SUPPLEMENTARY INFORMATION

Running title: NRPS and *Aspergillus fumigatus* ergot alkaloid biosynthesis

Non-Ribosomal Peptide Synthetases *pesL* and *pes1* are essential for Fumigaclavine C production in *Aspergillus fumigatus*

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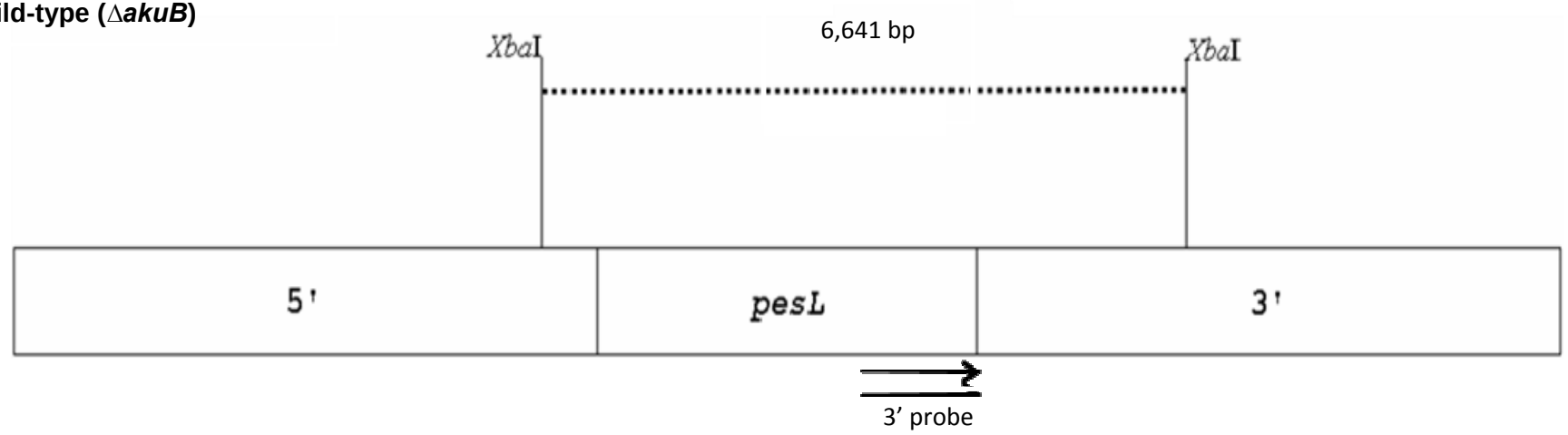
1

A.

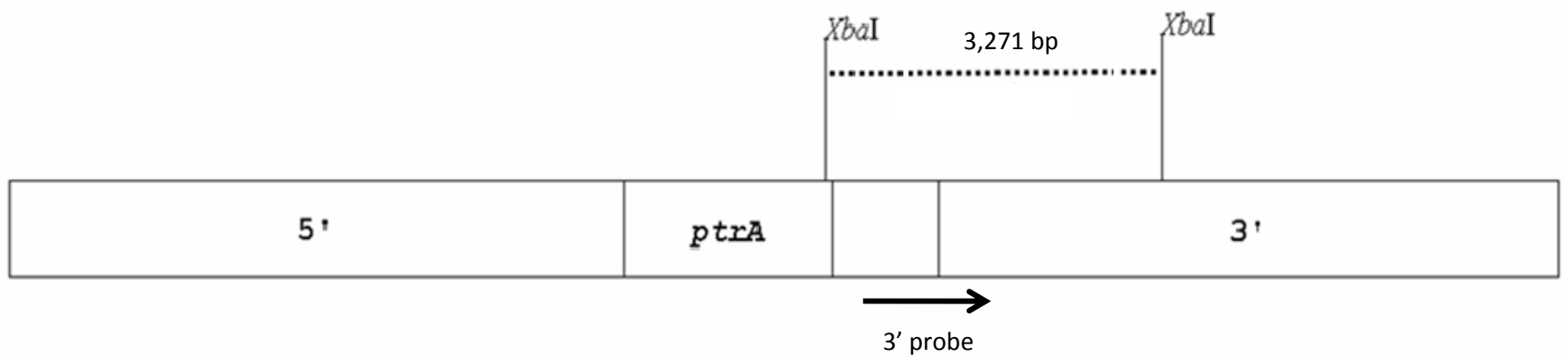
Wild-type (Δ*akuB*)



