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Identification of the first steps in phenalenone pigment biosynthesis in *Fusarium solani*

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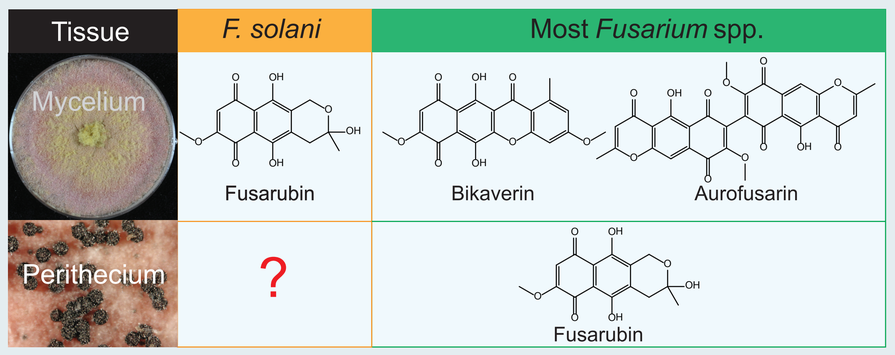
BACKGROUND - *FUSARIUM* PIGMENTS

- Most *Fusarium* Species produce bikaverin and aurofusarin for mycelium pigmentation and fusarubins for perithecial pigmentation [1].
- *Fusarium solani* produces fusarubins during mycelial growth and another unknown pigment during sexual reproduction. This unknown pigment is predicted to be synthesized by a non-reducing polyketide synthase (PKS35 = *pksN* [2]).
- **The aim of this study is to identify the pigment through heterologous production in yeast.**

