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Fusarium graminearum PKS14 is involved in orsellinic acid and orcinol synthesis



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ABSTRACT

The available genome sequences show that the number of secondary metabolite genes in filamentous fungi vastly exceeds the number of known products. This is also true for the global plant pathogenic fungus *Fusarium graminearum*, which contains 15 polyketide synthase (PKS) genes, of which only 6 have been linked to products. To help remedy this, we focused on *PKS14*, which has only been shown to be expressed during plant infections or when cultivated on rice or corn meal (RM) based media. To enhance the production of the resulting product we introduced a constitutive promoter in front of *PKS14* and cultivated two of the resulting mutants on RM medium. This led to the production of two compounds, which were only detected in the *PKS14* overexpressing mutants and not in the wild type or *PKS14* deletion mutants. The two compounds were tentatively identified as orsellinic acid and orcinol by comparing spectroscopic data (mass spectroscopy and chromatography) to authentic standards. NMR analysis of putative orcinol isolated from the *PKS14* overexpressing mutant supported our identification. Orcinol and orsellinic acid, not previously detected in *Fusarium*, have primarily been detected in lichen fungi. Orsellinic acid is hypothesized to be the PKS release product which is transformed to orcinol through decarboxylation. Phylogenetic analyses of PKSs placed *PKS14* in a subclade of known OA synthases. Expression analysis by microarray of 55 experiments identified seven genes near *PKS14* that were expressed in a similar manner. One of the seven genes encodes a predicted carboxylase, which could be responsible for transforming orsellinic acid to orcinol.

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1. Introduction

The filamentous fungus *Fusarium graminearum* has the genetic potential of producing a wide array of secondary metabolites (Hansen et al., 2012b). Whole-genomic analysis have identified 15 polyketide synthases (PKS1–15) (Kroken et al., 2003; Gaffoor and Trail, 2006), where six have been linked to metabolites: *PKS3* – perithecial pigment (Gaffoor et al., 2005), *PKS4/13* – zearalenone (Kim et al., 2005; Gaffoor and Trail, 2006), *PKS9* – fusarielins (Sørensen et al., 2012), *PKS10* – fusarin C (Gaffoor et al., 2005) and *PKS12* – aurofusarin (Frandsen et al., 2006). The remaining PKSs have not yet been assigned products, even though they are expressed under tested conditions (Gaffoor et al., 2005). As *F. graminearum* infects cereal grains, there is a risk that these unidentified metabolites can contaminate our food sources

(Burlakoti et al., 2008), which emphasizes the need to determine the full metabolic capacity of *F. graminearum*.

The genes involved in the syntheses of polyketides are often found in gene clusters encoding the synthase and tailoring enzymes, transporters and local cluster activating transcription factor(s) (Keller and Hohn, 1997). Gene clusters, which have not been linked to secondary metabolites, can be activated by many different approaches including changing the cultivation practice according to the OSMAC (one strain, many compounds) method, where light, air, nutrition sources and pH are varied (Bode et al., 2002) or co-cultivation with other competing microorganism (Schroeckh et al., 2009). Secondary metabolic pathways have also been induced through chromatin modification using chemical agents or manipulation of chromatin modifying proteins like LaeA (Fisch et al., 2009) or by the overexpression of any transcription factor encoding genes located proximal to the PKSs (Bergmann et al., 2007). The low success rate of these strategies is illustrated by our efforts to over express putative transcription factors near

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PKS3, 5, 7, 9, 11 and 15 gene clusters in *F. graminearum*, which was only successful in the case of PKS9 leading to the production of novel fusarielins (Sørensen et al., 2012). In *Aspergillus nidulans* overexpression of transcription factors near 17 secondary metabolite gene clusters led to induction of only five products (Ahuja et al., 2012). However, when Ahuja et al. (2012) used overexpression of the PKS genes directly, they managed to identify the release products of eight non-reducing (NR)-PKSs of which downstream metabolites were known or identified for six.

PKS14 is the only NR-PKS with a claisen-type cyclase (CLC) domain in *F. graminearum* that has not been linked to synthesis of a chemical product (Hansen et al., 2012b). CLC domains are responsible for releasing the product from the synthase and the presence of this domain suggests that PKS14 is not dependent on other enzymes for releasing its product. The gene appears to be induced by plant stimuli as it is transcribed under wheat and barley infection (Lysøe et al., 2011) and when *F. graminearum* is cultivated on rice or corn media (Gaffoor et al., 2005). Gaffoor et al. (2005) deleted PKS14, which resulted in reduced growth. However, no change in metabolite profile was detected in the mutant compared to the wild-type. As there is no transcription factor in the proximity of PKS14, we overexpressed the PKS14 gene itself using the same strategy as Ahuja et al. (2012), which led to production of orsellinic acid and orcinol.

