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Genes associated with and mechanism of biological control of grapevine crown gall by *Agrobacterium vitis* strain F2/5

A. vitis causes crown gall disease in grapevine, which can be severe in grapevine growing regions worldwide. Vitis vinifera L. cultivars are highly susceptible to freeze injury, providing wounds important for infection (Burr and Otten 1999; Otten and Burr 2006). Phytohormone concentration within the plant appears to play a role in tumor development, as does natural wound healing. Via an unknown mechanism, the nontumorigenic strain, F2/5, prevents crown gall in grapevine tissue (Creasap et al. 2005). M852, a Tn5 mutant of F2/5, lacks the biological control ability, does not induce an HR on tobacco, and is reduced in its ability to cause grapevine necrosis (Herlache et al. 2001). The disrupted gene has been identified as homologous to an ATP-dependent Clp protease, *clpA*, with similarities to A. tumefaciens (86%), and Sinorhizobium meliloti Rm1021 (83%) clpA genes. With primers designed from the genome sequence of A. vitis strain S4, the ORF of clpA was amplified from F2/5 DNA, sequenced, and confirmed similar to *clpA* in S4 (97%). Screening of this site-directed mutant has shown that this gene is involved in biological control, and this *clpA* homolog will be cloned into an expression vector and used to complement M852 and the site-directed mutant $\mu clpA$. Additionally, to determine whether *clpA* is solely responsible for the lack of biological control, the genes immediately upstream and downstream have been sequenced and identified as a *clpS* homolog and ORFD, respectively. Sitedirected mutants of these genes have been tested for biological control, HR, and necrosis.

Additional work in *Ricinus* determined that F2/5 prevented tumor development by tumorigenic strain K306. This indicates that the biocontrol activity is not grapevine specific. *Ricinus* stems were inoculated with either the tumorigenic K306 *A. vitis* strain carrying p35Sgusint or K306(p35Sgusint) and F2/5. Isolation of mRNA from inoculated *Ricinus* stems indicated that F2/5 prevents expression of the gus mRNA (Zäuner et al. 2006).

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