

### OE 3 - Structure-function Studies of CBP21, a Non-catalytic Chitin-binding Protein Promoting Chitin Degradation

**G. Vaaje-Kolstad<sup>(1)</sup>**, S.J. Horn<sup>(1)</sup>, D.R. Houston<sup>(2)</sup>, B. Synstad<sup>(1)</sup>, D.M.F. Van Aalten<sup>(2)</sup>

<sup>(1)</sup>*Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences -*

<sup>(2)</sup>*Wellcome Trust Biocentre, University of Dundee, Scotland, U.K.*

The Gram-negative soil bacterium *Serratia marcescens* employs three family 18 chitinases to degrade chitin, an abundant insoluble carbohydrate polymer composed of  $\beta(1,4)$ -linked units of N-acetylglucosamine. During chitin degradation, *S. marcescens* also secretes a small non-catalytic chitin-binding protein, CBP21, which binds to the insoluble crystalline substrate. The structure of CBP21 was determined at 1.55 Å resolution and showed a budded fibronectin type-III fold with a patch of conserved, mainly hydrophilic, residues on the surface. Site-directed mutagenesis was used to construct single point mutants of selected residues on the conserved surface patch. Analysis of these mutants showed that CBP21 binds its substrate through specific, mostly polar interactions, which disrupts the chitin structure. Chitin degradation assays using combinations of the *S. marcescens* chitinases and CBP21 showed that the chitin-binding protein strongly promoted hydrolysis of crystalline  $\beta$ -chitin by chitinases A and C, while it was essential for the complete degradation of chitin by chitinase B. Interestingly, homologues of CBP21 occur in most chitin-degrading microorganisms, suggesting a general mechanism by which chitin-binding proteins enhance chitinolytic activity. Homologues also occur in chitinase-containing insect viruses, whose infectiousness is known to depend on chitinase efficiency.