 µM

1.1.8 Chemical extraction from small scale *P. paxilli* cultures

Mycelia were isolated from fermentation broths by filtration through a blue cloth and washed with sterile water. Indole diterpenes (IDTs) were extracted from 850 mg of wet weight mycelia or 50 mg of freeze-dried mycelia via homogenisation with 500 µl EtOAc (ThermoFisher, E-0900-17) in a bead beating apparatus (MPBio FastPrep24 5G bead beater grinder and lysis system, 40 s, 8 m/s). The extract was recovered by centrifuging the homogenised sample at 13,000 rpm for 10 minutes and solvent removed in vacuo using a speedvac (Labconco Centrivap DNA concentrator). Extracts were resuspended in MeCN (150 µl) (ThermoFisher, M/4000/PC17), filtered using 0.2 µm syringe filters prior to LC-MS analysis.

1.1.9 Chemical extraction from *E. festucae* cultures

Freeze-dried plant pseudostem from 13-week Δ*ltmS* or wildtype samples (50-200 mg dry weight) were ground into fine powder using MPBio FastPrep24 5G bead beater grinder and lysis system (40 s, 6m/s) and resuspended in 1 ml 1% HCl. Indole diterpenes were extracted with 3 ml (for 25 ml mycelial cultures) or 0.5 ml (for plant pseudostem samples) dichloromethane (DCM) and resuspended in acetonitrile (MeCN).

1.1.10 Liquid Chromatography - Mass Spectrometry (LC-MS):

LC-MS was performed on an Agilent 1260 Infinity II LC-MS system with DAD and electrospray ionisation. Either a Phenomenex C18 Kinetex column (2.6 µ, 100 Å, 50 × 2.1 mm) equipped with a Phenomenex C18 guard cartridge or a Phenomenex C18 Kinetex column (2.6 µ, 100 Å, 3.0 x 100 mm) with a Phenomenex C18 guard cartridge was used. The columns were maintained at 40 °C and eluted with a mobile phase of A: H_2O and B: MeCN, both containing 0.1% formic acid (ThermoFisher, A117-50). For the 50 × 2.1 mm column an injection volume of 10 µl and flow rate of 0.4 ml/min were used. The gradient was as follows: 0–1 min 40% B, 1–5 min 40–60% B, 5–24 min 60–90% B, 24–25 min 90–100% B, 25–27 min 100% B. For the 3.0 x 100 mm column an injection volume of 10 µl and flow rate of 0.6 ml/min were used. The gradient was as follows: 1–5 min 40–60% B, 5–24 min 60–90% B, 24–24.10 min 90–100% B, 24.10-27 min 100% B.

1.1.11 MIDAS protocols

MIDAS cloning was performed as described in van Dolleweerd et al.[7] with the following modifications:

- **Protocols for MIDAS Level-1 module cloning** – Esp3I (NEB, R0734L) was used as alternative to BsmBI in some reactions. All reactions were incubated overnight at 37 °C.
- **Protocols for MIDAS Level-2 TU assembly** – 80 ng of pML2 was combined with level 1 vectors at 1:2 molar ratio. All reactions were incubated overnight at 37 °C.
- **Protocols for MIDAS Level-3 multigene assembly** – Esp3I and PaqCI (NEB, R0745L) were used as alternatives to BsmBI and AarI in some reactions. When PaqCI was used 0.5 µl of PaqCI activator was added to the reaction. 80 ng of the pML3 destination vector was combined with level 2 plasmids at a 1:2 molar ratio. Reactions were incubated overnight at 37 °C. In certain cases, an alternative level 3 destination vector with ampicillin resistance was utilised instead of the standard level 3 destination vector.

1.1.12 General Molecular biology – *P. paxilli*

MIDAS cloning reagents including restriction endonucleases and were purchased from New England Biolabs (NEB), except AarI, which was purchased from Thermo Fisher Scientific. T4 DNA Ligase, 10× T4 DNA Ligase buffer, and 10 mM ATP were from NEB. Primers were synthesized by Macrogen and synthetic MIDAS level 1 constructs ordered from Twist Bioscience. Monarch® Kits (NEB) were used for plasmid purification and PCR products were purified using the NucleoSpin Gel and PCR Clean-up Mini kit for gel extraction and

PCR clean up (Machery-Nagel). Genomic DNA from *P. paxilli* was isolated using the ZR Fungal/Bacterial DNA MicroPrep Kit (Zymo Research). All PCRs for the construction of the MIDAS source, shuttle, and destination vectors and for amplification of MIDAS modules were performed using Phusion® High-Fidelity PCR Master Mix with HF Buffer (NEB). PCRs for fungal strain screening were performed with OneTaq® DNA Polymerase (NEB), LongAmp® Taq DNA Polymerase or Phusion® High-Fidelity PCR Master Mix with HF Buffer. PCR products were sequenced using the linear/Amplicon sequencing service from Plasmidsaurus. CRISPR-Cas9 ribonucleoproteins (RNPs) were generated using the reaction mixture described in Table S1. A separate reaction was performed for each sgRNA. Reactions were incubated for 15 min at room temperature prior to transformation into fungal protoplasts.

Table S1: RNP reaction mixture

Reagent	Volume
NEBuffer™ r3.1	1 µl
sgRNA (5 µM)	2 µl
EnGen® Spy Cas9 NLS (NEB, M0386M)	0.5 µl
Milli-Q H_20	6.5 µl
Total	10 µl

1.1.13 General molecular biology – *E. festucae*

Endophyte infection of plants was determined by PCR. Briefly, plant tissues were lysed with the MPBio FastPrep24 5G bead beater in lysis buffer (400 mM Tris-HCl pH 8, 60 mM EDTA, 150 mM NaCl) (Liu et al., 2000) and SDS was added to a concentration of 1% and mixed by inversion, followed by addition of precipitation solution (3 M potassium acetate, 10% glacial acetic acid, pH 4.8) and centrifugation at 17,000 x g for 5 min. An equal volume of isopropanol was added to the supernatant and DNA was precipitated by centrifugation, washed with 75% (v/v) ethanol and resuspended in 100 µl Tris-EDTA buffer. 1 µl of DNA was used as template for multiplex PCR reactions using primers YL705/YL706 (*E. festucae* ribosomal protein S22; 671 bp product size) and YL501F/YL501R (*L. perenne* cinnamoyl-CoA reductase 1; 112 bp product size) using the OneTaq® Quick-Load® 2X Master Mix with Standard Buffer (NEB).

1.2 Generation and analysis of *ΔpaxA* and reconstruction strains

1.2.1 Generation of *ΔpaxA* strains using CRISPR-Cas9:

ΔpaxA strains were engineered using a plasmid (pRC337) designed to facilitate homologous recombination leading to the replacement of the *paxA* coding sequence (CDS) with a nourseothricin selection cassette. RC337 was created using a MIDAS level 2 reaction by combining level 1 plasmids containing homology arms (HAs) that spanned 542 bp upstream of the *paxA* start codon (HA1), 752 downstream of the *paxA* stop codon (HA2) and a nourseothricin selection cassette (Figure S1) To increase the efficiency of homologous recombination at the *paxA* locus Cas9 was added along with two sgRNAs (RCC7 and RCC8), to generate cuts at both ends of the *paxA* locus. RCC7 and RCC8 RNPs were transformed into wildtype (PN2013) *P. paxilli* protoplasts with 100 ng of RC337 and Cas9 protein as described in 1.1.12. Ten transformants were selected and grown up following standard methods as described in (1.1.4 and 1.1.5).

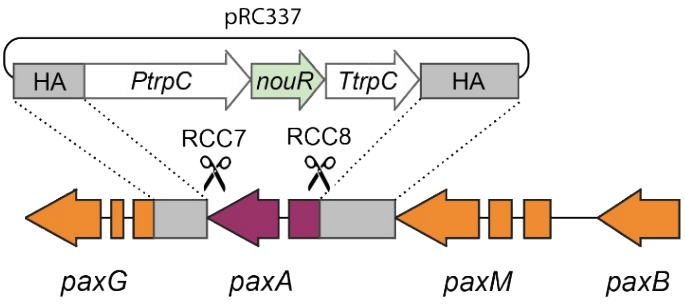


Figure S1: Schematic of *ΔpaxA* strain design.

1.2.2 Confirmation of *ΔpaxA* by PCR and sequencing

Ten nourseothricin resistant transformants were screened for the presence of the *paxA* CDS using PCR. The complete *paxA* CDS was amplified with paxA_F and paxA_R primers. The *paxQ* CDS was also amplified with paxQ_frag1_F and paxQ_frag4_R primers as control. A *paxA* amplicon was only observed in the wildtype sample with no amplification of *paxA* in any of the ten transformants (Figure S2). The expected *paxQ* control band was present in 9/10 transformants.

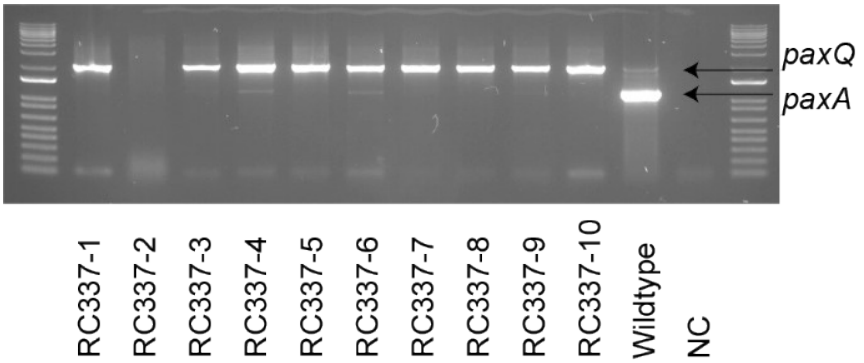


Figure S2: PCR of *paxA* and *paxQ* from Δ*paxA* transformants.
A 1.5% agarose gel showing PCR amplicons for *paxA* (1131 bp) and *paxQ* (2087 bp) from DNA of ten Δ*paxA* transformants as well as a wildtype control. A no DNA control (NC) was also included.

Further PCR screening was performed to confirm RC337 had integrated as expected within the *paxA* locus. A 6952 bp fragment that included 173 bp upstream of the left homology arm and 2876 bp downstream of the right homology arm was amplified with the paxG_frag1_R and paxB_F primers. 10/10 transformants had a band of the expected size (Figure S3) Amplicons were sequenced from RC337-6, 8 and 10 confirming integration of RC337 into the *paxA* locus as expected. The sequence for RC337-6 is provided in section 1.9.3. A band of the expected size (5488 bp) was present in the wildtype sample.

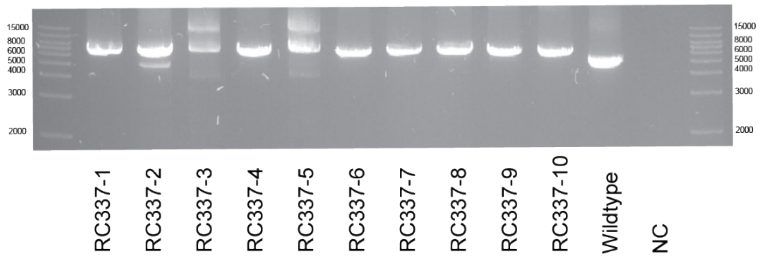


Figure S3: PCR from Δ*paxA* transformants.
1.5% agarose gel showing PCR amplicons that span the integration site in Δ*paxA* strains and wildtype. A no DNA control (NC) was also included.

1.2.3 LC-MS analysis of Δ*paxA* transformants

Extracts were obtained from ten Δ*paxA* (RC337) strains and analysed by LC-MS (as described in 1.1.8 and 1.1.10). Extracted ion chromatograms (EICs) are shown for *M*422.3 (3′4′-epoxyemindole SB and paspaline) (Figure S4) and *M*436.3 (paxilline) (Figure S5). Dilution and injection volumes are listed for each sample where they differ from what is described in 1.1.10. All ten RC337 transformants had a *M*422.3 peak at 9.4 min that corresponds to 3′4′-epoxyemindole SB; this peak is absent in wildtype (PN2013). A peak at 12.8 min that corresponds to paspaline is also present in all transformants. A paxilline peak (*M*436.3, 7.2 min) was not detected in 9/10 RC337 transformants and RC337-9 had a greatly reduced paxilline peak compared to wildtype. The accumulation of 3′4′-epoxyemindole SB and reduction of paxilline in *ΔpaxA* strains indicates that PaxA is important in the conversion of 3′4′-epoxyemindole SB to paspaline and that without it paxilline biosynthesis is inefficient.

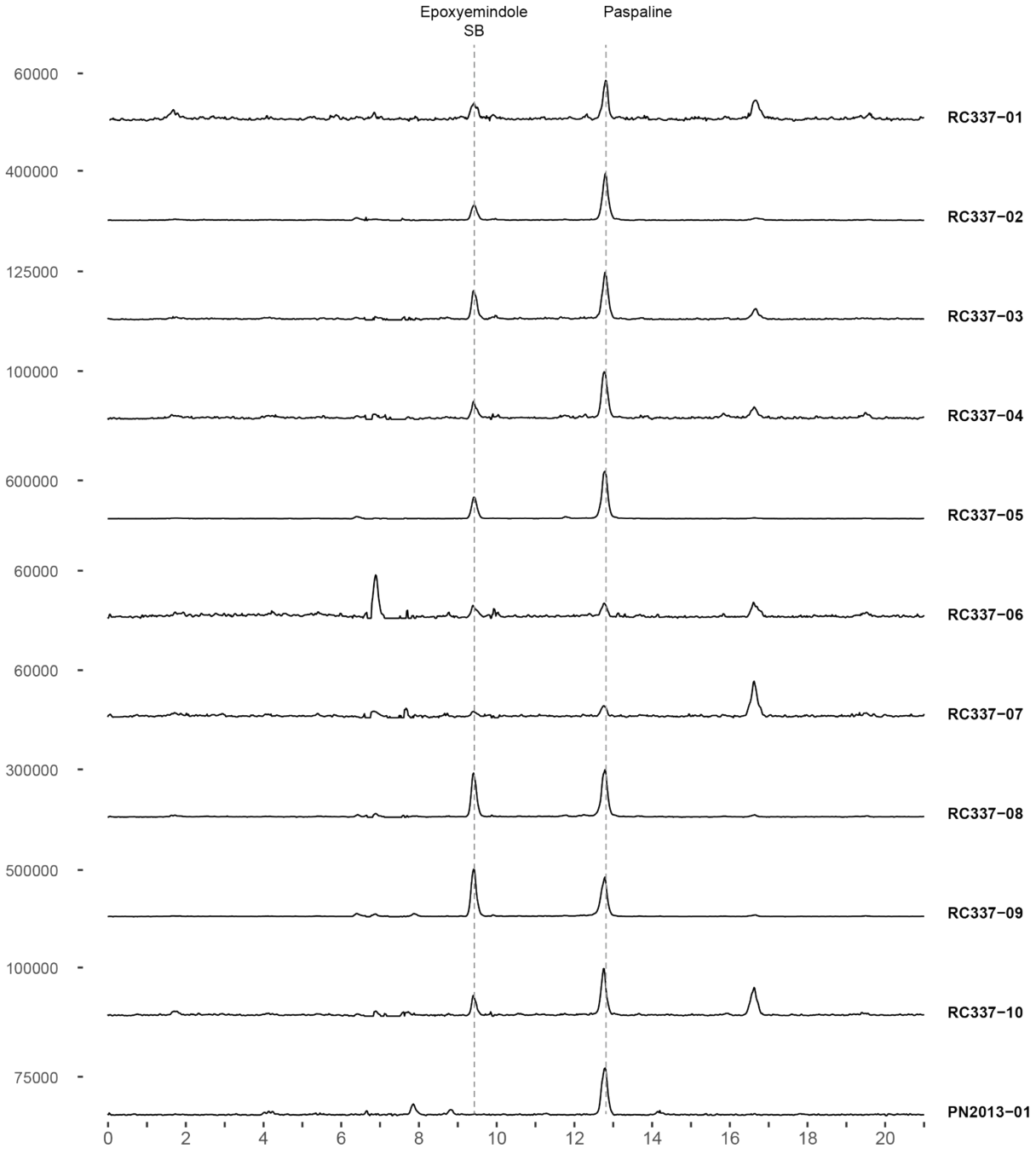


Figure S4: LC-MS analysis of RC337 (*ΔpaxA*) and PN2013 (wildtype), 422

Metabolite: 3′4′-epoxyemindole SB and paspaline

Dilution: RC337: undiluted, PN2013: 1/50

LC-MS trace: EIC (*M*422.3)

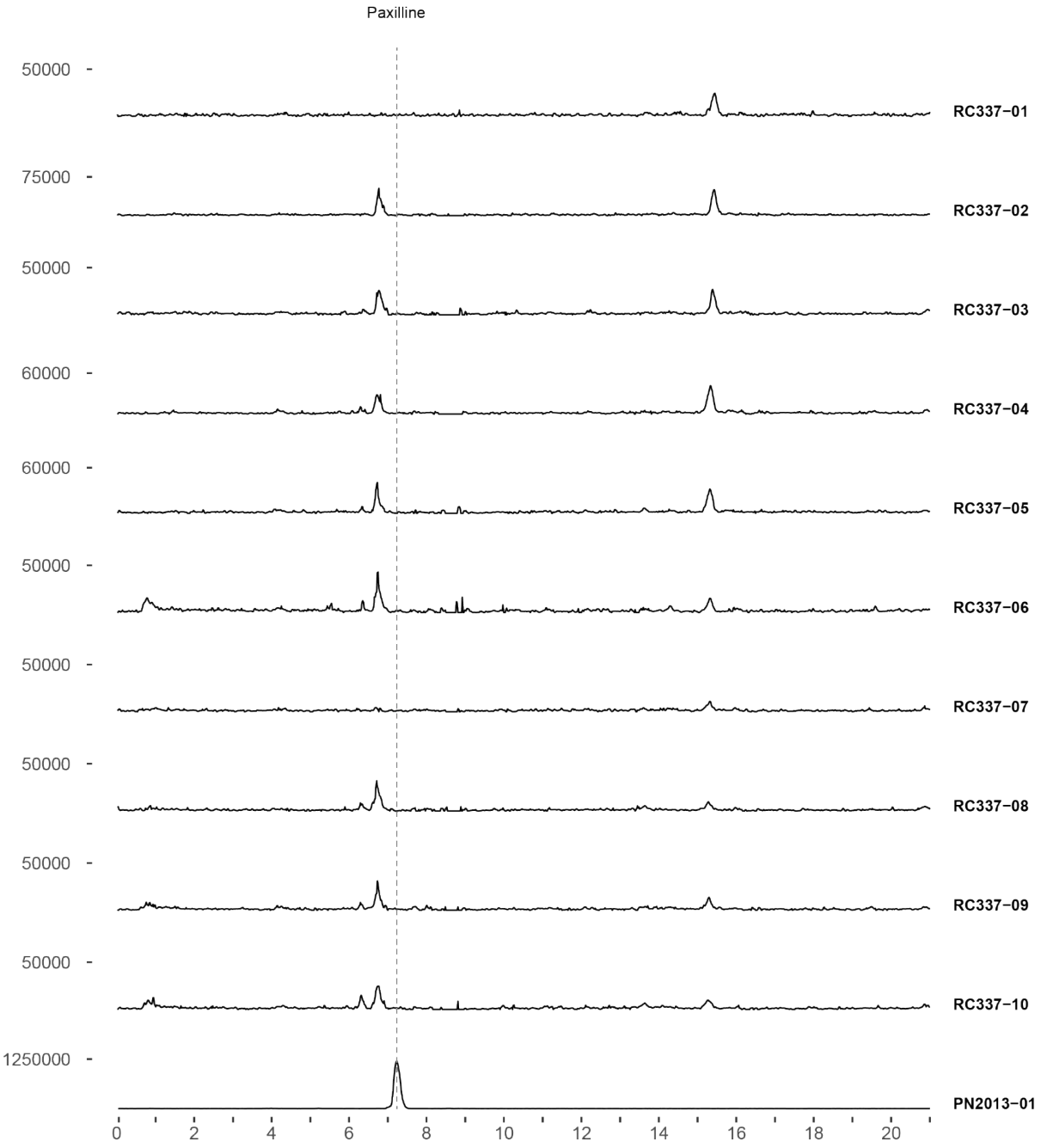


Figure S5: LC-MS analysis of RC337 (*ΔpaxA*) and PN2013 (wildtype), 436

Metabolite: paxilline

Dilution: RC337: undiluted, PN2013: 1/50

LC-MS trace: EIC (*M*436.3)

1.2.4 LC-MS analysis of *ΔpaxA::paxA* transformants

To confirm that the presence of the 3′4′-epoxyemindole SB peak and reduction in paxilline production was due to the loss of PaxA, a *ΔpaxA* strain (RC337-6) was complemented by random integration with a plasmid containing the *paxA* CDS driven by the *paxA* promoter (RC356). Extracts were obtained from ten RC356 strains and analysed by LC-MS (as described in 1.1.8 and 1.1.10). Extracted ion chromatograms (EICs) are shown for *M*422.3 (3′4′-epoxyemindole SB and paspaline) (Figure S6) and *M*436.3 (paxilline) below (Figure S7). Dilutions and injection volumes are listed for each sample where they differ from what is described in 1.1.10. Eight out of ten RC356 transformants had no observable 3′4′-epoxyemindole SB peak. Instead, a paxilline peak was present in these transformants at 7.2 min (*M*436.3), suggesting a restoration of a wildtype-like phenotype.

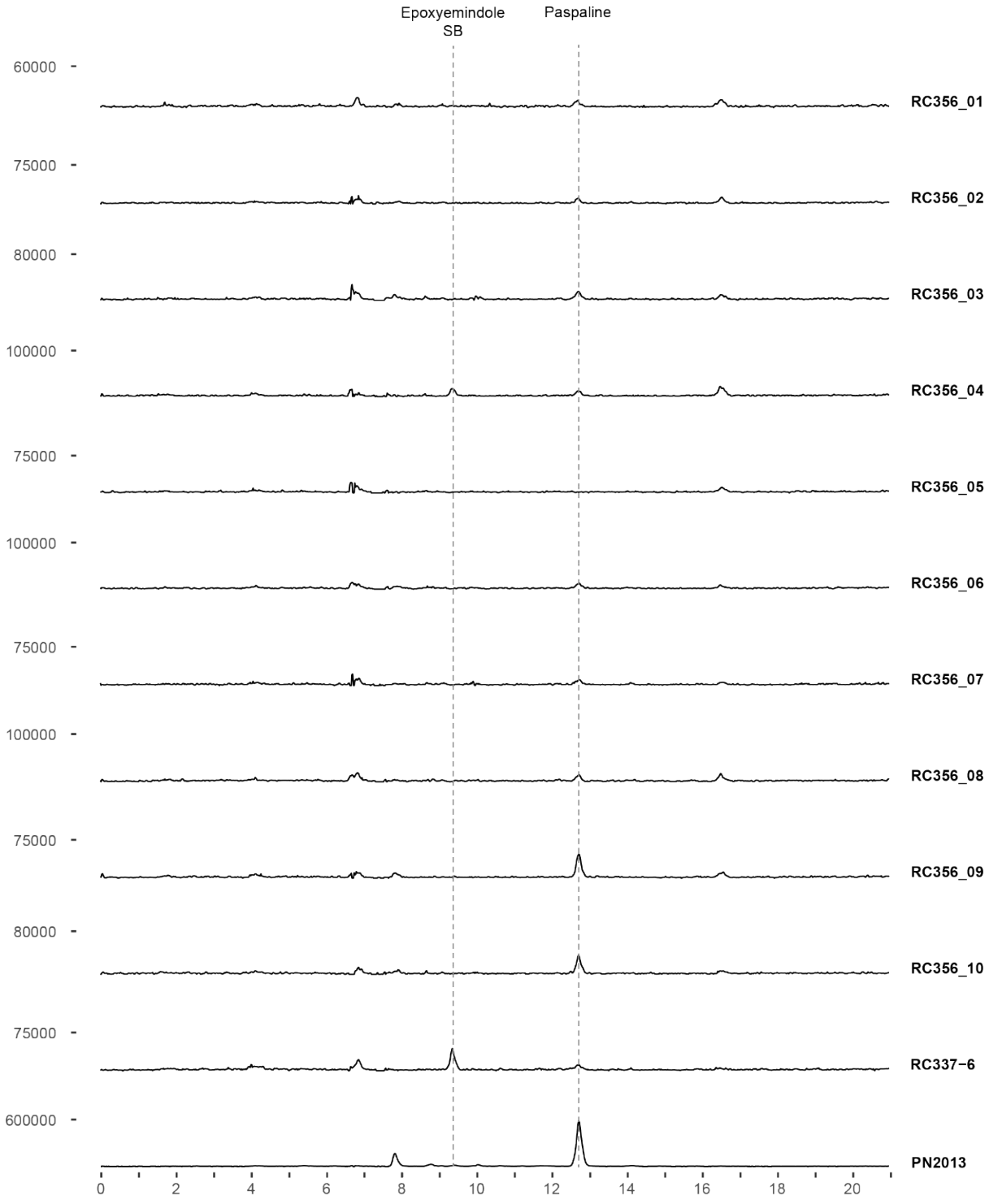


Figure S6: LC-MS analysis of RC356 (*ΔpaxA::paxA*), RC337-6(*ΔpaxA*) and PN2013 (wildtype), 422

Metabolite: 3′,4′-epoxyemindole SB and Paspaline

Dilution: 1/5 (1 µl injection)

LC-MS trace: EIC (*M*422.3)

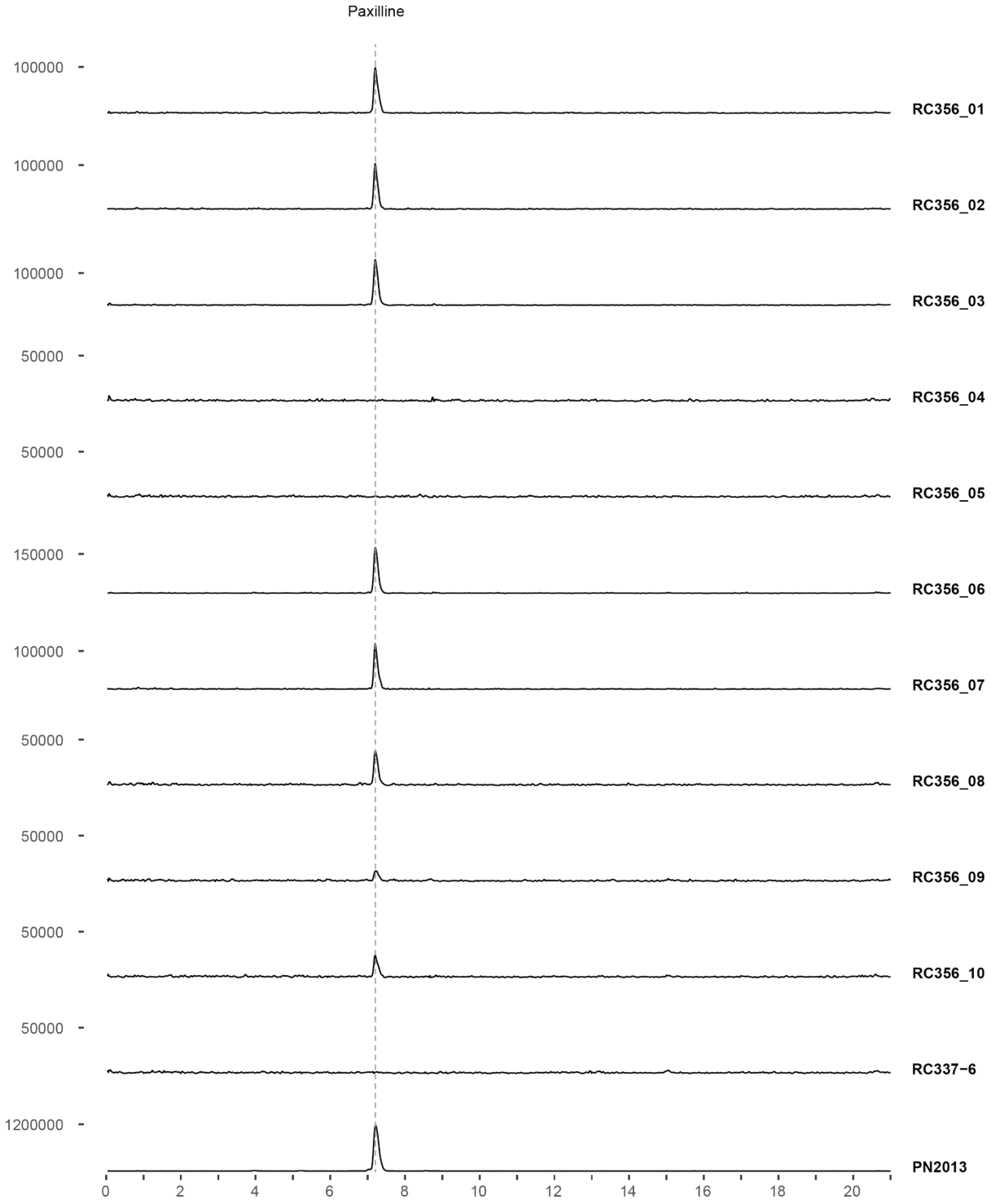


Figure S7: LC-MS analysis of RC356 (*ΔpaxA::paxA*), RC337-6 (*ΔpaxA*) and PN2013 (wildtype), 436

Metabolite: Paxilline

Dilution: 1/5 (1 µl injection)

LC-MS trace: EIC (*M*446.3)

1.2.5 Generation of reconstruction strains

To further investigate the role of PaxA, plasmids were constructed containing either *paxG, paxB, paxC*, and *paxM* (pMH17) or *paxG, paxB, paxC, paxM*, and *paxA* (pMH45). These plasmids were then integrated into a *ΔPAX* strain (LS293), which has the entire paxilline biosynthetic gene cluster replaced with a hygromycin resistance cassette and a thymidine kinase gene from herpes simplex virus. The plasmids were engineered with homology arms (HAs) consisting of the sequences flanking the hygromycin resistance and thymidine kinase gene cassettes in the *ΔPAX* locus of LS293, facilitating their targeted integration into this genomic region normally occupied by the paxilline biosynthetic gene cluster. pMH17 and pMH45 were transformed into LS293 protoplasts along with RNPs generated (as described in 1.1.12) from two sgRNAs (sgRNA_LJS1 and sgRNA_LJS2). Transformants were selected using 5-fluorodeoxyuridine for negative selection against the thymidine kinase gene to enhance integration into the target location[8], and nourseothricin for positive selection.

