

**Title** *Agrobacterium tumefaciens*-mediated genetic transformation of the phytopathogenic fungus *Penicillium digitatum*

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### Abstract

*Penicillium digitatum* (Pers.:Fr) Sacc. is the causal agent of citrus green mold, which is the most widespread and destructive disease of postharvest citrus. Despite the economic importance of this fungus, little is known about the genetic basis of pathogenicity, which is necessary to develop a disease control strategy. Understanding the molecular mechanism of pathogenesis of *P. digitatum* is hampered by the lack of a readily accessible genetic tool to efficiently generate and recover mutant genes. Thus, the development of an efficient system to generate tagged insertional mutants in *P. digitatum* will greatly aid the efforts to characterize gene(s) involved in pathogenicity. *Agrobacterium tumefaciens*-mediated transformation (ATMT) is widely used to create insertional mutants in a wide variety of plants. Recently, increasing numbers of fungal species have been transformed with *A. tumefaciens*. We report a method of *A. tumefaciens*-mediated genetic transformation of *P. digitatum* using hygromycin phosphotransferase gene (*hph*) as a selected marker. A transformation efficiency up to 60 transformants per 10<sup>6</sup> conidia of *P. digitatum* was achieved. Southern blot analysis of 6 randomly selected transformants verified the integration of the *hph* gene into the fungal genome. Therefore showing that the *hph* gene was randomly integrated as single copy into the fungal genome of *P. digitatum*. The 6 transformants tested were also shown to be mitotically stable. Thus *Agrobacterium tumefaciens*-mediated transformation of *P. digitatum* is a practical genetic tool for conducting insertional mutagenesis in *P. digitatum* to search for a gene(s) responsible for its pathogenicity and other characters.