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NOTE

Reaction Route for Enzymatic Production of Neofructo-oligosaccharides from Sucrose Using *Penicillium citrinum* Cells

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The production of oligosaccharides using *Penicillium citrium* cells at high sugar concentrations was investigated at 50°C and pH 5.0. Both 1-kestose and neokestose were produced from sucrose, while both nystose and tetrasaccharide were produced from 1-kestose. However, no reaction product was obtained from neofructo-oligosaccharides such as neokestose. Based on these experimental results, a hypothetical reaction route was proposed to illustrate how neofructo-oligosaccharides are formed from 1-kestose.

Key words: disproportionation reaction, neofructo-oligosaccharides, *Penicillium citrinum*, reaction route

Fructo-oligosaccharides have been used in health foods since the 1980s (4,9). The chemical structures of various types of fructo-oligosaccharides (β2→1 and β2→6) are shown in Fig. 1. In ealier publications (5, 6, 9), the enzymatic production of inulin-type fructo-oligosaccharides such as 1-kestose (GF_2) and nystose (GF_3) from sucrose (GF) by *Aureobasidium pullulans* has been reported.

Neofructo-oligosaccharides such as neokestose (FGF) can be extracted from vegetables (for example, onions, garlic, and asparagus) (1) or cereals (e.g. oats, barley, and rye) (7). The enzymatic production of neofructo-oligosaccharides with fungal enzymes has also been reported (2, 8). These oligosaccharides are synthesized by a fructose-transferring reaction with enzymes, such as β-fructofuranosidase (EC 3.2.1.26), from plants and microorganisms (8).

In a previous work (3), it was found that inulin-type fructo-oligosaccharides together with neofructo-oligosaccharides could be produced simultaneously via a number of enzyme reaction steps from sucrose using *Penicillium* cells. The present investigation examined the production of neofructo-oligosaccharides by *Penicillium* cells at high sugar concentrations, and a possible reaction route for the formation of these oligosaccharides is proposed.

Penicillium citrinum FERM P-15944 was used throughout the current study. The medium composition and culture conditions were reported previously (3). The cells were harvested by centrifugation using a table-top centrifuge (2000 rpm) at room temperature and washed twice with deionized water prior to use. The fructose-transferring activity in the free cells was determined by measuring the release of glucose (G) in the reaction mixture described below. One unit was defined as the amount of enzyme activity required to produce one micromole of glucose per minute under the following conditions: pH 5.0, temperature 55°C, and reaction mixture consisting of 200 ml of 500 g/l sucrose plus 0.5 g wet cells (approximately 80% moisture). The reaction was carried out for 30 minutes, and then stopped by heating at 100°C for 10 minutes. Thereafter, the released glucose was measured by the method described below. Unless otherwise specified, 1.5 units per g sucrose of free cells were transferred to a 1 liter bioreactor (Takasugi, Tokyo, Japan) containing a substrate, then a batch operation was carried out at 100 rpm for several days at 50°C and pH 5.0. The reaction products, including glucose, were analysed by an HPLC (Waters Associates Model 244, equipped with a differential refractometer RI-401 detector) using a Daisopak SP-120-5-ODS-B column (150 mm × 4.6 mm I.D.) (Daiso, Osaka, Japan). A mixture of acetonitrile/distilled water (75 : 25, v/v) was used as the mobile phase. An HPLC chromatogram was reported previously (3).

Table 1 summarizes the effect of the sucrose concentration on the composition of the initial reaction products. It was found that both 1-kestose and neokestose were pro-

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Table 1. Data for initial reaction products with various sucrose concentrations[a]

Substrate (g/l)	Glucose (g/l)	1-Kestose (g/l)	Neokestose (g/l)	Neokestose / (1-Kestose + Neokestose)	Molar ratio[b]
620	12.3	27.5	6.9	0.20	1.0
790	14.2	29.4	10.3	0.26	1.0
940	16.9	30.7	16.5	0.35	1.0

[a]Reactions were carried out for 8 h at 45°C.
[b]Molar ratio of glucose to 1-kestose plus neokestose.

