Characterization of a Novel β(1→3) Galactosidase from *Streptococcus pneumoniae* R6

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Sequence and structural information of the glycan are essential to understand its biological functions. Enzymatic hydrolysis by employing various glycosidases is one of the powerful tool for determining the sequence and structure of glycan. β-galactosidases (EC 3.2.1.23), which hydrolyze the terminal non-reducing galactose from oligosaccharide, are often used for such purpose and obtained from diverse organisms ranging from bacteria to plants and mammals. It was previously reported that *bgaA* gene of *Streptococcus pneumoniae* encodes a surface-associated β (1→4) galactosidase, which is involved in degalactosylation of human glycoconjugates for colonization and/or pathogenesis. Based on the whole genome sequence of *S. pneumoniae*, a putative β-galactosidase gene, *bgaC*, was also predicted. The *bgaC* encodes a polypeptide showing amino acid sequence homology with β-galactosidases of glycosyl hydrolase family 35, which mainly includes enzymes of higher eukaryotes.

In this study, the *bgaC* gene of *S. pn*eumoniae was cloned and expressed in *E. coli*. The recombinant BgaC was purified and its catalytic properties were investigated by measuring the hydrolysis of *p*-nitrophenyl-D-galactopyranoside and *o*-nitrophenyl-D-galactopyranoside. BgaC has an optimal activity at 30℃ at pH 6.5. Further, regiospecificity and sugar specificity of BgaC were also determined by measuring hydrolysis ability to various commercially available glycan sub-

strates with HPLC or MALDI-TOF. Obtained results indicated that the BgaC of *S. pneumoniae* is a novel β-galactosidase which specifically recognizes and hydrolyzes the terminal galactose linked to GlcNAc by a β(1→3) linkage. Amino acid sequence alignment result and existence of NHL repeat motif homologous suggest that BgaC may be acquired by horizontal gene transfer from their eukaryotic host.

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