## Electronic Supplementary Information

# Filipins: the first antifungal "weed killers" identified from bacteria isolated from the trap-ant

Hong Gao[a, b], Sabine Grüschow[a, b], Jörg Barke[c], Ryan F. Seipke[c], Lionel M. Hill[d], Jérôme Orivel[e], Douglas W. Yu[c, f], Matthew Hutchings[c] and Rebecca J.M. Goss[a, b, *]

[a] School of Chemistry, University of St. Andrews, St. Andrews, Fife, KY16 9ST, UK

[b] School of Chemistry, University of East Anglia ,Norwich Research Park, Norwich, Norfolk, NR4 7TJ, UK

[c] School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich, Norfolk, NR4 7TJ, UK

[d] John Innes Centre, Norwich Research Park, Norwich, Norfolk, NR4 7UH, UK

[e] CNRS, UMR Ecologie des Forêts de Guyane, Campus Agronomique, BP 316, 97379 Kourou Cedex, France

[f] State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Kunming, Yunnan, 650223, China

* Email: rjmg@st-andrews.ac.uk; Tel: +44 (0) 1334 463856.

## Materials and Methods

### Bacterial strains and culture conditions

The Streptomyces strain FG26 was isolated by Seipke *et al* [1]. The strain was streaked on SFM agar plates (2.0% soy flour, 2.0% mannitol, and 2.0% agar) and incubated at 28 ˚C for 7 d. Then a single colony was picked up to inoculate ISP2 media (0.4% yeast extract, 1.0% malt extract and 0.4% glucose; pH 7.2) as the starter culture. Following 3 d of growth at 28 ˚C and 180 rpm shaking, (incubator throw 19 mm), the starter culture was inoculated into the fermentation media (Table S1) at 1% culture volume to produce antifungal compounds and was grown for 8 d at 28 ˚C and 180 rpm shaking (incubator throw 19 mm).

### Isolation and detection of filipins complex

After fermentation, the whole cell culture was extracted with an equal volume of ethyl acetate, then the organic solvent phase was evaporated. The solid residue from 50 mL of culture was dissolved in 1 mL of methanol.

The extract was fractionated by column chromatography (Sephadex, 3 cm * 40 cm column, mobile phase: methanol), 60 fractions were collected, and the volume for each fraction was 10 mL. The resulting fractions were analyzed for antifungal activity.

Samples were analyzed by HPLC with a Phenomenex Luna 5a C18(2) column. For HPLC analysis, the column was developed using a gradient of 10-95% acetonitrile in water (0.01% trifluoroacetic acid added) for 6 min at a flow rate of 0.6 mL $min^{-1}$.

For LC-MS analysis samples were re-dissolved in water: methanol 50:50 and separated using a Surveyor LC

(Thermo Finnigan) on a Phenomenex Luna C18(2) column (100×2mm, 3µm) with a linear gradient of 20 – 95 % methanol against 0.1 % formic acid in water over 25 min at a flow rate of 0.24 ml $min^{-1}$.
A LCQ DecaPlus$^{XP}$ ion trap (Thermo Finnigan) was used for nominal mass LC-MS/MS, and a LTQ Orbitrap mass spectrometer (Thermo Scientific) was used at 60,000 resolution for HR mass analysis. The assignment of individual filipins (II, III, IV) is based on comparison to the published relative retention times [2].

**Antifungal Assay**

The antibacterial activity of the crude extracts and pure compounds was determined using the paper disc diffusion method[3]. Briefly, crude extracts and nystatin (as positive control) was reconstituted in an appropriate volume of MeOH, after which they were dispensed into paper bioassay discs. An equal volume of MeOH solvent, which was used as a negative control, was also applied onto a disc. *Candida albicans* was grown in LB medium at 37 ˚C overnight, then 100 µl of culture was dispensed into LB agar medium. The dried discs were placed onto agar plates and incubated at 37 ˚C overnight. The diameter of the zone of inhibition was used to evaluate the antibacterial activity. All assays were performed in triplicate.

**Identification and characterization of the filipins' biosynthetic cluster cluster**

Following genome sequencing, genes coding for type I polyketide synthases (PKS) were identified and analyzed by software Open Reading Frame Finder protocol of the NCBI (ORF Finder, NCBI)[4]. The corresponding deduced proteins were compared with the known proteins for filipin biosynthesis in *Streptomyces avermitilis*[5] using available BLAST methods (http://www.ncbi.nlm.nih.gov/blast).

**Table S1.** Fermentation media used in this study

| Medium No. | Medium composition per litre | Reference |
|---|---|---|
| M1 | 10 g soluble starch, 4 g yeast extract, 2 g peptone | 6 |
| M2 | 1 g L-arginine, 1 g $K_2HPO4$, 0.5 $MgSO_4 \cdot 7H_2O$, 6 ml of 100% glycerol | |
| M3 | ISP-2: 4 g yeast extract, 10 g malt extract, 4 g glucose | 7 |
| M4 | 0.1 g asparagine, 0.5 g $K_2HPO_4$, 0.001 g $FeSO_4 \cdot 7H_2O$, 0.1 g $MgSO_4 \cdot 7H_2O$, 2 g peptone, 4 g sodium propionate | 8 |
| M5 | 10 g beef extract, 4 g peptone, 10 g brain heart infusion, 5 g yeast extract, 5 g glucose, 15 g $K_3PO_4$, 1 g starch, 1 g $(NH_4)_2SO_4$, 1 g cysteine, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.01 g $CaCl_2$ | |
| M6 | 4 g beef extract, 4 g peptone, 1 g yeast extract, 10 g glucose | |
| M7 | 2 g peptone, 0.1 g asparagine, 4 g sodium propionate, 4 g $K_2HPO_4$, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.001 g $FeSO_4 \cdot 7H_2O$, 5 g glycerol | |
| M8 | 4 g yeast extract, 15 g soluble starch, 1 g $K_2HPO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$ | |
| M9 | 20 g soluble starch, 1 g $KNO_3$, 0.5 g $K_2HPO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$ | |
| M10 | Terrific broth (Sigma) | - |
| M11 | TSB medium (Difco) | - |
| M12 | SFM medium | 9 |

