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A Polyketide Synthase Is Required for Fungal Virulence and Production of the Polyketide T-Toxin

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Race T of the fungal pathogen *Cochliobolus heterostrophus* is highly virulent toward Texas male sterile (T) maize and differs from its relative, race O, at a locus (*Tox1*) that is responsible for the production of T-toxin, a family of linear long-chain (C_{35} to C_{41}) polyketides. In a previous study, the restriction enzyme–mediated integration procedure was used to mutagenize and tag *Tox1*. Here, we report that the DNA recovered from the insertion site of one mutant encodes a 7.6-kb open reading frame (2530 amino acids) that identifies a multifunctional polyketide synthase (PKS)–encoding gene (*PKS1*) with six catalytic domains arranged in the following order, starting at the N terminus: β-ketoacyl synthase, acyltransferase, dehydratase, enoyl reductase, β-ketoacyl reductase, and acyl carrier protein. *PKS1* is interrupted by four apparent introns (74, 57, 49, and 41 bp) and exists in the genome as a single copy surrounded by highly repetitive, A+T-rich DNA. When *PKS1* in race T was inactivated by targeted gene disruption, T-toxin production and high virulence were eliminated, indicating that this PKS is required for fungal virulence. Race O strains, which do not produce T-toxin, lack a detectable homolog of *PKS1*, suggesting that race T may have acquired *PKS1* by horizontal transfer of DNA rather than by vertical inheritance from an ancestral strain.

INTRODUCTION

Cochliobolus heterostrophus, a heterothallic ascomycete, was first described in 1925 as a pathogen of maize (Drechsler, 1925). This biotype of the fungus was designated race O in 1970 to distinguish it from a new form, race T, characterized by extreme virulence to maize carrying Texas male sterile (T) cytoplasm. Race T first appeared in the United States in 1969 (Ullstrup, 1970). Race T (but not race O) produces T-toxin (Figure 1), a family of linear long-chain (C_{35} to C_{41}) polyketide molecules (Kono and Daly, 1979; Kono et al., 1981) that specifically bind to a protein (URF13) unique to the inner mitochondrial membrane of T-cytoplasm maize (Rhoads et al., 1995). URF13 is encoded by a mosaic gene (*T-urf13*) carried only on the T-cytoplasm mitochondrial chromosome (Levings et al., 1995). Binding of T-toxin to URF13 causes the formation of pores in the inner membrane (Siedow et al., 1995) and leakage of NAD^+ along with other solutes necessary for normal mitochondrial function (Matthews et al., 1979). Thus, specific interaction between a unique protein in the plant and a unique polyketide from the fungus leads to susceptibility. Plant resistance is due to the absence of URF13 (which results in T-toxin–insensitive mitochondria) rather than to an active defense mechanism. No plant other than T-cytoplasm maize contains URF13, and only *C. heterostrophus* is known to produce T-toxin, although a distantly related fungal maize pathogen (*Mycosphaerella zeae-maydis*) makes a polyketide (PM-toxin) that is structurally and functionally similar to T-toxin (Yoder et al., 1997).

The production of T-toxin by *C. heterostrophus* is controlled by a genetically defined locus, *Tox1*, that is inseparably linked to the break points of a pair of reciprocally translocated chromosomes (Tzeng et al., 1992). Analyses of pairs of strains (near isogenic except for heterozygosity at *Tox1*) have shown that the essential difference between race T and race O is determined by *Tox1*. Therefore, an understanding of the molecular nature of *Tox1* should help to unravel the genetic event(s) that gave rise to race T. Cloning and manipulation of the gene(s) encoded at *Tox1* will allow a rigorous evaluation of the role of T-toxin in pathogenesis by *C. heterostrophus*. A simple hypothesis (Yoder et al., 1993) is that *Tox1* encodes at least one polyketide synthase (PKS), because T-toxin is a polyketide and polyketides generally depend on PKS activity for their biosyntheses (Hopwood and Sherman, 1990). The goal of our study was to test this hypothesis. Tagged mutations causing a T-toxin–deficient phenotype were introduced at *Tox1* by the restriction enzyme–mediated integration (REMI) procedure (Lu et al., 1994). We report here the recovery of DNA flanking the tag in one of the mutants and show that it encodes a PKS that is indeed necessary for both T-toxin production and high virulence.

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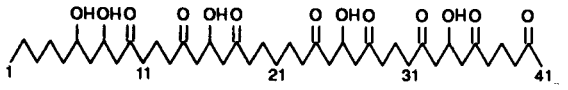

Figure 1. Structure of T-Toxin.

The molecule shown (C_{41}) represents the most abundant class in a family of 10 to 15 homologs. Each is linear, has an odd number of carbons, and lacks a terminal carboxyl group.

RESULTS

Identification of *PKS1* at the Tagged Site

Analysis of the combined sequences of pF5P1 and pF6P1 (see Methods and Figure 2) revealed a 7.6-kb open reading frame (ORF; after splicing of four putative introns), 1323 bp of 5′ flanking DNA, and 692 bp of 3′ flanking DNA. A BLAST search of NCBI nonredundant protein data bases with the putative translation product (2530 amino acids; Figure 3) revealed highest similarity to PKSs from the prokaryotes *Mycobacterium leprae*, *Saccharopolyspora erythraea*, *M. tuberculosis*, and *Streptomyces hygroscopicus* and to fatty acid synthase from rat; lower similarity to fungal PKSs was noted.

Six conserved motifs (Figures 3 and 4) representing known type I PKS enzymatic domains were identified by their "signature sequences" (Figure 4); alignments centered on known PKS catalytic sites are shown in Figure 4. Recognizable motifs included one each of β-ketoacyl synthase (KS), acyltransferase (AT), dehydratase (DH), enoyl reductase (ER), β-ketoacyl reductase (KR), and acyl carrier protein (ACP), with the order being (N terminus–KS-AT-DH-ER-KR-ACP–C terminus). Although a thioesterase (TE) domain was not found, the presence of the other six motifs suggested that the 7.6-kb ORF, designated *PKS1*, could encode a multifunctional PKS. Confirmation of PKS function was obtained by targeted gene disruption (see below), which led to the loss of T-toxin, a known polyketide (Figure 1). Sequence analysis also revealed that the REMI plasmid pUCATPH inserted into the ACP domain of *PKS1* (Figure 2A).

Four putative introns (41 to 74 bp) were suggested by their conserved 5′ and 3′ border sequences and branch sites; three (74, 57, and 41 bp) are in the region of the KS domain, and one (49 bp) is near the AT domain (Figure 3). Splicing of these introns eliminates stop codons that would otherwise interrupt the ORF.

The G+C content of *PKS1* is ~50%, which is lower than that of most *Cochliobolus* genes (54 to 62%) and of PKS-encoding bacterial genes (62 to 74%). Codon usage was evaluated by comparing, with the aid of chi-square contingency tables ($P = 0.01$), the tendency of *PKS1* and other genes to use a C residue in the third position; filamentous fungi (e.g., *Neurospora*) tend to have a C residue in the third position more frequently than any other nucleotide (Edelmann and Staben, 1994). The analysis was limited to the eight amino acids that have the option of all four bases in the third position, that is, Ala, Arg, Gly, Leu, Pro, Ser, Thr, and Val. *PKS1* was compared with the ORFs (without introns) of sequenced genes from *Cochliobolus* spp (gene symbols and accession numbers are as follows: *HTS1*, L48797; *TRP1*, X70035; *MAT-2*, X68398; *MAT-1*, X68399; *GPD1*, X63516; *EXG1*, L48994; *ALP1*, U39500; *PGX1*, L48982; *PGN1*, M55979; *XYL3*, U58916; *CEL1*, U25129) and with codon usage tables compiled from several other fungi (*Neurospora crassa*, *Aspergillus niger*, *A. parasiticus*, *Coprinus cinereus*, *Fusarium oxysporum*, *Magnaporthe grisea*, and *Penicillium chrysogenum*). In addition, the tendency to use a C residue in the third position was evaluated for the subset of bacterial PKS-encoding genes most similar to *PKS1*, for example, those in Figure 4. *PKS1* had no preference for a C residue in the third position. *PKS1* also differs in its use of a C residue in the third position, from compiled codon usage tables and from all *Cochliobolus* genes tested, except for *C. heterostrophus TRP1* (involved in tryptophan biosynthesis), *MAT-1* and *MAT-2* (which control mating), and *C. carbonum HTS1* (encoding a cyclic peptide synthetase). The frequency of a C residue in the third position was different from that of all PKS-encoding bacterial genes, except for *Mycobacterium leprae pksE*, which encodes part of the polyketide synthase that has the greatest similarity to PKS1 of any protein in the data base.