2. Materials and methods

2.1. Phylogenetic analyses

Amino acid sequences of KS domains from selected NR-PKSs were retrieved from the *Fusarium* comparative database (<http://www.broad.mit.edu/annotation/fungi/fusarium>), the *Aspergillus* comparative database (www.broadinstitute.org/annotation/genome/aspergillus_group) and GenBank (<http://www.ncbi.nlm.nih.gov>). The PKS14 PT domain was identified by BlastP analyses using the PT domain of PKSA from *Aspergillus parasiticus* as previously described by Li et al. (2010). The PKS14 PT domain was then used to identify PT domains in the remaining NR-PKSs. The sequences were aligned by clustalW and a phylogeny tree was constructed by the neighbor joining (NJ) algorithm with 1000 bootstrap replicates in the Molecular Evolutionary Genetics Analysis software (MEGA 5.2) (Tamura et al., 2011).

2.2. Expression analyses and cluster prediction

Hierarchical clustering was performed in CLC Genomics Workbench 6.5.1, using 175 microarray samples from 55 experiments; a combination of published results found in the plant expression database (plexdb.com) and unpublished results. The variable conditions in the experiments include plant infection, sexual – and asexual development as well as different media with varying nitrogen sources. The Affymetrix CEL files were processed through the “affy” package in Bioconductor and normalized using the quantile method in CLC Genomics Workbench. The hierarchical clustering of features was performed with single linkage using manhattan distance. The function of the proteins encoded by the cluster genes was predicted based on comparison to proteins in the NCBI Conserved Domain Database (Marchler-Bauer et al., 2011).

2.3. Construction of PKS14 overexpression and deletion mutants

The flanking regions of PKS14 was PCR amplified from *F. graminearum* PH-1 using primers PKS14-KO1–PKS14-KO4 listed in Supplementary Table 1 and Pfu polymerase (Stratagene, La Jolla, CA, USA). The amplified fragments were introduced into the

pRF-HU2 vector flanking the hygromycin B resistance cassette by USER cloning (Frandsen et al., 2008). Overexpression of PKS14 was achieved by introducing the constitutive promoter *gpdA* from *A. nidulans* in front of PKS14 using a similar approach as described previously. A fragment upstream of PKS14 and a fragment from the beginning of the gene were amplified by PCR, using primers PKS14-O1–PKS14-O4, and cloned into the linearized pRF-HU2E vector containing the *gpdA* promoter. Transformation of *F. graminearum* was carried out by *Agrobacterium tumefaciens* mediated transformation as described previously (Malz et al., 2005) and the resulting mutants were initially verified by diagnostic PCR using a forward primer annealing to gDNA outside the border region and a reverse primer annealing to the hygromycin resistance gene. The number of inserts was determined by Southern blotting (Hansen et al., 2012a). Genomic DNA was extracted from the transformed fungi and subjected to a restriction digestion using *HindIII* fast digest (Fermentas, Thermo Fisher Scientific, Inc., Denmark) followed by hybridization with probe of 588 bp corresponding to a part of the hygromycin resistance gene.

2.4. Transcription analyses

Extraction of RNA from *F. graminearum* was adapted from the method used by Droce et al. (2013). In brief, *F. graminearum* was cultivated for 9 days on solid rice meal (RM) medium (75 g/L rice meal, 20 g/L agar and 1 mL/L trace solution (1.0 g ZnSO₄ and 0.5 g CuSO₄ in 100 mL H₂O) pH 6.7). Total RNA was extracted with RNeasy plant mini-kit (Qiagen, Hilden, Germany) following the recommended protocol including the QIAshredder column (Qiagen). First-stranded cDNA was synthesized using poly(dT) primer (15 bp) and Superscript III reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer including the RNaseOUT step. Expression of genes in the PKS14 cluster was determined by PCR using the primers listed in the Supplementary Table 1 and paq5000 DNA polymerase (Stratagene). The amplified region included a predicted intron which would result in a 60 bp between gDNA and cDNA. Translation elongation factor 1 α (TEF-1 α) and β -tubulin were included as controls.

2.5. Extraction and purification of secondary metabolites

F. graminearum was grown on a solid RM medium in petri dishes for two weeks at 25 °C. The microscale extraction of metabolites was adapted from (Smedsgaard, 1997). Nine agar plugs were sonicated for 45 min with 1 mL ethyl acetate:dichloromethane:methanol (3:2:1) buffered with 1% formic acid. The extract were lyophilized and dissolved ultrasonically in 600 μ L methanol.

