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Communication

4-*O*-Acetylation and 3-*O*-Acetylation of Trichothecenes by Trichothecene 15-*O*-Acetyltransferase Encoded by *Fusarium Tri3*

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In the biosynthesis of *Fusarium* trichothecenes, the C-3 hydroxyl group of isotrichodermol must be acetylated by TRI101 for subsequent pathway genes to function. Despite the importance of this 3-*O*-acetylation step in biosynthesis, *Tri101* is both physically and evolutionarily unrelated to other *Tri* genes in the trichothecene gene cluster. To gain insight into the evolutionary history of the cluster, we purified recombinant TRI3 (rTRI3), one of the two cluster gene-encoded trichothecene *O*-acetyltransferases, and examined to determine whether this 15-*O*-acetyltransferase can add an acetyl to the C-3 hydroxyl group of isotrichodermol. When a high concentration of rTRI3 was used in the assay (final concentration, 50 μM), we observed 3-*O*-acetylation activity against isotrichodermol that was more than 10^5 times less efficient than the known 15-*O*-acetylation activity against 15-deacetylcalonectrin. The rTRI3 protein also exhibited 4-*O*-acetylation activity when nivalenol was used as a substrate; in addition to 15-acetylnivalenol, di-acetylated derivatives, 4,15-diacetylnivalenol, and, to a lesser extent, 3,15-diacetylnivalenol, were also detected at high enzyme concentrations. The significance of the trace trichothecene 3-*O*-acetyltransferase activity detected in rTRI3 is discussed in relation to the evolution of the trichothecene gene cluster.

Key words: biosynthesis gene cluster; fungal secondary metabolism; *Fusarium graminearum* (*Gibberella zeae*); sesquiterpene; trichothecene mycotoxins

Fungal genes involved in secondary metabolism often compose a unit with all the genes necessary for production of the metabolite clustered together in the host genome. To explain this self-contained nature of secondary metabolite gene clusters, Walton hypothesized that a selective advantage is conferred on the genes that comprise the cluster by the clustering, separately from any advantage that the product of that cluster confers on the host organism.[1)] Occasionally, the secondary metabolite genes are duplicated, separated, and/or only partially clustered due to known genomic forces, such as translocation, inversion, and unequal crossing-over. Examples include the genes for HC-toxin biosynthesis in *Cochliobolus carbonum*[2)] and the genes for T-toxin biosynthesis in *Cochliobolus heterostrophus*.[3)] Nevertheless, all the genes necessary for toxin production are thought to have occurred originally in a single gene cluster in either case. Hence, the ancestral secondary metabolite gene clusters are thought to have been self-contained (complete) when the producing fungi acquired them by horizontal gene transfer.

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*** TT initially made all the new findings presented in this paper. NTA re-confirmed the findings by further analysis and obtained the main data for presentation.

Abbreviations: 3-ADON, 3-acetyldeoxynivalenol; 15-ADON, 15-acetyldeoxynivalenol; 3-ANIV, 3-acetylnivalenol; 4-ANIV, 4-acetylnivalenol; 15-ANIV, 15-acetylnivalenol; CAL, calonectrin; 15-deCAL, 15-deacetylcalonectrin; 3,4-diANIV, 3,4-diacetylnivalenol; 3,15-diADON, 3,15-diacetyldeoxynivalenol; 3,15-diANIV, 3,15-diacetylnivalenol; 4,15-diANIV, 4,15-diacetylnivalenol; DON, deoxynivalenol; ITD, isotrichodermin; NIV, nivalenol; 3,4,15-triANIV, 3,4,15-triacetylnivalenol

In the case of trichothecene biosynthesis in *Fusarium* species, the extant trichothecene gene cluster is not self-contained.[4,5] Most of the pathway genes cannot function unless the C-3 hydroxyl group of isotrichodermol, the first pathway intermediate with a toxic trichothecene skeleton, is acetylated by a pathway enzyme encoded by *Tri101*.[6,7] This gene is a non-cluster trichothecene gene with a different evolutionary history from other *Tri* genes;[8,9] that is, the possibility that *Tri101* was originally in the gene cluster and later separated from other *Tri* genes was unambiguously excluded.[8,10,11] Its functional homolog has also been found in several other ascomycetes, but mostly with much less catalytic activity than the trichothecene-producer TRI101.[11] Therefore, only few fungal species appear to serve as suitable hosts for the persistence of the non-self-contained (incomplete) trichothecene gene cluster. Given the importance of complete clustering to the persistence of secondary metabolite genes,[1] it might be necessary to consider a scenario alternative to the fortuitous acquisition and propagation of a *Tri101*-dependent gene cluster.