Inactivation of *PKS1* by Targeted Gene Disruption

An EcoRV digest of plasmid pF5P1 (Figure 2C) was transformed into wild-type *Tox1*$^+$ strain C4. Gel blot analysis of DNA from 14 hygromycin B–resistant transformants, probed with the 1.6-kb BamHI-HindIII fragment of pF5P1 (Figure 2C), showed that in 11 cases, the 5.1-kb EcoRV fragment (including vector sequences) integrated by homologous recombination with genomic DNA at *PKS1*; two examples are shown in Figure 5A. All 11 transformants with homologous integrations were T-toxin negative in the microbial bioassay (examples are shown in Figure 5B) and had reduced virulence typical of race O on T-cytoplasm maize (Figure 5C). DNA from the three remaining transformants had uninterpretable DNA gel blot hybridization patterns. Each retained the 4.6-kb native band (Figure 5A, lane 1), was Tox$^+$ in the microbial assay, and caused symptoms on T-cytoplasm maize that were indistinguishable from those of the *Tox1*$^+$ control strain C4 (data not shown).

PKS1 Is Absent from the Genome of Race O

The gel blot of genomic DNAs from race T strain C4 and race O strain C5, probed with the 1.6-kb BamHI-HindIII fragment (Figure 5A, lanes 1 and 2) or with the 7-kb EcoRI-HindIII fragment of pF5P1 containing all or part of each enzymatic domain (Figure 2C; data not shown), revealed that *PKS1* is present as a single copy in race T but is not detectable in the genome of race O.

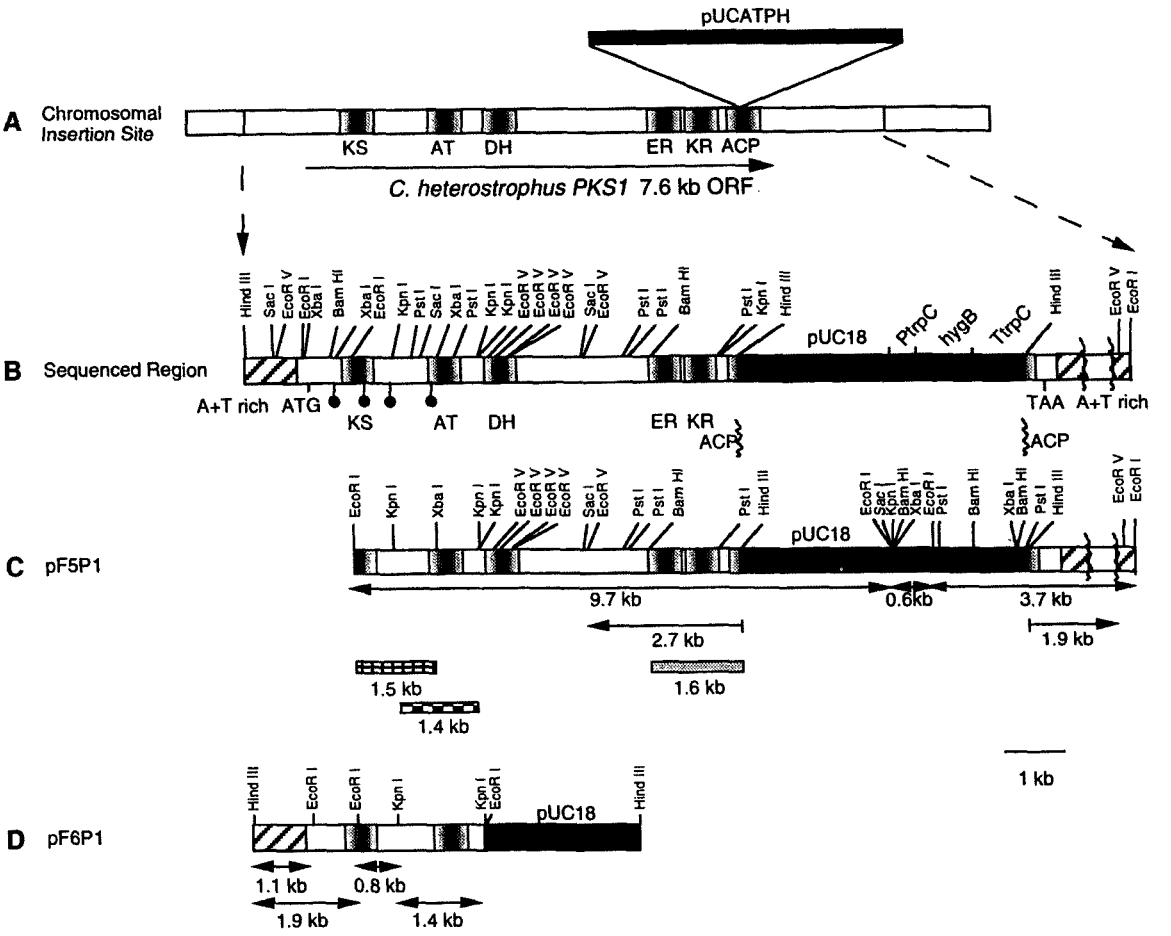

Figure 2. Transformation Vector pUCATPH Inserted at the *Tox1* Locus in REMI Transformant R.C4.350L.

Cloning and sequencing of DNA flanking the insertion site revealed a 7.6-kb ORF. When translated, it included six PKS enzymatic motifs (KS, AT, DH, ER, KR, and ACP). Each is represented by a shaded box centered on the motif active site (borders of enzymatic domains are not defined). Note that the vector disrupted the ACP domain.

(A) Open bar, chromosomal region of vector insertion; solid bar, vector; shaded boxes, approximate locations of PKS enzymatic domains. The solid arrow shows the limits of the ORF and the direction of transcription. Arrows with broken lines indicate expanded view.

(B) Map of the sequenced region showing sites of all six nucleotide recognition sites for restriction enzymes referred to in the text. Solid circles indicate the approximate locations of four introns. DNA on both flanks of the ORF, designated by slashed boxes, is A+T rich; the break in the 3′ box indicates a region of ~900 bp that has not yet been sequenced. PtrpC and TtrpC represent the *A. nidulans trpC* promoter and terminator, respectively; hygB is the *Escherichia coli* gene for hygromycin B resistance.

(C) Plasmid pF5P1 shown linearized at one of the three EcoRI sites. Lengths of vector-disrupted EcoRV and three EcoRI fragments are indicated (by arrows below the bar) to aid in the interpretation of Table 1 and Figure 5A. The textured bars below the plasmid represent fragments described in the text. The 1.5-kb EcoRI-XbaI fragment (open boxes) was used for screening cosmid libraries. The 1.4-kb KpnI fragment (checkerboard box) was used for targeted integration into the wild-type genome to permit the cloning of sequences 5′ of pF5P1. The 1.6-kb BamHI-HindIII fragment (stippled box) was used as a probe in Figure 5A.

(D) Plasmid pF6P1, containing 1.9 kb of sequence 5′ of pF5P1 (indicated as a HindIII-EcoRI region), 2.2 kb of the 5′ end of pF5P1 (which includes the 0.8-kb EcoRI-KpnI and 1.4-kb KpnI fragments), and pUC18, linearized at the HindIII site. The 1.1-kb HindIII-EcoRI fragment is also shown. Limits of all restriction fragments are indicated by two-headed arrows.

