Plant Pathol. J. 16(2) : 90-97 (2000)

The Plant Pathology Journal

Symposium

Molecular Plant-Microbe Interactions

December 10, 1999, Taejon, Korea

Molecular Genetics of Polyketide Toxin Production by Two Fungal Corn Pathogens

Sung-Hwan Yun*

Plant Resources Major, Division of Life Sciences, Soonchunhyang University, Asan city, Choongnam 336-745, Korea

(Received on March 9, 2000)

Plant disease is the outcome of interaction between a pathogen and its host. During disease development, both partners produce a variety of molecules which interact and determine the outcome: susceptibility or resistance. In this regard, it has been postulated that molecules produced by pathogens play crucial roles in disease. To test this, various experimental methods to inactivate or eliminate the pathogen-produced molecules in plant-pathogen interactions have been used in order to determine what change occurs in disease development. Recently, molecular manipulations, especially targeting the gene encoding the molecule in question, have provided critical evidence either for or against roles of fungal molecules in pathogenesis. In plant diseases caused by fungal pathogens, two classes of fungal molecule have been shown to have roles in the infection process (Yoder and Turgeon, 1996). One class of molecule is involved in adhesion and penetration of fungal pathogens into plant surfaces (hydrophobin, melanin, glycerol, cutinase, and components of signal transduction pathway), whereas the other class is required for fungal colonization inside the plant tissue (various toxins and enzymes).

Among the identified fungal molecules, are host specific or selective toxins, secondary metabolites toxic only to the host of the producing fungal pathogen (Yoder, 1980). Due to the extreme specificity toward only susceptible plants, these unusual metabolites have been extensively studied as specific disease determinants of certain fungal pathogens. Besides biochemical and pathological aspects of host specific toxins, biosyntheses of the toxins have been studied with an eye toward understanding evolution of new pathogenic fungal races as well as molecular mechanisms of pathogenesis.

My focus in this review is the host specific polyketide toxins produced by two unrelated fungal corn pathogens: T-toxin of *Cochliobolus heterostrophus* and PM-toxin of *Mycosphaerella zeae-maydis*. Despite taxonomical or mycological differences between these ascomycetous fungi, they show strikingly similar features based on diseases they cause. In the late 1960s, two different corn diseases caused by these fungi appeared concurrently in the U.S.A.: Southern corn leaf blight and yellow corn leaf blight by a new race (T) of *C. heterostrophus* and *M. zeae-maydis*, respectively. Interestingly, both exhibited high virulence only on corn containing Texas male-sterile (T-) cytoplasm (Arny et al., 1970; Ayers et al., 1970; Scheifele et al., 1969). Furthermore, this rare case of cytoplasmically controlled disease susceptibility was found to be associated with polyketides produced by these causal fungi, which are chemically similar to each other (Kono et al., 1980; Kono et al., 1983; Kono et al., 1985). The sudden and concurrent appearance of these new toxin-producing fungi, therefore, suggests i) similarities in biosynthetic pathways of the two polyketides and ii) a common genetic step for evolution of these fungi, all of which could be investigated, in this case, by exploring the biosynthetic pathways of the polyketide toxins. This review describes progress in comparative genetics of the polyketide production by these fungi.

Polyketide Toxins Produced by *C. heterostrophus* and *M. zeae-maydis* and Their Receptor on Plant

Structural analyses have revealed that both T- and PM-toxins are mixtures of unusual linear polyketols: T-toxin is a complex of C_{35} to C_{45} components (Kono et al., 1980; Kono et al., 1981). PM-toxin is composed of a series of 10-12 chemically analogous components that have linear alkane chains ranging from C_{33} to C_{35} in length (Kono et al., 1983; Kono et al., 1985). Major components of each toxin (C_{39} and C_{41} components of T-toxin and C_{33} and C_{35} of PM-toxin, respectively) shown in Fig. 1 have certain functional

*Corresponding author.
Phone) +82-418-530-1288, Fax) +82-418-530-1287
E-mail) sy14@sch.ac.kr

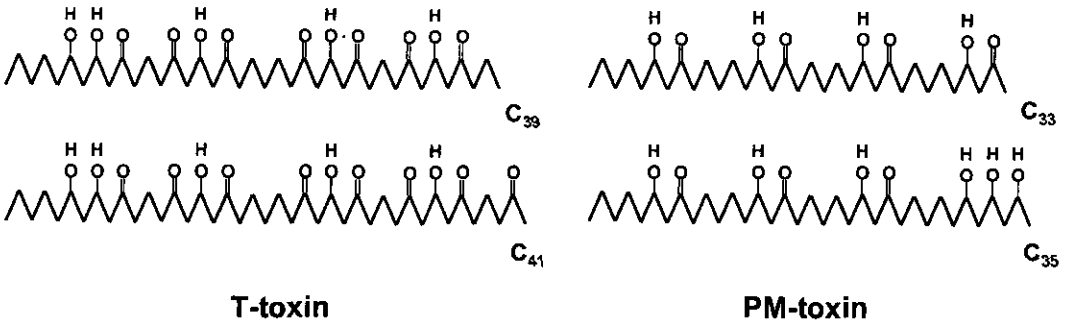

Fig. 1. Structures of major components in the T-toxin and PM-toxin families. Like T-toxin, PM-toxin is a linear polyketide, but contains shorter chain lengths and fewer oxygen-containing functional groups (Kono et al., 1983).

groups in common: 3,5-dihydroxyl ketone and 3-hydroxyl-1,5-diketone in T-toxin and 3-hydroxy ketone, and 1,3-diol or 1,3,5-triol functions in PM-toxin. Thus, both T-toxin and PM-toxin contain several 3-hydroxyl ketone groups with the same absolute configuration (Kono et al., 1985). These native toxin mixtures at extremely low concentrations affect several physiological processes in only T-cytoplasm corn: ion leakage (Payne et al., 1980a), altered respiration (Arntzen et al., 1973), mitochondrial oxidation (Miller and Koeppe, 1971; Payne et al., 1980b), and photosynthesis (Daly and Barna, 1980).

