

Thesis Title	Roles of <i>RDS2</i> and <i>PDR5</i> Genes in Conferring Resistance to <i>Xylaria</i> Extracts in <i>Saccharomyces cerevisiae</i>
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ABSTRACT

In *Saccharomyces cerevisiae*, the expression of drug resistant genes is regulated by a group of proteins called zinc cluster or zinc binuclear cluster proteins. The zinc cluster Pdr1 and Pdr3 are the master transcriptional regulators that control expression of various drug transporter genes, including *PDR5*, *SNQ2*, and *YOR1*. Recently, the phenotypic analysis performed with different drugs showed that another zinc cluster protein called Rds2 is also associated with antifungal ketoconazole drug sensitivity. Moreover, Rds2 is found at the promoter of *PDR5* during the glycerol shift. However, the role of Rds2 in drug resistance/sensitivity remains unclear. In this work, the goal is to characterize the function of Rds2 regulator in mediating drug resistance/sensitivity and to find new natural products with antifungal properties. The antifungal activities of four *Xylaria* species were investigated through the clear zone of inhibition assay using the *S. cerevisiae* wild-type and the deletion $\Delta rds2$ and $\Delta pdr5$ strains. All *Xylaria* extracts including *Xylaria cubensis*, *Xylaria globosa*, *Xylaria obovata*, and *Xylaria* sp. showed some clear zones of inhibition which were increased when tested in combination with ketoconazole. The antifungal activities of four *Xylaria* extracts were further confirmed by minimal inhibitory/fungicidal concentration (MIC and MFC) assays. The *Xylaria* sp. extract produced the lowest MIC₅₀ values of 0.10, 0.03, and 0.02 mg/ml for the wild-type and deletion $\Delta rds2$ and $\Delta pdr5$ strains, respectively. The $\Delta pdr5$ strain showed greatest sensitivity to all extracts. The involvement of Rds2 regulator on expression of *PDR5* gene was then investigated by quantitative realtime-polymerase chain reaction (qRT-PCR) analysis. The results indicated that Rds2 is not responsible for *PDR5*

expression upon *Xylaria* sp. extract treatment although the previous assays showed increased sensitivity of the $\Delta rds2$ strain to *Xylaria* sp. extract. Thus, the overall results suggested that there are additional drug resistance genes whose expression is controlled by Rds2 and that *PDR5* expression may be regulated by other unknown transcription factors. Nevertheless, the important results of this study showed that *Xylaria* extracts possess promising antifungal activity against *S. cerevisiae*.

Keywords: Antifungal activity / Drug resistance / *PDR5* / Rds2 regulator/ *Saccharomyces cerevisiae* / *Xylaria* extract