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

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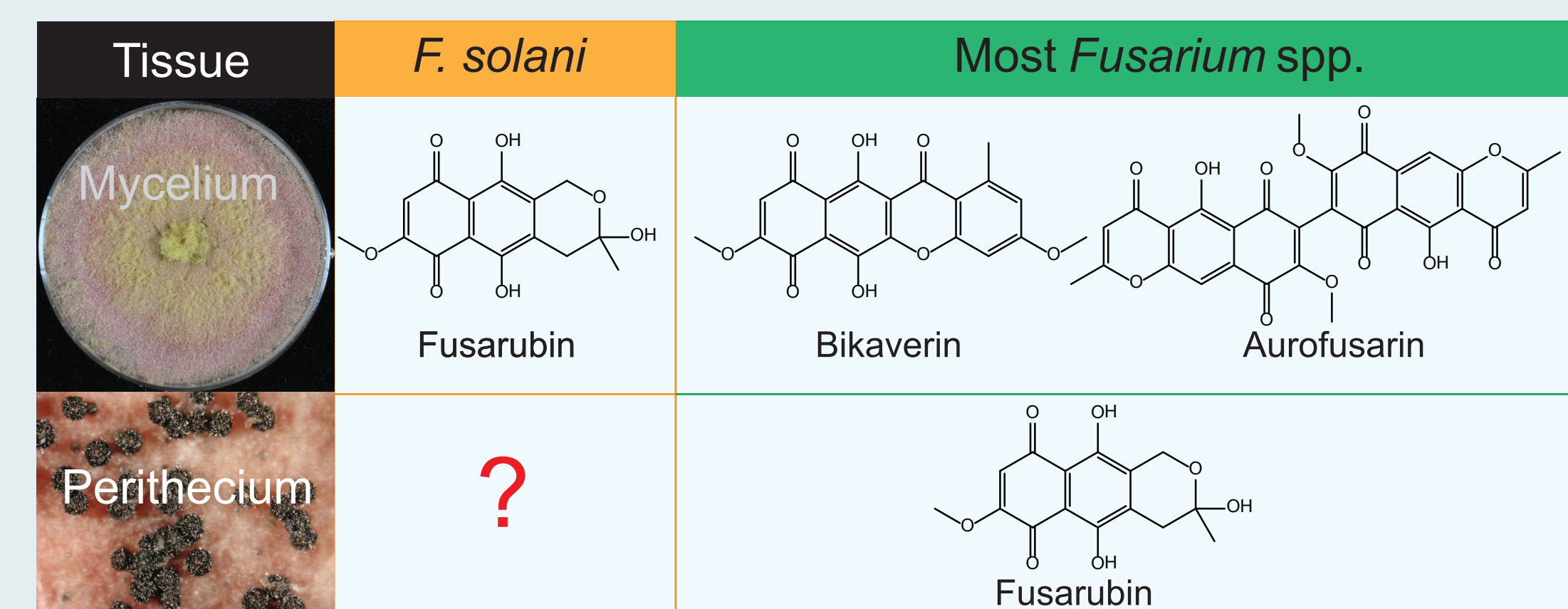
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## BACKGROUND - FUSARIUM PIGMENTS

- 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 = *pkS*N [2]).
- The aim of this study is to identify the pigment through heterologous production in yeast.



