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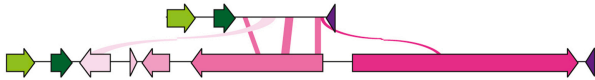
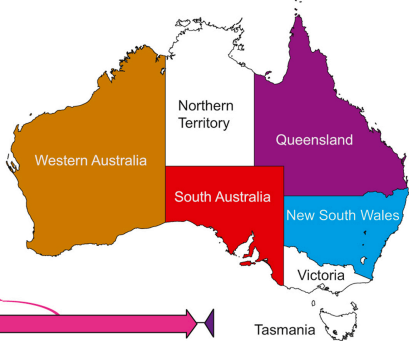
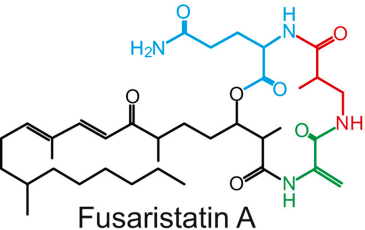
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1 **There it is! *Fusarium pseudograminearum* did not lose the fusaristatin**  
2 **gene cluster after all.**

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21 **Summary**

22 *Fusarium pseudograminearum* is a significant pathogen of cereals in arid regions worldwide and  
23 has the ability to produce numerous bioactive secondary metabolites. The genome sequences of  
24 seven *F. pseudograminearum* strains have been published and in one of these strains, C5834, we  
25 identified an intact gene cluster responsible for biosynthesis of the cyclic lipopeptide fusaristatin A.  
26 The high level of sequence identity of the fusaristatin cluster remnant in strains that do not produce fusaristatin  
27 suggests that the absence of the cluster evolved once, and subsequently the resulting locus with the cluster  
28 fragments became widely dispersed among strains of *F. pseudograminearum* in Australia. We examined a  
29 selection of 99 Australian *F. pseudograminearum* isolates to determine how widespread the ability  
30 to produce fusaristatin A is in *F. pseudograminearum*. We identified 15 fusaristatin producing  
31 strains, all originating from Western Australia. Phylogenetic analyses could not support a division  
32 of *F. pseudograminearum* into fusaristatin producing and nonproducing populations, which could  
33 indicate the loss has occurred relatively recent.

34

35 **Keyword:** Secondary metabolites; polyketides; non-ribosomal peptides; *Fusarium* Crown Rot;  
36 evolution

## 37 **Introduction**

38 *Fusarium pseudograminearum* is the primary cause of Fusarium crown rot (FCR) of wheat and  
39 barley in the arid cereal growing regions of the world including Australia (Burgess et al. 2001),  
40 Southern Europe (Balmas 1994), Northern Africa (Gargouri et al. 2011), South Africa (Lamprecht  
41 et al. 2006), China (Ji et al. 2016; Li et al. 2012; Xu et al. 2017) and the United States of America  
42 (Smiley et al. 2005). The disease is one of the most severe in cereals in Australia with yearly  
43 economic losses of approximately 100 million Australian dollars (Murray and Brennan 2009, 2010).  
44 *F. pseudograminearum* is heterothallic (Aoki and O'Donnell 1999b; Summerell et al. 2001) and was  
45 initially recognized as a population within the *F. graminearum* species group (Group 1) based on  
46 cultivation and its inability to form homothallic perithecia (Burgess et al. 1975; Francis and Burgess  
47 1977). Later, the two species were formally segregated by molecular analyses (Aoki and O'Donnell  
48 1999a) and further sequence analyses suggested that *F. pseudograminearum* is a single globally  
49 occurring species (Scott and Chakraborty 2006), while *F. graminearum* can be divided into more  
50 than 16 phylogenetically distinct species (Aoki et al. 2012; O'Donnell et al. 2000). *F. graminearum*  
51 is involved in Fusarium head blight (FHB) in cereals, a disease which *F. pseudograminearum* has  
52 only been observed to cause in Australia (Backhouse et al. 2004) and China (Ji et al. 2016). Both  
53 species are known producers of the trichothecene mycotoxin deoxynivalenol (and derivatives) and  
54 of the mycoestrogen zearalenone (Sydenham et al. 1991).

55 Comparative analyses of the first genome sequenced strains of *F. graminearum* (NRRL 31084) and  
56 *F. pseudograminearum* (CS3096) revealed only minor differences in the composition of polyketide  
57 synthases (PKSs) and non-ribosomal peptide synthetases (NRPSs) (Hansen et al. 2015). The two  
58 strains differ, however, in their ability to produce polyketide lipopeptides: in their ability to produce  
59 two polyketide lipopeptides: *F. graminearum* NRRL 31084 produces fusaristatin A but not W493,

60 while *F. pseudograminearum* CS3096 produces W493 but not fusaristatin A (**Figure 1**; (Sørensen  
61 et al. 2014a)).

