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Production of inulo-oligosaccharides using endo-inulinase from a pseudomonas sp.

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Inulo-oligosaccharides were produced from inulin by using high activities of an endo-acting inulinase. The total yields of oligosaccharide were slightly decreased as the concentration of inulin increased from 50 to 200 g/l. Under the optimal reaction conditions, the products consist of inulo-oligosaccharides ranging from DP (degrees of polymerization)2 to DP7, where the major oligosaccharides are 29.8% DP2, 21.4% DP3, and 8.1% DP4 oligomer, respectively. The maximum yield was 75.6% when 50 g inulin/l and 15 units/g substrate were used.

Introduction

Inulin is a linear β-(2→1)-linked fructose polymer that occurs as a reserve carbohydrate in plants of Jerusalem artichoke, chicory and dahlias (Bacon and Edelman, 1951; Flood *et al.*, 1967). Inulin represents a source for the production of ultra-high-fructose syrup through enzymatic hydrolysis by either single exo-inulinase (EC 3.2.1.26; β-D-fructofuranosidase) or cooperative action with endo-inulinase (EC 3.2.1.7; β-fructan-fructanohydrolase) (Lee *et al.*, 1988; Park *et al.*, 1991; Vandamme and Derycke, 1983). Inulin also can be a very promising source for the production of oligosaccharides when strong action of endo-inulinase is acted on inulin, excluding exo-inulinase activity. Many oligosaccharides such as fructo-, isomalto-, and galatooligosaccharides are known as 'functional sweeteners' because of their various health-promoting properties (Kuriki *et al.*, 1992, 1993; Yun *et al.*, 1990, 1992, 1993, 1994a,b). Most microbial inulinases are exoactings, which split off terminal fructose units successively from the non-reducing end of the inulin molecule. Thus many researchers have focused on the production of high fructose syrup rather than oligosaccharide production (Byun *et al.*, 1978; Zittan, 1981).

Recently, inulo-oligosaccharides have been investigated making an attempt to use them as new functional sweeteners like other oligosaccharides that have already been commercially produced (Norman and Hojer-Perderson, 1989).

In the present study, we propose a useful process for the production of inulo-oligosaccharides from inulin using the strong endo-inulinase activity derived from a new isolate *Pseudomonas* sp. No. 65 (Lee *et al.*, 1988).

Materials and methods

Materials

Pure, non-hydrolyzed inulin from dahlia tubers (Sigma) was used.

Enzyme preparation

Pseudomonas sp. No.65, a new strain isolated from soil, was cultivated at 45°C for 60 h in a 250 ml flasks containing 50 ml medium composed of (as g/l) 10 inulin, 8 $(NH_4)_2HPO_4$, 15 corn steep liquor, 0.5 KCl, 0.5 $MgSO_4 \cdot 7H_2O$, and 0.03 $FeSO_4 \cdot 7H_2O$. After removal of the cells by centrifugation (10,000 g), the supernatant was dialyzed and then treated by use of a membrane (cut off 30,000 Da), and the resulting crude enzyme solution was used throughout the experiments.

Enzyme reaction

Unless otherwise specified, enzyme reactions were carried out with 100 g inulin/l and 15 units/gram inulin at 55°C for 25 h in a shaking water bath.

Enzyme assay

Endo-inulinase activity was assayed by incubating 2 ml enzyme solution with 2% (w/v) inulin prepared in 0.1 M sodium acetate buffer (pH 5.5) at 55°C for 60 min. One enzyme unit was defined as the amount of hydrolyzed inulin (μmol) per min under the above conditions. All reaction products were analyzed by HPLC system using the Aminex HPX-42C column (cationic ion exchanger, 0.78 × 30 cm) and a refractive

index detector. The column was at 85°C and water was used as the mobile phase at a flow rate of 0.6 ml/min. For identifying the degree of polymerization of the products, thin layer chromatography was carried out on silica gel 60 plates. The plates were developed with a solvent system of propan-2-ol/ethyl acetate/water (6:2:2 by vol). Sugars were visualized by spraying the plates with sulfuric acid/phenol reagent and heating 120°C for 5 min.

Results and discussion

Effect of enzyme concentration

To determine the optimum enzyme dosage, enzyme reactions were carried out for 30 h with several enzyme concentrations using 200 g inulin/l which was the most acceptable concentration within the solubility limit under the reaction temperature. As shown in Fig. 1, the maximum yield of total oligosaccharides of each reaction mixture was 63% without any significant effect of enzyme dosage. Although enzyme concentrations higher than 15 units/g substrate greatly shortened the reaction time (from 25 to 5 h) to reach maximum yield, final total oligosaccharide concentrations were significantly reduced as reactions proceeded. In contrast, with 15 units/g substrate a reaction time of 20–30 h was needed to reach maximal yield.