1.2.6 LC-MS analysis of reconstruction strains

Extracts were obtained from thirteen MH17 and MH45 strains and analysed by LC-MS (as described in 1.1.8 and 1.1.10). Extracted ion chromatograms (EICs) are shown for *M*422.3 (3′4′-epoxyemindole SB and paspaline). Dilutions and injection volumes are listed for each sample where they differ from what is described in 1.1.10. The retention times differ in this experiment from previous experiments due to the use of the 50 × 2.1 mm column rather than the 3.0 x 100 mm column. Twelve of the thirteen MH17 transformants had a *M*422.3 peak at 8.8 min that corresponded to 3′4′-epoxyemindole SB (Figure S8). The 3′4′-epoxyemindole SB peak was not observed in the *ΔpaxP* strain (PN2258), which carries a mutation in the *paxP* gene, resulting in an accumulation of paspaline (*M*422.3, 11.9 min). In contrast, all thirteen MH45 transformants lacked an 3′4′-epoxyemindole SB peak and twelve of the thirteen transformants had a paspaline peak at 11.9 min. This provides further evidence that PaxA has a role in the conversion of 3′4′-epoxyemindole SB to paspaline as without PaxA an accumulation of 3′4′-epoxyemindole SB is consistently observed.

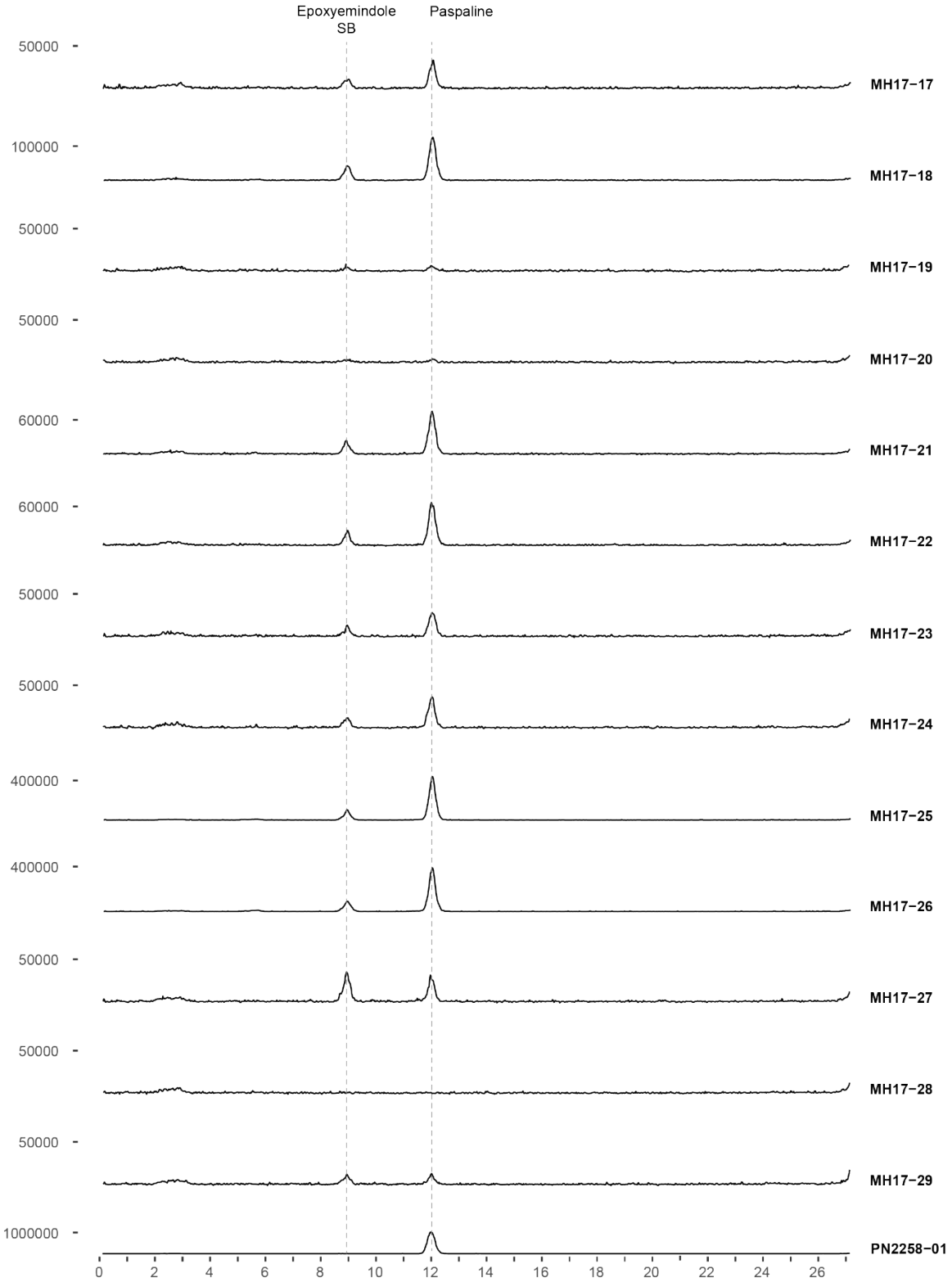


Figure S8: LC-MS analysis of MH17 (*paxG*, *paxB*, *paxC* and *paxM*) and PN2258 (*ΔpaxP*), 422

Metabolite: 3′,4′-epoxyemindole SB and paspaline

Dilution: 1/20

LC-MS trace: EIC (*M*422.3)

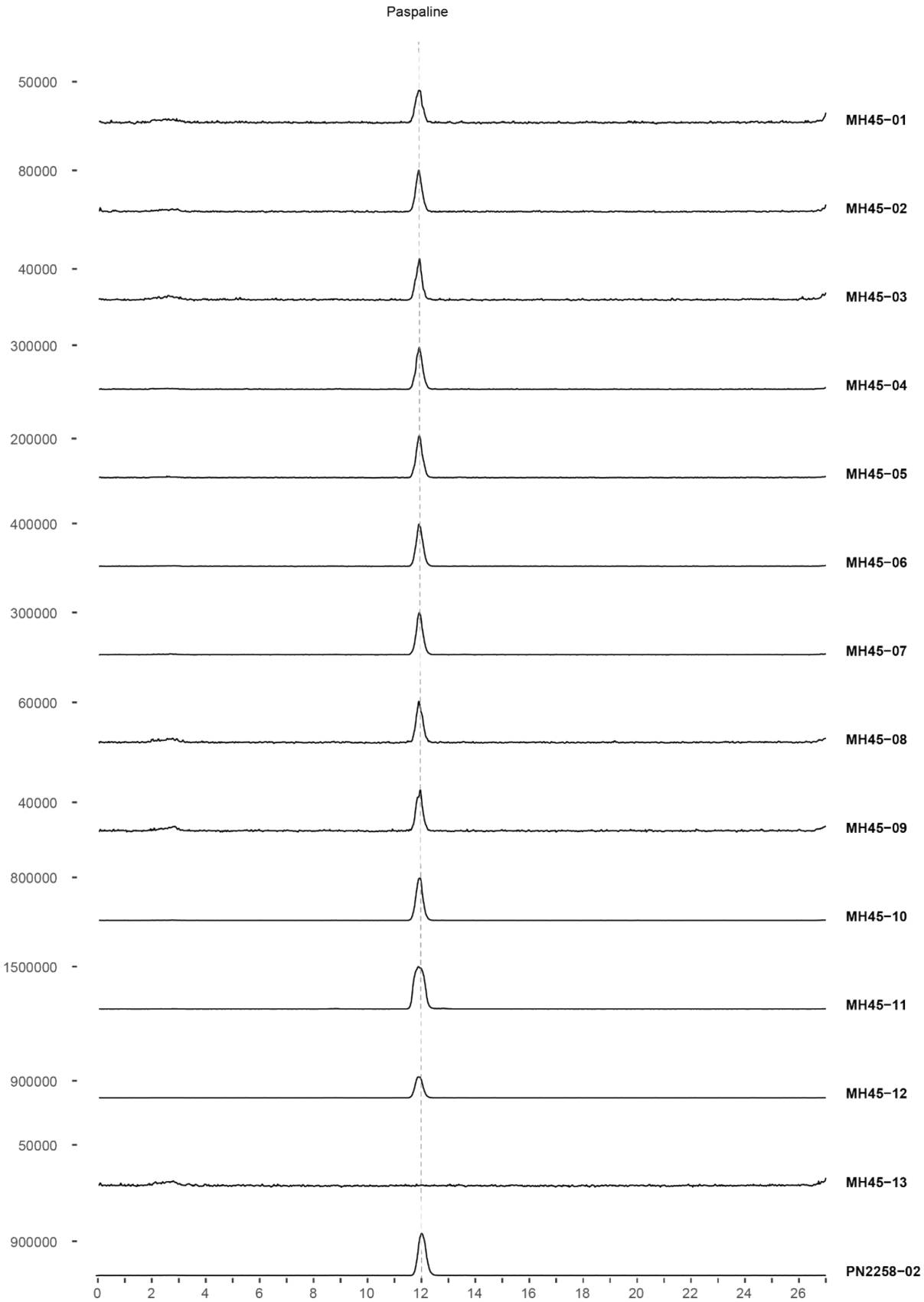


Figure S9: LC-MS analysis of MH45 (*paxG*, *paxB*, *paxC*, *paxM* and *paxA*) and PN2258 (*ΔpaxP*), 422

Metabolite: 3′,4′-epoxyemindole SB and paspaline

Dilution:1/20

LC-MS trace: EIC (*M*422.3)

1.3 Isolation and characterisation of 3′,4′-epoxyemindole SB

1.3.1 Isolation of 3′,4′-epoxyemindole SB

A *ΔpaxA* strain was grown using standard growth conditions for *P. paxilli*. Spores were used to inoculate 2 x 2 L shake flasks each containing 500 mL of CDYE liquid media and incubated at 28 °C and 200 rpm for 8 days. Mycelia (approx. 100 g) was separated from media by vacuum filtration and subsequently extracted overnight with ethyl acetate (approx. 300 mL). The extract was collected by vacuum filtration, aqueous layer removed, and organic layer concentrated in vacuo. The sample was then dissolved in MeCN (4 mL) and purified using an Agilent 1260 Infinity II preparative HPLC system equipped with DAD and a Phenomenex Luna C18 250x15 mm 100 Å 5 µm column. The mobile phase was A: H_2O and B: MeCN and followed a linear gradient from 60-98% B over 30 min at 15 mL min^{-1}. A peak eluting at 16.5 min was collected and concentrated under vacuum to give a white powder (2.1 mg). Analysis by 2D NMR spectroscopy which confirmed the compound was 3′,4′-epoxyemindole SB.

1.3.2 High-resolution mass spectrometric data and MS/MS spectra

High-resolution mass spectrometric data and MS/MS spectra were obtained with an Agilent 6530 Accurate Mass Q-TOF fitted with an electrospray ion source and equipped with an Agilent 1260 Infinity II LC system. Chromatography was carried out using an Agilent Accucore C18 2.0 µm 50 × 2.1 mm column eluted with a mobile phase of A: H_2O and B: MeCN, both containing 0.1% formic acid. The flow rate was 0.3 mL/min and injection volume 5 µL. The gradient used was as follows: 0–1 min 40% B, 1–30 min 40–100% B, 30–35 min 100% B. The mass spec parameters used were: positive ion mode, mass range 100–1000 Da, acquisition rate 2 scans/s, capillary temperature 300 °C, capillary voltage 3500 V, fragmentor voltage 175 V, drying gas flow 8 L/min, sheath gas temp 350 °C, sheath gas flow 11 L/min, and a nebulizer pressure of 35 psi. MS/MS data were acquired for selected masses using CID with an isolation width of M 1.6 and collision energy of 30 eV.

Compound	Chemical Formula	Exact Mass	Calc. for $[M + H]^+$	HRMS Obs. (Δppm)
Epoxy-Emindole SB	$C_{28}H_{39}O_2N_1$	421.2981	422.3054	422.3054 (0.58)

1.3.3 NMR spectroscopy

NMR spectroscopy was conducted on a JEOL JNM-ECZ600R with a nitrogen cooled 5 mm SuperCOOL cryogenic probe (600 MHz for ^1H nuclei and 150 MHz for ^{13}C nuclei). The residual solvent peak was used as an internal reference for 1H [δH 7.26, $CDCl_3$] and ^{13}C [δC 77.16, $CDCl_3$] chemical shifts.

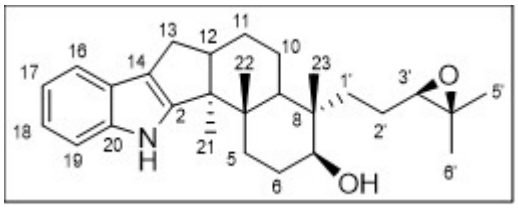


Table S2: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for 3′,4′-epoxyemindole SB in $CDCl_3$

Position	^{13}C	^{1}H	COSY	HMBC
1 (N)	-	7.69 (1H, s)	-	2, 14, 15, 20
2	150.7	-	-	-
3	53.0	-	-	-
4	39.3	-	-	-
5a	33.3	1.57 (1H, m)	6, 5b	-
5b		1.90 (1H, m)	6, 5a	-
6	27.3	1.83 (2H, m)	5a, 5b, 7	-
7	72.9	3.52 (1H, dd, J = 7.9, 7.9 Hz)	6	23
8	41.0	-	-	-
9	40.0	1.66 (1H, m)	10a, 10b	-
10a	22.7	1.45 (1H, m)	9, 10b, 11b	-
10b		1.57 (1H, m)	9, 10a	-
11a	25.0	1.58 (1H, m)	12, 11b	-
11b		1.77 (1H, m)	10a, 11a, 12	-
12	48.6	2.76 (1H, m)	11a, 11b, 13a, 13b	-
13a	27.2	2.32 (1H, dd, J = 13.2, 10.6 Hz)	12, 13b	2, 11, 12, 14
13b		2.67 (1H, dd, J = 13.2, 6.4 Hz)	12, 13a	2, 3, 12, 14
14	118.4	-	-	-
15	125.3	-	-	-
16	118.5	7.42 (1H, m)	17, 18	14, 15, 18, 20
17	119.5	7.07 (1H, m)	16, 18, 19	15, 18, 19
18	120.4	7.07 (1H, m)	16, 17, 19	16, 17, 20
19	111.3	7.29 (1H, m)	17, 18	15, 17
20	140.2	-	-	-
21	14.5	1.00 (3H, s)	-	2, 3, 4, 12
22	19.0	1.11 (3H, s)	-	3, 4, 5, 9
23	16.3	0.87 (3H, s)	-	7, 8, 9, 1'
1'a	33.4	1.36 (1H, m)	1'b	-
1'b		1.73 (1H, m)	1'a, 2'a	-
2'a	22.3	1.35 (1H, m)	1'a, 2'b, 3'	3', 4'
2'b		1.64 (1H, m)	2'a, 3'	-
3'	64.6	2.73 (1H, dd, J = 6.1, 6.1 Hz)	2'a, 2'b	1', 2', 4', 6'
4'	58.6	-	-	-
5'	18.6	1.31 (3H, s)	-	3', 4', 6'
6'	24.8	1.33 (3H, s)	-	3', 4', 5'

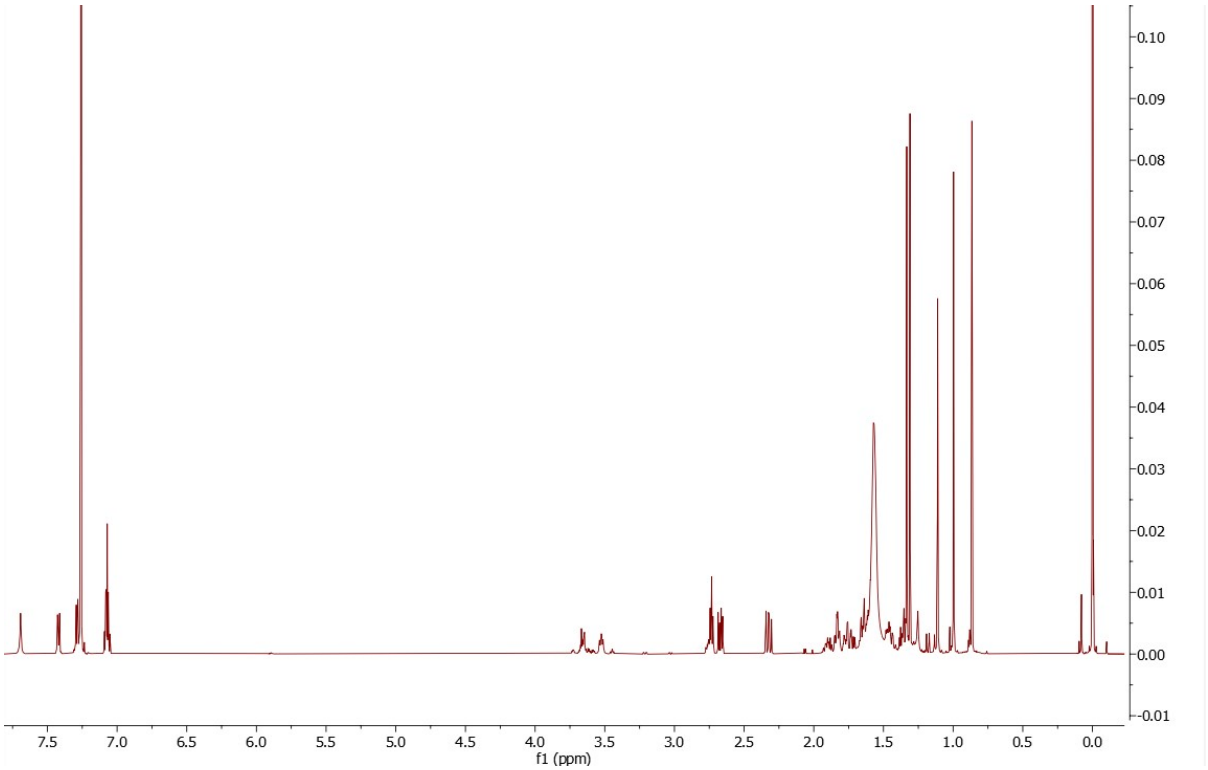


Figure S10: ^{1}H NMR spectrum of 3′,4′-epoxyemindole SB (600 MHz)

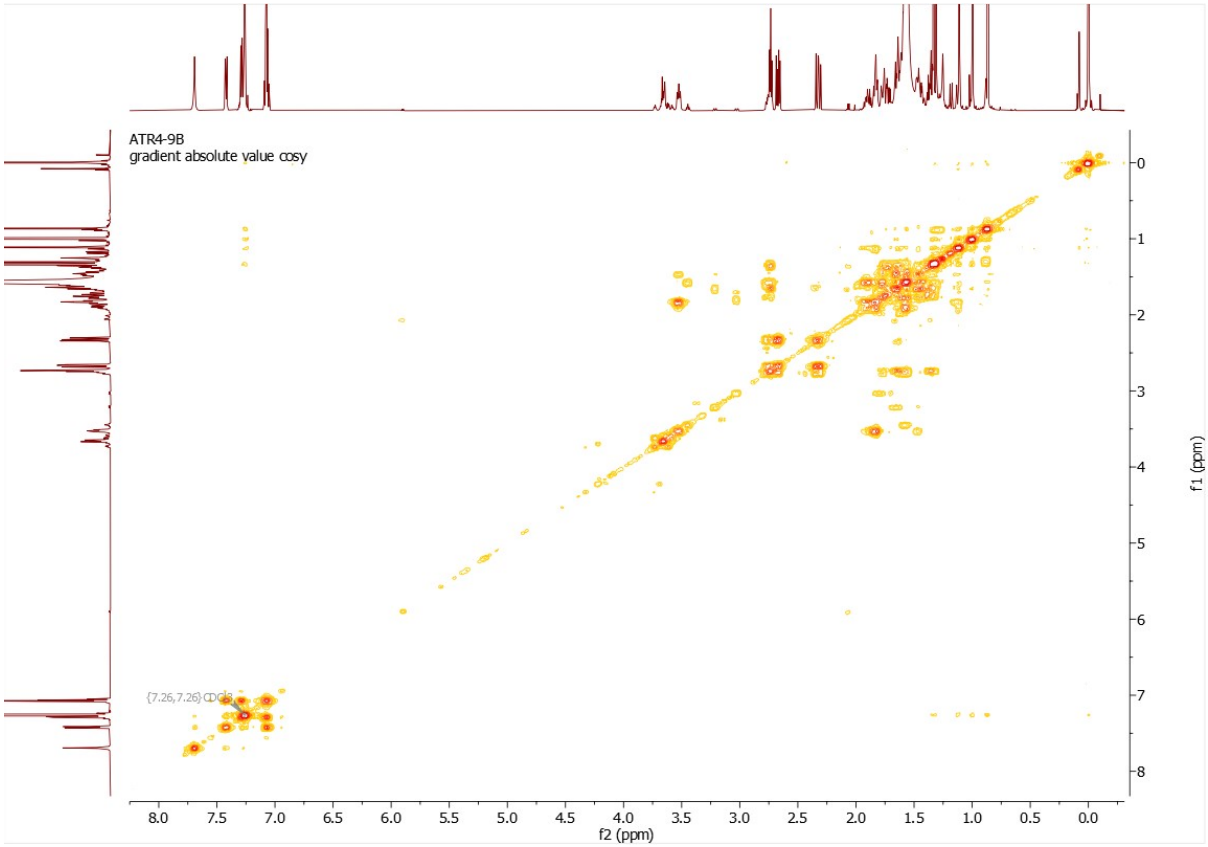


Figure S11: ^{1}H-^{1}H COSY spectrum of 3′,4′-epoxyemindole SB (600 MHz)