To determine whether traces of trichothecene 3-*O*-acetyltransferase activity exist among known acetylases encoded by the trichothecene cluster genes, we have undertaken expression of *Tri3* (known as the trichothecene 15-*O*-acetyltransferase gene responsible for acetylation at C-15 of 15-deacetylcalonectrin, 15-deCAL, in the biosynthesis of trichothecenes)[12] and *Tri7* (known as the trichothecene 4-*O*-acetyltransferase gene)[13] in *Escherichia coli*. *Fusarium graminearum* MAFF 111233 (*F. graminearum* lineage 6, newly named *Fusarium asiaticum*)[14] was used as the source of trichothecene biosynthetic genes in this study. In the construction of the expression vector, cDNA was synthesized from RNA isolated from mycelia grown in RF medium[15] using a Superscript III™ First-Strand Synthesis system (Invitrogen, Carlsbad, CA). Among the two acetyltransferases, *Tri7* failed to be expressed at a detectable level by any means. Hence, we focused on the purification and characterization of recombinant TRI3, whose cDNA was successfully expressed at a high level in *E. coli*, as follows: (i) the entire coding region of *Tri3* was amplified by PCR with primers MFTri3ATG/Nde (5′-TCATATGAGCGCTTCATCCTCCGCCTTG-3′) and MFTri3TAA/Bam (5′-TGGATCCTTACAATTTGAA-TGCCAGCATGA-3′) using KOD-plus DNA polymerase (Toyobo, Osaka, Japan), (ii) the *Nde*I-*Bam*HI fragment of the amplified *Tri3* product was inserted between the corresponding sites of a pCold III™ expression vector (Takara Bio, Kusatsu, Japan), and (iii) the resulting vector, pColdIII-MF*Tri3*, was transformed to *E. coli* Rosetta™ 2 (DE3) competent cells (Novagen, Madison, WI) for production of the recombinant enzyme. The recombinant TRI3 protein produced from this vector (hereafter referred to as rTRI3) contains *N*-terminal extensions of six amino acid residues derived from the pCold III™ vector. Its theoretical M_r and pI have been calculated to be 57,878 and 5.12 respectively.

After overexpression of *Tri3* following the protocol of the cold shock expression system (Takara Bio), a recombinant protein fraction was prepared from disrupted bacterial cells as described previously.[16] The sample was then applied to a HiTrap S HP (5 ml) column (GE Healthcare UK, Buckinghamshire, UK) equilibrated with buffer A (20 mM Tris–HCl, pH 6.5). While most proteins were trapped to the cation exchange column at this pH, rTRI3 readily passed through the column. The flow-through fraction was concentrated with Centriprep-30/Centricon-30 (Millipore, Billerica, MA) and further purified by gel permeation chromatography with a Superdex 75 HR10/30 column (GE Healthcare UK) using buffer A containing 0.1 M NaCl. The rTRI3 protein was eluted as a single band at about 9.6 ml (M_r 55 k) (Supplemental Fig. 1A; see *Biosci. Biotechnol. Biochem.* Web site). The enzyme concentration was determined using the BCA protein assay kit (Thermo Fisher Scientific, Rockford, IL). When deoxynivalenol (DON) was used as the substrate, the purified enzyme showed maximal C-15 acetylation activity at pH 6.5–7.5 (Supplemental Fig. 1B), and at 15–37 °C (Supplemental Fig. 1C).