The samples were initially analyzed on an Agilent 1260 LC system (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector. Five μ L sample were injected and separated on a 100 \times 2.1 mm kinetex 2.6 μ m Hexyl-Phenyl (Phenomenex, Torrance, CA, USA) using a flow of 0.400 mL/min with a linear water-acetonitrile gradient, where both eluents were buffered with 50 ppm trifluoroacetic acid. The gradient started at 15% acetonitrile and reached 100% acetonitrile in 20 min, which were held for 5 min. The extracts were subsequently analyzed by ultra-high-performance liquid chromatography combined with high resolution mass spectrometry (UHPLC–HRMS) on an Agilent 1290 UHPLC system (Agilent technologies, Santa Clara, CA, USA) equipped with a 25 cm, 2 mm ID, 2.6 μ m Agilent Poroshell phenyl hexyl column, and coupled to an Agilent 6550 quadrupole Time of Flight (qTOF) high resolution mass spectrometer equipped with an electrospray source. The qTOF was operated at 2 GHz in the extended dynamic range mode at a resolution of 30,000 FMWH. Subsamples of 1 μ L were separated at 60 °C with a flow rate of 0.35 mL/min using a linear water-acetonitrile system both

containing 20 mM formic acid. The acetonitrile gradient started from 10% and was raised to 100% in 15 min, and held for 3 min prior to returning to the start conditions. The qTOF was operated in positive mode making full scans from m/z 100 to 1700 10 times per sec. Furthermore, MS/MS spectra was collected at 10, 20, and 40 eV (m/z 30–1700) from all major chromatographic peaks with mass in the range m/z 120–1700 (Nielsen et al., 2013). UV/Vis data and the full scan MS data were compared to the data listed in Nielsen et al. (2011).

For structure elucidation large scale extraction and purification was carried out. Metabolites were extracted from 37 RM plates of two weeks old cultures by ultrasonic treatment for 45 min in ethyl acetate buffered with 1% formic acid. The extract was evaporated to dryness on a rotary evaporator, resuspended in 20 mL methanol

and the metabolites were then fractionated on a Strata C18-E (55 μ m, 70 Å), 50 g/150 mL, Giga Tube (Phenomenex, Torrance, CA, USA). The extract were loaded on the column in a 10% methanol solution, washed with 200 mL 20% methanol and eluted with 200 mL 40% acetonitrile. The final purification was performed by multiple cycles on an Agilent 1260 semi-preparative HPLC system equipped with a 150 \times 10 mm Gemini 5 μ m C6-Phenyl 110 Å column (Phenomenex, Torrance, CA, USA). 30 μ L crudely purified metabolites were injected on the column using a flow of 5.000 mL/min with a linear water-acetonitrile gradient, where both were buffered with 50 ppm trifluoroacetic acid. The gradient started at 0% acetonitrile and reached 60% acetonitrile in 10 min, which were held for 1 min before reverting to 0% acetonitrile.