DISCUSSION

Role of *PKS1* in Fungal Virulence

We reported previously the efficacy of the REMI procedure to mutate and tag genes of interest in *C. heterostrophus* (Lu et al., 1994). Here, we describe the recovery of DNA flanking a REMI insertion point at the *Tox1* locus. Sequencing of this DNA has confirmed our hypothesis that the *Tox1* locus carries a gene, designated *PKS1*, encoding a PKS. The identification is based on the observation that disruption of *PKS1* causes loss of ability to produce the polyketide T-toxin. The reduced

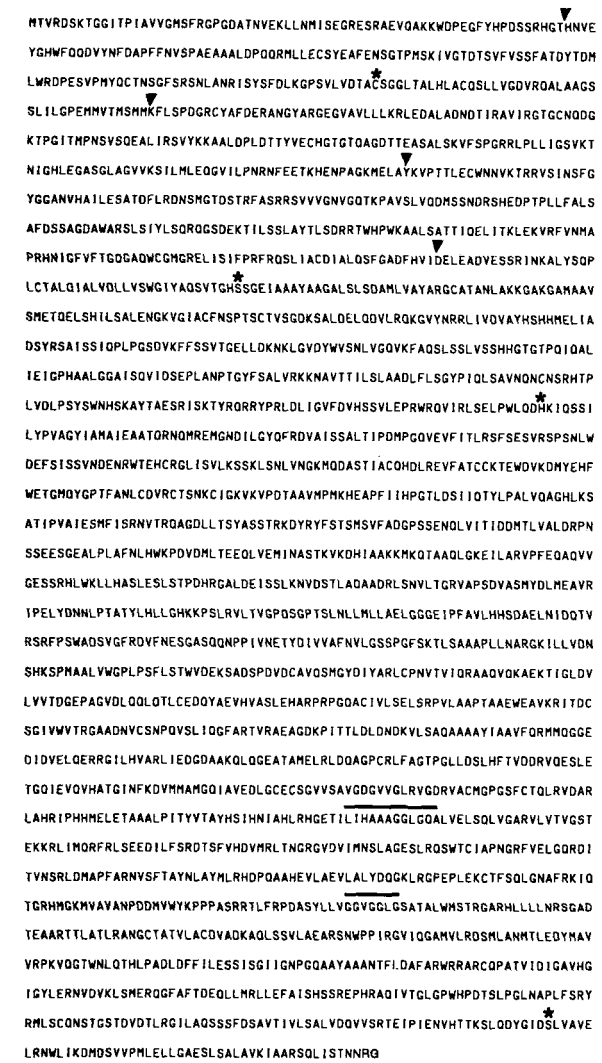

Figure 3. Amino Acid Sequence of *PKS1*.

Arrowheads indicate the positions of introns, and the asterisks designate the active sites (signature sequences, 5′ to 3′, are in parentheses) of the KS acyl binding cysteine (DXACSSSL), the AT pantotheine binding serine (PXXXXGHSXG), the DH active histidine (HXXXXXXXXP), and the ACP phosphopantetheine binding serine (GXDSL) domains; overlines show the NADPH binding sites in the ER (LIHXXXGGXGXA) and KR (GGXGXLG) domains. The nucleotide sequence of *PKS1* has GenBank accession number U68040.

virulence to T-cytoplasm maize that accompanies *PKS1* disruption indicates that T-toxin is an essential factor in pathogenesis of *C. heterostrophus* race T.

Features of PKS1 and Other PKSs

Polyketide synthesis resembles that of fatty acids catalyzed by fatty acid synthases (FASs). For the latter, CoA thioesters of acetate or malonate are condensed via stepwise decarboxylation reactions. Each step adds two carbons to the growing chain. After each condensation, the distal β-keto group is processed by a reduction, a dehydration, and another reduction to yield a hydroxyl, an enoyl, and finally an alkyl function. This cycle is repeated until the nascent fatty acid reaches full length, when it is released from the FAS. Linear fatty acids are commonly 16 or 18 carbons long and are fully reduced except for a terminal carboxyl group.

PKSs perform similarly except that their products (polyketides) are more complex because of (1) use of extender and starter units other than acetate or malonate; (2) nonuniform processing of the β-keto groups that results in the possibility of keto, hydroxyl, and/or enoyl functions in the chain, in addition to alkyl groups; (3) chiral centers in the chain caused by incomplete processing of β-keto groups or incorporation of branched chain extender units; and (4) condensations within or between polyketide chains that create cyclic compounds. Additional enzymatic reactions, such as reductions, hydroxylations, epoxidations, and glycosidations, may be required to produce a mature polyketide (Hopwood and Sherman, 1990; Hopwood and Khosla, 1992). The structure of T-toxin (Figure 1) provides no evidence that the latter activities are involved in its biosynthesis. PKS1 itself could be sufficient for assembly of the toxin molecule because it contains all of the domains required for condensation (KS, AT, and ACP) and complete processing of the β-carbon (KR, DH, and ER).

Both PKSs and FASs typically have an ACP domain serving as an attachment point for the growing carbon chain and five catalytic activities: an AT to initiate synthesis by transferring acetate from acetyl-CoA to the pantotheine arm of the ACP, a KS to condense by decarboxylation an extender unit (e.g., malonate) and the enzyme-bound acetate, a KR to reduce the β-keto group to a hydroxyl, a DH to produce an enoyl function by removing water, and an ER to reduce the enoyl to an alkyl function (Wakil, 1989; Hopwood and Sherman, 1990; Hopwood and Khosla, 1992; Katz and Donadio, 1993). PKSs do not always use KR, DH, and ER domains (and may not even have them), thus leaving any given β-keto group unchanged or reduced to a hydroxyl, enoyl, or alkyl function. After processing, the nascent chain is transferred to KS, allowing AT to charge ACP with the next extender unit, at which point the cycle is repeated. When the chain reaches full length, it is released from ACP by the action of a TE or a palmityltransferase (PT) or by an unknown mechanism. Because *C. heterostrophus PKS1* encodes neither a TE nor a PT domain, the carbon chain release must occur either by an unknown mechanism or by the action of a TE or PT domain encoded elsewhere in the genome.

PKSs and FASs are classified as either of two types (McCarthy and Hardie, 1984; Hopwood and Sherman, 1990; Hopwood and Khosla, 1992). Type I enzymes (vertebrate FASs and bacterial and fungal PKSs) are large multifunctional polypeptides, each encoding all of the necessary enzymatic domains for one or more cycles of condensation and β-keto processing. Type II enzymes (plant and bacterial FASs and

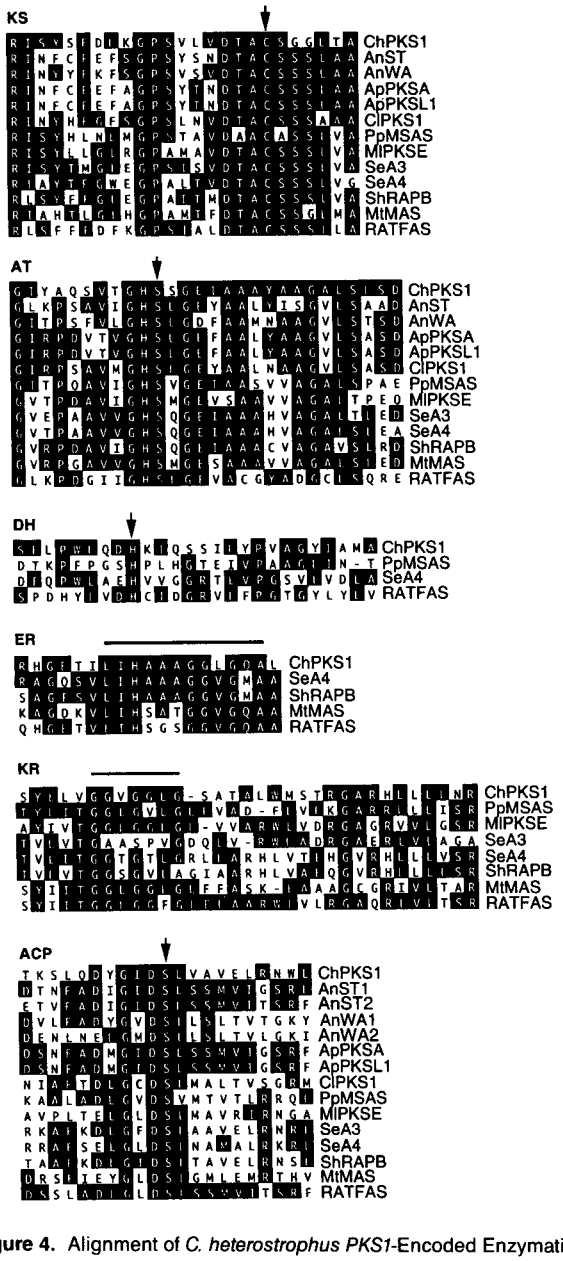

Figure 4. Alignment of *C. heterostrophus PKS1*-Encoded Enzymatic Domains with Those of PKSs (and Rat FAS) from Other Organisms.

