Title A molecular mechanism of azoxystrobin resistance in *Penicillium digitatum* UV mutants and a PCR-based assay for detection of azoxystrobin-resistant strains in packing- or store-house isolates

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Citation International Journal of Food Microbiology, Volume 131, Issues 2-3, 31 May 2009, Pages 157-161

Keywords *Penicillium digitatum*; Citrus green mold; Azoxystrobin; Resistance risk; Mechanism of azoxystrobin-resistance; Allele-specific PCR

Abstract

Sixty-five isolates of *Pencillium digitatum* (Pers.:Fr) Sacc., a causative agent of green mold of postharvest citrus, were collected from various locations in Zhejiang province in 2000, 2005 and 2006, and assayed for their sensitivity to the quinone outside inhibitor (QoI) fungicide azoxystrobin. The results showed that azoxystrobin is highly effective against *P. digitatum*, *in vitro*, and that the effective concentrations resulting in reduction of conidial germination and mycelial growth by 50% (EC_{50}) averaged 0.0426 μg/ml and 0.0250 μg/ml, respectively. Twenty-eight azoxystrobin-resistant mutants were obtained by UV mutagenesis and subsequent selection on medium amended with azoxystrobin (12 μg/ml) and salicylhydroxamic acid. All obtained mutants were highly resistant to azoxystrobin and their resistance was genetically stable. Analysis of the cytochrome *b* gene structure of *P. digitatum* (*Pdcyt b*) showed the absence of type I intron in the first hot spot region of mutation. These results indicate that *P. digitatum* is likely to evolve high levels of resistance to azoxystrobin after its application. Analysis of partial sequences of *Pdcyt b* from both the azoxystrobin-sensitive parental isolate and the 28 azoxystrobin-resistant mutants revealed that a point mutation, which leads to the substitution at code 143 of alanine for glycine (G143A), is responsible for the observed azoxystrobin resistance in the laboratory mutants. Based on this point mutation, two allele-specific PCR primers were designed and optimized for allele-specific PCR detection of azoxystrobin-resistant isolates of *P. digitatum*.