Effect of initial concentration of inulin

The effect of the initial concentration of inulin on the production of inulo-oligosaccharides was studied. Enzyme reactions were conducted for 48 h under various concentrations of inulin. As illustrated in Table 1, the maximum yield (75.6%) in total inulo-oligosaccharides was observed when 50 g inulin/l was used. It was found that the products consist of inulo-oligosaccharides ranging from DP2 to DP7 being mainly DP2 and DP3 by thin layer chromatography analysis (data not shown). Moreover, considerable amounts of fructose and glucose and trace quantity of sucrose, regarded as by-products, were released. Figure 2 shows a typical reaction pattern for inulo-oligosaccharide production using 100 g inulin/l as a substrate. Inulin was completely hydrolyzed leading to formation of DP2, DP3 and other higher DP oligosaccharides successively (see also HPLC chromatogram in Fig. 3). It should be noted here that the concentration of DP2 was continuously increasing towards the end of the reaction, whereas higher oligosaccharides, namely DP3, DP4 and other products of higher DP were gradually hydrolyzed after reaching their maxima. Considering that more rapid decrease occurs in the concentration of the higher DP oligosaccharides as the reaction proceeds, it appears that the enzyme preferentially acts on the higher DP of oligosaccharides than on DP3 and DP4 at all initial inulin concentrations examined. On the basis of data from substrate specificity (Lee *et al.*, 1988); i.e., endo-inulinase used in this work cannot hydrolyze sucrose (GF), 1-kestose (GF_2), nystose (GF_3) but acts on 1^F-fructofuranosyl nystose (GF_4), it is likely that early product F_4 is hydrolyzed to F_3 and F, GF_4 to GF and F_3, F_5 to F_3 and F_2. Similar modes of endo-inulinase action have been reported from a fungal endo-inulinase (Nakamura *et al.*, 1994). More detailed kinetic studies with purified enzyme will be conducted in our laboratory.

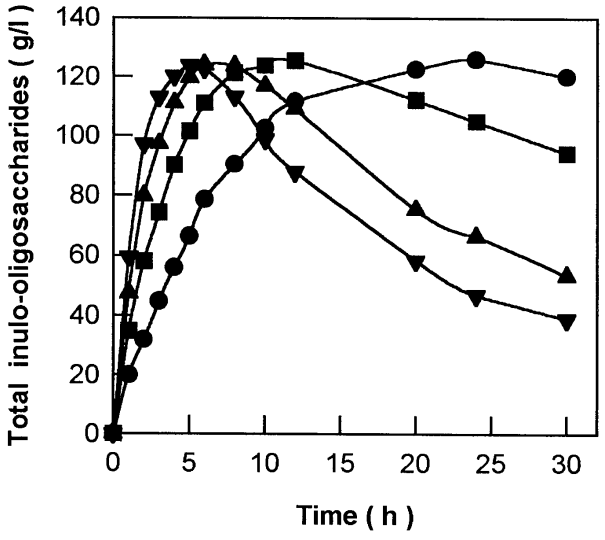

Figure 1 The effect of enzyme concentration with 200 g inulin/l as an initial substrate: Enzyme dosage (unit/g inulin); (●) 15, (■) 31, (▲) 47, (▼) 62.

Table 1 Composition of inulo-oligosaccharides produced from different initial concentrations of inulin (g/l)

	Composition (%, w/w)[a]			
Carbohydrates	50	100	150	200
Inulin	4.0	4.5	9.0	8.8
Glucose	8.5	8.2	7.9	8.7
Fructose	9.5	9.7	11.3	12.7
Oligosaccharides				
DP2	29.8	28.7	25.1	26.1
DP3	21.4	21.4	19.8	19.6
DP4	8.1	7.9	7.8	7.4
>DP4	16.3	17.1	16.9	14.5
Total oligosaccharides	75.6	75.1	69.6	67.6

[a]Product compositions are given at the reaction time of maximum oligosaccharide formation.

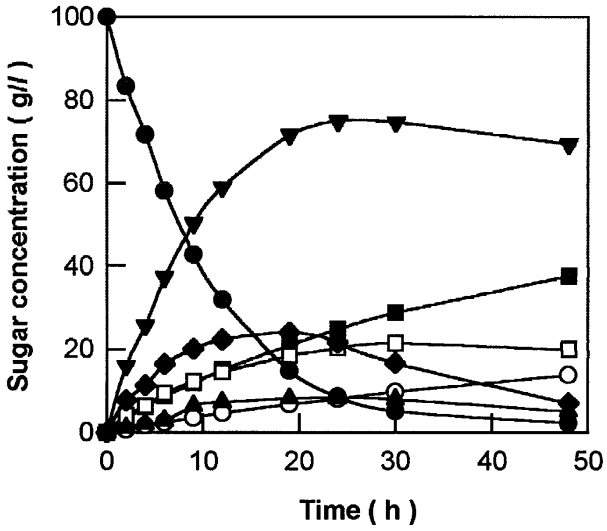

Figure 2 Typical time course of enzymic reaction with 100 g inulin/l as an initial substrate. (●) inulin, (○) fructose, (■) DP2, (□) DP3, (▲) DP4, (◆) oligosaccharides of higher than DP4, (▼) total inulo-oligosaccharides.