Areas boxed in black indicate amino acid identities; arrows and overlines identify conserved amino acids at catalytic sites (see Figure 3). Abbreviations for proteins (in alphabetical order), accession numbers for corresponding genes, and references are as follows: AnST, *A. nidulans pksST* for sterigmatocystin biosynthesis, L39121 (Yu and Leonard, 1995); AnWA, *A. nidulans wA* for biosynthesis of a conidial pigment, X65866 (Mayorga and Timberlake, 1992); ApPKSA, *A. parasiticus pksA* for biosynthesis of aflatoxin, Z47198 (Chang et al., 1995); ApPKSL1, *A. parasiticus pksL1* for biosynthesis of aflatoxin, L42765 (Feng and Leonard, 1995); ChPKS1, *C. heterostrophus PKS1* for biosynthesis of T-toxin, U68040 (this report); ClPKS1, *Colletotrichum lagenarium PKS1* for biosynthesis of melanin, D83643 (Takano et al., 1995); MlPKSE, *M. leprae pksE* for a polyketide synthase, U00023 (GenBank entry); MtMAS, *M. tuberculosis mas* for biosynthesis of mycocerosic acid, M95808 (Mathur and Kolattukudy, 1992); PpMSAS, *P. patulum MSAS1* for biosynthesis of patulin, X55776 (Beck et al., 1990); RATFAS, *Rattus norvegicus* FAS for fatty acid biosynthesis, M84761 (Amy et al., 1989); SeA3 and SeA4, *Saccharopolyspora erythraea eryA* modules for biosynthesis of erythromycin, M63677 (Donadio et al., 1991); ShRAPB, *Streptomyces hygroscopicus rapB* for biosynthesis of rapamycin, X86780 (Schwecke et al., 1995).

PKSs) consist of several single function (or bifunctional) polypeptides in a loosely associated complex. The structure of *C. heterostrophus PKS1*, containing six enzymatic domains, indicates that it encodes a type I PKS.

Type 1 PKSs are divided into two subtypes. The first subtype includes those translated from a single transcript encoding a single set of condensation (KS, AT, and ACP) and β-keto processing (DH, ER, and KR) domains used iteratively to perform all of the cycles of chain extension; 6-methylsalicylic acid synthase (MSAS) from *P. patulum* (Hopwood and Khosla, 1992) is an example. The second subtype includes those translated from one or more transcripts, each encoding multiple sets of condensation and β-keto processing domains; the PKS encoded by *eryA* of *Saccharopolyspora erythraea* (Donadio et al., 1991; Donadio and Katz, 1992) is an example. In the latter subtype, each set of chain extension domains (typically organized as N terminus–KS-AT-DH-ER-KR-ACP–C terminus) is called a synthase unit, and each synthase unit is encoded by a module, resulting in the designation "modular PKS." Genes encoding modular PKSs are generally clustered. Each synthase unit appears to catalyze one cycle of chain extension at a defined point in the biosynthetic process and does not necessarily contain all or any of the β-keto processing domains. Thus, a modular PKS is "programmed" to produce a polyketide of defined length with a predetermined pattern of functional groups at alternate carbons, that is, the more modules, the more synthase units and the longer the polyketide chain. For example, the *eryA* locus of *S. erythraea* (which makes erythromycin) is 35 kb long, contains six modules, and produces a C_{13} polyketide (Donadio et al., 1991; Donadio and Katz, 1992), whereas the *avr* locus of *Streptomyces avermitilis* (which makes avermectin) is 65 kb long, contains 12 modules, and produces a C_{25} polyketide (MacNeil et al., 1992).

Mode of PKS1 Action

Whether the PKS encoded by *C. heterostrophus PKS1* is iterative or modular is unclear. The T-toxin molecule (Figure 1) includes three of the four functional groups produced by a PKS, that is, keto, hydroxyl, and alkyl (there are no enoyl functions). This structure indicates that at least six enzymatic activities are required for its assembly: KS, AT, and ACP for chain extension and KR, DH, and ER for β-keto group processing. *PKS1*

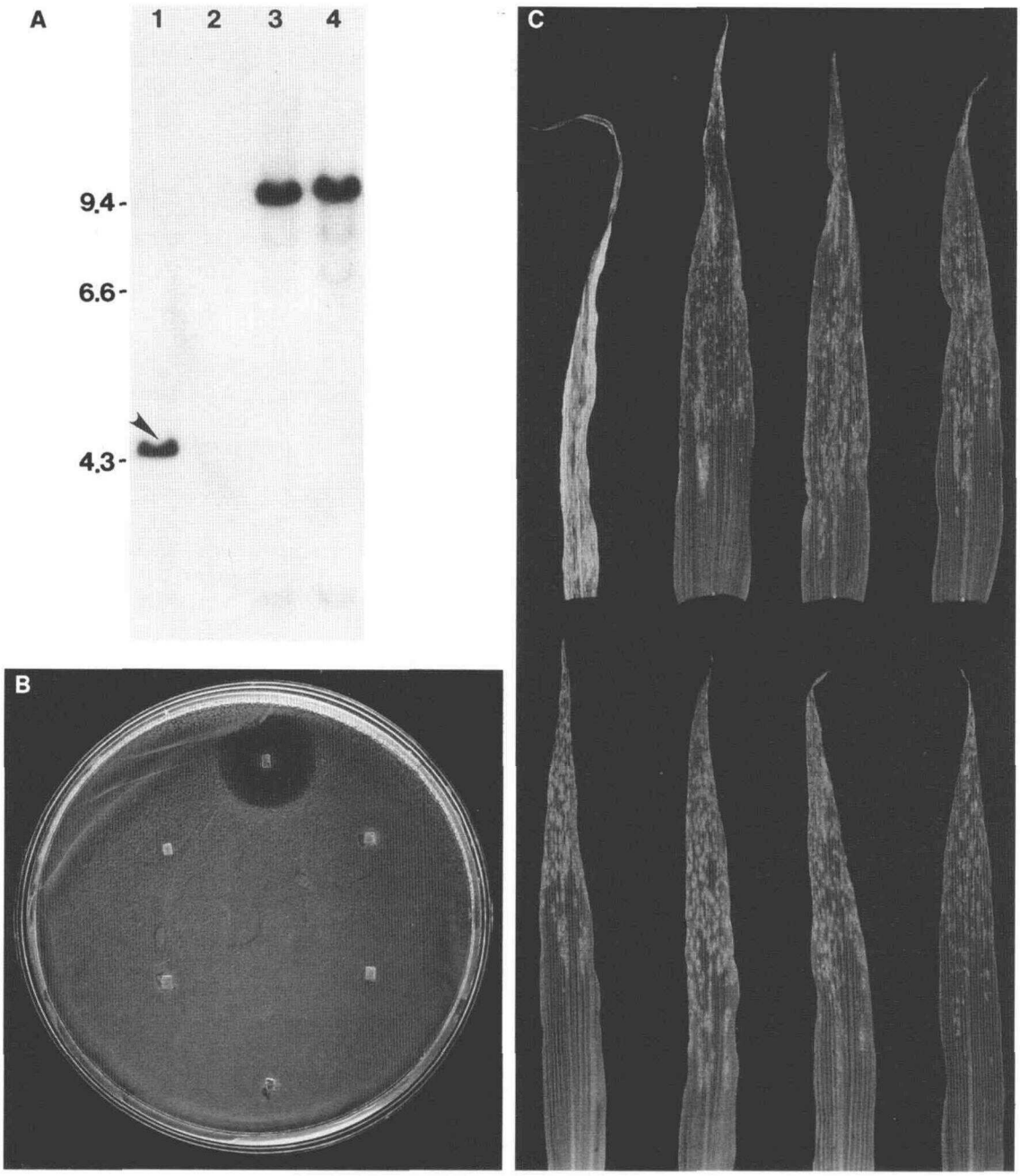

Figure 5. Disruption of *PKS1* Causes Loss of T-Toxin Production, Resulting in Low Virulence for T-Cytoplasm Maize.

(A) Gel blot of EcoRV-digested genomic DNAs probed with the 1.6-kb BamHI-HindIII fragment of pF5P1 (Figure 2C). Lanes 1 and 2 contain wild-type *Tox1*$^+$ (strain C4) and *Tox1*$^-$ (strain C5), respectively, as controls; lanes 3 and 4 contain transformants TX51RV-2 and TX51RV-3, respectively, each with *PKS1* inactivated by targeted gene disruption. In the DNA of the transformants, the 4.6-kb (2.7 kb plus 1.9 kb; Figure 2C) native band (lane 1, arrowhead) is replaced by a 9.7-kb band representing insertion of the 5.1-kb vector (Figure 2C) into the native 4.6-kb fragment. Note the lack of a signal in lane 2 containing DNA from strain C5 (race O). Numbers at left are length markers in kilobases.