62 Biosynthesis of W493 and fusaristatin A are suggested to follow similar routes starting with  
63 production of a partially reduced polyketide which serves as a substrate for a NRPS that catalyzes  
64 the condensation of the polyketide and amino acids before the compounds are released by  
65 cyclization (Sørensen et al. 2014a). The key enzymes involved in biosynthesis of W493 are PKS32,  
66 which produces a reduced polyketide (C<sub>14</sub>) chain and NRPS40, which catalyzes condensation of six  
67 amino acids (threonine, alanine, alanine, glutamine, tyrosine and valine/isoleucine (W493-A/  
68 W493-B)). Fusaristatin biosynthesis is initiated by production of a reduced polyketide (C<sub>24</sub>) by  
69 PKS6 prior to incorporation of three amino acids (dehydroalanine,  $\beta$ -aminoisobutyric acid and  
70 glutamine) by NRPS7.

71 The fusaristatin gene cluster has also been identified in the more distantly related *Botrytis*  
72 *fuckeliana*, *Cochliobolus heterostrophus* and *Pyrenophora teres* (Sieber et al. 2014). Following the  
73 first genome release of a *F. pseudograminearum* strain, six additional strains were published  
74 (Gardiner et al. 2017; Moolhuijzen et al. 2013). In one of these strains, CS5834, we identified the  
75 intact fusaristatin gene cluster and the aim of the current study was to determine how common this  
76 cluster is in *F. pseudograminearum* and whether its presence or absence arose from a gain or loss  
77 event.

78

## 79 **Materials and methods**

### 80 *Fungal strains*

81 Ninety-nine strains of *F. pseudograminearum* were obtained from the CSIRO collection in Brisbane  
82 Australia. These strains were isolated from four different Australian states; New South Wales (42  
83 strains), Queensland (18 strains), South Australia (4) and Western Australia (35).

84

### 85 *Fusaristatin gene cluster analyses*

86 The available genome sequences of seven *F. pseudograminearum* strains (CS3096, CS3220,  
87 CS3270, CS3427, CS3487, CS5834 and RBG5266) were screened for presence of the fusaristatin  
88 gene cluster using the published gene cluster from *F. graminearum* (Sørensen et al. 2014a).  
89 Remnant fragments of the fusaristatin gene cluster were identified though BlastN analyses (Altschul  
90 et al. 1990) using the fusaristatin gene cluster from *F. pseudograminearum* CS5834 against the  
91 whole-genome sequence (WGS) database of the six other *F. pseudograminearum* strains.

92

### 93 *Analyses of W493-B and fusaristatin A production*

94 For secondary metabolite analyses the 99 *F. pseudograminearum* strains were cultivated on solid  
95 yeast extract sucrose (YES) agar medium (Sørensen and Sondergaard 2014) and corn meal agar  
96 (CM; corn meal 60 g/L, ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 10 mg/L, CuSO<sub>4</sub> x 5 H<sub>2</sub>O 5 mg/L, agar 20 g/L) medium for  
97 two weeks in the dark at 25 °C. The extraction of secondary metabolites were performed as  
98 previously described (Smedsgaard 1997). The resulting extracts were analyzed on a Hitachi Elite  
99 LaChrom HPLC system equipped with a 150 x 4.6 mm Ascentis Xpress 2.7 µm phenyl-hexyl  
100 column (Sigma-Aldrich, USA) and coupled to a high resolution mass spectrometer (compact qTOF,  
101 Bruker, Germany) with an electrospray source using a 3:97 flowsplitter. 40 µL extract was

102 separated using a flow of 1 mL/min with a linear water–acetonitrile gradient, with both eluents  
103 buffered with 0.1% formic acid. The gradient started at 10% acetonitrile and reached 100% in 20  
104 min, which was held for 5 min.

105

#### 106 *Determination of presence or absence of the fusaristatin gene cluster*

107 The fungal strains were cultivated in 30 mL liquid Czapek dox (Sigma-Aldrich) medium prior to  
108 DNA extraction. The cultivated fungi were filtered through sterile MiraCloth (Calbiochem®) and  
109 ground in liquid nitrogen before genomic DNA was extracted with the DNeasy® Plant Mini Kit  
110 (Qiagen, Hilden, Germany) (Droce et al. 2013). The isolated genomic DNA served as template in a  
111 polymerase chain reaction (PCR) targeting *PKS6* with primers PKS6conFw (5'-3': CTG TTG TTG  
112 GCA TGA GTT GC) and PKS6conRv (5'-3': TGG CCC ATG CGA GGA TAC TG), which  
113 amplify a 1751 bp product in strains with intact *PKS6* and 1564 bp product in strains with *PKS6*  
114 remnants. The PCR reactions were performed in 50 µL volume using the Phusion Hot Start II DNA  
115 Polymerase (Thermo Fisher Scientific) according to manufactures protocol. The resulting PCR  
116 products were run on 1% agarose gels with 1 kbp plus DNA ladder (Thermo Fisher Scientific).

