Solving the Polyketide Pigmentation Puzzle in Fusarium solani

Linking biosynthetic genes to compounds

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SOLVING THE POLYKETIDE PIGMENTATION PUZZLE OF *FUSARIUM SOLANI*

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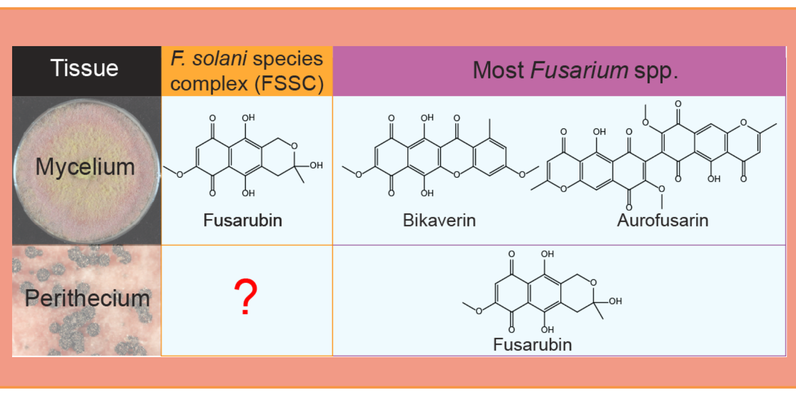
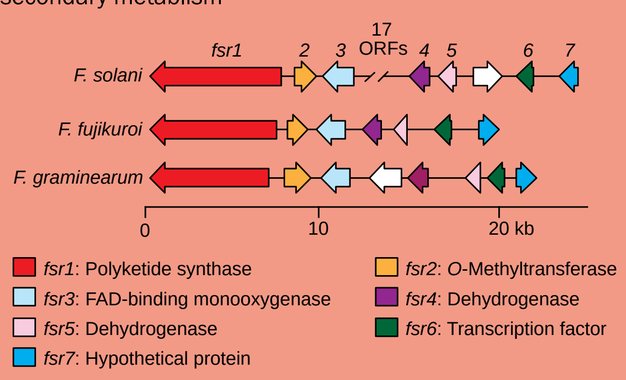
Introduction

Fusarium pigmentation is dictated by a set of two polyketide synthase (PKS) biosynthethic gene clusters (BGC) where one is expressed during mycelial growth and the other during peritheical development. In the vast majority of *Fusarium* species, peritheical pigmentation relies on the PKS3 (*fsr1*) cluster reponsible for biosynthesis of the red and purple naphtoquinone pigments fusarubin and bostrycoidin [1, 2]. However, the situation is different for members of the *Fusarium solani* species complex (FSSC), where mycelial pigmentation is controlled by the PKS3 cluster, while the clade-restricted PKS35 (*pksN*) [3] is responsible for perithecial pigmentation [4, 5], although no actual compounds(s) has ever been associated with the latter. In this study, we seek to associate the two *F. solani* polyketide pigmentation clusters to their respective compounds by an experimantal approach. We aim to describe a set of novel pigment compounds – a category of secondary metabolites previously associated with structiral diversity and biological function. We hope the techniques and methods applied can aid linking other fungal biosynthetic genes to their respective products in future studies.

