# **Electronic Supplementary Information**

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

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# **Materials and Methods**

#### Bacterial strains and culture conditions

The Streptomyces strain FG26 was isolated by Seipke *et al*<sup>1</sup>. 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<sup>-1</sup>.

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 ( $100 \times 2mm$ ,  $3\mu m$ ) 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<sup>-1</sup>. A LCQ DecaPlus<sup>XP</sup> 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 <sup>2</sup>.

#### **Antifungal Assay**

The antibacterial activity of the crude extracts and pure compounds was determined using the paper disc diffusion method<sup>3</sup>. 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)<sup>4</sup>. The corresponding deduced proteins were compared with the known proteins for filipin biosynthesis in *Streptomyces avermitilis*<sup>5</sup> using available BLAST methods (<u>http://www.ncbi.nlm.nih.gov/blast</u>).

Medium	Medium composition per litre	Referenc
No.		e
M1	10 g soluble starch, 4 g yeast extract, 2 g peptone	6
M2	1 g L-arginine, 1 g K <sub>2</sub> HPO4, 0.5 MgSO <sub>4</sub> ·7H <sub>2</sub> O, 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 <sub>2</sub> HPO <sub>4</sub> , 0.001 g FeSO <sub>4</sub> ·7H <sub>2</sub> O,	8
	0.1 g MgSO <sub>4</sub> ·7H <sub>2</sub> O, 2 g peptone, 4 g sodium propionate	
M5	10 g beef extract, 4 g peptone, 10 g brain heart infusion,	
	5 g yeast extract, 5 g glucose, 15 g K <sub>3</sub> PO <sub>4</sub> , 1 g starch, 1	
	g (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , 1 g cysteine, 0.2 g MgSO <sub>4</sub> ·7H <sub>2</sub> O, 0.01 g	
	CaCl <sub>2</sub>	
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 <sub>2</sub> HPO <sub>4</sub> , 0.1 g MgSO <sub>4</sub> ·7H <sub>2</sub> O, 0.001 g FeSO <sub>4</sub> ·7H <sub>2</sub> O, 5	
	g glycerol	
M8	4 g yeast extract, 15 g soluble starch, 1 g $K_2$ HPO <sub>4</sub> , 0.5 g	
	MgSO <sub>4</sub> ·7H <sub>2</sub> O	
M9	20 g soluble starch, 1 g KNO <sub>3</sub> , 0.5 g K <sub>2</sub> HPO <sub>4</sub> , 0.5 g	
	MgSO <sub>4</sub> ·7H <sub>2</sub> O, 0.01 g FeSO <sub>4</sub> ·7H <sub>2</sub> O	
M10	Terrific broth (Sigma)	-
M11	TSB medium (Difco)	-
M12	SFM medium	9

Table S1. Fermentation media used in this study



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 S6: MS<sup>2</sup> spectra for filipin III (A) and II (B)

NL: 8.44E6 21b06#1081-1186 RT: 20.58-22.02 AV: 3 F: + c d Full ms2 677.36@cid35.00 [175.00-1365.00]

NL: 8.04E6 21b04#1126-1172 RT: 20.60-20.93 AV: 2 F: + c d Full ms2 677.36@cid35.00 [175.00-1365.00]

NL: 7.25E6

21b06#1219-1260 RT: 23.24-23.30 AV: 2 F: + c d Full ms2 661.41@cid35.00 [170.00-1335.00]

NL: 5.31E6

21b04#1239-1294 RT: 23.22-23.27 AV: 2 F: + c d Full ms2 661.38@cid35.00 [170.00-1335.00]

