

PE 11 - Chitosanase from *Alternaria alternata*

M. Kohlhoff^(1,2), **N.E. El Gueddari**⁽¹⁾, E.N. Oliveira Jr.^(1,2), T.T. Franco⁽²⁾, B.M. Moerschbacher⁽¹⁾

⁽¹⁾Department of Plant Biochemistry and Biotechnology, University of Münster, Hindenburgplatz 55, 48143 Münster, Germany - ⁽²⁾School of Chemical Engineering, State University of Campinas, caixa postal 6066, 13081-970 Campinas, Brasil

Chitosan, the only known polycationic biopolymer, has a number of interesting material properties and biological activities. Among these, the antimicrobial activities of chitosan are well described. A broad range of taxonomically different bacteria and fungi have been shown to be inhibited in their growth by chitosan. In our recent investigation into the effect of the degrees of polymerisation (DP) and acetylation (DA) of chitosan polymers and oligomers on their antimicrobial activity, we found that some fungi tolerated rather high concentrations of even the most antimicrobially active chitosans, and that they even grew better in the presence of low amounts of chitosans. We found that these fungi secreted rather high activities of chitosanase in their culture media, explaining both their insensitivity to chitosans as an antibiotic and their ability to use chitosans as a carbon and/or nitrogen source. One fungal species with particular tolerance towards chitosans was a strain of *Alternaria alternata*, a pathogen with a broad host range affecting many crop plants, fruits, and vegetables. *A. alternata* produced at least five different chitosanolytic activities having neutral to basic isoelectric points. All of these enzymes degraded chitosans with intermediate DA better than glycol chitin or chitosan with a very low DA. The most prominent of the chitosanases was purified from the culture medium of the fungus using cation exchange chromatography. The enzyme had an apparent molecular mass of ca. 17 kDa. Currently, the enzyme is being characterised concerning pH and temperature optimum and stability, substrate specificity, cleavage mechanism, and product pattern. In addition, sequencing of the protein is in progress in order to identify and clone the corresponding gene.