117

#### 118 *Phylogenetic analyses of F. pseudograminearum strains*

119 For phylogenetic analyses the primers PHO1 (5'-3': ATC TTC TGG CGT GTT ATC ATG) and  
120 PHO6 (5'-3': GAT GTG GTT GTA AGC AAA GCC C) were used to amplify a fragment of the  
121 Phosphate permease gene (FPSE\_11047 in *F. pseudograminearum* CS3096) (Scott and  
122 Chakraborty 2006) by PCR. The PCR products were purified with the QIAquick PCR purification  
123 kit (Qiagen, Hilden, Germany) and sequenced at Eurofins Genomics (Ebersberg, Germany) using  
124 the forward primer PHO1. The sequences were aligned with by multiple alignment using fast

125 fourier transform (MAFFT) at the T-REX web server (Boc et al. 2012). The alignments were  
126 analysed with CLC main workbench (CLC Bio, Qiagen, Germany) using maximum likelihood with  
127 1000 bootstraps and visualized with EvolView (<http://evolgenius.info/evolview>) (Zhang et al.  
128 2012).

129

### 130 *Whole-Genome Sequencing*

131 With minor modifications, genomic DNA was extracted from strains CS3894, CS3900, CS5541,  
132 CS7093, CS7108, CS7081, CS7088, CS7065 and CS7060 using the FastDNA<sup>TM</sup> SPIN kit for Soil  
133 (MP Biomedicals, USA). Following clean-up with Agencourt AMPure XP beads (Beckman  
134 Coulter, USA), 2 µg DNA was used as input for the SQK-LSK8 ligation sequencing kit protocol  
135 (NBE\_9006\_v103\_revQ\_21Dec2016). The protocol was modified to allow for barcoding with the  
136 Native Barcoding Kit (EXP-NBD103, Oxford Nanopore Technologies, UK) directly following the  
137 end-prep step and for downstream compatibility with sequencing on the PromethION alfa/beta  
138 sequencer (Oxford Nanopore Technologies, UK). Briefly, 10 µL Native barcode (NB01-NB9) was  
139 mixed with 30 µL end-prepped DNA mix (2 µg DNA), 10 µL nuclease-free water, 40 µL Ultra II  
140 ligation master mix (New-England Biolabs, USA), 1 µL ligation enhancer (New-England Biolabs,  
141 USA) and incubated at room temperature for 10 minutes before being further processed according  
142 to the PromethION SQK-LSK9 protocol (GDLE\_9056\_v109\_revE\_02Feb2018). Approximately  
143 600 ng of pooled DNA was loaded onto a primed FLO-PRO001 flow-cell (Oxford Nanopore  
144 Technologies, UK) and sequenced on the PromethION alfa/beta sequencer with live base-calling  
145 enabled. Approximately 60 Gbp reads were demultiplexed and trimmed in Porechop version 0.2.3  
146 and subsequently mapped to the reference genome of *F. pseudograminearum* CS3096 (Gardiner et  
147 al. 2017) in CLC Genomics Workbench version 9.5.5 (CLC Bio, Qiagen, Germany). Consensus  
148 sequences from the complete genes of beta-tubulin (FPSE\_03337), translation elongation factor 1-

149 alfa (FPSE\_11980), trichothecene 3-O-acetyltransferase (FPSE\_11049), ammonia-ligase  
150 (FPSE\_11050) and phosphate permease (FPSE\_11047) were finally extracted for phylogenetic  
151 analysis (O'Donnell et al. 2000). The alignment was executed with MUSCLE (Edgar 2004). A few  
152 nucleotides (1-3 pr. sequence) resulting in non-sense mutation were excluded from the final  
153 alignments to eliminate Nanopore sequencing-biases (in some homopolymeric nucleotide-region).  
154 The alignments were fused and analysed using the same approach as for the phosphate permease  
155 gene. CANU version 1.7 was used to assemble the genome of CS3894 with default settings  
156 (genome size set at 36 gbp) (Koren et al. 2017).

157

## 158 **Results and discussion**

159 *The fusaristatin cluster is conserved in F. pseudograminearum CS5834*

160 The predicted fusaristatin cluster in *F. pseudograminearum* CS5834 was initially compared to the  
161 published clusters in *F. graminearum* and *F. avenaceum* (Sørensen et al. 2014a; Sørensen et al.  
162 2014b). The comparison showed that the hypothetical proteins are of comparable length and  
163 identity (**Table 1**) suggesting that the gene cluster is also functional in *F. pseudograminearum*  
164 CS5834. Based on their phylogenetic relationship (Kristensen et al. 2005; O'Donnell et al. 2013) it  
165 was not surprising that a higher identity was observed to *F. graminearum* (94-98 %) than to *F.*  
166 *avenaceum* (73-86 %).