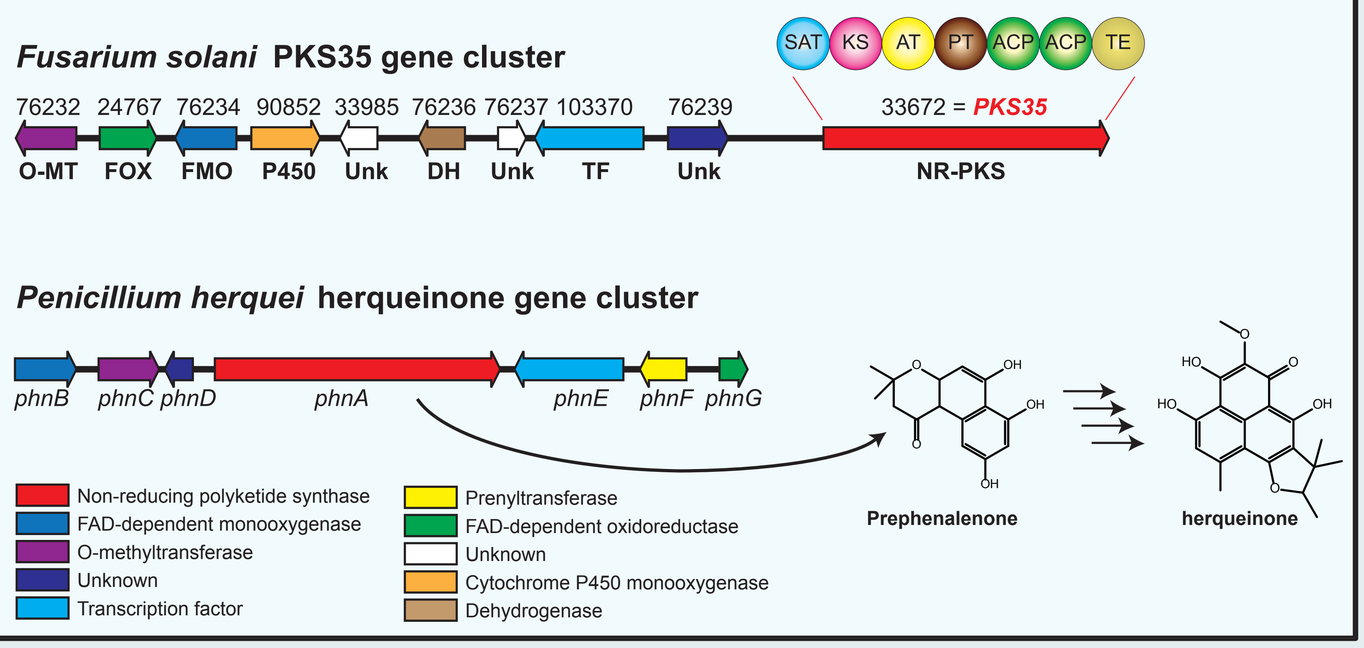


Pigments in Fusarium

2

PKS35 GENE CLUSTER

- Comprised of 10 genes (NECHADRAFT_76332 - 33672)
- Orthologs of six genes are also present in the herqueinone cluster
- The first step in herqueinone biosynthesis is prephenalenone, which is cyclized to a tricyclic phenalenone ring structure by a FAD-dependant monooxygenase [3].



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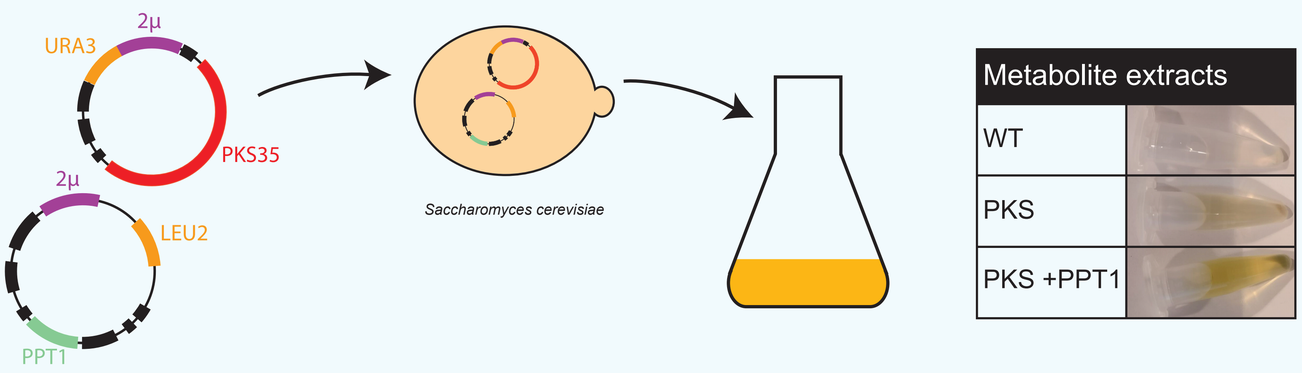
HETEROLOGEOUS PRODUCTION

- Intronless *PKS35* was cloned into a 2µ vector and put under control of a galactose inducible promoter P_{GAL1}.
- A Sfp-Type 4′-Phosphopantetheinyl Transferase (PPT1) was also expressed from another 2µ vector to facilitate polyketide formation.
- The transformed yeast strain was cultivated under induced conditions in liquid cultures for five days.