2

3

ΔpesL



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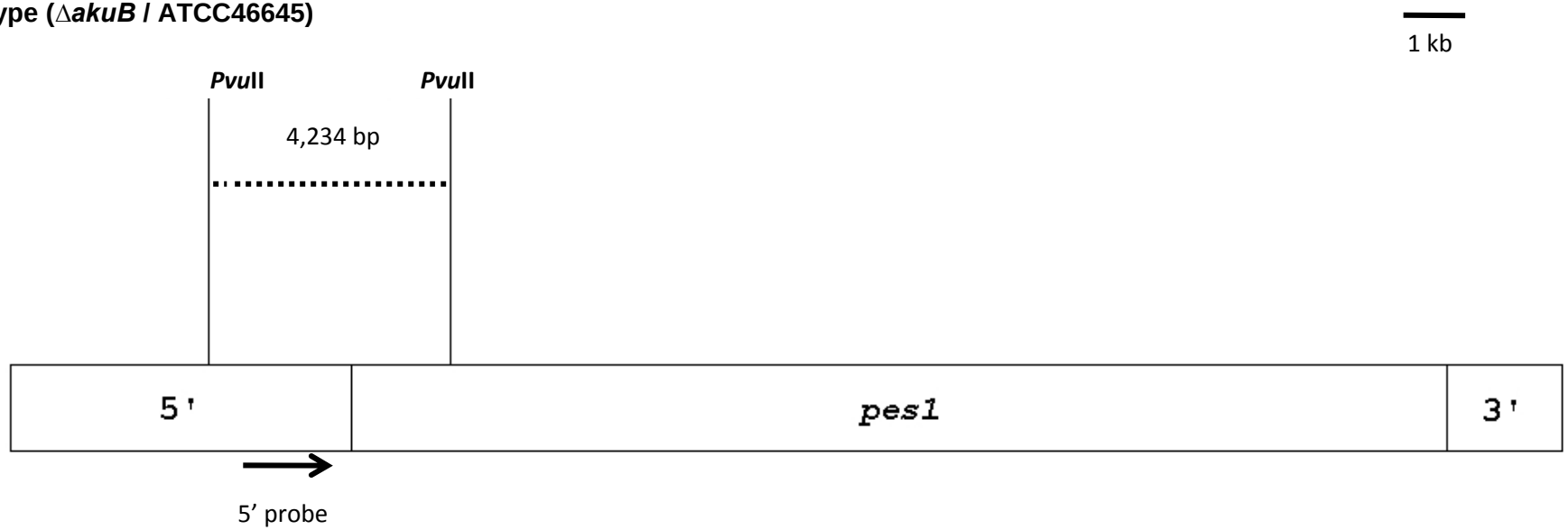
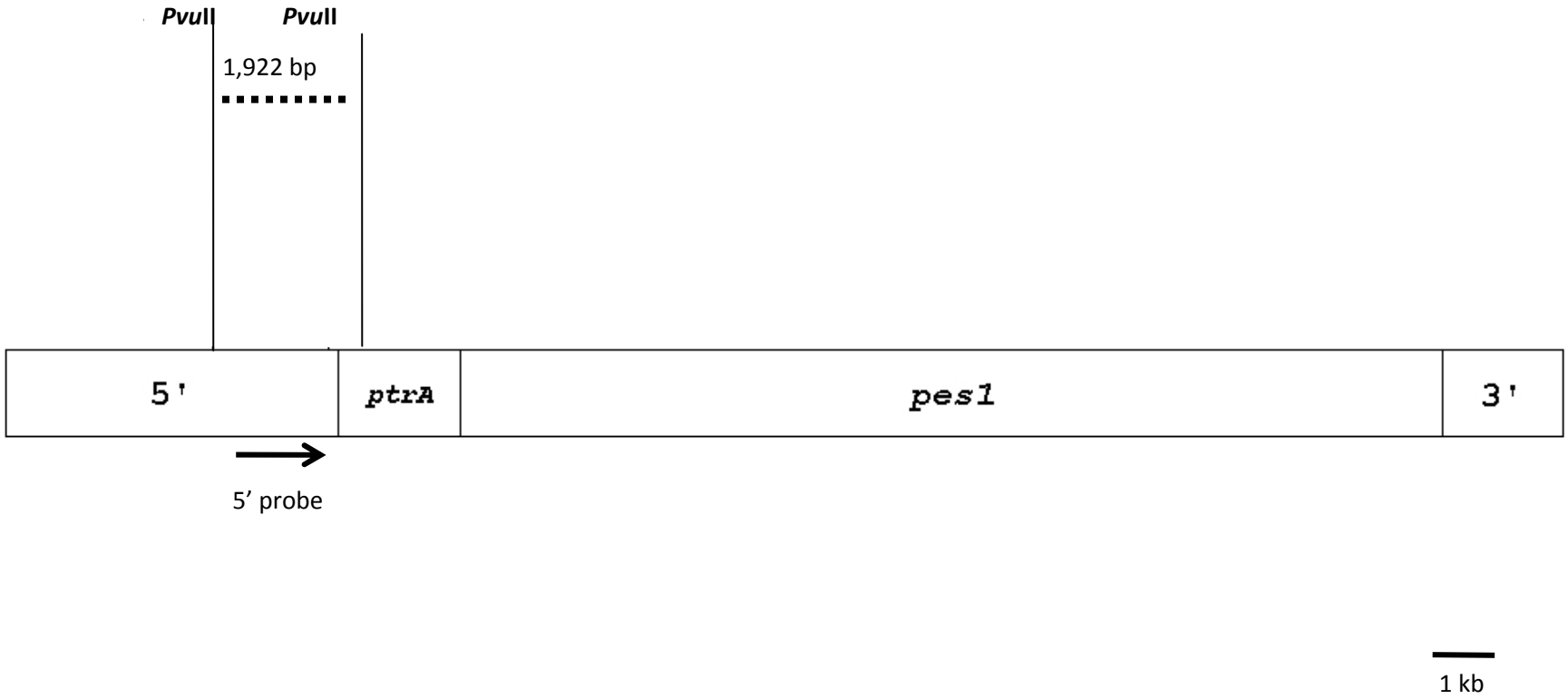
Wild-type (Δ*akuB* / ATCC46645)



