PE 8 - Characterisation and Heterologous Expression of Chitin-De-N-Acetylases from *Fusarium graminearum* in *Schizosaccharomyces pombe*

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A number of fungal CDA have been characterised, mostly from the *Zygomycetes* which are characterised by a chitosan containing cell wall. In these fungi, CDA 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. In these *Ascomycetes* and *Basidiomycetes* fungi, hyphal tip growth appears to require chitin syn-thesis, and deacetylation appears to occur in the subapical cell wall. Consequently, CDA of these fungi should act on chitin polymers within the cell wall and, thus, might differ in their enzymic properties from *Zygomycetes* CDA.
In this study, we present the characterisation of five novel chitin-de-N-acetylase(CDA) genes from *Fusarium graminearum*, an *Ascomycete* pathogen of wheat and maize. Full length cDNAs of four of the genes have been cloned, two of them with and without their secretory signal peptide, and these have been expressed heterologously in *E. coli*. Recombinant protein was obtained in all cases but as the products were insoluble, no enzymatic activity was detected.
We, therefore, used *Schizosaccharomyces* pombe as a eukaryotic expression system providing post-translational modifications potentially needed for the functional expression of the fungal genes. Although we cloned the CDA genes in two versions, with and with-out its wildtype signal peptide, the product was found in the intracellular fraction in both cases. As a limited compatibility of secretion signals between species has been re-ported previously, we decided to construct a versatile secretion vector for the *S. pombe* system. This vector will allow the fusion of a homologous signal peptide without the addition of linker amino-acids to any protein to be produced. Using this system, we expect efficient secretion of recombinant proteins into the yeast medium, correct post-translational processing of the recombinant products and, thus, a substantial increase in the overall yield of functional enzyme.