The great difference in toxin sensitivity between T-cytoplasm and other types of corn suggested that only T-cytoplasm corn possesses a putative receptor for these toxins. It has been found that the site for action of both toxins in T-cytoplasm is a 13 KD protein (URF13) unique to the inner mitochondrial membrane of T-cytoplasm corn (Levings and Siedow, 1992). URF13 is encoded by the gene *T-urf13* on the T-cytoplasm mitochondrial DNA. It is a chimeric gene created by complex multiple rearrangements of the mitochondrial chromosome. Specific binding of URF13 to toxin (Braun et al., 1990) enlarges pores created in the inner mitochondria membrane by the toxin/URF13 complex (Kaspi and Siedow, 1993; Korth et al., 1991; Siedow et al., 1995), which results in leakage of essential metabolites (Matthews et al., 1979), nearly instantaneous cessation of ATP synthesis (Walton et al., 1979), and subsequent cell death. Other cytoplasmic male sterile and normal cytoplasm corn not containing URF13 are not affected by either toxin even at higher concentrations. These types of corn are not highly susceptible to either *C. heterostrophus* race T or *M. zeae-maydis*. Thus, susceptibility in these diseases is an active process, resulting from the specific interaction between unique polyketide toxins from the fungal pathogens and a unique protein, URF13 from the susceptible corn plant. In this case, disease resistance results from the absence of either or both components of the interaction rather than from a defense mechanism of the host plant. Heterologous expression of URF13 also confers toxin sensitivity to *E. coli* (Dewey et al., 1988), yeast (Glab et al., 1990; Huang et al., 1990), transgenic tobacco (von Allmen et al., 1991), and insect cells and larvae (Korth and Levings, 1993). *E. coli* cells expressing URF13, which are sensitive to both toxins, have been used as an efficient and reliable microbial assay for these toxins (Ciuffetti et al., 1992).

Comparative Genetics of Polyketide Production by *C. heterostrophus* and *M. zeae-maydis*

T-toxin production by *C. heterostrophus* race T. Since *C. heterostrophus* has been developed as a model organism for molecular genetic study of filamentous ascomycetes (Yoder, 1988), genetics of T-toxin production by this fungus has been extensively studied. The progress in this area includes the identification of the genetic locus (*Tox1*) involved in biosynthesis of T-toxin using Mendelian genetics, attempts to generate *Tox*$^-$ mutants, and cloning of the genes at the *Tox1* locus.

To determine how many genes were required for T-toxin production by *C. heterostrophus*, crosses between a toxin producing race (T) and a non-toxin producing race (O) were made (Lim and Hooker, 1971). Progeny segregated 1:1 for T-toxin production, defining a single locus (designated *Tox1*) (Leach et al., 1982) which controls toxin production. Moreover, all toxin-producing progeny showed high virulence toward T-cytoplasm corn, suggesting that this host specificity and toxin production are controlled by the same genetic locus (Yoder and Gracen, 1975). With respect to the number of genetic loci essential for T-toxin production, these genetic analyses did not identify any locus other than *Tox1*. In addition, genetic studies on ascospore abortion found in crosses between strains heterozygous for *Tox1* and in backcrosses of the heterozygous strains suggested that the *Tox1* locus might be associated with a chromosomal rearrangement (Taga et al., 1985). More genetic evidence for the reciprocal translocation was obtained when an RFLP map was constructed using prog-

eny of a cross between a *Tox*⁻ field isolate and a *Tox*⁺ lab strain (Tzeng et al., 1992), which showed a four-armed linkage group, indicative of a reciprocal translocation (Fincham et al., 1979; Perkins, 1977). *Tox1* mapped to the intersection of this linkage group, indicating that it is inseparably linked to the translocation breakpoints (Tzeng et al., 1992). Electrophoretic karyotype analysis using near isogenic race O and race T strains provided physical evidence for the translocation: all chromosomes from the two races were the same except for a unique pair in each. The unique pair of chromosomes in race T is reciprocally translocated with respect to the unique pair in race O. RFLP markers specific to different sides of the translocation breakpoint on each race T chromosome in the four armed linkage group hybridized to different chromosomes in race O but the same one in a race T (Kodama et al., 1999). Furthermore, this study revealed that the genome of race T has about 1.2 megabases more DNA than that of race O, and that *Tox1* is associated with highly repetitive sequences since 25% of all probes repetitive in the genome mapped near the translocation breakpoint.

The genetic evidence that there is a single locus (*Tox1*) controlling T-toxin production by *C. heterostrophus* race T led to attempts to clone *Tox1*. The first step was to induce toxin deficient mutants of race T. Using EMS mutagenesis, no *Tox*⁻ mutants were found among approximately 10,000 isolates screened. Several alternative strategies were employed including complementation of a *Tox*⁻ strain with genomic DNA of a *Tox*⁺ strain, chromosome walking using a RFLP marker (B88) thought to be closely linked to the *Tox1* locus, and genomic subtraction between *Tox*⁻ and *Tox*⁺ strains. All were, however, unsuccessful.

