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Further Characterization of Dothistromin Genes in the Fungal Forest Pathogen *Dothistroma septosporum*

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Hong ping Jin

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ABSTRACT

Dothistroma septosporum is a forest pathogen that causes a disease called Dothistroma needle blight. The symptoms are thought to be due to the accumulation of dothistromin toxin produced by *D. septosporum*. Dothistromin is characterized as a difuranoanthraquinone and shows remarkable similarity to the aflatoxin (AF) and sterigmatocystin (ST) precursor versicolorin B. The similar structure to AF/ST suggests that dothistromin biosynthesis shares biosynthetic steps with the AF/ST pathway. The AF gene cluster in *Aspergillus parasiticus* and ST gene cluster in *A. nidulans* have been well characterized. Nine putative dothistromin biosynthetic genes have been identified. One of them, *dotA* was previously characterized by gene disruption and shown to have a similar function to homologous genes in AF/ST biosynthesis.

Two additional putative dothistromin biosynthetic genes, *pksA* and *epoA,* were characterized by gene disruption in this study. The inability of the *pksA* mutants to produce dothistromin indicated that the *pksA* is a key gene in dothistromin biosynthesis. The feeding of intermediates confirmed that *pksA* gene product is required for a very early step of dothistromin biosynthesis. The *pksA* mutants also showed reduced sporulation compared to wildtype, suggesting a relationship between dothistromin production and sporulation. The *epoA* gene replacements were also obtained successfully by homologous recombination. Both Southern blot and northern hybridization confirmed that the *epoA* gene was disrupted. However, the *epoA* mutants did not show any difference to the wild type in three analyses (growth rate, sporulation rate, dothistromin biosynthesis). However it was not possible to rule out a role for EpoA at a very late stage of dothistromin biosynthesis.

RACE analysis of the nine identified dothistromin genes characterized the transcription start and stop sites of the genes. Analyzing the putative regulatory protein binding motifs in the untranscribed region of the genes provided clues about the regulation of dothistromin biosynthesis and suggested there might be an *aflR*-like gene that governs dothistromin biosynthesis.

Both the *pksA* gene disruption and the RACE results suggested that the dothistromin biosynthetic pathway is homologous to that of AF/ST biosynthesis. Further work on the dothistromin gene cluster will help us to understand the evolution of fungal toxin gene clusters.

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Abbreviations

amp^r:	ampicillin resistance
bp:	base pair
cDNA:	complementary deoxyribonucleic acid
cm:	centimeter
°C:	degree celsius
CHEF:	contour-clamped homogeneous electric field
DNA:	deoxyribonucleic acid
dCTP:	deoxycytidine triphosphate
DEPC:	diethyl pyrocarbonate
DMSO:	dimethyl sulphoxide
DNase:	deoxyribonuclease
dNTP:	deoxynucleotide triphosphate
Fig:	figure
g :	gram
IPTG:	Isopropyl-β-d-thiogalactoside
kb:	kilobase pair
L:	litre
M:	mole per litre
ml:	milliliter
mM:	millimole per litre
OD_{600}	optical density at 600 nm
RNase:	ribonuclease
RNA:	ribonucleic acid
SDS:	sodium dodecyl sulfate
μl:	microlitre
μM:	micromole per litre
μg:	microgram
v/v:	volume per volume
w/v:	weight per volume
X-Gal:	5- bromo-4-chloro-3-indolyl-β-D-galactopyranoside