1

Δpes1

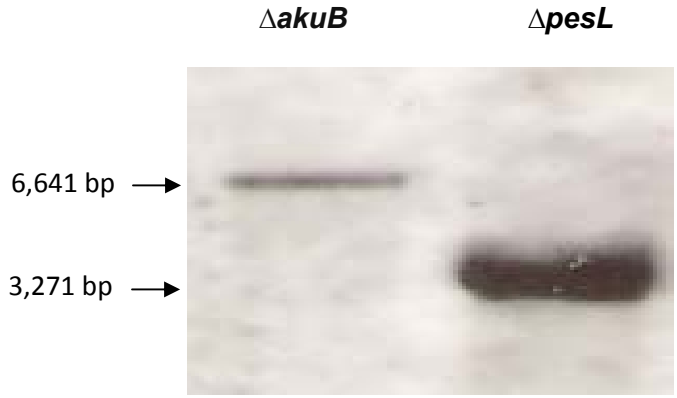


2

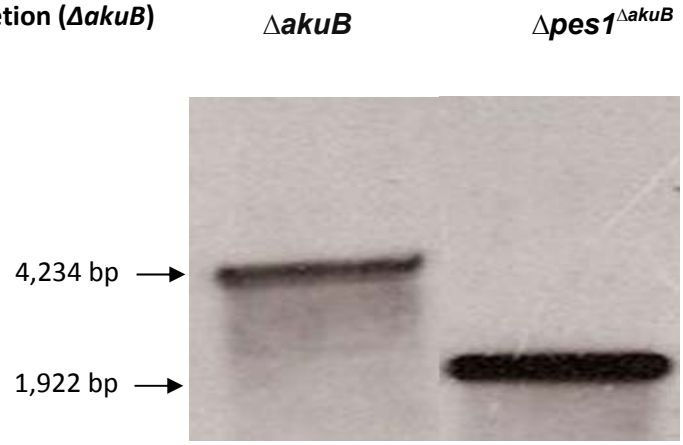
3

B.

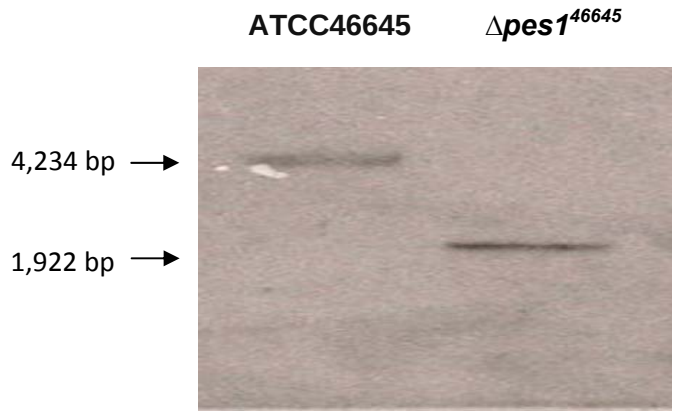
(a) *pesL* deletion



(b) *pes1* deletion (*ΔakuB*)



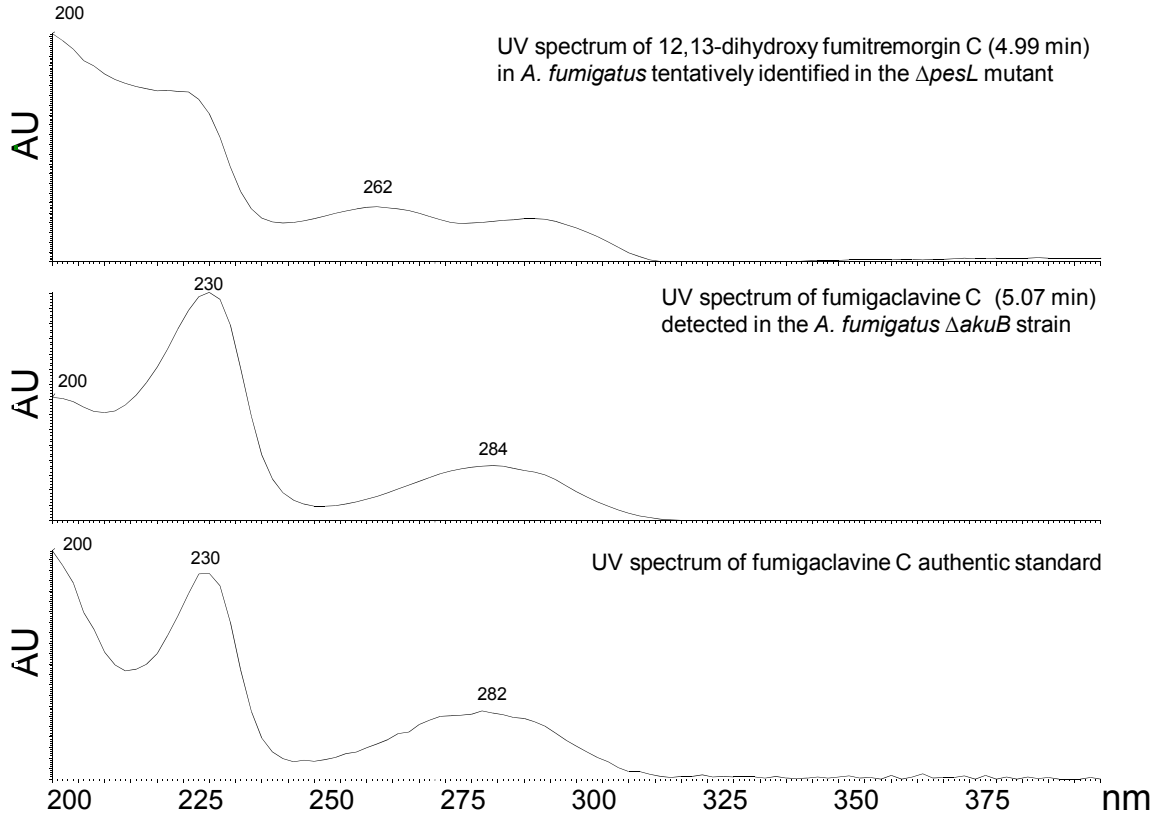
(c) *pes1* deletion (ATCC46645)



