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Co-immobilization of dextransucrase and dextranase in alginate

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ABSTRACT

Dextransucrase from *Leuconostoc mesenteroides* and dextranase from *Penicillium lilacinum* were co-immobilized and used to produce isomaltooligosaccharides from sucrose. The enzymes were co-immobilized by encapsulating soluble dextransucrase and dextranase covalently attached to Eupergit C in alginate (beads, fibers, and capsules). The alginate capsule co-immobilization was done in the presence of soluble starch and resulted in a high immobilization yield (71%), and the enzymes retained their activities during 20 repeated batch reactions and for a month in storage at 4 °C. The presence of starch was essential for the stability of dextransucrase in alginate capsules. Furthermore, it is important that the dextranase be pre-immobilized prior to alginate capsule co-immobilization to prevent dextranase leakage and inactivation of dextransucrase. The co-immobilized enzymes formed oligosaccharides from sucrose, which can be used as prebiotics. In addition, the oligosaccharides that were produced after the addition of sucrose reacted with the alginate fiber-encapsulted dextransucrase, thus increasing the amount of prebiotics. Co-immobilization in alginate fiber and beads also resulted in high yields (70 and 64%), but enzymatic activities decreased by 74 and 99%, respectively, after a month in storage at 4 °C. The newly developed alginate capsule method for co-immobilization of dextransucrase and dextranase is simple yet effective and has the potential for industrial-scale production of isomaltooligosaccharides.

1. Introduction

Sucrose is the most commonly used sweetener; however, it contributes significantly to the formation of dental plaque and caries. In the oral cavity, *Streptococcus mutans* converts some of the glucose in sucrose into glucan. This glucan binds to teeth and helps *S. mutans* adhere to teeth surfaces. The bacteria ferment the resulting fructose to lactic acid, which is held close to the tooth surface where it attacks the enamel, leading to dental caries [1]. Some carbohydrates, including isomaltooligosaccharides, reduce the harmful cariogenic effects of sucrose when consumed with sucrose [2]. Also, some components of isomaltooligosaccharides (isomaltose and isomaltotriose) are partially digested by the small intestine. Isomaltooligosaccharides are larger than isomaltotriose and could serve as prebiotics, enhancing the growth of bifidobacteria in the human large intestine [3]. Bifidobacteria occur naturally in the human intestine and provide many health benefits, including resistance to gastrointestinal infections, restoration of bowel flora, and improvement of liver enzymes in human alcohol-induced liver injury [4–6]. In addition to isomaltooligosaccharides, other prebiotics include fructooligosaccharides and galactooligosaccharides. Solutions of isomaltooligosaccharides administered intravenously to humans could prevent the dextran-antibody precipitation reaction, which is sometimes observed when a solution of dextran is given to humans as a plasma substitute [7]. Isomaltooligosaccharides can be produced either by acceptor reactions of dextransucrase or hydrolysis of dextran by dextranase. *Leuconostoc mesenteroides* B-512 FM dextransucrase catalyzes the polymerization of the glucosyl moiety of sucrose to form dextran, which has 95% α-(1 → 6) linkages and 5% α-(1 → 3) branch linkages. Dextran is hydrolyzed by dextranase, forming isomaltooligosaccharides (Fig. 1). Dextransucrase and dextranase have been used together to produce these oligosaccharides from sucrose because their optimum pHs are the same (pH 5.4). Soluble dextransucrase and soluble dextranase were reacted with sucrose to produce syrup containing isomaltose and isomaltotriose [8]. Goulas et al. also studied in detail the synthesis of isomaltooligosaccharides and oligodextrans by the combined use of soluble dextransucrase and soluble dextranase [9]. In a different work, dextransucrase treated with glutaraldehyde was immobilized in alginate and used with soluble dextranase to synthesize isomaltooligosaccharide from sucrose with little success; sucrose conversion dropped to 60–70% from 90% by the sixth batch [10]. Recently, a fusion enzyme of dextransucrase from *L. mesenteroides* B-1299CB4 and dextranase from *A. oxydans* was constructed and used successfully for one-step synthesis of isomaltooligosaccharides from sucrose [11].

Acceptor reactions have been widely studied for the production of isomaltooligosaccharides from glucose and maltose. In these