After these failures, a new strategy to generate *Tox*⁻ mutants was attempted based on knowledge of the molecular basis of toxin sensitivity to T-cytoplasm corn (Yang et al., 1994). A wild type *Tox*⁺ strain (C4) was transformed with the *T-urf13* gene encoding the 13 kDa T-toxin-binding protein (URF13) from the mitochondria of T-cytoplasm corn. *T-urf13* was fused to the polygalacturonic acid-inducible promoter from the *Aspergillus nidulans pelA* gene. Growth of the transformants was inhibited on inducing medium (PGA medium) because the transformants expressing URF13 were sensitive to the T-toxin they produced. Therefore, enrichment for *Tox*⁻ mutants involved treatment of conidia of the transformants with EMS and plating on inducing medium. Of 362 survivors, nine were Tox⁻: one was a null mutant (no toxin production at all), whereas the other eight showed leaky phenotypes (producing less toxin than the wild type *Tox*⁺ strain). Genetic analysis revealed that each of these mutants carried a single mutation and each mutation mapped at the *Tox1* locus. The mutant showing a tight Tox⁻ phenotype was the first *Tox*⁻ mutant induced from a *Tox*⁺ strain. However, attempts to clone the gene by complementing this mutant with a genomic library of the wild type *Tox*⁺ strain failed (Yang, unpublished data).

Another strategy for cloning used the procedure called restriction enzyme mediated integration (REMI), which was developed in yeast (Schiestl and Petes, 1991) and the slime mold *Dictyostelium discoideum* (Kuspa and Loomis, 1992) for tagging random mutations in the genome with a selectable gene. The REMI procedure was successful not only in inducing *Tox*⁻ mutants carrying tagged mutations but also in cloning genes required for T-toxin production. Among 1,300 recovered REMI transformants, two *Tox*⁻ mutants were isolated and genetic analysis indicated that each carries a single mutation at the *Tox1* locus, which was tagged with the fungal selectable marker *hygB*^{R} (Lu et al., 1994). Sequence analysis of genomic DNA flanking the tagged site revealed a gene (designated *PKS1*) encoding a polyketide synthase (PKS). Further molecular study confirmed that *PKS1* is unique in the genome of race T but is missing in race O, and required for T-toxin production and host specificity by race T (Yang et al., 1996).

While the REMI strategy was being pursued (but before success in cloning of *PKS1*), another strategy was initiated for cloning *Tox1*, which yielded a second gene essential for T-toxin production. This strategy was based on knowledge from molecular genetics of polyketide biosynthesis. Since T-toxin is a polyketide, it had been postulated that a PKS should be required for T-toxin biosynthesis. To clone this gene, degenerate primers were made to conserved regions of an enzymatic domain (acyl transferase, AT) from known PKSs and used in a PCR reaction with genomic DNA of race T as a template. Four 300 bp PCR products with similarity to known AT domains were amplified and cloned, but found, by targeted gene disruption, to be dispensable for T-toxin production. However, one *Tox*⁻ mutant obtained in the course of these experiments became the source for cloning the second gene. Genetic analysis indicated that, while this mutant was confirmed to carry an ectopic (not at the 'AT' site) integration of the transforming vector, the *Tox*⁻ mutation in this mutant was tagged with *hygB*^{R} and mapped at the *Tox1* locus. Moreover, CHEF gel analysis showed that this mutant sustained a large deletion in the chromosome carrying the plasmid insertion, rendering the chromosome about 100 kb smaller than the respective chromosome in race T. This difference also made a clear polymorphism when genomic DNAs of both strains were digested with the rare cutting enzyme, *Not*I. In the mutant, a 540 kb *Not*I fragment was replaced by a 440 kb fragment carrying the plasmid insertion. These results indicated that a large deletion occurred during transformation, and that the deleted DNA fragment could be responsi-

ble for T-toxin production. To clone single copy genes located on the missing DNA, a subtractive hybridization procedure was used. Probing a cDNA library of the wild type *Tox*$^+$ strain with both DNA fragments (the 440 kb and 540 kb fragments from the deletion mutant and its *Tox*$^+$ progenitor) revealed two genes, *DEC1* and *RED1*. DNA gel blot analysis indicated that both genes were unique in the wild type strain. Site-specific disruption analysis indicated that only *DEC1* was required for T-toxin production and high virulence on T-cytoplasm corn. The sequence of *DEC1* matched only one gene in the current data base, acetoacetate decarboxylase of *Clostridium acetobutylicum* (Rose, 1996).