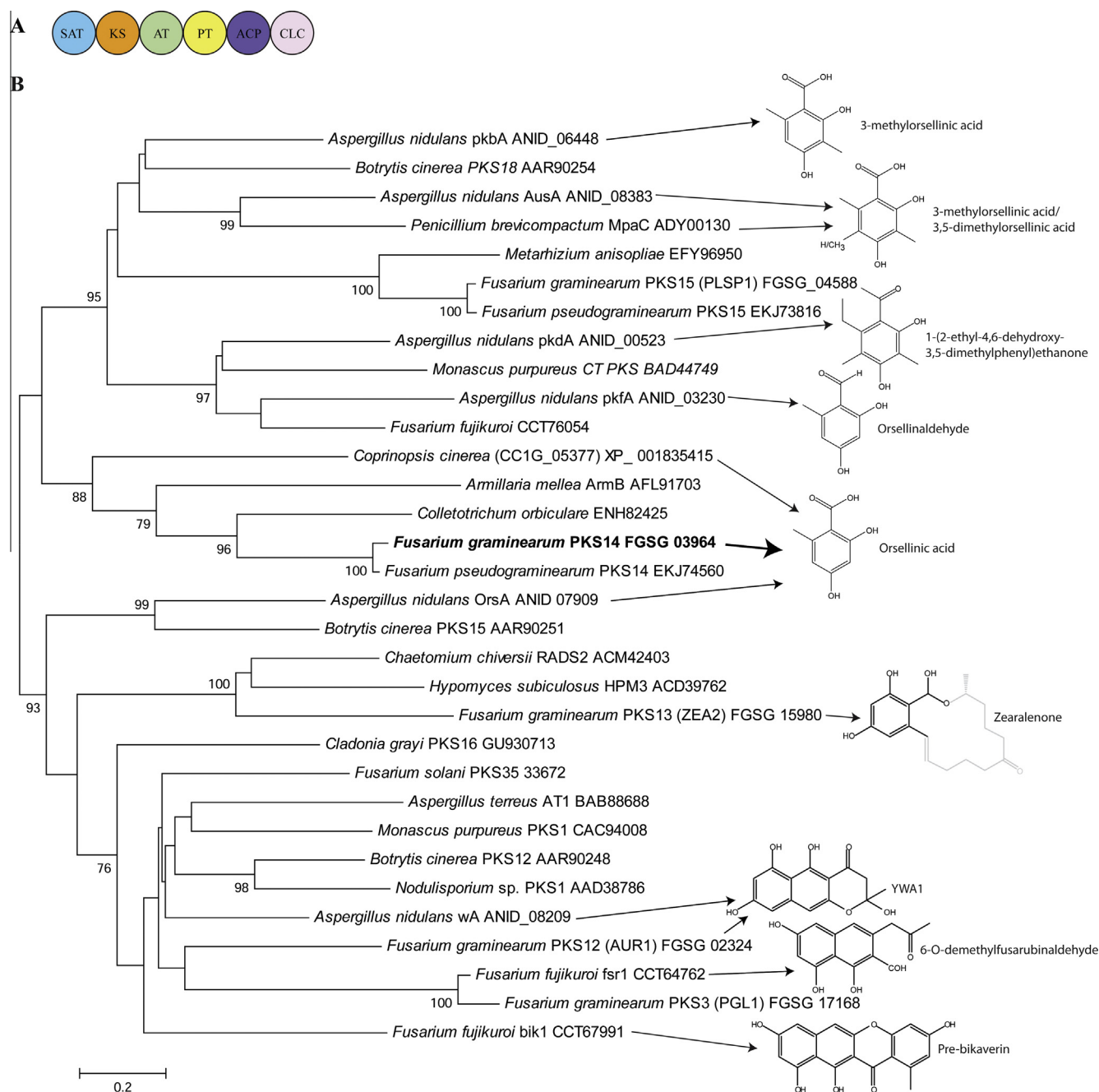


Fig. 1. (A) Domain organization of PKS14 containing a starter acetyltransferase (SAT), ketosynthase (KS), acyl transferase (AT), product template (PT), acyl carrier protein (ACP) and a claisen-type cyclase (CLC) domain. (B) Phylogenetic tree NR-PKSs obtained by neighbor joining analyses of PT domain amino acid sequences. The numbers at the internal nodes indicate percentage bootstrap support (>65) from 1000 bootstrap replications. The chemical structural of known PKS products are indicated, where PKS13 in *F. graminearum* hypothetically produces the highlighted part of zearalenone (Zhou et al., 2008).

A well resolved peak eluting at 5.4 min, corresponding to the retention time of authentic orcinol, was collected.

2.6. Structural verification

The structure was verified using nuclear magnetic resonance spectroscopy (NMR) on a Bruker AVIII-600 spectrometer (Bruker, Karlsruhe, Germany). Approximately 5 µg of the compound was dissolved in 600 µL CDCl₃ and analyzed with ¹H, ¹³C and 2D-[¹H-¹³C]-HSQC at 291 K. Spectra were recorded and analyzed with TopSpin 3.2 (Bruker). All chemical shifts are relative to internal tetramethylsilane (TMS).

3. Results and discussion

3.1. Phylogenetic analyses of PKS14

PKS14 contains a starter acetyltransferase (SAT), ketosynthase (KS), acyl transferase (AT), product template (PT), acyl carrier protein (ACP) and a claisen-type cyclase (CLC) domain (Fig. 1A). To gain insight into the chemical product of PKS14, we performed a phylogenetic analysis of the predicted amino acids corresponding to the PT and KS domain from PKS14 and 31 other NR-PKSs (Fig. 1B and Supplementary Fig. 1). The PKSs include five from *A. nidulans* shown to produce orsellinic acid or derivatives (Ahuja et al., 2012), a PKS from *Cladonia grayi* hypothesized to be responsible for production of orsellinic acid in route to the

synthesis of the orcinol depsidone grayanic acid (Armaleo et al., 2011), and a PKS from *Coprinopsis cinerea* shown in *Saccharomyces cerevisiae* to produce orsellinic acid (Ishiuchi et al., 2012). The resulting genealogy resolved the 32 PKSs into three major clades each with 100% bootstrap support. PKS14 was present in a clade with the orsellinic acid synthase from *C. cinerea* and a PKS (armB) from the orsellinic acid producer *Armillaria mellea* (100% bootstrap support in analyses with KS domains, 88% in analyses with PT domains). Compared to the *A. nidulans* orsellinic acid synthase (anid_07909), the *C. cinerea* synthase lacks the SAT domain and the second copy of ACP. As observed by Lackner et al. (2012), the two synthases do not cluster together in KS based phylogenetic analyses. This suggests that PKS14 from *F. graminearum* is a member of orsellinic acid synthases, which are polyphyletic and can have variable domain structures.