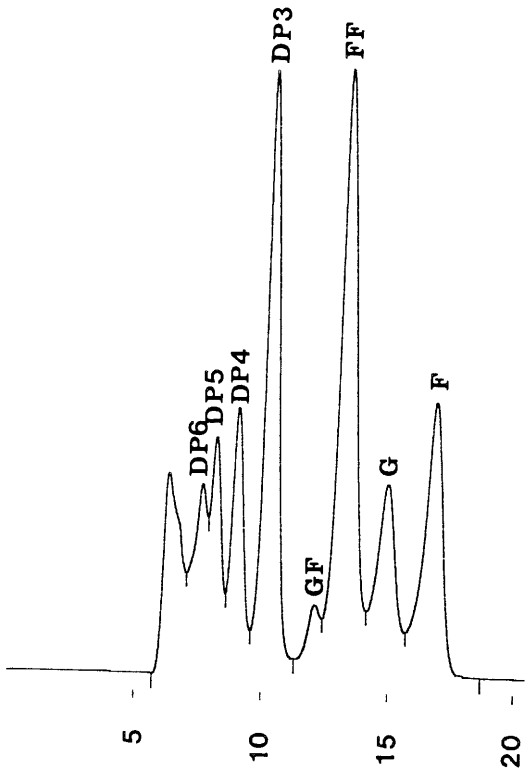

Figure 3 An HPLC chromatogram of inulin hydrolyzates: Numbers in the left mean retention time (min) and DPs refer to degree of polymerization of the products.

In conclusion, the endo-inulinase described in this work seems to have an industrial potential, in that it has no serious side functions such as invertase and exo-inulinase activities, which allows to produce high level oligosaccharides over 75% in dry sugar basis. Further studies including functionalities of inulo-oligosaccharides, enyme kinetics and process development for continuous production are under doing in our laboratory.

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References

Azhari, R., Szlak, A.M., Ilan, E. Sideman, S. and Lotan, N. (1989). *Biotechnol. Appl. Biochem.* 11, 105–117.

Bacon, J.S.D. and Edelman, J. (1951). *Biochem. J.* 48, 114–126.

Byun, S.M. and Nahm, B.H. (1978). *J. Food Sci.* 43, 1871–1873.

Flood, A.E., Rutherford, P.P. and Weston, E.W. (1967). *Nature*, 214, 1049–1050.

Kuriki, T., Tsuda, M. and Imanaka, T. (1992). *J. Ferment. Bioeng.* 73, 198–202.

Kuruki, T., Yanase, M., Takata, H., Takesada, Y., Imanaka, T. and Okada, S. (1993). *Appl. Environ. Microbiol.* 59, 953–959.

Lee, T.K., Shin, H.C., Choi, Y.J. and Yang, H.C. (1988). *Kor. J. Appl. Microbiol. Biotechnol.* 16, 484–488.

Nakamura, T., Nagamoto, Y., Hamada, S., Nishino, Y. and Ohta, K. (1994). *J. Ferment. Bioeng.* 78, 134–139.

Norman, B.E. and Hojer-Perderson, B. (1989). *Denpun Kagaku*, 36, 103–111.

Park, S.G. and Choi, Y.J. (1991). *Kor. J. Appl. Microbiol. Biotechnol.* 19, 52–56.

Vandamme, E.J. and Derycke, D.G. (1983). *Adv. Appl. Microbiol.* 29, 139–176.

Yun, J.W., Jung, K.H., Oh, J.W. and Lee, J.H. (1990). *Appl. Biochem. Biotechnol.* 24/25, 299–308.

Yun, J.W., Jung, K.H., Jeon, Y.J. and Lee, J.H. (1992). *J. Microbiol. Biotechnol.* 2, 98–101.

Yun, J.W. and Song, S.K. (1993). *Biotechnol. Lett.* 15, 573–576.

Yun, J.W., Lee, M.G. and Song, S.K. (1994a). *J. Ferment. Bioeng.*, 77, 159–163.

Yun, J.W., Lee, M.G. and Song, S.K. (1994b). *Biotechnol. Lett.* 16, 359–362.

Zittan, L. (1981). *Starch* 33, 373–377.

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