167 Further analyses of the available *Fusarium* genome sequences revealed that the fusaristatin gene  
168 cluster is present with conserved synteny in *F. pseudograminearum* CS5834, *F. graminearum*, *F.*  
169 *culmorum*, *F. meridionale*, *F. asiaticum*, *F. langsethiae*, *F. acuminatum* and *F. avenaceum* (**Figure**  
170 **2**). The flanking genes were, however, different in *F. avenaceum* and *F. acuminatum* compared to  
171 the other *Fusarium* species, indicating that the cluster is present in a different genomic location

172 these two species. The identical location of the fusaristatin cluster in *F. pseudograminearum*  
173 CS5834 and the majority of other *Fusarium* species suggests that CS5834 did not acquire the  
174 cluster through horizontal gene transfer. This in turn suggests that the fusaristatin cluster was  
175 present in *F. pseudograminearum* after it diverged from other fusaria but was subsequently lost. To  
176 further investigate the nature of the loss, we examined the genomic region between the flanking  
177 genes of the fusaristatin gene cluster by which five conserved remnant fragments (88-95 %  
178 sequence identity) of the cluster could be found in all six *F. pseudograminearum* strains (**Figure**  
179 **2A**). One of the fragments (R1; 897 bp) originates from a predicted aminotransferase gene  
180 (BN849\_0052070), three other fragments (R2-R4; 120, 446 and 273 bp, respectively) originate  
181 from *PKS6* (BN849\_0052040) while a fifth fragment (R5; 407 bp) originates from *NRPS7*  
182 (BN849\_0052030). To illustrate that the fragments originate from *PKS6* the three remnant  
183 fragments of *PKS6* in *F. pseudograminearum* CS3096 were translated into amino acid sequences  
184 and aligned against the functional *PKS6* of *F. pseudograminearum* CS5834 (**Figure 2B**). In these  
185 alignments, a high sequence identity was observed for the three fragments as R2 had 90% (60  
186 amino acids), R3 had 89% (148 amino acids) and R4 had 82% identity (91 amino acids). The  
187 presence of conserved remnant fragments suggests that the missing fusaristatin gene cluster is a  
188 result of a deletion event in a common ancestor.

189

190 *Fusaristatin-producing F. pseudograminearum strains are geographically co-localized*

191 The distribution of the fusaristatin-producing ability in Australian *F. pseudograminearum* strains  
192 was further investigated through chemical analyses of the 99 strains, which originated from five  
193 different states (New South Wales, Queensland, South Australia and Western Australia). The  
194 analyses showed that while nearly all strains (except CS3002 and CS5897) were able to produce  
195 W493-B only 15 strains produced fusaristatin when cultivated on solid YES or CM medium (**Table**



196 2). The ability to produce fusaristatin seemed to be geographically confined, because all 15  
197 fusaristatin A-producing strains were isolated from Western Australia. Although a slight decrease in  
198 W493-B levels was observed in the fusaristatin A producers, this difference was not significant  
199 ( $P>0.05$ ; Supplementary Figure 1).

200

201 *Fusaristatin producing isolates do not form a unique lineage*

202 The phosphate permease gene was partially sequenced (807 of 1851 bp) to investigate whether  
203 fusaristatin-producing and nonproducing strains constitute phylogenetically distinct lineages of *F.*  
204 *pseudograminearum*. Assumedly, this locus is inherited independently of the fusaristatin gene  
205 cluster, as they are located on two different chromosomes. The phosphate permease gene is located  
206 near the middle of chromosome IV, while the fusaristatin gene cluster is located near and end of  
207 chromosome II. Phylogenetic analyses of the resulting sequences resulted in a tree with two major  
208 clades (**Figure 3A**), separated by 26 variable sites (3%). The first clade contained the majority of  
209 the strains isolated from New South Wales (40/42) and Queensland (16/18). Three nonproducers of  
210 fusaristatin A from Western Australia were also present in clade I, while the remaining thirty-two  
211 strains were located in the second clade. This second clade consisted of two different sequence  
212 types, sharing 805 of 807 nucleotides and contained both fusaristatin producers and nonproducers  
213 without any signs of segregation.

214 In a further attempt to achieve a phylogenetic separation of fusaristatin producers and nonproducers,  
215 we performed a multiplexed genome sequencing of four producers (CS5541, CS7108, CS7081, and  
216 CS7060) and five nonproducers (CS3894, CS3900, CS7065 CS7088, CS7093). In addition to the  
217 phosphate permease gene, sequences of five genes were extracted ( $\beta$ -tubulin, translation elongation  
218 factor 1 $\alpha$ , trichothecene 3-O-acetyltransferase and ammonia-ligase) and used to generate an  
219 additional phylogenetic tree. The resulting tree failed to separate fusaristatin producers and

220 nonproducers, although this combination of genes has previously been used to separate *F.*  
221 *graminearum* into different phylogenetic species (**Figure 3C**). Due to the inadequacy of this  
222 multigene approach, future studies could focus on full genome analyses in order to determine  
223 whether producers and nonproducers of fusaristatin can be separated into two groups.

224 The lack of fusaristatin production in a strain does not necessarily mean that the strain does not  
225 have a functional fusaristatin gene cluster, because lack of production can also be caused by too low  
226 production levels or repression under the tested conditions. A PCR based strategy was used to  
227 determine the presence or absence of a functional *PKS6* yielding predicted products of 1751 bp in  
228 strains with an intact *PKS6* and 1564 bp in strains with *PKS6* remnant fragments. Thus, the two  
229 fragments are markers for the two alternative alleles of the locus (i.e., an intact and a deleted gene  
230 cluster) based on available genome sequence data. The results showed that the PCR of the 15  
231 fusaristatin producing strains resulted in amplified fragments of the expected size for the intact and  
232 functional *PKS6* (**Figure 3B**). The PCR fragments for all the nonproducing strains, except CS3894,  
233 were smaller, which corresponds to the presence of the *PKS6* remnant region. The slightly larger  
234 PCR fragment in CS3894 was investigated further using the full genome sequence of CS3894,  
235 which showed that overall the sequence was very similar to the nonproducing CS3096 remnant  
236 region with the exception of an additional 100 bp (**Supplementary Figure 2**) which accounts for  
237 the intermediate size of the band observed for this isolate (**Figure 3B**).