3.2. Prediction of the PKS14 gene cluster

To examine the extent of the putative PKS14 gene cluster, the transcription of PKS14 and eight upstream and downstream genes were analyzed (Fig. 2A). The expression profile of PKS14 clustered together with a decarboxylase (FGSG_03965) as well as six other genes. These six genes are predicted to encode two proteins with unknown functions (FGSG_03958 and FGSG_16177), an amidase/hydrolase (FGSG_03963), a hydrophobin protein (FGSG_03960), a cytochrome P450 monooxygenase (FGSG_12342) and a carboxypeptidase (FGSG_03967) (Fig. 2B). These co-regulated genes are

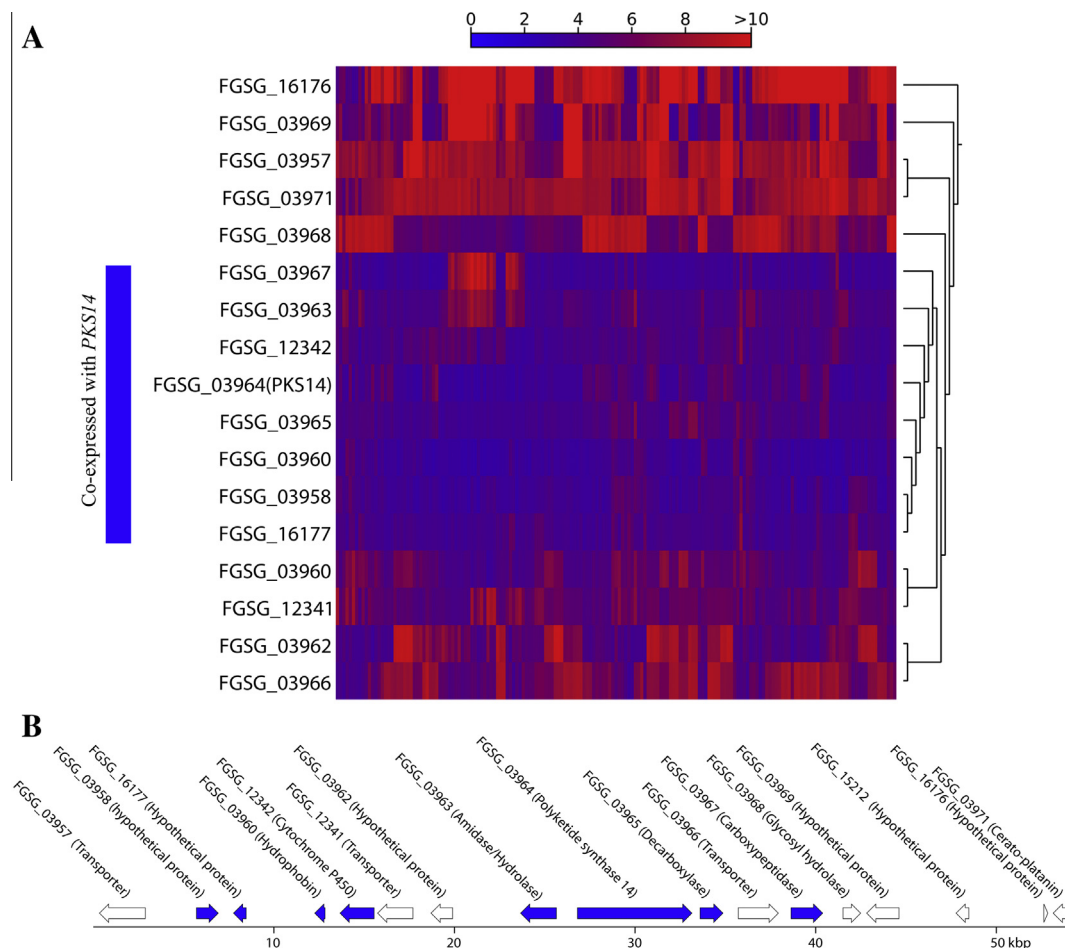


Fig. 2. (A) Hierarchical clustering of PKS14 and eight down- and upstream genes based on expression profiles in 175 microarray samples from 55 experiments (Lysøe et al., 2011; plexdb.com and unpublished data). (B) Genome map of PKS14 and neighboring genes. The genes co-expressed with PKS14 are labeled blue and comprise the predicted cluster. The predicted function of the genes was determined through the NCBI Conserved Domain Database (Marchler-Bauer et al., 2011).

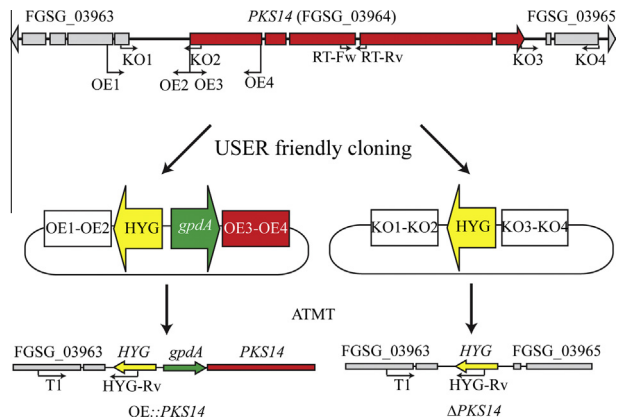


Fig. 3. Generation of overexpressing and deletion mutants of *PKS14*. Overexpressing mutants are obtained by cloning amplified regions OE1–OE2 and OE3–OE4 into a p-HU2E vector containing the *HYG* gene (hygromycin resistance) and the *gpdA* constitutive promoter. Deletion mutants are obtained by cloning amplified regions KO1–KO2 and KO3–KO4 into p-HU2. Both vectors were introduced into *F. graminearum* by *A. tumefaciens* mediated transformation and inserted into the genome by homologous recombination.