Figure S7. UV absorption of filipin III



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

PteA5-KR13		GTVLITGGTGGVGRHLARWL-AGAGAOHLVLTSRSGPDAPGAOELHAELTALGAEVTIAA	59
FlpA5-KR13		GTVLTTGGTGGVGRHLARWL-AGAGARHLVLTSRRGPDAPGARELHAELTALGVEVTTAA	59
PteA1-KR1		GTVLVTGGTGALGGOVARWIAGAGAEHLVI.TSRRGPDAPGAAELKAEI.EELGAOVTVVA	59
FlpA1-KR1		GTVLVTGGTGALGGHVARWIAGAGAEHLVLVSRRGPEAPGAAELRAELESGVRVTVAA	59
P+015-KB12		CTVLTTCCTCALCCHVARWI - ARCCAFHLVLTSRRCADAPCAAALRDFLFVLCARVTFAA	59
FlpA5-KR12		GTVL.VTGGTGALGGHVARWIARGGAEHLVLTSRRGADAPGAAALRDELAALGTOVTLAA	59
PtoAd-KR8		CTVLTTCCTCALCCHVARWIACACAEHLVLTSRRCADAPCATALKAELEELCARVTLAV	59
FloAd=KP8			59
D+07/=KD0			59
FIDA/-KDQ			50
D+oA/-VD11			50
FIDA/-KP11			59
P+oN/=KP10			59
FlpA4-KR10		CTVLTTCCTCALCCHVARWL-ACACAEHLVLTSRRCLDAPCAAELTAFLEESCVRVTVAA	59
P+073-KD7			60
Fold3-KR7		CTVLLTCATCCLCRSLARHLVAFRCARRLLLASRRCPAAFCVCFLVAFLSCLCAHTDVAA	60
PtoA2=KR5		CTVLLTCA ACALCKTLARHLVTCHCVRRLLLVSRRCADA PCA ADLKAFLAALCAFATWAA	60
FloA2_KR5			60
PteA1-KR2		GTVLLTGASGGLGGLFARHLVAEHGVRSLLLVSRRGGDAPGAAELTADLTAOGADVTWAA	60
FlpA1-KR2			60
PteA1-KR3		GPVLLTGASGALGGLVARHLVAEHGVRSLLLLSRRGAEAPGAVELEAELAAWGAEVRWAA	60
FlpA1-KR3		CVVLLTCA SCALCCLVARHLVAFHCVRSLLLLSRCAFA PCAVELFAFLTAWCAEVNWAA	60
PteA2-KR6		GRVLLTGASGALGGLVARHLVAEHGLRSLLLMSRGADAEGAAELOEELTAAGAEVLWAA	60
FlpA2-KR6		GSVLLTGA SGALGGLVARHLVAEHGVRRLLLMSRGADAEGAAELOAELTAAGAEVRWAA	60
PteA1-KR4		GPVLLTGASGALGGLVARHLVAEHGVRST.I.I.I.SRRGAEAPGAVELEAEI.AAWGAEVRWAA	60
FlpA1-KR4		GTVLVTGASGSLGALVARHLVTAHKVRRLLLASRRGADAPGAADLAAELAAOGAETTWAA	60
1 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	*	**•** •* •* ** * • *•* * * * * * *	00
PteA5-KR13	A	CDMADRAAVARLLAGIESRHPLTAVI. <mark>HAAGTARSSMI,ADAGI</mark> ,DEFAEAAASKVTGAIHID	119
FlpA5-KR13	A	CDIADRAALARLLAGIESRHPLTAVI HAAGTARSSLLADADLOEFADAAAAKVTGAVHLD	119
PteA1-KR1	в	CDAADRDALAELFGRYIVNAVVHTAGVLDDGLVESLTPERIMENTVIONVHDD	115
FlpA1-KR1	в	CDAADRDALAELFARHPVNAVVHTAGILDDGLIDSLTPERLDGVLRPKADAALHLH	115
PteA5-KR12	А	CDVADRDAVAALLAOHVFTAVVHAAGVADAGTVDATTPAAFAAALAAKVGGAAHLD	115
FlpA5-KR12	А	CDLADRDAVAALLAEHALTAVVHAAGVADPGMLDATTPDAFAAALAAKADGAAHLD	115
PteA4-KR8	A	CDVADRDALAALLAEHTFTSVFHAAGVEOFAPFDELTPADFARTMAAKAHGAAHLD	115
FlpA4-KR8	А	CDLADRDAVAALLAEHTCTAVFHAAGVPOFTPFGELTADDFARTLAAKAHGATHLD	115
PteA4-KR9	А	CDAADRDALRALLAOHPVNAVVHAAGVGDHVMIEDSDPAGFAGSVAAKAAGATHLD	115
FlpA4-KR9	A	CDMADRAAVAALLAEHPVNAVV <mark>HAAGVGDHAMIEDSDP</mark> AGFARTVSAKAAGAIHLD	115
PteA4-KR11	А	CDVADREALAVLLAEHPVNAVVHTAGTAEAGMLAETSLGDFAATVAAKALGAVHLH	115
FlpA4-KR11	А	CDVADRAALAALLAEHPVDAVVHAAGTAEAGMLAETNLGDFAATVAPKALGALHLH	115
PteA4-KR10	А	CDVADREALAALLAEHPVNAVVHTAGVDHMEPLEAMTPGACADVLSAKAAGALHLD	115
FlpA4-KR10	А	CDVADREALAALLAEHPVNAVVHTAGVDHLDPLETMTPGAFADVLSAKAAGALHLD	115
PteA3-KR7	в	CDIADRDALAELLASVPAEHPLVAVV <mark>HAAAALDDGVITALTP</mark> GRLDTVLRPKADGALHLH	120
FplA3-KR7	в	CDIADRDALAALLASVPAAHPLVAVV <mark>HAAGALDDGVITALTP</mark> RRLDTVLRPKADGALNLH	120
PteA2-KR5	в	CDLAERDALARLLAATPVDSVVHTAGVLDDGVIAALTPERVGAVLAPKADAVLNLD	116