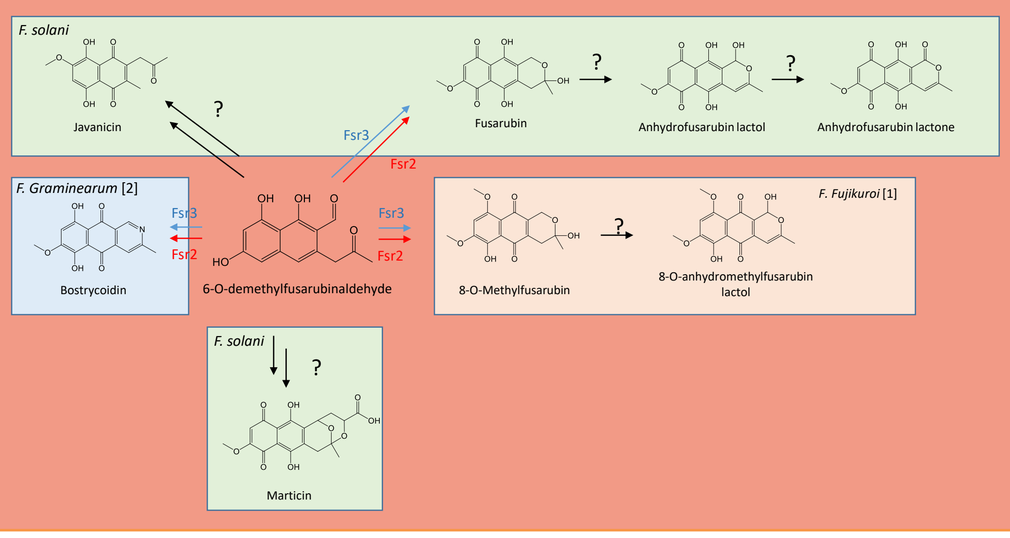


Mycelial pigmentation of *F. solani*

- *PKS3 / pgl1 / fsr1* [1, 2] BGC present in all Fusaria [3] – the BGC is often associted with peritheical pigmentation in species outside the FSSC. Responsible for mycilial pigmentation in members of FSSC
- The *F. solani* PKS3 BGC shares seven genes with similar clusters in F. graminearum and F. fujikuroi. However, it differs from these clusters by containing several additional genes, some with predictied function related to secondary metablism

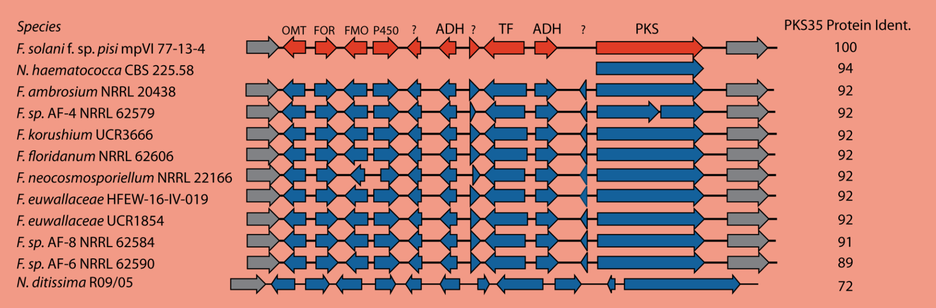


- The PKS3-related naphtoquinones produced by members of the FSSC comprise fusarubin, javanicin, bostrycoidin, marticin, and derivatives of these molecules [5]

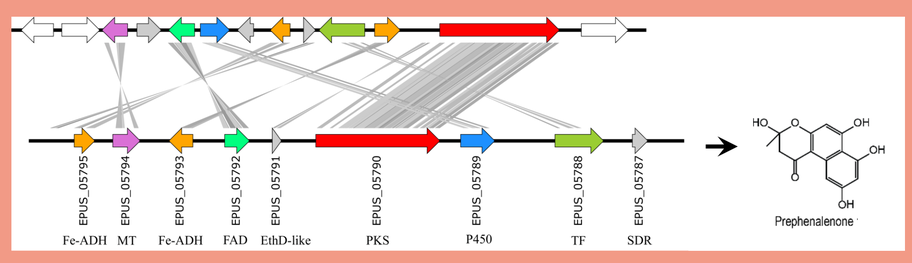


Perithecial pigmentation of *F. solani*

- The *pksN* /PKS35 BGC is associated with producing a red/orange perithecial pigment [4, 6] that is characteristic of FSSC species with known sexual reproduction [5].
- The PKS35 cluster might be unique to the FSSC, and has not been reported in other Fusaria [3]. 11 cluster genes are highly conserved in sequenced members of the FSSC [5, 7]

