Title Overexpression of a redox-regulated cutinase gene, *MfCUT1*, increases virulence of the brown rot pathogen *Monilinia fructicola* on *Prunus* spp.

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Abstract

A 4.5-kb genomic DNA containing a *Monilinia fructicola* cutinase gene, *MfCUT1*, and its flanking regions were isolated and characterized. Sequence analysis revealed that the genomic *MfCUT1* carries a 63-bp intron and a promoter region with several transcription factor binding sites that may confer redox regulation of *MfCUT1* expression. Redox regulation is indicated by the effect of antioxidants, shown previously to inhibit *MfCUT1* gene expression in cutin-induced cultures, and in the present study, where H_2O_2 enhanced *MfCUT1* gene expression. A β-glucuronidase (GUS) reporter gene (*gusA*) was fused to *MfCUT1* under the control of the *MfCUT1* promoter, and this construct was then used to generate an MfCUT1-GUS strain by *Agrobacterium* spp.-mediated transformation. The appearance of GUS activity in response to cutin and suppression of GUS activity by glucose in cutinase-inducing medium verified that the MfCUT1-GUS fusion protein was expressed correctly under the control of the *MfCUT1* promoter. MfCUT1-GUS expression was detected following inoculation of peach and apple fruit, peach flower petals, and onion epidermis, and during brown rot symptom development on nectarine fruit at a relatively late stage of infection (24 h postinoculation). However, semiquantitative reverse-transcriptase polymerase chain reaction provided sensitive detection of *MfCUT1* expression within 5 h of inoculation in both almond and peach petals. *MfCUT1-GUS* transformants expressed *MfCUT1* transcripts at twice the level as the wild type and caused more severe symptoms on *Prunus* flower petals, consistent with MfCUT1 contributing to the virulence of *M. fructicola*.