Table of contents

Page	Chapter content
1	**CHAPTER ONE: INTRODUCTION**
2	1.1 *DOTHISTROMA* NEEDLE BLIGHT AND *DOTHISTROMA PINI*
2	1.1.1 *Dothistroma* needle blight
2	1.1.2 *Dothistroma septosporum*
3	1.1.3 The infection process
3	1.1.4 Disease control
4	1.2 DOTHISTROMIN AND DOTHISTROMIN BIOSYNTHESIS GENE CLUSTER
4	1.2.1 Dothistromin
5	1.2.2 Aflatoxin and aflatoxin biosysthesis
5	1.2.3 Aflatoxin gene cluster
6	1.2.4 Dothistromin gene cluster
11	1.2.5 Polyketide biosynthesis
12	1.2.6 The putative *epoA* gene
13	1.3 GENE DISRUPTION
14	1.3.1 Gene disruption construct
14	1.3.2 Fungal transformation methods
16	1.3.3 Identification and purification of mutants
17	1.3.4 Characterization of the mutants
18	1.4 AIMS AND OBJECTIVES
19	**CHAPTER TWO: MATERIALS AND METHODS**
20	2.1 FUNGAL AND BACTERIAL STRAINS, LAMBDA CLONES AND PLASMIDS.
20	2.2 GROWTH AND MAINTENANCE OF CULTURES
20	2.2.1 Growth and maintenance of *E. coli* cultures
20	2.2.2 Growth and maintenance of *D. septosporum* cultures
20	2.2.2.1 Single spore purification
20	2.2.2.2 Growth of *D. septosporum* from transformed protoplasts
23	2.2.2.3 General growth and maintenance of *D. septosporum* cultures
23	2.3 DNA ISOLATION, PURIFICATION AND QUANTIFICATION
23	2.3.1 Small scale genomic DNA isolation from *D. septosporum* cultures
23	2.3.2 Large scale DNA isolation from *D. septosporum* cultures
24	2.3.3 Isolation of plasmid DNA from *E. coli*
24	2.3.4 Agarose gel purification of DNA fragments
24	2.3.5 Purification of PCR products from a PCR reaction
25	2.3.6 Purification of DNA by phenol/chloroform extraction
25	2.3.7 Agarose gel electrophoresis of DNA
25	2.3.8 Determination of DNA concentration by fluorometric assay
25	2.3.9 Determination of DNA concentration by gel electrophoresis
26	2.3.10 Determination of DNA molecular weights
26	2.4 DNA ISOLATION, LIGATION AND CLONING

26 2.4.1 Restriction endonuclease digestion of DNA
26 2.4.2 Standard ligation reactions
27 2.4.3 Gateway recombination reactions
27 2.4.3.1 BP recombination
27 2.4.3.2 LR recombiantion
28 2.5 TRANSFORMATION PROTOCOL
28 2.5.1 Transformation of *E. coli* competent cells
28 2.5.1.1 Preparation of competent cells and transformation by electroporation
29 2.5.1.2 Preparation and transformation of $CaCl_2$ competent cells
29 2.5.2 Transformation of *D. septosporum*
29 2.5.2.1 Preparation of competent *D. septosporum* protoplasts
30 2.5.2.2 Transformation of *D. septosporum* protoplasts
31 2.6 AMPLIFICATION OF DNA BY THE POLYMERASE CHAIN REACTION (PCR)
31 2.6.1 Oligonucleotide primers
31 2.6.2 Reagents and cycling conditions for basic PCR
31 2.6.3 *E. coli* colony PCR
34 2.7 DNA SEQUENCING
34 2.8 SOUTHERN BLOTTING AND HYBRIDISATION
34 2.8.1 Southern blotting (Capillary)
34 2.8.2 DIG labeling of DNA probe
34 2.8.3 Southern blot hybridization of DIG labeled probe
35 2.8.4 Immunological detection
35 2.8.5 Stripping
36 2.9 RACE (Rapid Amplification of cDNA Ends)
37 2.10 RNA MANIPULATION PROCEDURES
37 2.10.1 Isolation of total RNA
37 2.10.2 DNase treatment of RNA
37 2.10.3 Quantification of RNA
38 2.10.4 Reverse transcription and RT-PCR
38 2.10.4.1 Reverse transcription
38 2.10.4.2 RT-PCR
38 2.10.5 Agarose gel electrophoresis of RNA
38 2.10.5.1 SDS/Agarose gel electrophoresis of RNA
39 2.10.5.2 Formaldehyde gel electrophoresis of RNA
39 2.11 NORTHERN BLOTTING AND HYBRIDIZATION
39 2.11.1 Northern blotting
39 2.11.2 Probe labeling
40 2.11.3 Hybridization
40 2.12 ISOLATION OF SECONDARY METABOLITES FROM MYCELIUM OF *D. SEPTOSPORUM*
41 2.13 DETECTION OF AFLATOXIN INTERMEDIATES
41 2.14 INTERMEDIATE FEEDING STUDY
42 2.15 QUANTIFICATION OF DOTHISTROMIN USING COMPETITIVE ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)
43 2.16 GROWTH RATE ANALYSIS
43 2.17 EXAMINATION OF CONIDIA FORMATION

