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Identification of the Biosynthetic Gene Clusters for the Lipopeptides Fusaristatin A and W493 B in *Fusarium graminearum* and *F. pseudograminearum*

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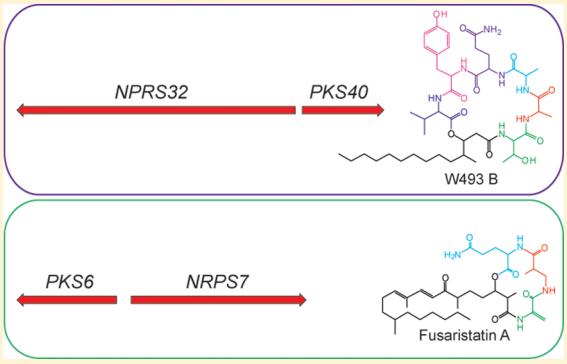
Supporting Information

ABSTRACT: The closely related species *Fusarium graminearum* and *Fusarium pseudograminearum* differ in that each contains a gene cluster with a polyketide synthase (PKS) and a nonribosomal peptide synthetase (NRPS) that is not present in the other species. To identify their products, we deleted *PKS6* and *NRPS7* in *F. graminearum* and *NRPS32* in *F. pseudograminearum*. By comparing the secondary metabolite profiles of the strains we identified the resulting product in *F. graminearum* as fusaristatin A, and as W493 A and B in *F. pseudograminearum*. These lipopeptides have previously been isolated from unidentified *Fusarium* species. On the basis of genes in the putative gene clusters we propose a model for biosynthesis where the polyketide product is shuttled to the NPRS via a CoA ligase and a thioesterase in *F. pseudograminearum*. In *F. graminearum* the polyketide is proposed to be directly assimilated by the NRPS.



Filamentous fungi are capable of producing a wealth of bioactive secondary metabolites, of which one significant class is the lipopeptides. These are cyclic or linear compounds with a fatty acid attached to the N-terminal amino acid.[1] The peptide part consists of proteinogenic and nonproteinogenic amino acids, which are assembled by nonribosomal peptide synthetases (NRPSs). The lipid part, which is attached to the peptide core, can be provided by different sources such as primary lipid metabolism or via polyketide synthases (PKSs).[1] In the biosynthesis of lipopeptides, the fatty acyl chain is incorporated into the peptidyl backbone by a process known as lipoinitiation.[2] PKSs and NRPSs are both organized as assembly lines containing multiple catalytic domains, which are responsible for chain elongation and modification.[3,4]

The enzymes used for biosynthesis of polyketides and nonribosomal peptides are generally encoded from gene clusters containing one or more PKSs and/or NRPSs and optional tailoring enzymes, transporters, and regulators.[5] Genome analyses have shown that fungi possess many secondary metabolite gene clusters where the resulting metabolites have yet to be determined. Some pathways can furthermore result in several different end products. This is also the case for the extensively studied plant pathogen *Fusarium graminearum*, where six of the 15 PKSs and three of the 19 NRPSs have a known product.[6] One of these clusters comprises an *NRPS* (*NRPS7*) and *PKS* (*PKS6*), which was unique to this species compared to the genome sequences of *F. oxysporum, F. solani*, and *F. verticillioides*.[6] The gene cluster is also absent from the *F. pseudograminearum* genome, which contains a PKS and NRPS cluster absent in other sequenced *Fusarium* species.[7]

The numerical classification system for *PKSs* and *NRPSs* in *Fusarium* originally listed 35 PKSs and 30 NRPSs,[6] which has subsequently been expanded by the addition of four *PKSs* and one *NRPS* from *F. fujikuroi*.[8] Following this system we named the new *F. pseudograminearum* genes *PKS40* and *NRPS32*.