(B) Assay for T-toxin on a lawn of *E. coli* cells. Strains shown are wild-type *Tox1*$^+$ (top; halo surrounding inoculum indicates T-toxin) and *Tox1*$^-$ (bottom; no halo) controls. The four remaining inocula are (left to right and top to bottom) *pks1*$^-$ REMI transformant R.C4.350L and transformants TX51RV-2, TX51RV-3, and TX51RV-12, each with *PKS1* inactivated by targeted gene disruption and with no halo.

(C) Virulence of *C. heterostrophus* toward maize plants. At top is T-cytoplasm maize. At bottom is N (normal, fertile)-cytoplasm maize. Within each grouping, leaves were inoculated with, from left, the wild-type *Tox1*$^+$ (showing complete tissue necrosis on T-cytoplasm only) and *Tox1*$^-$ (showing fully green leaves bearing small necrotic flecks) control strains, *pks1*$^-$ REMI transformant R.C4.350L, and transformant TX51RV-2 with *PKS1* inactivated by targeted gene disruption. Symptoms caused by the latter two strains are identical on both N- and T-cytoplasm maize and are indistinguishable from those caused by the *Tox1*$^-$ control strain. As described in the text, similar results were obtained with the remaining 10 transformants that had *PKS1* inactivated by targeted gene disruption (data not shown).

encodes each of these six essential domains and therefore contains all of the information necessary for any of the steps in polyketide construction. Thus, *PKS1* could act by an iterative mechanism, and it alone could account for construction of the T-toxin carbon chain. Alternatively, it is possible that additional PKS-encoding genes are required for T-toxin assembly and that *Tox1* represents a modular mechanism of PKS activity in which an individual module is needed for each extension of the polyketide chain. Because T-toxin is a C_{41} molecule, there could be as many as 20 modules necessary for its biosynthesis, and it is likely that the corresponding genes would be clustered, as they are in other organisms (Hopwood and Sherman, 1990), although no *PKS* homologs have yet been found in the vicinity of *PKS1*. One way to investigate whether PKS1 acts by an iterative or modular mechanism would be to inactivate its ER domain in vivo by site-specific mutation and to analyze the T-toxin molecules that result. Introduction of one enoyl function into the molecule would suggest a modular mechanism, whereas the appearance of several enoyl functions would imply an iterative mechanism; absence of enoyl groups would suggest that the ER domain of PKS1 is not usually functional and that at least one other PKS is involved in T-toxin biosynthesis—a result that would be consistent with the hypothesis that PKS1 is modular.

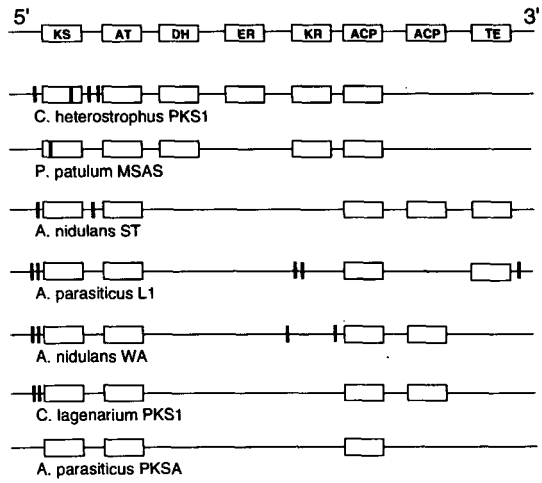

Figure 6. Organization of Fungal PKS Genes.

The drawing at the top represents the order of domains in a generic PKS. Vertical bars indicate the approximate positions of introns. Note that all genes encode the domains for polyketide chain extension (KS, AT, and ACP), but only *C. heterostrophus PKS1* contains all three domains (DH, ER, and KR) required for complete processing of β-carbons. Gene designations are given in the legend to Figure 4.

PKSs in Fungi

Polyketides are commonly produced by filamentous fungi; to date, nucleotide sequences have been reported for six fungal *PKS* genes, all of which encode type I PKSs. A comparison of enzymatic domains and intron positions of fungal PKS-encoding genes is shown in Figure 6. The *MSAS1* gene of *P. urticae* (Wang et al., 1991) and *P. patulum* (Beck et al., 1990) encodes MSAS, which catalyzes the synthesis of the tetraketide 6-methylsalicylic acid from one acetyl- and three malonyl-CoAs. *MSAS1* encodes a PKS of 1735 amino acids that has the domain organization N terminus–KS-AT-DH-KR-ACP–C terminus (Beck et al., 1990) and operates by the iterative mechanism (Hopwood and Khosla, 1992). The *wA* gene of *A. nidulans* encodes a PKS of 1986 amino acids with the organization N terminus–KS-AT-ACP-ACP–C terminus (Mayorga and Timberlake, 1992). The product of the *wA* PKS is unknown but is likely a phenolic component of the green pigment found in *A. nidulans* conidia. A homolog of *wA* (but containing a single ACP domain), isolated from *A. parasiticus* and designated *pksA*, encodes a polypeptide that is required for the production of a yellow pigment and for biosynthesis of aflatoxin B-1, a potent carcinogen (Chang et al., 1995). The product of the *A. parasiticus pksL1* gene is 2109 amino acids long, has four conserved domains (KS, AT, ACP, and TE), and is required for the production of several related aflatoxins and their biosynthetic precursors (Feng and Leonard, 1995). *A. nidulans* requires the gene *pksST* for the production of the polyketide-derived mycotoxin sterigmatocystin. *pksST* encodes a polypeptide of 2181 amino acids containing the enzymatic domains KS, AT, and TE and two copies of ACP (Yu and Leonard, 1995). Another apparent homolog of the *A. nidulans wA* gene has been isolated from the plant pathogenic fungus *Colletotrichum lagenarium* (Takano et al., 1995). This gene (*PKS1*) encodes a polypeptide of 2187 amino acids that, like the *A. nidulans wA* product, contains KS, AT, and two copies of ACP. *C. lagenarium* PKS1 is required for the production of the pentaketide precursor of melanin, a pigment essential for infectivity of the fungus toward plants. Interestingly, three of the six fungal PKSs are involved in production of pigments; in contrast, *C. heterostrophus pks1*$^-$ mutants are as darkly melanized as wild-type strains, indicating that PKS1 does not have a role in pigment production.

Genetic Organization of the *Tox1* Locus

Although the cloning of *C. heterostrophus PKS1* has provided insight into the molecular nature of *Tox1*, it is clear from our recent observations (Turgeon et al., 1995) that *Tox1* is surprisingly complex. It is in fact not a single locus at all but rather two different loci on two different chromosomes. These loci, designated *Tox1A* and *Tox1B*, appear to be linked in crosses between race T and race O (Turgeon et al., 1995) because they are located at the break points of the pair of chromosomes that are reciprocally translocated in race T with respect to race O (Tzeng et al., 1992). *PKS1* maps at *Tox1A*, which is on the translocated chromosome designated 12;6, and is flanked immediately on both sides by A+T-rich repeated DNA. The significance of the repeats is not known; interestingly, repeated

DNA is also found at the *Tox2* locus of *C. carbonum* (Panaccione et al., 1992). Genes at *Tox1B*, which is on the translocated chromosome designated 6;12, are currently being investigated: preliminary results show that one of these genes encodes a decarboxylase that is required for modification of the T-toxin carbon chain and is unique to race T strains (M.S. Rose, unpublished data).

Because neither of the *Tox1* loci appears to be represented in the genome of race O (Figure 5), there are no known naturally occurring recessive alleles of genes at these loci. Therefore, gene symbols designating dominant (*TOX1*) and recessive (*tox1*) alleles, as recommended generally for genetic nomenclature (Yoder et al., 1986), do not seem appropriate in this case. Instead, the locus symbol itself (*Tox1*) is used to designate relevant genotypes of the *C. heterostrophus* races, that is, $Tox1^+$ for race T and $Tox1^-$ for race O. Alleles of genes identified at *Tox1* follow the normal rules, for example, *PKS1* and *pks1* for dominant (wild-type) and recessive (induced mutant) alleles, respectively, of the gene encoding the PKS described in this paper.

