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Role of Histone Deacetylase in Plant Pathogenic Fungus *Cochliobolus carobum* Pathogenesity

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The filamentous fungus *Cochliobolus carbonum* causes a leaf spot and ear rot of corn. Isolates of the ascomycetes *Cochliobolus carbonum* that produce the cyclic tetrapeptide HC-toxin (cyclo[D-Pro-L-Ala-D-Ala-L-Aeo], where Aeo stands for 2-amino-9,10-epoxi-8-oxodecanoic acid) are specifically virulent on maize lines that are homozygous for the recessive allele of *Hm*. Isolates of *C. carbonum* that do not produce HC-toxin ($Tox2^-$) are only weakly pathogenic regardless of the host genotype, and maize lines with the dominant allele of *Hm* are insensitive to HC-toxin and resistant to HC-toxin-producing ($Tox2^+$) isolates of the fungus. Several genes (*HTS1, TOXA, TOXC, TOXD, TOXE, TOXF,* and *TOXG*) that had an established or putative role in HC-toxin biosynthesis were identified. These genes are only present in Tox2+ isolates.

In other hand, it was shown that HC-toxin inhibits maize histone deacetylases (HDAC). Reversible histone acetylation is a major mechanism by which chromatin structure and gene regulation are integrated. The level of acetylation of histones in chromatin is a product of the balance between the activities of two enzymes, hisotne acetyltransferase (HAT) and histone deacetyloase (HDAC), and both enzymes have been demonstrated to be necessary for the correct expression of a number of genes. HC-toxin inhibits all three types of maize HDAC, the inhibition is host-selective in vivo but not in vitro. Hyperacetylation of histone H3 and H4 is dectable 96 hr after inoculation by $Tox2^+$, but not $Tox2^-$ isolates of *C. carbonum*, consistent with HDAC's being a biologically relevant site of action of HC-toxin during infection.

A fundamental question that arises in light of HDAC's being the site of action of HC-toxin is how the fungus prevents killing itself. It was found that the HDAC activity in crude extracts of *C. carbonum* $Tox2^+$ isolates is insensitive to HC-toxin. HDACs from other organisms (plants, animals, insects, protozoans, and other fungi) are sensitive. HDAC activity of another fungus, *Diheterospora chalmydospora*, which produces an HDAC inhibitor closely related to HC-toxin (chalmydocin), is also insensitive. Furthermore, HDAC activities in at least some *C. carbonum* natural $Tox2^-$ isolates are sensitive. It indicated that the basis of HDAC resistance is, at least in part, an extrinsic factor made only by $Tox2^+$ isolates. We thought that the firs extrinsic factor would be the HDAC of *C. carbonum*.

To understand the function of HADCs in *C. carbonum*, especially in relation to the fact that this fungus produces a potent HDAC inhibitor, the structure and function of the HDAC genes of *C. carbonum* was investigated. A gene, HDAC which is related to the *Saccharomyces cerevisiae* HDAC gene *HOS2* was isolated from *C. carbonum*. Engineered mutants of HDC1 had smaller and less septate conidia and exhibited an ~ 50% reduction in total HDAC activity. Mutants were strongly reduced in virulence as result of reduced penetration efficiency. Growth of hdc1 mutants in vitro was normal on glucose, slightly decreased on sucrose, and reduced by 30 to 73 % on other simple and complex carbohydrates. Extracellular depolymerase activities and expression the corresponding genes were downregulated in hdc1 mutant strains. Except for altered conidial morphology, the phenotypes of hdc1 mutants were similar to those of *C. carbonum* strains mutated in ccSNF1 encoding a protein kinase necessary for expression of glucose-repressed genes. These results show that HDC1 has multiple functions in filamentous fungus and is required for full virulence of *C. carbonum* on maize.