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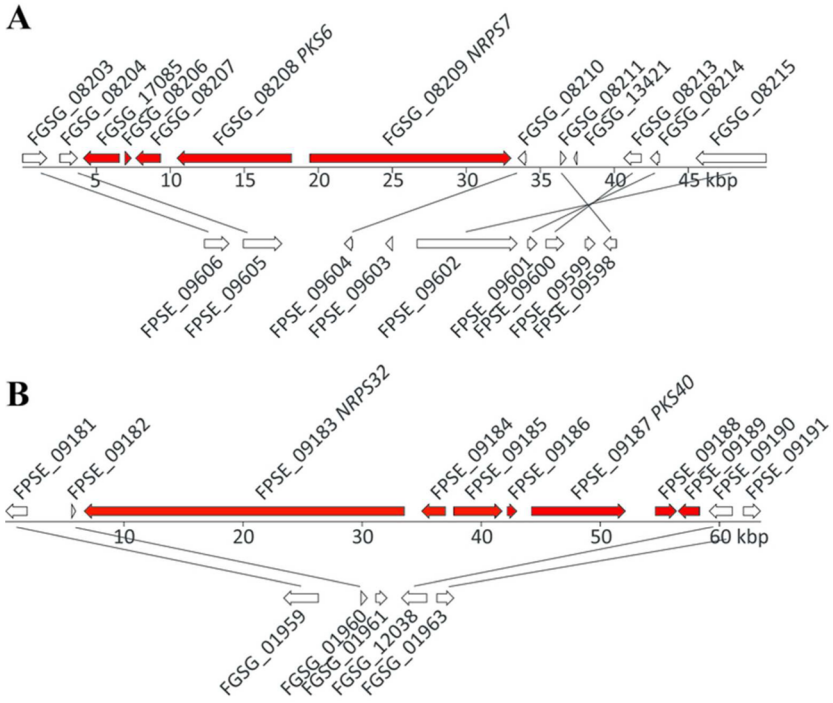

Figure 1. Prediction of gene clusters. (A) Conservation of neighboring genes of *PKS6*/*NRPS7* from *F. graminearum* against *F. pseudograminearum*. (B) Conservation of neighboring genes of the *PKS40*/*NRPS32* gene cluster from *F. pseudograminearum* in *F. graminearum*.

In this paper a reverse genetic approach was used to identify the products of lipopeptides from the two different PKS/NRPS clusters in *F. graminearum* and *F. pseudograminearum*.

RESULTS AND DISCUSSION

PKS6 and *PKS40* are two reducing PKSs with similar domain architectures comprising a β-ketosynthase (KS) for catalyzing the condensation of acyl-CoA units, an acyl-transferase (AT) for delivery of the correct substrate to the enzyme, a dehydrogenase (DH) for reduction of the hydroxy group to an enoyl, a methyltransferase (MET) for C-methylation, an enoyl reductase (ER) for reduction of the enoyl to an alkyl, a β-ketoreductase (KR) for reduction of the ketone to a hydroxy group, and an acyl-carrier protein (ACP) domain for facilitating the movement of substrates and products between the different active sites.[9] A phylogenetic analysis of KS domains of all PKSs from the genome-sequenced *F. graminearum*, *F. pseudograminearum*, *F. fujikuroi*, *F. verticillioides*, *F. oxysporum*, and *F. solani* placed PKS6 and PKS40 in the same subclade (Figure S1 in the Supporting Information). They share 65% identity on the aa level in the KS domain (overall 43%), which is below the limit of 70% that has been used to determine when genes are orthologs. The phylogenetic analysis suggests that the two PKSs produce different products.

NRPS7 is a three-module synthetase, where each module contains the three core domains: the condensation (C) domain responsible for peptide bond formation, an adenylation (A) domain for selecting the amino acid substrate, and a peptide acyl carrier domain (T). The third module contains an additional condensation domain, which could be responsible for cyclization of the final product.[10] NRPS32 on the other hand consists of six modules each containing the three core domains, where modules 1, 2, and 5 contain an epimerization domain (E) to change the epimeric form of the amino acid substrate. The first module contains an additional T domain, which could be responsible for accepting the incoming polyketide as suggested for the biosynthesis of the PKS/NRPS lipopeptide, emericellamide, in *Aspergillus nidulans*.[11]