All previously obtained *Tox*$^-$ mutations mapped at the *Tox1* locus, and thus would expected to be closely linked to each other. Analysis of progeny of a cross between the mutant described in the preceding paragraph and other these *Tox*$^-$ mutants, however, revealed an unexpected result. Progeny of crosses between two previously obtained *Tox*$^-$ mutants induced by REMI mutagenesis were 100% *Tox*$^-$; *hygB*^{R} (Lu et al., 1994), indicating that the two mutations map to the same locus, whereas 25% of progeny were *Tox*$^+$ when either of the two REMI mutants was crossed to the deletion mutant (Rose, 1996). These results indicated that while mutations sustained by the two REMI mutants are closely linked, there is no detectable linkage between either REMI mutation and the deletion mutation. Physical evidence for the lack of linkage between these mutations was obtained by CHEF gel analysis: probes of transforming vectors hybridized to two different chromosomes in the two mutants (Rose, 1996). The explanation for this apparent discrepancy between the original genetic data indicating that the *Tox1* is a single locus and the newer data is based on the observation that the *Tox1* locus is associated with the breakpoint of a reciprocal translocation. Thus, *Tox1* is two loci (designated *Tox1A* carrying *PKS1* and *Tox1B* carrying *DEC1* and *RED1*, respectively) located at or near the breakpoints of the reciprocally translocated chromosomes in race T. Therefore, they appear linked to each other when the translocation breakpoints are heterozygous in a cross *i.e.,* in any cross between race T and race O although these two loci are located on different chromosomes.

In summary, molecular genetic analyses using *Tox*$^-$ mutants have revealed several features of the *Tox1* locus (Yoder et al., 1997): 1) it is genetically inseparable from the breakpoints of the reciprocal translocation, 2) it is associated with highly repeated DNA (some of which is A+T rich), 3) there is more DNA in the *Tox*$^+$ genome than in a near isogenic *Tox*$^-$ genome, 4) it is not a single locus, but two loci carrying *PKS1* and *DEC1*, respectively, which are required for T-toxin production, 5) it is missing in all *Tox*$^-$ isolates.

PM-toxin Production by *M. zeae-maydis*

Unlike *C. heterostrophus*, *M. zeae-maydis* has several characteristics which made molecular genetic analysis difficult: homothallism, no non-toxin producing strain available, and lack of a stable genetic transformation system. Due to these deficiencies, even the role of PM-toxin in pathogenesis by *M. zeae-maydis* has not been evaluated.

As the first step to determine the pathological role of PM-toxin, the REMI procedure that has been shown to efficiently work in *C. heterostrophus* was adapted using a newly developed transformation procedure to generate *Tox*$^-$ mutants of *M. zeae-maydis*. Unexpectedly, REMI yielded a high frequency (six of 504) of transformants that failed to produce PM-toxin (Yun et al., 1998). All genetically stable *Tox*$^-$ mutants lost the ability to cause disease on T-cytoplasm corn, establishing a role of PM-toxin in pathogenesis (Yun et al., 1998). However, one of the merit of the REMI procedure which introduces tagged mutations into fungal genomes, making it easy to clone the gene(s) under investigation, was not realized in the case of the *M. zeae-maydis* *Tox*$^-$ mutants. Molecular analysis of five *Tox*$^-$ insertion mutants of *M. zeae-maydis* did not result in the cloning of genes involved in PM-toxin biosynthesis, in contrast to the case of insertion mutants of *C. heterostrophus* deficient in T-toxin biosynthesis (Rose et al., 1996; Yang et al., 1994). Examination of genomic DNAs from the five mutants revealed that only one of them was the result of a "REMI event", *i.e.,* the transformation vector linearized with *Hin*dIII inserted into a *Hin*dIII site in the fungal genome. However, no ORF was found at the insertion site of this mutant; a nearby transposon like sequence was shown by retransformation analysis to have no direct involvement in PM-toxin biosynthesis. Similarily, no ORFs were found at the sequences recovered from the single insertion sites of two other transformants, none of which was shown to be responsible for toxin production. For technical reasons, sequences flanking insertion sites were not recovered from the remaining two *Tox*$^-$ mutants. Interestingly, genomic DNAs from three of the five mutants including the real REMI one did not hybridized to a homolog of the *C. heterostrophus PKS1* (*ChPKS1*), indicating that they sustained deletions (Yun et al., 1998). Although these deletions are as yet uncharacterized, they all appear to involve in the same genomic region, tempting to speculate that the region of the genome represented by the *ChPKS1* homolog (*MzPKS1*) is essential for PM-toxin production. Despite the uncertainty regarding their origin, these deletions have been valuable in facilitating the cloning *MzPKS1* from *M. zeae-maydis* so that its role in PM-toxin biosynthesis can be assessed (Yun, 1998).

The first step of the cloning strategy that took advantage

of these deletions was to amplify a part of *MzPKS1* from *M. zeae-maydis* genomic DNA using degenerate primers derived from conserved regions of known *PKS* or fatty acid synthase (*FAS*) genes. The ketoacyl (KS) domain in PKS was specifically targeted for design of degenerate primers since the KS domain is known to be the most highly conserved among all enzymatic domains of FASs and PKSs (Siggaar-Anderson, 1993). One pair of degenerate primers amplified the expected size bands from *C. heterostrophus* race T and *M. zeae-maydis* wild type genomic DNAs, but not from a non-toxin producing race (O) of *C. heterostrophus*. Furthermore, one PCR product cloned from a single 290 bp band amplified from *M. zeae-maydis* genomic DNA not only showed 82% amino acid identity to the KS domain of *ChPKS1*, but also was missing in *MzPKS1* deletion mutants. Targeted disruption of the genomic copy of the PCR product revealed that the amplified KS-encoding PCR product was required for PM toxin production by *M. zeae-maydis*. These results proved that it is the part of the KS domain in *MzPKS1* (Yun, 1998).