On HPLC analysis, it is not possible to monitor the trichothecene pathway's intermediates (which lack a keto at C-8) by measuring UV absorbance at 254 nm. Hence, TLC was used to estimate roughly the amount of the acetylated trichothecene product. Samples were developed on a TLC plate using ethyl acetate/toluene (3:1) as the solvent, and trichothecenes separated on the plate were visualized by a color reaction using a chromogenic reagent, 4-(*p*-nitrobenzyl)pyridine (NBP).[17] Substrates trichothecene and acetyl-CoA (final concentrations, 0.1 mg/ml and 2 mM respectively) were mixed with various concentrations of rTRI3 in 100 mM Tris–HCl, pH 7.0, and the reaction was carried out at 30 °C. The enzyme concentration and incubation time were adjusted so that the reaction mixture contained only similar low levels of acetylated product on a TLC plate.

When the assay was carried out using a high concentration of rTRI3 protein (50 μM), acetylation of a small fraction of isotrichodermol giving isotrichodermin (ITD) was observed with 18 h of incubation (see Fig. 1A). This result indicates that TRI3 has trace activity against the C-3 hydroxyl group of isotrichodermol. To control the acetylation reaction to a level as low as that obtained as above using the regular substrate 15-deCAL, the enzyme concentration and incubation time had to be reduced to 5 nM ($1/10^4$ concentration) and 1 h ($1/18$ time) (Fig. 1B). This means that the 3-*O*-acetylation activity against isotrichodermol was lower than the 15-*O*-acetylation activity against 15-deCAL on the order of 10^5 or more. When 3-acetyldeoxynivalenol (3-ADON) and DON were used as substrate and the reaction was allowed to proceed for 1 h, the amount of

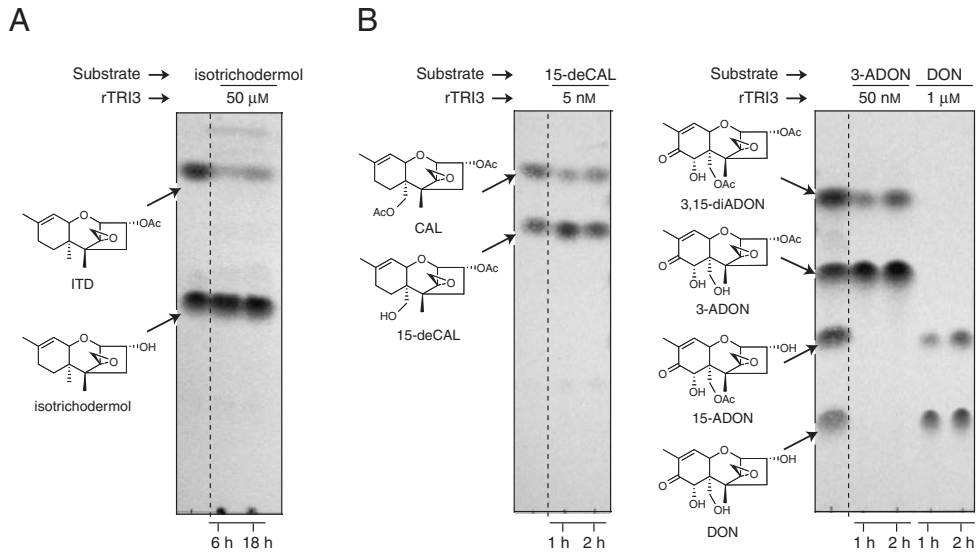

Fig. 1. The TRI3 Enzyme Exhibits Trace Acetylation Activity against the C-3 Hydroxyl Group of Isotrichodemol.

After the enzyme reaction, trichothecenes were extracted with ethyl acetate, and aliquots (approximately 25 μg) were separated on TLC plates. Substrate trichothecenes and enzyme concentrations are indicated at the top, and incubation times are indicated at the bottom. Trichothecene standards loaded on the TLC plates are indicated by arrows. A, 3-*O*-Acetylation of isotrichodermol by rTRI3. Isotrichodermol was purified from the *Tri101*$^{-}$ mutant of *F. graminearum*.[20] Formation of ITD was also confirmed by GC-MS analysis (data not shown). B, 15-*O*-Acetylation of DON-type trichothecenes by rTRI3. Three known substrates, 15-deCAL, 3-ADON, and DON, were used in the acetylation assay to roughly evaluate the 3-*O*-acetylation activity against isotrichodermol. 15-deCAL, calonectrin (CAL), 3,15-diacetyldeoxynivalenol (3,15-diADON), and 15-acetyldeoxynivalenol (15-ADON) were purified from the *Tri*$^{-}$ mutants of *F. graminearum*,[20] and 3-ADON and DON were purchased from Wako Pure Chemical Industries (Osaka, Japan).