To determine the putative gene cluster in *F. graminearum*, we searched for orthologs of the neighboring genes in *F. pseudograminearum*, and the reverse approach was applied to determine the cluster in *F. pseudograminearum*. Five genes (*PKS6*, *NRPS7*, FGSG_17085, FGSG_08206, and FGSG_08207) were present in *F. graminearum* but not in *F. pseudograminearum* and are hypothesized to be part of the putative gene cluster (Figure 1A). One gene is predicted to encode a cytochrome P450 monooxygenase, the second gene an aminotransferase, and the third gene a hypothetical protein containing a stress-responsive A/B barrel domain (Table 1). Using a similar approach the *PKS40*/*NRPS32* cluster was identified in *F. pseudograminearum* and hypothesized to comprise five additional genes (Figure 1B). The genes are predicted to encode an acyl-CoA ligase, a transporter, a thioesterase, and two transcription regulators (Table 1). Some of the neighboring genes could also be involved in the biosynthesis of fusaristatin or W493. This would imply that the gene clusters have been lost rather than gained during evolution.

To identify the chemical products, the *NRPS7*/*PKS6* genes were deleted in *F. graminearum*, and the *NRPS40* gene was disrupted in *F. pseudograminearum* (Figure S2 in Supporting Information). Correct integration of the transformation vectors in the resulting mutants was verified by diagnostic PCR. One representative mutant of each gene deletion was selected and subjected to transcription and metabolite analysis.

Transcription analysis of *F. graminearum* showed that *NRPS7* and *PKS6* were expressed in the wild-type strain grown on YES (Figure 2A). No transcripts were detected in the corresponding mutants, which confirmed that the genes had been correctly deleted. Transcription analysis of *NRPS40* and *PKS34* in *F.*

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Table 1. Fusaristatin and W493 Gene Clusters and Predicted Function in *F. graminearum* and *F. pseudograminearum*

gene name (locus tag[a])	length (aa)	predicted function
FGSG_08211	124	hypothetical protein
FGSG_08210	155	hypothetical protein
FGSG_08209 (*NRPS7*)	**4423**	**nonribosomal peptide synthetase**
FGSG_08208 (*PKS6*)	**2554**	**polyketide synthase**
FGSG_08207	**495**	**cytochrome P450 monooxygenase**
FGSG_08206	**138**	**hypothetical protein[b]**
FGSG_17085	**437**	**aminotransferase**
FGSG_08204	364	hypothetical protein
FGSG_08203	538	oxidoreductase
FPSE_09191	475	dehydrogenase kinase
FPSE_09190	406	argininosuccinate synthase
FPSE_09189	**500**	**transcriptional regulator (TenA)**
FPSE_09188	**406**	**transcriptional regulator (bZIP)**
FPSE_09187 (*PKS40*)	**2564**	**polyketide synthase**
FPSE_09186	**250**	**thioesterase**
FPSE_09185	**1305**	**ABC transporter**
FPSE_09184	**579**	**acyl-CoA ligase**
FPSE_09183 (*NRPS32*)	**8892**	**nonribosomal peptide synthetase**
FPSE_09182	71	peptidase inhibitor
FPSE_09181	536	cytochrome P450 monooxygenase

[a]Genes in bold belong to the predicted gene clusters. [b]Contains a stress-responsive A/B barrel domain.

pseudograminearum showed that the two genes were expressed in cultures of the wild-type strain (Figure 2B) and that both transcripts were absent in the Δ*NRPS40* mutant.