**Figure S1: LC-MS of filipins complex produced in FG26**

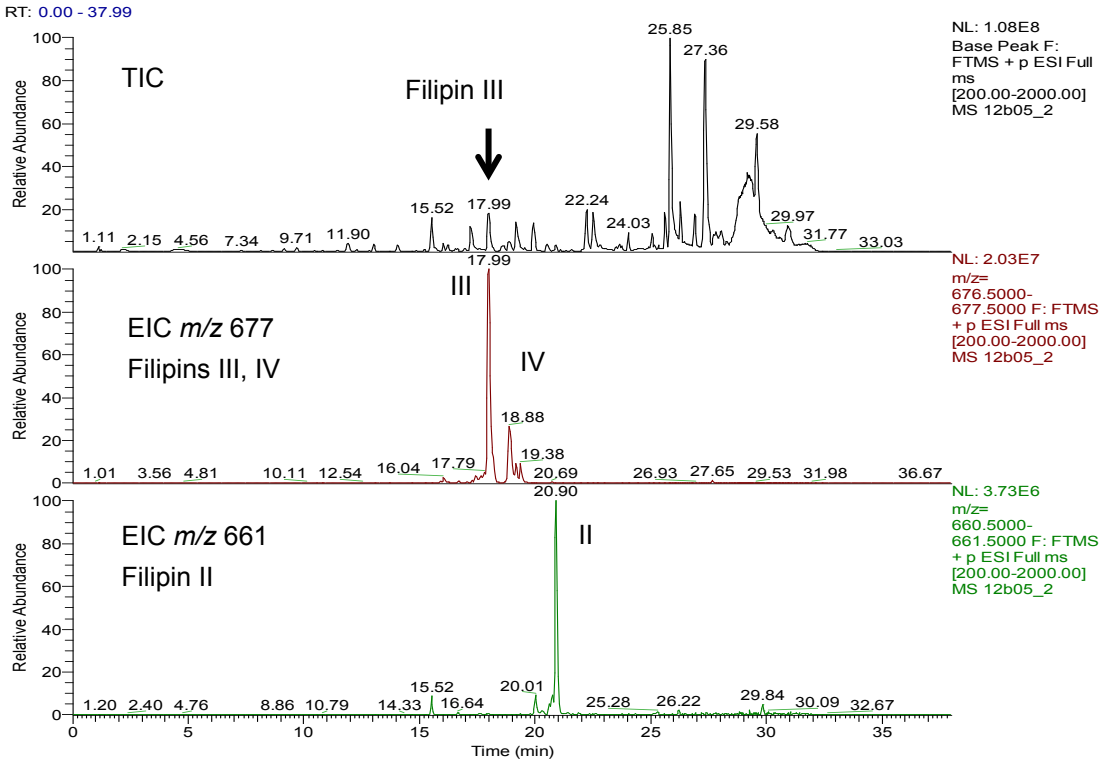


**Figure S2: HR-MS of filipin III produced in FG26**

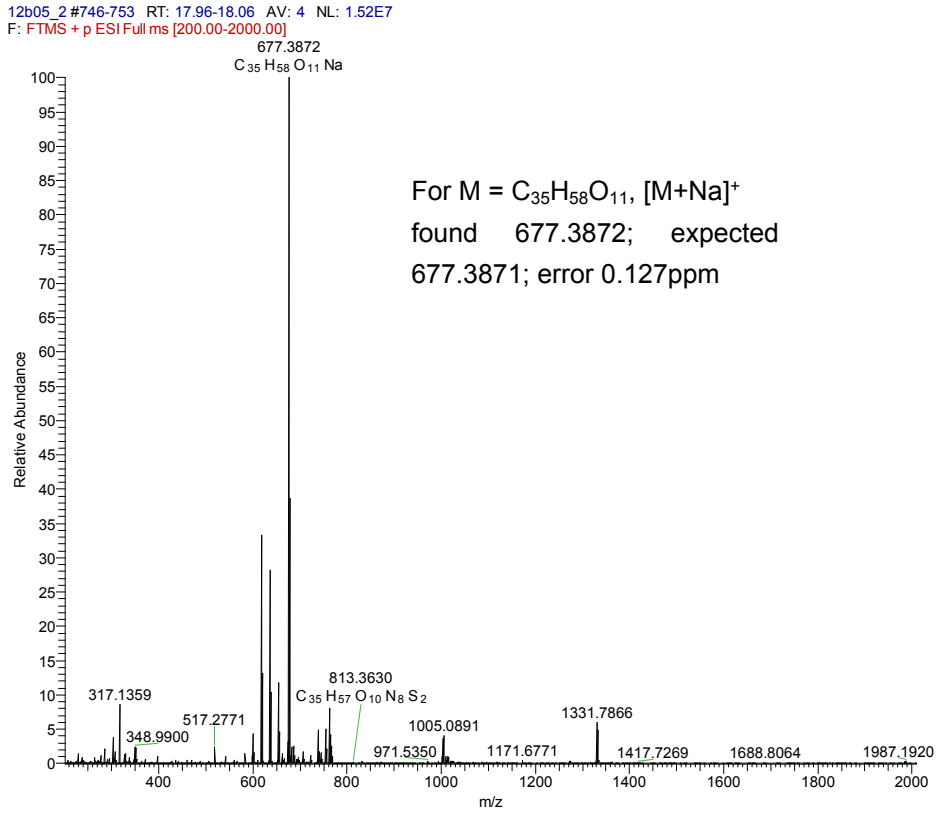


**Figure S3: HR-MS of filipin IV produced in FG26**

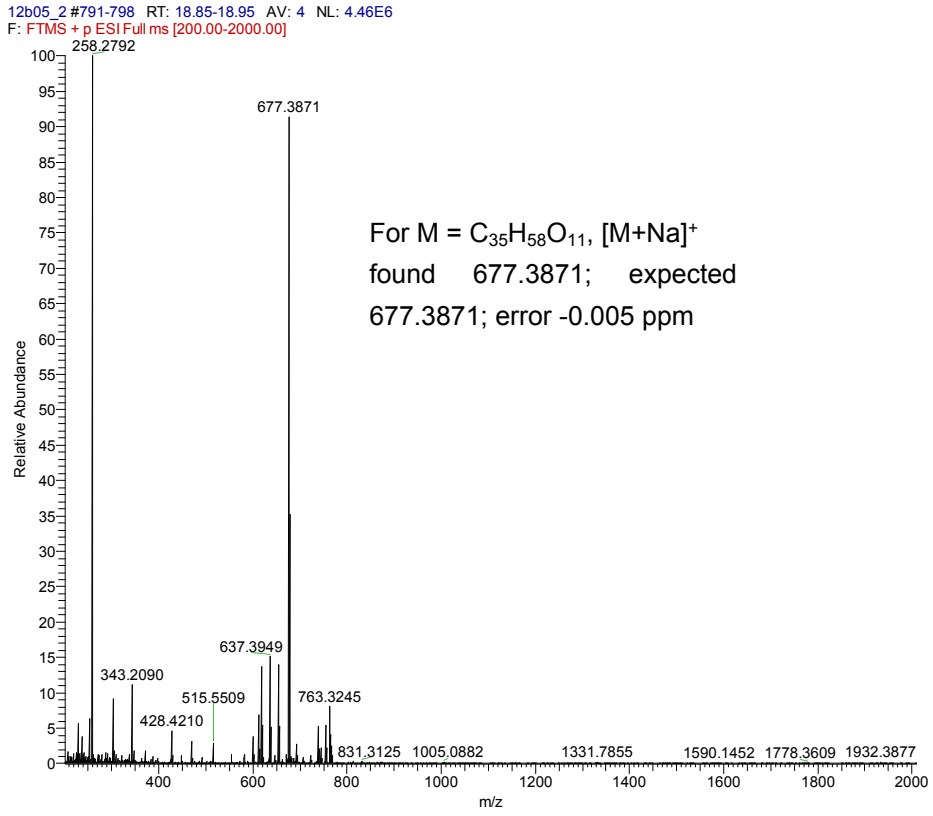