Evolution of Race T

The origin of *PKS1* remains to be determined. The observation that *PKS1* has no homolog in race O (Figure 5; M.S. Rose, unpublished results) was a surprise. Because race O was described 45 years before race T was first detected in the field, it has been hypothesized that race O is the progenitor of race T. This theory is consistent with the fact that race T appeared suddenly in the field population in the late 1960s (Ullstrup, 1970). Moreover, in the early 1970s, the race T field population was predominantly *MAT-1* (Leonard, 1973; Alcorn, 1975), whereas race O *MAT-1* and *MAT-2* existed at equal frequencies. By 1975, *MAT-1* and *MAT-2* were found at equal frequency in the field population of race T, implying that race T arose by a mutation in a *MAT-1* race O strain. How might this have happened? Several possibilities are based on the premise that a mutation occurred in DNA already present in the race O genome. (1) Race O might usually produce T-toxin but metabolize it to a nontoxic product (Yoder, 1980). A block in the toxin biosynthetic pathway would cause accumulation of T-toxin. (2) A mutation in a race O gene might result in a new enzymatic activity that leads to T-toxin biosynthesis. (3) Novel sequences may have been created at the *Tox1*-linked break points associated with the reciprocal translocation that distinguishes race T from race O. All three possibilities are ruled out by the observation that *PKS1*, which is essential for T-toxin production, is not present in the race O genome.

We are left with the following alternatives. (1) Race O arose from race T by deletion of *PKS1* and perhaps other genes. Although this possibility has not been formally eliminated, it seems unlikely in view of the data on the *MAT* gene cited above. (2) Race T arose from race O by insertion of *PKS1* after horizontal transfer of DNA from an alien organism. It is perhaps significant that the "pathogenicity islands" containing genes required for pathogenicity in bacteria appear to have been acquired from heretofore unidentified organisms (Barinaga, 1996). Thus, a tenable scenario is that race T arose as a result of two mutational events in a race O strain. One event would be the insertion of foreign DNA carrying *PKS1* into the genome of race O. The other would be the reciprocal translocation that placed *Tox1* on two different chromosomes. Although the existence of reciprocal translocation is well documented for all race T strains examined to date (Chang and Bronson, 1996), a physical association between *PKS1* and the translocation break point has not been established.

If race T is distinguished from race O by the presence of "alien" DNA, from where did it come? The G+C content and presence of introns in *PKS1* suggest that it has a fungal origin. Yet codon usage of *PKS1* is unlike that of most other fungal genes, including the majority of those from *Cochliobolus* spp. This raises the prospect of a prokaryotic source, although the only evidence for this is the observation that the highest similarities to PKS1 in the data bases are to bacterial PKSs. Significantly, codon usage analysis for the *HTS1* gene of *C. carbonum* reveals the same lack of preference for a C residue in the third position, as was observed for *PKS1*. *HTS1* encodes a cyclic peptide synthetase required for the biosynthesis of the cyclic peptide HC-toxin, a pathogenicity factor in the maize leaf spot disease (Panaccione et al., 1992). Unlike *PKS1*, *HTS1* contains no introns, a characteristic consistent with the possibility of a nonfungal source. Like *PKS1*, *HTS1* is unique to the genome of toxin-producing strains, suggesting that it too is of "alien" origin. That these two related fungi appear to have acquired genes for pathogenesis by horizontal transfer, that other pathogenic fungi may have done the same (Miao et al., 1991; Vankan et al., 1991; Masel et al., 1993), and that bacterial pathogens of both plants and animals maintain "alien" DNA to support their pathogenic activities (Barinaga, 1996) suggest that this mechanism for evolution of virulence could be prominent among microbes.

METHODS

Strains, Media, Crosses, and Transformation

Cochliobolus heterostrophus wild-type strains C4 ($Tox1^+$; *MAT-2*) and C5 ($Tox1^-$; *MAT-1*) (Leach et al., 1982) and restriction enzyme–mediated integration (REMI) transformant R.C4.350L ($Tox1^-$; *MAT-2*; Lu et al. 1994) were recovered from storage and grown as described previously (Lu et al., 1994). *Escherichia coli* DH5α, used for transformation, was grown on Luria-Bertani agar or liquid medium and supplemented with 50 μg of ampicillin per mL when appropriate. Fungal crosses (Leach et al., 1982) and transformation (Turgeon et al., 1993) were performed as described previously.

DNA Manipulations

Genomic DNA from *C. heterostrophus* was prepared by standard procedures (Yoder, 1988). Isolation of plasmid and cosmid DNAs, agarose

gel electrophoresis, screening of cosmid libraries, and DNA gel blot hybridizations were performed as described by Turgeon et al. (1993). DNA fragments recovered from gels were purified by using GeneClean (Bio-101 Inc., La Jolla, CA).

Two vectors, pBluescript KS+ (Stratagene, La Jolla, CA) and pUCATPH, were used for subcloning and for constructing *C. heterostrophus* transformation vectors. pUCATPH carries the 2.4-kb SalI fragment from pDH25 (Cullen et al., 1987) containing the *E. coli* hygromycin B resistance gene fused to the *Aspergillus nidulans trpC* promoter and terminator in the SalI site of pUC18 (Lu et al., 1994). A list of clones used in this study is found in Table 1.

DNA sequences were determined at the Cornell University DNA Services Facility using TaqCycle automated sequencing with DyeDeoxy terminators (Applied Biosystems, Foster City, CA). Primers used in sequencing were designed with the Genetics Computer Group (Madison, WI) primer program and synthesized by the Cornell DNA Services Facility. Sequences were assembled using the SeqEd program from Applied Biosystems Inc. and aligned with the LaserGene program MegAlign (DNASTAR Inc., Madison, WI). BLAST (Altschul et al., 1990) searches were done against the National Center for Biotechnology Information/GenBank data bases.

Recovery of Tagged DNA at the *Tox1* Locus

Genomic DNA (3 μg) of tagged *Tox1*⁻ mutant R.C4.350L (Lu et al., 1994; Figure 2A) was partially digested with EcoRI (which has two sites in REMI vector pUCATPH; Figure 2C), purified by phenol extraction and ethanol precipitation, and ligated overnight at 12°C in 1 × T4 DNA ligase buffer (New England Biolabs, Beverly, MA), with 10 mM ATP and T4 DNA ligase. The volume (400 μL) of the reaction mixture and the concentration (1.5 units per μL) of T4 ligase were both high to promote intramolecular ligation. The DNA was ethanol precipitated, dissolved in 10 μL of water, and electroporated (Lindeberg and Collmer, 1992) into 100 μL of *E. coli* DH5α cells. Gel blot analysis of EcoRI-digested miniprep (Sambrook et al., 1989) plasmid DNA, probed with

Table 1. Vectors and Clones Used in This Study

Designation	Length (kb)	Relevant Characteristics	References
Cloning vectors			
pBluescript KS+	2.9		Stratagene
pUCATPH	5.1	The 2.4-kb SalI fragment from pDH25 (Cullen et al., 1987) containing the *E. coli hygB* gene fused to the *A. nidulans trpC* promoter and terminator in the SalI site of pUC18	Lu et al. (1994)
Clones recovered by recircularization of genomic DNA[a]			
pF5P1	14	Fragment from a partial EcoRI digest of REMI transformant R.C4.350L genomic DNA containing the plasmid-tagged site, including the entire vector pUCATPH, 7 kb of the left flank, and 1.9 kb of the right flank	This study
pF5P4	9.7	EcoRI fragment from an EcoRI digest of transformant R.C4.350L genomic DNA containing 7 kb of the left flank and pUC18	This study
pF5P3	10.3	Fragment from a partial EcoRI digest of transformant R.C4.350L genomic DNA containing 7 kb of the left flank, the pUC18 vector, the *trpC* promoter, and a short segment of the *hygB* gene	This study
Clones derived from pF5P1[a]			
pF5P5	4.3	Recircularized BamHI fragment containing 1.6 kb of the left flank and pUC18	This study
pF5P7	8.2	Recircularized XbaI fragment containing 5.5 kb of the left flank and pUC18	This study
pF5P9	5.2	The 2.2-kb XbaI-EcoRI fragment, including the 1.9-kb right flank and the adjacent 0.3-kb XbaI-HindIII pUCATPH fragment in pBluescript KS+	This study
pF5P12	5.6	Recircularized SacI fragment containing 2.9 kb of the left flank and pUC18	This study
pF5P28	4.0	Recircularized fragment after deletion of two PstI fragments (66 bp and 1.5 kb) from the left flank of pF5P12	This study
Additional clones[a]			
pF5P42(+)	6.5	The 1.4-kb KpnI fragment of pF5P4 inserted in the KpnI site of pUCATPH	This study
pF6P1	6.5	HindIII fragment from a transformant of strain C4 carrying pF5P42(+), with the HindIII fragment containing 1.9 kb of DNA 5′ of the 5′ flank of pF5P1, 2.2 kb of the 5′ end of pF5P1, and pUC18	This study
pF6P2	3.9	The 1.1-kb HindIII-EcoRI fragment of pF6P1 in pBluescript KS+	This study

[a] For laboratory record keeping, the prefix for each plasmid is R.C4.350L.

pUCATPH, showed that 17 of 22 transformants carried a 9.7-kb EcoRI fragment containing the *C. heterostrophus* genomic sequence along with the pUC18 portion of pUCATPH (Figure 2C). This fragment was identified previously at the tagged mutation site of R.C4.350L (Figure 5 in Lu et al., 1994). Five of the 17 plasmids contained two additional EcoRI fragments (3.7 and 0.6 kb; Figure 2C), which were also observed in the earlier study (Lu et al., 1994). Each of these five plasmids included the entire vector (pUCATPH) and both sides of the genomic insertion site. One plasmid (14 kb, designated pF5P1; Table 1) was chosen for further analysis (Figure 2C).