the enzyme necessary to achieve similar levels of 15-*O*-acetylation increased on the order of 10 (50 nM) and 200 (1 μM) respectively, as compared to that obtained with 15-deCAL (Fig. 1B). Nevertheless, the 15-*O*-acetylation activities against these type B trichothecenes were still much greater than the 3-*O*-acetylation activity against isotrichodermol. The extremely weak activity of TRI3 against isotrichodermol does not contradict a previous report that *Tri101*$^{-}$ mutants (carrying a functional copy of *Tri3*) of *Fusarium sporotrichioides* were blocked in their ability to produce T-2 toxin and accumulated isotrichodermol.[7]

To examine the enzymatic properties of TRI3 in more detail, we used nivalenol (NIV) next as a substrate. The reaction was carried out for 12 h with different concentrations of the enzyme. As shown in Fig. 2A, the C-15 hydroxyl group of NIV was first acetylated to give 15-acetynivalenol (15-ANIV). Completion of C-15 acetylation was then followed by C-4 acetylation or, to a lesser extent, C-3 acetylation, to give 4,15-diacetylnivalenol (4,15-diANIV) and 3,15-diacetylnivalenol (3,15-diANIV) respectively, albeit with much less efficiency than C-15 acetylation. This result indicates that TRI3 is a multifunctional enzyme that can acetylate two of the three hydroxyl groups, the C-15 and C-4 hydroxyl groups or, to a lesser extent, the C-15 and C-3 hydroxyl groups, attached to the trichothecene skeleton. The acetylation of two hydroxyl groups is similar to the action of chloramphenicol acetyltransferase (CAT), which acetylates the antibiotic at one (chloramphenicol 3-acetate) or both (chloramphenicol 1,3-diacetate) of its two hydroxyl groups. However, CAT can add an acetyl only to the first acetylation site of chloramphenicol, and the second acetylation is based on re-acetylation of the first site during the non-enzymatic interconversion of mono-acetylated derivatives at higher pH values.[18] In this respect, TRI3 is a novel type of multifunctional acetylase distinct from CAT and its related acetylases, although it belongs to the CAT class of acetyltransferases containing the consensus sequence HXXXDG.[19]

We also used all possible mono- and di-acetylated NIV derivatives as substrate in subsequent analyses: 3-acetylnivalenol (3-ANIV), 4-acetylnivalenol (4-ANIV), 15-ANIV, 3,4-diacetylnivalenol (3,4-diANIV), 3,15-diANIV, and 4,15-diANIV (see Fig. 2B). Based on the enzyme concentration and incubation time used in the assay, the following properties of TRI3 were identified: (i) the presence of an acetyl at C-3 significantly accelerates 15-*O*-acetylation (compare lane 3-ANIV and Fig. 2A), (ii) the presence of an acetyl at C-4 blocks 15-*O*-acetylation (see lane 4-ANIV), (iii) the presence of both C-3 and C-4 acetyls allows an extremely limited level of 15-*O*-acetylation (see lane 3,4-diANIV), and (iv) neither C-4 nor C-3 can be acetylated if the other two positions are occupied by acetyls (see lanes 3,15-diANIV and 4,15-diANIV). Considering that the biosynthetic pathway of NIV-type trichothecenes 4,15-diANIV/4-ANIV proceed *via* 3,15-diANIV and 3,4,15-triacetylnivalenol (3,4,15-triANIV),