FlpA2-KR5	в	CDLADRAAVARLLAAHPVDSVVHTAGVLDDGVIAALTPORLRAVLAPKTDAVLHLD	116
PteA1-KR2	в	CDVADRAAVRALLAG-PG-OSLSAII <mark>HTAGVLDDGIIGSLTP</mark> ERLDAVFRPKVDAALNLH	118
FlpA1-KR2	в	CDVADRDAVRALLAG-PG-OKLSAIV <mark>HTAGVLDDGIIGSLTP</mark> ERLDTVFRPKVDAALNLH	118
PteA1-KR3	в	CDAADRDALAEVLAGTPVTAVV <mark>HAAGVLDDGVIAALTP</mark> ERMEKVLRPKTDAVLNLH	116
FlpA1-KR3	в	CDAADRDALAEVLSGTPVTGVV <mark>HTAGVLDDGVIASLTP</mark> ERMAKVLRPKVDAVLNLH	116
PteA2-KR6	в	CDVADRDALAEVLSGTPVTAVV <mark>HTAGVLDDGVIGSLTP</mark> ERMEMVLRPKVDAVLNLH	116
FlpA2-KR6	в	CDVADRDALAEALSGVPVTAVV <mark>HTAGVLDDGVLSSLTP</mark> ERLDTVLRPKADAVLHLH	116
PteA1-KR4	в	CDVADREAVSGMLNG-LGEQSLSAVV <mark>HTAGVLGDGIVASLTP</mark> ERMREVFRPKVDAVLNLH	119
FlpA1-KR4	в	CDVADREAVSALLDGRRLSAVV <mark>HTAGVLDDGVVGSVTP</mark> ERMREVFRPKVDAVLNLH	116
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PteA5-KR13	Α	ELLDGRELDAFVL <mark>FASGAGVWGSGGQASY</mark> ASANAFLDALALRRRARGLTATSVAWGGW	177
FlpA5-KR13	A	ELLDGRALDAFVL <mark>FASGAGVWGSGGQGSY</mark> AGANAFLDALALRRRARGLTATSVAWGGW	177
PteA1-KR1	в	ELTRDR-QDLDAFVL <mark>FSSMTGVWGNGGQGAY</mark> GAANAFLDALAEHRRAQGVPALAVAWGSW	174
FlpA1-KR1	в	ELTRDR-QDLDAFVL <mark>FSSMTGVWGNGGQGAY</mark> GAANAFLDALAEQRRSQGLPALAVAWGSW	174
PteA5-KR12	Α	FLLCDOFLDAFVLFSSTSCVWCSCCOAAYAACNAFLDCLARORRDRCLTATAVSWCPW	173
FlpA5-KR12			
	Α	ELLGDQELDAFVLFSSISGVWGSGGQAAYAAGNAFLDALARCRRDRGRTATAVSWGPW	173
PteA4-KR8	A A	ELLGDQELDAFVLFSSISGVWGSGGQAAYAAGNAFLDALARCRRDRGTATAVSWGPW ELLGDRELDAFVLFSSIAGVWGSGLQTAYAAGNAFLDGLAARRRARGLTATAIAWGPW	173
PteA4-KR8 FlpA4-KR8	A A A	ELLGDQELDAFVLFSSISGVWGSGGQAAYAAGNAFLDALARCRRDRGRTATAVSWGPW ELLGDRELDAFVLFSSIAGVWGSGLQTAYAAGNAFLDGLAARRRARGLTATAIAWGPW DLLGDRDLDAFVLFSSIAGVWGSGRQTAYAAANAHLDGLAARRRARGLTATSIAWGPW	173 173 173
PteA4-KR8 FlpA4-KR8 PteA4-KR9	A A A A	ELLGDQELDAFVL <mark>FSSISGV@GSGQQAY</mark> AAGNAFLDALARCRRDRGRTATAVS&GFW ELLGDRELDAFVL <mark>FSSISGV@GSGQQTAY</mark> AAGNAFLDGLAARRRARGLTATAIA&GFW DLLGDRDLDAFVL <mark>FSSIAGV&amp;GSGRQTAY</mark> AAANAHLDGLAARRRARGLTATSIA&GFW ELLAGQELDAFVM <mark>FSSGAGI&amp;GGAGQGAY</mark> SAANAYLDALAEHRRAHGRTALAVS&GGW	173 173 173 173
PteA4-KR8 FlpA4-KR8 PteA4-KR9 FlpA4-KR9	A A A A A	ELLGDQELDAFVL <b>FSSISGVWGSGGQAA</b> YAAGNAFLDALARCRRDRGRTATAVSWGFW ELLGDRELDAFVL <b>FSSISGVWGSGLQTAY</b> AAGNAFLDGLAARRRARGLTATAIAWGFW DLLGDRDLDAFVL <b>FSSIAGVWGSGRQTAY</b> AAANAHLDGLAARRRARGLTATSIAWGFW ELLAGQELDAFVM <mark>FSSGAGIWGGAGQGAY</mark> SAANAYLDALAEHRRAHGRTALAVSWGGW ELLAGQELDAFVM <mark>FSSGAGIWGGAGQAAY</mark> AAANACLDALAEHRRAHGRTALAVSWGGW	173 173 173 173 173
PteA4-KR8 FlpA4-KR8 PteA4-KR9 FlpA4-KR9 PteA4-KR11	A A A A A A	ELLGDQELDAFVL <b>FSSISGVMGSGGQA</b> YAAGNAFLDALARCRRDRGRTATAVSWGPW ELLGDRELDAFVL <b>FSSISGVWGSGQQTAY</b> AAGNAFLDGLAARRRARGLTATAIAWGPW DLLGDRDLDAFVL <b>FSSIAGVWGSGRQTAY</b> AAANAHLDGLAARRRARGLTATSIAWGPW ELLAGQELDAFVH <mark>FSSGAGIWGGAGQGAY</mark> SAANAYLDALAEHRRAHGRTALAVSWGGW ELLAGQELDAFVL <b>FSSISGVWGGGQQAAY</b> AAANACLDALAEHRRARGRTALAVSWGGW ELLGDQELDAFVL <b>FSSISGVWGGGGQAAY</b> SAANAFLDGLAQHRRARGRTALAVSWGGW	173 173 173 173 173 173