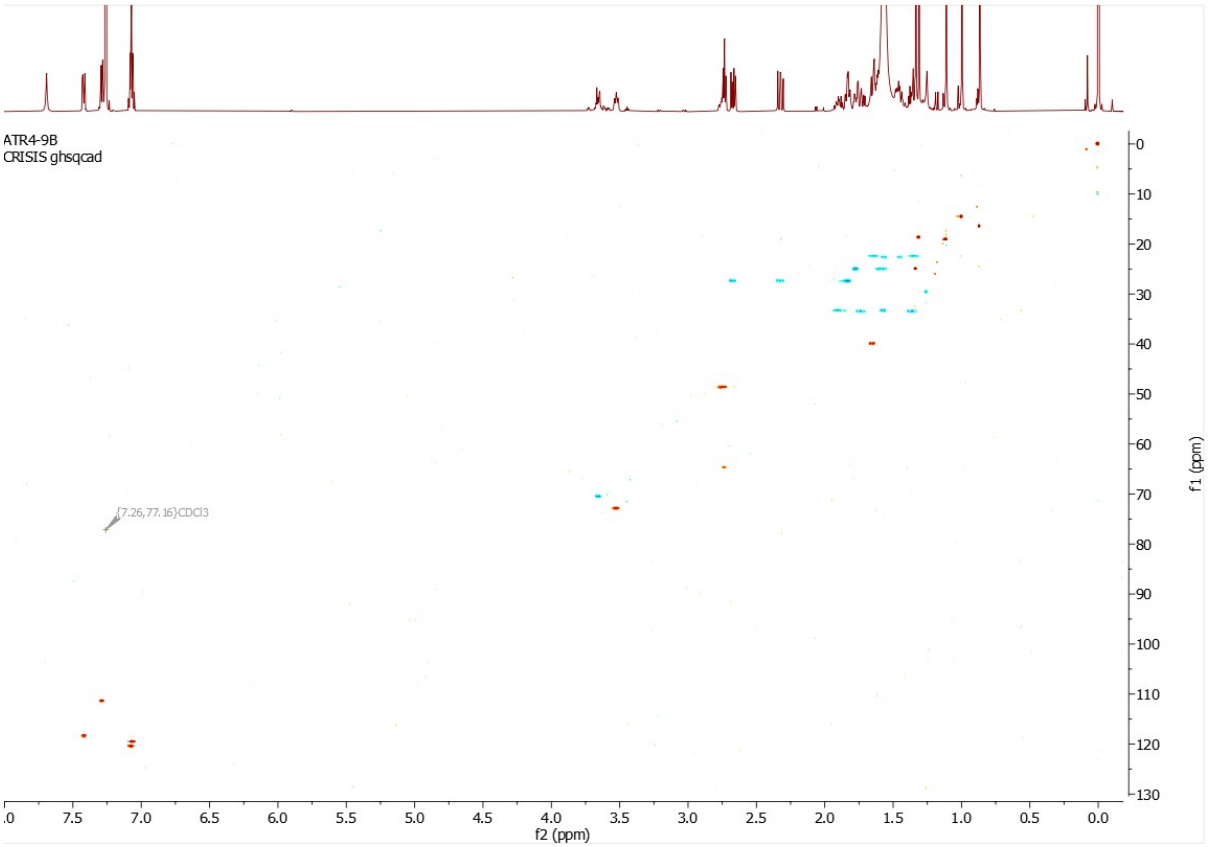


Figure S12: HSQC spectrum of 3′,4′-epoxyemindole SB (600 MHz)

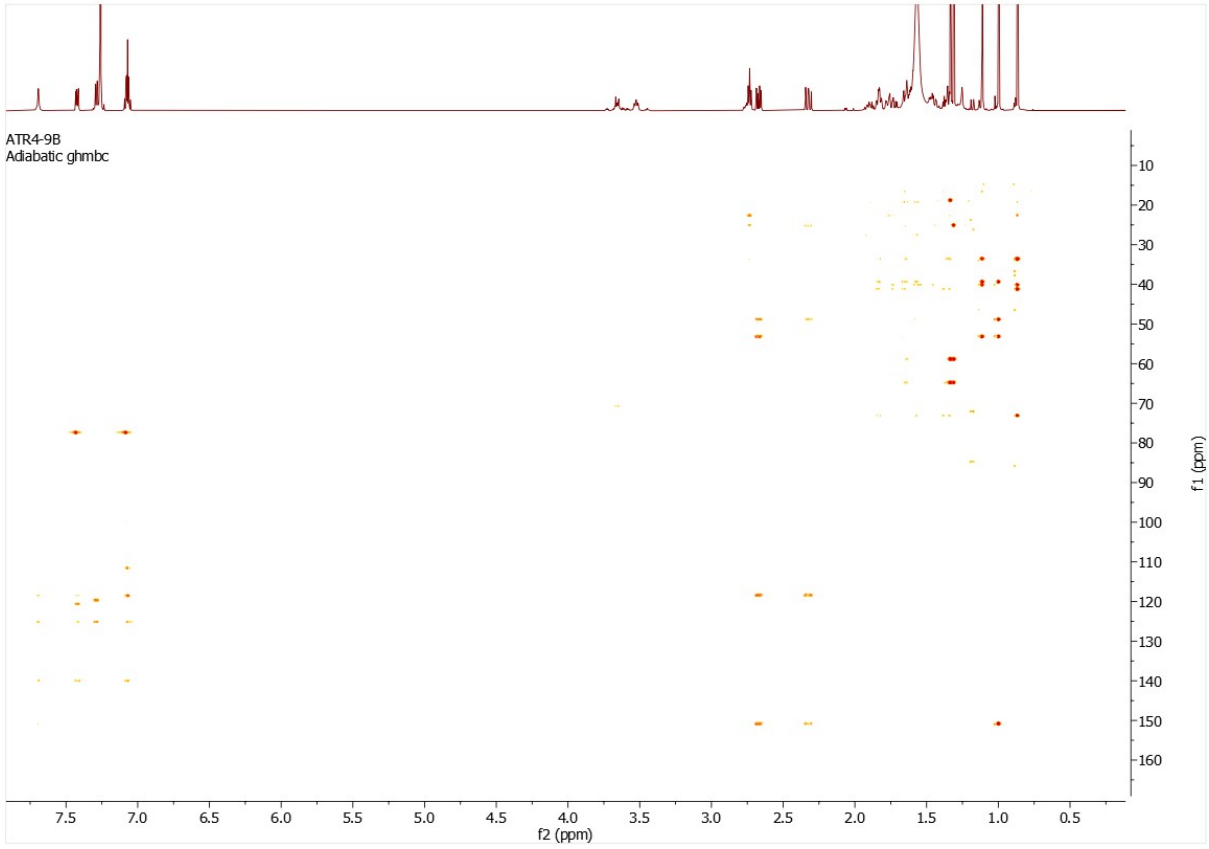


Figure S13: HMBC spectrum of 3′,4′-epoxyemindole SB (600 MHz)

1.4 In Vivo Feeding Studies:

1.4.1 Generation of strains for feeding

To further interrogate the role of PaxA a feeding study was performed. All strains for feeding were built from LS239 (*ΔPAX*). Plasmids were engineered using the same HAs as described in 1.2.5 and contained either *paxM* (pRC370) alone, *paxM* and *paxB* (pRC379) or *paxM* and *paxA* (pRC380). RNPs were generated from sgRNA_LJS1 and sgRNA_LJS2 to facilitate replacement of the hygromycin resistance and thymidine kinase gene cassettes with *PAX* genes. PCR screening was used to ensure strains contained the target genes (Figure S14).

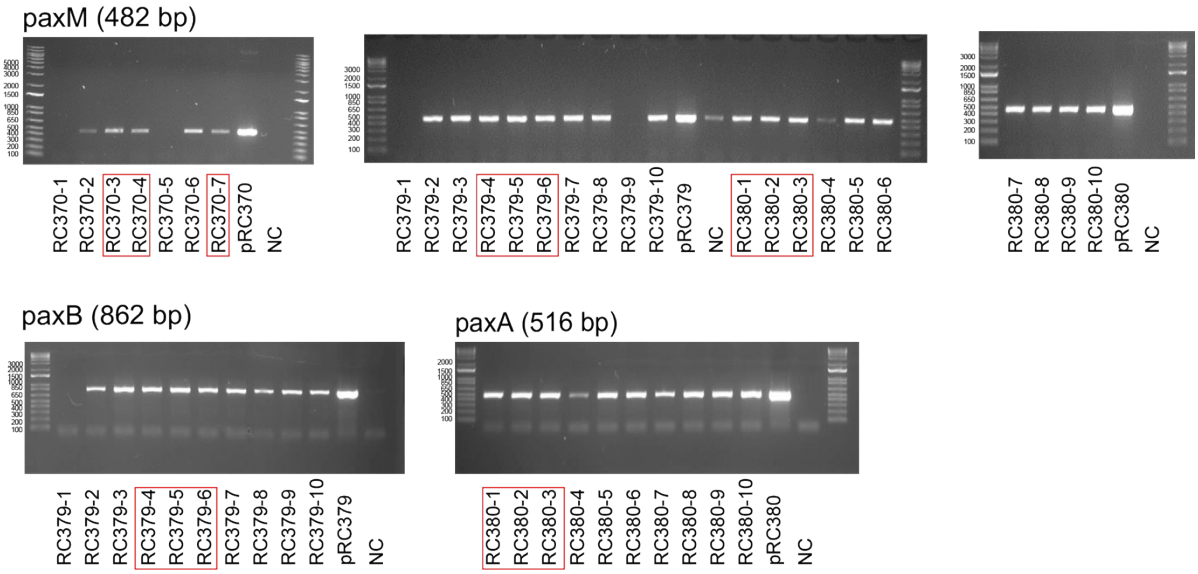


Figure S14: PCR of *paxM*, *paxB* and *paxA* from strains for feeding.1.5% agarose gel to confirm the presence of target genes in strains for feeding with emindole SB. Strains selected for feeding are indicated with red boxes. Expected amplicon sizes are shown in brackets for each gene. A no DNA control (NC) was also included.

1.4.2 In vivo Feeding

Three strains of RC370 (3,4 and 7), RC379 (4,5 and 6), (1,2 and 3) and LS293 were grown under standard conditions (1.1.5). Cultures were fed with 100 µl of 1.4 µg/ml emindole SB in methanol on days zero and four. Emindole SB was selected for feeding instead of 3′,4′-epoxyemindole SB to prevent issues with compound stability.

1.4.3 LC-MS analysis of feeding strains

Extracts were obtained from three RC370, RC379, RC380 and LS293 strains and analysed by LC-MS (as described in 1.1.8 and 1.1.10). EICs for *M*406.3 (emindole SB) and *M*422.3 (3′,4′-epoxyemindole SB and paspaline) were combined and shown below (Figure S15).

Residual emindole SB was seen in all strains with LS293 strains having the largest peaks, as expected due to a lack of enzymes to create any downstream products. RC380-2 also contained a large 3′,4′-epoxyemindole SB peak, but this is likely due to a problem with gene expression in that strain as DNA from the target genes was shown to present (Figure S14). The 3′,4′-epoxyemindole SB peak was only present in RC370 and RC379 with RC380 having no evidence of 3′,4′-epoxyemindole SB. This indicates efficient conversion of 3′,4′-epoxyemindole SB to paspaline only occurs when *paxA* is present. A *M*422.3 paspaline peak was present in RC370 and RC379 indicating spontaneous conversion of 3′4′-epoxyemindole SB to paspaline.

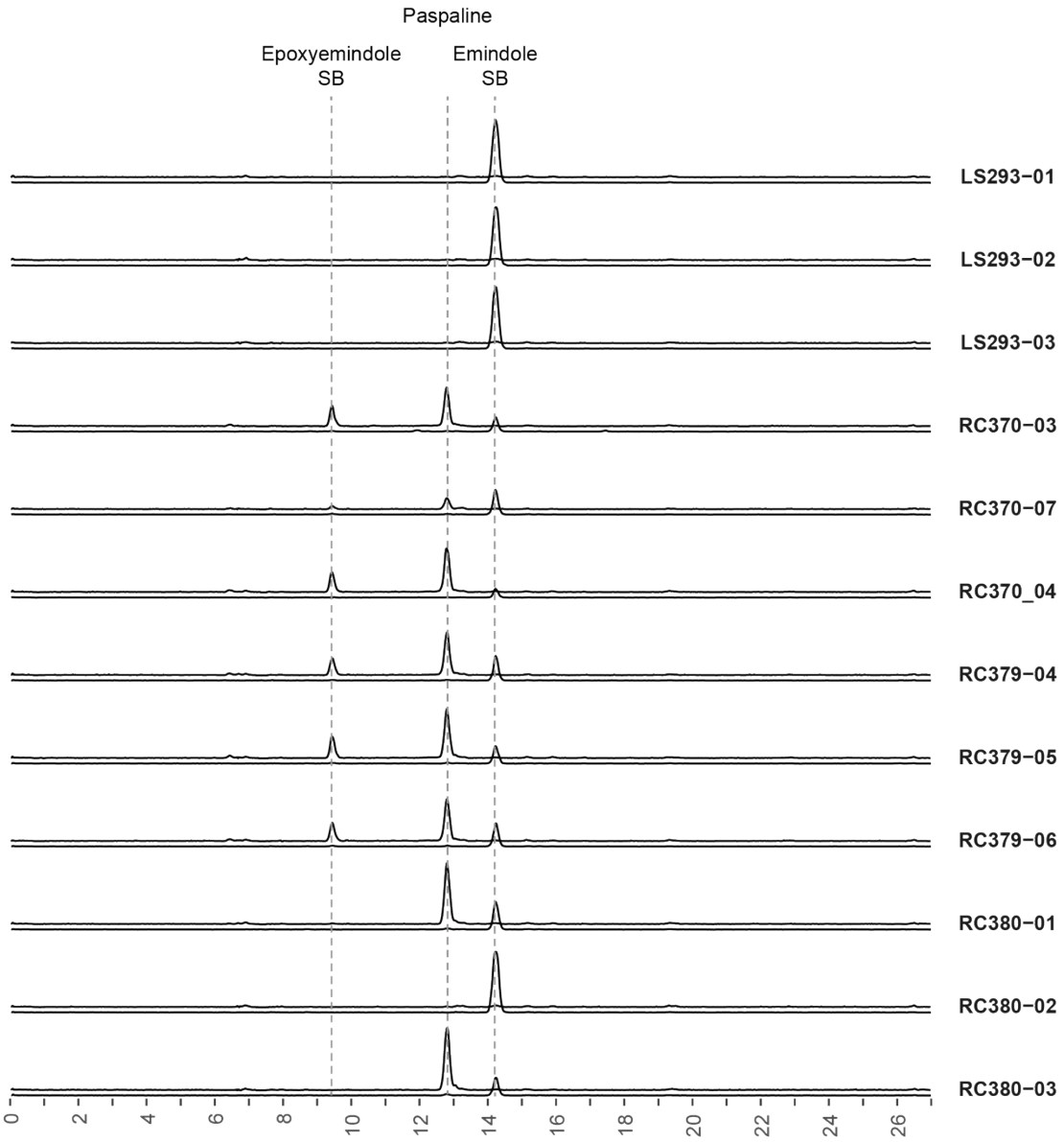


Figure S15: LC-MS analysis of Emindole SB FeedingLS239 (Δ*PAX*), RC370 (*paxM*), RC79 (*paxM*, *paxB*), and RC380 (*paxM*, *paxA*)Metabolites: emindole SB, 3′,4′-epoxyemindole SB and paspaline. LC-MS trace: EIC (*M*406.3 and *M*422.3)

Y-axis: 0 – 1×10^6

1.5 Catalytic role of conserved aspartic acid in IdtAs

To understand the role of PaxA (AF-E3UBL5-F1-v4) structural models were obtained from the AlphaFold Protein Structure Database [9-10]. Protein sequences were prepared in Maestro 13.6.122 using the Protein Preparation Workflow (default parameters), with the pKa values of ionisable groups predicted using PROPKA.[11] Substrate docking simulations were performed by preparing ligand structures (3′,4′-epoxyemindole SB and paspaline) using Maestro LigPrep (OPLS4 force field; generating possible states at pH 7.0 ± 2.0 using Epik; chirality defined by the input structure) then docking these to the prepared protein structure using Maestro Induced Fit Docking (Ligand settings: sampling ring conformations within a 2.5 kcal/mol energy window, penalising non-planar amide bond conformations; Glide settings: receptor van der Waals scaling = 0.50, ligand van der Waals scaling = 0.50, maximum 20 poses; Prime settings: residues refined within 5.0 Å of ligand poses, side chains optimised, using implicit membrane of thickness 44.5 with atom specification language set to helices; Glide redocking settings: redock structures within 30.0 kcal/mol of the best and within the top 20 structures overall, XP precision). The receptor box centre of the PaxA model was set to the centroid of the residues that line the putative binding site (residues 20, 21, 30, 33, 34, 37, 69, 70, 124-129, 275, 276 and 279) and ligands were docked within a length setting of ≤ 20 Å. Docked poses were then manually assessed for plausibility based on the proximity of epoxy functional group of of 3′,4′-epoxyemindole SB or the alcohol functional group of paspaline to the putative active site Asp279 residue (Figure 5).

Both PaxA and the IdtA homologs identified in this study possess a conserved aspartic acid residue, which is believed to play a key role in catalytic function. This residue has been previously demonstrated to be essential for the catalytic activity of several terpene cyclase enzymes involved in analogous chemical reactions (Figure S16).[12-15] To investigate whether D279 is essential for PaxA function, we used CRISPR-Cas9 genome editing to create a PaxA substitution strain, replacing the aspartic acid at position 279 with an alanine residue.

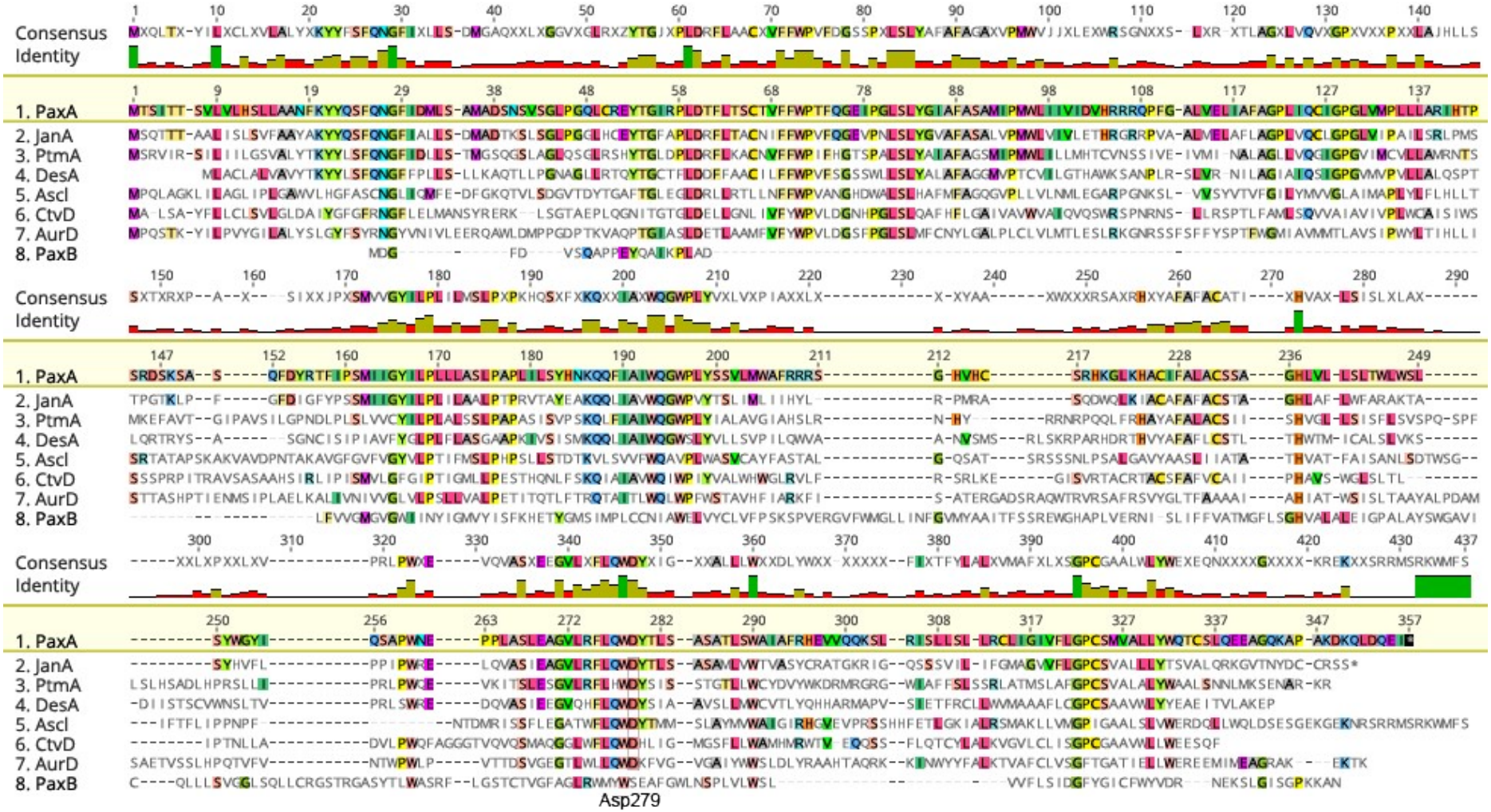


Figure S16: Clustal Omega alignment of IdtA proteins (PaxA, PtmA, JanA and DesA) with other known terpene cyclases that perform similar reactions; AscI (*Acremonium egyptiacum*), CtvD (*Aspergillus terreus*) and AurD (*Calcarisporium arbuscula*), and PaxB. PaxA was used as a reference sequence.

	PaxA	JanA	PtmA	DesA	AscI	CtvD	AurD	PaxB
PaxA		50.142%	38.005%	31.339%	21.705%	23.944%	18.394%	7.916%
JanA	50.142%		36.658%	33.048%	20.735%	24.507%	18.701%	7.652%
PtmA	38.005%	36.658%		37.950%	21.410%	24.390%	20.157%	7.235%
DesA	31.339%	33.048%	37.950%		21.526%	24.860%	19.189%	5.882%
AscI	21.705%	20.735%	21.410%	21.526%		24.390%	25.445%	8.418%
CtvD	23.944%	24.507%	24.390%	24.860%	24.390%		28.457%	8.289%
AurD	18.394%	18.701%	20.157%	19.189%	25.445%	28.457%		9.669%
PaxB	7.916%	7.652%	7.235%	5.882%	8.418%	8.289%	9.669%	

Figure S17: Identity table based on alignment (Figure S16).

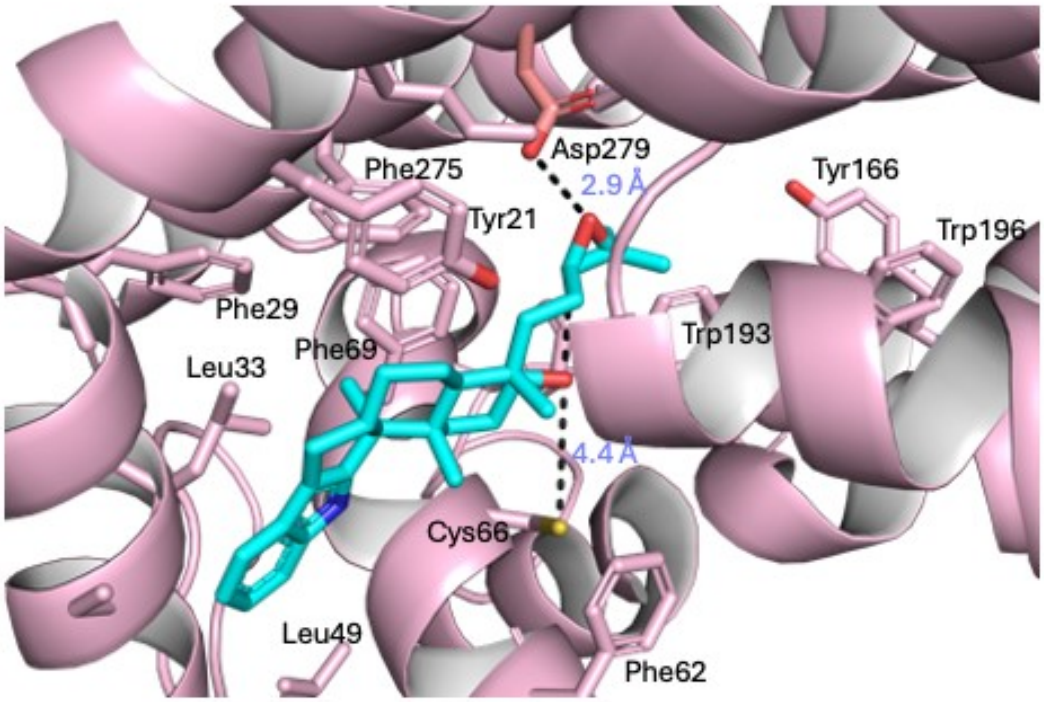


Figure S18: Model of PaxA with 3′,4′-epoxyemindole SB showing conserved IdtA residues located within 5 Å of the ligand. The model file is available as a supplementary file (epoxEmSB_model.pdb).

LC-MS of PaxA D279A:

Extracts were obtained from two PaxA D279A substitution strains and compared to a *ΔpaxA* strain (RC337-8) and the wild-type strain (PN2013). All extracts were analysed by LC-MS (as described in 1.1.8 and 1.1.10). Extracted ion chromatograms (EICs) are shown for *M*422.3 (3′4′-epoxyemindole SB and paspaline) (Figure 5) and *M*436.3 (paxilline) (Figure S19). Both PaxA D279A transformants displayed a chemotype comparable the *ΔpaxA* strain, with peaks corresponding to 3′4′-epoxyemindole SB (14.6 min) and paspaline (18.4 min) observed. In contrast, the wild-type strain showed no evidence of the epoxyemindole SB peak. All *paxA* mutants exhibited a significant reduction in paxilline production compared to the wild-type strain (Figure S18). While the wild-type strain was diluted 1:50, all other strains were analysed undiluted. The substitution of aspartic acid with an alanine results in a loss-of-function phenotype for PaxA.

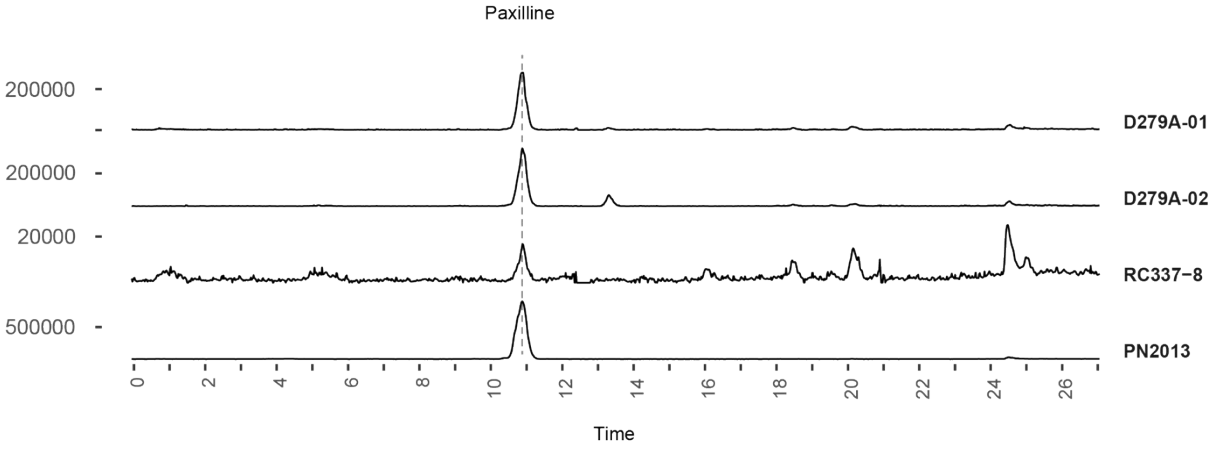


Figure S19: LC-MS analysis of PaxA D279, and RC337-8 (*ΔpaxA*) and PN2013 (wildtype), 436

Metabolites: 3′4′-epoxyemindole SB and paspaline

Dilution: D279A1, 2 and RC337-8: undiluted, PN2013: 1/50

LC-MS trace: EIC (*M*436.3)

1.6 Non-enzymatic THP ring formation

To determine whether 3′,4′-epoxyemindole SB could spontaneously cyclise to form paspaline a sample of the purified epoxide was dissolved in MeCN and analysed by LC-MS (Figure S20). After 48 h at room temperature the sample was re-analysed and showed that approx. 50% of the epoxide had been converted to paspaline and a small amount of dihydroxy emindole SB had also been formed. Addition of 1% formic acid to the sample led to approx. 90% conversion of the epoxide to paspaline within 0.5 h. Increasing the concentration of formic acid to 10% did not appear to convert the remaining epoxide to paspaline within 1 h of its addition. These results clearly demonstrate that cyclisation of the THP ring can occur spontaneously in a non-enzymatic fashion and can be catalysed by the addition of acid.