**Figure S4: HR-MS of filipin II produced in FG26**

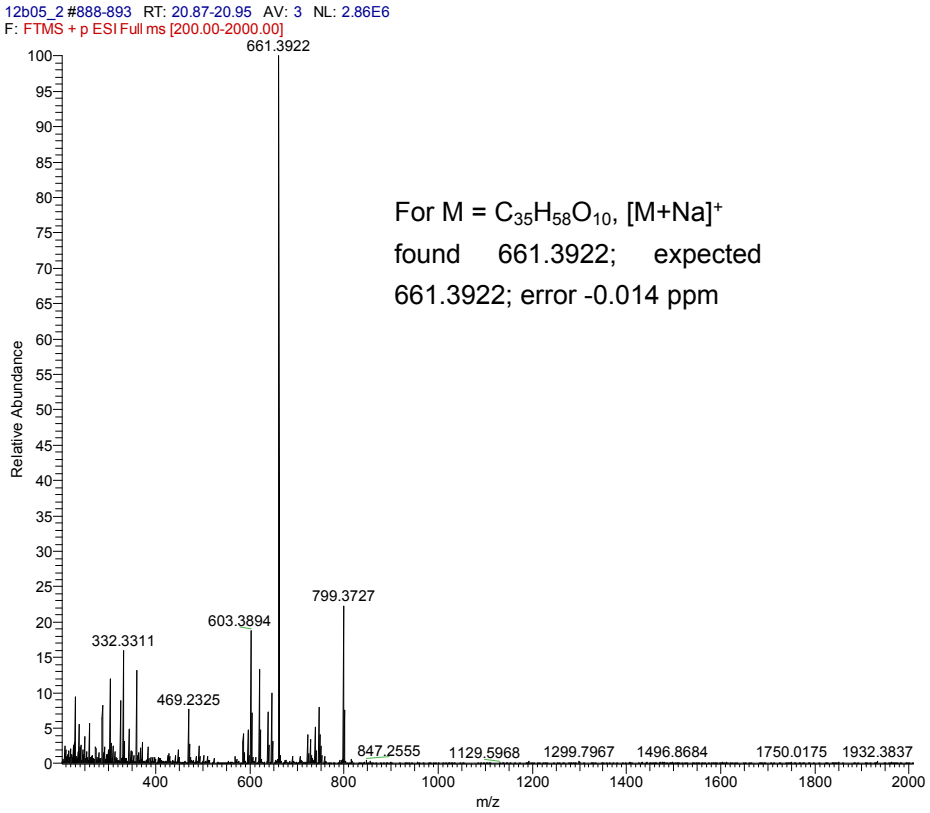


**Figure S5: LC-MS of a co-injection of FG26 extract and filipin complex standard. The intensity of the peak of filipin III was increased after co-injected with filipin complex standard.**

