Isolation and Functional Analysis of a Secreted Protein Gene LAC2 Required for Fungal Pathogenicity of Colletotrichum orbiculare

Purpose Laccase has been detected in a variety of organisms such as bacteria, fungi, plants, and insects. Fungal laccases are believed to play a variety of roles, such as, morphogenesis, pathogenesis, and lignin degradation. As an oxidase, laccase plays important roles in catalyzing phenolic compounds. Collectotrichum orbiculare develops specific infection structures called appressoria. Appressoria are known to be pigmented with melanin, which is critical for appressorium function. Melanin biosynthesis involves oxidation of 1,8-dihydroxynaphthalene (1,8-DHN), which is the former product of melanin. There is a hypothesis that laccases are involved in oxidizing 1,8-DHN into melanin. However, there is no direct evidence that laccases are involved in appressorial melanization. In this study, I report a putative laccase gene (LAC2) is involved in conidial pigmentation, appressorial melanization and pathogenicity of C. orbiculare.

Methods I identified a putative laccase gene (LAC2) from a partial genome data of C. orbiculare. To investigate function of LAC2, lac2 null mutants were created, and the phenotype was examined. To assess the gene expression of LAC2 and its protein localization, I generated multiple GFP-based reporter strains of C. orbiculare. To further understand the evolutionary relationship of LAC2, LAC2 homologs in other fungi were introduced into the lac2 mutant for complementation assays.

Results and Discussion Pigmentation of conidia on colony was impaired in the lac2 mutants, which appeared dark-brown colored. The lac2 mutants also showed loss of pathogenicity on host cucumber, however, was able to grow invasively inside host plant tissue. Phenotypic analysis revealed that the lac2 mutants had a defect in appressorial melanization. GFP-based reporter assays unveiled that LAC2 was preferentially expressed during appressorial melanization, and the LAC2 protein was secreted extracellularly. These results indicate a strong link of the LAC2-encoded laccase with appressorium melanization and pathogenicity, which implies that LAC2 oxidizes 1,8-DHN to melanin in appressoria. The LAC2 homologs of other fungi, located at the same phylogenetic clade as LAC2, complemented conidial pigmentation, appressorial melanization, and pathogenicity of the lac2 mutant. Interestingly, the LAC2 homolog of Magnaporthe oryzae, located at a distinct clade from the LAC2 clade, was able to complement conidial pigmentation but not appressorium melanization of the lac2 mutant. The findings suggest that the substrates of LAC2 for conidial pigmentation and appressorial melanization are distinct, and that genes having homology with LAC2 may be broadly presented among fungi, but LAC2 homologs functioning in appressorium melanization may be conserved only within Colletotrichum species.