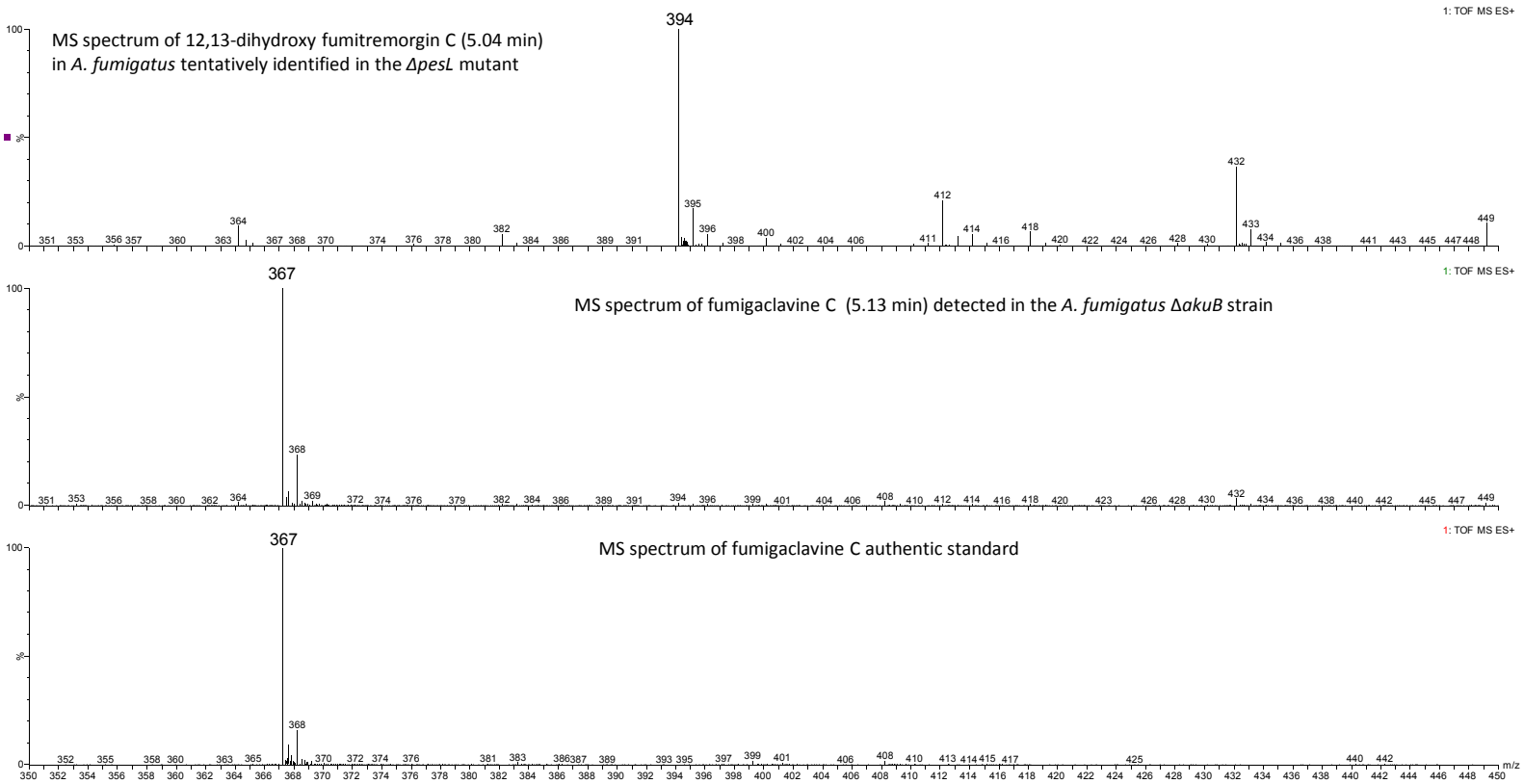
Supplementary Figure 1. Confirmation of *pesL* and *pes1* deletion. A. Schematic representation of Southern blots and probe hybridisation for *A. fumigatus* Δ*akuB* and

ΔpesL, or *A. fumigatus* wild-type and *Δpes1* is indicated, displaying the *pesL* or *pes1* locus in wild-type and respective mutant strains. ***pesL:*** *Xba*I restricted DNA was subjected to hybridisation with a 1,171 bp DIG-labelled probe corresponding to a region at the 3' end of *pesL* leading to fragments of 6,641 bp in Δ*akuB* and 3,271 bp in *ΔpesL*. For ***pes1,*** *Pvu*II restricted DNA was hybridised with a 1,236 bp 5' probe, leading to fragments of 4,234 bp in Δ*akuB* and ATCC46645 and 1,922 bp in Δ*pes1*. Probe hybridisation regions are indicated with black arrows. **B.** Southern blot analysis of *A. fumigatus* wild-type and mutant strains confirms *pesL* and *pes1* deletions in *A. fumigatus*, by the presence of the expected hybridisation patterns as described in (A), where (a) shows analysis of *A. fumigatus* Δ*akuB* and Δ*pesL* (b) shows analysis of *A. fumigatus* Δ*akuB* and $\Delta pes1^{\Delta akuB}$ and (c) shows analysis of *A. fumigatus* ATCC46645 and $\Delta pes1^{46645}$.