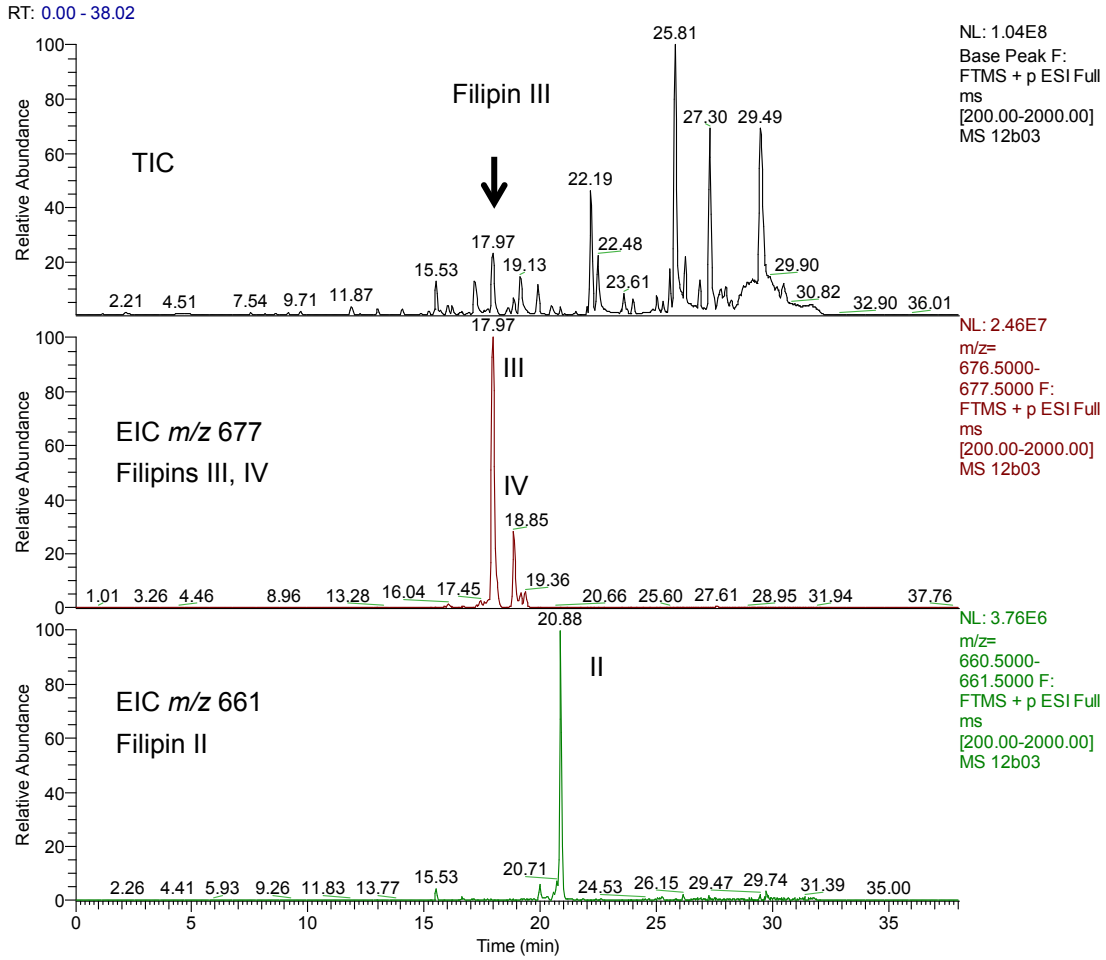


**Figure S6: $MS^2$ spectra for filipin III (A) and II (B)**

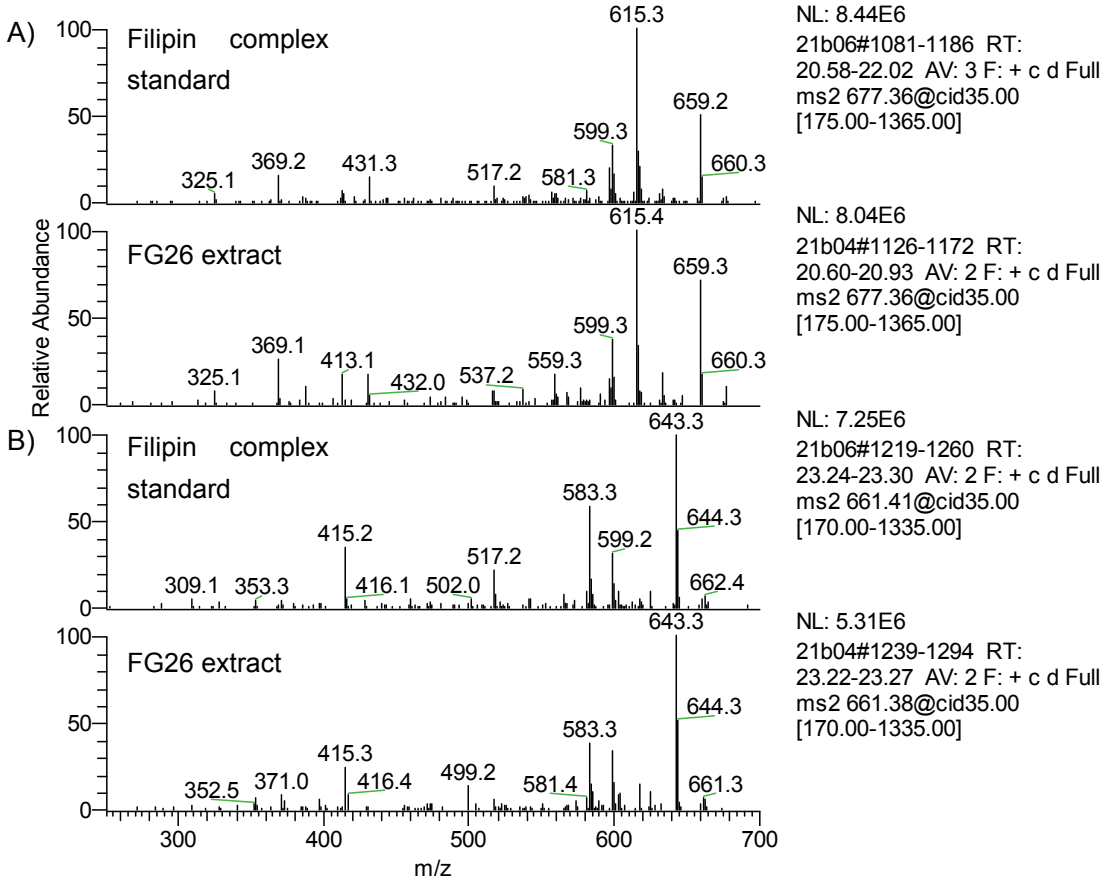


**Figure S7. UV absorption of filipin III**

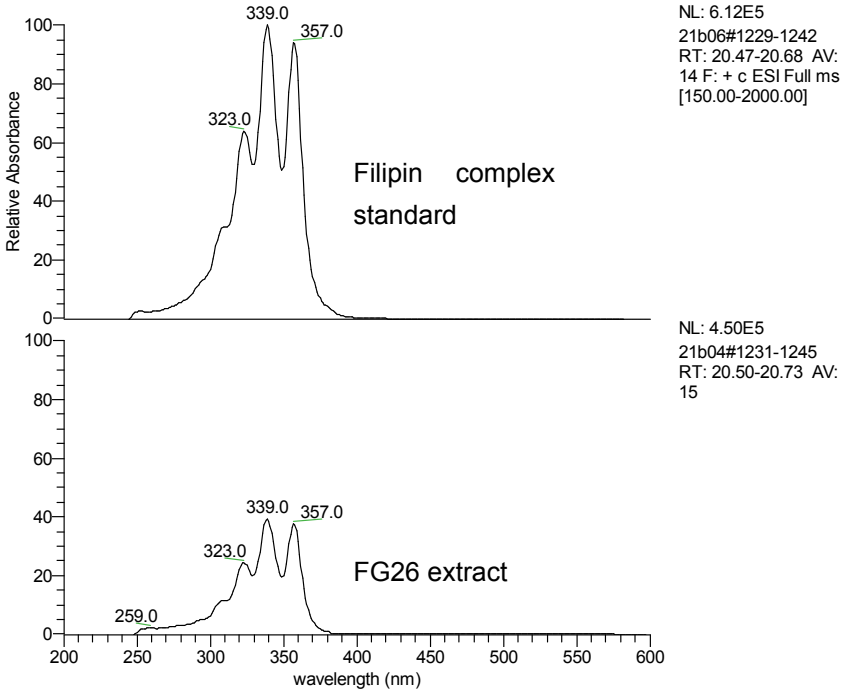


**Figure S8. ClustalW sequence alignment of filipin PKS KR domains**

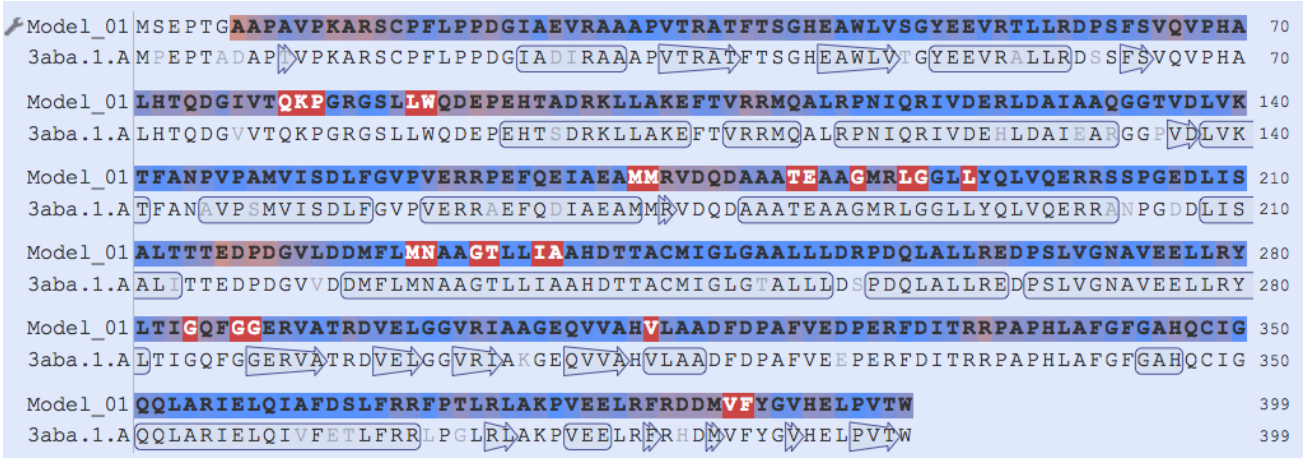
```
PteA5-KR13     GTVLITGGTGGVGRHLARWL-AGAGAQHLVLTSRSGPDAPGAQELHAELTALGAEVTIAA   59
FlpA5-KR13     GTVLITGGTGGVGRHLARWL-AGAGARHLVLTSRRGPDAPGARELHAELTALGVEVTIAA   59
PteA1-KR1      GTVLVTGGTGALGGQVARWL-AGAGAEHLVLTSRRGPDAPGAAELKAELEELGAQVTVVA   59
FlpA1-KR1      GTVLVTGGTGALGGHVARWL-AGAGAEHLVLVSRRGPEAPGAAELRAELEESGVRVTVAA   59
PteA5-KR12     GTVLITGGTGALGGHVARWL-ARGGAEHLVLTSRRGADAPGAAALRDELEVLGARVTFAA   59
FlpA5-KR12     GTVLVTGGTGALGGHVARWL-ARGGAEHLVLTSRRGADAPGAAALRDELAALGTQVTLAA   59
PteA4-KR8      GTVLITGGTGALGGHVARWL-AGAGAEHLVLTSRRGADAPGATALKAELEELGARVTLAV   59
FlpA4-KR8      GTVLVTGGTGALGGHIARWL-AATGAEHLVLTSRHGADAPGAPALAAELAELGARVTLAA   59
PteA4-KR9      GTVLITGGTGALGGHVARWL-AGAGAEHLVLTSRRGPAAPGAAELVAELEELGAAATVVA   59
FlpA4-KR9      GTVLITGGTGALGGHVARWL-AGAGAEHLVLTGRRGLDAPGAAELQAELEESGVRVTVAA   59
PteA4-KR11     GTVLITGGTGALGGHVARWL-AGAGAEHLVLTSRRGPAAPGAAGLKAELEEAGVRVTVAA   59
FlpA4-KR11     GTVLITGGTGALGGHVARWL-AGAGAEHLVLTGRRGLDAPGAAELQAELQESGVRVTVAA   59
PteA4-KR10     GTVLITGGTGALGGHVARWL-AGAGAEHLVLTSRRGPDAPGAAGLVAELEATGVRVTVAA   59
FlpA4-KR10     GTVLITGGTGALGGHVARWL-AGAGAEHLVLTSRRGLDAPGAAELTAELEESGVRVTVAA   59
PteA3-KR7      GTVLLTGATGGLGRSLARHLVAERGARRLLLASRRGPAAEGVGELVAALSELGAHVDVAA   60
FplA3-KR7      GTVLLTGATGGLGRSLARHLVAERGARRLLLASRRGPAAEGVGELVAELSGLGAHIDVAA   60
PteA2-KR5      GTVLLTGAAGALGKTLARHLVTGHGVRRLLLVSRRGADAPGAADLKAELAALGAEATWAA   60