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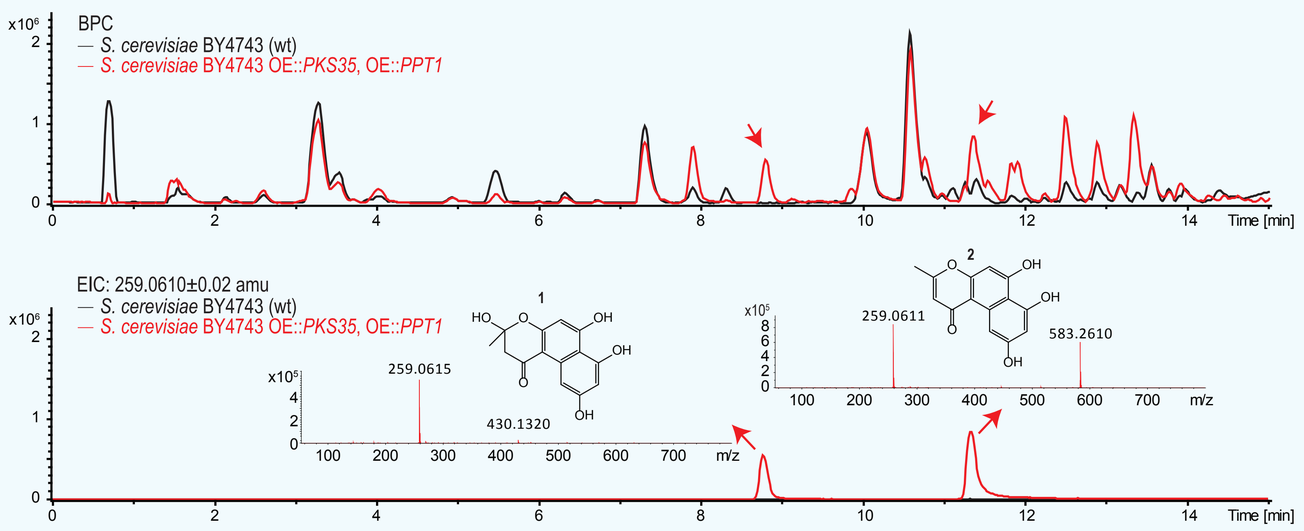
IDENTIFICATION OF PKS PRODUCT

- Production of secondary metabolites was analyzed by high-resolution mass spectrometry (HRMS).
- The yeast strain produced prephenalenone; the first step of the herqueinone pathway [3].
- We also detected dehydroxyprephenalenone, which is formed by spontaneous dehydration.



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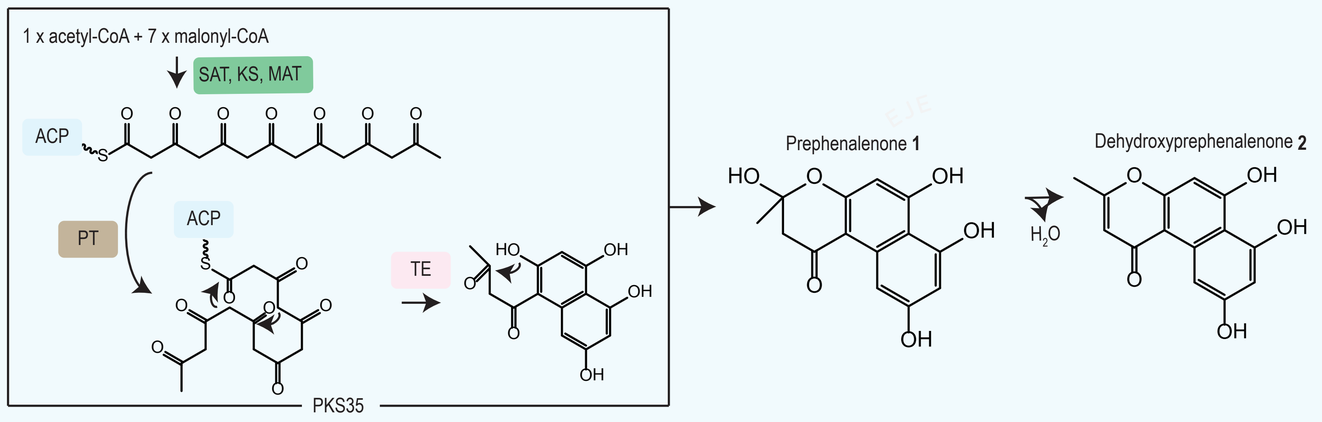
BIOSYNTHETIC PATHWAY AND OUTLOOK

- PKS35 produce prephenalenone from a single acetyl-CoA and seven malonyl-CoA units
- Prephenalenone is expected to be cyclized and additionally modified to a phenalenone pigment in *F. solani*.
- Future experiments will include heterologous production of additional genes in yeast and constitutive expression of the TF in *F. solani*



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3. Gao SS, Duan A, Xu W et al (2016): J Am Chem Soc, 138: 4249-4259.

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