A.


200
UV spectrum of 12,13-dihydroxy fumitremorgin C (4.99 min)
in A. fumigatus tentatively identified in the ΔpesL mutant
AU
262
230
UV spectrum of fumigaclavine C (5.07 min)
detected in the A. fumigatus ΔakuB strain
200
AU
284
200
230
UV spectrum of fumigaclavine C authentic standard
AU
282
200
225
250
275
300
325
350
375
nm

B.



Supplementary Figure 2. A. UV spectra of 12,13-dihydroxy fumitremorgin C (top) at 4.99 min peak, fumigaclavine C (middle) at 5.07 min peak, both in FIG. 2, compared to the UV spectrum of an authentic standard of fumigaclavine C. **B.** MS spectra of 12,13-dihydroxy fumitremorgin C (top) at 5.04 min peak, fumigaclavine C (middle) at 5.13 min peak, both in FIG. 2, compared to the MS spectrum of an authentic standard of fumigaclavine C

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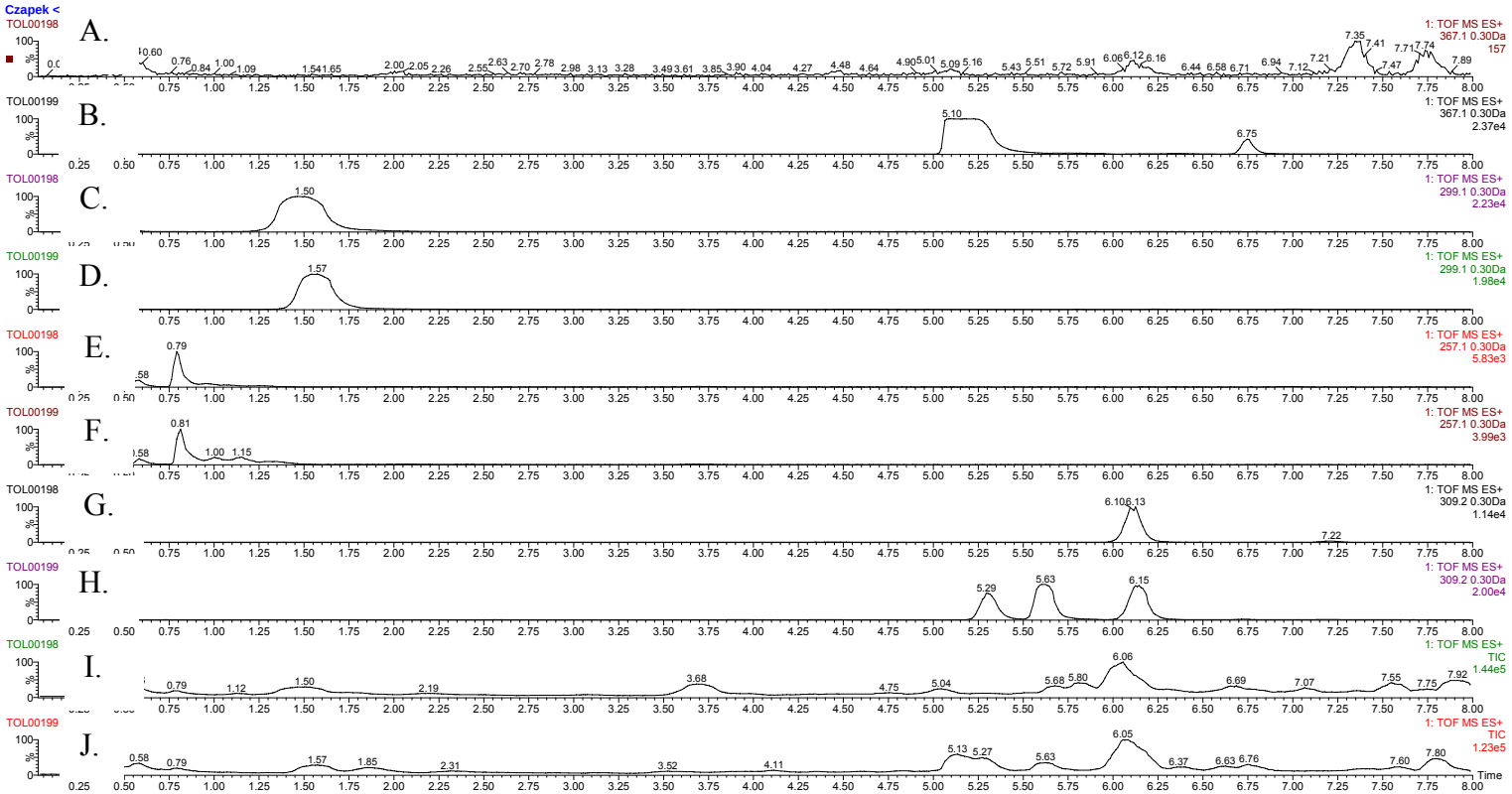

Czapek <
TOL00198
A.
1: TOF MS ES+
367.1 0.30Da
157
TOL00199
B.
1: TOF MS ES+
367.1 0.30Da
2.37e4
TOL00198
C.
1: TOF MS ES+
299.1 0.30Da
2.23e4
TOL00199
D.
1: TOF MS ES+
299.1 0.30Da
1.98e4
TOL00198
E.
1: TOF MS ES+
257.1 0.30Da
5.83e3
TOL00199
F.
1: TOF MS ES+
257.1 0.30Da
3.99e3
TOL00198
G.
1: TOF MS ES+
309.2 0.30Da
1.14e4
TOL00199
H.
1: TOF MS ES+
309.2 0.30Da
2.00e4
TOL00198
I.
1: TOF MS ES+
TIC
1.44e5
TOL00199
J.
1: TOF MS ES+
TIC
1.23e5
Time

Supplementary Figure 3. Mass ion profiling analysis of fumigaclavines in *Aspergillus fumigatus* Δ*akuB* strain (TOL00199) and Δ*pesL* (TOL00198).
A: Ion trace (m/z 367.1 Da, $[M+H]^+$) illustrating absence of fumigaclavine C in *Aspergillus fumigatus* in the Δ*pesL* mutant.