The production of secondary metabolites was analyzed by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS), and the base peak chromatograms (BPCs) show that the *F. graminearum* wild-type strain produces a compound ($[M + H]^+$: 659.437 83) that is absent in the Δ*NRPS7* and Δ*PKS6* mutants (Figure 2C). The most likely elemental composition of the compound was estimated to be $C_{36}H_{36}N_4O_7$, which is identical to the lipopeptide fusaristatin A. This compound has previously been isolated from an unidentified *Fusarium* strain and consists of a polyketide chain linked to three amino acid residues [glutamine (Gln), dehydroalanine, and β-aminoisobutyric acid].[12]

When examining the metabolite profiles of the *F. pseudograminearum* strains, two compounds ($[M + H]^+$: 874.528 43 and $[M + H]^+$: 888.544 08) were identified, which were present in the wild-type stain and not in the Δ*NRPS32* strain (Figure 2D). The elemental composition of the two compounds was estimated to be $C_{44}H_{71}N_7O_{11}$ and $C_{45}H_{73}N_7O_{11}$, which corresponds to the two lipopeptides, W493 A and B, previously isolated from an unidentified *Fusarium* strain.[13] W493 A and B consist of six amino acid residues [D-*allo*-threonine (Thr), L-alanine (Ala), D-Ala, L-Gln, D-tyrosine (Tyr), and L-valine/isoleucine (Val/Ile)] linked to a polyketide chain 3-hydroxy-4-methyltetradecanoic acid. These two compounds were most likely part of an antifungal mixture of compounds isolated from *F. acuminatum*[14] together with a third compound, acuminatum, which has the same elemental composition as W493 B, as it contains a leucine instead of isoleucine.[15] To determine whether *F. pseudograminearum* produces W493B or acuminatum, the isolated compound was analyzed by 2D-[^{1}H,^{13}C]-HSQC-NMR. The results revealed that the Cb of the residue is a methine group, while the Cg is a methylene group. Thus, the residue cannot be leucine, but must be isoleucine. The chemical shifts matched the published assignments, except for some trivial errors in the published assignment of some atoms. These atoms and their correct shifts (in ppm, relative to internal TMS) are Ile5 H^{δ} 0.76 ppm and Ile5 $C^{\gamma 2}$14.8 ppm. Further, literature assignments on Gln 3-H^{β}/C^{β} and H^{γ}/C^{γ} are missing, and the correct shifts of these atoms (in ppm, relative to internal TMS) are Gln3 H^{β} 1.882 and 1.764, Gln3 C^{β} 25.8, Gln3 H^{γ} 2.054 and 1.986, and Gln3 C^{γ} 31.2 ppm. Nihei et al. (1998) showed that W493 B inhibited growth of *Venturia inaequalis*, *Monilinia mali*, and *Cochliobolus miyabeanus*, but was inactive against *F. fujikuroi* and *Alternaria mali*. We examined the antifungal properties of the isolated fusaristatin A and W493 B against *F. solani*, *Saccharomyces cerevisiae*, *Penicillium chrysogenum*, and *Scopulariopsis brevicaulis* at concentrations of 1, 10, and 100 μM. Neither of the compounds inhibited the growth of any of the four species (data not shown).

The biosynthetic pathways for fusaristatin A and W493 B can be proposed based on a model for emericellamide synthesis in *A. nidulans*.[11] The biosynthesis starts with formation of a linear polyketide chain by the reducing PKS EasB (AN2547) (Figure