PteA4-KR8 FlpA4-KR8 PteA4-KR9 FlpA4-KR9 PteA4-KR11 FlpA4-KR11	A A A A A A A	ELLGDQELDAFVLFSSISGVWGGGGQAAYAAGNAFLDALARCRRDRGRTATAVSWGFW ELLGDRELDAFVLFSSISGVWGSGCQTAYAAGNAFLDGLAARRRARGLTATAIAWGFW DLLGDRDLDAFVLFSSIAGVWGSGRQTAYAAANAHLDGLAARRRARGLTATSIAWGFW ELLAGQELDAFVMFSSGAGIWGGAGQGAYSAANAYLDALAEHRRAHGRTALAVSWGGW ELLAGQELDAFVMFSSGAGIWGGAGQAAYAAANACLDALAEHRRARGRTALAVSWGGW ELLGDQELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ELLGERELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW	173 173 173 173 173 173 173 173
PteA4-KR8 FlpA4-KR8 PteA4-KR9 FlpA4-KR9 PteA4-KR11 FlpA4-KR11 PteA4-KR10	A A A A A A A	ELIGDQELDAFVLFSSISGVWGSGGQAAYAAGNAFLDALARCRRDRGRTATAVSWGFW ELLGDRELDAFVLFSSISGVWGSGCQTAYAAGNAFLDGLAARRRARGLTATAIAWGFW DLLGDRDLDAFVLFSSIAGVWGSGRQTAYAAANAHLDGLAARRRARGLTATSIAWGFW ELLAGQELDAFVHFSSGAGIWGGAGQGAYSAANAYLDALAEHRRAHGRTALAVSWGGW ELLAGQELDAFVHFSSISGVWGGGGQAAYAAANACLDALAEHRRARGRTALAVSWGGW ELLGDQELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ELLGERELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ALLAGQELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW	173 173 173 173 173 173 173 173 173
PteA4-KR8 FlpA4-KR8 PteA4-KR9 FlpA4-KR9 PteA4-KR11 FlpA4-KR11 FlpA4-KR10 FlpA4-KR10	A A A A A A A A A	ELIGDQELDAFVLFSSISGVWGSGGQAAYAAGNAFLDALARCRRDRGRTATAVSWGFW ELLGDRELDAFVLFSSISGVWGSGCQTAYAAGNAFLDGLAARRRARGLTATAIAWGFW DLLGDRDLDAFVLFSSIAGVWGSGRQTAYAAANAHLDGLAARRRARGLTATSIAWGFW ELLAGQELDAFVLFSSIAGVWGGGQGAYSAANAYLDALAEHRRAHGRTALAVSWGGW ELLAGQELDAFVHFSSGAGIWGGAGQAAYAAANACLDALAEHRRAHGRTALAVSWGGW ELLGDQELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ELLGERELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ALLAGQELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ALLAGQELDAFVLFSSIAGVWGSGHQAAYAAANALLDGLAERRRAQGLPATAVAWGFW ALLAGRELDAFVLFSSIAGVWGGGHQAAYAAANALLDGLAERRRAQGLPATAVAWGFW	173 173 173 173 173 173 173 173 173 173
PteA4-KR8 FlpA4-KR8 PteA4-KR9 FlpA4-KR9 PteA4-KR11 FlpA4-KR10 FlpA4-KR10 PteA3-KR70 PteA3-KR70	A A A A A A A B	ELLGDQELDAFVLFSSISGVWGSGGQAAYAAGNAFLDALARCRRDRGRTATAVSWGFW ELLGDRELDAFVLFSSISGVWGSGGQAAYAAGNAFLDGLAARRRARGLTATAIAWGFW DLLGDRDLDAFVLFSSIAGVWGSGRQTAYAAANAHLDGLAARRRARGLTATSIAWGFW ELLAGQELDAFVLFSSIAGVWGSGRQGAYSAANAYLDALAEHRRAHGRTALAVSWGGW ELLAGQELDAFVHFSSGAGIWGGAGQAAYAAANACLDALAEHRRAHGRTALAVSWGGW ELLGDQELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ELLGERELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ALLAGQELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ALLAGQELDAFVLFSSIAGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ALLAGQELDAFVLFSSIAGVWGGGGQAAYAAANALLDGLAERRRAQGLPATAVAWGFW ALLDGRELDAFVLFSSIAGVWGGGHQAAYAAANALLDGLAERRRAQGLPATAVAWGFW	173 173 173 173 173 173 173 173 173 173