B: Ion trace (m/z 367.1 Da, $[M+H]^+$) illustrating presence of fumigaclavine C in *Aspergillus fumigatus* Δ*akuB*.

C: Ion trace (m/z 299.1 Da, $[M+H]^+$) illustrating presence of fumigaclavine A in Δ*pesL*.

D: Ion trace (m/z 299.1 Da, $[M+H]^+$) illustrating presence of fumigaclavine A in Δ*akuB*.

E: Ion trace (m/z 257.1 Da, $[M+H]^+$) illustrating presence of fumigaclavine B in Δ*pesL*.

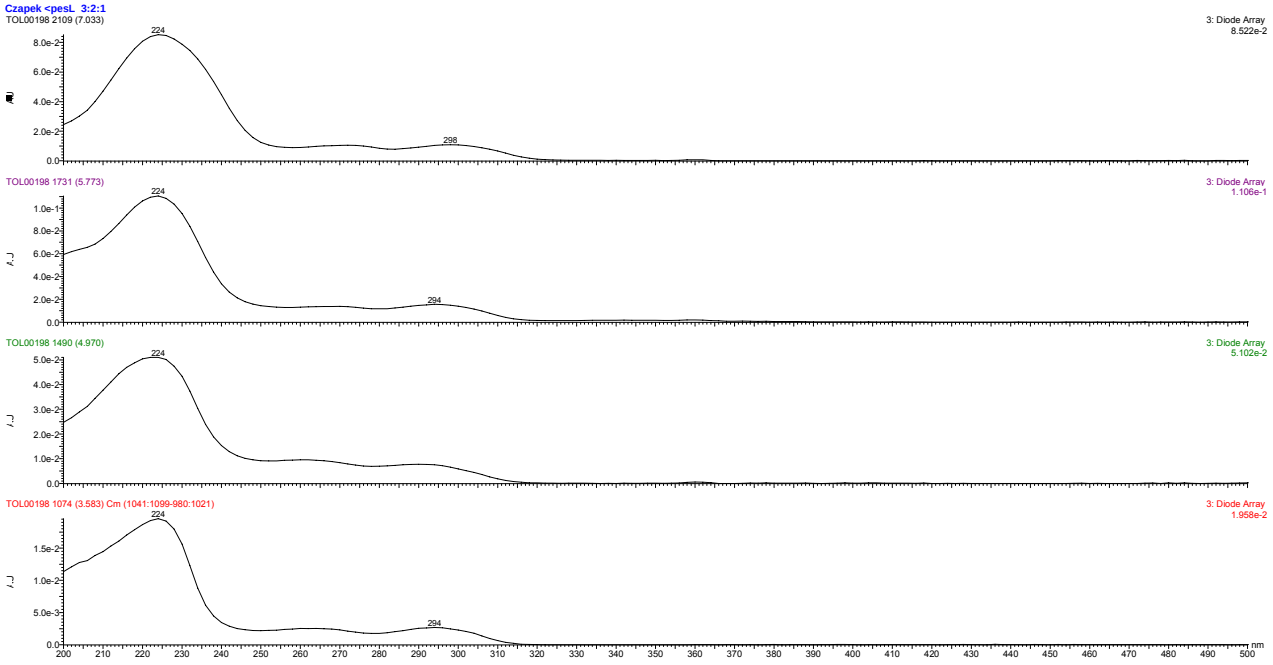
F: Ion trace (m/z 257.1 Da, $[M+H]^+$) illustrating presence of fumigaclavine B in Δ*akuB*.

G: Ion trace (m/z 309.2 Da, $[M+H]^+$) illustrating absence of two likely isomers (RT: 5.29 and 5.63 min) of 9-deoxy fumigaclavine C in Δ*pesL*.

H: Ion trace Ion trace (m/z 309.2 Da, $[M+H]^+$) illustrating presence of two likely isomers (RT: 5.29 and 5.63 min) of 9-deoxy fumigaclavine C in Δ*akuB*.

I: Total ion chromatogram (TIC) of metabolite extracts of the *Aspergillus fumigatus* Δ*pesL* from growth on Czapek medium.