proposed to make up the *PKS14* gene cluster. The remaining three genes which are located inside the cluster, but are not co-regulated, are predicted to encode a protein with unknown function and two putative transporters. The expression profile of these three genes clustered together to form a separate subclade. The remaining genes surrounding the predicted cluster are more abundantly expressed under most of the conditions examined and are not considered part of the predicted *PKS14* cluster.

3.3. Generation and characterization of *PKS14* deletion and overexpression mutants

Deletion mutants of *PKS14* were successfully generated by gene replacements using *Agrobacterium* mediated transformation (Fig. 3). The constitutive *A. nidulans* promoter *gpdA* was introduced in front of *PKS14* for *in locus* overexpression of the gene. Correct integration of the transformation vectors into the genome was verified with PCR and single transformation events confirmed by Southern blot analysis (Supplementary Fig. 2). Two overexpression mutants and one deletion mutant were selected for further studies.

These mutant strains were grown on rice medium (RM), potato dextrose agar (PDA) and yeast extract sucrose (YES) medium to examine whether deletion of *PKS14* had an inhibiting effect on growth rate as reported by Gaffoor et al. (2005). In our experiments, we did not observe any significant differences in the radial

growth of the strains after five days (Fig. 4A). After seven days of growth the mycelium had covered the growth medium and exhibited similar morphology to that of the wild type (Fig. 4B).

Gaffoor et al. (2005) have previously shown that growth on rice medium induces expression of *PKS14*. To examine changes in the transcription level of the *PKS14* cluster in the mutant strains, the mutants and wild type were, grown on this medium and RNA was extracted. The RT-PCR showed that *PKS14* was transcribed by the OE::*PKS14* mutants and the wild type strain, while no transcript was observed in the Δ *PKS14* mutant (Fig. 4C). The remaining genes were all expressed in the different strains, although there was a tendency that the intensity was weaker for some of the genes in the Δ *PKS14* mutant compared to the wild type.

3.4. Metabolite analyses

To detect changes in the production of secondary metabolites, extracts of strains grown on RM medium for two weeks were analyzed. The extracts were initially screened by reverse phase HPLC-UV, where we observed an early eluting peak from a compound, which was present in OE::*PKS14* mutants, but not in the wild type or the Δ *PKS14* mutant. The samples were subsequently analyzed by UHPLC-HRMS, where the compound produced by the OE::*PKS14* mutants was detected as a very poorly ionizing peak with $[M+H]^+$ at a m/z 125.0587 (and no ionization in ESI^-) corresponding to an elemental composition of $C_7H_8O_2$ (calculated $[M+H]^+$ 125.0602) (Fig. 5A). The compound was identified as orcinol by comparing the elution time, accurate mass and fragmentation pattern to a commercial reference standard. Another fungal metabolite with this elemental composition, 3-methylbenzene-1,2-diol, was acquired (Sigma–Aldrich) and co-analyzed showing that this positional isomers was easily separated from orcinol.

Orcinol can be produced by decarboxylation of orsellinic acid (Pettersson, 1965; Mosbach and Ehrensward, 1966), and we were also able to detect this compound by UHPLC-HRMS in extracts from the two OE::*PKS14* mutants (Fig. 5B). The identity of orsellinic acid was verified by comparing retention time, HRMS using extracted ion-chromatograms $m/z \pm 0.01$ and by measuring the accurate mass of $[M+H]^+$ as 169.0492 (Calculated 169.0495, deviation -2.0 ppm). To verify the identity of orcinol, the compound was isolated by preparative HPLC and analyzed by NMR. The levels of orsellinic acid produced by the mutant strains were too low to obtain satisfactory amounts for NMR validation. The 1H and ^{13}C NMR spectra of the isolated orcinol identified six H-atoms and at least five C-atoms (Table 1, Supplementary Fig. 3). The measured chemical shifts matched those of orcinol reported by Richter et al. (2011), although the chemical shifts for the two hydroxyl groups were not obtained, due to rapid exchange.

