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Characterization of two novel bacterial type A *exo*-chitobiose hydrolases having C-terminal 5/12-type carbohydrate-binding modules

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Abstract Type A chitinases (EC 3.2.1.14), GH family 18, attack chitin ((1 → 4)-2-acetamido-2-deoxy-β-D-glucan) and chito-oligosaccharides from the reducing end to catalyze release of chitobiose (*N*,*N*′-diacetylchitobiose) via hydrolytic cleavage of *N*-acetyl-β-D-glucosaminide (1 → 4)-β-linkages and are thus "*exo*-chitobiose hydrolases." In this study, the chitinase type A from *Serratia marcescens* (SmaChiA) was used as a template for identifying two novel exo-chitobiose hydrolase type A enzymes, FbalChi18A and MvarChi18A, originating from the marine organisms *Ferrimonas balearica* and *Microbulbifer variabilis*, respectively. Both FbalChi18A and MvarChi18A were recombinantly expressed in *Escherichia coli* and were confirmed to exert *exo*-chitobiose hydrolase activity on chito-oligosaccharides, but differed in temperature and pH activity response profiles. Amino acid sequence comparison of the catalytic β/α barrel domain of each of the new enzymes showed individual differences, but ~69% identity of each to that of SmaChiA and highly conserved active site residues. Superposition of a model substrate on 3D structural models of the catalytic domain of the enzymes corroborated *exo*-chitobiose hydrolase type A activity for FbalChi18A and MvarChi18A, i.e., substrate attack from the reducing end. A main feature of both of the new enzymes was the presence of C-terminal 5/12 type carbohydrate-binding modules (SmaChiA has no C-terminal carbohydrate binding module). These new enzymes may be useful tools for utilization of chitin as an *N*-acetylglucosamine donor substrate via chitobiose.

Keywords Chitinase · *Ferrimonas balearica* · *Microbulbifer variabilis* · C-terminal CBM

Introduction

Chitin, a β-(1,4)-linked polymer of *N*-acetylglucosamine moieties (GlcNAc), is the second most abundant natural polysaccharide in the world besides cellulose. Chitin serves as the structural component in the exoskeleton of the arthropods (including crustaceans such as crabs and shrimp) and is also present in the cell walls of fungi and yeast (Synowiecki and Al-Khateeb 2003; Rinaudo 2006). Partial deacetylation of chitin results in chitosan; in contrast to chitin, chitosan is soluble