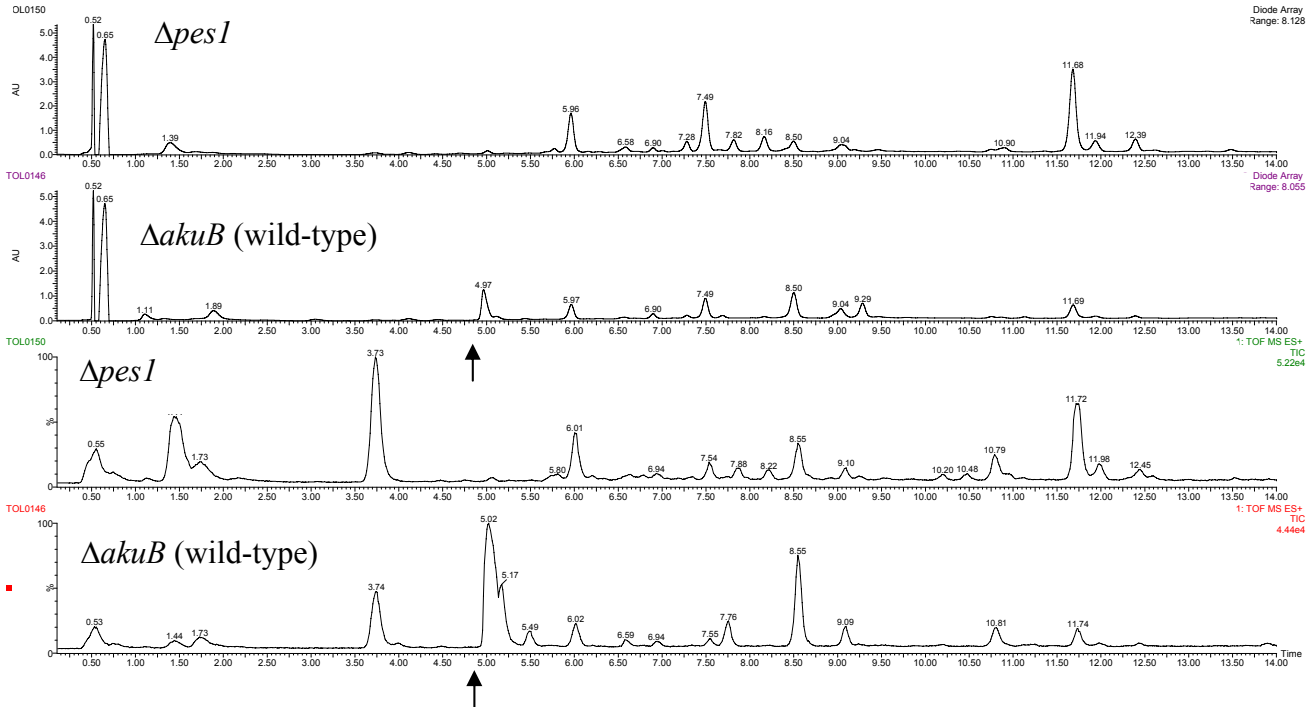
J: Total ion chromatogram (TIC) of metabolite extracts of the *Aspergillus fumigatus* Δ*akuB* from growth on Czapek medium.



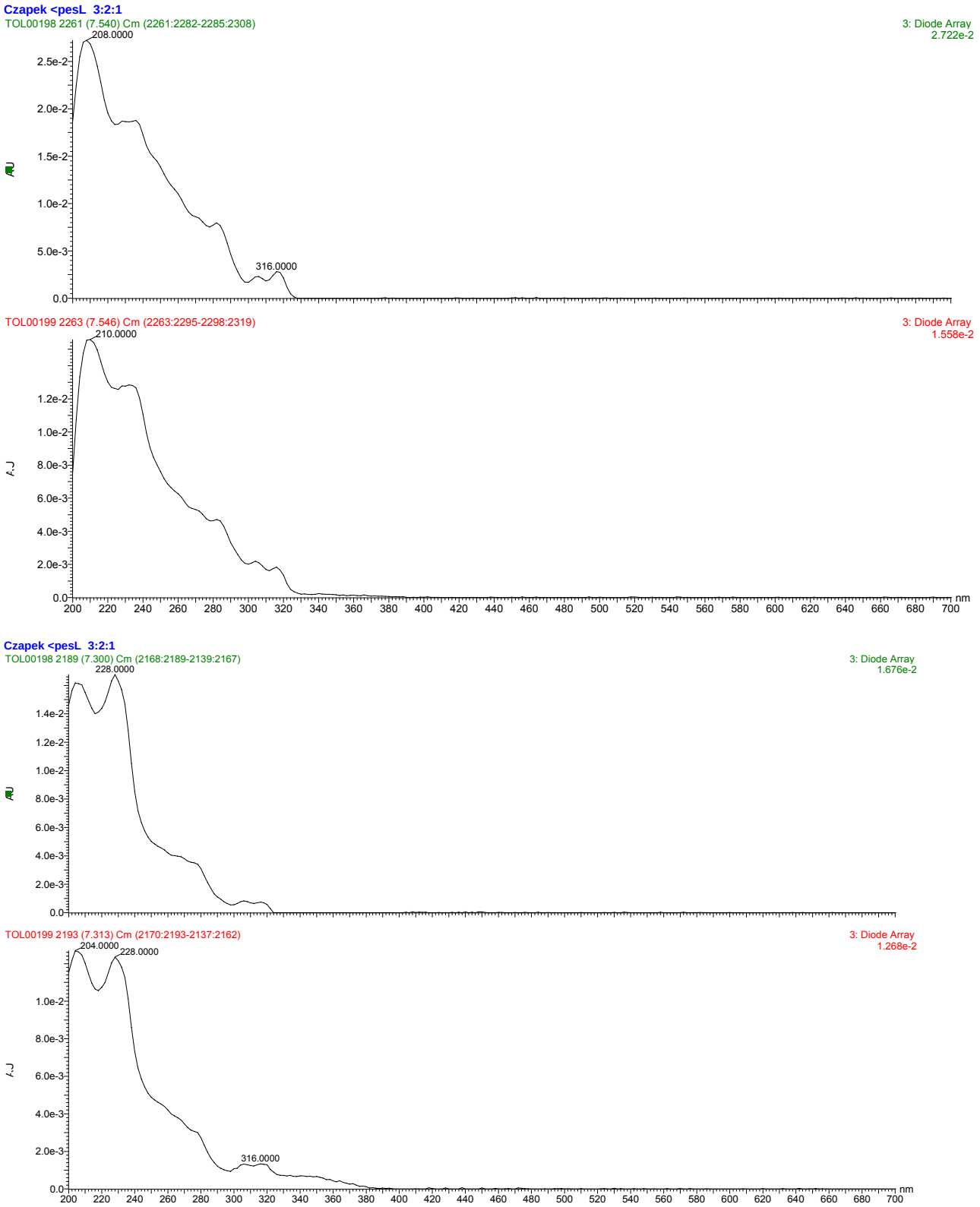
Supplementary Figure 4. UV spectra of likely biosynthetically related compounds present in both *A. fumigatus* Δ*akuB* and Δ*pesL* conidial extracts (FIG. 3 manuscript). All UV spectra are very similar and indicate fumitremorgins (1).