Comparison of *M. zeae-maydis* and *C. heterostrophus Tox* loci

MzPKS1 encodes a multifunctional polypeptide (type I PKS) similar to *ChPKS1* (Yang et al., 1996). Like *ChPKS1*, it carries six enzymatic domains with the same order (Fig. 2). Three of the four putative intron positions are identical in both *PKS* genes. These similarities suggest that *MzPKS1* is a homolog of *ChPKS1*. Targeted disruption of *MzPKS1* indicated that *MzPKS1*, like *ChPKS1*, is required for biosynthesis of a polyketide toxin. The genes do, however, differ in major respects. First, they share only 59% overall amino acid identity. Second, DNAs flanking these two genes are different from each other. No additional genes have been found in the 5′ or 3′ flanking regions of *ChPKS1*, furthermore these regions are repetitive A+T rich (66.0-68.0%) sequences, whereas the corresponding regions of *MzPKS1* are not A+T rich. Instead, additional ORFs, *RED1* and *RED2*, were found 5′ of *MzPKS1* (Fig. 2), which showed high similarity to reductases necessary for various fatty acid or polyketide synthetic processes (Yun, 1998). Most sequences found to be similar to both RED1 and RED2 in the databases were β-ketoacyl-ACP reductases of various FASs or PKSs, which reduce β-keto groups generated by condensation steps in fatty acid or polyketide biosyntheses. Interestingly, RED2 also showed high similarity to the ketoreductases encoded by *A. nidulans verA* or *A. parasiticus ver-1*, which are located 5′ of PKS genes (*pksST* of *A. nidulans* and *pksL1* of *A. parasiticus*) required for biosyntheses of polyketide mycotoxins (sterigmatocystin and aflatoxin, respectively) in these two fungi, and involved in processing the polyketide backbones produced by these PKSs (Brown et al., 1996). Like *MzPKS1*, both *RED1* and *RED2* are essential for PM-toxin production (Yun, 1998). Their roles in polyketide biosynthesis, however, are not clear; they are thought to be involved in modification of a polyketide backbone and therefore may function in a fashion similar to that of *verA* and *ver-1* of *A. nidulans* and *A. parasiticus*, respectively. Five more ORFs (encoded by *Mzt1* to *Mzt5*), all showing similarity to fungal transposases, were found on the flanks of *MzPKS1* (Fig. 2). All *Mzt*s could belong to the *Fot1* family of class II fungal DNA transposons (Daboussi and Langin, 1994; Daboussi et al., 1992) (assumed to transpose directly through DNA copies and characterized by presence of terminal inverted repeats). These structural differences between *Tox* loci of *C. heterostrophus* and *M. zeae-maydis* are important in deter-

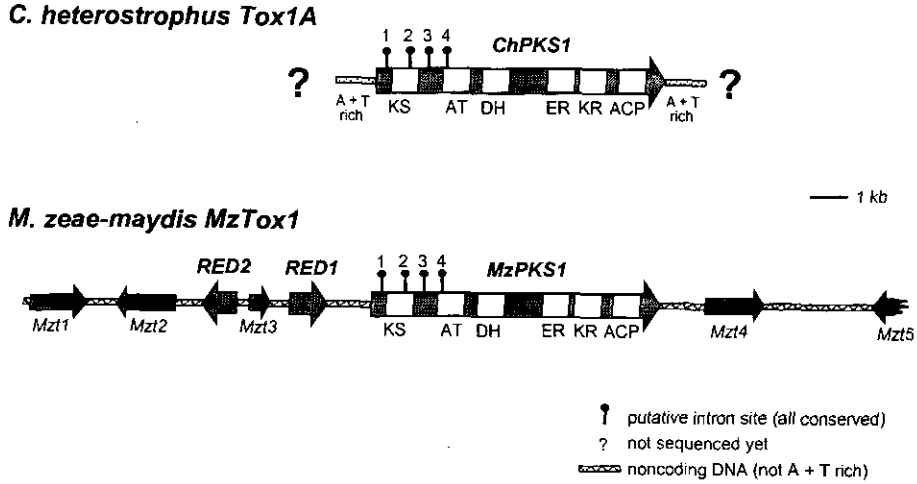

Fig. 2. Comparison of genomic organization of the two *Tox* loci. Abbreviations of each enzymatic domain: KS (β-ketoacyl synthase), AT (acyl transferase), DH (dehydratase), ER (enoyl reductase), KR (ketoreductase), and ACP (acyl carrier protein).

mining whether or not these two fungi have identical pathways for their polyketide biosyntheses or if they evolved independently (Yun, 1998).

Evolution of Polyketide Production by *C. heterostrophus* and *M. zeae-maydis*

The sudden appearance of these fungi as corn pathogens at the same time has led to speculation that a common genetic event may have occurred in both fungi. Thus, comparing PKS genes for biosyntheses of their polyketide toxins should provide an insight into understanding the possible genetic mechanisms. Previously, a possible evolutionary history for race T of *C. heterostrophus* has been hypothesized based on analysis of the genes required for T-toxin biosynthesis (Yang et al., 1996; Rose, 1996). Since genes for T-toxin production (*ChPKS1* and *DEC1*) are not present in the non-toxin producing race (O) or in other *Cochliobolus* spp., it has been suggested that race T arose from race O by acquiring these genes *via* horizontal transfer from an alien organism (Yang et al., 1996; Rose, 1996).

