

**Title** Identification of *Penicillium digitatum* genes putatively involved in virulence/pathogenicity towards citrus fruit

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#### **Abstract**

*Penicillium digitatum* is the major post harvest pathogen of citrus fruit, being responsible for up to 80% of the losses due to decay. This necrotrophic fungus shows a very restricted host range, infecting only citrus fruit. Despite this fact, our knowledge of the factors involved in pathogenicity is very scarce; therefore we aimed to study at the molecular level the mechanisms involved in pathogenicity and virulence of this fungus. We have applied the Suppression Subtractive Hybridization (SSH) technique to obtain a cDNA library enriched in *P. digitatum* genes that are upregulated during infection using a mixture of RNAs from uninfected fruit and *in vitro* grown fungus as a “driver” and RNA from infected fruit tissue as “tester”. After two rounds of PCR amplification subtracted cDNA fragments were ligated into plasmid pCRII and cloned into *E. coli* competent cells. DNA inserts from 1440 clones together with positive and negative controls were PCR-amplified and spotted onto replicate nylon membranes. Using this macroarray we have identified *P. digitatum* genes that are upregulated during infection as compared to *in vitro* growth conditions and thus are good candidates to be involved in pathogenicity. The results of these hybridizations together with sequence analysis of a number of clones constitute a first step to elucidate the virulence/pathogenicity determinants of this important citrus pathogen. We will also present a more detailed Northern blot analysis of selected *P. digitatum* genes.