A. DAD

B. TIC



Supplementary Figure 5. Pes1 is essential for fumigaclavine C biosynthesis. A. Diode array detection (DAD) based chromatogram of *A. fumigatus* Δ*pes1* (upper). Notice the absence of the peak for fumigaclavine C (Rt: 4.97 min). Diode array based chromatogram of the Δ*akuB* (lower) fumigaclavine C (Rt: 4.97 min) is present. **B.** Total ion chromatogram (TIC) of the Δ*pes1* mutant. Notice the absence of the peak for fumigaclavine C (Rt: 5.02 min). Total ion chromatogram of Δ*akuB* confirms the presence of fumigaclavine C (Rt: 5.02 min) (lower TIC).



Supplementary Figure 6. UV spectra of fumiquinazonines C/D (Rt: ca. 7.5 min) and fumiquinazolines A/B (Rt: ca. 7.3 min) in *Aspergillus fumigatus* Δ*akuB* (TOL00199) and Δ*pesL* mutant (TOL00198).

SUPPLEMENTARY TABLE 1. OLIGONUCLEOTIDE PRIMERS USED DURING THIS STUDY

Primer Name	Sequence 5'-3'
opesL 1	GTCTATCAGCACACCCTTACCG
opesL 2	AACTCCGCTTCACAGACC
opesL 3	CCTGTTGCTCGACATTCC
opesL 4	AATCTGCAGGACAACGCAGCATCAAGG
opesL 5	GATTCTGCCTTGGATGCG
opesL 6	TCAGGTCCCTTCTCACAC
opes1 1	GTCGGCATCGGACATCTAC
opes1 2	CTGTAGCTTCTGGCCGAG
opes1 3	GCGGTACCCAAGGCATTGGTCTCACTG
opes1 4	GACGATCGGTACCATCTGCCACTCAC
opes1 5	CATGCAATCAAGGATATGG
opes1 6	CCTTGCACTACCAATGCTG
OPtrA1	GAGGACCTGGACAAGTAC
OPtrA2	CATCGTGACCAGTGGTAC
pes1 RT-F	TACCCATGGACCCAAGTCAT
pes1 RT-R	TTGTGGGAAGATCTGGAAGG
pesL RT-F	GGGCCGCTATATACCACAGA
pesL RT-R	AAGAGGAGTGCCACCAACAC
AFUA_6G12040 RT-F	TCTATGCCACGGTTGGTGTA
AFUA_6G12040 RT-R	ATTGCCCGAATCGACATTAT
AFUA_6G12060 RT-F	GTGTCTTTGCGTTTCCCAAT
AFUA_6G12060 RT-R	ATGTGTCCTCCACCCGATAA
AFUA_6G12070 RT-F	TCATGGGGTCCAATGAAGAT

AFUA_6G12070 RT-R	TGGCTGCATCTGTTCTTCTG
AFUA_6G12080 RT-F	TCATGGGGTCCAATGAAGAT
AFUA_6G12080 RT-R	TGGCTGCATCTGTTCTTCTG
calm F	CCGAGTACAAGGAAGCTTTCTC
calm R	GAATCATCTCGTCGATTCGTCGTCTCAGT

SUPPLEMENTARY TABLE 2. SUMMARY OF THE FUMIQUINAZOLINE COMPOUNDS

Compound	Molecular formula	M/Z	Retention time (RP-HPLC) – this study
Fumiquinazoline A	$C_{24}H_{23}N_5O_4$	445.1744	7.32-7.34 min
Fumiquinazoline B	$C_{24}H_{23}N_5O_4$	445.1744	7.32-7.34 min
Fumiquinazoline C	$C_{24}H_{21}O_4N_5$	443.1588	7.6 min
Fumiquinazoline D	$C_{24}H_{21}O_4N_5$	443.1588	7.6 min
Fumiquinazoline F	$C_{21}H_{18}O_2N_5$	358.1430	6.12-6.15 min
Fumiquinazoline G	$C_{21}H_{18}O_2N_5$	358.1430	6.12-6.15 min

References

1. Larsen, T. O., J. Smedsgaard, K. F. Nielsen, M. A. Hansen, R. A. Samson, and J. C. Frisvad. 2007. Production of mycotoxins by *ASPERGILLUS LENTULUS* and other medically important and closely related species in section Fumigati. Med. Mycol. **45:**225-232.