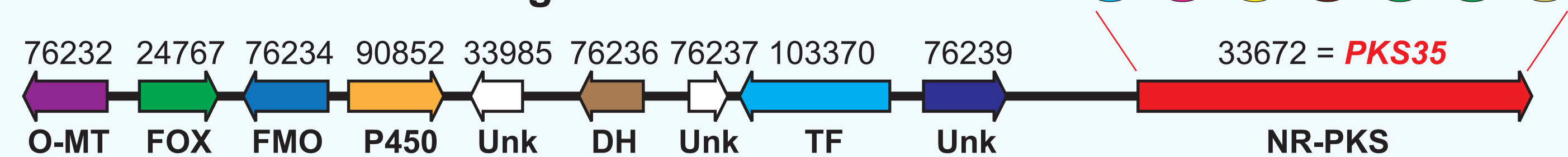
Pigments in *Fusarium*

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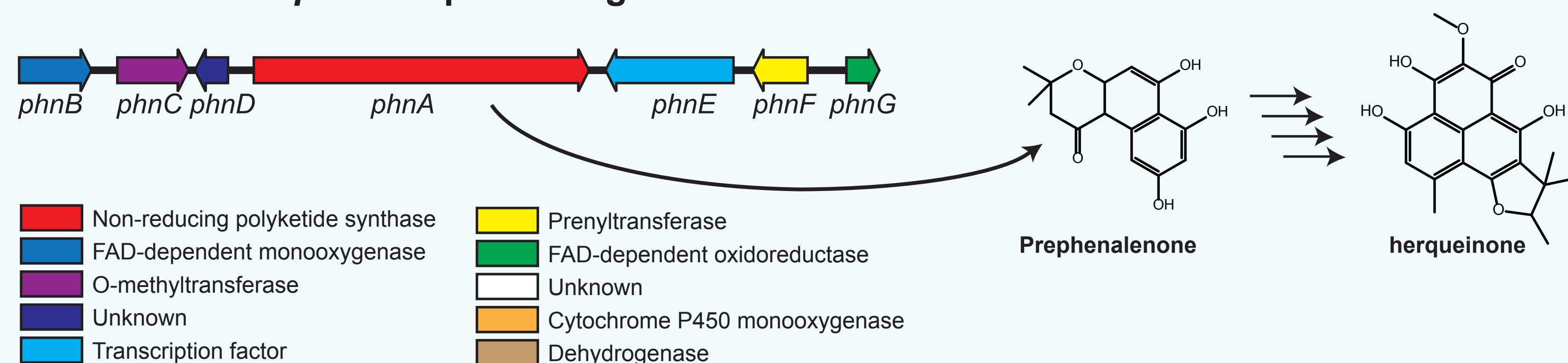
## PKS35 GENE CLUSTER

- 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].

### *Fusarium solani* PKS35 gene cluster



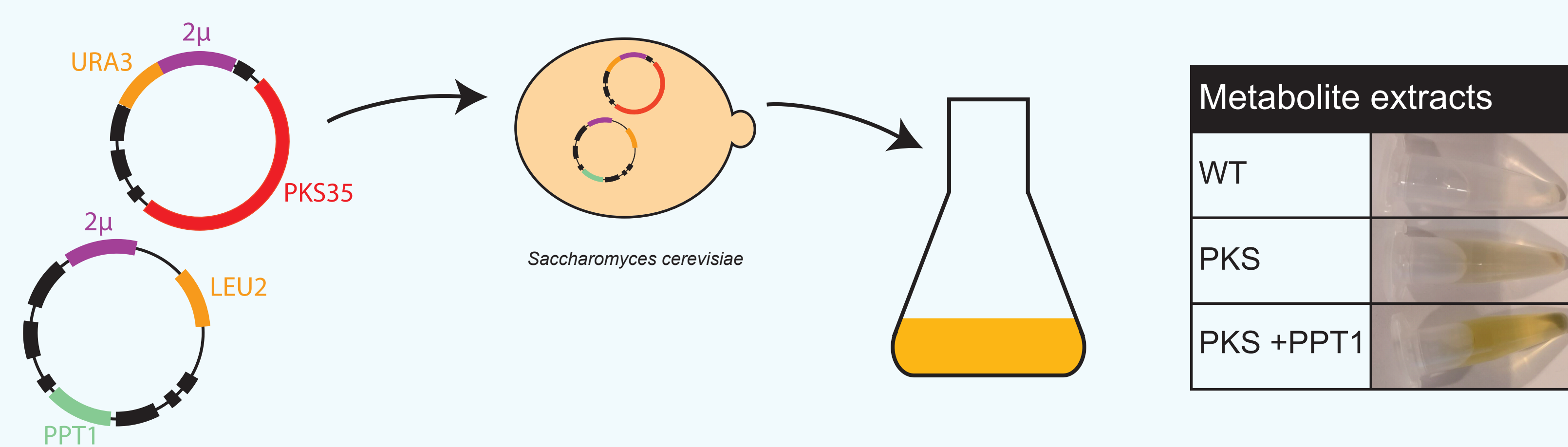
### *Penicillium herquei* herqueinone gene cluster



