1-40. The phosphoinositide-specific phospholipase C gene, *MPLC1*, of *Magnaporthe grisea* is required for fungal development and plant colonization

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Magnaporthe grisea, the casual agent of rice blast, forms an appressorium to penetrate its host. Much has been learned about environmental cues and signal transduction pathways, especially those involving cAMP and MAP kinases, on appressorium formation during the last decade. More recently, pharmacological data suggest that calcium/calmodulin-dependent signaling system is involved in its appressorium formation. To determine the role of phosphoinositide-specific phospholipase C (PI-PLC) on appressorium formation, a gene (*MPLC1*) encoding PI-PLC was cloned and characterized from *M. grisea* strain 70-15. Sequence analysis showed that *MPLC1* has all five conserved domains present in other phospholipase C genes from several filamentous fungi and mammals. Null mutants (*mplc1*) generated by targeted gene disruption exhibited pleiotropic effects on conidial morphology, appressorium formation, fertility and pathogenicity. *mplc1* mutants developed nonfunctional appressoria and are also defective in infectious growth in host tissues. Defects in appressorium formation and pathogenicity in *mplc1* mutants were complemented by a mouse PLCdelta-1 cDNA under the control of the *MPLC1* promoter. These results suggest that cellular signaling mediated by *MPLC1* plays crucial and diverse roles in development and pathogenicity of *M. grisea*, and functional conservation between fungal and mammalian PI-PLCs.

1-41. Molecular characterization of yeast *Snf1* homologue (sucrose non-fermenting gene) from *Magnaporthe grisea*

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Magnaporthe grisea causes the devastating blast disease of rice. Extensive research has been conducted on infection mechanisms, particularly on appressorium formation and penetration, of this fungus during the last decade. However, the role(s) of cell-wall-degrading enzymes (CWDEs) on pathogenesis is not clearly demonstrated at molecular level. Many CWDEs in plant pathogenic fungi including *M. grisea* are redundant; that is, there are multiple genes encoding enzymes with a similar or overlapping spectrum of activities. It is laborious to isolate all of the genes encoding related enzymes and to construct mutants lacking all of them. Thus, we considered alternative strategies to address the role of CWDEs in pathogenesis. Since expression of CWDE genes is repressed by a simple sugar, as the first step, we cloned a *Snf1* (sucrose non-fermenting) gene (*MgSnf1*) from *M. grisea*. The predicted amino acid sequence showed a high identity with other *Snf1* genes from various fungi. To elucidate molecular function of *MgSnf1*, a transformant lacking *Mgsnf1* was created by targeted gene replacement. In glucose, sucrose, and xylan the *MgSnf1* mutant grew normally but in pectin and complex media, it grew slower than wild type. Expression of various CWDEs in *MgSnf1* mutant was investigated and found that expression of some CWDEs is repressed. However, no significant difference was observed in conidial germination, appressorium formation, and pathogenicity in *MgSnf1* mutant. However, *MgSnf1* functionally complemented a yeast *Snf1* mutant. These results suggest that *MgSnf1* is involved in regulation of CWDEs and *MgSnf1* is dispensable in pathogenicity of *M. grisea*.

1-42. Shifting reproductive mode of a mycotoxin producing-fungus by manipulation of mating-type genes

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In most ascomycetes, a single mating type locus, *MAT*, with two alternate forms (*MAT1-1* and *MAT1-2*) called idiomorphs, controls mating ability. In heterothallic ascomycetes these alternate idiomorphs reside in different nuclei. In contrast, most homothallic ascomycetes carry both *MAT1-1* and *MAT1-2* in a single nucleus, usually closely linked. An example of the latter is *Gibberella zeae*, a producer of mycotoxins such as trichothecene and zearalenone that threaten human and animal health. We asked if *G. zeae* could be made strictly heterothallic by manipulation of *MAT*. Targeted gene replacement was used to differentially delete *MAT1-1* or *MAT1-2* from a wild type haploid *MAT1-1;MAT1-2* strain, resulting in *MAT1-1;mat1-2*, *mat1-1;MAT1-2* strains that were self-sterile, yet able to cross to wild type testers and more importantly, to each other. These results indicated that differential deletion of *MAT* idiomorphs eliminates selfing ability of *G. zeae*, but the ability to outcross is retained. To our knowledge, this is the first report of complete conversion of fungal reproductive strategy from homothallic to heterothallic by targeted manipulation of *MAT*. Practically, this approach opens the door to simple and efficient procedures for obtaining sexual recombinants of *G. zeae* that will be useful for genetic analyses of mycotoxin production and other traits, such as ability to cause disease.

1-43. Insertional mutagenesis of *fusarium graminearum* for characterization of genes involved in disease development and mycotoxin production

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Fusarium graminearum is an important pathogen of cereal crops in many areas of the world causing head blight and ear rot of small grains. In addition to serious economic losses, this fungus produces mycotoxins, such as trichothecenes and zearalenone on diseased crops and has been a potential threat to human and animal health. To massively identify pathogenesis-related genes