duced simultaneously from sucrose together with the liberation of glucose. The ratio of neokestose to 1-kestose plus neokestose was found to be dependent on the sucrose concentration. The value tended to increase with an increase in the sucrose concentrations. As can be seen from Table 1, the molar ratio of glucose to 1-kestose plus neokestose was found to be about 1.0 irrespective of the sucrose concentration, indicating that a disproportionation reaction was involved. A similar disproportionation reaction (viz. $GF_n + GF_n \rightarrow GF_{n+1} + GF_{n-1}$) was previously observed in the production of inulin-type fructo-oligosaccharides from sucrose with *Aureobasidium pullulans* (6). It is interesting to note that with *Aureobasidium pullulans*, no neofructo-oligosaccharides were produced but rather inulin-type fructo-oligosaccharides were.

The batch reaction kinetics with 940 g/l sucrose are shown in Fig. 2. For this experiment, free cells equivalent to 1.5 units per g sucrose were employed. The oligosaccharides formed from this time-course reaction were found to be 1-kestose, neokestose, nystose and tetrasaccharide (FGF_2 or F_2GF), and the liberation of glucose occurred simultaneously. The total fructo-oligosaccharides (inulin-type plus neo-type) formed accounted for 55% of the total sugars after 7 days.

Fig. 3 shows the batch reaction kinetics with 940 g/l 1-kestose. Free cells equivalent to 1.5 units per g 1-kestose were used. As can be seen from Fig. 3, significant amounts of nystose and tetrasaccharide were produced. However, when neofructo-oligosaccharides, such as neokes-

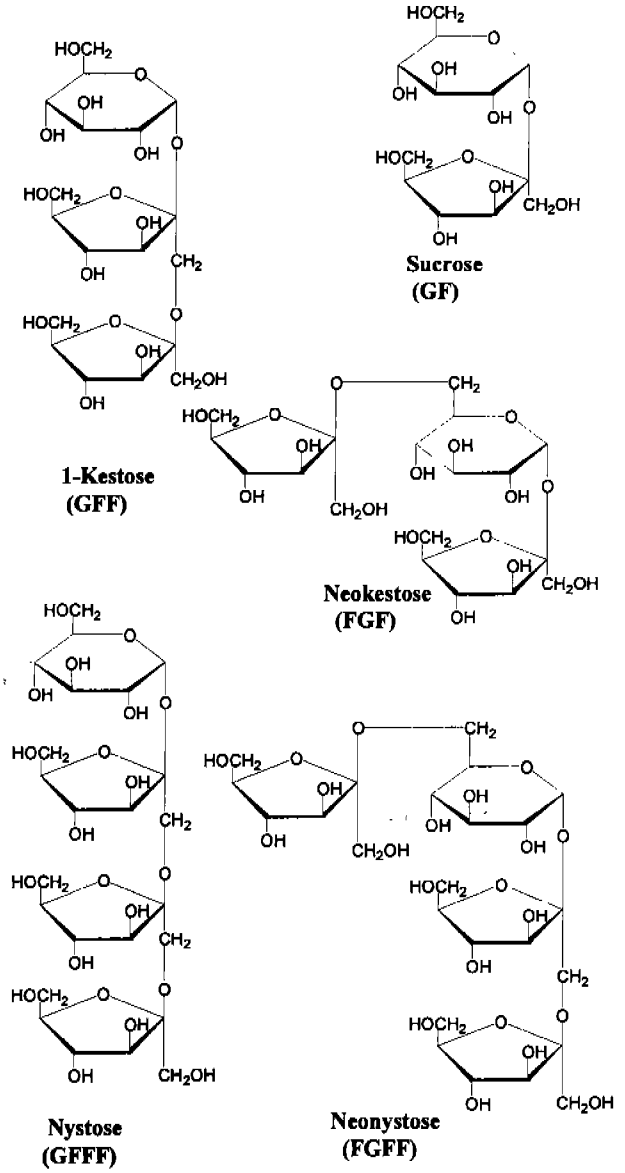

Fig. 1. Chemical structures of various types of fructo-oligosaccharides.