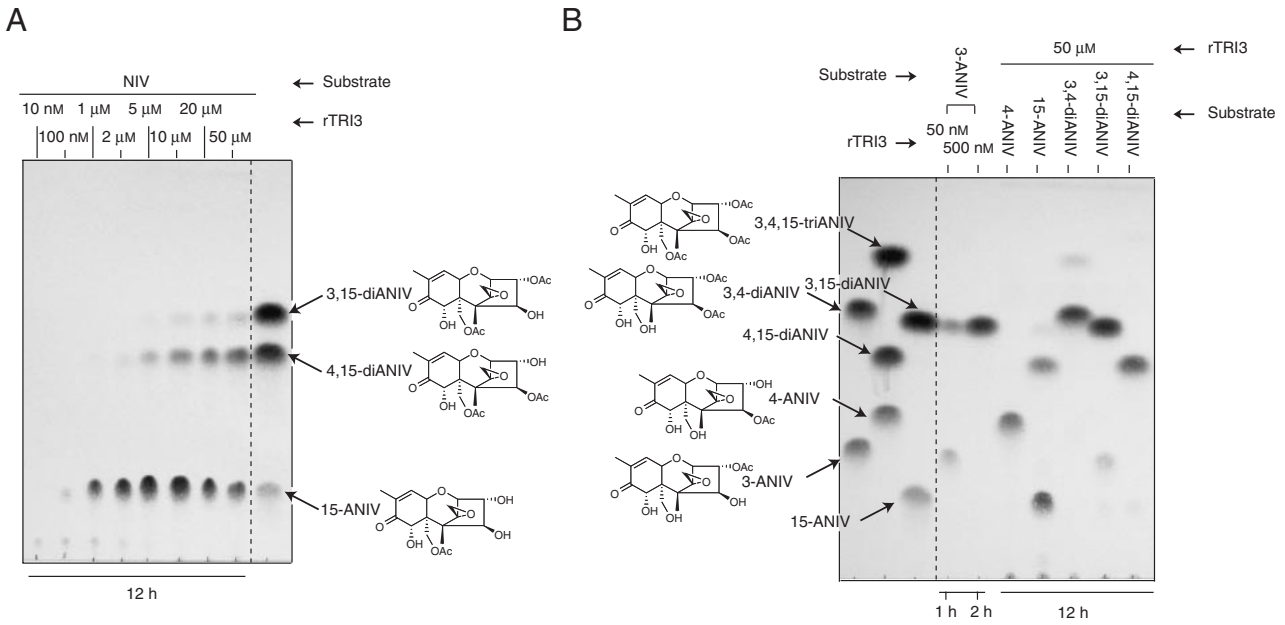

Fig. 2. The TRI3 Enzyme Acetylates the Hydroxyl Groups at C-15, C-4, and/or C-3 of NIV-Type Trichothecenes.

After the enzyme reaction, trichothecenes were extracted with ethyl acetate, and aliquots (approximately 25 μg) were separated on TLC plates. Substrate trichothecenes and enzyme concentrations are indicated at the top, and incubation times are indicated at the bottom. Trichothecene standards loaded on the TLC plates are indicated by arrows. A, Acetylation of NIV by rTRI3. The substrate NIV (Wako) was hardly detected on the TLC plate, because only a limited amount of this mycotoxin is extractable with ethyl acetate. The reaction products were also analyzed by GC-MS to confirm their structures (data not shown). B, Acetylation of mono- and di-acetylated NIV derivatives by rTRI3. A small amount of 3-ANIV was produced when 3,15-diANIV was used as the substrate. This reverse reaction is catalyzed by the TRI3 enzyme and not by an endogenous deacetylase derived from *E. coli*, because no such product was detected in the control reaction mixture (data not shown). 4-ANIV (fusarenone X) was purchased from Wako. 3-ANIV and 3,4-diANIV were prepared by *in vitro* acetylation of NIV and 4-ANIV respectively by TRI101.[6] 4,15-diANIV was purified from wild-type *F. graminearum* MAFF 111233, and 15-ANIV and 3,15-diANIV were purified from the *Tri7*$^{-}$ mutant.[20]

in that order,[20] the inability of TRI3 to acetylate the three positions at the same time, as indicated in (iv), necessitated the involvement of *Tri7* at the last acetylation step at C-4. This is in consistent with the results of previous targeted gene-disruption experiments, in which deletion of *Tri7* (with a functional copy of *Tri3*) caused the C-4 hydroxyl group of trichothecenes no longer to be acetylated.[13,21]