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## HETEROLOGEOUS PRODUCTION

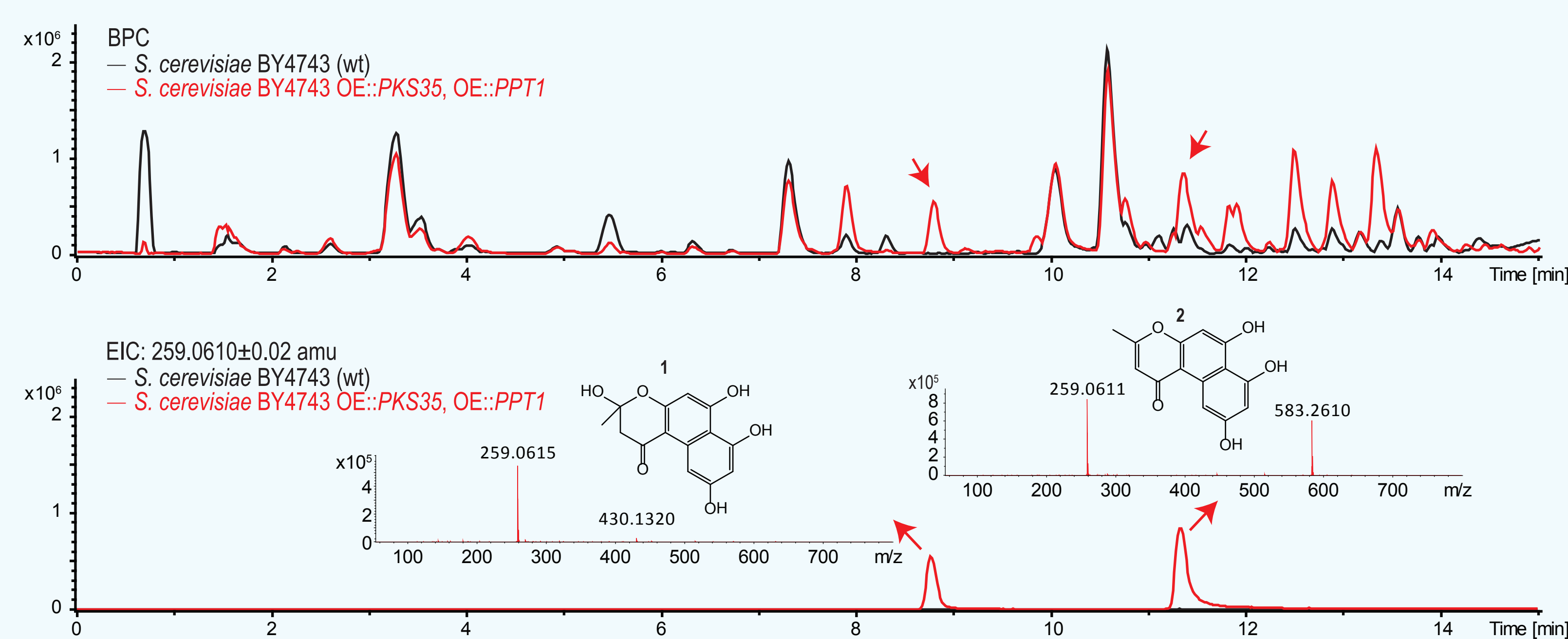
- Intronless *PKS35* was cloned into a 2 $\mu$  vector and put under control of a galactose inducible promoter *P*<sub>GAL1</sub>.
- A Sfp-Type 4'-Phosphopantetheinyl Transferase (*PPT1*) was also expressed from another 2 $\mu$  vector to facilitate polyketide formation.
- The transformed yeast strain was cultivated under induced conditions in liquid cultures for five days.



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## IDENTIFICATION OF PKS PRODUCT

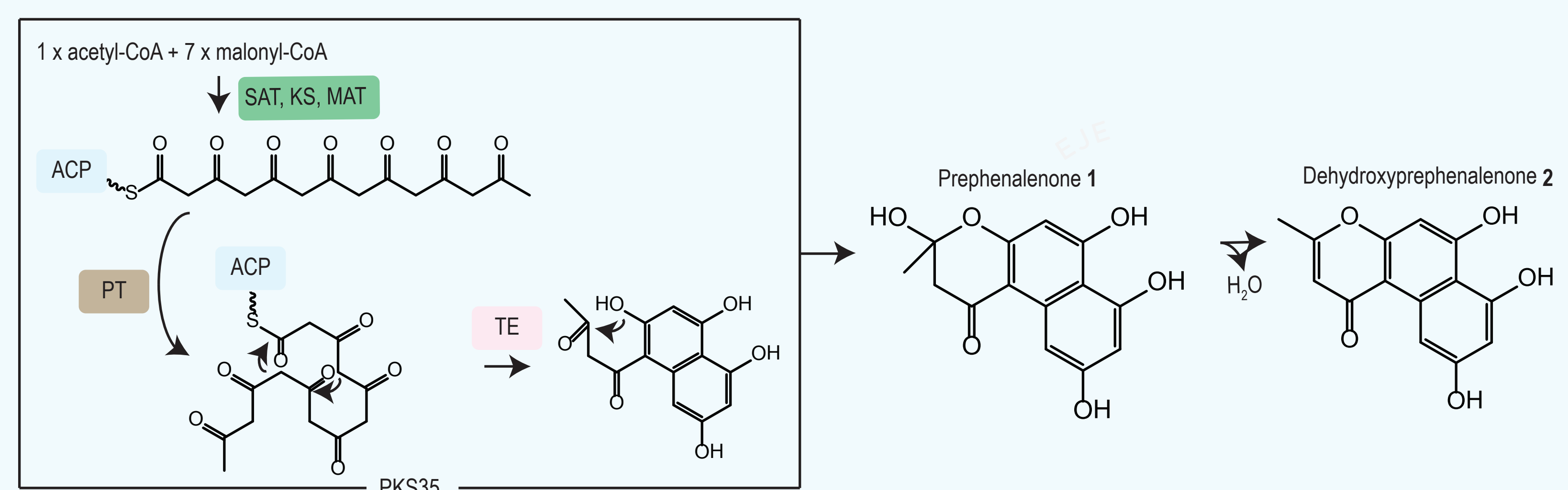
- Production of secondary metabolites was analyzed by high-resolution mass spectrometry (HRMS).
- The yeast strain produced prephenalenone; the first step of the herqueinone pathway [3].
- We also detected dehydroxyprephenalenone, which is formed by spontaneous dehydration.



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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*



## REFERENCES

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