FlpA2-KR5      GTVLLTGASGALGGTLARHLVTGHGVRRLLLVSRRGADAPGAADLADDLAALGAEATWAA   60
PteA1-KR2      GTVLLTGASGGLGGLFARHLVAEHGVRSLLLVSRRGGDAPGAAELTADLTAQGADVTWAA   60
FlpA1-KR2      GTVLLTGASGGLGGLLARHLVAEHGVRSLLLVSRRGGDAPGAADLTAELTARGADITWAA   60
PteA1-KR3      GPVLLTGASGALGGLVARHLVAEHGVRSLLLLSRRGAEAPGAVELEAELAAWGAEVRWAA   60
FlpA1-KR3      GVVLLTGASGALGGLVARHLVAEHGVRSLLLLSRRGAEAPGAVELEAELTAWGAEVNWAA   60
PteA2-KR6      GRVLLTGASGALGGLVARHLVAEHGLRSLLLMSRRGADAEGAAELQEELTAAGAEVLWAA   60
FlpA2-KR6      GSVLLTGASGALGGLVARHLVAEHGVRRLLLMSRRGADAEGAAELQAELTAAGAEVRWAA   60
PteA1-KR4      GPVLLTGASGALGGLVARHLVAEHGVRSLLLLSRRGAEAPGAVELEAELAAWGAEVRWAA   60
FlpA1-KR4      GTVLVTGASGSLGALVARHLVTAHKVRRLLLASRRGADAPGAADLAAELAAQGAEITWAA   60
               * **:**.:*.:*  .** * :    . *:* .* *  * *.  *   *   *.   ..

PteA5-KR13 A   CDMADRAAVARLLAGIESRHPLTAVLHAAGTARSSMLADAGLDEFAEAAASKVTGAIHLD   119
FlpA5-KR13 A   CDIADRAALARLLAGIESRHPLTAVLHAAGTARSSLLADADLQEFADAAAAKVTGAVHLD   119
PteA1-KR1  B   CDAADRDALAELF----GRYIVNAVVHTAGVLDDGLVESLTPERLNHVLRPKVDAALHLH   115
FlpA1-KR1  B   CDAADRDALAELF----ARHPVNAVVHTAGILDDGLIDSLTPERLDGVLRPKADAALHLH   115
PteA5-KR12 A   CDVADRDAVAALL----AQHVFTAVVHAAGVADAGTVDATTPAAFAAALAAKVGGAAHLD   115
FlpA5-KR12 A   CDLADRDAVAALL----AEHALTAVVHAAGVADPGMLDATTPDAFAAALAAKADGAAHLD   115
PteA4-KR8  A   CDVADRDALAALL----AEHTFTSVFHAAGVEQFAPFDELTPADFARTMAAKAHGAAHLD   115
FlpA4-KR8  A   CDLADRDAVAALL----AEHTCTAVFHAAGVPQFTPFGELTADDFARTLAAKAHGATHLD   115
PteA4-KR9  A   CDAADRDALRALL----AQHPVNAVVHAAGVGDHVMIEDSDPAGFAGSVAAKAAGATHLD   115
FlpA4-KR9  A   CDMADRAAVAALL----AEHPVNAVVHAAGVGDHAMIEDSDPAGFARTVSAKAAGAIHLD   115
PteA4-KR11 A   CDVADREALAVLL----AEHPVNAVVHTAGTAEAGMLAETSLGDFAATVAAKALGAVHLH   115
FlpA4-KR11 A   CDVADRAALAALL----AEHPVDAVVHAAGTAEAGMLAETNLGDFAATVAPKALGALHLH   115