PteA4-KR8 FlpA4-KR8 PteA4-KR9 FlpA4-KR9 PteA4-KR11 FlpA4-KR10 FlpA4-KR10 PteA3-KR7 FplA3-KR7	A A A A A A A B B B	ELLGDQELDAFVLFSSISGVWGSGGQAAYAAGNAFLDALARCRRDRGRTATAVSWGFW ELLGDRELDAFVLFSSISGVWGSGCQTAYAAGNAFLDGLAARRRARGLTATAIAWGFW DLLGDRDLDAFVLFSSIAGVWGSGRQTAYAAANAHLDGLAARRRARGLTATSIAWGFW ELLAGQELDAFVLFSSIAGVWGSGRQGAYSAANAYLDALAEHRRAHGRTALAVSWGGW ELLAGQELDAFVLFSSISGVWGGGGQAAYAAANACLDALAEHRRAHGRTALAVSWGGW ELLGDQELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ALLGERELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ALLAGQELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ALLAGQELDAFVLFSSIAGVWGSGHQAAYAAANALLDGLAERRRAQGLAATAIAWGFW ALLAGQELDAFVLFSSIAGVWGGGHQAAYAAANALLDGLAERRRAQGLPATAVAWGFW ALLDGRELDAFVLFSSIAGVWGGGHQAAYAAANALLDGLAERRRARGLAATAIAWGFW ELTRDL-NLSAFVLFSSIAGVWGSGAAGYAAANAFLDGLARQRRAAGLPATSLAWGFW	173 173 173 173 173 173 173 173 173 173
PteA4-KR8 FlpA4-KR8 PteA4-KR9 FlpA4-KR9 PteA4-KR11 FlpA4-KR10 FlpA4-KR10 PteA3-KR7 FplA3-KR7 PteA2-KR5	A A A A A A B B B B	ELLGDQELDAFVLFSSISGVWGSGGQAAYAAGNAFLDALARCRRDRGRTATAVSWGFW ELLGDRELDAFVLFSSISGVWGSGCQAYAAGNAFLDALARCRRDRGRTATAVSWGFW ELLGDRDLDAFVLFSSIAGVWGSGRQTAYAAANAHLDGLAARRRARGLTATSIAWGFW ELLAGQELDAFVLFSSIAGVWGSGRQTAYAAANAYLDALAEHRRAHGRTALAVSWGGW ELLAGQELDAFVLFSSISGWGGGGQAAYAAANACLDALAEHRRAHGRTALAVSWGGW ELLGDQELDAFVLFSSISGVWGGGGQAAYAAANACLDALAEHRRARGRTALAVSWGGW ELLGDQELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ALLAGQELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ALLAGQELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ALLAGQELDAFVLFSSIAGVWGSGHQAAYAAANALLDGLAERRRAQGLPATAVAWGFW ALLDGRELDAFVLFSSIAGVWGGGHQAAYAAANALLDGLAERRRAQGLPATAVAWGFW ELTRDL-NLSAFVLFSSIAGVUGSAGAAGYAAANAFLDGLARQRRAAGLPGTSLAWGIW ELTRNL-DLSAFVLFSSIAGVUGSAGAAGYAAANAFLDGLARQRRAAGLPGTSLAWGIW ELTRDSTTFVLFSSAAGVFGNFQQGYAAANAFLDAFAHRHAQGRPTVSLAWGIW	173 173 173 173 173 173 173 173 173 173
PteA4-KR8 FlpA4-KR8 PteA4-KR9 FlpA4-KR9 PteA4-KR11 FlpA4-KR10 FlpA4-KR10 FlpA4-KR10 PteA3-KR7 FplA3-KR7 FteA2-KR5 FlpA2-KR5	A A A A A A B B B B B B	ELIGDQELDAFVLFSSISGVWGSGGQAAYAAGNAFLDALARCRRDRGRTATAVSWGFW ELLGDRELDAFVLFSSISGVWGSGGQAYAAGNAFLDALARCRRDRGRTATAVSWGFW DLLGDRDLDAFVLFSSIAGVWGSGRQTAYAAANAHLDGLAARRRARGLTATSIAWGFW ELLAGQELDAFVLFSSIAGVWGSGRQGAYSAANAYLDALAEHRRAHGRTALAVSWGGW ELLAGQELDAFVHFSSGAGIWGGAGQAAYAAANACLDALAEHRRARGRTALAVSWGGW ELLGDQELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ALLAGQELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ALLAGQELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ALLAGQELDAFVLFSSIAGVWGGGGQAAYAAANALLDGLAERRRAQGLPATAVAWGFW ALLDGRELDAFVLFSSIAGVWGGGGQAAYAAANALLDGLAERRRAQGLPATAVAWGFW ELTRDLNLSAFVLFSSIAGVWGSGGQAAYAAANAFLDGLARRRARGLPATAVAWGFW ELTRDLNLSAFVLFSSIAGVUGSAGAAGYAAANAFLDGLARQRRAAGLPGTSLAWGLW ELTRNLDLSAFVLFSSIAGVLGSAGAAGYAAANAFLDGLAHRRRAAGLPATSLAWGLW ELTGPSTTFVLFSSAAGVFGNPGQGNYAAANAFLDAFARHRRAQGRPTVSLAWGIW ELTGDGTFFVLFSSAAGVFGNPGQGNYAAANAFLDAFARHRRAQGRPTVSLAWGLW	173 173 173 173 173 173 173 173 173 173