If this is true, what happened in *M. zeae-maydis*? The differences between the PKS genes from *M. zeae-maydis* and *C. heterostrophus* rule out the possibility of recent gene transfer to both fungi from the same source or from one to the other. Thirty years are not enough to cause the substantial divergence between two PKS genes. Alternative possibilities are that both fungi acquired their *PKS* genes recently from different sources or they obtained them from the same source (or one from the other) in the distant evolutionary past (Yun, 1998). Several observations support the recent gene transfer hypothesis (Yang et al., 1996; Rose, 1996) in *C. heterostrophus*: 1) these genes are not in the *Cochliobolus* relatives, 2) race T contains ~1 Mb more DNA than race O, this extra DNA is located on the pair of chromosomes reciprocally translocated with respect to race O, the *Tox* genes map to these chromosomes, 3) analysis of mating type gene distribution in the field (Leonard, 1973; Leonard, 1977; Alcorn, 1975) in the early 1970s indicated all race T isolates were *MAT-1*, while *MAT-1* and *MAT-2* were equally distributed in race O. By 1975 the *MAT* genes were equally distributed in race T also. Although it is not possible to do the same analysis in *M. zeae-maydis*, since there is no known naturally occurring *Tox*$^-$ field isolates, it would be reasonable to speculate that there was a recent horizontal transfer of genes into the *M. zeae-maydis* genome from a species different from the donor for *C. heterostrophus*. In this case, non-toxin producing strains of *M. zeae-maydis* might not be recognized as corn pathogens because their virulence is so low. Analysis of close *M. zeae-maydis* fungal relatives for the presence of the PM-toxin genes should be necessary.

The presence of transposon-like sequences at *MzTox1* may reflect the mechanism by which horizontal gene transfer occurred in *M. zeae-maydis*. If that is true, the five *Mzt*s dispersed in the *MzTox1* locus could be remnants of horizontal gene transfer event(s) as suggested for the *C. carbonum TOX2* gene; in this case a putative transposon (*Fcc1*) flanks both 5′ and 3′ ends of the *Tox2* locus which is unique to toxin producing strains and is required for biosynthesis of the cyclic peptide HC-toxin (Panaccione et al., 1996). There is, however, no experimental evidence to tell whether or not they were directly involved in the horizontal transfer, or which *Mzt*s, if so, were responsible (Yun, 1998).

Recently, horizontal gene transfer has been thought to play an important role in microbial evolution (Hacker, et al., 1997). Since acquisition of a DNA fragment by this mechanism may generate new variants of microbes quickly, it has been proposed as a possible genetic step for sudden appearance of new pathogenic races . Molecular analysis of new virulence factors of bacterial pathogens revealed that they were located in large chromosomal DNA regions called "pathogenicity islands" (PGI) (Blum, et al., 1995; Carniel, et al., 1996; Hacker, et al., 1997). Several characteristics of pathogenicity islands have been identified (Hacker, et al., 1997): i) large "alien" DNA fragment carrying a cluster of virulence factors unique to pathogenic strains, ii) usually flanked by direct repeats or mobility genes such as an integrase or transposase, and iii) the mobility genes seem to undergo a high rate of mutation, often leading to stop codons as a mechanism to maintain the stability of the newly acquired genes. Interestingly, new pathogenic races of fungal plant pathogens discussed here seem to have some of these general features of PGI. *C. carbonum* race 1 has a unique *Tox2* locus encoding several genes required for HC toxin production (Ahn and Walton, 1996), which are surrounded by putative transposases carrying stop codons (Panaccione et al., 1996). *C. heterostrophus* race T has unique *Tox1* loci carrying *PKS1* and *DEC1*, which are surrounded by highly repetitive A+T rich sequences. Like these two fungi, *M. zeae-maydis* has the *MzTox1* locus (its uniqueness remains to be determined) carrying a gene cluster required for polyketide toxin production, which is flanked by five *Mzt*s, each containing several stop codons. Therefore, horizontal gene transfer could be a common mechanism for evolution of new fungal virulent races or species (Yun, 1998).

References

Ahn, J. H. and Walton, J. D. 1996. Chromosomal organization of *TOX2*, a complex locus controlling host-selective toxin biosynthesis in *Cochliobolus carbonum*. *Plant Cell* 8:887-897.

Alcorn, J. L. 1975. Race-mating type association in Australian

population of *Cochliobolus heterostrophus*. *Plant Dis. Reprtr.* 59:708-711.

Arntzen, C. J., Haugh, M. F. and Bobick, S. 1973. Induction of stomatal closure by *Helminthosporium maydis* pathotoxin. *Plant Physiol.* 52:569-574.

Arny, D. C., Worf, G. L., Ahrens, R. W. and Lindsey, M. F. 1970. Yellow leaf blight of maize in Wisconsin: its history and the reactions of inbreds and crosses to the inciting fungus (*Phyllosticta* sp.). *Plant Dis. Reptr.* 54:281-285.

Ayers, J. E., Nelson, R. R., Koons, C. and Scheifele, G. L. 1970. Reaction of various maize inbreds and single crosses in normal and male-sterile cytoplasm to the Yellow Leaf Blight organism (*Phyllosticta* sp.). *Plant Dis. Reptr.* 54:277-280.

Blum, G., Falbo, B., Caprioli, A. and Hacker, J. 1995. Gene clusters encoding the cytotoxic necrotizing factor type 1, Prs-fimbriae and a-heolysin form the pathogenicity island II of the uropathogenic *Escherichia coli* strain J96. *FEMS Microb. Lett.* 126:189-196.