PteA4-KR10 A   CDVADREALAALL----AEHPVNAVVHTAGVDHMEPLEAMTPGACADVLSAKAAGALHLD   115
FlpA4-KR10 A   CDVADREALAALL----AEHPVNAVVHTAGVDHLDPLETMTPGAFADVLSAKAAGALHLD   115
PteA3-KR7  B   CDIADRDALAELLASVPAEHPLVAVVHAAAALDDGVITALTPGRLDTVLRPKADGALHLH   120
FplA3-KR7  B   CDIADRDALAALLASVPAAHPLVAVVHAAGALDDGVITALTPRRLDTVLRPKADGALNLH   120
PteA2-KR5  B   CDLAERDALARLLAA----TPVDSVVHTAGVLDDGVIAALTPERVGAVLAPKADAVLNLD   116
FlpA2-KR5  B   CDLADRAAVARLLAA----HPVDSVVHTAGVLDDGVIAALTPQRLRAVLAPKTDAVLHLD   116
PteA1-KR2  B   CDVADRAAVRALLAG-PG-QSLSAIIHTAGVLDDGIIGSLTPERLDAVFRPKVDAALNLH   118
FlpA1-KR2  B   CDVADRDAVRALLAG-PG-QKLSAIVHTAGVLDDGIIGSLTPERLDTVFRPKVDAALNLH   118
PteA1-KR3  B   CDAADRDALAEVLAG----TPVTAVVHAAGVLDDGVIAALTPERMEKVLRPKTDAVLNLH   116
FlpA1-KR3  B   CDAADRDALAEVLSG----TPVTGVVHTAGVLDDGVIASLTPERMAKVLRPKVDAVLNLH   116
PteA2-KR6  B   CDVADRDALAEVLSG----TPVTAVVHTAGVLDDGVIGSLTPERMEMVLRPKVDAVLNLH   116
FlpA2-KR6  B   CDVADRDALAEALSG----VPVTAVVHTAGVLDDGVLSSLTPERLDTVLRPKADAVLHLH   116
PteA1-KR4  B   CDVADREAVSGMLNG-LGEQSLSAVVHTAGVLGDGIVASLTPERMREVFRPKVDAVLNLH   119
FlpA1-KR4  B   CDVADREAVSALLDG----RRLSAVVHTAGVLDDGVVGSVTPERMREVFRPKVDAVLNLH   116
               ** *:* *:   :         .:.*:*.     .           *. .. .*.

PteA5-KR13 A   ELLDG--RELDAFVLFASGAGVWGSGGQASYASANAFLDALALRRRARGLTATSVAWGGW   177
FlpA5-KR13 A   ELLDG--RALDAFVLFASGAGVWGSGGQGSYAGANAFLDALALRRRARGLTATSVAWGGW   177
PteA1-KR1  B   ELTRDR-QDLDAFVLFSSMTGVWGNGGQGAYGAANAFLDALAEHRRAQGVPALAVAWGSW   174
FlpA1-KR1  B   ELTRDR-QDLDAFVLFSSMTGVWGNGGQGAYGAANAFLDALAEQRRSQGLPALAVAWGSW   174
PteA5-KR12 A   ELLGD--QELDAFVLFSSISGVWGSGGQAAYAAGNAFLDGLARQRRDRGLTATAVSWGPW   173
FlpA5-KR12 A   ELLGD--QELDAFVLFSSISGVWGSGGQAAYAAGNAFLDALARCRRDRGRTATAVSWGPW   173