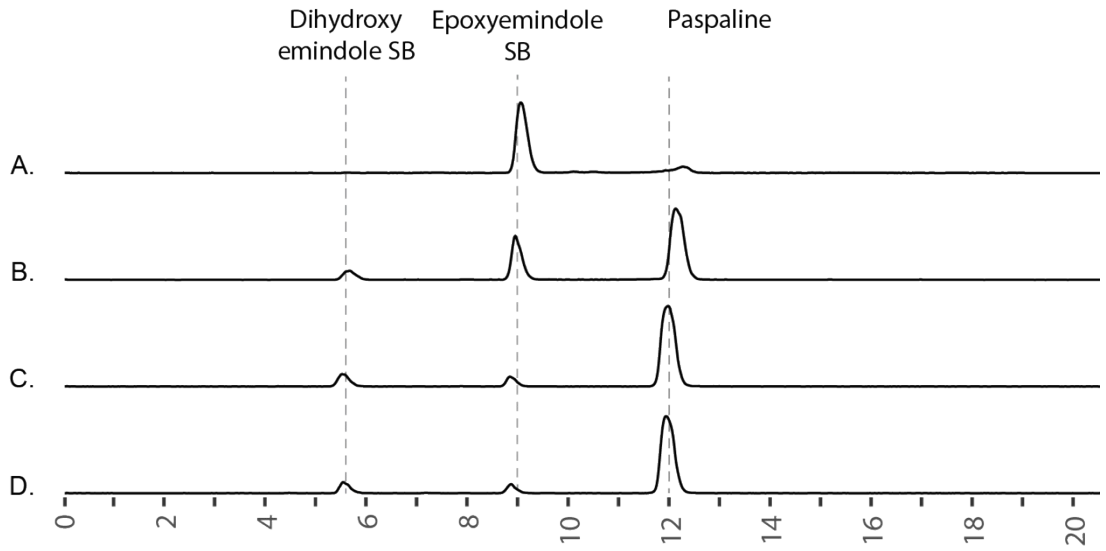


Figure S20: Spontaneous cyclisation of 3′,4′-epoxyemindole SB to paspaline.(A) Purified epoxy emindole SB analysed immediately after purification. B) Epoxyemindole SB after 48 h at room temperature. C) Epoxyemindole SB + 1% formic acid and 0.5 h incubation. D) Epoxyemindole SB + 10% formic acid and 1 h incubation.

1.7 *ΔpaxA* complementation:

1.7.1 Generation of *ΔpaxA* complementation strains

To confirm that other known *idtAs* are orthologs of *paxA* we complemented the *ΔpaxA* strain; RC337-6 with *idtAs* from IDT biosynthetic clusters known to produce THP-ring containing compounds. Plasmids were engineered to contain either *ptmA* (pRC389), *janA* (pRC383) or *desA* (pRC391) from the biosynthetic gene clusters that produce penitrems, shearinines and emindole DB respectively. Genes were introduced by random integration and transformants selected following standard methods (1.1.3 and 1.1.4).

1.7.2 LC-MS analysis of *ΔpaxA* complementation strains

IDTs were isolated from ten strains for each *idtA* gene and analysed by LC-MS using standard methods (1.1.8 and 1.1.10). Extracted ion chromatograms (EICs) are shown for *M*422.3 (3′,4′-epoxyemindole SB and paspaline) and *M*436.3 (paxilline) below. Dilutions and injection volumes are listed for each sample where they differ from what is described in 1.1.10. Eight out of nine *ΔpaxA::ptmA* (RC389) strains lacked the *M*422.3 peak at 9.4 min that corresponds to 3′,4′-epoxyemindole SB (Figure S17). This peak is present in the *ΔpaxA* strain (RC337-6). A paxilline peak was also observed in seven of the ten RC389 strains (Figure S18), indicating that PtmA is able to complement the function of PaxA and restore paxilline production. A similar result was observed for *ΔpaxA::janA* (RC383) with all strains lacking an 3′,4′-epoxyemindole SB peak and seven out of ten having a paxilline peak (Figure S19 and S20) A similar result was also obtained for *desA* (RC391) with no observable 3′,4′-epoxyemindole SB peak in any of the strains (Figure S21) and a paxilline peak present in eight out of ten strains (Figure S22).

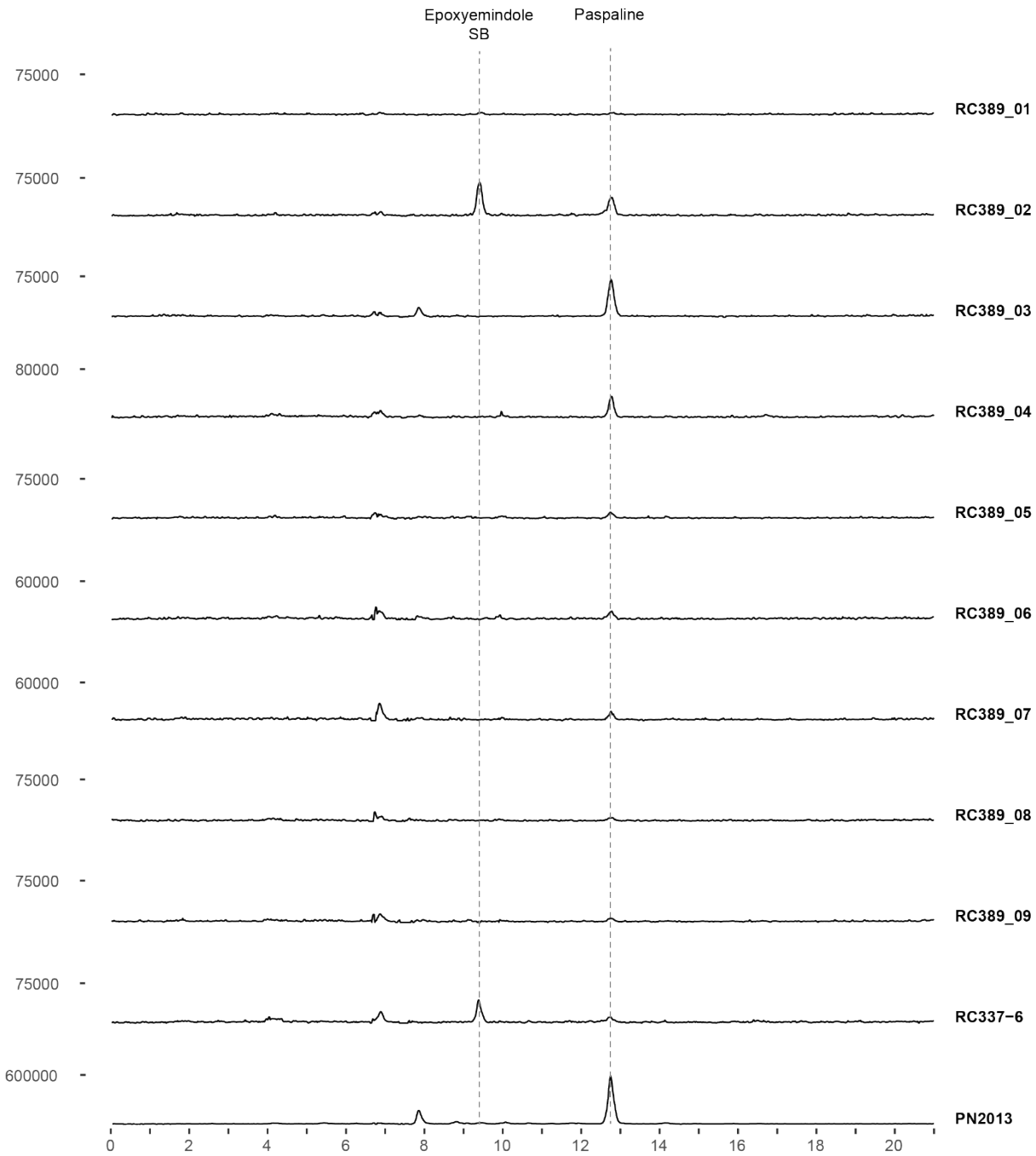


Figure S21: LC-MS analysis of RC389 (*ΔpaxA*::*ptmA*), RC337-6 (*ΔpaxA*) and PN2013 (wildtype), 422

Metabolites: 3′,4′-epoxyemindole SB and paspaline

Dilution: 1/5

LC-MS trace: EIC (*M*422.3)

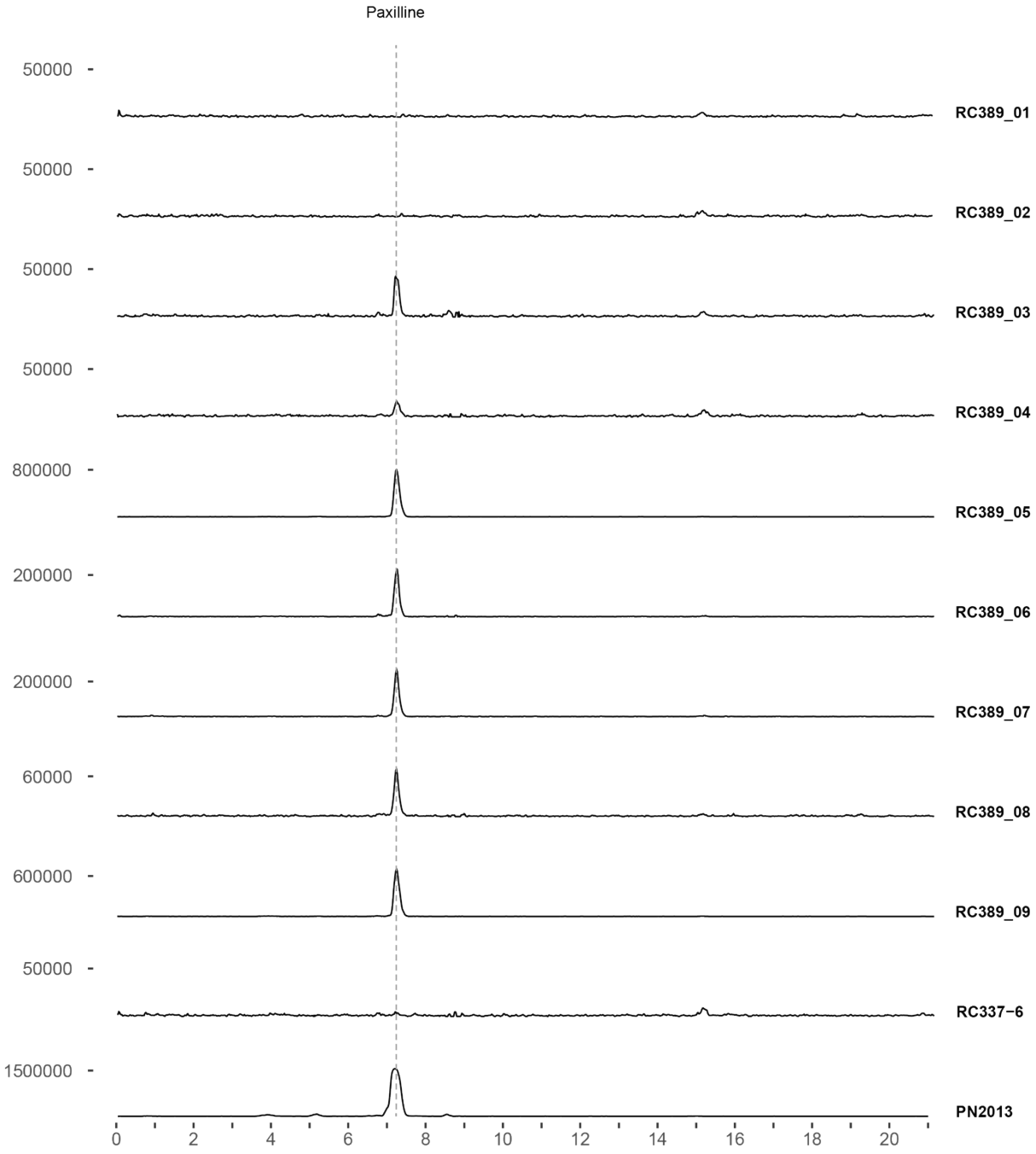


Figure S22: LC-MS analysis of RC389 (*ΔpaxA*::*ptmA*), RC337-6 (*ΔpaxA*) and PN2013 (wildtype), 436

Metabolite: paxilline

Dilution: 1/5

LC-MS trace: EIC (*M*436.3)

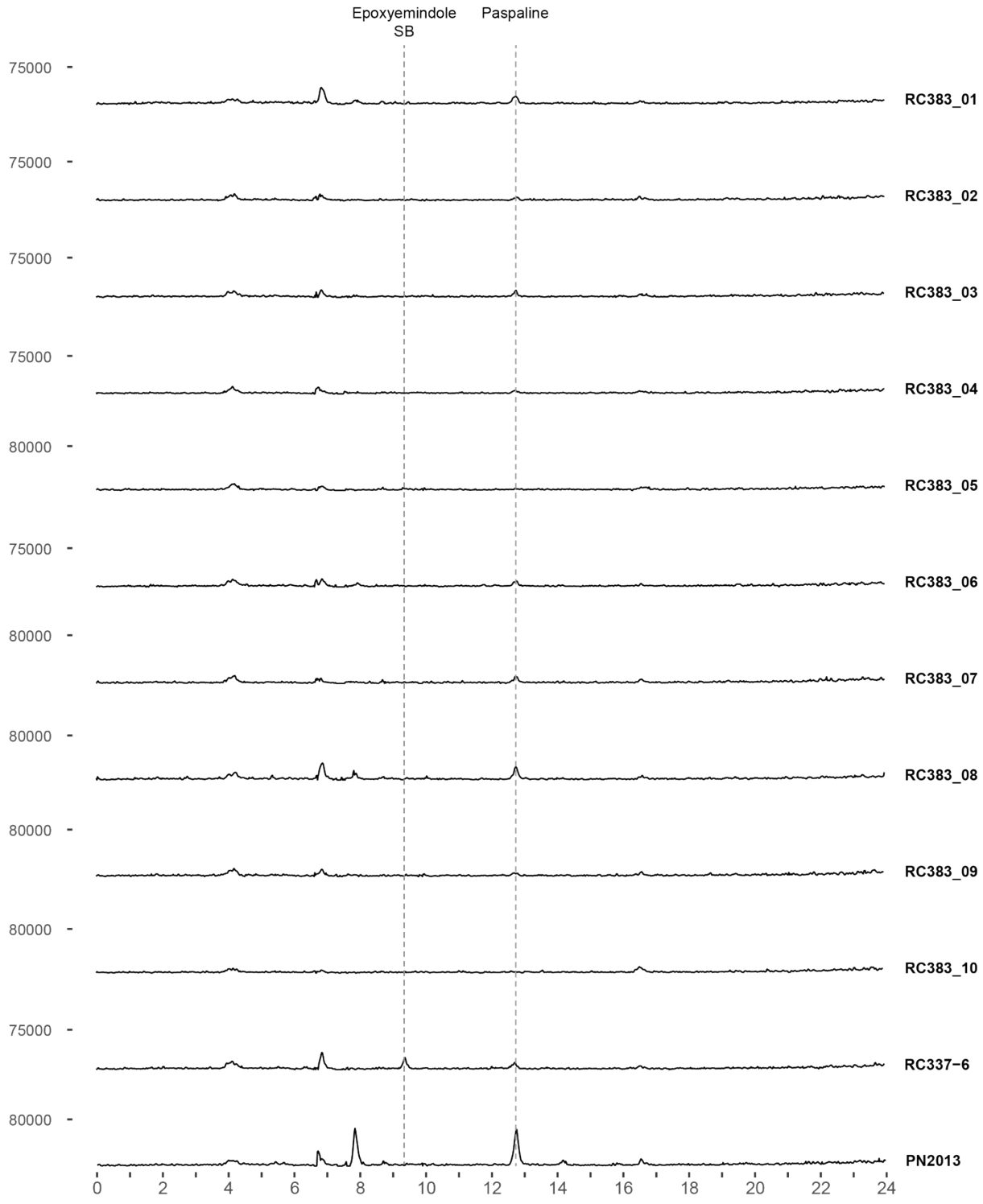


Figure S23: LC-MS analysis of RC383 (*ΔpaxA*::*janA*), RC337-6 (*ΔpaxA*) and PN2013 (wildtype), 422

Metabolites: 3′,4′-epoxyemindole SB and paspaline

Dilution: 1/5

LC-MS trace: EIC (*M*422.3)

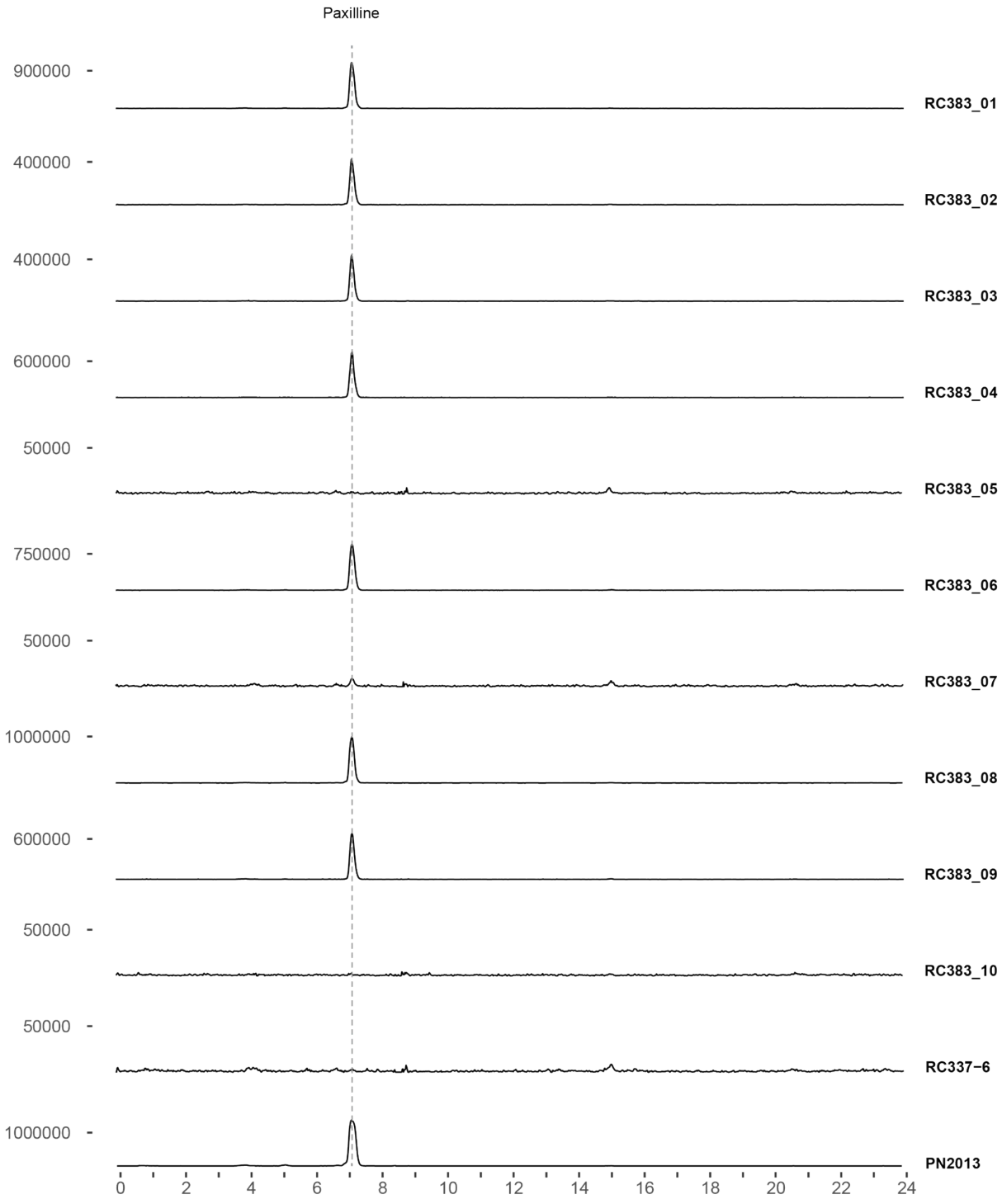


Figure S24: LC-MS analysis of RC383 (*ΔpaxA*::*janA*), RC337-6 (*ΔpaxA*) and PN2013 (wildtype), 436

Metabolite: Paxilline

Dilution: 1/5

LC-MS trace: EIC (*M*436.3)

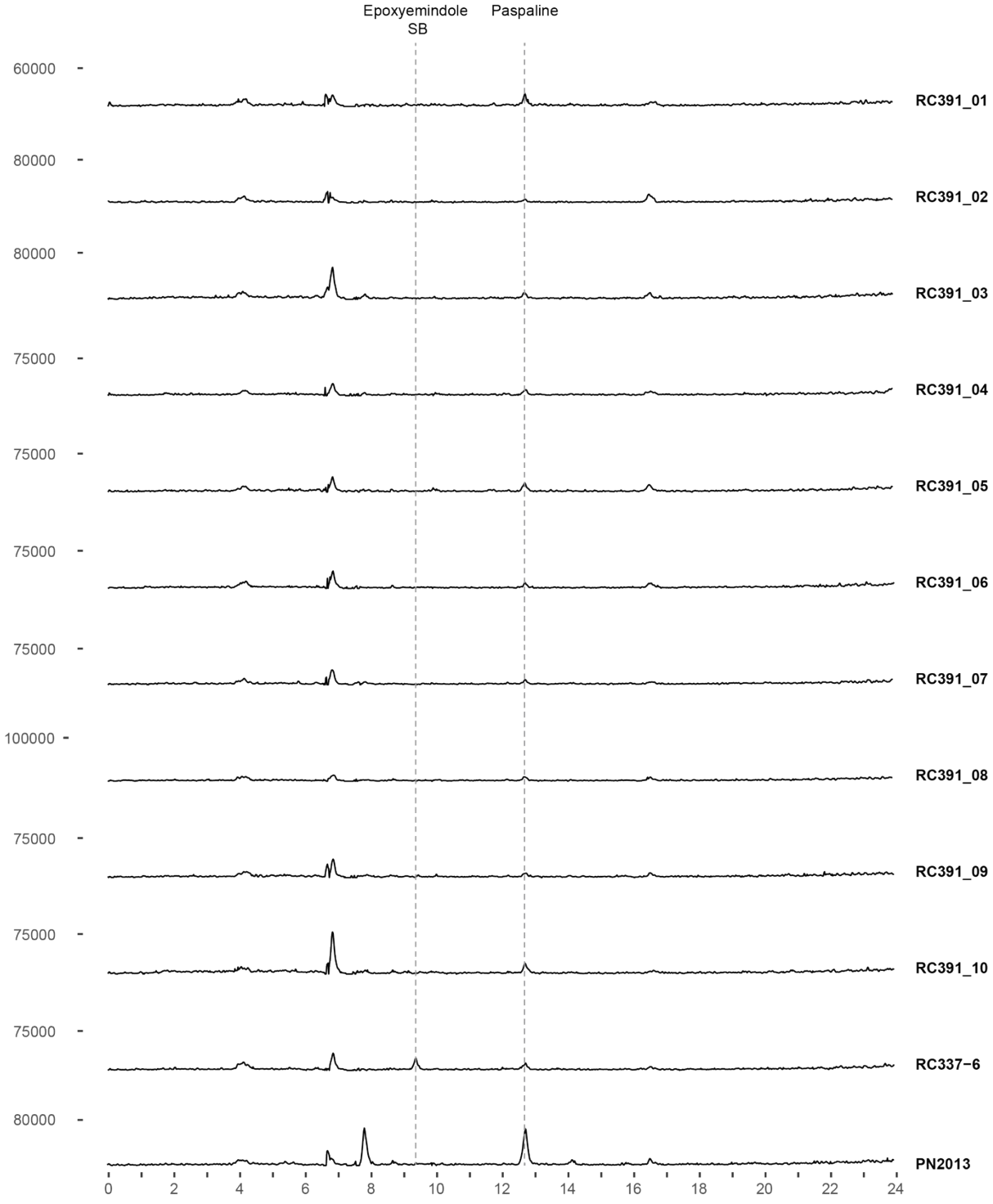


Figure S25: LC-MS analysis of RC391 (*ΔpaxA*::*desA*), RC337-6 (*ΔpaxA*) and PN2013 (wildtype), 422 Metabolites: 3′,4′-epoxyemindole SB and paspaline

Dilution: 1/5

LC-MS trace: EIC (*M*422.3)

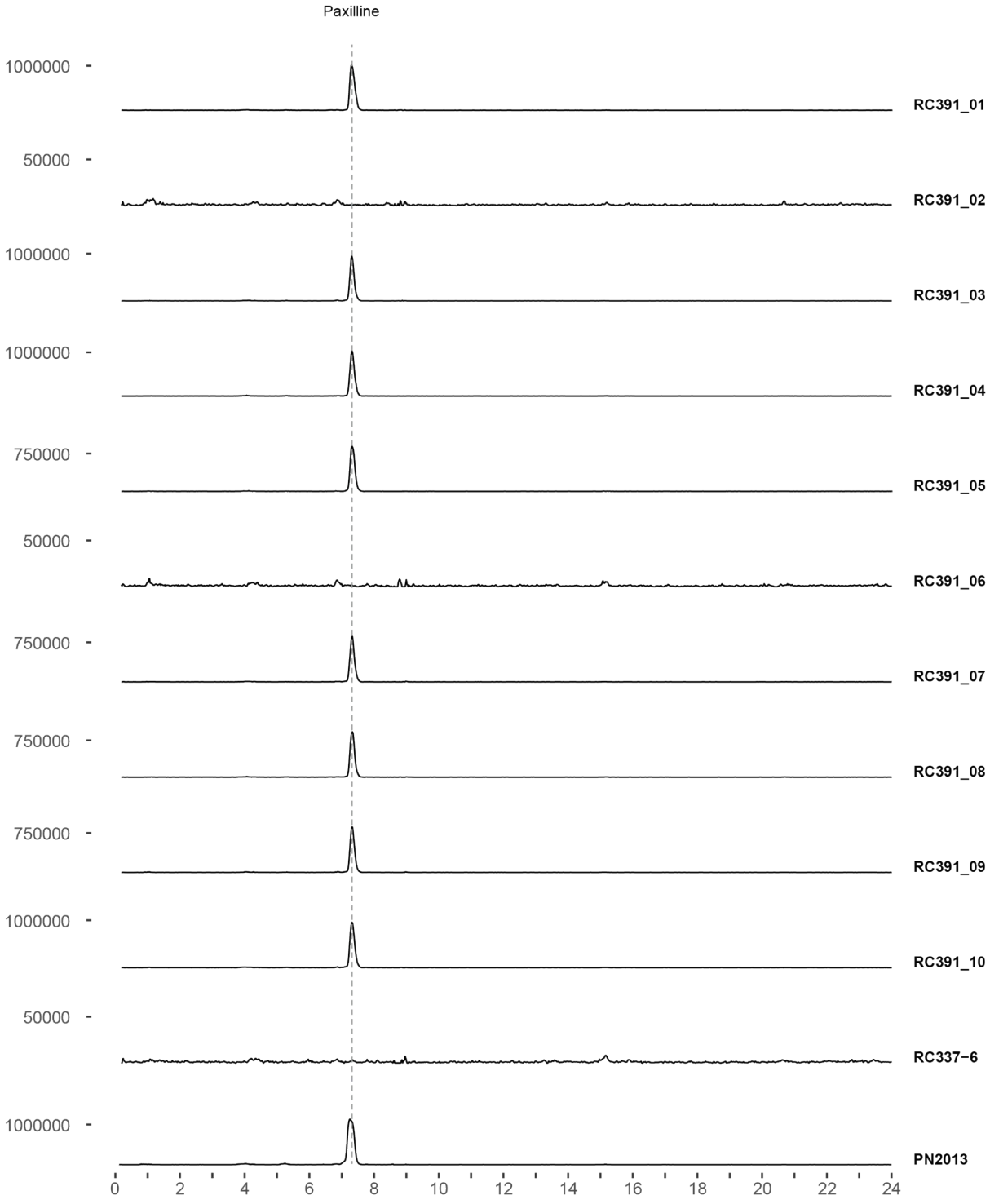


Figure S26: LC-MS analysis of RC391 (*ΔpaxA*::*desA*), RC337-6 (*ΔpaxA*) and PN2013 (wildtype), 436

Metabolite: paxilline

Dilution: 1/5
LC-MS trace: EIC (*M*436.3)

1.8 *ΔltmS* and *ΔpaxA*::*ltmS* complementation

1.8.1 Comparison of IdtA and IdtS

There are several examples of IDT BGCs from Sordariomycete fungi that do not contain an *idtA* ortholog but are known to specify THP-ring containing IDTs including the BGCs that produce lolitrems and terpendoles (Figure 2). These BGCs contain another biosynthetic gene that has not been functionally annotated; *idtS*. To determine if the *idtS*-encoded protein IdtS shares any sequence similarity with IdtAs an alignment was generated between several known IdtA and IdtSs using Clustal Omega 1.2.3[16]. While the IdtAs and IdtSs share sequence similarity within their respective families, there is no discernable sequence similarity between these families (Figure S27A). Structural models for PaxA (AF-E3UBL5-F1-v4) and LtmS (AF-J7FJH0-F1-v4) were obtained from the AlphaFold Protein Structure Database.[9-10] These structures were aligned using Maestro version 13.6.122[17] (Figure S6B). Both PaxA and LtmS are predicted transmembrane proteins. The structures of PaxA and LtmS share eight helices in common, with PaxA containing an additional three helices.