238 Together the molecular analyses suggests that the presence of the fusaristatin gene cluster is  
239 reflected to some extent in the phylogenetic analyses of genes used in the present study. However,  
240 the genes do not contain sufficient variation to segregate the strains into clades reflecting the ability  
241 to produce fusaristatin A. A phylogenetic analysis of *F. pseudograminearum* based on the  
242 phosphate permease, reductase, translation elongation factor-1 $\alpha$  and  $\beta$ -tubulin genes concluded that  
243 *F. pseudograminearum* is a single monophyletic species (Scott and Chakraborty 2006). The high

244 sequence conservation within *F. pseudograminearum* is also reflected in the RNA polymerase II  
245 largest (RPB1) and second largest subunit (RPB2) genes, which have been successfully used for  
246 separating closely related *Fusarium* species (O'Donnell et al. 2013). In these genes CS3096 and  
247 CS5834 share high sequence identity (1604/1606 and 901/902).

248 The loss of the fusaristatin gene cluster in *F. pseudograminearum* could represent an evolutionary  
249 development where the compound is not needed for spread and survival. Biosynthesis of huge  
250 proteins, like PKS6 and NRPS7, represent a significant energy cost for the fungus; thus, losing the  
251 redundant gene cluster can result in an improved fitness.

252 One of the reasons for losing the fusaristatin gene cluster could be due to an overlapping mode of  
253 action for W493 and fusaristatin A, which is not an unlikely scenario given their similar  
254 biosynthetic background and structural similarities. The high level of identity of the sequence of the  
255 remnant fusaristatin cluster in strains CS3096, CS3220, CS3270, CS3427, CS3487 and RBG5266  
256 suggests that presence a deletion event occurred in one strain or lineage of the fungus rather than  
257 multiple times in multiple strains or lineages. However, the presence of the additional region in  
258 CS3894 suggests that some modifications has occurred locus where the fusaristatin gene cluster was  
259 lost. Understanding when this loss event occurred may provide some indication of the evolutionary  
260 reason for the absence of the cluster in most strains. The climatic conditions (and native grass  
261 populations) in WA can be drastically different to the eastern states of Australia. The restricted  
262 geographic location of isolates containing the fusaristatin cluster may suggest different evolutionary  
263 pressures exist in WA but the widespread (and overlapping) presence of isolates carrying the cluster  
264 loss in the same location and the absence of obvious lineages are contrary to this scenario.

265 Although *Fusarium* crown rot has likely been present in WA for a long time, it has only recently  
266 emerged as a significant economic impediment to wheat production in this area (Murray and  
267 Brennan 2009). Further complicating our understanding of the evolutionary pressures that have

268 shaped the *F. pseudograminearum* genome is the likelihood that *F. pseudograminearum*, like  
269 *F. graminearum*, has not co-evolved with wheat (Lofgren et al. 2018) and can be considered an  
270 opportunistic pathogen of wheat. Thus, it will be extremely challenging to pinpoint the reason for  
271 loss of the cluster or even whether maintaining the clusters provides some advantage in the WA  
272 environment.

273

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284

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413 **Figure legends**

414 **Figure 1.** Structures of W493-B and fusaristatin A highlighting the reduced polyketide (black) and  
415 peptide (colored) parts.

416

417 **Figure 2.** Comparative analysis of the fusaristatin gene cluster and remnant fragments in *Fusarium*.

418 **A.** Illustration of the intact cluster in *F. pseudograminearum* CS5834 (BN849\_0052030 -

419 BN849\_0052070) and seven other *Fusarium* species. Only five remnant fragments (R1-R5) are

420 present in *F. pseudograminearum* CS3096, CS3220, CS3487, CS3270, CS3427 and RBG5266. **B.**

421 Predicted amino acid sequence of regions corresponding to PKS6 fragments R2 - R4 in *F.*

422 *pseudograminearum* strains CS3096 (lacks intact cluster) and CS5834 (has intact cluster). Amino

423 acids are represented by standard single-letter abbreviations, and two letters stacked one on top of

424 the other indicate a difference in the sequence of the two strains.

425

426 **Figure 3.** Molecular analyses of the *F. pseudograminearum* strains. **A.** Phylogenetic analyses of the

427 99 *F. pseudograminearum* strains (orange: Western Australia; red: South Australia; blue: New

428 South Wales; purple: Queensland) and of selected genome sequenced *Fusarium* strains with *F. poae*

429 strain 2516 as outgroup. Numbers indicate bootstrap values from 1000 replications. **B.** 1% agarose

430 gels visualizing the PCR products for determining the presence (●) and absence (○) of *PKS6* of

431 strains located in clade II. **C.** Multi-locus phylogeny of 16 *F. pseudograminearum* isolates with and

432 without the fusaristatin gene cluster. Numbers indicate bootstrap values from 1000 replications.