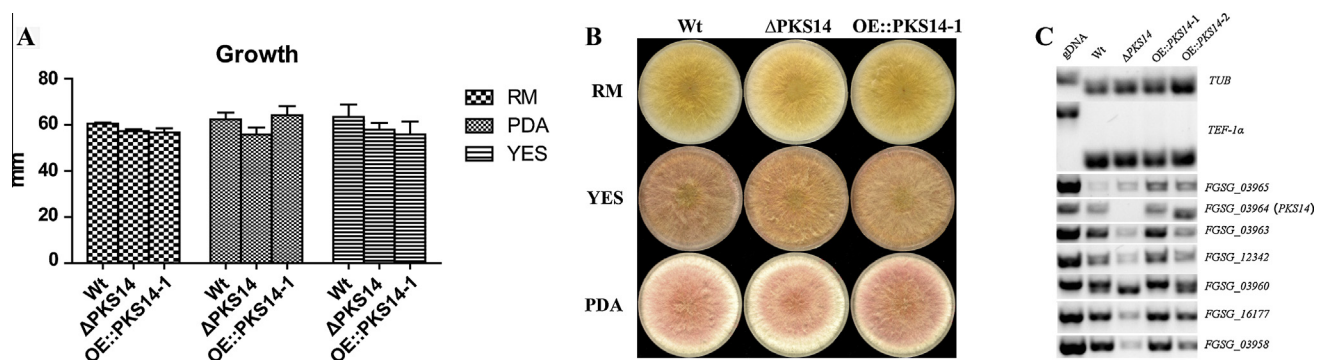


Fig. 4. (A) Radial growth (mm) of wild type (WT), OE::*PKS14* and Δ *PKS14* mutants on rice meal (RM), potato dextrose agar (PDA) and yeast extract sucrose (YES) medium after five days at 25 °C in the dark. (B) Representative plates of strains grown on RM, PDA and YES after five days. (C) Transcription analyses of genes in the putative *PKS14* cluster, translation elongation factor 1 α (TEF-1 α) and β -tubulin (β -tub) in the WT, OE::*PKS14* and Δ *PKS14* mutants.

