

CHARACTERISATION AND HETEROLOGOUS EXPRESSION OF CHITIN DE-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 production 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 applications 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 performed 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 characterised 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 biotrophic plant pathogenic fungi belonging to the *Ascomycetes* and *Basidiomycetes*, and these may represent a source for novel CDA genes and enzymes with potentially differing and interesting enzymic properties. In particular, such enzymes may be able to act on crystalline chitin which is not efficiently de-N-acetylated by the currently known CDA, severely limiting their biotechnological uses.

We have, therefore, 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 profiling 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 expression in *Escherichia coli* and *Aspergillus niger*. In the case of *E.coli*, only truncated products were observed, 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 promoter in *U. maydis*. The enzymes will be expressed as fusion proteins bearing an N-terminal tag for purification.