433

434 **Supplementary figure 1.** Production of W493-B by *Fusarium pseudograminearum* strains

435 collected in New South Wales (NSW), Queensland (QLD) and Western Australia (WA). Strains



436 from WA have been divided in fusaristatin producers (+) and nonproducers (-). The box plot  
437 illustrate minimum and maximum; first and third quartile and mean peak areas.

438

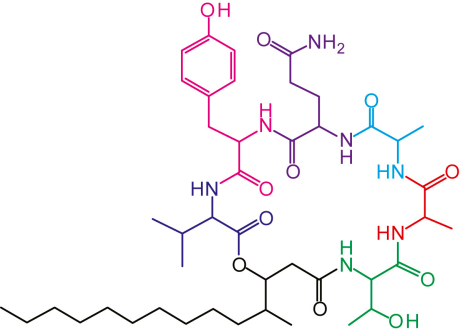
439 **Supplementary Figure 2.** Alignment of *F. pseudograminearum* CS3096 and CS3894 in the region  
440 where the fusaristatin gene cluster has been lost. A highlighted 100 bp region is present in CS3894,  
441 but absent in CS3096.

**Table 1.** Description of genes in the fusaristatin cluster in *F. pseudograminearum* CS5834 and comparison (% identity on amino acid level) to *F. graminearum* NRRL 31084 and *F. avenaceum* Fa05001.

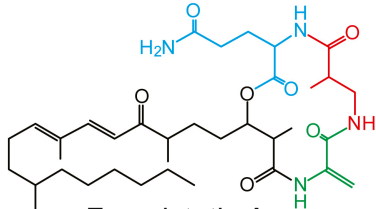
<i>F. pseudograminearum</i>	Length	Function	<i>F. graminearum</i>	<i>F. avenaceum</i>
BN849_0052030	4355 aa	Non-ribosomal peptide synthetase	FGSG_08209 (94%)	FAVG1_08708 (73%)
BN849_0052040	2554 aa	Polyketide synthase	FGSG_08208 (98%)	FAVG1_08709 (84%)
BN849_0052050	520 aa	Cytochrome P450 monooxygenase	FGSG_08207 (98%)	FAVG1_08710 (86%)
BN849_0052060	138 aa	Hypothetic protein	FGSG_08206 (96%)	FAVG1_08711 (79%)
BN849_0052070	511 aa	Aminotransferase	FGSG_08205 (96%)	FAVG1_08712 (78%)

**Table 2.** Production<sup>a</sup> of W493-B and Fusaristatin A (Fst A) by *F. pseudograminearum* strains collected from New South Wales (NSW), Queensland (QLD), South Australia (SA) and Western Australia (WA).