PteA4-KR8  A   ELLGD--RELDAFVLFSSIAGVWGSGLQTAYAAGNAFLDGLAARRRARGLTATAIAWGPW   173
FlpA4-KR8  A   DLLGD--RDLDAFVLFSSIAGVWGSGRQTAYAAANAHLDGLAARRRARGLTATSIAWGPW   173
PteA4-KR9  A   ELLAG--QELDAFVMFSSGAGIWGGAGQGAYSAANAYLDALAEHRRAHGRTALAVSWGGW   173
FlpA4-KR9  A   ELLAG--QELDAFVMFSSGAGIWGGAGQAAYAAANACLDALAEHRRARGRTALAVSWGGW   173
PteA4-KR11 A   ELLGD--QELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGPW   173
FlpA4-KR11 A   ELLGE--RELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGPW   173
PteA4-KR10 A   ALLAG--QELDAFVLFSSIAGVWGSGHQAAYAAANALLDGLAERRRAQGLPATAVAWGPW   173
FlpA4-KR10 A   ALLDG--RELDAFVLFSSIAGVWGGGHQAAYAAANALLDGLAERRRARGLPATAVAWGPW   173
PteA3-KR7  B   ELTRDL--NLSAFVLFSSLAGVLGSAGAAGYAAANAFLDGLARQRRAAGLPGTSLAWGLW   178
FplA3-KR7  B   ELTRNL--DLSAFVLFSSLAGVLGSAGAAGYAAANAFLDGLAHRRRAAGLPATSLAWGLW   178
PteA2-KR5  B   ELTGP----STTFVLFSSAAGVFGNPGQGNYAAANAFLDAFARHRHAQGRPTVSLAWGLW   172
FlpA2-KR5  B   ELTGD----GTPFVLFSSAAGVFGNPGQGNYAAANAFLDAFARHRRAQGRPTVSLAWGLW   172
PteA1-KR2  B   DLAGELSGDLSAFVLFSSVAGTLGTPGQANYAAANTFLDALAEHRRAAGQRATSLAWGLW   178
FlpA1-KR2  B   DLAAELCGDLSAFVLFSSVAGTLGTPGQGNYAAANTFLDALAEHRRALGLRATSLAWGLW   178
PteA1-KR3  B   ELTSD----LSAFVVFSSVSGILGSAGQGNYAAANAFLDAFAEARRARGLPATSLAWGLW   172
FlpA1-KR3  B   ELTGE----LSAFVVFSSVSGILGSAGQGNYAAANTFLDAFAGFRRDQGLPATSLAWGLW   172
PteA2-KR6  B   ELAGD----VSAFVLFSSLAGTLGTPGQANYAAANAFLDAFARRRRAQGLPAVSLPWGLW   172
FlpA2-KR6  B   ELVGD----VSAFVVFSSLAGTLGTPGQANYAAANAYLDAFAAQRRAQGLPAVSLPWGLW   172
PteA1-KR4  B   ECTRDM--GLAAFVVFSSVAGMVGSAGQASYAAANSFLDAFSAHRRREGLPAISLAWGVW   177
FlpA1-KR4  B   ECTRDM--GLAAFVVFSSVAGLIGGAGQASYAAANTFLDAFAGSRRAQGLPATSLAWGVW   174
                     **:*:*   :*     *               *...*:   **.::     *:     *          ::   **   *
```