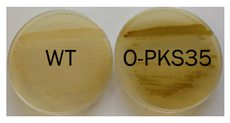


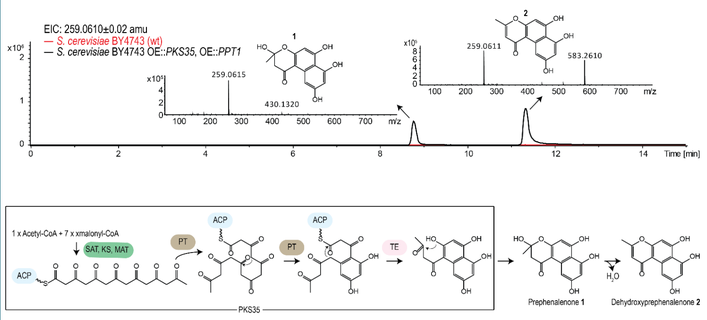
- A cluster in *Neonectria ditissima* shares high synteny to the PKS35 cluster. The genus is known for producing corymbiferan lactone E [8], a compound closely resembling herquinone[10]
- The PKS35 BGC shares eight ORFs with the lichen forming Endocarpon pusillum cluster PKS23. This cluster has preivously been associated with the formatiotion of prehenalenone and dehydroxyprephenalenone [9]



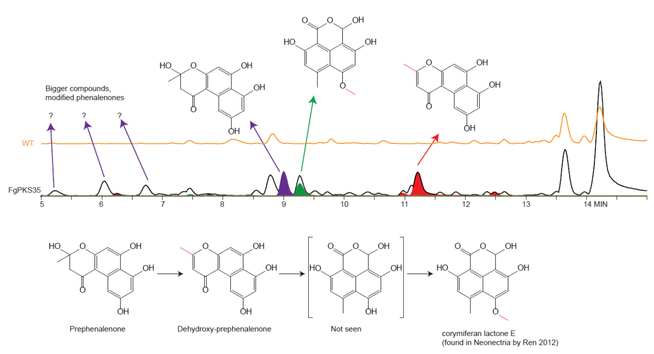
- Recently, a study on FSSC species *F. neocosmosporiellum* [6] reported high similarity to the herquinone producing *phn* BGC characterized in *P. herquei* [10]. The product initially released from the PKSN ortholog PhnA was identified as prephenalenone.
- Phylogenetic analysis of PT domains from NR-PKSs grouped *F. solani pksN* together with *P. herquei phnA* in a clade of polyketides perfoming C4-C9 cyclization (not shown)

Heterologous expression of *pksN* in *S. cerevisiae*





Heterologous expression of 10 PKSN cluster genes in *F. graminearum*

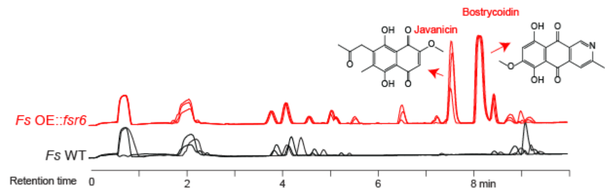


Wild type

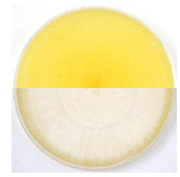
F. solani f.sp. *pisi* 77-13-4

Overexpression of Fsr cluster specific transcriptional regulator *fsr6* [11]

O-*fsr6*



Control: Deletion of *fsr1*

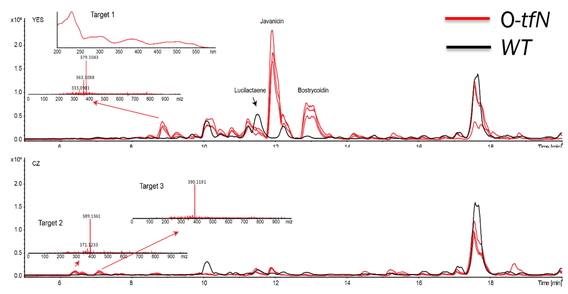


O-*fsr6* Δ*fsr1*

- Δ*fsr1* gives an albino phenotype cementing *fsr6* promotes the transcription of *fsr1* which is responsible for the formation of red mycilial pigments