Since both LtmS and PaxA exhibit similarities in their predicted protein structures (Figure 23), and given the presence of *idtS* genes in THP-ring-specifying BGCs that lack *idtA* genes, we hypothesized that IdtS and IdtA likely perform analogous functions in IDT biosynthesis. To confirm the ability of ltmS to perform the same function as PaxA a plasmid was engineered to containing *ltmS* (pRC358). *ltmS* was introduced into a *P. paxilli ΔpaxA* strain by random integration following standard fungal methods (1.1.8).

A.

	PaxA	AceA	JanA	DesA	PtmA	PenA	AtmA	NomA	LtmS	IdtS_Pi	IdtS_Cp	IdtS_At
PaxA *Penicillium paxilli*		51%	50%	32%	39%	37%	29%	29%	8%	10%	9%	11%
AceA *Aspergillus alliaceus*	51%		59%	36%	41%	40%	26%	28%	12%	12%	12%	11%
JanA *Penicillium janthinellum*	50%	59%		34%	37%	36%	28%	28%	11%	11%	11%	10%
DesA *Aspergillus desertorum*	32%	36%	34%		38%	39%	30%	29%	9%	10%	11%	11%
PtmA *Penicillium simplcissimum*	39%	41%	37%	38%		91%	32%	31%	9%	9%	9%	11%
PenA *Penicillium crustosum*	37%	40%	36%	39%	91%		31%	31%	9%	9%	10%	11%
AtmA *Aspergillus flavus*	29%	26%	28%	30%	32%	31%		73%	10%	9%	10%	11%
NomA *Aspergillus nomius*	29%	28%	28%	29%	31%	31%	73%		12%	11%	12%	12%
LtmS *Epichloë festucae*	8%	12%	11%	9%	9%	9%	10%	12%		67%	49%	45%
IdtS *Periglandula ipomoeae*	10%	12%	11%	10%	9%	9%	9%	11%	67%		51%	49%
IdtS *Claviceps purpurea*	9%	12%	11%	11%	9%	10%	10%	12%	49%	51%		52%
IdtS *Aciculosporium take*	11%	11%	10%	11%	11%	11%	11%	12%	45%	49%	52%	

B.

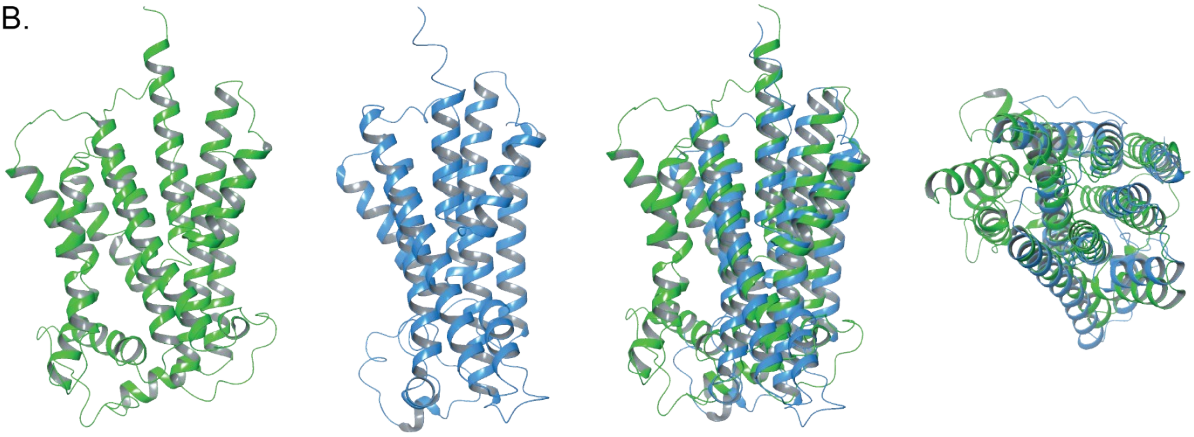


Figure S27: Alignments of IdtAs and IdtSs.(A) Sequence similarity between IdtA and IdtSs based on a Clustal Omega alignment. (B) Predicted protein structures for PaxA and LtmS. In the side-on views, the protein helices are oriented perpendicular to the cellular membrane.

1.8.2 LC-MS analysis of *ΔpaxA*::*ltmS*

Extracts from the ten *ΔpaxA::ltmS* strains were analysed by LC-MS using standard methods (1.1.10). For each of the *ΔpaxA::ltmS* (RC358) strains the extracted ion chromatograms (EICs) are shown for *M*422.3 (3′,4′-epoxyemindole SB and paspaline) and *M*436.3 (paxilline). Dilution and injection volumes are listed for each sample where they differ from what is described in 1.1.10. No observable 3′,4′-epoxyemindole SB peak was present in eight out of ten RC358 strains and five out of ten had a clear paxilline peak with a further two out of ten showing some evidence of paxilline production (Figure S24 and S25). The restoration of paxilline production when *ltmS* was introduced to the *ΔpaxA* strain confirms that LtmS can catalyse the conversion of 3′,4′-epoxyemindole SB to paspaline.

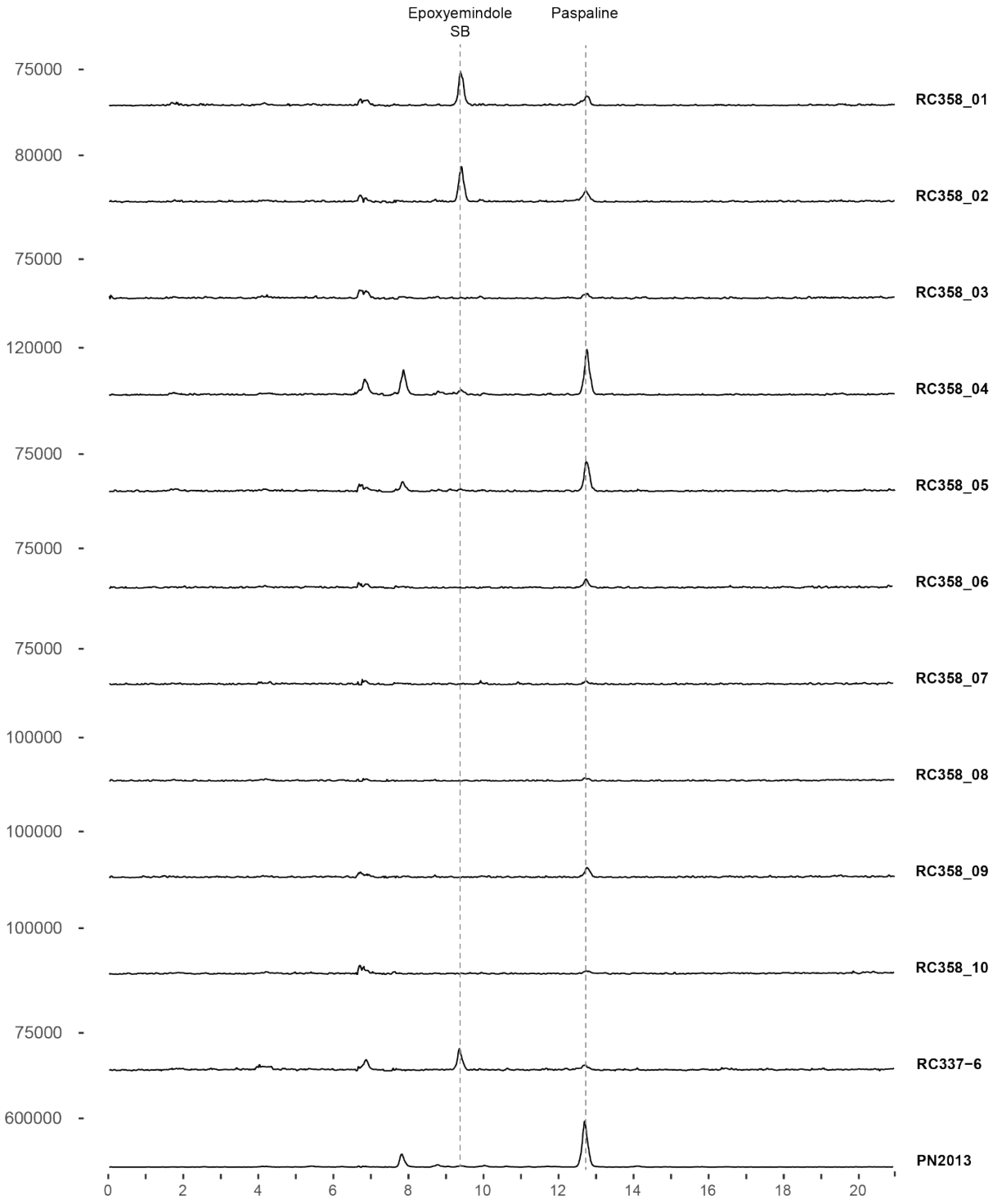


Figure S28: LC-MS analysis of RC358 (*ΔpaxA*::*ltmS*), RC337-6 (*ΔpaxA*) and PN2013 (wildtype), 422

Metabolites: 3′,4′-epoxyemindole SB and paspaline

Dilution: 1/5

LC-MS trace: EIC (*M*422.3)

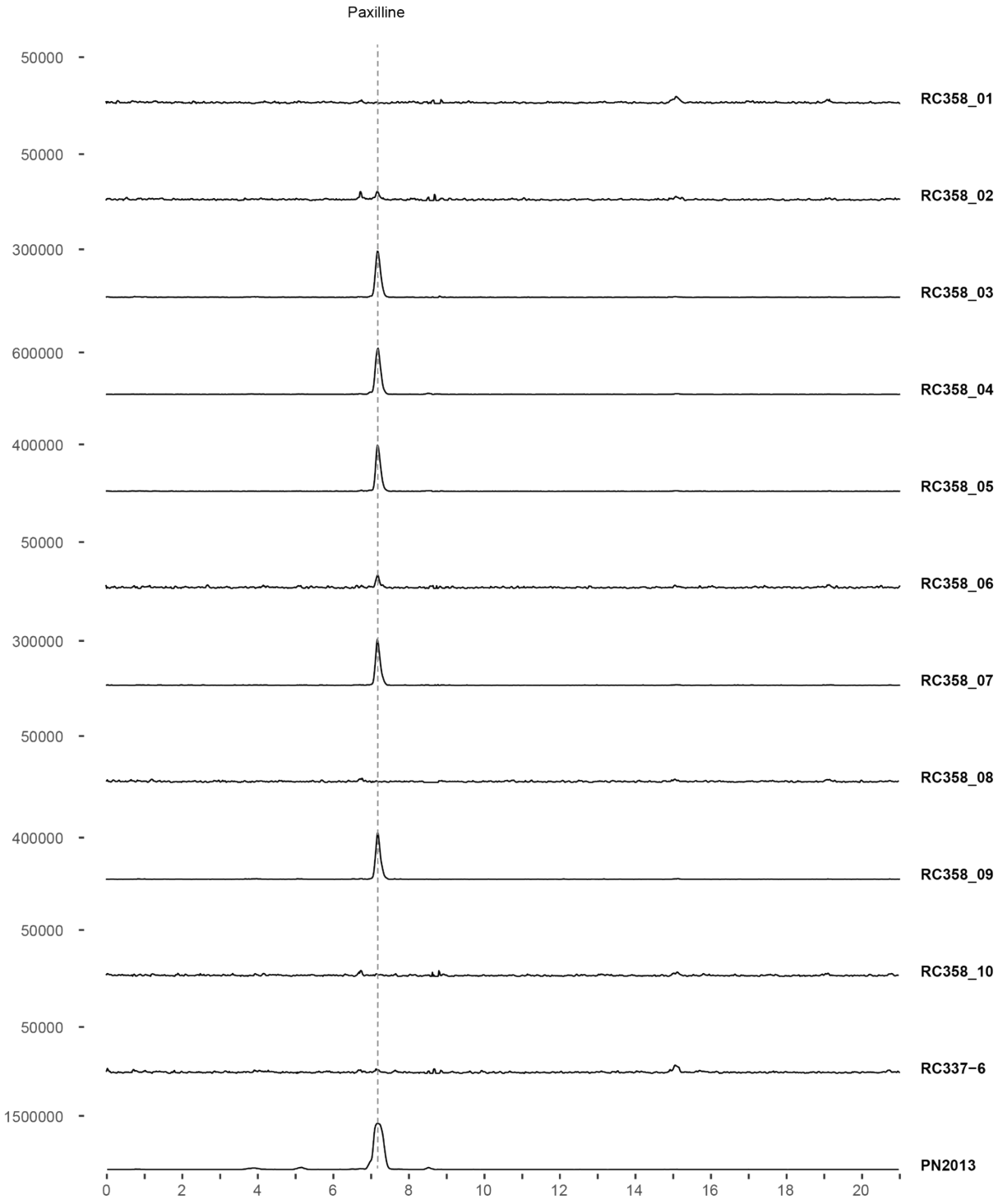


Figure S29: LC-MS analysis of RC358 (*ΔpaxA*::*ltmS*), RC337-6 (*ΔpaxA*) and PN2013 (wildtype), 436

Metabolite: paxilline

Dilution: 1/5 (1 µl injection)

LC-MS trace: EIC (*M*436.3)

1.8.3 Investigating the role of LtmS *in planta*

To confirm the role of IdtS in a native BGC, a *ΔltmS* strain of *Epichlöe festucae* was generated as described in 1.1.6. The wildtype *Epichlöe festucae* strain Fl1 contains a BGC that specifies production of lolitrems, with lolitrem B as the final product. The lolitrem BGC contains an *idtS* gene (*ltmS*) and is known to generate paspaline as an intermediate of the pathway.

1.8.4 LC-MS analysis of *ΔltmS*

Extracts from fourteen plants infected with an *E. festucae ΔltmS* strain and twelve plants infected with wildtype *E. festucae* strain Fl1 strains were analysed by LC-MS using methods described in 1.1.9, 1.1.10. Extracted ion chromatograms (EICs) are shown for *M*422.3 metabolites (3′4′-epoxyemindole SB and paspaline) and for *M*686.3 metabolites (lolitrem B). A large peak was observed for 3′4′-epoxyemindole SB in *ΔltmS* strains, and lolitrem B production was reduced compared to wildtype (Figures S26, 27, 28 and 29).

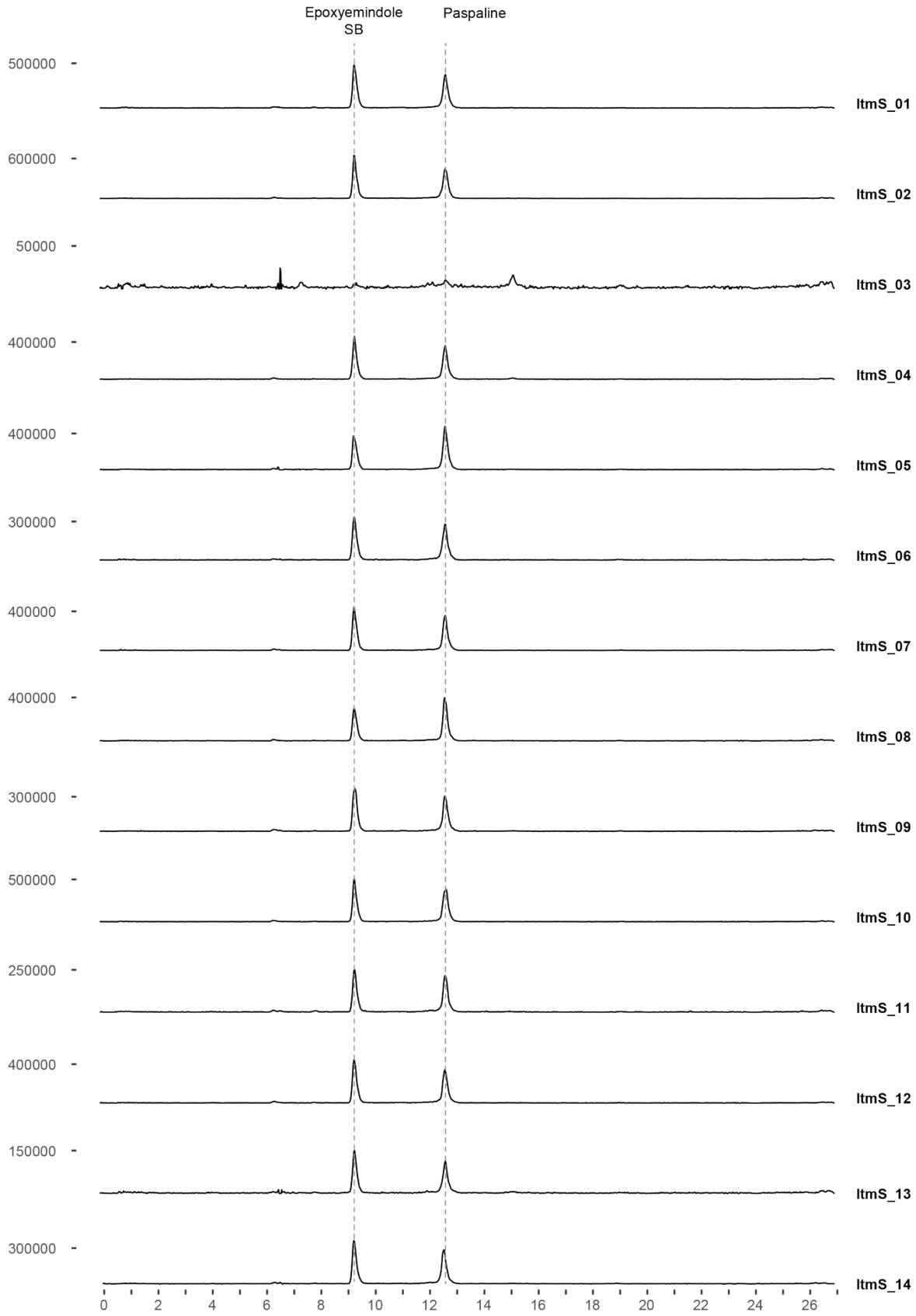
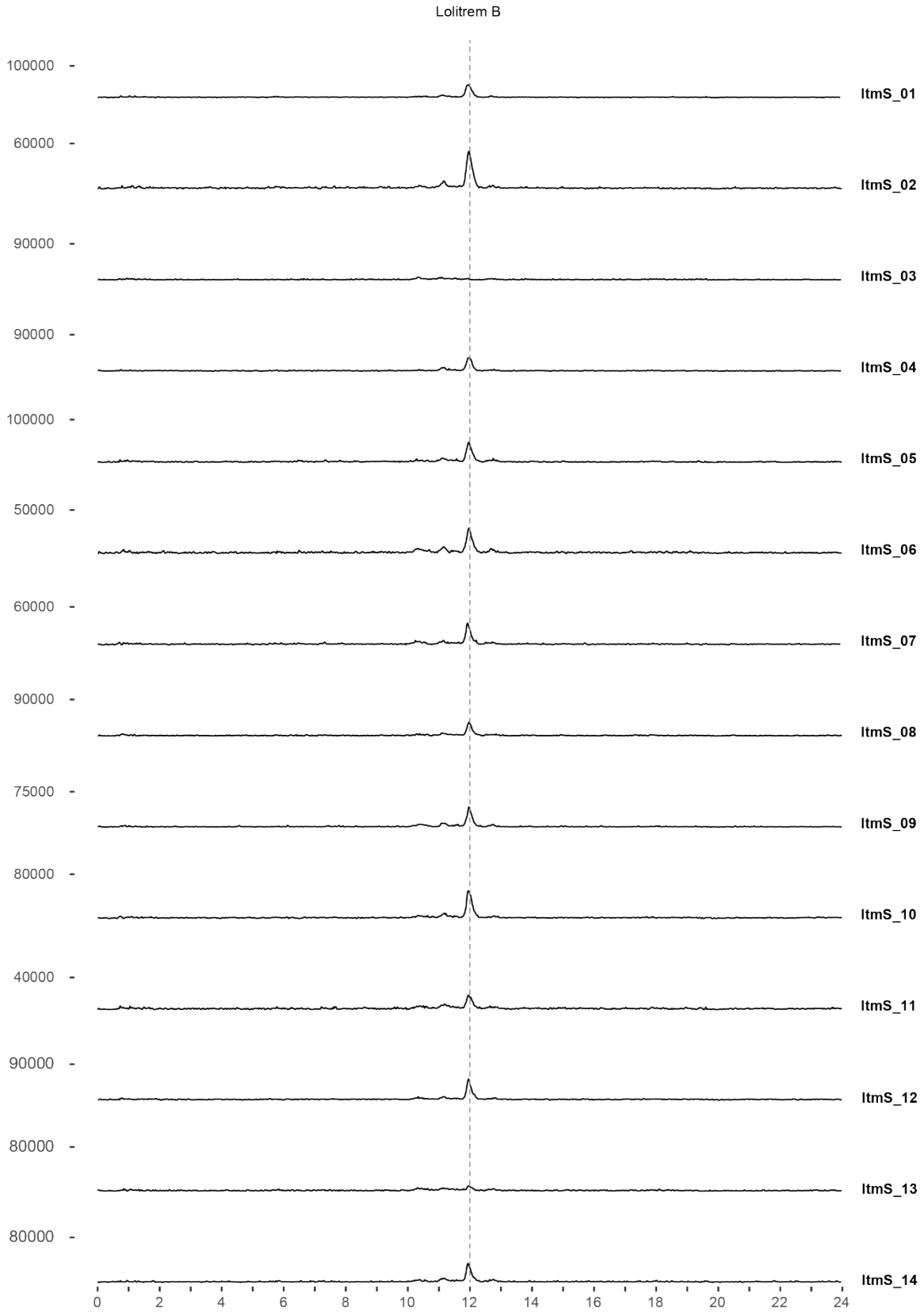


Figure S30: LC-MS analysis of *ΔltmS*, 422

Metabolites: 3′,4′-epoxyemindole SB and paspaline

LC-MS trace: EIC (*M*422.3)


