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Synthesis of galactooligosaccharides by CBD fusion β-galactosidase immobilized on cellulose

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ABSTRACT

The β-galactosidase gene (*bga*L3) was cloned from *Lactobacillus bulgaricus* L3 and fused with cellulose binding domain (CBD) using pET-35b (+) vector in *Escherichia coli*. The resulting fusion protein (CBD-BgaL3) was directly adsorbed onto microcrystalline cellulose with a high immobilization efficiency of 61%. A gram of cellulose was found to absorb 97.6 U of enzyme in the solution containing 100 mM NaCl (pH 5.8) at room temperature for 20 min. The enzymatic and transglycosylation characteristics of the immobilized CBD-BgaL3 were similar to the free form. Using the immobilized enzyme as the catalyst, the yield of galactooligosaccharides (GOS) reached a maximum of 49% (w/w) from 400 g/L lactose (pH 7.6) at 45 °C for 75 min, with a high productivity of 156.8 g/L/h. Reusability assay was subsequently performed under the same reaction conditions. The immobilized enzyme could retain over 85% activity after twenty batches with the GOS yields all above 40%.

1. Introduction

Galactooligosaccharides (GOS) are among the most attractive prebiotics that selectively stimulate the proliferation of intestinal probiotics. It is reported that GOS cause the largest decrease in harmful Clostridia, higher short-chain fatty acid generation and lower gas production when compared with Raftilose P95, Raftiline LS, lactulose, xylo-, isomalto-, and soybean oligosaccharides (Rycroft et al., 2001). Also, GOS are able to act as decoy agents for the pathogen instead of host cell surface oligosaccharides and help to flush the pathogen from the gastrointestinal tract (Searle et al., 2009). As a result, a great deal of attention has been devoted to GOS synthesis, especially via enzymatic transglycosylation since chemical synthesis of GOS is very tedious.

GOS can be synthesized by β-galactosidase (EC3.2.1.23) from lactose by glycosyl transfer of a D-galactose residue onto lactose itself, or its hydrolysis products, D-galactose or D-glucose, or the newly-produced oligosaccharides. Both free and immobilized β-galactosidases from different microorganisms have been employed for GOS synthesis (Panesar et al., 2010; Gosling et al., 2010; Park and Oh, 2010). Considering industrial application, the immobilized enzymes are more advantageous for enzyme reusability, simplified processing, and effective catalysis. Various strategies for β-galactosidase immobilization have been successfully applied in GOS synthesis. In many cases, however, the enzymes used for immobilization are either involved in a tedious purification procedure or limited in sources commercially available. Also the modifications required for covalent binding of the enzyme to the matrix result in the loss of enzyme activity, as well as the introduction of toxic organic compounds (e.g., glutaraldehyde used for protein cross-linking) (Berger et al., 1995; Shin et al., 1998; Albayrak and Yang, 2002; Maugard et al., 2003; Matella et al., 2006; Gaur et al., 2006; Neri et al., 2009; Pan et al., 2009; Huerta et al., 2011).

Recently, the inclusion of an affinity tag fused to the target protein by genetic engineering has been proven to be very useful to make purification and immobilization of protein in simply one step. Also this strategy offers additional advantages, including the oriented attachment and the minimization of conformational changes (Andreescu et al., 2006). Many affinity systems are now in use. Unfortunately, they share the common drawback of the high cost of the affinity matrix. In contrast, the use of biospecific affinity of cellulose binding domain (CBD) to cellulose offers a series of industrially attractive advantages: (i) the cellulose is abundant and inexpensive, with excellent chemical and physical properties for use in food or pharmaceutical applications; (ii) CBD binds to crystalline cellulose spontaneously and noncovalently, avoiding the use of possible toxic compounds in the immobilization; (iii) the high affinity of specific CBDs for cellulose, combined with the high tolerance of desorption, enables essentially irreversible immobilization (Shoseyov and Warren, 1997; Boraston et al., 2002; Shoseyov et al. 2006). The CBD fusion

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