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PRODUCTION OF OLIGOSACCHARIDES BY GLUTARALDEHYDE TREATED AND IMMOBILIZED BETA-GALACTOSIDASES FROM BACILLUS CIRCULANS

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Summary. The oligosaccharide-producing activity of beta-galactosidase (beta-D-galactoside galactohydrolase, EC 3.2.1.23) preparation of Bacillus circulans was increased from 21% to 40% after glutaraldehyde treatment or immobilization onto porous silicagel(Merckogel) by crosslinkage with glutaraldehyde.

Introduction. The enzyme preparation of Bacillus circulans contain mainly two major beta-galactosidases, designated beta-galactosidase-1 and beta-galactosidase-2, with different transgalactosidation activity (Mozaffar et al., 1984). In a previous paper (Mozaffar et al., 1987) we reported that oligosaccharide-producing activity of purified beta-galactosidase-1 was considerably increased after glutaraldehyde treatment. In this paper we reports an increase in the oligosaccharide-producing activity of the commercially available beta-galactosidase preparation of B. circulans after glutaraldehyde treatment or immobilization onto Merckogel.

Materials and Methods. Materials. Crude beta-galactosidase enzyme preparation from B. circulans was kindly supplied by Daiwa Kasei K.K. (Osaka, Japan). Lactose was purchased from BDH Chemicals Ltd., England. A Glucostat reagent kit and a galactose ultraviolet test kit were obtained from Worthington

Biochemical Corp. and Boehringer Mannheim GmbH, respectively. Merckogel (pore size 500 Å) was purchased from E. Merck, A.G. Glutaraldehyde was obtained from Sigma Chemical Company (USA). All of the other reagents were of analytical grade and were obtained either from BDH Chemicals, Ltd. or E. Merck.

Preparation of glutaraldehyde treated enzyme. The crude enzyme preparation, a mixture of beta-galactosidase-1 and beta-galactosidase-2, in the assay buffer (100 mM sodium phosphate buffer, pH 6.0) was treated with glutaraldehyde (final concentration, 3%) at 4°C for 2 h as described previously (Mozaffar et al., 1987). The activity of the native or glutaraldehyde treated enzyme was assayed, with 4.56% lactose as the substrate dissolved in the assay buffer, as described by Nakanishi et al. (1983). One unit of beta-galactosidase activity was defined as the amount of enzyme producing 1 μmol of D-glucose per minute at 40°C and pH 6.0.

Preparation of immobilized enzyme. Immobilized enzyme was prepared by adsorption of the enzyme onto Merckogel, and crosslinkage was done with glutaraldehyde solution (final concentration, 3%) as reported previously (Mozaffar et al., 1986a). One unit of immobilized beta-galactosidase activity was defined as the amount of wet immobilized enzyme producing 1 μmol of D-glucose (from 4.56% lactose solution) per minute at 40°C and pH 6.0.

Analysis of saccharides. The concentrations of D-glucose and D-galactose produced during the hydrolysis of lactose were assayed with Glucostat reagent and a Galactose UV test kit, respectively. Lactose and other oligosaccharides were separated by paper chromatography and the amounts were measured as described previously (Mozaffar et al., 1984; Mozaffar et al., 1985).

Results and Discussion. We have reported earlier (Mozaffar et al., 1984) that the enzyme preparation of B. circulans contain mainly two major beta-galactosidases, beta-galactosidase-1 and beta-galactosidase-2, with different oligosaccharide-producing activity. Furthermore, In a previous investigation (Mozaffar et al., 1987) we observed that oligosaccharide-producing activity of the beta-galactosidase-1 was increased from 6% to 40% after glutaraldehyde treatment or immobilization onto Merckogel by crosslinkage with glutaraldehyde. Free of immobilized beta-galactosidase-2 produces 40% oligosaccharides during hydrolysis of 4.56% lactose (Mozaffar et al., 1984; Mozaffar et al., 1986a). In this investigation, we tried to increase oligosaccharide production by using commercial

enzyme preparation treated with glutaraldehyde or immobilized onto Merckogel by crosslinkage with glutaraldehyde. Initially, we treated the enzyme preparation with 3% glutaraldehyde, the same concentration as used for immobilization or modification of the beta-galactosidase-1 (Mozaffar et el., 1987). The modified enzyme preparation produced oligosaccharides with a maximum yield of 40% at around 60% conversion of lactose (Table 1). The yield of oligosaccharides and their R_f values on paper chromatography were the same as those of modified or immobilized beta-galactosidase-1 during hydrolysis of 4.56% lactose at 40°C and pH 6.0 (Mozaffar et al., 1987). Free enzyme preparation produced 21% oligosaccharides at 38% conversion of lactose (Table 1). We immobilized the enzyme preparation onto Merckogel with glutaraldehyde as reported previously (Mozaffar et al., 1986a). Similar to modified enzyme, the immobilized enzyme also produced 40% oligosaccharides at 55% conversion of lactose (Table 1). The formation of oligosaccharides during the hydrolysis of lactose with immobilized beta-galactosidase from B. circulans was also observed by Nakanishi et al. (1983). However, they reported less oligosaccharide production than this study. This could be because formation of disaccharides other than lactose and pentasaccharides were not analyzed in their study. Also, they used different carrier (Duolite ES 762) with high immobilized enzyme units (170 units/g of wet gel). In this study we used Merckogel with low enzyme units (10 units/g of wet gel). We observed that properties of carrier and immobilized enzyme units has great influence on production of oligosaccharides(Mozaffar et al., 1986a; Mozaffar et al., 1986b).

This study is very useful from the viewpoint of practical application of the enzyme. We showed that, oligosaccharide-producing activity of the commercial enzyme preparation of B. circulans was increased after glutaraldehyde treatment or immobilization of the enzyme with glutaraldehyde.

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Table 1. Concentration of saccharides produced during hydrolysis of lactose with beta-galactosidases from Bacillus circulans.

Saccharides	Saccharides (mg/ml) produced with the		
	Free enzyme (38%)[a]	Glutaraldehyde treated enzyme (60%)[a]	Immobilized enzyme (55%)[a]
Glucose	6.1	8.8	7.6
Galactose	2.6	1.8	1.4
Disaccharide-1	0.0	1.9	1.7
Disaccharide-2	0.0	1.7	1.6
Trisaccharide	8.3	9.4	9.8
Tetrasaccharide	1.2	4.2	4.0
Pentasaccharide	0.0	0.9	1.0

[a] Percent conversion of lactose where maximum oligosaccharides are detected.

The 4.56% lactose was hydrolyzed at 40°C and pH 6.0 with beta-galactosidase as a free enzyme (final enzyme units, 5 units/ml), treated with 3% glutaraldehyde (final enzyme units, 10 units/ml), and immobilized onto Merckogel (10 units/g of wet gel).

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