In view of the highly unstable nature of fungal genomes,[22] secondary metabolite pathway genes in complete gene clusters have greater chances of persistence than dispersed pathway genes, because the former can be transmitted horizontally: if genes for a novel metabolic process can move by horizontal gene transfer, then they have a reasonable chance of conferring a new selective advantage on the new host, which promotes the survival of the new host and also of the genes.[1] In addition to these advantages of clustering, secondary metabolite gene clusters might be maintained by the advantage that they are subject to efficient transcriptional regulation, presumably at the level of chromatin structure.[23] To date, the only scenario for the evolution of the trichothecene gene cluster has been that an ancestral *Fusarium* species, possessing a strong trichothecene 3-*O*-acetyltransferase activity for trichothecene resistance, fortuitously acquired the non-self-contained trichothecene gene cluster. However, secondary metabolite genes in an incomplete gene cluster are evolutionarily less advantageous for persistence than those in a complete gene cluster.[1] An alternative scenario is that the ancestral trichothecene gene cluster originally contained all the genes necessary for the biosynthesis of its product. Although the contribution of *Tri3* in extant trichothecene-producing *Fusarium* is too small to feature in the biosynthetic step carried out by *Tri101* (isotrichodermol → ITD), the trace 3-*O*-acetylation activity discovered in this study suggests the self-contained nature of the ancestral trichothecene gene cluster. If this is the case, the presence of two functional enzymes for 3-*O*-acetylation (TRI101 and TRI3) led to a relaxation of selective constraints, which eventually resulted in the loss of the trichothecene 3-*O*-acetyltransferase activity of TRI3. In either case, the 3-*O*-acetylation step and its enzymes are unique and important in the biosynthesis of *Fusarium* trichothecenes.