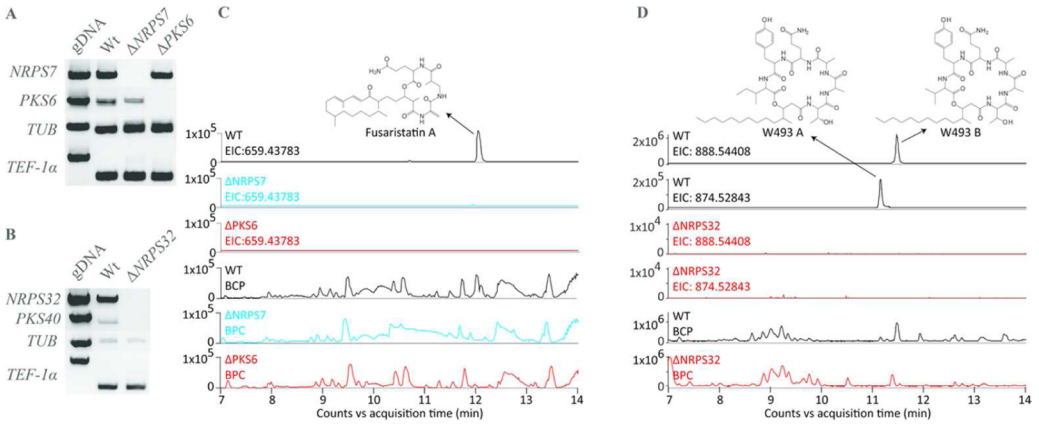

Figure 2. Transcription and metabolite analyses. (A) RT-PCR of *PKS6* and *NRPS7* in *F. graminearum* and (B) *PKS40* and *NRPS32* in *F. pseudograminearum* with *translation elongation factor 1α* (*TEF-1α*) and *β-tubulin* (*TUB*) as control. (C) Base peak chromatograms (BCP) and extracted ion chromatogram (EIC) for fusaristatin A of *F. graminearum* wild-type strain and Δ*PKS6* and Δ*NRPS7*. (D) BPC and EIC of W493 A and B for *F. pseudograminearum* wild type and Δ*NRPS32*.

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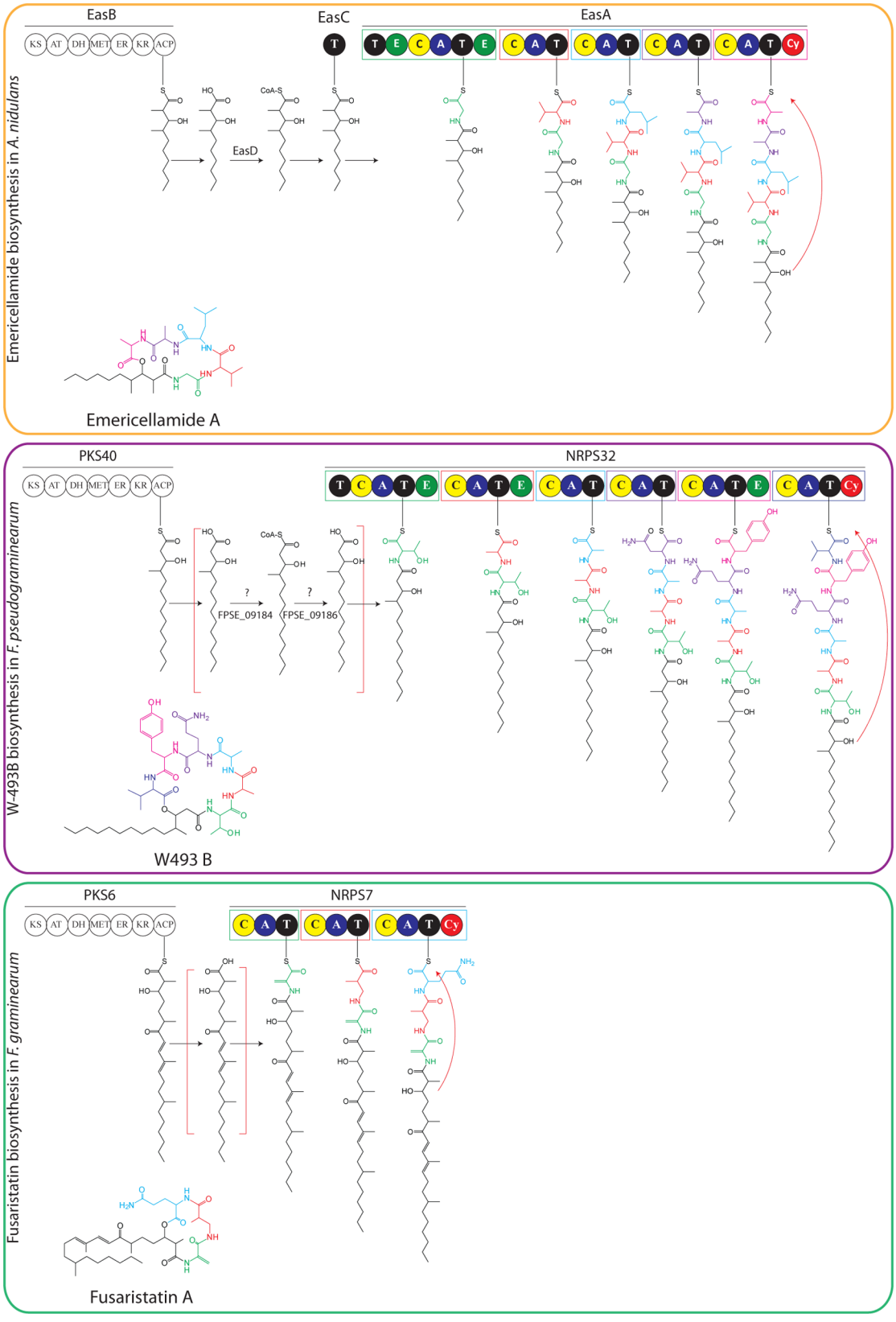