PteA4-KR8 FlpA4-KR8 PteA4-KR9 FlpA4-KR11 FlpA4-KR11 PteA4-KR10 FlpA4-KR10 PteA3-KR7 FplA3-KR7 PteA2-KR5 FlpA2-KR5 FlpA2-KR5 PteA1-KR2	A A A A A A B B B B B B B B B B B B B B	ELIGDQELDAFVLFSSISGVWGSGGQAAYAAGNAFLDALARCRRDRGRTATAVSWGFW ELLGDRELDAFVLFSSISGVWGSGGQAYAAGNAFLDALARCRRDRGRTATAVSWGFW DLLGDRDLDAFVLFSSIAGVWGSGCQTAYAAANAHLDGLAARRRARGLTATSIAWGFW ELLAGQELDAFVLFSSIAGVWGSGRQTAYAAANAHLDGLAARRRARGLTATSIAWGFW ELLAGQELDAFVLFSSIAGVWGGGQQAAYAAANACLDALAEHRRAHGRTALAVSWGGW ELLGDQELDAFVLFSSISGVWGGGGQAAYAAANACLDALAEHRRARGRTALAVSWGGW ELLGDQELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ALLAGQELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGFW ALLAGRELDAFVLFSSIAGVWGGGGQAAYAAANALLDGLAERRRAQGLPATAVAWGFW ALLDGRELDAFVLFSSIAGVWGGGHQAAYAAANALLDGLAERRRAQGLPATAVAWGFW ELTRDLNLSAFVLFSSIAGVWGGGHQAAYAAANALLDGLAERRRAGLPATAVAWGFW ELTRDLSTFVLFSSIAGVLGSAGAAGYAAANAFLDGLARQRRAAGLPGTSLAWGLW ELTRNLDLSAFVLFSSIAGVLGSAGAAGYAAANAFLDGLAHRRRAAGLPATSLAWGLW ELTGDSTFVLFSSAAGVFGNPGQGNYAAANAFLDAFARHRAQGRPTVSLAWGLW ELTGDGTPFVLFSSAAGVFGNPGQGNYAAANAFLDAFARHRRAQGRPTVSLAWGLW DLAGELSGDLSAFVLFSSVAGTLGTFGQANYAAANAFLDAFARHRRAAGQRATSLAWGLW	173 173 173 173 173 173 173 173 173 173
PteA4-KR8 FlpA4-KR9 FlpA4-KR9 FlpA4-KR11 FlpA4-KR10 FlpA4-KR10 FlpA4-KR10 PteA3-KR7 FplA3-KR7 FlpA2-KR5 FlpA2-KR5 FlpA2-KR5 FlpA1-KR2 FlpA1-KR2	A A A A A A A B B B B B B B B B B B B B	ELIGDQELDAFVL <b>FSSISGVWGSGGQAAY</b> AAGNAFLDALARCRRDRGRTATAVSWGFW ELLGDRELDAFVL <b>FSSISGVWGSGCQTAY</b> AAGNAFLDGLAARRRARGLTATAIAWGFW DLLGDRDLDAFVL <b>FSSIAGVWGSGCQTAY</b> AAANAHLDGLAARRRARGLTATSIAWGFW ELLAGQELDAFVL <b>FSSIAGVWGSGCQAY</b> AAANAYLDALAEHRRAHGRTALAVSWGGW ELLAGQELDAFVH <b>FSSGAGIWGGAGQAAY</b> AAANACLDALAEHRRARGRTALAVSWGGW ELLGDQELDAFVL <b>FSSISGVWGGGGQAAY</b> AAANACLDALAEHRRARGRTALAVSWGGW ALLGDQELDAFVL <b>FSSISGVWGGGGQAAY</b> AAANAFLDGLAQHRRARGLAATAIAWGFW ALLAGQELDAFVL <b>FSSISGVWGGGGQAAY</b> AAANAFLDGLAQHRRARGLAATAIAWGFW ALLAGRELDAFVL <b>FSSIAGVWGGGGQAAY</b> AAANALLDGLAERRRAQGLPATAVAWGFW ALLDGRELDAFVL <b>FSSIAGVWGGGGQAAY</b> AAANALLDGLAERRRAQGLPATAVAWGFW ELTRDLNLSAFVL <b>FSSIAGVWGGGGQAAY</b> AAANALLDGLAERRRARGLPATAVAWGFW ELTRNLDLSAFVL <b>FSSIAGVWGGGHQAAY</b> AAANAFLDGLARQRRAAGLPGTSLAWGLW ELTRNLDLSAFVL <b>FSSIAGVWGSAGAAGY</b> AAANAFLDGLAHRRRAAGLPATSLAWGLW ELTGPSTTFVL <b>FSSAAGVFGNPGQGNY</b> AAANAFLDAFARHRAQGRPTVSLAWGLW DLAGELSGDLSAFVL <b>FSSVAGTLGTPGQQNY</b> AAANTFLDALAEHRRAAGQRATSLAWGLW DLAAELCGDLSAFVL <b>FSSVAGTLGTPGQQNY</b> AAANTFLDALAEHRRAAGLRATSLAWGLW	173 173 173 173 173 173 173 173 173 173
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PteA4-KR8 FlpA4-KR9 FlpA4-KR9 PteA4-KR11 FlpA4-KR11 PteA4-KR10 PteA3-KR7 FlA3-KR7 FteA2-KR5 FlpA2-KR5 FlpA1-KR2 FlpA1-KR2 FlpA1-KR3 FlpA1-KR3 FlpA1-KR3 FlpA2-KR6 FlpA2-KR6 FlpA2-KR6	A A A A A A A A B B B B B B B B B B B B	ELIGD - QELDAFVLFSSISGVWGSGCQAAYAAGNAFLDALARCRRDRGRTATAVSWGPW ELLGD - RELDAFVLFSSISGVWGSGCQTAYAAGNAFLDGLAARCRRDRGRTATAVSWGPW DLLGD - RELDAFVLFSSIAGVWGSGCQTAYAAGNAFLDGLAARCRRARGLTATSIAWGPW DLLGD - RDLDAFVLFSSIAGVWGSGCQTAYAAANAHLDGLAARCRARGLTATSIAWGPW ELLAG - QELDAFVLFSSIAGVWGSGCQAAYAAANALDGLAAERRARGLTATSIAWGPW