Overexpression of PKSN cluster specific transcriptional regulator *tfN*

O-*tfN*



PKS35-related candidate compounds

Strain	[M+H]+	Formula	Putative compound
Sc PKS35	259.0615	$C_{14}H_{10}O_5$	Dehydroxy-prephenalenone
Fs O-tfN	379.1043	$C_{18}H_{18}O_9$	Candidate 1
	389.1361	$C_{19}H_{20}N_2O_7$	Candidate 2
	390.1191	$C_{19}H_{19}NO_8$	Candidate 3
Fg PKS35	417.1769	$C_{22}H_{26}NO_7$	Candidate 4
	261.0763	$C_{14}H_{12}O_5$	Corymbiferan lactone E

Control: Deletion of *fsr1*

Δ*fsr1*

- Δ*fsr1* gives albino phenotype
- *fsr1* is responsible for the formation of 6-O-demethylfusarubinalde hyde, the polyketide precurser of red mycylial pigments

Control: Deletion of *pksN*

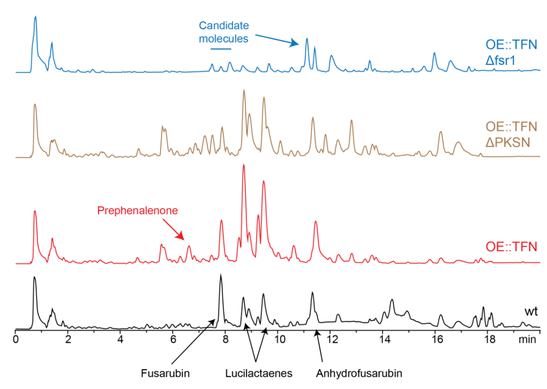
Δ*pksN*

- No significant changes to phenotype or metabolite profile observed

Construction of double mutants enables detection of PKS35-related compounds

O-*tfN* Δ*pksN*

O-*tfN* Δ*fsr1*



Conluding remarks on *F. solani* pigmentation

- The mycelial pigmentation is controlled by *fsr1* which releases 6-O-methylfusarubinaldehyde, the precurser for a wide range of coloured naphtoquinones. The *F. solani* PKS3 BGC comprises several additional genes in comparison to that of other Fusaria. Meanwhile, additional PKS3-derived compounds are produced only in species within the FSSC e.g. javanicin.
- Perithecial pigmentation is drived by the conserved *pksN*. Nucleotide analysis suggests the initial release product is prehenalenone. A mass fitting prephenalenone was, together with larger masses, observed in overexpressing and heterologously expressing mutants. Curiously, an increase in PKS3-derived metabolties was observed when overexpressing the PKS35 intrinsic TF. This observation indicates the regulation of both clusters is somehow connected
- In this study we applied several strategies to map the products formed from the PKS35 BGC: Overexpression, gene deletion and heterologous expression. Concerning future studies, we will recommend applying a mixed methods approach to increase the likelihood of isolating novel compounds.

References

[1] Studt et al. Applied Environmental Microvbiology (2012) Jun;28(12):4468-80
[2] Frandsen et al. Nature Scientific Reports (2016) 6:26206
[3] Brown & Proctor. Fungal Genetics and Biology (2016) 89:37-51
[4] Graziani et al. Applied and Environmental Microbiology (2004) 70(5) 2984-2988
[5] Coleman. Molecular Plant Pathology (2016) 17(2) 146-158
[6] Kim et al. Frontiers in Microbiology (2019) 10:1247
[7] Coleman et al. PLoS Genetics (2009) Aug 5(8): e1000618
[8] Ren et al. Organic Letters (2012) Dec 21;14(24):6226-9
[9] Harvey et al. PMC Scientific Advances (2018) 11;4(4):eaar5459
[10] Gao et al. Journal of American Chemical Society (2016) Mar 30;138(12):4249-59
[11] Nielsen et al. Fungal Biology and Biotechnology (2019) Dec 2019 6:25