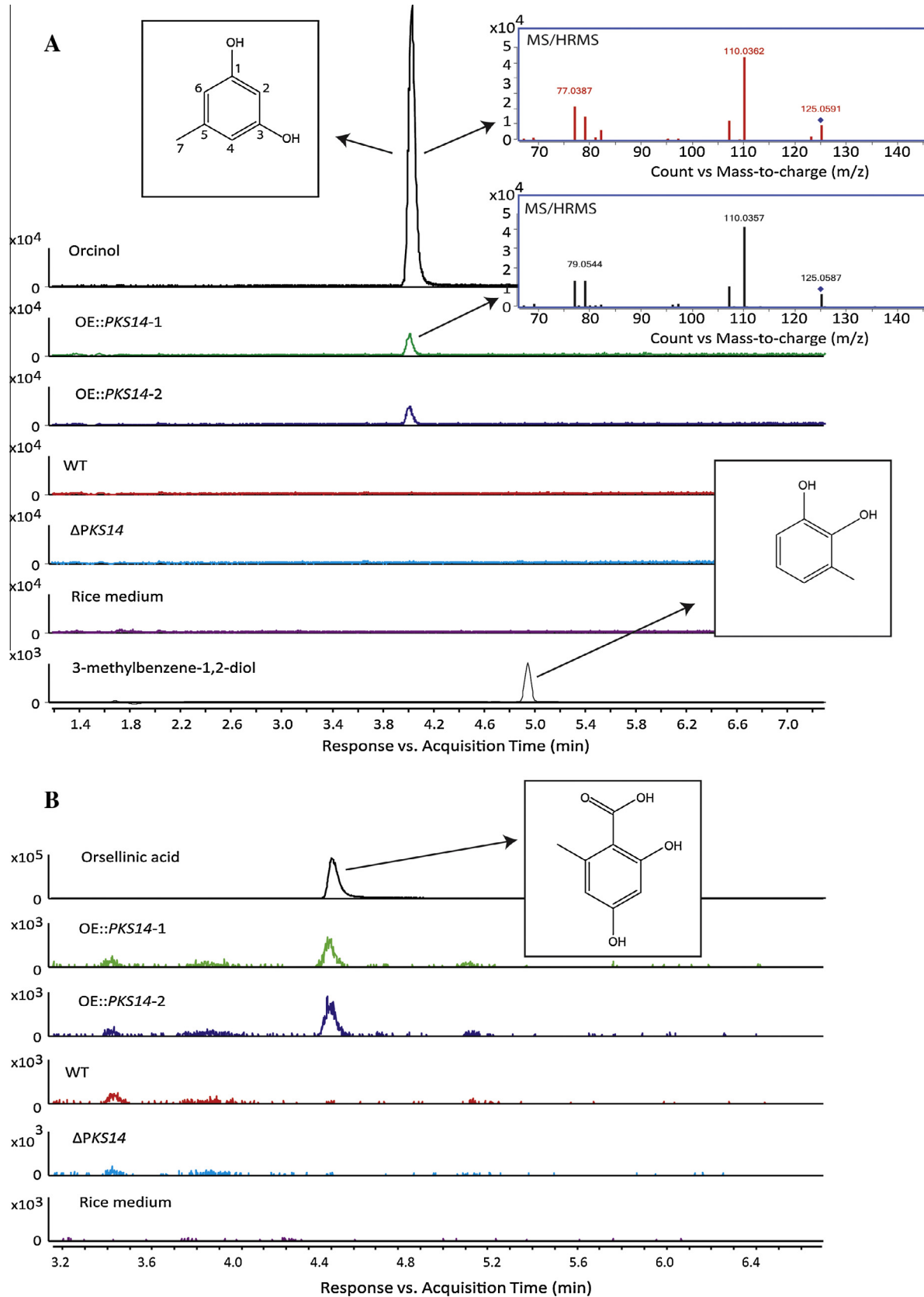


Fig. 5. (A) Detection of orcinol by extracted ion chromatogram (ESI⁺) of m/z 125 ± 0.3 of (WT), OE::PKS14 (1 and 2) and Δ PKS14. MS/MS spectra is inserted for OE::PKS14-1 and an orcinol reference standard. 3-methylbenzene-1,2-diol was also co-analyzed in the sequence showing that these positional isomers can easily be separated. (B) Detection of orsellinic acid in the stains by ion chromatogram (ESI⁺) of m/z 169.0495 ± 0.01 compared to a reference standard.

Table 1
Chemical shifts of the isolated orcinol in CDCl₃ at 291 K.

| Atom | ¹³ C ^a | ¹ H ^a |
|------|------------------------------|-----------------------------|
| 1,3 | 141.0/156.6 | – |
| 2 | 99.9 | 6.16 |
| 4,6 | 108.7 | 6.24 |
| 5 | 141.0/156.6 | – |
| 7 | 21.4 | 2.24 |

^a Chemical shifts are relative to internal tetramethylsilane (TMS).

Orsellinic acid represents the archetypal phenolic polyketide because it is the simplest acetate-derived aromatic metabolite and is a precursor in numerous biosynthetic pathways (Schroeckh et al., 2009). It is interesting that orsellinic acid can be produced by synthases which do not have same domain architecture and release mechanism. The orsellinic acid synthase (*orsA*) in *A. nidulans* has a thioesterase (TE) release domain, whereas *PKS14* has a CLC domain. Furthermore, *PKS14* and the *orsellinic acid synthase* from *C. cinerea* (CC1G_05377) have one ACP domain, whereas *orsA* has two.

Orcinol has been isolated from numerous lichen fungi (Robiquet, 1829) and can be synthesized by decarboxylation of orsellinic acid in *Umbilicaria papulosa* and *Gliocladium roseum* (Pettersson, 1965; Mosbach and Ehrensvärd, 1966). *Umbilicaria* spp. can furthermore produce numerous orsellinic acid-derived depsides and tridepsides (Narui et al., 1998), whereas *G. roseum* produce gliorosein, derived from orsellinic acid (Packter and Steward, 1967). These compounds were however not detected in the *PKS14* overexpressing mutants. The putative decarboxylase (FGSG_03965), which is present directly upstream of *PKS14*, is likely to catalyze the step from orsellinic acid to orcinol. This hypothesis is supported by the microarray data and RT-PCR results, which showed that the decarboxylase is co-regulated with *PKS14*. The decarboxylases from *U. papulosa* and *G. roseum* have not been sequenced and it therefore unknown whether the putative *F. graminearum* orsellinic acid decarboxylase are homologues. The orsellinic acid gene cluster in *A. nidulans* contains a putative decarboxylase (AN7911) and tyrosinase/polyphenol oxidase (AN7912) and in a wild type strain the cluster is responsible for production of lecanoric acid F-9775A and B (Schroeckh et al., 2009; Sanchez et al., 2010). Deletion of the tyrosinase and decarboxylase in *A. nidulans* resulted in accumulation of gerfelin and diorcinol, which was not detected in the wild type strain (Sanchez et al., 2010). Whether the decarboxylase is involved in biosynthesis of diorcinol is still hypothetical.

As our expression analyses suggests that *PKS14* belongs to a gene cluster, orcinol might not be the true end product of the biosynthetic pathway in *F. graminearum* as some of the putative cluster genes can potentially perform additional modifications to orcinol. Furthermore, orcinol is a substructure found in many fungal compounds, but as we only detected orcinol in the extracts from the overexpression mutants further investigations are needed to elucidate the full biosynthetic pathway of the *PKS14* gene cluster.

4. Conclusions

In the present study, we show that *F. graminearum* is capable of producing orsellinic acid and orcinol when *PKS14* was overexpressed. Although the wild type strain does not produce the compounds in detectable amounts under the tested conditions it is possible they may be found during infection of wheat, where transcriptome analyses have shown that the gene is expressed (Lysøe et al., 2011). The entire biosynthetic pathway of *PKS14* has probably not been fully revealed in the present study and further studies

are needed to identify the fate and function of orsellinic acid and orcinol in *F. graminearum*.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fgb.2014.06.008>.

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