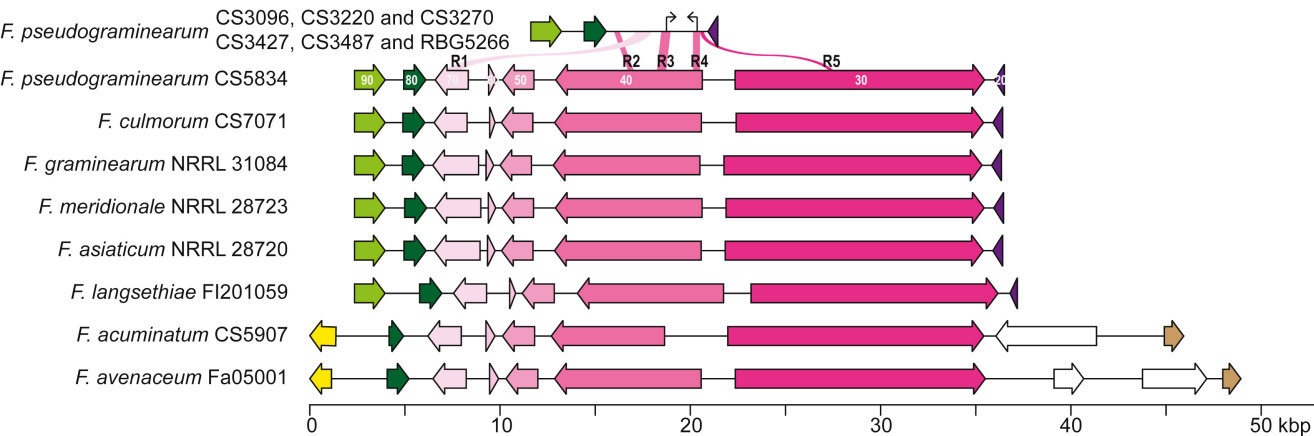
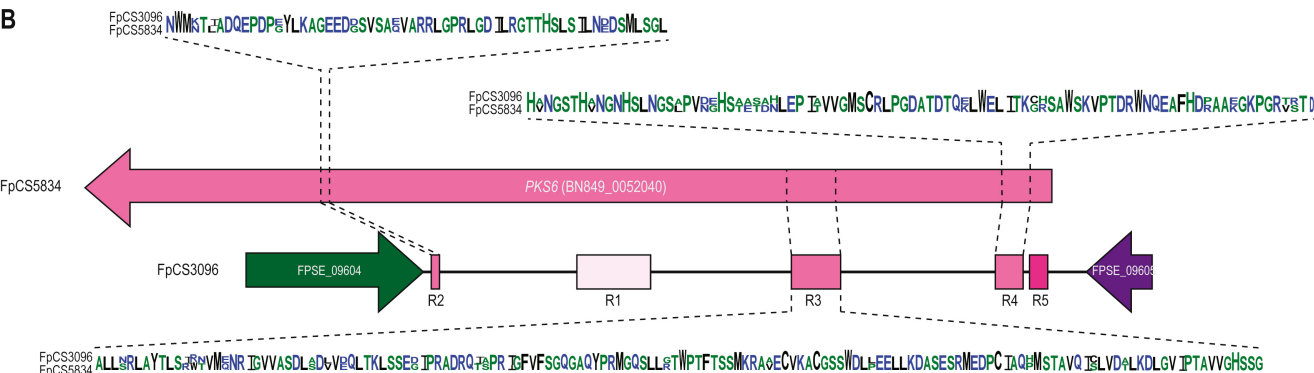
Strain <sup>a</sup>	W493-B <sup>b</sup>	Fst A <sup>b</sup>	Location	State	Strain	W493-B	Fst A	Location	State
CS3096	●		Moree	NSW	CS7114	●		Bowenville	QLD
CS3164	●		Qurindi	NSW	CS7118	●		Marmadiaz	QLD
CS3166	●		Qurindi	NSW	CS7124	●		Hannaford	QLD
CS3173	●		Qurindi	NSW	CS7126	●		Hannaford	QLD
CS3184	●		Bladeville	NSW	CS7133	●		Toobeak	QLD
CS3220	●		Liverpool Plains	NSW	CS7139	●		Toobeak	QLD
CS3270	●		Liverpool Plains	NSW	CS7145	●		Wyaga	QLD
CS3293	●		Boggabri	NSW	CS7147	●		Wyaga	QLD
CS3319	●		Boggabri	NSW	CS7149	●		Warra	QLD
CS3361	●		Bellata	NSW	CS7153	●		Warra	QLD
CS3768	●		North Stat	NSW					
CS3784	●		North Stat	NSW	CS3891	●		Foolunga Street	SA
CS3941	●		Cooper Creek K	NSW	CS3894	●		Foolunga Street	SA
CS3950	●		Cooper Creek K	NSW	CS3900	●		Angus Valley	SA
CS3965	●		9 Miles Road	NSW	CS3907	●		Angus Valley	SA
CS3967	●		9 Miles Road	NSW					
CS3983	●		Livingstone Farm	NSW	CS5541	●	●	Stockdale	WA
CS3986	●		Livingstone Farm	NSW	CS5573	●	●	Stockdale	WA
CS7291	●		Nombi 1	NSW	CS5588	●	●	Tammin	WA
CS7302	●		Spring Ridge 1	NSW	CS5703	●	●	Tammin	WA
CS7305	●		Spring Ridge 1	NSW	CS5834	●	●	Tammin	WA
CS7311	●		Nombi 1	NSW	CS5877	●		Farm 3	WA
CS7313	●		Nowbi 1	NSW	CS5894	●	●	Jerramungub	WA
CS7319	●		Spring Ridge 2	NSW	CS5897	●	●	Jerramungub	WA
CS7344	●		Nowbi 2	NSW	CS7054	●	●	Lake Grace	WA
CS7350	●		Nowbi 2	NSW	CS7055	●	●	Boxwood Hill	WA
CS7358	●		Tambar Springs	NSW	CS7056	●	●	Boxwood Hill	WA
CS7374	●		Tambar Springs	NSW	CS7060	●	●	Lake Grace	WA
CS7385	●		Spring Ridge 3	NSW	CS7062	●	●	Lake Grace	WA
CS7391	●		Spring Ridge 3	NSW	CS7065	●		Mettler	WA
CS7405	●		Spring Ridge 4	NSW	CS7066	●	●	Wellstead	WA
CS7407	●		Bladeville	NSW	CS7069	●		Wellstead	WA
CS7420	●		Spring Ridge 5	NSW	CS7078	●		Lake Grace	WA
CS7427	●		Spring Ridge 5	NSW	CS7080	●		Lake Grace	WA
CS7436	●		Spring Ridge 2	NSW	CS7081	●	●	Carnamagh	WA
CS7453	●		Spring Ridge 6	NSW	CS7082	●		Lake King	WA
CS7460	●		Werris Creek	NSW	CS7084	●		Lake King	WA
CS7461	●		Werris Creek	NSW	CS7085	●	●	Lake King	WA
CS7463	●		Kelvin	NSW	CS7088	●		Lake King	WA
CS7464	●		Kelvin	NSW	CS7089	●		Grasspatch	WA
CS7465	●		Caroona 4	NSW	CS7090	●		Grasspatch	WA
CS7467	●		Caroona 4	NSW	CS7091	●		Grasspatch	WA
					CS7093	●		Grasspatch	WA
CS3002				QLD	CS7094	●		Grasspatch	WA
CS3427	●		Wilga Downs	QLD	CS7098	●		Grasspatch	WA
CS3438	●		Wilga Downs	QLD	CS7099	●		Salmon Gums	WA
CS3442	●		Coondiwindi	QLD	CS7100	●		Salmon Gums	WA
CS3744	●		Kentare	QLD	CS7104	●		Salmon Gums	WA
CS3752	●		Kentare	QLD	CS7105	●		Lake Grace	WA
CS3910	●		Westfield	QLD	CS7108	●	●	Lake Grace	WA
CS7113	●		Bowenville	QLD					

