

Title *PdCYP51B*, a new putative sterol 14 α -demethylase gene of *Penicillium digitatum* involved in resistance to imazalil and other fungicides inhibiting ergosterol synthesis

Author Xuepeng Sun, Jiye Wang, Dan Feng, Zhonghua Ma and Hongye Li

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Abstract

Penicillium digitatum, causing green mold decay, is the most destructive postharvest pathogen of citrus fruits worldwide. The phenotypes and genotypes of 403 isolates of *P. digitatum*, collected from packing houses and supermarkets in Zhejiang, China, during 2000 to 2010, were characterized in terms of their imazalil sensitivity. The frequency of detected imazalil-resistant (IMZ-R) isolates increased from 2.1% in 2000 to 60–84% during 2005–2010. Only 6.5% and 4.5% of the collected IMZ-R isolates belong to the previously described IMZ-R1 and IMZ-R2 genotypes, respectively. To determine the resistance mechanism of the predominant and novel IMZ-R isolates of *P. digitatum* (termed IMZ-R3), genes *PdCYP51B* and *PdCYP51C*, homologous to the sterol 14 α -demethylase encoded gene *PdCYP51*, were cloned from six IMZ-R3 and eight imazalil-sensitive (IMZ-S) isolates of *P. digitatum*. A unique 199-bp insertion was observed in the promoter region of *PdCYP51B* in all IMZ-R3 isolates examined but in none of the tested IMZ-S isolates. Further analysis by PCR confirmed that this insertion was present in all IMZ-R3 isolates but absent in IMZ-S, IMZ-R1, and IMZ-R2 isolates. Transcription levels of *PdCYP51B* in three IMZ-R3 isolates were found to be 7.5- to 13.6-fold higher than that in two IMZ-S isolates of *P. digitatum*. Introduction of another copy of *PdCYP51B*^S (from IMZ-S) into an IMZ-S isolate decreased the sensitivity of *P. digitatum* to 14 α -demethylation inhibitors (DMIs) only to a small extent, but introduction of a copy of *PdCYP51B*^R (from IMZ-R3) dramatically increased the resistance level of *P. digitatum* to DMIs. Regarding *PdCYP51C*, no consistent changes in either nucleotide sequence or expression level were correlated with imazalil resistance among IMZ-R and IMZ-S isolates. Based on these results, we concluded that (1) the CYP51 family of *P. digitatum* contains the *PdCYP51B* and *PdCYP51C* genes, in addition to the known gene *PdCYP51A* (previously *PdCYP51*); (2) *PdCYP51B* is involved in DMI fungicide resistance; and (3) overexpression of *PdCYP51B* resulting from a 199-bp insertion mutation in the promoter region of *PdCYP51B* is responsible for the IMZ-R3 type of DMI resistance in *P. digitatum*.