Figure 3. Proposed biosynthetic pathway for W493 B in *F. pseudograminearum* and fusaristatin in *F. graminearum* using emericellamide as a model.[11]

3). The carboxylic acid residue of the polyketide chain is converted to a CoA thioester by an acyl-CoA ligase (EasD, AN2549) and loaded onto an acyl-transferase (EasC, AN2548), which shuttles the product to the NRPS (EasA, AN2545). *PKS40* in *F. pseudograminearum* is predicted to produce a polyketide that differs by a missing C2 methylation. The gene cluster in *F. pseudograminearum* contains a putative acyl-CoA ligase (FPSE_09184) for formation of a CoA thioester

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polyketide. The thiol bond could be hydrolyzed by the putative thioesterase (FPSE_09186) and then accepted by the first T domain in module 1 of NRPS32. The second T domain is responsible for accepting a threonine, which is adenylated by the A domain and epimerized to the D-*allo*-threonine formed by the E domain. The five successive modules incorporate Ala, Ala, Gln, Tyr, and Val/Ile into the final product, which is released by cyclization. The *PKS40/NRPS32* gene cluster contains two putative transcription factors (FPSE_09188 and FPSE_09189), which could be responsible for orchestrating expression of the cluster genes. The gene cluster also encodes a transporter for excretion or internal transport and two putative transcription factors, which may be involved in regulation of the cluster.

The gene cluster in *F. graminearum* does not contain an acyl-CoA ligase or an acyl-transferase, and it is therefore predicted that the polyketide is transferred directly to NRPS7. Most knowledge on lipoinitiation is derived from bacterial studies, where the polyketide has to be activated (e.g., by an acyl CoA ligase) before assimilated by the NRPS. This is probably also the case in fungi, but the required enzymes are not encoded by any of the other cluster genes. It is therefore unknown how the polyketide is incorporated into fusaristatin.[2]

Modules 1−3 incorporate dehydro-Ala, Gln, and β-amino-isobutyric acid in the compound, which is released by cyclization. The β-aminoisobutyric acid units are most likely not freely available to the NRPS, but can be synthesized from thymine, which requires a dehydrogenase, a monooxygenase, and an aminotransferase.[16] The *PKS6/NRPS7* cluster contains a cytochrome P450 monooxygenase (FGSG_08207) and an aminotransferase (FGSG_17085), which theoretically can perform two of the enzymatic steps. The enzymes may however also be involved in biosynthesis of dehydroalanine or modification of the polyketide. The last gene of the cluster encodes a protein with an A/B barrel domain found in variable enzymes, which hampers functional prediction. The dehydro-Ala residue can be a result of cyclization, where serine is dehydrated as previously described.[17]

The 10 residues that define the signature sequence of A domain selectivity[18] were identified in *NRPS7* and *NRPS32* with NRPSpredictor.[19] The amino acid substrates for the A domains were predicted using NRPSpredictor and the PKS/NRPS analysis Web site (Table S1 in the Supporting Information).[20] The software tools were unable to predict the substrates for the three adenylation domains in *NRPS7* and the first five in *NRPS32,* and only the terminal domain of *NRPS32* was correctly predicted to recruit a valine, isoleucine, or leucine. The poor prediction of the amino acid substrates emphasizes the need to provide links between fungal NRPSs and their products for the development of reliable prediction tools. The present tools are developed from bacterial information.