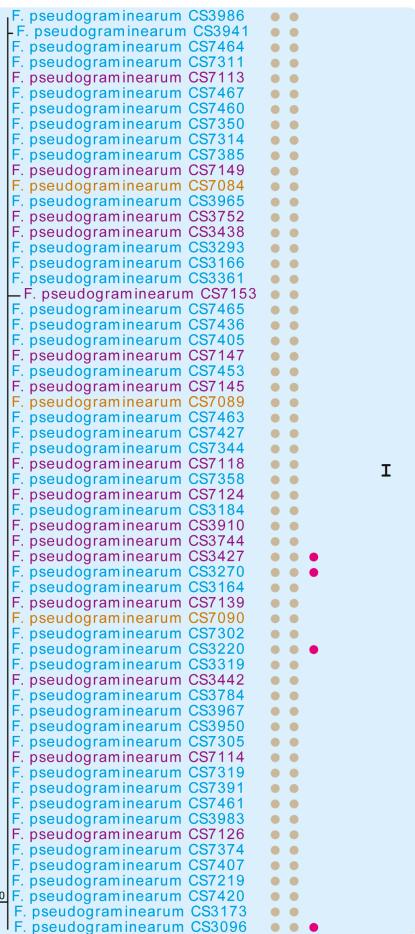


W493 B

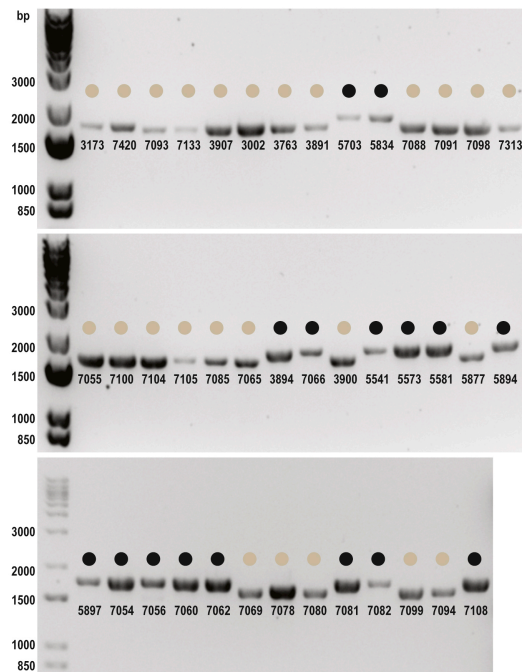


Fusaristatin A

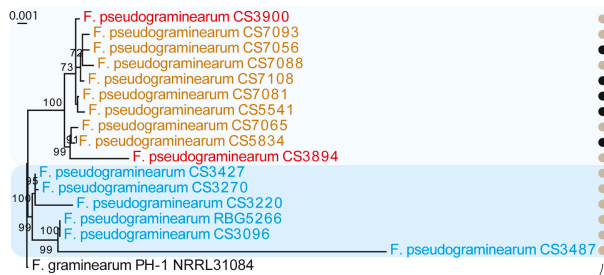
**A****B**



B



C



*F. pseudograminearum* CS3986  
*F. pseudograminearum* CS3941  
*F. pseudograminearum* CS7464  
*F. pseudograminearum* CS7311  
*F. pseudograminearum* CS7113  
*F. pseudograminearum* CS7467  
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*F. pseudograminearum* CS7350  
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*F. pseudograminearum* CS7149  
*F. pseudograminearum* CS7084  
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*F. pseudograminearum* CS7089  
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*F. pseudograminearum* CS3744  
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*F. pseudograminearum* CS7100  
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*F. pseudograminearum* CS7108  
*F. graminearum* NRRL31084  
*F. graminearum* CS3005  
*F. asiaticum* NRRL28720  
*F. meridionale* NRRL28721  
*F. culmorum* CS7071  
*F. praegramineum* NRRL39664  
*F. poae* strain 2516

Genome sequenced (● published, ● this study)

PKSG (● present, ● absent)

Fusaristatin A (● produced, ● not produced)

Fusaristatin gene cluster (● present, ● absent)

**Highlights**

Ancestral *Fusarium pseudograminearum* strains produced fusaristatin

Fusaristatin-producing *F. pseudograminearum* are confined to Western Australia

Remnant fragments of fusaristatin cluster was found in non-producers

ACCEPTED MANUSCRIPT