DNA Sequence at the Tagged Site

Sequencing of the DNA flanking the vector insertion site was initiated with primers corresponding to the ends of pUCATPH in pF5P1 (Figure 2C). The M13-40 primer was used for the left flank, and primer gy39, which hybridizes with a sequence in the *A. nidulans trpC* terminator region of pUCATPH (Figure 2B), was used for the right flank (left and right refer to the corresponding sides of pUCATPH in Figure 2). Subsequently, sequences were extended by designing primers that corresponded to a previously determined sequence. Certain fragments of pF5P1 were also subcloned to facilitate sequencing (Table 1).

To extend the sequence into the region to the left of pF5P1 (the β-ketoacyl synthase domain is truncated in pF5P1; see Figure 2C), we employed a targeted integration and walking strategy; attempts to recover this DNA by probing three independent cosmid libraries of race T genomic DNA with the 1.5-kb EcoRI-XbaI fragment of pF5P1 (Figure 2C) failed to yield any hybridizing clones. A transformation vector, pF5P42(+) (Table 1), was constructed by inserting the 1.4-kb KpnI fragment of pF5P4 (derived from pF5P1; Figure 2C and Table 1) into the KpnI site of pUCATPH (one particular orientation was chosen to facilitate subsequent cloning; Table 1). This vector was transformed into wild-type *Tox1*$^+$ strain C4, and transformants were screened on DNA gel blots for homologous integration of the transforming DNA into the 1.4-kb KpnI target region. Genomic DNA of one such transformant (TX1.F5P42) was digested with HindIII, recircularized, and transformed into *E. coli*, selecting for ampicillin resistance. Analysis of recovered plasmids revealed some that carried an additional 1.9 kb of DNA to the left of pF5P1; one such plasmid was designated pF6P1 (Figure 2D and Table 1). The map of HindIII, EcoRI, and XbaI sites in pF6P1 was consistent with hybridizing bands on a genomic DNA gel blot of the region probed with the 1.5-kb EcoRI-XbaI fragment of pF5P1 (Figure 2C), indicating that the recovered DNA in pF6P1 mapped to the left of pF5P1 and not elsewhere in the genome.

Sequencing of the 1.9 kb of additional DNA in pF6P1 was initiated with the M13-40 primer and with a primer (gy6) designed to hybridize with the 0.8-kb EcoRI-KpnI fragment at the left end of pF5P1 (Figure 2C). A subclone (pF6P2) of pF6P1 (Table 1) was also sequenced using the M13-70R primer. Subsequently, sequences were extended with primers targeted to a previously determined sequence. To confirm that the 1.9-kb fragment was adjacent to pF5P1, we sequenced the region spanning the junction between pF5P1 and pF6P1 in both directions. The overlapping sequences matched perfectly. Details of the sequencing strategy are available upon request.

Bioassays

To test for T-toxin (Ciuffetti et al., 1992), we spread cells (2 mL) of *E. coli* DH5α carrying the *T-urf13* gene from T-cytoplasm maize mitochondrial DNA (Dewey et al., 1988) evenly on plates containing Luria-Bertani medium supplemented with 50 μg of ampicillin per mL by tilting the plates from side to side and then decanting the excess and allowing the surfaces to dry in a laminar flow clean air cabinet. Agar cylinders bearing fungal mycelia were placed upside down on the film of *E. coli* cells before overnight incubation at 32°C. T-toxin–producing fungal strains cause halos by inhibiting growth of *E. coli* cells; nonproducing strains do not cause halos.

Plant assays were performed as described previously (Yoder and Gracen, 1975), using T- and N (normal, fertile)-cytoplasm maize (inbred W64A) grown to the three-leaf stage (~2 weeks). Conidial suspensions (10^5 per mL) were atomized onto plants with a pressurized Preval Spray Gun Power Unit thin layer chromatography sprayer (Alltech Associates, Deerfield, IL). Plants were incubated for 2 to 3 days in a growth chamber (24°C). Symptoms were then evaluated, and leaves were photographed.

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REFERENCES

Alcorn, J.L. (1975). Race-mating type associations in Australian populations of *Cochliobolus heterostrophus*. Plant Dis. Rep. **59,** 708–711.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990). Basic local alignment search tool. J. Mol. Biol. **215,** 403–410.

Amy, C.M., Witkowski, A., Naggert, J., Williams, B., and Randhawa, Z. (1989). Molecular cloning and sequencing of cDNAs encoding the entire rat fatty acid synthase. Proc. Natl. Acad. Sci. USA **86,** 3114–3118.

Barinaga, M. (1996). A shared strategy for virulence. Science **272,** 1261–1263.

Beck, J., Ripka, S., Siegner, A., Schiltz, E., and Schweizer, E. (1990). The multifunctional 6-methylsalicylic acid synthase gene of *Penicillium patulum*—Its gene structure relative to that of other polyketide synthases. Eur. J. Biochem. **192,** 487–498.

Chang, H.R., and Bronson, C.R. (1996). A reciprocal translocation and possible insertion(s) tightly associated with host-specific virulence in *Cochliobolus heterostrophus*. Genome **39,** 549–557.

Chang, P.K., Cary, J.W., Yu, J., Bhatnagar, D., and Cleveland, T.E. (1995). The *Aspergillus parasiticus* polyketide synthase gene *pksA*, a homolog of *Aspergillus nidulans wA*, is required for aflatoxin B-1 biosynthesis. Mol. Gen. Genet. **248,** 270–277.

Ciuffetti, L.M., Yoder, O.C., and Turgeon, B.G. (1992). A microbiological assay for host-specific fungal polyketide toxins. Fungal Genet. Newsl. **39,** 18–19.

Cullen, D., Leong, S.A., Wilson, L.J., and Henner, D.J. (1987). Transformation of *Aspergillus nidulans* with the hygromycin-resistance gene, *hph*. Gene **57,** 21–26.

Dewey, R.E., Siedow, J.N., Timothy, D.H., and Levings III, C.S. (1988). A 13-kilodalton maize mitochondrial protein in *E. coli* confers sensitivity to *Bipolaris maydis* toxin. Science **239,** 293–295.

Donadio, S., and Katz, L. (1992). Organization of the enzymatic domains in the multifunctional polyketide synthase involved in erythromycin formation in *Saccharopolyspora erythraea*. Gene **111,** 51–60.

Donadio, S., Staver, M.J., McAlpine, J.B., Swanson, S.J., and Katz, L. (1991). Modular organization of genes required for complex polyketide biosynthesis. Science **252,** 675–679.

Drechsler, C. (1925). Leafspot of maize caused by *Ophiobolus heterostrophus* n. sp., the ascigerous stage of a *Helminthosporium* exhibiting bipolar germination. J. Agric. Res. **31,** 701–726.

Edelmann, S.E., and Staben, C. (1994). A statistical analysis of sequence features within genes from *Neurospora crassa*. Exp. Mycol. **18,** 70–81.

Feng, G.H., and Leonard, T.J. (1995). Characterization of the polyketide synthase gene (*pksL1*) required for aflatoxin biosynthesis in *Aspergillus parasiticus*. J. Bacteriol. **177,** 6246–6254.

Hopwood, D.A., and Khosla, C. (1992). Genes for polyketide secondary metabolic pathways in microorganisms and plants. In Secondary Metabolites: Their Function and Evolution, D.J. Chadwick and J. Whelan, eds (Chichester, UK: John Wiley and Sons), pp. 88–112.

Hopwood, D.A., and Sherman, D.H. (1990). Molecular genetics of polyketides and its comparison to fatty acid biosynthesis. Annu. Rev. Genet. **24,** 37–66.