Lolitrem B
100000
ltmS_01
60000
ltmS_02
90000
ltmS_03
90000
ltmS_04
100000
ltmS_05
50000
ltmS_06
60000
ltmS_07
90000
ltmS_08
75000
ltmS_09
80000
ltmS_10
40000
ltmS_11
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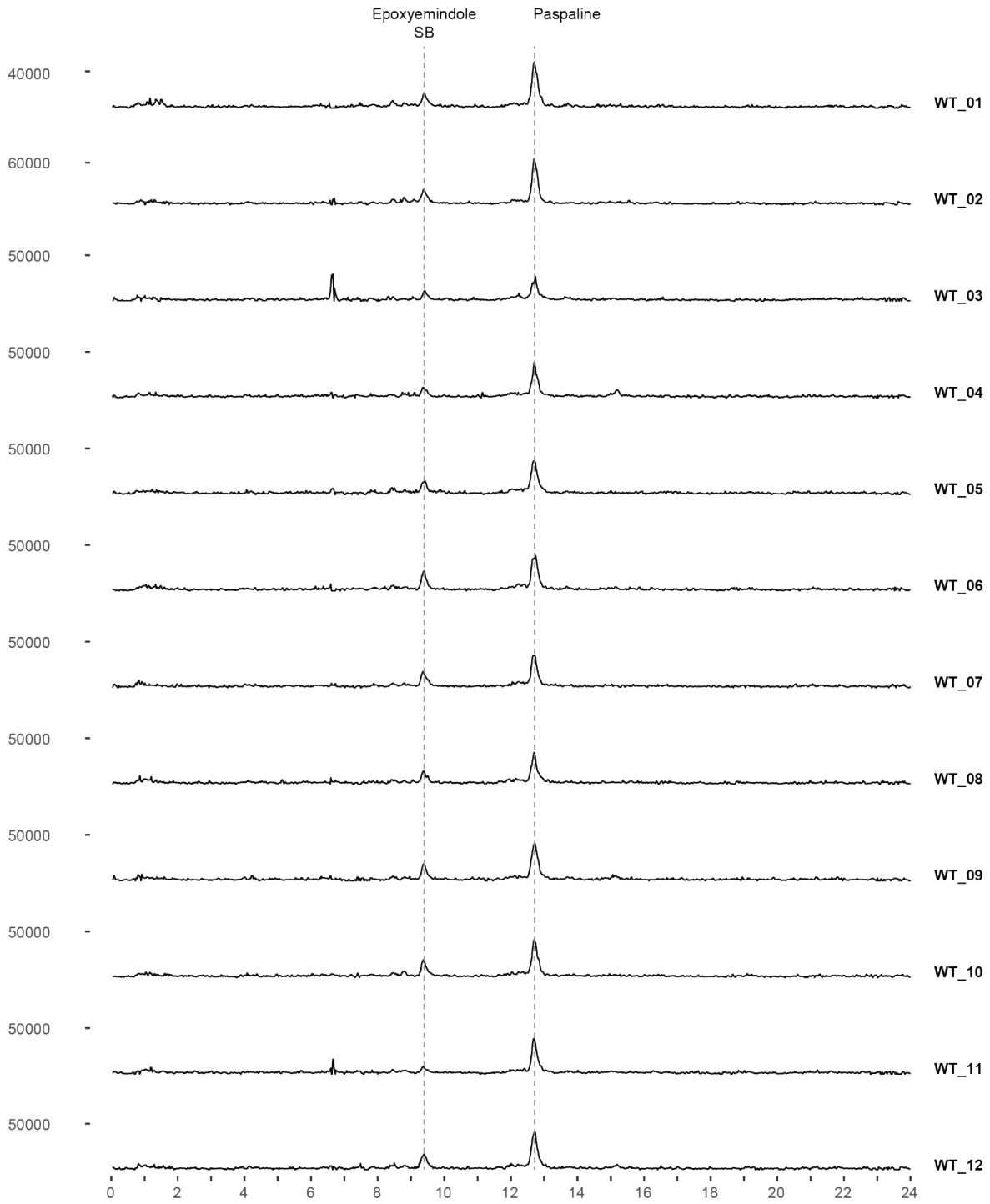

Figure S31: LC-MS analysis of Δ*ltmS*, 686

Metabolite: lolitrem B

LC-MS trace: EIC (*M*686.3)

Figure S32: LC-MS analysis of Wildtype (*Epichloë lolii*), 422

Metabolites: 3′,4′-epoxyemindole SB and paspaline

LC-MS trace: EIC (*M*422.3)

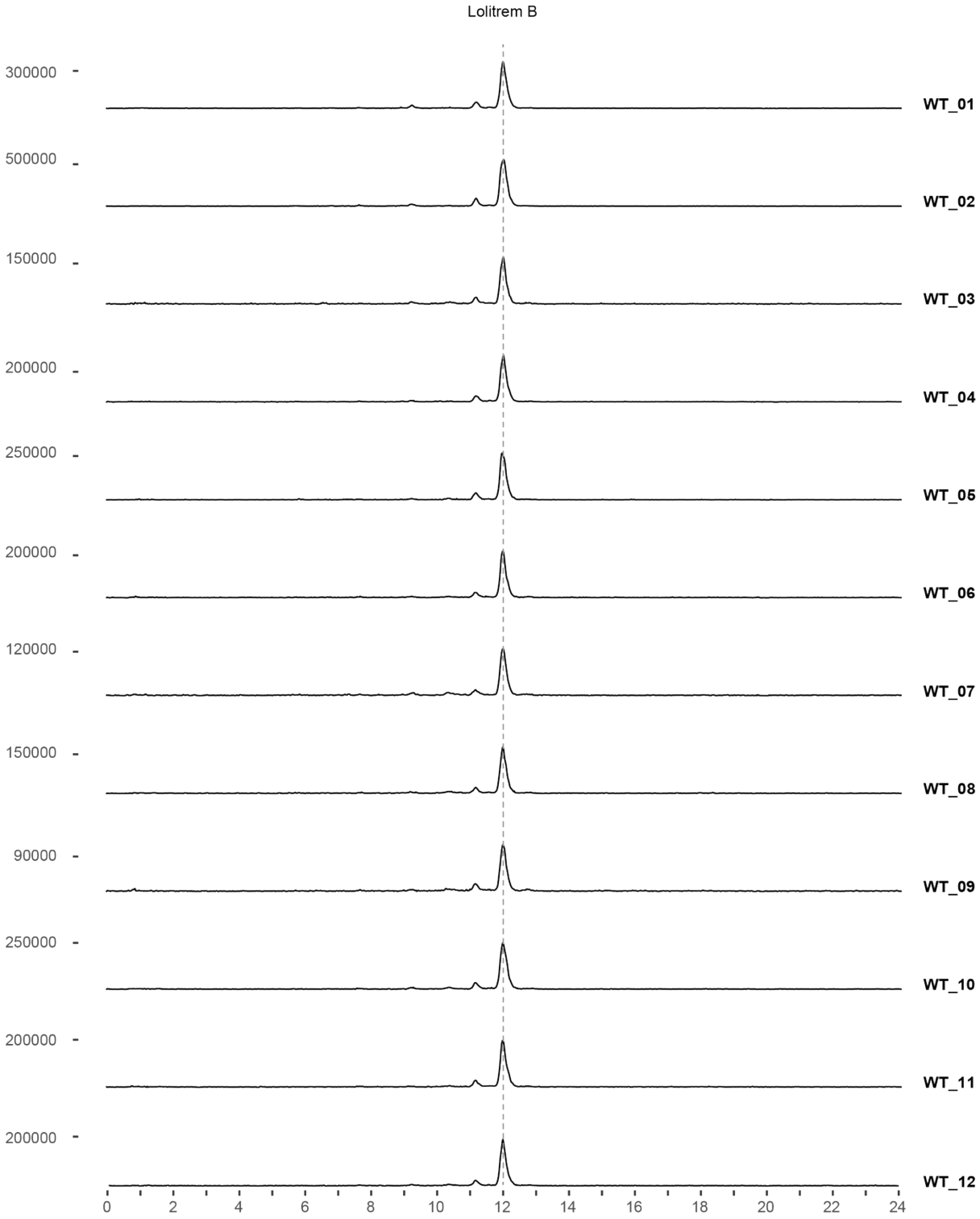

Figure S33: LC-MS analysis of Wildtype (*Epichloë lolii*), 686
Metabolite: lolitrem B
LC-MS trace: EIC (*M*686.3)

1.9 Sequence information.

1.9.1 sgRNAs:

>RCC7

GUGUAAAACAAGAACACUCG

>RCC8

UAAAGAUAAACAACUGGACC

>sgRNA_LJS1

AGCAAACGGUGGUCAAAGGA

>sgRNA_LJS2

CGGCACUGGCAAUAACAAGC

>YLsgRNA1

CCGTGACTTCCATATTACGCC

>YLsgRNA2

ACAAGATCTAAAAAGGACTG

1.9.2 Primers

>paxG_frag1_R (8)

CTTCTACGTCTCGTACTGTTCTAATCGTGCTTGGTG

>paxB_F (21)

CGATGTACGTCTCACTCGAATGGACGGTTTTGATGTTTCCCAA

>paxA_F

CGATGTACGTCTCACTCGAATGACATCCATCACCACGAGTG

>paxA_R

GACCTTTCGTCTCTGTCTCAAAGCCTAGATCTCCTGGTCCAGTTGTTTATCTTTAGCC

> paxQ_frag1_F

CGATGTACGTCTCACTCGAATGGATTTCGTGTTATCAGCCTTAC

> paxQ_frag4_R

GACCTTTCGTCTCTGTCTCAAAGCTCATGCCGAGACAGACTTTCTG

Table S3: Plasmid components

Name	Promoter	Gene	Terminator	Homology arm
pRC337	-	-	-	HA1
	P*trpC*	*nouR*	T*trpC*	
	-	-	-	HA2
pRC356	P*paxA*	*paxA*	T*aceB*	-
pMH17				HA3
	P*paxG*	*paxG*	T*paxG*	
	P*paxM*	*paxM*	T*paxM*	
	P*paxB*	*paxB*	T*paxB*	
	P*paxC*	*paxC*	T*paxC*	
	-	-	-	HA4
pMH45	-	-	-	HA3
	P*paxG*	*paxG*	T*paxG*	-
	P*paxM*	*paxM*	T*paxM*	-
	P*paxB*	*paxB*	T*paxB*	-
	P*paxC*	*paxC*	T*paxC*	-
	P *paxA*	*paxA*	T*paxA*	-
	-	-	-	HA4
pRC370	-	-	-	HA3
	P*trpC*	*neoR*	T*trpC*	-
	P*janO*	*paxM*	T*paxC-P*	-
	-	-	-	HA4
pRC379	-	-	-	HA3
	P*trpC*	*neoR*	T*trpC*	-
	P*janO*	*paxM*	T*paxC-P*	-
	P*janO*	*paxB*	T*paxC-P*	-
	-	-	-	HA4
pRC380	-	-	-	HA3
	P*trpC*	*neoR*	T*trpC*	-
	P*janO*	*paxM*	T*paxC-P*	-
	P*janO*	*paxA*	T*paxC-P*	-
	-	-	-	HA4
pLS293	-	-	-	HA3
	P*tefA*	*hphR*	T*tubB*	-
	P*tefA*	*tk*	T*tubB*	-
	-	-	-	HA4
pRC389	P*trpC*	*neoR*	T*trpC*	-
	P*janO*	*ptmA*	T*aceB*	-
pRC383	P*janO*	*janA*	T*aceB*	-
	P*trpC*	*neoR*	T*trpC*	-
pRC391	P*janO*	*desA*	T*aceB*	-
	P*trpC*	*neoR*	T*trpC*	-
pRC358	P*janO*	*ltmS*	T*aceB*	-