45 **RESULTS & DISCUSSION**

46 **CHAPTER THREE: *PKSA* GENE DISRUPTION**

47 3.1 CHARACTERIZATION OF THE PUTATIVE DOTHISTROMIN GENE, *PKSA*
47 3.2 TRANSFORMATION OF *D. SEPTOSPORUM*
49 3.3 IDENTIFICATION OF THE *PKSA* GENE DISRUPTION MUTANTS
49 3.3.1 PCR to screen the transformants
51 3.3.2 Purification of the *pksA* mutants
53 3.3.3 Southern hybridization to confirm the putative *pksA* mutants
58 3.4 CHARACTERIZATION OF THE *PKSA* MUTANTS
58 3.4.1 Analysis of dothistromin production of the *pksA* mutants (TLC and ELISA)
61 3.4.2 Intermediate feeding of the *pksA* mutants
65 3.4.3 Growth rate of the *pksA* mutants
66 3.4.4 Sporulation rate of the *pksA* mutants
67 3.5 DISCUSSION
67 3.5.1 Transformation
69 3.5.2 Identification of the mutants
71 3.5.3 Characterization of the *pksA* mutants
72 3.5.4 The relationship of the secondary metabolites and sporulation
73 3.5.5 The dothistromin gene cluster

75 **CHAPTER FOUR: *EPOA* GENE DISRUPTION**

76 4.1 TARGETED REPLACEMENT AND CHARACTERIZATION OF A PUTATIVE DOTHISTROMIN GENE, *EPOA*
77 4.2 CONSTRUCT *EPOA* GENE DISRUPTION VECTOR
78 4.2.1 Step 1 PCR to add *attB* sites
78 4.2.2 Step 2 BP (attB: attP) recombination to generate entry clones
79 4.2.3 Step 3 LR recombination to create final disruption vector pR246
81 4.2.4 Factors affecting transformation efficiency
82 4.3 TARGETED DELETION OF THE PUTATIVE GENE IN *D. SEPTOSPORUM*
83 4.3.1 Pre-screening *epoA* deletion mutants by PCR
85 4.3.2 Southern blot hybridization analysis
87 4.4 CHARACTERIZATION OF THE *EPOA* MUTANTS
87 4.4.1 TLC analysis of the *epoA* mutant
88 4.4.2 ELISA analysis of the *epoA* mutants
89 4.4.3 Growth rate of the *epoA* mutants
90 4.4.4 Sporulation rate of the *epoA* mutants
91 4.4.5 Northern hybridization
92 4.5 DISCUSSION
92 4.5.1 Construction of the *epoA* disruption vector
93 4.5.2 Transformation of *D. septosporum* protoplasts
94 4.5.3 Identification of the *epoA* mutants
95 4.5.4 Characterization of the *epoA* mutants
96 4.5.5 The possible functions of *epoA*

99 CHAPTER FIVE: RACE RESULTS
100 5.1 RACE ANALYSIS
100 5.2 RNA PURIFICATION AFTER EACH REACTION OF RACE PROCEDURE
101 5.3 RACE OF *DOTA, DOTB, DOTC* AND *DOTD* GENES
106 5.4 RACE AND cDNA OF THE FIVE NEWLY IDENTIFIED DOTHISTROMIN GENES
108 5.4.1 The 3' RACE of the newly identified dothistromin genes
112 5.4.2 The 5' RACE of the newly identified dothistromin genes
116 5.5 THE REGULATORY MOTIFS OF THE IDENTIFIED DOTHISTROMIN GENES
117 5.6 DISCUSSION
117 5.6.1 RNA purification with PCR column
118 5.6.2 RACE PCR
118 5.6.3 RACE of the dothistromin genes
119 5.6.4 The regulatory motifs of the identified dothistromin genes

121 CHAPTER SIX: CONCLUSIONS AND FUTURE WORK
122 6.1 CONCLUSIONS AND FUTURE WORK

126-131 REFERENCE

132 Appendix I MEDIA
133 Appendix II COMMON BUFFERS AND SOLUTIONS
134-137 Appendix III PARTIAL SEQUENCE OF THE EPOA GENE DISRUPTION VECTOR
138-154 Appendix IV* RACE SEQUENCES
155-161 Appendix V* THE PUTATIVE REGULATORY BINDING MOTIFS OF THE IDENTIFIED DOTHISTROMIN GENES

*The coloured version of Appendices IV and V are shown on CD.
GeneRacer^{TM} kit and Multi-site gateway Three Fragment vector construction kit manuals are shown on CD as Appendices VI and VII.