In summary we have successfully deleted key enzymes of two different *PKS/NRPS* gene clusters in *F. graminearum* and *F. pseudograminearum*. Through metabolite profiling we identified the resulting product in *F. graminearum* as fusaristatin A and W493 A and B in *F. pseudograminearum*. We have proposed a model for biosynthesis of both compounds based on the genes available in the two gene clusters. These compounds may enter the food and feed chain through infected cereal crops. A preliminary study of the *NRPS32* deletion mutant did not indicate that W493 B is important for pathogenicity in *F. pseudograminearum* (data not shown).

EXPERIMENTAL SECTION

General Experimental Procedures. Analysis of secondary metabolites was performed on an ultra-high-performance liquid chromatograph 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 i.d., 2.6 μm 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.

Fusaristatin A and W493 B were isolated on an Agilent 1260 semipreparative HPLC system equipped with a 150 × 10 mm Gemini 5 μm C_6−Phenyl 110 Å column (Phenomenex, Torrance, CA, USA). The identity of fusaristatin A and W493 B was verified by NMR spectroscopy on a Bruker AVIII-600 spectrometer (Bruker, Karlsruhe, Germany). ^{1}H and [^{1}H, ^{13}C]-HSQC spectra were recorded at 298.1 K (fusaristatin A) and 308.1 K (W493 B), respectively.

Cluster Prediction and Analysis. DNA and protein sequences of *F. pseudograminearum* were downloaded from NCBI (AFNW00000000),[7] whereas the *F. graminearum* sequences were obtained from MIPS (Munich Information Center for Protein Sequences) using the latest annotations.[21]

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 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) as described previously.[22]

Definition of the *PKS6* and *NRPS7* gene clusters in *F. graminearum* was obtained by comparative analysis to the *F. pseudograminearum* genome sequence by BLASTp to identify missing genes. The same approach was applied to the *F. pseudograminearum NRPS32/PKS40* gene cluster. The function of the neighboring genes was predicted through the NCBI Conserved Domain Database.[23] Domain architectures of PKSs and NRPSs were identified by the PKS/NRPS analysis Web site[20] and NRPSpredictor.[19]

Generation of Mutant Strains. For generation of *PKS6* and *NRPS7* knock-out mutants in *F. graminearum* (PH-1) flanking regions (800−1418 bp) were amplified by PCR using the primers listed in Table S2 in the Supporting Information. The PCR fragments were cloned into a linearized pRF-HU2E vector (carrying a hygromycin resistance cassette) by a four-fragment cloning step using the USER enzyme (New England Biolabs, Ipswich, MA, USA).[24] The vectors were verified by sequencing at Eurofins MWG Operon (Ebersberg, Germany) and transformed into *F. graminearum* by *Agrobacterium tumefaciens*-mediated transformation.[25,26] Correct integration of the vectors was verified by diagnostic PCR using one primer specific for the hygromycin gene and another corresponding to a flanking region outside the insertion.

For deletion of *NRPS32* in *F. pseudograminearum* an internal fragment was amplified (8.8 kb product) with the primers DG636 and DG637 and cloned into the pCR8/GW/TOPO vector. A 625 bp fragment of the cloned region was cut out by *Bam*HI, blunt ended, and a gpdA-NEO-hsvtk (G418 resistance) cassette (*Nhe*I-*Hind*III blunt ended) was cloned into the vector. The vector was used for transformation of *F. pseudograminearum* (CS3096) protoplasts as previously described.[7] The resulting mutants were selected on G418 and verified by PCR using a three-primer PCR assay with DG589, DG662, and DG725. DG589 binds to the antibiotic cassette (the terminator) and DG662 to *NRPS32* and was designed to amplify a 616 bp product. DG725 binds to the region deleted from *NRPS32* and with DG662 amplifies a 424 bp product. Absence of this DG725−662 product was used as confirmation of successful deletion of *NRPS32* (data not shown).