	P*trpC*	*hygR*	T*trpC*	-
pYL413	-	-	-	HA5
	P*trpC*	*neoR*	T*trpC*	-
	-	-	-	HA6

1.9.3 Sequences:

```
>Homology arm 1 (HA1)
GGCGTCGAACTTGATGAAGTTTTCTCTGGCGTGAACCTGAATTCAAGAGCACTATTTTTATAACCCTATGATGAA
AGAAATGGGACTGGAAGAAATTCTTTTCTATTTTGCAGAGTAGGATTGCAAAATTGCGATGAAGGAAATTCCGGG
GTATGGGTAGGGTTCGAGAGTAAACGACACCGGATCCTTGTATTGATTTATGCCGCTAAAAATCCGTTCCCAACT
ACCCCAGTTAAATAACTCTCTATAATTACAACTGCGGTACAGTCATGTTTCAAATTCTCGAAAATAAATCCCTCT
GCCCCCACTTGAGAACTCCGTATTATAGACAAGGCGTGCGAACATGGATGACATGATGCAACGATTATGGTTGCG
TTGTAATACATCCATGACTGGGTGATTCGTATTCACAAAGCGAGATGTAATTCTGTGTACGGAAGTAAACTCCAT
AGATTATACGTGCCAGATCTCGTGCATAGTATTCAACACAACACCCAGTAAGTTTTAAAATTGATAACCTTGACT
AGTCACAGGTCGTGAA

>Homology arm 2 (HA2)
TTTGGCTTACTTCAGTTTAACTGTTTTGCTAATTGGCCGATTTCCGAGAATAGAGTTGCAAAACAATG
GGCATCTGATACAATAGCCTGGTGAGATCTCCCTTCTTAAATCCCCGATAGAATGAAACATGGCCCAA
AAAGACGAGGAATTATAGCTAGGAGGAAATTGGATCTTTCATCGGTGTAACTGACATCCATTACAGCT
GACGAAATTACCAAGTTCGCTGGCTGGACTCGATTGCTTGTGGGTCTACGGCCACCCAGGTAATTCTT
CTGTCGTAGAGGTGGATTAGGACTTGCATTATGATACTAACCTTGGAACATTGCTTATATATTATGAG
ACACTGTCCGGCCTTGATTTGCCACATTTAATATAGAATTTCGTTTTCGGAGTTGACCCAGTGTAGTA
GAAGGTGGTGAAGGAATGTCGATTGTCAATTTCGAAAGTTCTATCTTCCATCGTACTGGTCAGTCAGA
GTACCCCTCTGCAGTAGAAATATTTCGTAGAAAAAGTAGGTATGGATAGTGTATGGAATTTTCATAGT
ATGGCTTACTCAAGCTGTGAGTATTCGCTGCCCATTTGAGATCGCTTGAATGCTCTCGCAGGGAGCCT
GAAGACGGTTTAAAACAAAGCGGCTTAACGATATAGTACTAATTGAACTCAAATCTAGTGATGTTTCT
TGAAAGCAAGATAGGTGACAATCAACAGCTAGACACTGGAAAGAAAGGAAAACCAAAAATTGCTCCAA
TGGT

>Homology arm 3 (HA3)
GGAGACACAGAGATGTTACAGTGGAGGAAAGCGTTGATAAAGATACTTTACAACAAGGCACTACGTTT
ATAATTATCTTGATTATTGAACCAACATAAAAGATCAAAACAAGTTGAATTTTGAGAACAAATAATGT
AATATTCATGGAAGCTGTAGTAGGTGGGGGTCAAAGTCTCTCAGAAGTAGTGATCTGTGAGTCTTTGC
TTAAGGATAATCTATATGCAAGAACCTTCTTTAGGTCGCTGCAACACTTCTTATGGGCTGTTTATCTG
TAATCTGGGAACATAATCCGTCATTCAGTGGCCTTGTGATGCCAATGAGATCCTTACAGGGGGTATCC
CAGTTGCAAAAGGCATTGATCGAGCTCTTTGCATATCAATGCGGTTGGCTTGGAGAGATGCGAGTTTG
GACTCTTCCGATAGGTTGAGCTGAATTAACTGGACATGCCACGAAGACCAGTCAACCTTTATCCTGTC
TCTAATAAGAGAATCTTTGATATTTAATCACAAATTGGTCATGTCATCTGACTTGGCGACGCTTGTGC
ACTGCGCATTACGAGAAGCAATGAGACTTGACCAAGCATGCTCCTTGAAGCATGTTTAACTGAACGTG
TCTATGGAATTATCAACAATCCTTGGGCTTTAATCTAACAATAGTTCCTACCTAGATATCGATAAACA
AGCCAAATAATGAAAGAGCTGAAAGGTACTACTGTTAACACTCTGTGCAGCGAAAAAACTCGGGATCT
TGAACCATGAAGGGAGTGTTATAACACGCTTAAATATAGTAGTACCTACTATCTGCGAAATTTCATTT
TGAAAATTTTGCAACGGAATTGTGAAAATCTCAGTGTATAGTTCTACCACTTTTTGTTGAGTTCTACG
GCATCTCAAATAAAGATGCACAGATATTCTGTCCTTCACTCCCGAGCAATATTGCTAAAATAAAGTGG
GTCGGTGATCGTTTTACCGAATGAAAATGTCGCATTTCCACTTTAAGAGGT

>Homology arm 4 (HA4)
GGAGGAGCCGACAAAGTCGAAGCGAAAGCTAGCGCACAGAGTGGAAGTGATGATCGAAGCATCTTGAA
TGTGTGAAGTATGTTGGTACTAGAAAGTCCAGATCTGAGGTCACGCCGGCATTGATAAATAGAGGAAC
AAATCTTCAGCTTTGTTCATGAAAAAGCGCGTCATGTTGATCTTGGACTGCGCCCCTCTGTGAGTGAC
AGAAATAGTTTCAGGCCAACCGCGAAGTGCCGATCGCTGTCAAAACATGGGCTTTGAGCTAAGCCAAG
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GCCAGGGGGGGTTCTGTTCCGGCCAATTCATCCCATGGGGTGCAGTCAGATGGAAATAATCATGAATA
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CCTGGTGGGTATATTTAGTACTATTTTGTATGAATTAGTGACATAGTCGCTTTCACCGTGAACTCAAG
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CACGTCACTTCTGCTTCTCCTTCATCTTCCTCCTCCACTTCCTCCTCCTCTCATGAATAGCGAAATGA
ATTATTTGGAGCCTCTTCGTAAGGCTCATTCTGTCTTCACATCGGACTCGGAACTTCAGCTG

>Homology arm 5 (HA5)
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ATTACGTTTTGTCTAGACCACAAAAATTGGGTTTATTCTGATGTAAGATAGGAGAGTGGTTATAGTCC
TATACTAAAATCCACCTCCAAAACAACTCCAAATCAATATTTTATTAGTGGTATTGCATGTGTCTGTG
AGCTTTAGTAGGATTCACGAATCTGCACAAAGACAAAAACTGTCTTTATTGTGGCTTGGGTATTGACA
GGGGTGTGTTTATTAAGCACACCCTTATGCGTAAATAGCACCCCTGAATGTAGCTAAGGTAGCTGCAA
TAACTGTCAGGGAGAGCTCCCTTAAAGCCTAGGATGTATGAGGCAGCGCGCATTGACTAGGATAAAAC
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TTGCCGAAAGATAATGACGACACGGGTTAGGGATGGAGAGGGGGTCGGGTGCTCAAGCCAATTCTTTG
CGTCAGTCGCACTCGGCATTCTTCCGGTTTCCTATTGCCAAGATTTCTCAGCCCCTATAAAGAAACAG
CTTAGTCTCTCGCTTTGACGATATCATGACGATTGTACGGATTATGCTTCTGGCCTTTCCCAATATTG
CATGAAACTGGACCTAAAGTTATTATCAGTTTTCCTCTATTTTTCCC

>Homology arm 6 (HA6)
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GATAATTAGCAACCTAGTATGGTTTTCCTACTACAAAGCAACATCACGGCAGGAGTACTGGTTAATGG
CATAAATTGCTATCTAGGGTAAATCATATAATATAATATATTCATATATTGATAGATATTGTGTGGCT
AGCATTGTGACCTCTCGAGTCCTCTCTCACAAGACCTCGGGCCCCTAGACCTTGGCTTCGTCAGATCT
ATTGGCTTGTCAGATCAATTCGGCCACATCGGATCCCTAGTCATGTGACCTCCTAGCTAGATAAAACC
CCTAGATTCCCTCCCTTTAAATAGAAACTTTACTCTCCCTTCGTTGCCTTAGGCTTGGATTGTTTAAT
TAATAAAATAACCTATTATTAGAAAAAGACCCTACCCCCTAGCTTGAATCCTAGGCGTGGATCGGAAA
CCTCGCAGACCTAGATCCCGATATCTAGGTTCAATTGCTAATATCGTCAAATTCGTTAGCTTTGTTGT
TAGTGTCATCGTCAAATCCGTCGTTTTCGGGTGGTTGTTTTCGCCTGACAAAATATAAAGGGATTTTT
CTTCTTATTTAAGGGTTTTCCCTTTTAACTAACTAGAGGCAGTACTAGAACTAGCCCTACATAGAGCT
AGCTAGAGTATACAATATATATATATATATTTAGGGATAAGGCTAGGATTAAGAATACACTTTATATT
AATTCTTCAATTGGGTTTATCTACGAGAAGGGAAGAAGGAAAGAAAAGTCTATTATAACTAGTAAAAA
AAACTTAGTGTTTTACGACTAAAAAAAGAATGCACCT

>P*trpC* – promoter of *trpC* gene from *Aspergillus nidulans*
GAATTCATGCCAGTTGTTCCCAGTGATCTTCGTTTCGAAGATGGACACTCCCAATTTGTGCAAGTTAT
TCGGCCTACCTGGCTGTGGCCGAGGCGCGTTATCATGACCGTCGCTGTTCAAAGATAAGGCGAGAAGT
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GTTGATTTCATCAATCCCAGCCTTTTCGCTCAACCAAAGAGCATCGGCTTTGATTTCCTTCAGGTCAT
ACGAGGCTTGTGCAATGGTCTgCGCATGGATCGCTGCTGTTCTCCTATCAAACTCGGATTTTGTCTTA
GGGGATGGCGTAGGAAAGACGCTGCCGCGGTTCAGAAGCACCTCGATGCTATCAGGATGTGACAAAAA
CGACTCGAAAACCCGGGATTCATCGGTGATGCTTTCGGGATCGCAAGCGTAAAGAAAGACTCTCTTCC
AAGACCTAGAAGTATAGCAAAATCAGCAGCAGACCATCAATGTATAGCGAATGCGCCCATACAAAAGC

TGAACGTCCCCGGAGAAGCACTTGTCCAGGGACGGGAAATAGGCTTCCGGAACGGGAGCCATTGGCAG
CACAGCTATATCATTCTAAGTAAACAAATGTAATGAGCAAGCGGACGGAGTGCTGAAACCTCCGTATG
CCTGAAGCCGACGAAAGCGCGTTGGATTAGAGGTCGACAGAAGATGATATTGAAGGAGCACTTTTTGG
GCTTGGCTGGAGCTAGTGGAGGTCAACAATGAATGCCTATTTTGGTTTAGTCGTCCAGGCGGTGAGCA
CAAAATTTGTGTCGTTTGACAAGATGGTTCATTTAGGCAACTGGTCAGATCAGCCCCACTTGTAGCAG
TAGCGGCGGCGCTCGAAGTGTGACTCTTATTAGCAGACAGGAACGAGGACATTATTATCATCTGCTGC
TTGGTGCACGATAACTTGGTGCGTTTGTCAAGCAAGGTAAGTGAACGACCCGGTCATACCTTCTTAAG
TTCGCCCTTCCTCCCTTTATTTCAGATTCAATCTGACTTACCTATTCTACCCAAGCATCGAT

>P*paxA* - promoter of *paxA* gene from *Penicillium paxilli*
GGAGCCATTGGAGCAATTTTTGGTTTTCCTTTCTTTCCAGTGTCTAGCTGTTGATTGTCACCTATCTT
GCTTTCAAGAAACATCACTAGATTTGAGTTCAATTAGTACTATATCGTTAAGCCGCTTTGTTTTAAAC
CGTCTTCAGGCTCCCTGCGAGAGCATTCAAGCGATCTCAAATGGGCAGCGAATACTCACAGCTTGAGT
AAGCCATACTATGAAAATTCCATACACTATCCATACCTACTTTTTCTACGAAATATTTCTACTGCAGA
GGGGTACTCTGACTGACCAGTACGATGGAAGATAGAACTTTCGAAATTGACAATCGACATTCCTTCAC
CACCTTCTACTACACTGGGTCAACTCCGAAAACGAAATTCTATATTAAATGTGGCAAATCAAGGCCGG
ACAGTGTCTCATAATATATAAGCAATGTTCCAAGGTTAGTATCATAATGCAAGTCCTAATCCACCTCT
ACGACAGAAGAATTACCTGGGTGGCCGTAGACCCACAAGCAATCGAGTCCAGCCAGCGAACTTGGTAA
TTTCGTCAGCTGTAATGGATGTCAGTTACACCGATGAAAGATCCAATTTCCTCCTAGCTATAATTCCT
CGTCTTTTTGGGCCATGTTTCATTCTATCGGGGATTTAAGAAGGGAGATCTCACCAGGCTATTGTATC
AGATGCCCATTGTTTTGCAACTCTATTCTCGGAAATCGGCCAATTAGCAAAACAGTTAAACTGAAGTA
AGCCAAA

>P*paxG* - promoter of *paxG* gene from *Penicillium paxilli*
AGATTCACGACCTGTGACTAGTCAAGGTTATCAATTTTAAAACTTACTGGGTGTTGTGTTGAATACTATGCACGA
GATCTGGCACGTATAATCTATGGAGTTTACTTCCGTACACAGAATTACATCTCGCTTTGTGAATACGAATCACCC
AGTCATGGATGTATTACAACGCAACCATAATCGTTGCATCATGTCATCCATGTTCGCACGCCTTGTCTATAATAC
GGAGTTCTCAAGTGGGGGCAGAGGGATTTATTTTCGAGAATTTGAAACATGACTGTACCGCAGTTGTAATTATAG
AGAGTTATTTAACTGGGGTAGTTGGGAACGGATTTTTAGCGGCATAAATCAATACAAGGATCCGGTGTCGTTTAC
TCTCGAACCCTACCCATACCCCGGAATTTCCTTCATCGCAATTTTGCAATCCTACTCTGCAAAATAGAAAAGAAT
TTCTTCCAGTCCCATTTCTTTCATCATAGGGTTATAAAAATAGTGCTCTTGAATTCAGGTTCACGCCAGAGAAAA
CTTCATCAAGTTCGACGCCA

>P*paxM* - promoter of *paxM* gene from *Penicillium paxilli*
ACAATGCATTATCTATGGAGGGATGAGAAAAAGTAAAATGAAATTGAAGAAAAGAAAAGAAAATTAGAAACAAAA
ATACACGTTTACTTTTTGAAATAACCTTGGAAACTAAGATCCGAAAAAAGAAATCTTTTCTTCATGCATTTCTGC
CAGTGCTAGATTGAAATTGAAGAAAAGTACATACGATGTACTTTGCTTACGATACGCGTAACGTGCATGTCCAAG
GCGACCCAATCCATGATATAATTGGCACGTTTTAGTAGTAAGCAATGATCATTTGTGGCTGACACTATGCAATCT
GTATCATATGATATTAGTATGCAAAGGTTGATTCTGAGTTTGAATTTCAACCACTGTTCATCGTTCCTCATTGTT
AACCTTATTCATATTTCACCTTTTGCTTTATTCTTTTCATGTATCTTTAAGATTCAGAAACCA

>P*paxB* - promoter of *paxB* gene from *Penicillium paxilli*
GTACCTTAATAATTCCGGTTTCTCCGTGATGCAGATATCAAACTATGCAGTAGTAATTAATCTTTTATTTTTTCT
TCATACCAGCCCCTGTAATGACAACATCACGCCTAAATAGGTTAAGCGCAGCAACGCCTCAAGATCCTTCATGCA
GTGAGAAAAATAAGAATTATAATATTAATTAAAAAAGAGGGAAAGTAACGAAACTTTTGCCCTGATCCACATCGA
CGCCTCGATTTTCCAGGCGCACCGTACTCAAATCGGCTTTCATTCCTTTGAGCCATTTTCTTCACTTGGCAGTAA
GCCTAAACTCTGCCTATCCAAAGACAGCTAATTGTTGCGACGCAGTTCGTCTGAATTCCATGGCAAGAATGACTG
CATACTAGCTTTCGTTGGCGAAATAGACTAGTTTAGATCTTGCAGGATCTCCTCGTGGACTGCATCTACAGAGAT
TTCCGGGAGTGTCCATCTCCCGCTGCGGGCTAACCGATGAGATAATGCAGTTTCATTAACAATTCAATATTCCCA
TTTCATCCACAGGGCATAAATAGACGTACATTTAGCCGTTAATACGCATCATGGTCAAATCCTTCAATCTATAGC
TATTCAACGAGTGACTCTAATTTCTTTTTCCCACGTCAACCTTAGAAACTA

>P*paxC* - promoter of *paxC* gene from *Penicillium paxilli*

GGAGAGAATCGAAAGAGTATTCATGTTGACTTGGAACAGAGAATTTGGTATTGACTAACGTATTCTCGAGTTCTC
TCAAGGAAAGGAAAAATGAAATGAGTCGAGTAGCTCTGCTTGGTAGGCTGTTGAGATTCGGCGCAATACATCCCC
TCCATATAATAAGTAATGCAAGGAGTATACTTTCCCAGGTGTCCAATCGGCGGCTGCAGCTATCACAATTAGAAA
AAGCAGTGGGATTCATCGCGTTCAGCATTTTCGCCCCACAATTGTCAGCTTTCAGGCGTGATCCCAAAAGTGATA
CATGTTACTAAATTTACGGATTGACCTCATTCAGCGATGTCTACGATGAGCAACGAGGTAGTAGTATCAAGAGGG
GCTGTCGGTAC

>P*janO* - promoter of *janO* gene from *Penicillium janthinellum*
AGTTTAAGATGATCTTGAGCTTGATGAAGTGGAAAGATTAGGTTTGGTCAAACAAGGTGCTCGCTGAG
TGCACCCCACGACTAATCAGATTTATATGAGAAAGAAGGGTGAGTGATGTTGACAAATTACAGTATCT
ATTGATGAAGATCAAAGGACCATGATTGACATCTGGTATCTGGTCAACGGTATCATGCATGTCGTATT
ACCCCACTTTCGCAGGATGAAAAGTCGAAGAACTACAGTCAGCCCGGATGTATAGCAGCTCTATACGC
AAATCAGAATGTGCGGTGGGCCCCTGTGATCTATCAATTACCTTCGAAGCCATGATAAGAATCCCGAC
TCCTGTGCAGGAAAATAGGACGAGATTCATACAGCTAGTGGTATTGTGTATTCCATACCGTACAGAAC
GTGTAGGGTTACACGTTGAGTGAAGCCCAATCTGCCCGAATGCATATGCCAATTACTAGCTCATAGCG
TTATGCGAGGCGGAAATAGTTTGATGGGCTACCTGCGCCGAGAATCTGCATTTCTTTTCTATGCCAAG
TGACACGTAGCTAAATTACATCTGATATTCGAGTGCATAAACTATAGCTGCAATGCAGGAAACATAGT
GATTGAGATTGCGTCTGCATGGGCTAATGTGGACTGCAGTGAAACGTTGCAATCAACATGAACACGAG
AGCTGATCTAGAGAATCGAGTCTTTACCGATGAGGTAGAGGTGAGACAGGTCCAGAGCTGTGCAGCAC
CTAGGAGGTGTTCAGTTTCCTTATTGCGTAATTTTACCTTATGTTTTTGGGGTCAATAGGTAGCTGAT
AGGAGAATCTCCTTGGCCAGTAGGGCTCATGAACACTTAAATACCTATATCACTTCGCCTTGCCCACT
ATCTTTTCACTTCCTTGGAATACCACTCAAACAAATTTGCTGGCTGAATGCAAGTACTTCAGTTTCTT
TTTTCTTTCTGTTAATAAGCATTACTTGAACCCCCTACAGCCTTCCTCGTGCCCTTGACATCTATTTA
AGCCCGATCTCTGAGTTTTCGTTGAGAAAAAAGGACGGGTAAG

>P*tefA* – promoter of *tefA* from *Aureobasidium pullulans*
GGTAGCAAACGGTGGTCAAAGGATGGTTCAGATACAAATTAGCAACAGGCCAGGCTAGACGCGCGACT
ATCCACTGCGGCAAATGGTGAGCTGCAAGCAACGGTAAGATGTGACAGGACGAGCGGTGTGCCGGGAA
AAAAATTGGAGGAGCGCAAAGCGGCGGCTGTCCCTCAGTGGTGCCCAAACGTTATCGATAGTACACCA
AGCATGGGCAGTGAGCGGCTATACAGAGGGAATAATAGGCATATCGGCACGACTAGATTCGGTAGAAA
GCATCGAAGAGCAATTCATTGAGCATATTATCACGTGGAATGCGATAGCTGTGGCCAGGTTGAGACAC
CGCAAGTGAAAGATACACACATAGATTCTCGATTCGAGCGGTTTGCCTCCGCCACCGCAGTGCATAGC
AAGCAAAGAAACGACAGTTGGCTCATCATCCGTTACATCATTTTTTCTACTGGCTCCGCTCGGTGGGC
TCCCAACGAAGCAGCAAAAAAGTGAGAGAAAAAAACTAGCTTGGCGGGGCAACAGAAGCTAGACCCTT
TGGCTCGCTTAGTCAGTGCGCCCACTCACTCACACTCAAAAAGGCCACCCCTCCCGCACCCTCTTCTC
ATCACCGTCTTCATACCACGGTTCGTCAAGCAATCGTATCTGGTAAGCTTTGACCTCCTCGAGCGGGC
TCCACTTTGCTATTTCTTGGATCTGCTCTTTCTTTTCTCTCTACCTCTTTTTCTAACCTCTCTTCAGA
AAGTTCAACCGTACTTCACTCAATCTTCCATACATCACCGTCAAACC

>*natR* – gene from *Streptomyces noursei*
ATGACCACTCTTGACGACACGGCTTACCGGTACCGCACCAGTGTCCCGGGGGACGCCGAGGCCATCGAGGCACTG
GATGGGTCCTTCACCACCGACACCGTCTTCCGCGTCACCGCCACCGGGGACGGCTTCACCCTGCGGGAGGTGCCG
GTGGACCCGCCCCTGACCAAGGTGTTCCCCGACGACGAAAGCGACGACGAATCGGACGCCGGGGAGGATGGCGAC
CCGGACTCCCGGACGTTCGTCGCGTACGGGGACGACGGCGACCTGGCGGGCTTCGTGGTTGTCTCGTACTCCGGC
TGGAACCGCCGGCTGACCGTCGAGGACATCGAGGTCGCCCCGGAGCACCGTGGGCACGGGGTCGGGAGAGCGTTG
ATGGGGCTCGCGACTGAGTTCGCTCGCGAGCGAGGCGCCGGGCACCTCTGGCTGGAGGTCACCAACGTCAACGCA
CCGGCGATCCACGCGTACCGGCGGATGGGGTTCACCCTCTGCGGCCTGGACACCGCCCTGTACGACGGCACCGCC
TCGGACGGCGAGCAGGCGCTCTACATGAGCATGCCCTGCCCCTGA

>*neoR* – gene from the Tn5 transposon of *Escherichia coli*
ATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGA
CTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGG
TTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCG

TGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTG
GCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTAT
CCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAA
GCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGA
CGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCG
AGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCT
GGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGA
TATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCG
ATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAG

>*hphR* gene from *Escherichia coli*
ATGAAAAAGCCTGAACTCACCGCGACGTCTGTCGAGAAGTTTCTGATCGAAAAGTTCGACAGCGTGTCCGACCTG
ATGCAGCTCtcGGAGGGCGAAGAATCTCGTGCTTTCAGCTTCGATGTAGGAGGGCGTGGATATGTCCTGCGGGTA
AATAGCTGCGCCGATGGTTTCTACAAAGATCGTTATGTTTATCGGCACTTTGCATCGGCCGCGCTCCCGATTCCG
GAAGTGCTTGACATTGGGGAGTTCAGCGAGAGCCTGACCTATTGCATCTCCCGCCGTGCACAGGGTGTCACGTTG
CAAGACCTGCCTGAAACCGAACTGCCCGCTGTTCTCCAGCCGGTCGCGGAGGCGATGGATGCGATCGCTGCGGCC
GATCTTAGCCAGACGAGCGGGTTCGGCCCATTCGGACCGCAAGGAATCGGTCAATACACTACATGGCGTGATTTC
ATATGCGCGATTGCTGATCCCCATGTGTATCACTGGCAAACTGTGATGGACGACACCGTCAGTGCGTCCGTCGCG
CAGGCTCTCGATGAGCTGATGCTTTGGGCCGAGGACTGCCCCGAAGTCCGGCACCTCGTGCATGCGGATTTCGGC
TCCAACAATGTCCTGACGGACAATGGCCGCATAACAGCGGTCATTGACTGGAGCGAGGCGATGTTCGGGGATTCC
CAATACGAGGTCGCCAACATCCTCTTCTGGAGGCCGTGGTTGGCTTGTATGGAGCAGCAGACGCGCTACTTCGAG
CGGAGGCATCCGGAGCTTGCAGGATCGCCGCGCCTCCGGGCGTATATGCTCCGCATTGGTCTTGACCAACTCTAT
CAGAGCTTGGTTGACGGCAATTTCGATGATGCAGCTTGGGCGCAGGGTCGATGCGACGCAATCGTCCGATCCGGA
GCCGGGACTGTCGGGCGTACACAAATCGCCCGCAGAAGCGCGGCCGTCTGGACCGATGGCTGTGTAGAAGTACTC
GCCGATAGTGGAAACCGACGCCCCAGCACTCGTCCGAGGGCAAAGGAATAG

>Thymidine kinase(*tk*)- gene from herpes simplex virus
ATGGCTTCGTACCCCTGCCATCAACACGCGTCTGCGTTCGACCAGGCTGCGCGTTCTCGCGGCCATAGCAACCGA
CGTACGGCGTTGCGCCCTCGCCGGCAGCAAGAAGCCACGGAAGTCCGCCTGGAGCAGAAAATGCCCACGCTACTG
CGGGTTTATATAGACGGTCCTCACGGGATGGGGAAAACCACCACCACGCAACTGCTGGTGGCCCTGGGTTCGCGC
GACGATATCGTCTACGTACCCGAGCCGATGACTTACTGGCAGGTCCTGGGGGCTTCCGAGACAATCGCGAACATC
TACACCACACAACACCGCCTCGACCAGGGTGAGATATCGGCCGGGGACGCGGCGGTGGTAATGACAAGCGCCCAG
ATAACAATGGGCATGCCTTATGCCGTGACCGACGCCGTTCTGGCTCCTCATATCGGGGGGGAGGCTGGGAGCTCA
CATGCCCCGCCCCCGGCCCTCACCCTCATCTTCGACCGCCATCCGATCGCCGCCCTCCTGTGCTACCCGGCCGCG
CGATACCTTATGGGCAGCATGACCCCCCAGGCCGTGCTGGCGTTCGTGGCCCTCATCCCGCCGACCTTGCCCGGC
ACAAACATCGTGTTGGGGGCCCTTCCGGAGGACAGACACATCGACCGCCTGGCCAAACGCCAGCGCCCCGGCGAG
CGGCTTGACCTGGCTATGCTGGCCGCGATTCGCCGCGTTTACGGGCTGCTTGCCAATACGGTGCGGTATCTGCAG
GGCGGCGGGTCGTGGCGGGAGGATTGGGGACAGCTTTCTGGAACGGCCGTGCCTCCACAGGGTGCCGAGCCTCAG
AGCAACGCGGGCCCACGACCCCATATCGGGGACACGTTATTTACCCTGTTTCGGGCCCCCGAGTTGCTGGCCCCC
AACGGCGACCTGTACAACGTGTTTGCCTGGGCCTTGGACGTCTTGGCCAAACGCCTCCGTCCCATGCACGTCTTT
ATCCTGGATTACGACCAATCGCCCGCCGGCTGCCGGGACGCCCTGCTGCAACTTACCTCCGGGATGGTCCAGACC
CACGTCACCACCCCCGGCTCCATACCGACGATCTGCGACCTGGCGCGCACGTTTGCCCGGGAGATGGGGGAGGCT
AACTGA

>*paxA* – gene from *Penicillium paxilli*
ATGACATCCATCACCACGAGTGTTCTTGTTTTACACTCGCTACTTGCGGCAAACTTCAAGTATTACCAATCCTTT
CAAAATGGCTTCATCGACATGTTATCAGCAATGGCCGACAGCAATTCCGTTTCGGGACTTCCCGGACAACTCTGC
CGCGAGTATACAGGAATAAGGCCCCTTGATACATTCTTGACAAGCTGTACTGTATTCTTCTGGCCAACCTTTCAG
GGTGAGATTCCCGGATTGTCCCTTTATGGAATCGCTTTCGCTAGTGCCATGATTCCGATGTGGTTGATAATCGTG
ATCGATGTACATCGGCGCCGTCAGCCGTTTGGCGCCTTGGTGGAGTAAGCCCGCGTGCAGATCATCGGTATAGCG
ACCCAAGCTGACAGGAATCGCTTCGATAGGCTCATAGCTTTTGCCGGACCTTTGATTCAATGTATTGGACCGGGC
CTTGTAATGCCACTTCTCTTAGCAAGGATTCATACACCAAGTCGCGACAGCAAATCAGCTTCTCAATTCGATTAT
CGCACATTCATCCCCTCAATGATCATCGGATACATCTTACCTTTACTTCTCGCCTCACTCCCAGCTCCACTTATC
CTTTCGTATCATAATAAGCAGCAATTTATTGCGATCTGGCAAGGATGGCCCCTGTACAGTTCCGTTCTCATGTGG
GCCTTCCGTCGCCGCTCGGGCCACGTACATTGTTCGCGCCATAAGGGGCTAAAGCATGCATGCATATTTGCCTTG
GCTTGCTCATCTGCAGGCCACCTGGTTCTTCTGTCATTGACTTGGCTCTGGAGTTTGTCCTATTGGGGTTACATT

CAATCTGCCCCATGGAACGAACCTCCATTGGCCAGTCTAGAAGCTGGCGTACTCCGCTTCCTTCAGTGGGACTAC
ACGTTATCGGCTTCAGCCACGTTGAGCTGGGCGATTGCTTTCCGACACGAAGTAGTTCAACAAAAGTCCCTCCGC
ATTTCGTTACTCTCACTCCTTCGTTGCCTAATTGGGATCGTTTTTCTTGGGCCATGCAGCATGGTGGCCTTGTTG
TATTGGCAAACATGCTCACTGCAGGAGGAAGCTGGGCAAAAAGCGCCGGCTAAAGATAAACAACTGGACCAGGAG
ATCTAG

>*paxB* - gene from *Penicillium paxilli*
ATGGACGGTTTTGATGTTTCCCAAGCTCCTCCGGAGTACCAGGCGATTAAGCCATTGGCTGATCTTTTTGTTGTT
GGCATGGGAGTAGGATGGATCATAAACTACATCGGAATGGTCTACATCTCGTTCAAACATGAAACTTACGGAATG
TCGATTATGCCCCTCTGTTGCAACATCGCGTGGGAACTGGTCTACTGTCTGGTCTTTCCCTCGAAAAGCCCGGTG
GAGAGGGGTGTATTCTGGATGGGCCTCCTCATCAACTTTGGTGTCATGTACGCAGCAATAACGTTTTCATCCCGA
GAGTGGGGCCATGCGCCGCTTGTTGAGCGCAATATTTCCTTGATATTTTTCGTCGCGACCATGGGCTTTCTCTCT
GGGCATGTTGCATTGGCCCTTGAAATCGGGCCTGCGCTGGCATATTCATGGGGGGCCGTCATTTGTCAACTTCTT
CTAAGCGTTGGTGGATTGAGTCAACTGTTGTGTCGAGGCAGTACGCGTGGCGCATCCTATACTCTATGGTATGTC
TGATCTCATTTCATGCCCGATGAATCGGAATTTCTTCCCCAAAGTGACAGAAATACAAGTACTCATCTTCTGTAC
TATAGGGCTTCCCGCTTCTTAGGATCAACATGTACAGTTGGGTTTGCCGGCTTACGTTGGATGTATTGGTCGGAA
GCATTCGGTTGGCTGAATAGTCCTTTGGTACTGTGGAGTCTTGTGGTGTTTTTATCAATCGATGGGTTTTATGGA
ATTTGTTTCTGGTACGTCGACCGTAACGAAAAGTCACTTGGCATAAGCGGGCCGAAAAAAGCAAATTGA

>*paxM* - gene from *Penicillium paxilli*
ATGGAAAAGGCCGAGTTTCAAGTTATCATTGTGGGCGGGTCGATCGGAGGATTGACATTGGCACATTG
TCTACATCGTGCGGGAATAAAACATGTTGTCCTGGAAAAGGCCAGCGATCCAGCACCACAGATTGGAG
CATCCATCGGCATTCTGCCGAATGGAGCTCGCGTGCTGGATCAGCTTCAGCTCTATGATCAAGTTGAA
GAGCATATCGAACCGCTAAGCAAAGCTACAATTGGGCTTCCTGATGGGTTCAACTTCAGCAGCTCATA
TCCAAAGATCATCGATCAAAGGTCAGTAATTATCTTGATCTTATACAGTTTTGCATTATCATCTAAAA
TGGCACCCGTGAGGCAACAGGTTCGGTTTTCCTATAGCGTTTCTAGATCGACAGAAGATGCTCGAAAT
TCTCTACAAAGGGTATCCAGATCCCAGCAAAATACGTCTGGGCCAAAGAGTTACATCAATTGAATCAT
TGGATGATGGAGTGCTAATTACCACCACAACTGGACACGTTTATCGTGGGGATCTTCTTGTCGGCGCA
GATGGAGTCCACAGCATCGTCCGCAGAGAGATATGGAAGGCAAGGGGAATTGCCAGACGGGTATCAAA
GATCAAACAAGATAGTTCCAGTGAGTGGATAGCATCAACAGGCCTCATGTCTTTGCATTCATGCTAAC
AAGTACGAAGAGCTTACGGTCGAGTTTCGCTGTATTTTCGGCATTTCATCGGCGATGCCGGGATTAAA
ACTTGGCGAGCAAGTCAACGCTTTATTCGATGGACTCACTATTGTCACAATCCATGGAAAAGACGGTC
