

## CHARACTERISATION AND HETEROLOGOUS EXPRESSION OF CHITIN-DE-N-ACETYLASES FROM *Puccinia graminis* AND *Fusarium graminearum*

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A number of fungal CDAs have been characterised, mostly from the *Zygomycetes* which are characterised by a chitosan containing cell wall. In these fungi, CDAs are believed to act in tandem with chitin synthases, deacetylating the nascent chitin chain to produce chitosan. While *Zygomycetes* cell walls were long regarded as the sole biogenic source of chitosan, we have recently shown that some plant pathogenic fungi switch from chitin to chitosan containing cell walls upon penetration into their host tissues [1]. In these *Ascomycetes* and *Basidiomycetes* fungi, hyphal tip growth appears to require chitin synthesis, and deacetylation appears to occur in the subapical cell wall. Consequently, CDAs of these fungi should act on chitin polymers within the cell wall and, thus, might differ in their enzymic properties from *Zygomycetes* CDAs.

In this study, we present the characterisation of two novel chitin-de-N-acetylase (CDA) genes from *Puccinia graminis* and *Fusarium graminearum*, pathogens causing tremendous losses in wheat and maize agriculture.

Full length cDNAs and the genomic loci of both genes have been cloned and characterised, revealing a clear match in both candidates for the polysaccharide deacetylase-HMM pattern as deposited in PFam. Additionally, the *Fusarium* gene harbours three chitin binding domains.

As several attempts failed to heterologously express these genes in conventional systems, we developed a new expression platform based on *Schizosaccharomyces pombe* as a eucaryotic host. Focussing on efficient secretion, this expression system assures proper folding and posttranslational modification of the recombinant enzymes.

Once inserted into this novel system, we could show both CDA-genes to be efficiently expressed and their products accumulating in the culture medium, from where we could easily purify them. The purified CDAs were then characterised in terms of their pH-, temperature- and cofactor-dependency as well as their enzyme kinetics.

Besides understanding plant-pathogen interactions, a major focus of our work is to use such enzymes as tools for the design of tailor-made, highly bioactive and/or biocompatible chitosans for phyto-protective or medical applications. With that in mind, our work focusses on determining the exact modes of action of the two CDAs, which will then probably help us to control and fully understand the third dimension of chitosan characteristics, the pattern of acetylation.

[1] El Gueddari N.E., U. Rauchhaus, B.M. Moerschbacher, H.B. Deising (2002) Developmentally regulated conversion of surface-exposed chitin to chitosan in cell walls of plant pathogenic fungi. *New Phytol.* **156**: 103-112