ELLGG - QELDAFVLFSSISGVWGGGGQAAYAAANACLDALAEHRRARGRTALAVSWGGW ELLGD - QELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGPW ALLGG - RELDAFVLFSSIAGVWGSGCQAAYSAANAFLDGLAQHRRARGLAATAIAWGPW ALLAG - QELDAFVLFSSIAGVWGSGCQAAYAAANALLDGLAERRRAQGLPATAVAWGPW ALLAG - QELDAFVLFSSIAGVWGSGCQAAYAAANALLDGLAERRRAQGLPATAVAWGPW ALLDG - RELDAFVLFSSIAGVWGSGCQAAYAAANALLDGLAERRRAQGLPATAVAWGPW ELTRDL - NLSAFVLFSSIAGVWGSGCQAAYAAANAFLDGLARQRRAAGLPGTSLAWGLW ELTRDL - DLSAFVLFSSLAGVLGSAGAAGYAAANAFLDGLAHRRRAAGLPATSLAWGLW ELTGD STTFVLFSSLAGVLGSAGAAGYAAANAFLDAFARHRAQGRPTVSLAWGLW DLAGELSGDLSAFVLFSSVAGTLGTPGQANYAAANAFLDAFARHRAAGQRATSLAWGLW DLAAELCGDLSAFVLFSSVAGTLGTPGQQNYAAANAFLDAFARHRAAGQRATSLAWGLW ELTSD LSAFVVFSSVSGILGSAGQGNYAAANAFLDAFAERRARGLPATSLAWGLW ELTGE LSAFVVFSSVSGILGSAGQGNYAAANAFLDAFAERRRAGGRATSLAWGLW ELAGD VSAFVLFSSVSGILGSAGQQNYAAANAFLDAFAERRRAGGRATSLAWGLW ELAGD VSAFVVFSSVSGILGSAGQQNYAAANAFLDAFAERRRAQGLPAVSLPWGLW ELAGD VSAFVVFSSVSGILGSAGQQNYAAANAFLDAFAARRRAQGLPAVSLPWGLW ELAGD VSAFVVFSSVSGILGSAGQQNYAAANAFLDAFAARRRAQGLPAVSLPWGLW ELAGD VSAFVVFSSVSGILGSAGQQNYAAANAFLDAFAARRRAQGLPAVSLPWGLW	173 173 173 173 173 173 173 173 173 173
PteA4-KR8 FlpA4-KR9 FlpA4-KR9 FlpA4-KR10 FlpA4-KR10 FlpA4-KR10 FlpA4-KR10 PteA3-KR7 PteA2-KR5 FlpA2-KR5 FlpA1-KR2 FlpA1-KR2 FlpA1-KR3 FlpA1-KR3 PteA2-KR6 FlpA2-KR6 FlpA1-KR4 FlpA1-KR4	A A A A A A A A B B B B B B B B B B B B	ELIGDQELDAFVLFSSISGVWGSGCQAAYAAGNAFLDALARCRRDRGRTATAVSWGPW ELLGDRELDAFVLFSSISGVWGSGCQTAYAAGNAFLDGLAARRRARGLTATAIAWGPW DLLGDRDLDAFVLFSSIAGVWGSGRQTAYAAANAHLDGLAARRRARGLTATSIAWGPW ELLAGQELDAFVLFSSIAGVWGSGRQGAYSAANAYLDALAEHRRAHGRTALAVSWGGW ELLAGQELDAFVLFSSISGVWGGGGQAAYAAANACLDALAEHRRAHGRTALAVSWGGW ELLGDQELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGPW ALLGCRELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGPW ALLAGQELDAFVLFSSISGVWGGGGQAAYSAANAFLDGLAQHRRARGLAATAIAWGPW ALLAGQELDAFVLFSSIAGVWGSGHQAAYAAANALLDGLAERRRAQGLPATAVAWGPW ALLAGQELDAFVLFSSIAGVWGGGGQAAYAAANALLDGLAERRRAQGLPATAVAWGPW ALLDGRELDAFVLFSSIAGVWGSGHQAAYAAANALLDGLAERRRAQGLPATAVAWGPW ALLDGRELDAFVLFSSIAGVUGSAGAAGYAAANAFLDGLARQRRAAGLPGTSLAWGLW ELTRDLDLSAFVLFSSLAGVLGSAGAAGYAAANAFLDGLAHRRRAAGLPATSLAWGLW ELTGDSTTFVLFSSAAGVFGNPGQGNYAAANAFLDAFARHRAQGRPTVSLAWGLW DLAGELSGDLSAFVLFSSVAGTLGTPGQANYAAANAFLDAFARHRRAQGRATSLAWGLW DLAAELCGDLSAFVLFSSVAGTLGTPGQANYAAANAFLDAFARHRRAGQRATSLAWGLW ELTSDLSAFVVFSSVSGILGSAGQGNYAAANAFLDAFAERRRAGLPATSLAWGLW ELTGCVSAFVVFSSVSGILGSAGQGNYAAANAFLDAFAERRRAGLPATSLAWGLW ELTGCVSAFVVFSSVSGILGSAGQGNYAAANAFLDAFAERRRAGGLPATSLAWGLW ELTGDVSAFVVFSSVSGILGSAGQGNYAAANAFLDAFAERRRAGGLPATSLAWGLW ELTGCVSAFVVFSSVSGILGSAGQGNYAAANAFLDAFAERRRAGGLPATSLAWGLW ELTGCVSAFVVFSSVSGILGSAGQGNYAAANFFLDAFAGFRRDQGLPATSLAWGLW ELTGCVSAFVVFSSVSGILGSAGQGNYAAANFFLDAFAERRRAGGLPAVSLPWGLW ELTGDVSAFVVFSSVSGILGSAGQGNYAAANFFLDAFAERRRAGGLPAVSLPWGLW ELTGDVSAFVVFSSVSGILGSAGQANYAAANFFLDAFAERRRAGGLPAVSLPWGLW ELTGDVSAFVVFSSVSGILGSAGQANYAAANFFLDAFAERRRAGGLPAVSLPWGLW	173 173 173 173 173 173 173 173 173 173