GCATATATTGGTTCGTCATTCAAAAGCTGGGCAAGAAATACGTGTATCCTGACAGCCCCCGCTACACG
TCCCATGAAACATCTATTGCGGCAGAAGAGATCAGGGATGTCAAGTTTTACGAAAACATTACTTTTGG
CGAGCTCTGGGACAAAAGAGAGACATCTTCAATGACTGCTCTGGAAGAAAATACATTCAAAGTCTGGC
ACCATGGCCGCTGCGTGCTCTTAGGAGACAGTGTCCACAAAATGACTCCCAATGTTGGTCAAGGGGCT
AATATGGCCATTGAGGATGCCGCTGCTCTGGCAAATCTCCTTCGAAAGATGCGGATTTCCTCTGGACC
ATACTTTCCCACCTCCTCACAAATGGAGTTCCTTTTGCAAAAATATCGCGACCTTCGATATGAGCGCG
TCAATACTATCTACCAGAGCTCTCGATTCTTGGTTCGTTTTCAGGTTCGTGATGGCATAATCTATAGC
CTGCTAAGCCGGTATTGGGCACCATACGCTGGTGACCTGCCTGCTGATATGGCGTCAAAAACGATTGC
TGATGGCACAATGTGTGATTTCCTCCCTACGCCAAAGCGAAGTGGTGGTGGATGGGAGAAGTATAGCA
AACAGGGGCGAAGCTGGAGTTATTTGACTCAACTTATGATCTATCTATTTGGACTAACCATTGTCTAT
ACCTCTCTTACAATGATGTTCGACCTCGAGGGTGCCCTGAAGTTTTATTTTCTTCAAGTTTAA

>*paxC* - gene from *Penicillium paxilli*
ATGGGCGTAGCAGGGAGCGGAGTTCTTTACTTTCTTTTCAACAATGTCCCGAGTCCTCGCTTCTGGTTGAAGAAA
ACCCAGTTGATAGGAACCGAGAACCCAGAAGGCATAACTGGCTACGAATGCCCTTATGAATATCTGCGGAAGTCA
TACGGCAAGCATCACTGGGCAGCGTTCGTTGACAAGCTATCGCCCAACCTTCAAAATGAGGATCCAGCTAAATAC
CGCATGGTGCTTGAAACAATGGATGTCATTCACCTATGCTTGATGATGGTCGACGATATCTCCGATGGAAGCGAA
TATCGCAAAGGAAAGCCGGCTGCGCACAAAATTTATGGTGCGCCTGAAACAGCGAATCGGGCTTATTATCGAGTT
ACACAAATATTGGCTCAAACTGCGACAGAATTCCCACGTCTTTCGCCTTGGTTGATGACTGATCTTCGGGATATT
CTCGAGGGTCAAGATATGTCCCTTGTCTGGCGCCGTGACGGAGTCAATGGGTTCCCTGGAACTGCATCGGAGAGA
ACTGCTGCTTACAAGCGCATGGTTCTGCTAAAGACAGGTGGACTATTTCGTCTACTCGGGCATCTCACTCTCGAG
AACAATTCCATGGATGAAGCCTTTAGCACCCTTGGCTGGCATTCGCAATTGCAAAATGACTGCAAGAATGTCTAC

TCGTCGGAATATGCCAAGATGAAGGGCGTTGTAGCAGAAGATTTGCTCAATCGTGAGATGACATACCCCATCGTA
CTCGCACTGGACGCCTCTGGTGGTCATTGGGTAGAGGCAGCTCTAAAGTCGCCCTCTCGGCGAAACGTCGGAAAT
GCCTTGAAGATAATACAGTGCGACTATGTTCGAGATGTTTGCATGGCAGAGCTGGCGAGATCTGGTGCCCCGGTT
AAGGAATGGTTGAAGTTATGGAAACGGGAGGAGAAGCTTGACCTGAAGGCATGA

>*paxG* - gene from *Penicillium paxilli*
ATGTCCTACATCCTTGCAGAAGCTCTGAACTTCGTTCGTCGAGGAATATCTCATCTTAATTATTGGGGAGCTTCA
CACTCATTATCTGCTGATAATTACTGGGAATCAAACTTCCAAGGATTCCCAAGACTCCTGAGTGATTCCAGCAAA
GCACCAAGCACGATTAGAACAGTACAGGTGCTAGAAGATGACGTTGACGATATTGCTATTCAGTATAACAAGATT
GTTCGTGGACCTCTAGACTATCTTCTAGCGATTCCGGGAAAAGACATCCGAAGCAAGCTGATCGACTCCTTCAAC
ATATGGCTCCAGCTTCCAGAGGAGAAGTTGTCTATTGTGAAAGATATAATAAACTTACTTCACACTGCATCCCTT
CTTATCGATGATATTCAAGATGCATCACGGCTCCGGAGAGGAAAGCCTGTGGCTCATGATGTATACGGTGTAGCA
CAAACTATCAACTCAGCCAACTATGCCTACTATCTCCAACAAGCAAGATTGAAAGAGATTGGTGACCCCCGCGCC
TTCGAAATATTTACAAGGTCACTGCTAGATCTACATCTTGGCCAGGGCATGGACCTATATTGGCGTGATATGGTG
GTTTGTCCGACCGAGGAGGAATACACCCGGATGGTTATGTACAAAACTGGAGGTCTTTTCAACCTGGCCCTCGAT
TTGATGCGTATCCAATCCCGCAAGAATACTGACTTTTCTAAACTTGTCGAGCTATTGGGTGTTATATTCCAAATT
CGTGATGACTATATGAATCTGCAGAGCGGACTCTATGCTGAGAAAAAAGGGCTGATGGAAGATTTGACGGAGGGG
AAGTTCTCCTATCCTATCATACACAGCATCCGTGCATCTCCAGAGAGCTCCGAGCTTCTCGATATTCTAAAGCAA
CGCACAGAGGACGAAGCAGTCAAAATCCGGGCTGTGAAGATCATGGAGTCGACGGGGAGCTTCCAATATACTAGG
GAAACCCTAAGTCGACTTAGTGCGGAGGCTCGTGGCTATGTAAAGAAGCTGGAGACTTCCTTAGGGCCCAATCCT
GGAATTCATAAGATTCTCGATCTACTTGAAGTGGAGTACCCTACTAATGAGAAAGGAAGAGTTTAA

>*ptmA* gene from *Penicillium crustosum*
ATGTCGCGTGTCATACGATCTATATTGATCATTCTCGGATCCGTGGCCCTATATACAAAGTATTATTTATCCTTT
CAAAATGGCTTCATTGATCTTCTGTCGACCATGGGCTCTCAGGGCTCTCTTGCTGGACTTCAAAGTGGTCTTCGG
TCACATTATACTGGCTTGGACCCACTTGATAGATTCCTAAAGGCATGCAACGTTTTCTTCTGGCCAATTTTCCAT
GGTACCTCGCCTGCTTTATCCCTCTATGCGATTGCATTCGCGGGCTCTATGATCCCGATGTGGCTGATCCTCCTC
ATGCATACGTGCGTGAACAGCTCAATTGTGGAAATAGTCATGATGTACGTATGTACTCACATTGTATAGCGTTAT
ACTGATCTTCCAAAATTAGAAATGCCCTCGCCGGTCTGCTGGTGCAAGGAATCGGTCCAGGGGTGATTATGTGTG
TTCTACTAGCAATGAGAAATACATCCATGAAAGAGTTTGCCGTCACCGGTATACCAGCGGTATCTATACTGGGGC
CAAATGATCTTCCACTGTCACTGGTCGTCTGTTATATACTGCCCTTAGCCTTGAGCAGCCTTCCAGCGCCTGCAA
GCATATCTGTTCCGTCTAAGCAATTATTTATTGCCATTTGGCAAGGATGGCCCCTTTATATTGCACTTGCCGTTG
GAATCGCACACTCCTTAAGGAATCACTATAGACGAAATCGCCCACAGCAACTGTTCAGGCATGCTTACGCCTTTG
CCCTTGCGTGTTCCATCATCAGCCACGTTGGTCTTCTTTCGATCTCATTCCTTTCGGTATCTCCTCAGAGCCCTT
TCTTGTCACTGCACTCGGCAGACCTTCACCCACGGAGCCTTTTGATCCCCAGACTTCCATGGCAGGAGGTGAAAA
TTACATCTCTAGAGTCTGGAGTCCTTAGGTTTTTGCACTGGGACTATAGTATCTCATCTACGGGAACTCTGCTGT
GGTGCTATGATGTGTATTGGAAAGACAGAATGAGAGGCAGGGGGTGGATCGCTTTTTTCTCTCTTTCATCTCGAC
TAGCTACAATGAGCCTGGCGTTTGGTCCTTGTAGTGTAGCACTTGCACTCTACTGGGCAGCATTGTCAAATAATT
TGATGAAGAGCGAGAATGCAAGGAAGAGATAA

>*janA* gene from *Penicillium janthinellum*
ATGTCGCAAACAACAACAGCCGCTCTGATTTCACTCTCTGTCTTTGCGGCCTATGCCAAGTACTACCAGTCATTC
CAAAACGGCTTTATTGCTCTCTTATCTGATATGGCGGACACTAAGTCTCTATCTGGGCTACCTGGTGGACTGCAC
TGCGAGTACACGGGATTTGCTCCTCTGGATCGATTCCTGACCGCATGCAATATATTCTTCTGGCCAGTATTCCAG
GGAGAAGTTCCTAATTTGTCGCTATACGGAGTGGCATTCGCTAGTGCCCTAGTACCAATGTGGTTGGTGATCGTA
CTCGAGACACATCGAGGAAGACGTCCCGTTGCTGCATTGATGGAGTAAGTTTTTGTCCGGTTTGCCGACGCATTG
AGGTGTATTGACCGAGTTCTATACGACAGGCTAGCTTTTCTAGCCGGCCCACTAGTCCAATGCCTTGGCCCAGGA
CTTGTGATACCTGCGATATTGTCGAGATTGCCTATGTCAACTCCTGGCACCAAGTTGCCTTTTGGATTCGATATA
GGCTTCTATCCCTCGTCGATGATCATTGGATACATTCTCCCACTCATCCTCGCTGCATTACCCACCCCGCGTGTC
ACTGCATATGAAGCAAAGCAGCAACTGATCGCAGTTTGGCAGGGTTGGCCTGTTTACACGTCACTTATCATGTTG
ATCATCCACTACCTACGCCCTATGCGAGCCTCGCAAGATTGGCAGCTGAAGATAGCATGTGCGTTTGCCTTTGCA
TGTTCCACTGCTGGACATCTGGCGTTTCTGTGGTTTGCTCGAGCCAAGACTGCCTCGTATCACGTTTTTCTTCCA
CCGATTCCCTGGAGGGAACTGCAAGTGGCGAGTATCGAGGCGGGAGTGTTGCGATTTCTCCAATGGGACTATACG
TTATCTGCATCGGCTATGCTGGTCTGGACAGTTGCCTCCTACTGTCGGGCAACCGGAAAAAGGATTGGTCAGTCT
TCTTCGGTTATACTGATTTTCGGAATGGCCGGCGTGGTCTTTCTAGGGCCTTGCAGTGTGGCTTTGTTGCTGTAC
ACTTCGGTTGCGTTGCAGAGAAAGGGCGTCACGAACTATGACTGTTGCAGATCATCATGA

>*desA* gene from *Aspergillus desertorum*
ATGGATGTCTGGCACAAATTGAGAATCATGCTGGCGTGCCTGGCGCTGGTGGCAGTCTACACCAAGTATTATTTG
TCCTTTCAAAACGGTTTTTTTCCATTGCTCTCCTTACTCAAGGCTCAGACATTACTTCCTGGGAACGCAGGGCTG
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TTCAGTGGAAGTTCATGGTTGTTGTCACTCTACGCCCTCGCCTTTGCGGGTGGCATGGTACCAACTTGTGTCATA
CTGGGCACCCATGCCTGGAAGAGCGCGAATCCACTCAGGTCCCTCGTTCGGTACGTCACAACTGACCAAATACTT
GATCGGCATGTTGATATGTCAAAACAGAAACATACTCGCGGGCATTGCAATTCAGAGCATAGGCCCTGGAGTAAT
GGTCCCCGTCCTCTTGGCCTTGCAGTCGCCGACTTTGCAGCGCACACGTTACAGTGCTTCTGGGAACTGCATATC
CATTCCCATTGCCGTATTCTATGGATTGCCATTATTTCTGGCATCGGGTGCCGCTCCCAAAATAGTCTCAATCAG
CATGAAGCAACAGCTGATTGCTATTTGGCAAGGGTGGTCATTGTATGTCTTACTCTCGGTGCCCATATTGCAATG
GGTCGCAGCCAACGTGTCAATGTCTAGGTTGTCCAAACGACCTGCAAGACATGACAGAACGCATGTATATGCATT
TGCGTTTCTCTGCTCCACACTTACCCATTGGACTATGATCTGTGCTCTGTCTCTCGTCAAATCAGACATCATTTC
GACCAGTTGTGTTTGGAATTCCCTCACTGTGCCCAGGCTATCCTGGCGAGAGGATCAGGTGGCGAGTATTGAGGA
AGGTGTGCAGCATTTTCTCCAATGGGATTATAGCATTGCTGCTGTCTCGCTATTAATGTGGTGCGTGACTCTATA
CCAACACCACGCGAGAATGGCTCCGGTGTCCATCGAGACTTTTCGCTGTCTCCTGTGGGTGATGGCAGCGGCGTT
CCTTTGTGGGCCCTGTAGTGCGGCCGTATGGCTGTACTACGAAGCCGAGATTACCGTCTTAGCGAAGGAGCCATA
G

>*ltmS gene from Epichlöe festucae*
ATGTCGCGAAGTGATTGGATCTTTATCTCTCTGCAGGGTTTCTTTTGTTTAGCCGGCGTAATATGGAAGTCACGG
GAAGGATATCCGATCATTGACTTTCCCTGCCCATTACAGTTCATTGATTCTTCGGATGCTACATTAAGCTATGGA
ACCACATCACCATGGTTCGGGTTTCGCGCTGTTGCGTCGATGCAGTCCATTTGGGATAATGGGCCTTGGTTTTGG
CTTCACCTAATGCTTTACATTGCTCAGCTGGTTGGGCTCATTTTGATAATTCTGCATGAGACTGTACCGCATGGC
GCATTCCTCCGCAAGTTTGAAAGTTTGGCCGCACTAGGCTATCTCTCTTACACAGTTGGACTTTCCACTGCCTTT
CCCGTCTTTTCACTGTGGATCCTAAATCAATATCGTGCCGAGAAATTAGTAACAGCGTGGCCGAGGAGGCAAGAG
AAGGCTTTCCTAAGGACTATTTTCTGGTGCACTGGTATAAGCCACATTGGCATTTTTATGGTAGCGATCGTGGCG
ACTCTCCTTCACAGAGATGCCACTGCTCCCTTTCATATAGGGAATAGCCTCCTTGGTGTACCCGATTGCTCGCAG
TTTCCGTGTTCCGAGATCGCTGCACGACATGCTAGACTCAGACAGATTAATGAGATGACTGGTACATCAAGTGGA
TTTTTCTTGACCGTGGGGCTATTTTCTCAAGCACTTGAAGCAGAAAATAAACACTTGAGCTTACGAGTTATGGTA
AGGATGTTTTTTGTCAGTCTCATAGCTGGTCCAGCTGCAGGTAGCGCGGATGTGCTACTTCTACGAGACTCAATA
ACAAGATCTAAAAAGGACTGCGGGTAG

>T*trpC* – terminator of *trpC* gene from *Aspergillus nidulans*
GATCCACTTAACGTTACTGAAATCATCAAACAGCTTGACGAATCTGGATATAAGATCGTTGGTGTCGA
TGTCAGCTCCGGAGTTGAGACAAATGGTGTTCAGGATCTCGATAAGATACGTTCATTTGTCCAAGCAG
CAAAGAGTGCCTTCTAGTGATTTAATAGCTCCATGTCAACAAGAATAAAACGCGTTTCGGGTTTACCT
CTTCCAGATACAGCTCATCTGCAATGCATTAATGCATTGGACCTCGCAACCCTAGTACGCCCTTCAGG
CTCCGGCGAAGCAGAAGAATAGCTTAGCAGAGTCTATTTTCATTTTCGGcAGACGAGATCAAGCAGat
caacggtcgtcaacagaccTACGAGACTGAGGAATCCGCTCTTGGCTCCACGCGACTATATATTTGTC
TCTAATTGTACTTTGACATGCTCCTCTTCTTTACTCTGATAGCTTGACTATGAAAATTCCGTCACCAG
CCCCTGGGTTCGCAAAGATAATTGCACTGTTTCTTCCTTGAACTCTCAAGCCTACAGGACACACATTC
ATCGTAGGTATAAACCTCGAAAATCATTCCTACTAAGATGGGTATACAATAGTAACCATGGTTGCCTA
GTGAATGCTCCGTAACACCCAATACGCCGGCCGAAACTTTTTTACAACTCTCCTATGAGTCGTTTACC
CAGAATGCACAGGTACACTTGTTTAGAGGTAATCCTTCTTTCTAGA

>T*aceB* – terminator of *aceB* gene from *Aspergillus alliaceus*
GCTTTACACTGCGGTGATACAGAGATAGATGAGAAAAGTATCTTAAAAATAAAACTATTGAGCGGATG
AAGAAAAAGTAAACATTTGTATGGTTGACCAACGCGAAATGTAACGTTAGGTACGCAACTCTGCAGGA
AAATATATGGGTCAGCTGAAACCTACAGGTCCTAAACCTTGGCGAAACCAGGGTTTGGATTTCCAGTC
GGCGTCCGTCCAAATGCAAATGGTGTGATTTTACAGACACCTCTTTTTTTGCCGCTCCACTTTAGCTC
GTGAATCGGAGGTAGATCGCCATCAGAGAGCGTCGAGACTCTGCGTGGACACGCAGTTGGATTCATTA
AGTACTGCTAGCTTCCTATAGGGCCTGTGTGCCTTGAATCTCCCCGTCCAACCGTGCAAG

>T*paxG* – terminator of *paxG* gene from *Penicillium paxilli*

TCAATCGTGCTGCATTTCTCTTCCAGGATCGCTGTATCGCGGAGATAGAGATTCCTTTATGAATGAAA
TATTTTCCAGGCGTTTTTCAACCCATATTTTCCATTTATCGAATTTTTGCATAGAACTCGCAATAGTG
AAGCAAATGTACCTCGGACCGCGATCATTCAAATGTTTTGGCAAGGAAGTTGGTATCAAGTCTCACCA
TTCAGTAGTTAACCTTGGCGGTCATAAAATTAAATGACATGGACTTAACGATACTCTGAAAATGCAGA
GTTTATGCTCCAAATATTAGTAGCATTTATCAATATTGTCGTTGAGGTTCAAGTTATTCTATGAACTA
TTTTCAAGTTCGTGAGCTTGATGTGTTGTTAGATATACGGATAGTGCTTTTAACCAAGTGACACAAGA
AAAATGCCGAGGTTT

>T*paxM* – terminator of *paxM* gene from *Penicillium paxilli*
ACCATTGGAGCAATTTTTGGTTTTCCTTTCTTTCCAGTGTCTAGCTGTTGATTGTCACCTATCTTGCT
TTCAAGAAACATCACTAGATTTGAGTTCAATTAGTACTATATCGTTAAGCCGCTTTGTTTTAAACCGT
CTTCAGGCTCCCTGCGAGAGCATTCAAGCGATCTCAAATGGGCAGCGAATACTCACAGCTTGAGTAAG
CCATACTATGAAAATTCCATACACTATCCATACCTACTTTTTCTACGAAATATTTCTACTGCAGAGGG
GTACTCTGACTGACCAGTACGATGGAAGATAGAACTTTCGAAATTGACAATCGACATTCCTTCACCAC
CTTCTACTACACTGGGTCAACTCCGAAAACGAAATTCTATATTAAATGTGGCAAATCAAGGCCGGACA
GTGTCTCATAATATATAAGCAATGTTCCAAGGTTAGTATCATAATGCAAGTCCTAATCCACCTCTACG
ACAGAAGAATTACCTGGGTGGCCGTAGACCCACAAGCAATCGAGTCCAGCCAGCGAACTTGGTAATTT
CGTCAGCTGTAATGGATGTCAGTTACACCGATGAAAGATCCAATTTCCTCCTAGCTATAATTCCTCGT
CTTTTTGGGCCATGTTTCATTCTATCGGGGATTTAAGAAGGGAGATCTCACCAGGCTATTGTATCAGA
TGCCCATTGTTTTGCAACTCTATTCTCGGAAATCGGCCAATTAGCAAAACAGTTAAACTGAAGTAAGC
CAAAA

>T*paxB* – terminator of *paxB* gene from *Penicillium paxilli*
ACAATGCATTATCTATGGAGGGATGAGAAAAAGTAAAATGAAATTGAAGAAAAGAAAAGAAAATTAGA
AACAAAAATACACGTTTACTTTTTGAAATAACCTTGGAAACTAAGATCCGAAAAAAGAAATCTTTTCT
TCATGCATTTCTGCCAGTGCTAGATTGAAATTGAAGAAAAGTACATACGATGTACTTTGCTTACGATA
CGCGTAACGTGCATGTCCAAGGCGACCCAATCCATGATATAATTGGCACGTTTTAGTAGTAAGCAATG
ATCATTTGTGGCTGACACTATGCAATCTGTATCATATGATATTAGTATGCAAAGGTTGATTCTGAGTT
TGAATTTCAACCACTGTTCATCGTTCCTCATTGTTAACCTTATTCATATTTCACCTTTTGCTT

>T*paxC* – terminator of *paxC* gene from *Penicillium paxilli*
TTGGCCTTGTGAAATATGGGACTACGAAGTGAATCAGAAGTGAATATTGAGTATTTGAGTCTAGATAG
TTCACAATTTTACTTGCAATTACCAAACTTTGCAGAATTCTACCAAGCTAGACTACATGCGGGGTGTA
TCCTTCTTAGACTCATGTCTGCAGTTCAGTATACAACGAGGATGCCTTCTATAGGATATTGAATCTAC
GTTGAAGTATTCCTTATAGAGGTTGCAAAGTGCCCAGCAACATCTGATATCCGACATGACAGAGAT

>T*paxA* – terminator of *paxA* gene from *Penicillium paxilli*
ATTCACGACCTGTGACTAGTCAAGGTTATCAATTTTAAAACTTACTGGGTGTTGTGTTGAATACTATG
CACGAGATCTGGCACGTATAATCTATGGAGTTTACTTCCGTACACAGAATTACATCTCGCTTTGTGAA
TACGAATCACCCAGTCATGGATGTATTACAACGCAACCATAATCGTTGCATCATGTCATCCATGTTCG
CACGCCTTGTCTATAATACGGAGTTCTCAAGTGGGGGCAGAGGGATTTATTTTCGAGAATTTGAAACA
TGACTGTACCGCAGTTGTAATTATAGAGAGTTATTTAACTGGGGTAGTTGGGAACGGATTTTTAGCGG
CATAAATCAATACAAGGATCCGGTGTCGTTTACTCTCGAACCCTACCCATACCCCGGAATTTCCTTCA
TCGCAATTTTGCAATCCTACTCTGCAAAATAGAAAAGAATTTCTTCCAGTCCCATTTCTTTCATCATA
GGGTTATAAAAATAGTGCTCTTGAATTCAGGTTCACGCCAGAGAAAACTTCATCAAGTTCGACGCC

>T*paxC-P* - terminator of *paxC and paxP* genes from *Penicillium paxilli*
TTGGCCTTGTGAAATATGGGACTACGAAGTGAATCAGAAGTGAATATTGAGTATTTGAGTCTAGATAG
TTCACAATTTTACTTGCAATTACCAAACTTTGCAGAATTCTACCAAGCTAGACTACATGCGGGGTGTA
TCCTTCTTAGACTCATGTCTGCAGTTCAGTATACAACGAGGATGCCTTCTATAGGATATTGAATCTAC
GTTGAAGTATTCCTTATAGAGGTTGCAAAGTGCCCAGCAACATCTGATATCCGACATGACAGAGATGA
GCATGGATTACAATTTTGCGAGGGCGGCCATTCAATCTCAAGGCAATGTCCCTTTACAGAAGAACCAG

AAAAGGATGCCAACAAGGTTAATGCACTCAGGTATTCTGATCCGACTCACTGTTACAGAGAATAGAAG
ACATTCCTGAGGATTAATAAACAGAAAGCATAATTCAATTCATTCTAAGATGTAAAATCTATCTCCAG
TGAACGTCCACTGCCCACCACTGCGTTCAAAGACAACGAAAGCGACCGGCAACGTATCCCTAT

>T*tubB* - terminator of *tubB* gene from *Botrytis cinerea*
ATCGTTGAGAATCGTTTCATCGATCTCAAGTCCCGTGGATGTTATGAAACTCCTGGTATCACATGTCTCCGCTCC
GCCCACGTTGATCTCGAAGGTTTGGTTATGGACCGTGAAGTCCGTGCTCTTCGTGACCAATTCGTCACCTACAAC
TACTCTAAGATCCTTTACAATGGTCTTTACTTCTCTCCTGAGCGTGAGTTCATCGAGGAATCTATCGTTGCTTCC
CAGAAGAATGTCAATGGACAAGTCAGATGCCGTGTGTACAAGGGTACCTTCAGTGTCTTGGGTCGTTCCTCAGTG
ACCGAGAAGTTGTACGATGCAAGCGAGAGTTCAATGGACGAAATTGGTTCATTCGCTCCTGCGGATACTACTGGT
TTCATCAGCGTTCAATCTATCAGATTGAAGAAGTATGGTGAGGCTAAGGCAGCTGCTGGTGAAAGACTATAGATG
GATCTTGACACCATAACGGCTTGAATTTAGTCTGAGTCTTGGATTGGACCTGAGACATTCGGGAACTTGCAACTT
CGCACAAAATGCAGATGAGACACCGATTGCCTCCGGTCCTCGGAGTGCAGATGGGAATGGGAATATGTAAACTAA
AATGCTACACAAAAGTCGCATATGAATGAAAACAGACCAGCTTGTTATTGCCAGTGCCGCTTCAATTCATGATGA
TTTATGTGTTTGTTTCCAAGAAAAGACTTAATGATGATTACTAACAGA

>linear amplicon from transformant RC337-6
TAATCGTGCTTGGTGCTTTGCTGGAATCACTCAGGAGTCTTGGGAATCCTTGGAAGTTTGATTCCCAG
TAATTATCAGCAGATAATGAGTGTGAAGCTCCCCAATAATTAAGATGAGATATTCCTCGACGAACGAA
GTTCAGAGCTTCTGCAAGGATGTAGGACATGGCGTCGAACTTGATGAAGTTTTCTCTGGCGTGAACCT
GAATTCAAGAGCACTATTTTTATAACCCTATGATGAAAGAAATGGGACTGGAAGAAATTCTTTTCTAT
TTTGCAGAGTAGGATTGCAAAATTGCGATGAAGGAAATTCCGGGGTATGGGTAGGGTTCGAGAGTAAA
CGACACCGGATCCTTGTATTGATTTATGCCGCTAAAAATCCGTTCCCAACTACCCCAGTTAAATAACT
CTCTATAATTACAACTGCGGTACAGTCATGTTTCAAATTCTCGAAAATAAATCCCTCTGCCCCCACTT
GAGAACTCCGTATTATAGACAAGGCGTGCGAACATGGATGACATGATGCAACGATTATGGTTGCGTTG
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CCATAGATTATACGTGCCAGATCTCGTGCATAGTATTCAACACAACACCCAGTAAGTTTTAAAATTGA
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AAGAGCATCGGCTTTGATTTCCTTCAGGTCATACGAGGCTTGTGCAATGGTCTGCGCATGGATCGCTG
CTGTTCTCCTATCAAACTCGGATTTTGTCTTAGGGGATGGCGTAGGAAAGACGCTGCCGCGGTTCAGA
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CTATTTTGGTTTAGTCGTCCAGGCGGTGAGCACAAAATTTGTGTCGTTTGACAAGATGGTTCATTTAG
GCAACTGGTCAGGTCAGCCCCACTTGTAGCAGTAGCGGCGGCGCTCGAAGTGTGACTCTTATTAGCAG
ACAGGAACGAGGACATTATTATCATCTGCTGCTTGGTGCACGATAACTTGGTGCGTTTGTCAAGCAAG
GTAAGTGAACGACCCGGTCATACCTTCTTAAGTTCGCCCTTCCTCCCTTTATTTCAGATTCAATCTGA
CTTACCTATTCTACCCAAGCATCGATAATGACCACTCTTGACGACACGGCTTACCGGTACCGCACCAG
TGTCCCGGGGGACGCCGAGGCCATCGAGGCACTGGATGGGTCCTTCACCACCGACACCGTCTTCCGCG
TCACCGCCACCGGGGACGGCTTCACCCTGCGGGAGGTGCCGGTGGACCCGCCCCTGACCAAGGTGTTC
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CGCGTACGGGGACGACGGCGACCTGGCGGGCTTCGTGGTTGTCTCGTACTCCGGCTGGAACCGCCGGC
TGACCGTCGAGGACATCGAGGTCGCCCCGGAGCACCGTGGGCACGGGGTCGGGAGAGCGTTGATGGGG
CTCGCGACTGAGTTCGCTCGCGAGCGAGGCGCCGGGCACCTCTGGCTGGAGGTCACCAACGTCAACGC
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AAACCTCGAAAATCATTCCTACTAAGATGGGTATACAATAGTAACCATGGTTGCCTAGTGAATGCTCC
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ATGATACTAACCTTGGAACATTGCTTATATATTATGAGACACTGTCCGGCCTTGATTTGCCACATTTA
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AAAAAGTAGGTATGGATAGTGTATGGAATTTTCATAGTATGGCTTACTCAAGCTGTGAGTATTCGCTG
CCCATTTGAGATCGCTTGAATGCTCTCGCAGGGAGCCTGAAGACGGTTTAAAACAAAGCGGCTTAACG
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AGACACTGGAAAGAAAGGAAAACCAAAAATTGCTCCAATGGTTTAAACTTGAAGAAAATAAAACTTCA
GGGCACCCTCGAGGTCGAACATCATTGTAAGAGAGGTATAGACAATGGTTAGTCCAAATAGATAGATC
ATAAGTTGAGTCAAATAACTCCAGCTTCGCCCCTGTTTGCTATACTTCTCCCATCCACCACCACTTCG
CTTTGGCGTAGGGAGGAAATCACACATTGTGCCATCAGCAATCGTTTTTGACGCCATATCAGCAGGCA
GGTCACCAGCGTATGGTGCCCAATACCGGCTTAGCAGGCTATAGATTATGCCATCACGAACCTGAAAA
CGAACCAAGAATCGAGAGCTCTGGTAGATAGTATTGACGCGCTCATATCGAAGGTCGCGATATTTTTG
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CGCCCACAATGATAACTTGAAACTCGGCCTTTTCCATGGTTTCTGAATCTTAAAGATACATGAAAAGA
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CCAAGTGACTTTTCGTTACGGTCGACGTACCAGAAACAAATTCCATAAAACCCATCGATTGATAAAAA
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GTACTTGTATTTCTGTCACTTTGGGGAAGAAATTCCGATTCATCGGGCATGAAATGAGATCAGACATA

CCATAGAGTATAGGATGCGCCACGCGTACTGCCTCGACACAACAGTTGACTCAATCCACCAACGCTTA
GAAGAAGTTGACAAATGACGGCCCCCCATGAATATGCCAGCGCAGGCCCGATTTCAAGGGCCAATGCA
ACATGCCCAGAGAGAAAGCCCATGGTCGCGACGAAAAATATCAAGGAAATATTGCGCTCAACAAGCGG
CGCATGGCCCCACTCTCGGGATGAAAACGTTATTGCTGCGTACATGACACCAAAGTTGATGAGGAGGC
CCATCCAGAATACACCCCTCTCCACCGGGCTTTTCGAGGGAAAGACCAGACAGTAGACCAGTTCCCAC
GCGATGTTGCAACAGAGGGGCATAATCGACATTCCGTAAGTTTCATGTTTGAACGAGATGTAGACCAT
TCCGATGTAGTTTATGATCCATCCTACTCCCATGCCAACAACAAAAAGATCAGCCAATGGCTTAATCG
CCTGGTACTCCGGAGGAGCTTGGGAAACATCAAAACCGTCC

Table S4: *Penicillium paxilli* strains used in this study. Hyg^R, Gen^R Nou^R denote hygromycin, and nourseothricin resistance respectively.

***P. paxilli* strain**	**Description**	**Indole diterpene phenotype**			**Source**
		3′4′-epoxyemindole SB	**Paspaline**	**Paxilline**	
PN2013	Wild type *Penicillium paxilli*	-	+	+	Barry Scott, Massey University[4]
RC337	PN2013/deletion of *paxA* locus (*ΔpaxA*)::*PtrpC_nat_TtrpC; Nou^R*	+	+	-	This study
RC356	RC337-6 (*ΔpaxA)/PpaxA-paxA-TaceB)_PtrpC-neo^R-TtrpC; Gen^R*	-	+	+	This study
RC358	RC337-6 (*ΔpaxA)/PjanO_ltmS_TaceB*::*PtrpC_hph_TtrpC; Hyg^R*	-	+	+	This study
RC383	RC337-6 (*ΔpaxA)/PjanO_janA_TaceB*::*PtrpC_neo^R_TtrpC; Gen^R*	-	+	+	This study
RC389	RC337-6 (*ΔpaxA)/PjanO_ptmA_TaceB*::*PtrpC_neo^R_TtrpC; Gen^R*	-	+	+	This study
RC391	RC337-6 (*ΔpaxA)/PjanO_desA_TaceB*::*PtrpC_neo^R_TtrpC; Gen^R*	-	+	+	This study
MH17	PN2013/*HA3::PtrpC_neo^R_TtrpC::PpaxG_paxG_TpaxG::PpaxM_paxM_TpaxM::PpaxB_paxB_TpaxB::PpaxC_paxC_TpaxC::HA4*; *Gen^R*	+	+	-	This study
MH45	PN2013/*HA3::PtrpC_neo^R_TtrpC::PpaxG_paxG_TpaxG::PPaxA_paxA_TpaxA::PpaxM_paxM_TpaxM::PpaxB_paxB_TpaxB::PpaxC_paxC_TpaxC::HA4*; *Gen^R*	-	+	-	This study
PN2258	PN2013/ΔpaxP::*PtrpC_hph_TtrpC; Hyg^R*	-	+	-	Barry Scott, Massey University[18]
LS293	PN2013/deletion of paxilline biosynthesis locus (ΔPAX)::*Ptef_hph_Ttub::Ptef_tk_Ttub; Hyg^R*	-	-	-	This study

1.10 References

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