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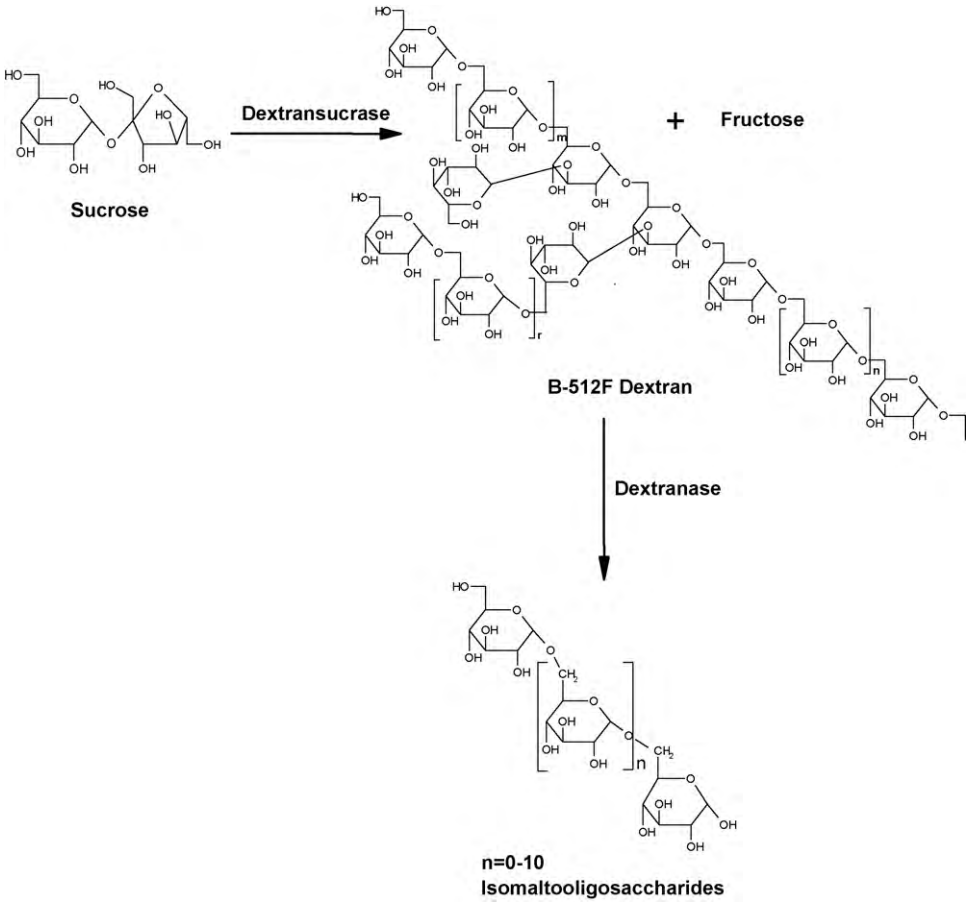

Fig. 1. Dextran formation from sucrose by dextransucrase and hydrolysis of dextran by dextranase.

studies, either soluble or alginate-immobilized dextransucrase was used [12–14]. Using immobilized enzymes makes production of isomaltooligosaccharides more economical. Studies on immobilization of dextransucrase in the literature have not been successful with respect to yield and stability. One reason is that the dextran associated with the enzyme masks the reactive groups of the enzyme. Kaboli and Reilly studied the immobilization of dextransucrase using different carriers and retained more than 10% activity upon immobilization using activated alkylamine porous silica [15]. Alcalde et al. studied the immobilization of native and dextran-free dextransucrase onto glutaraldehyde-activated porous silica, which resulted in 0.6 and 13% immobilization yields, respectively [14]. Dextran-free dextransucrase was also immobilized onto Eupergit C with a maximum activity recovery of 22%, and the work was patented [16,17]. In stability tests carried out at 30 °C, Eupergit C-immobilized dextransucrase lost more than 55% of the recovered activity. Thus, there are no reported successful covalent immobilization methods for dextransucrase. Encapsulation in alginate is the only method that results in high immobilization yield for this enzyme. There are several studies on immobilization of dextransucrase in alginate that result in immobilization yields up to 90% [13,14,18]. The encapsulation method can only be used for the production of oligosaccharides because dextran accumulates in alginate beads and causes them to rupture.

Dextranase can be used to produce isomaltooligosaccharides from dextran. The enzyme has been immobilized using different methods including glutaraldehyde-activated chitosan, which retains 63% of the original specific activity [19]. The enzyme was also immobilized on porous glass and bentonite [20,21]. Recently, dextranase was immobilized onto a commercially available matrix, Eupergit C, with high yield (90%) in our laboratory [22]. The immobilized enzyme was stable and could be used to produce isomaltooligosaccharides from dextran.

As of now, there has only been one report regarding the co-immobilization of dextransucrase and dextranase, in which dextranase pre-adsorbed onto bentonite and soluble dextransucrase were co-immobilized onto alginate beads for the production of isomaltooligosaccharides from sucrose. This method was not very successful due to the low immobilization yield for dextranase and the substantial loss in activity in the co-immobilized system during repeated batch reactions [23]. The co-immobilized enzymes were also used by the same group to synthesize isomaltose from sucrose [24]. The present study is on the successful co-immobilization of dextransucrase and dextranase in alginate matrices (beads, fibers, and capsules) to produce isomaltooligosaccharides from sucrose.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Dextran (5000 Da) was a gift from Dextran Products Limited (Ontario, Canada). Calcium acetate, sucrose, fructose, H_2SO_4, ethanol, acetonitrile, and calcium chloride were obtained from Merck. Sodium alginate (medium viscosity), maltose, soluble potato starch, and α-naphthol were from Sigma. Eupergit C was a gift from Roehm GmbH & Co. KG (Darmstadt, Germany).

2.1.2. Enzymes

Dextranase 50 L was a gift from Novozyme (Denmark). The activity of dextranase was determined as 63 IU/ml. The activity unit (IU) is defined as the amount of enzyme needed to produce 1 μmol isomaltooligosacharide (isomaltose and isomaltotriose) from a dextran solution (MW = 5000, 2% (w/v)) in 1 min at pH 5.4 and 30 °C.