**FEMS7-1825**
**Physiology / Biochemistry / Molecular Microbiology - Part III**

# DESIGN OF A REAL-TIME PCR TOOL TO STUDY CELL WALL STRESS IN FUNGI

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## Backgrounds

The cell wall integrity (CWI) pathway is responsible for the reparation and/or biosynthesis of the cell wall and is activated when changes on the cell surface occurred mainly under imposed stress. A new tool able to detect changes in stress-related genes would be useful to understand the action mechanism of some filamentous fungi against antifungal compounds.

## Objectives

The objective was to develop a real-time PCR (qPCR) using SYBR Green to monitor changes in the *Rho1* gene expression levels in moulds.

## Methods

Optimization of reaction conditions included evaluation of different primer pairs, primer concentrations and annealing temperatures/times. The final reaction mixture contained 200nM of each primer and an annealing temperature of 55ºC. The specificity of primers was demonstrated when amplified a unique qPCR product with a Tm value of 86ºC. The qPCR showed a $R^2$ value =0.9994 and amplification efficiency of 97.5%. The method was validated by treating *Aspergillus flavus* and *Penicillium polonicum* with the antifungal protein PgAFP. The PgAFP-resistant *P. polonicum* showed an overexpression of *Rho1*, while the opposite trend was detected in *A. flavus* with the antifungal treatment.

## Conclusions

This qPCR assay is a valuable tool to analyse intracellular responses linked to CWI pathway activation. This provides data to in-depth understand the ability of fungi to colonise different environments and to develop new antifungals

Acknowledgements: L. da Cruz Cabral is recipient of a pre-doctoral fellowship (BEC.ar call 2015). Dr. A. Rodríguez is supported by a Juan de la Cierva senior research fellowship (IJCI-2014-20666)