List of Figures

Page	Figure	
4	Fig 1.1	Chemical structures of aflatoxin B_1, versicolorin A, versicolorin B and dothistromin
8	Fig 1.2	Comparison between dothistromin, aflatoxin and sterigmatocystin gene cluster
11	Fig 1.3	The comparison of PKS proteins from *A. parasiticus, A. nidulans* to the predicted PksA from *D. septosporum*
15	Fig 1.4	Schematic diagram for Gateway system
50	Fig 3.1	PCR screening the *pksA* gene disruptants (a,b and c)
52	Fig 3.2	PCR results of the purified strains (a,b and c)
54	Fig 3.3	Southern hybridization results of *pksA* mutants
56	Fig 3.4	Southern hybridization of *KpnI* digestion
57	Fig 3.5	*KpnI* digestion to confirm P1-8 mutant
59	Fig 3.6	No dothistromin produced by the *pksA* mutant
60	Fig 3.7	Competitive ELISA results
61	Fig 3.8	AF biosynthesis
62	Fig 3.9	Intermediate feeding ELISA results
63	Fig 3.10	Intermediate feeding TLC results
64	Fig 3.11	ELISA results of the second time intermediate feeding
70	Fig 3.12	Possible integration of P1-8
74	Fig 3.13	The putative dothistromin biosynthetic steps
77	Fig 4.1	Experimental procedure for constructing the disruption vector
80	Fig 4.2	LR recombination
84	Fig 4.3	Preliminary screening of *epoA* transformants
86	Fig 4.4	Southern hybridization to confirm the identified *epoA* mutants
87	Fig 4.5	TLC results of the *epoA* mutant
88	Fig 4.6	The comparison of dothistromin production between *epoA* mutants and WT
89	Fig 4.7	Northern hybridization results
97	Fig 4.8	The reaction of the metabolism of aromatic hydrocarbons in mammal
98	Fig 4.9	The proposed dothistromin biosynthetic steps
100	Fig 5.1	The identified dothistromin genes
101	Fig 5.2	RNA on a formaldehyde gel
102	Fig 5.3	RACE of *dotA, dotB, dotC* and *dotD* genes
102	Fig 5.4	Nested RACE of *dotA, dotB, dotC* and *dotD* genes
104	Fig 5.5	The 5'RACE of *dotA*
105	Fig 5.6	The 3'RACE of *dotA*
108	Fig 5.7	The 3'nested RACE PCR products of the new identified dothistromin genes
109	Fig 5.8	The 3'RACE of *pksA*
112	Fig 5.9	Nested PCR of 5'RACE of *epoA, moxA, avfA, cypA* and *pksA*
114	Fig 5.10	The 5'RACE of *pksA*

List of Tables

Page	Table	
10	Table 1.1	Putative dothistromin genes and their homologs in AF/ST gene cluster
21	Table 2.1	Plasmids, fungal and bacterial strains
32	Table 2.2	Sequencing and PCR primers
48	Table 3.1	Transformation results of pR226
65	Table 3.2	Radial growth and unpaired T-test of *pksA* mutants
65	Table 3.3	Growth rate of *pksA* mutants
67	Table 3.4	Sporulation of *pksA* mutants
79	Table 4.1	The 5' and 3' elements constructs
81	Table 4.2	Comparison of the transformation results (transformants/ng vector DNA)
82	Table 4.3	Transformation results of pR226
89	Table 4.4	Unpaired T-test of dothistromin production of the *epoA* mutants
90	Table 4.5	Radial Growth of *epoA* mutants
91	Table 4.6	Sporulation of *epoA* mutants
107	Table 5.1	RACE results of *dotA, dotB, dotC* and *dotD* genes
115	Table 5.2	RACE results of *epoA, moxA, avfA, cypA* and *pksA* genes
117	Table 5.3	The putative regulatory motif of the dothistromin genes