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

А Model 01 MSEPTGAAPAVPKARSCPFLPPDGIAEVRAAAPVTRATFTSGHEAWLVSGYEEVRTLLRDPSFSVQVPHA 70 3aba.1.AMPEPTADAPDVPKARSCPFLPPDGIADIRAAAPVTRADFTSGHEAWLVTGYEEVRALLRDSSFSVQVPHA 70 Model 01 LHTQDGIVTQKPGRGSLIWQDEPEHTADRKLLAKEFTVRRMQALRPNIQRIVDERLDAIAAQGGTVDLVK 140 3aba.1.ALHTQDGVVTQKPGRGSLLWQDEPEHTSDRKLLAKEFTVRRMQALRPNIQRIVDEHLDAIEARGGPVDLVK 140 Model 01 TFANPVPAMVISDLFGVPVERRPEFQEIAEAMMRVDQDAAATEAAGMRLGGLLYQLVQERRSSPGEDLIS 210 3aba.1.ATFANAVPSMVISDLFGVPVERRAEFQDIAEAMMRVDQDAAATEAAGMRLGGLLYQLVQERRANPGDDLIS 210 Model 01 ALTTTEDPDGVLDDMFLMNAAGTLLIAAHDTTACMIGLGAALLLDRPDQLALLREDPSLVGNAVEELLRY 280 3aba.1.AAL]TTEDPDGVVDDMFLMNAAGTLLIAAHDTTACMIGLGTALLLDSPDQLALLREDPSLVGNAVEELLRY 280 Model\_01 LTIGQFGGERVATRDVELGGVRIAAGEQVVAHVLAADFDPAFVEDPERFDITRRPAPHLAFGFGAHQCIG 350 3aba.1.ALTIGQFGGERVATRDVELGGVRIAKGEQVVAHVLAADFDPAFVEEPERFDITRRPAPHLAFGFGAHQCIG 350 Model 01 **QOLARIELQIAFDSLFRRFPTLRLAKPVEELRFRDDMVFYGVHELPVTW** 399 3aba.1.AQQLARIELQIVFETLFRRLPGLRDAKPVEELRDRHDMVFYGVHELPVTW 399

### В

≁Model_01	MTETDIRHTG <mark>SEAPAFPQDRTCPYQPPQAYTEWRGESPLTRVTLFDGRPAWLITGHAEG</mark> RALLADPRLSS	70
3abb.1.A	MTETEIRLTGSPAPSFPQDRTCPYQPPKAYBERRGESPLTQVTLFDGRPAWLDTGHAEGRALLVDPRLSS	70
Model_01	DWGHPVFPVVVQRTEDRGGLAFPLIGVDDPLHARQRRMLIPSFGVKRMNAIRPSLQSLVERLLDDMLAKG	140
3abb.1.A	DWGHPDFPVVVRTEDRGGLAFPLIGVDDP <del>VHARQRRMLIPSF</del> G <mark>VKRMNAIRPRLQSLVDRLLDDMLA</mark> KG	140
Model_01	PVVDLVSAFALPVPSMAICELLGVPYDDHDFFEECSRDFVGAATSGDADAAFAKLYQYLHGLVAKKQAEP	210
3abb.1.A	PGADLVSAFALPVPSVAICELLGVPYGDHDFFEECSRNFVGAATSAEADAAFGELYTYLHGLVGRKQAEP	210
Model_01	GDGLLDELIARQL <mark>EE</mark> GGLDHNEVVMIALVLLVAGHETTVNAIALGALTLMQHPEQIEVLL <mark>ND</mark> PAAVPGVV	280
3abb.1.A	E DGLLDELIARQLEEG DLDH DEVVMIALVLLVAGHETTVNAIALGALTL DQHPEQI DVLLR DPGAVSGVV	280
Model_01	EELLRFTSVSDYMVRMAKEDIEVGGTTIRTGEAVLVSITLMNRDAKAYDDPDVFDARRNARHHVGFGHGI	350
3abb.1.A	EELLRFTSVSDHIVRMAKEDIEVGGATIKAGDAVLVSITLMNRDAKAYENPDIFDARRNARHHVGFGHGI	350
Model_01	HQCLGQNLARAELEIALGALFTRIPGLRLAVPLDEVPLKAGHDAQGPIELPVTW	404
3abb.1.A	HQCLGQNLARAELEIALGGLFARIPGLRLAVPLDEVPDKAGHDAQGDIELPV W	404

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