Braun, C. J., Siedow, J. N. and Levings, C. S., III. 1990. Fungal toxins bind to the Urf-13 protein in maize mitochondria and *Escherichia coli*. *Plant Cell* 2:153-162.

Brown, D. W., Yu, J. H., Kelkar, H. S., Fernandes, M., Nesbitt, T. C., Keller, N. P., Adams, T. H. and Leonard, T. J. 1996. Twenty-five coregulated transcripts define a sterigmatocystin gene cluster in *Aspergillus nidulans*. *Proc. Natl. Acad. Sci. USA* 93:1418-1422.

Carniel, E., Guilvout, I. and Prentice, M. 1996. Characterization of a large chromosomal 'high pathogenicity island' in biotype 1B *Yersinia enterocolitica*. *J. Bacteriol.* 178:6743-6751.

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.

Daboussi, M. J. and Langin, T. 1994. Transposable elements in the fungal plant pathogen *Fusarium oxysporum*. *Genetica* 93:49-59.

Daboussi, M. J., Langin, T. and Brygoo, Y. 1992. *Fot1* a new family of fungal transposable elements. *Mol. Gen. Genet.* 232:12-16.

Daly, J. M. and Barna, B. 1980. A differential effect of race T toxin on dark and photosynthetic CO_2 fixation by thin leaf slices from susceptible corn. *Plant Phyiol.* 66:580-583.

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

Fincham, J. R. S., Day, P. R. and Radford, A., 1979 Fungal Genetics, Blackwell Scientific Publications, Oxford.

Glab, N., Wise, R. P., Pring, D. R., Jacq, C. and Slonimski, P. 1990. Expression in *Saccharomyces cerevisiae* of a gene associated with cytoplasmic male sterility from maize: respiratory dysfunction and uncoupling of yeast mitochondria. *Mol. Gen. Genet.* 223:24-32.

Hacker, J., Blum-Oehler, G., Muhldorfer, I. and Tschape, H. 1997. Pathogenicity islands of virulent bacteria: structure, function and impat on microbial evolution. *Molecular Microbiology* 23:1089-1097.

Huang, J., Lee, S. H., Lin, C., Medici, R., Hack, E. and Myers, A. M. 1990. Expression in yeast of the T-*URF13* protein from texas male-sterile maize mitochondria confers sensitivity to methomyl and to texas-cytoplasm-specific fungal toxins. *EMBO* 9:339-348.

Kaspi, C. I. and Siedow, J. N. 1993. Cross-linking of the cms-T maize mitochondrial pore-forming protein Urf13 by N N′ dicyclohexylcarbodiimide and its effect on Urf13 sensitivity to fungal toxins. *J. Biol. Chem.* 268:5828-5833.

Kodama, M., Rose, M. S., Yang, G., Yun, S. H., Yoder, O. C. and Turgeon, B. G. 1999. The translocation-associated *Tox1* locus of *Cochliobolus heterostrophus* is two genetic elements on two different chromosomes. *Genetics* 151:585-596.

Kono, Y., Danko, S. J., Suzuki, Y., Takeuchi, S. and Daly, J. M. 1983. Structure of the host-specific pathotoxins produced by *Phyllosticta maydis*. *Tetrahedron Lett.* 24:3803-3806.

Kono, Y., Suzuki, Y., Takeuchi, S., Knoche, H. W. and Daly, J. M. 1985. Studies on the host-specific pathotoxins produced by *Helminthosporium maydis*, race T and *Phyllosticta maydis*: absolute configuration of PM-toxins and HMT-toxins. *Agri. Biol. Chem.* 49:559-562.

Kono, Y., Takeuchi, S., Kawarada, A., Daly, J. M. and Knoche, H. W. 1981. Studies on the host-specific pathotoxins produced in minor amounts by *Helminthosporium maydis* race T. *Bioorg. Chem.* 10:206-218.

Kono, Y., Takeuchi, S., Kawarda, A., Daly, J. M. and Knoche, H. W. 1980. Structure of the host-specific pathotoxins produced by *Helminthosporium maydis*, race T. *Tetrahedron Lett.* 21:1537-1540.

Korth, K. L., Kaspi, C. I., Siedow, J. N. and Levings, C. S., III. 1991. Urf13 a maize mitochondrial pore-forming protein is oligomeric and has a mixed orientation in *Escherichia coli* plasma membranes. *Proc. Natl. Acad. Sci. USA* 88:10865-10869.

Korth, K. L. and Levings, C. S. I. 1993. Baculovirus expression of the maize mitochondria protein URF13 confers insecticidal activity in cell cultures and larvae. *Proc. Natl. Acad. Sci.* USA 90:3388-3392.

Kuspa, A. and Loomis, W. F. 1992. Tagging developmental genes in *Dictyostelium* by restriction enzyme- mediated integration of plasmid DNA. *Proc. Natl. Acad. Sci. U S A* 89:8803-8807.

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.

Leonard, K. J. 1977. Races of *Bipolaris maydis* in the Southeastern U. S. from 1974-1976. *Plant Dis. Reptr.* 61:914-915.

Levings, C. S., III and Siedow, J. N. 1992. Molecular Basis of Disease Susceptibility in the Texas Cytoplasm of Maize. *Plant. Mol. Biol.* 19:135-147.

Lim, S. M. and Hooker, A. L. 1971. Southern corn leaf blight: genetic control of pathogenicity and toxin production in race T and race O of *Cochliobolus heterostrophus*. *Genetics* 69:115-

117.

