PE 9 - Characterisation and Heterologous Expression of Chitin N-Acetylases from *Ustilago maydis* in *Escherichia coli* and *Aspergillus niger*

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Chitin is the second most abundant biopolymer. It is a poly-N-acetyl-D-glucosamine, which is mainly found in insects, fungi, and marine invertebrates. The industrial produc-tion of chitin and its derivatives is mainly from the crustacean shells, namely shrimps and crabs. The most important of the derivatives of chitin is chitosan, with its applica-tions in the food, agricultural, cosmetics, and pharmaceutical industries. The critical step in the production of chitosan is the cleavage of the acetyl group from chitin. However, this de-N-acetylation is presently done chemically, with the limitations that the process of chain degradation cannot be prevented completely, and that only chitosans with a random pattern of acetylation are obtained. In contrast, this deacetylation is per-formed in nature by an enzyme called chitin de-N-acetylase (CDA). Usage of such an enzyme would represent an alternative, biotechnological process to convert chitin into chitosan, with the potential of yielding chitosans differing in their physico-chemical properties and, thus, biological activities, e.g. by the generation of non-random patterns of acetylation. Such novel, well-defined chitosans might possess an interesting potential for the development of applications in the biomedical and pharmaceutical industries.
CDA have been described from different sources, most notably *Zygomycetes* fungi cha-racterised by the presence of chitosan instead of chitin in their cell walls. However, we have recently described the de-N-acetylation of chitin in the cell walls of biotropic plant pathogenic fungi belonging to the *Ascomycetes* and *Basidiomycetes*, and these may re-present a source for novel CDA genes and enzymes with potentially differing and inter-esting enzymic properties. In particular, such enzymes may be able to act on crystalline chitin that is not efficiently de-N-acetylated by the currently known CDA, severely li-miting their biotechnological uses. Therefore, we have analysed the fully sequenced genome of the *Ascomycete* maize pathogen *Ustilago maydis* for the presence of potential CDA genes. Six putative CDA genes belonging to three different taxonomic classes were identified. Expression profi-ling revealed that one of these genes is up-regulated, a second one down-regulated during pathogenic growth in planta. These two genes were selected for heterologous ex-pression in *Escherichia coli* and *Aspergillus niger*. In the case of *E.coli*, only truncated products were onbserved, and in *A. niger*, no full-length integration was detectable. We have therefore now adopted a strategy to overexpress these genes homologously under the control of an arabinose- or nitrate-inducible promotor in *U. maydis*. The enzymes will be expressed as fusion proteins bearing an N-terminal tag for purification.