**Figure S9. Structure-based sequence alignments of PteC/FlpC (A) and PteD/FlpD (B) generated using SwissModel.[10] Substrate binding residues are highlighted.**

A




B


**References:**


1. R. F. Seipke, J. Barke, M. X. Ruiz-Gonzalez, J. Orivel, D. W. Yu and M. I. Hutchings, *Antonie Van Leeuwenhoek*, 2012, **101**, 443.
2. P. M. Kelly and K. A. Nabinger, *J. Antibiot.*, 1985, **38**, 485.
3. NCCLS (National Committee for Clinical Laboratory Standards), Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard M7-A4, NCCLS, Wayne, PA, 1997.
4. I.T. Rombel, K. F. Sykes, S. Rayner and S. A. Johnston, *Gene*, 2002, **282**, 33.
5. H. Ikeda, J. Ishikawa, A. Hanamoto, M. Shinose, H. Kikuchi, T. Shiba, Y. Sakaki, M. Hattori and S. Omura, *Nat. Biotechnol.*, 2003, **21**, 526.
6. R. Mwirichia, A. W. Muigai, B. Tindall, H. I. Boga and E. Stackebrandt, *Extremophiles*, 2010, **14**, 339.
7. E. B. Shirling and D. Gottlieb, *Int. J. Syst. Evol. Microbiol.*, 1966, **16**, 313.
8. H. Zhang, Y. K. Lee, W. Zhang and H. K. Lee, *Antonie Van Leeuwenhoek*, 2006, **90**, 159.
9. T. Kieser, M. J. Bibb, M. J. Buttner, K. F. Chater and D. A. Hopwood, 2000, John Innes Foundation.
10. K. Arnold, L. Bordoli, J. Kopp and T. Schwede, *Bioinformatics*, 2006, **22**,195.