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* by restriction enzyme-mediated integration. *Proc. Natl. Acad. Sci. USA* 91:12649-12653.

Matthew, D. E., Gregory, P. and Gracen, V. E. 1979. *Helminthosoprium maydis* race T toxin induces leakage of NAD+ from T cytoplasm corn mitochondria. *Plant Physiol.* 63:1149-1153.

Miller, R. and Koeppe, D. E. 1971. Southern corn leaf blight: susceptible and resistant mitochondria. *Science* 173:67-69.

Panaccione, D. G., Pitkin, J. W., Walton, J. D. and Annis, S. L. 1996. Transposon-like sequences at the *TOX2* locus of the plant-pathogenic fungus *Cochliobolus carbonum*. *Gene* 176:103-109.

Payne, G., Knoche, H. W., Kono, Y. and Daly, J. M. 1980. Biological activity of purified host-specific pathotoxin produced by *Bipolaris* (*Helminthosporium*) *maydis* race T. *Physiol. Plant Pathol.* 16:227-239.

Payne, G. A., Kono, Y. and Daly, J. M. 1980. A comparison of purified host-specific toxin from *Helminthosporium maydis*, race T, and its acetate derivative on oxidation by mitochondria from susceptible and resistant plants. *Plant Physiol.* 65:785-791.

Perkins, D. D. 1977. The manifestation of chromosome rearrangements in unordered asci of *Neurospora crassa*. *Genetics* 77:459-489.

Rose, M. S. 1996. Molecular genetics of polyketide toxin production in *Cochliobolus heterostrophus*. Ph.D. Dissertation, Cornell University, Ithaca, pp. 217.

Rose, M. S., Yoder, O. C. and Turgeon, B. G. 1996. A decarboxylase required for polketide toxin production and high virulence by *Cochliobolus heterostrophus*. 8th Int. Symp. Mol. Plant-Microbe Int., Knoxville p J-49.

Scheifele, G. L. and Nelson, R. R. 1969. The occurrence of *Phyllosticta* leaf spot of corn in Pennsylvania. *Plant Dis. Reptr.* 53:186-189.

Schiestl, R. H. and Petes, T. D. 1991. Integration of DNA fragments by Illegitimate recombination in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 88:7585-7589.

Siedow, J. N., Rhoads, D. M., Ward, G. C. and Levings, C. S., III. 1995. The relationship between the mitochondrial gene *T-urf13* and fungal pathotoxin sensitivity in maize. *Biochimica et Biophysica Acta.* 1271:235-240.

Siggaard-Anderson, M. 1993. Conserved residues in condensing enzyme domains of fatty acid synthases and related sequences. *Protein Seq. Data Anal.* 5:325-335.

Taga, M., Bronson, C. R. and Yoder, O. C. 1985. Nonrandom abortion of ascospores containing alternate alleles at the *Tox-1* locus of the fungal plant pathogen *Cochliobolus heterostophus*. *Can. J. Genet. Cytol.* 27:450-456.

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.

von Allmen, J. M., Rottmann, W. H., Gengenbach, B. G., Harvey, A. J. and Lonsdale, D. M. 1991. Transfer of methomyl and HmT-toxin sensitivity from T-cytoplasm maize to tobacco. *Mol. Gen. Genet.* 229:405-412.

Walton, J. D., Earle, E. D., Yoder, O. C. and Spanswick, R. M. 1979. Reduction of adenosine triphosphate levels in susceptible maize mesophyll protoplasts by *Helminthosporium maydis* race T toxin. *Plant Physiol.* 63:806-810.

Yang, G., Rose, M. S., Turgeon, B. G. and Yoder, O. C. 1996. A polyketide synthase is required for fungal virulence and production of the polyketide T-toxin. *Plant Cell* 8:2139-2150.

Yang, G., Turgeon, B. G. and Yoder, O. C. 1994. Toxin-deficient mutants from a toxin-sensitive transformant of *Cochliobolus heterostrophus*. *Genetics* 137:751-757.

Yoder, O. C. 1980. Toxins in pathogenesis. *Ann. Rev. Phytopathol.* 18:103-129.

Yoder, O. C., 1988. *Cochliobolus heterostrophus*, cause of Southern Corn Leaf Blight, In: *Genetics of Plant Pathogenic Fungi*, ed. by G. S. Sidhu. pp. 93-112, Academic Press, Sandiego.

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., Macko, V., Wolpert, T. J. and Turgeon, B. G., 1997. *Cochliobolus* spp. and their host-specific toxins, In: *The Mycota Vol. 5: Plant Relationships, Part A*, ed. by G. Caroll and P. Tudzynski. pp. 145-166, Springer-Verlag, Berlin.

Yoder, O. C. and Turgeon, B. G. 1996. Molecular-genetic evaluation of fungal molecules for roles in pathogenesis to plants. *J. Genet.* 75:425-440.

Yun, S. H. 1998. Molecular genetics and manipulation of pathogenicity and mating determinants in *Mycosphaerella zeae-maydis* and *Cochliobolus heterostrophus*. Ph.D. dissertation, Cornell University, Ithaca, NY, pp. 285.

Yun, S. H., Turgeon, B. G. and Yoder, O. C. 1998. REMI-induced mutants of *Mycosphaerella zeae-maydis* lacking the polyketide PM-toxin are deficient in pathogenesis to corn. *Physiol. Mol. Plant Pathol.* 52:53-66.