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References

1) Walton, J. D., Horizontal gene transfer and the evolution of secondary metabolite gene clusters in fungi: an hypothesis. *Fungal Genet. Biol.*, **30**, 167–171 (2000).
2) Ahn, J. H., Cheng, Y. Q., and Walton, J. D., An extended physical map of the *TOX2* locus of *Cochliobolus carbonum* required for biosynthesis of HC-toxin. *Fungal Genet. Biol.*, **35**, 31–38 (2002).
3) Turgeon, B. G., and Baker, S. E., Genetic and genomic dissection of the *Cochliobolus heterostrophus Tox1* locus controlling biosynthesis of the polyketide virulence factor T-toxin. *Adv. Genet.*, **57**, 219–261 (2007).
4) Kimura, M., Tokai, T., O'Donnell, K., Ward, T. J., Fujimura, M., Hamamoto, H., Shibata, T., and Yamaguchi, I., The trichothecene biosynthesis gene cluster of *Fusarium graminearum* F15 contains a limited number of essential pathway genes and expressed non-essential genes. *FEBS Lett.*, **539**, 105–110 (2003).
5) Brown, D. W., Dyer, R. B., McCormick, S. P., Kendra, D. F., and Plattner, R. D., Functional demarcation of the *Fusarium* core trichothecene gene cluster. *Fungal Genet. Biol.*, **41**, 454–462 (2004).
6) Kimura, M., Kaneko, I., Komiyama, M., Takatsuki, A., Koshino, H., Yoneyama, K., and Yamaguchi, I., Trichothecene 3-*O*-acetyltransferase protects both the producing organism and transformed yeast from related mycotoxins. Cloning and characterization of *Tri101*. *J. Biol. Chem.*, **273**, 1654–1661 (1998).
7) McCormick, S. P., Alexander, N. J., Trapp, S. E., and Hohn, T. M., Disruption of *TRI101*, the gene encoding trichothecene 3-*O*-acetyltransferase, from *Fusarium sporotrichioides*. *Appl. Environ. Microbiol.*, **65**, 5252–5256 (1999).
8) O'Donnell, K., Kistler, H. C., Tacke, B. K., and Casper, H. H., Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. *Proc. Natl. Acad. Sci. USA*, **97**, 7905–7910 (2000).
9) Kimura, M., Matsumoto, G., Shingu, Y., Yoneyama, K., and Yamaguchi, I., The mystery of the trichothecene 3-*O*-acetyltransferase gene. Analysis of the region around *Tri101* and characterization of its homologue from *Fusarium sporotrichioides*. *FEBS Lett.*, **435**, 163–168 (1998).
10) Kimura, M., Tokai, T., Matsumoto, G., Fujimura, M., Hamamoto, H., Yoneyama, K., Shibata, T., and Yamaguchi, I., Trichothecene nonproducer Gibberella species have both functional and nonfunctional 3-*O*-acetyltransferase genes. *Genetics*, **163**, 677–684 (2003).
11) Tokai, T., Fujimura, M., Inoue, H., Aoki, T., Ohta, K., Shibata, T., Yamaguchi, I., and Kimura, M., Concordant evolution of trichothecene 3-*O*-acetyltransferase and an rDNA species phylogeny of trichothecene-producing and non-producing fusaria and other ascomycetous fungi. *Microbiology*, **151**, 509–519 (2005).
12) McCormick, S. P., Hohn, T. M., and Desjardins, A. E., Isolation and characterization of *Tri3*, a gene encoding 15-*O*-acetyltransferase from *Fusarium sporotrichioides*. *Appl. Environ. Microbiol.*, **62**, 353–359 (1996).
13) Brown, D. W., McCormick, S. P., Alexander, N. J., Proctor, R. H., and Desjardins, A. E., A genetic and biochemical approach to study trichothecene diversity in *Fusarium sporotrichioides* and *Fusarium graminearum*. *Fungal Genet. Biol.*, **32**, 121–133 (2001).
14) O'Donnell, K., Ward, T. J., Geiser, D. M., Kistler, H. C., and Aoki, T., Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. *Fungal Genet. Biol.*, **41**, 600–623 (2004).
15) Tokai, T., Koshino, H., Kawasaki, T., Igawa, T., Suzuki, Y., Sato, M., Fujimura, M., Eizuka, T., Watanabe, H., Kitahara, T., Ohta, K., Shibata, T., Kudo, T., Inoue, H., Yamaguchi, I., and Kimura, M., Screening of putative oxygenase genes in the *Fusarium graminearum* genome sequence database for their role in trichothecene biosynthesis. *FEMS Microbiol. Lett.*, **251**, 193–201 (2005).
16) Igawa, T., Ochiai-Fukuda, T., Takahashi-Ando, N., Ohsato, S., Shibata, T., Yamaguchi, I., and Kimura, M., New TAXI-type xylanase inhibitor genes are inducible by pathogens and wounding in hexaploid wheat. *Plant Cell Physiol.*, **45**, 1347–1360 (2004).
17) Takitani, S., Asabe, Y., Kato, T., Suzuki, M., and Ueno, Y., Spectrodensitometric determination of trichothecene mycotoxins with 4-(*p*-nitrobenzyl)pyridine on silica gel thin-layer chromatograms. *J. Chromatogr.*, **172**, 335–342 (1979).
18) Thibault, G., Guitard, M., and Daigneault, R., A study of the enzymatic inactivation of chloramphenicol by highly purified chloramphenicol acetyltransferase. *Biochim. Biophys. Acta*, **614**, 339–342 (1980).
19) Alexander, N. J., McCormick, S. P., and Hohn, T. M., The identification of the *Saccharomyces cerevisiae* gene *AYT1*(ORF-YLL063c) encoding an acetyltransferase. *Yeast*, **19**, 1425–1430 (2002).
20) Kimura, M., Tokai, T., Takahashi-Ando, N., Ohsato, S., and Fujimura, M., Molecular and genetic studies of *Fusarium* trichothecene biosynthesis: pathways, genes, and evolution. *Biosci. Biotechnol. Biochem.*, **71**, 2105–2123 (2007).
21) Lee, T., Han, Y. K., Kim, K. H., Yun, S. H., and Lee, Y. W., *Tri13* and *Tri7* determine deoxynivalenol- and nivalenol-producing chemotypes of *Gibberella zeae*. *Appl. Environ. Microbiol.*, **68**, 2148–2154 (2002).
22) Sweigard, J. A., Carroll, A. M., Kang, S., Farrall, L., Chumley, F. G., and Valent, B., Identification, cloning, and characterization of *PWL2*, a gene for host species specificity in the rice blast fungus. *Plant Cell*, **7**, 1221–1233 (1995).
23) Keller, N. P., Turner, G., and Bennett, J. W., Fungal secondary metabolism—from biochemistry to genomics. *Nat. Rev. Microbiol.*, **3**, 937–947 (2005).