Transcription Analysis. The mycelium from *F. graminearum* and *F. pseudograminearum* wild-type and deletion strains grown on yeast extract sucrose (YES) agar medium for 2 weeks at 25 °C in the dark

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was harvested and lyophilized. RNA was extracted using RNeasy Plant Mini kit (QIAGEN, Hilden, Germnay) according to the protocol for isolation of RNA from fungi. First-stranded cDNA was synthesized using poly(dT) primer (18 bp) and Superscript III reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA, USA). Expression of *NRPS7* and *PKS6* in *F. graminearum* strains and *NRPS40* and *PKS34* in *F. pseudograminearum* was analyzed by PCR using the primers listed in Table S2 in the Supporting Information. The two housekeeping genes *Translation elongation factor 1α* (TEF-1α) and *β-tubulin* (TUB) were used as a reference in both species.

Metabolite Profiling. Strains of *F. graminearum* and *F. pseudograminearum* were grown for 2 weeks in the dark at 25 °C, and secondary metabolites were extracted as previously described.[27] The extracts were analyzed by UHPLC-MRMS, and the qTOF was operated at 2 GHz in the extended dynamic range mode at a resolution of 30 000 FMWH. Subsamples of 1 μL 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 second. Furthermore, MS/MS spectra were 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.[28] UV/vis data and the full-scan MS data were compared to the published data.[29]

Isolation and Structural Validation of Fusaristatin A and W493 B. Wild-type strains of *F. graminearum* and *F. pseudograminearum* were each grown on 50 Petri dishes (90 mm) with YES medium for 2 weeks at 25 °C in the dark. The plates were extracted with 1 L of ethyl acetate (1% formic acid), filtered through mira cloth, and evaporated to dryness. The metabolites were dissolved in 100 mL of methanol and washed twice with hexane. Primary separation was performed on a solid-phase extraction column (Strata C_{18}-E, 50 g/150 mL gigatube, Phenomenex, Torrance, CA, USA), which had been conditioned with 100 mL of methanol and 100 mL of water, sequentially. The compounds were eluted with 150 mL of acetonitrile, evaporated to dryness, and dissolved in 10 mL of methanol. Fusaristatin was isolated by multiple cycles on a semipreparative HPLC system using a flow of 5.000 mL/min and a linear water−acetonitrile gradient, where both were buffered with 50 ppm trifluoroacetic acid. The gradient started at 30% ACN, which was increased to 100% in 8 min, where fusaristatin A eluted after 7.5 min. W493 B was isolated from the *F. pseudograminearum* extract using another gradient starting at 10% ACN, which was increased to 100% in 16 min and with W493 B eluting after 12.1 min.

The isolated fusaristatin A (1.1 mg) and W493 B (1.2 mg) were dissolved in 600 μL of pyridine-d_5 and dimethyl sulfoxide (DMSO-d_6), respectively. The identity of the compounds was verified by NMR, and the recorded spectra were referenced to the known chemical shift of pyridine-d_5 solvent signals (fusaristatin A) and internal tetramethylsilane (TMS) in DMSO-d_6 (W493B), respectively. Spectra were processed with TopSpin 3.2 and analyzed with Amix 3.9.12 (Bruker).

Antifungal Assessment. Fusaristatin A and W493 B were dissolved in DMSO and used in an antifungal screen against *Penicillium chrysogenum*, *Saccharomyces cerevisiae*, *Scopulariopsis brevicaulis*, and *F. solani*. The experiments were performed in 96-well microtiter plates containing 200 μL of Czapek dox medium (Difco Sparks, MD, USA) and 5000 conidia per well. A 1 μL portion of fusaristatin A or W493 B in DMSO was added to the wells in a final concentration of 0, 1, 10, and 100 μM. The fungi were cultivated at 25 °C for 2 days, and growth was monitored and measured with oCelloScope (Unisensor A/S, Allerød, Denmark) using the algorithms described previously.[30]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Primers used to generate and verify mutants and for RT-PCR, phylogenetic analysis of KS domains, and transformation of *F. graminearum* and *F. pseudograminearum*. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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