Linking of Fusarium graminearum PKS3 to bostrycoidin production

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Members of the *Gibberella* genus are characterized by having dark violet perithecia. The chemical nature of the responsible pigments has so far remained unknown. Using targeted over-expression of *PKS3* (*PGL1*) and a cluster specific transcription factor *pglR*, we here identify the pigments as bostrycoidines, members of the fusarubin metabolite family.

Identification of a *PGL1* gene cluster

Previous studies [1,2] have shown that *PKS3* (*PGL1*) is essential for biosynthesis of the perithecial pigment(s) in *F. graminearum* and *F. verticillioides*.

Using comparative genome analysis (shuffle-LAGAN plots) of seven *Fusarium* species we have identified a conserved PKS gene cluster (Fig. 1A). The cluster encodes classical PKS tailoring enzymes: monoxygenase, O-methyltransferase, dehydrogenases and a Zn,Cys type transcription factor (*pglR*) (Fig. 1B). The cluster is only expressed in perithecial tissues.

Over-expression of *PGL1* and *pglR*

Based on the small size of perithecia and the classical difficulties of characterizing melanins (heterogenous polymers) we opted to activate the gene cluster in vegetative mycelium by TF and PKS over-expression hoping to identify the chemical nature of the monomers that makes up the perithecial pigment.

Over-expression of *PGL1* and *pglR* (TF) (Fig. 1C&D) resulted in the production and excretion of brown and yellow pigments (Fig. 2A).

Chemical analysis, UPLC-HRMS (Figure 2B) and NMR experiments, showed that the O-PKS3 strain produced 6-O-demethyl-5-deoxy-10-deketo-bostrycoidin (1), 6-O-demethyl-5-deoxy-bostrycoidin (2) and a purple dimer of 1 (named purpurfusarin (3)). While the yellow EO-pglR strain accumulated 5-deoxy-bostrycoidin (4), bostrycoidin (5) and 5-deoxy-10-deketo-bostrycoidin (6).

Biosynthetic model(s)

Based on structure of the accumulating intermediates we have formulated a model for biosynthesis of bostrycoidin in *F. graminearum* (Fig 3). Targeted deletion of *pglJ* and *pglM* has confirmed their involvement in the biosynthetic pathway (data not shown). The origin of the nitrogen atom in the C-ring is currently unknown. Interestingly the identified monomers all display yellow color at physiological pH while the dimer is pink/violet.

The study shows that when *PGL1* and the corresponding cluster is expressed in vegetative mycelium it results in the production of bostrycoidin pigments. To confirm that the perithecial pigments are bostrycoidins, and rule out an artifact of the overexpression in vegetative mycelium, chemical analysis of perithecia is ongoing.