Katz, L., and Donadio, S. (1993). Polyketide synthesis: Prospects for hybrid antibiotics. Annu. Rev. Microbiol. **47,** 875–912.

Kono, Y., and Daly, J.M. (1979). Characterization of the host-specific pathotoxin produced by *Helminthosporium maydis* race T affecting corn with Texas male sterile cytoplasm. Bioorg. Chem. **8,** 391–397.

Kono, Y., Knoche, H.W., and Daly, J.M. (1981). Structure: Fungal host-specific. In Toxins in Plant Disease, R.D. Durbin, ed (New York: Academic Press), pp. 221–257.

Leach, J., Lang, B.R., and Yoder, O.C. (1982). Methods for selection of mutants and *in vitro* culture of *Cochliobolus heterostrophus*. J. Gen. Microbiol. **128,** 1719–1729.

Leonard, K.J. (1973). Association of mating type and virulence in *Helminthosporium maydis*, and observations on the origin of the race T population in the United States. Phytopathology **63,** 112–115.

Levings III, C.S., Rhoads, D.M., and Siedow, J.N. (1995). Molecular interactions of *Bipolaris maydis* T-toxin and maize. Can. J. Bot. **73,** S483–S489.

Lindeberg, M., and Collmer, A. (1992). Analysis of eight *out* genes in a cluster required for pectic enzyme secretion by *Erwinia chrysanthemi*: Sequence comparison with secretion genes from other Gram-negative bacteria. J. Bacteriol. **174,** 7385–7397.

Lu, S.W., Lyngholm, L., Yang, G., Bronson, C., Yoder, O.C., and Turgeon, B.G. (1994). Tagged mutations at the *Tox1* locus of *Cochliobolus heterostrophus* using restriction enzyme–mediated integration. Proc. Natl. Acad. Sci. USA **91,** 12649–12653.

MacNeil, D.J., Occi, J.L., Gewain, K.M., MacNeil, T., Gibbons, P.H., Ruby, C.L., and Danis, S.J. (1992). Complex organization of the *Streptomyces avermitilis* genes encoding the avermectin polyketide synthase. Gene **115,** 119–125.

Masel, A.M., Irwin, J.A.G., and Manners, J.M. (1993). DNA addition or deletion is associated with a major karyotype polymorphism in the fungal phytopathogen *Colletotrichum gloeosporioides*. Mol. Gen. Genet. **237,** 73–80.

Mathur, M., and Kolattukudy, P.E. (1992). Molecular cloning and sequencing of the gene for mycocerosic acid synthase, a novel fatty acid elongating multifunctional enzyme from *Mycobacterium tuberculosis* var *Bovis bacillus* Calmette-Guerin. J. Biol. Chem. **267,** 19388–19395.

Matthews, D.E., Gregory, P., and Gracen, V.E. (1979). *Helminthosporium maydis* race T toxin induces leakage of NAD^+ from T cytoplasm corn mitochondria. Plant Physiol. **63,** 1149–1153.

Mayorga, M.E., and Timberlake, W.E. (1992). The developmentally regulated *Aspergillus nidulans wA* gene encodes a polypeptide homologous to polyketide and fatty acid synthases. Mol. Gen. Genet. **235,** 205–212.

McCarthy, A.D., and Hardie, D.G. (1984). Fatty acid synthase—An example of protein evolution by gene fusion. Trends Biochem. Sci. **9,** 60–63.

Miao, V.P., Covert, S.F., and VanEtten, H.D. (1991). A fungal gene for antibiotic resistance on a dispensable (B) chromosome. Science **254,** 1773–1776.

Panaccione, D.G., Scott-Craig, J.S., Pocard, J.A., and Walton, J.D. (1992). A cyclic peptide synthetase gene required for pathogenicity of the fungus *Cochliobolus carbonum* on maize. Proc. Natl. Acad. Sci. USA **89,** 6590–6594.

Rhoads, D.M., Levings III, C.S., and Siedow, J.N. (1995). URF13, a ligand-gated, pore-forming receptor for T-toxin in the inner membrane of cms-T mitochondria. J. Bioenerg. Biomembr. **27,** 437–445.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory).

Schwecke, T., Aparicio, J.F., Molnar, I., Konig, A., Khaw, L.E., Haydock, S.F., Oliynyk, M., Caffrey, P., Cortes, J., Lester, J.B., Bohm, G.A., Staunton, J., and Leadlay, P.F. (1995). The biosynthetic gene cluster for the polyketide immunosuppressant rapamycin. Proc. Natl. Acad. Sci. USA **92,** 7839–7843.

Siedow, J.N., Rhoads, D.M., Ward, G.C., and Levings III, C.S. (1995). The relationship between the mitochondrial gene T-urf13 and fungal pathotoxin sensitivity in maize. Biochim. Biophys. Acta **1271,** 235–240.

Takano, Y., Kubo, Y., Shimizu, K., Mise, K., Okuno, T., and Furusawa, I. (1995). Structural analysis of *PKS1*, a polyketide synthase gene involved in melanin biosynthesis in *Colletotrichum lagenarium*. Mol. Gen. Genet. **249,** 162–167.

Turgeon, B.G., Bohlmann, H., Ciuffetti, L.M., Christiansen, S.K., Yang, G., Schafer, W., and Yoder, O.C. (1993). Cloning and analysis of the mating type genes from *Cochliobolus heterostrophus*. Mol. Gen. Genet. **238,** 270–284.

Turgeon, B.G., Kodama, M., Yang, G., Rose, M.S., Lu, S.W., and Yoder, O.C. (1995). Function and chromosomal location of the *Cochliobolus heterostrophus Tox1* locus. Can. J. Bot. **73,** S1071–S1076.

Tzeng, T.H., Lyngholm, L.K., Ford, C.F., and Bronson, C.R. (1992). A restriction fragment length polymorphism map and electrophoretic karyotype of the fungal maize pathogen *Cochliobolus heterostrophus*. Genetics **130,** 81–96.

Ullstrup, A.J. (1970). History of southern corn leaf blight. Plant Dis. Rep. **54,** 1100–1102.

Vankan, J.A.L., Vandenackerveken, G.F.J.M., and DeWit, P.J.G.M. (1991). Cloning and characterization of cDNA of a virulence gene *avr9* of the fungal pathogen *Cladosporium fulvum*, causal agent of tomato leaf mold. Mol. Plant-Microbe Interact. **4,** 52–59.

Wakil, S.J. (1989). Fatty acid synthase: A proficient multifunctional enzyme. Biochemistry **28,** 4523–4530.

Wang, I.K., Reeves, C., and Gaucher, G.M. (1991). Isolation and sequencing of a genomic DNA clone containing the 3′-terminus of the 6-methylsalicylic acid polyketide synthetase gene of *Penicillium urticae*. Can. J. Microbiol. **37,** 86–95.

Yoder, O.C. (1980). Toxins in pathogenesis. Annu. Rev. Phytopathol. **18,** 103–129.

Yoder, O.C. (1988). *Cochliobolus heterostrophus*, cause of Southern corn leaf blight. In Genetics of Plant Pathogenic Fungi, G.S. Sidhu, ed (San Diego, CA: Academic Press), pp. 93–112.

Yoder, O.C., and Gracen, V.E. (1975). Segregation of pathogenicity types and host-specific toxin production in progenies of crosses between races T and O of *Helminthosporium maydis* (*Cochliobolus heterostrophus*). Phytopathology **65,** 273–276.

Yoder, O.C., Valent, B., and Chumley, F. (1986). Genetic nomenclature and practice for plant pathogenic fungi. Phytopathology **76,** 383–385.

Yoder, O.C., Yang, G., Adam, G., DiazMinguez, J.M., Rose, M., and Turgeon, B.G. (1993). Genetics of polyketide toxin biosynthesis by plant pathogenic fungi. In Industrial Microorganisms: Basic and Applied Molecular Genetics, R.H. Baltz, G.D. Hegeman, and P.L. Skatrud, eds (Washington, DC: American Society for Microbiology), pp. 217–225.

Yoder, O.C., Macko, V., Wolpert, T.J., and Turgeon, B.G. (1997). *Cochliobolus* spp. and their host-specific toxins. In The Mycota, Vol. 5: Plant Relationships, G. Carroll and P. Tudzynski, eds (Berlin: Springer–Verlag), in press.

Yu, J.H., and Leonard, T.J. (1995). Sterigmatocystin biosynthesis in *Aspergillus nidulans* requires a novel type I polyketide synthase. J. Bacteriol. **177,** 4792–4800.