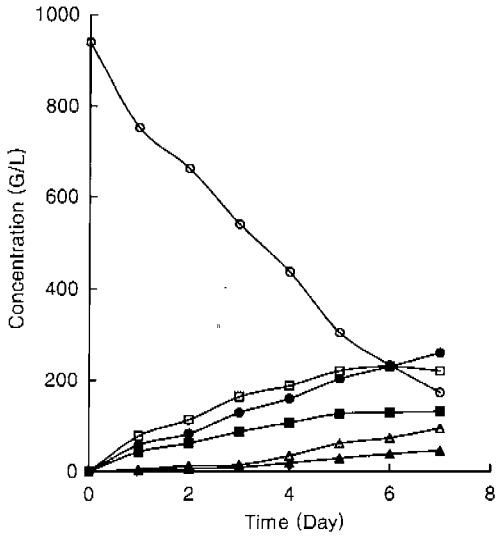

Fig. 2. Batch reaction kinetics with 940 g/l sucrose when using *P. citrinum* cells at 50°C and pH 5.0. Levels of sucrose (○), glucose (●), 1-kestose (□), neokestose (■), nystose (△), and tetrasaccharide (▲) were presented.

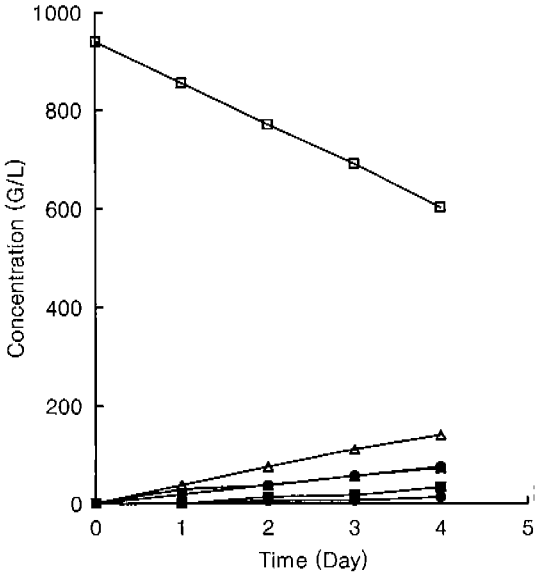

Fig. 3. Batch reaction kinetics with 940 g/l 1-kestose when using *P. citrinum* cells at 50°C and pH 5.0. Levels of sucrose (○), glucose (●), 1-kestose (□), neokestose (■), nystose (△), tetrasaccharide (▲) were presented.

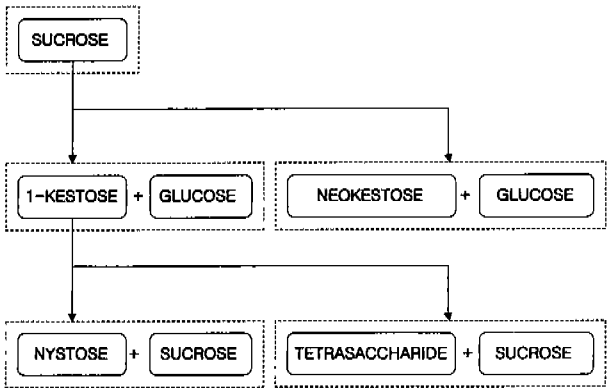

Fig. 4. Schematic reaction route for production of various oligosaccharides by *P. citrinum* cells.

tose or tetrasaccharide, were employed as the substrate, no reaction product was obtained (data not shown). This was confirmed repeatedly. Based on the experimental results described above, the reaction route is summarized in Fig. 4 to illustrate how neofructo-oligosaccharides are formed from inulin-type sugars, such as sucrose and 1-kestose. Since the reaction route for the production of neofructo-oligosaccharides has not yet been reported, the reaction route shown in Fig. 4 is a significant finding. Fig. 4 clearly shows how sucrose and glucose were both produced when 1-kestose was the substrate.

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