Title Cloning and primary functional analysis of *Pdmfs1*, a major facilitator superfamily transporter from the fungal pathogen *Penicillium digitatum*

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Abstract

Recent studies indicate that transporters in the major facilitator superfamily (MFS) in plant pathogens function in the secretion of endogenous fungal pathogenicity factors (e.g. toxins) and in protection against exogenous toxic compounds, such as plant defense compounds (e.g. phytoalexins) and fungicides. Therefore, transporters can act as virulent and fungicide-resistant factors. This study elucidates the function of MFS in the fungicide-resistance of *Penicillium digitatum* (Pers.:Fr) Sacc., the most important causal agent of postharvest decay of citrus. A gene (*Pdmfs1*) in the MFS was cloned by thermal asymmetric interlaced PCR (TAIL-PCR) following the an amplification of a conserved fragment using degenerated primers…Comparison of the sequence of genomic DNA and cDNA revealed that *Pdmfs1* contains a 1698-bp open reading frame (ORF) interrupted by 3 introns, encoding a protein with 566 amino acids. The nucleotide sequence of *Pdmfs1* is available at GenBank (Accesion No. AM412556). The Blast datable (http://www.ncbi.nlm.nih.gov/BLAST/) demonstrated that *Pdmfs1* is highly homologous with other fungal MFS transporters from *Neosartorya fischeri* (60%), *Aspergillus clavatus* (60%), and *Botrytis cinerea* (56%). A prediction of transmembrane (TMS) domains of *Pdmfs1* using a program of TMHMM (http://www.cbs.dtu.dk/services/TMHMM) suggested the presence of 14 TMS domains. The full length *Pdmfs1* ORF was colned into yeast expression vector pYES2, and then transformed to a hypersensitive *Saccharomyces cerevisiae* strain yorl (ΔLc64ΔLc65). The sensitivity comparison between the transformants with pYES2+*Pdmfs1* and with pYES2 (empty vector) indicated that the expression of *Pdmfs1* in yor1 resulted in an increase of its resistance against the 14-α-demethylation inhibitor fungicides imazalil, prochloraz, and myclobutanil. Expression analysis using real time PCR (RT-PCR) indicated that the expression of *Pdmfs1* in PDw03, a naturally imazalil-resistant isolate of *P. digitatum*, is about 10 times higher than that of the naturally imazalil-sensitive isolate Pd23. The expression level of PDw03 was activated by the treatment of 0.1 ppm imazalil for 30 min. Therefore, the data suggests that *Pdmfs1* might be involved in protection of *P. digitatum* against fungitoxic compounds.