PRODUCTION OF FRUCTO-OLIGOSACCHARIDES FROM SUCROSE SYRUP BY A MICROORGANISM TSC-FOS1

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Abstract
Fructosyltransferase activity from a microorganism (designated TSC-FOS1) capable of converting sucrose to fructo-oligosaccharide (FOS) was investigated. Using cultured cells, we were able to produce FOS from 50% sucrose syrup. Maximum cellular activity of FOS production was determined to be pH 6-7 and 65°C. Enzyme activity was stable within a very narrow pH range, pH 5; and it was sensitive to higher temperature. Optimum reaction condition for FOS production was determined to be at pH 5.5 and 55°C, after considering stability factors. The activity was inhibited by FeCl3, Al2O3, and methanol. The FOS content produced by the cultured cells was between 55-60% (FOS to total carbohydrates).

Introduction
Fructo-oligosaccharides (FOS) are considered a health food because of their special properties which lower serum lipids, increase intestinal calcium absorption, and indigestible ingredients (calorie-free and safe for diabetics) (Hidaka et al., 1986; Stamp, 1990; Tokunaga, 1989). They benefit the host by stimulating the growth of beneficial microflora (e.g. acidophilus and bifidum). FOS are composed mainly of 1-kestose (GF2), nystose (GF3), and fructofuranosyl nystose (GF4), in which fructosyl units (F) are bound at sucrose (GF). These bonds cannot be broken down by enzymes in the human digestive system. Because the sweet taste is similar to table sugar, they are alternative sweeteners for consumers desiring healthier and calorie-controlled foods.

FOS is produced by the conversion of sucrose through fructosyltransferase (EC 2.4.1.9). They are found in many higher plants, such as sugar beet, onion, and asparagus (Allen and Bacon, 1956; Henry and Darbyshire, 1980; Shiomi et al., 1976; 1979). Industrial-scale FOS production is done mainly with fungal enzymes from either Aureobasidium sp. (Yun et al., 1992; 1990) or Aspergillus niger (Hidaka et al., 1987; Hidaka et al., 1988). Meiji Seika Co. (Japan) was the first to commercially produce FOS (Neosugar) by Aspergillus niger enzyme.

In this study, we characterised the fructosyltransferase activity of an isolated microorganism that is capable of converting sucrose into FOS. This enzymatic system is used to manufacture FOS in commercial scale by the Taiwan Sugar Corporation.

Materials and methods
Cultivation conditions
A fungus (TSC-FOS1) from our microorganism banks had been identified for its ability to produce FOS. This microorganism was inoculated into a 1-L shake flask from a slant, and grown for two days at 27°C. The culture was subsequently inoculated into 5 L and 100 L fermenters containing selected media. At the end of fermentation, the broth was centrifuged to harvest cells. The moisture content of the collected cells was 70-72%. The cells were dried at 35°C and ground into powder for enzyme assay.

Enzyme assay
To determine the enzyme activity, harvested cells were added to a 50% (w/v) sucrose solution at various pHs and temperatures. At the end of the reaction, the enzyme was inactivated in boiled water for 10 min. The end products were diluted 20-fold with deionized water and filtered before being subjected to high performance liquid chromatography (HPLC) to determine the amount of each carbohydrate. The activity was defined as the ratio of FOS, which were produced in the reaction, to the total carbohydrates in solution. For pH-effect study on the enzyme, Malvaine buffer and Clark and Lubs buffer were used for the differing pH ranges.

High performance liquid chromatography (HPLC)
HPLC analysis was performed with a Sugar PAK-I column (Waters, USA). The injected samples were eluted with 500 mg/L Ca-EDTA (Sigma) at 0.6 mL/min. and detected with a refractive index detector (RI). The column temperature was kept at 90°C during separation. Total FOS produced by the cells was calculated based on the deduction of the residual sucrose, glucose, and fructose at the end of the reaction from the initial sucrose.

Results
Maximal FOS production activity
In order to obtain maximum FOS production from this microorganism, we sought to determine the optimum reaction conditions. Because the majority of the fructosyltransferase existed in the
cells (data not shown), only the intracellular activity was investigated. Cells harvested from the fermentation process were dried at low temperature and kept at room temperature until use. The effects of pH and temperatures on enzymatic activity are shown in Figure 1. Temperatures from 30 to 70°C were tested for FOS production for one hour as described in the materials and methods section. As shown in Figure 1A, maximum activity was found at 65°C. The effects of pH on the enzyme were performed at pHs between 3 and 9 for one hour. As shown in Figure 1B, pH 6 to 7 resulted in maximum activity. The activity rapidly dropped off at pH above 8.

**Stability test**

The effect of temperature on the stability of the enzyme was studied by incubating the cells in pH 5.5 at various temperatures from 40 to 70°C for one hour, then subjected to FOS-production assay for one hour. The activity rapidly decreased as the cells incubated at higher temperature (Figure 2A). This indicated that the enzyme is temperature-sensitive. After incubation at 55°C for one hour, 22% (w/w) FOS was synthesized; it was only 13% at 60 to 65°C. Therefore, 55°C was chosen for further studies, although the maximum activity was found at 65°C. The cells were also incubated at 55°C for one hour with various pH (3 to 9) conditions, then subjected to the reaction described above for stability studies. As shown in Figure 2B, the enzyme is most stable within a narrow pH range, around 5.

**Effect of chemicals**

Investigation of reaction mixtures containing various chemicals at pH 5.5 and 55°C was conducted for one hour as described in the materials and methods section. As shown in Table 1, the activity was greatly inhibited by FeCl₃, Al₂O₃, and methanol.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>Relative activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>0.40%</td>
<td>69.55</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>0.1 mM</td>
<td>52.02</td>
</tr>
<tr>
<td>CaSO₄·2H₂O</td>
<td>0.1 mM</td>
<td>82.97</td>
</tr>
<tr>
<td>FeCl₃·6H₂O</td>
<td>0.1 mM</td>
<td>31.87</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.1 mM</td>
<td>96.88</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>100.00</td>
</tr>
</tbody>
</table>

FOS production. The reaction profile of FOS production by the cells is shown in Figure 3. During the reaction, sucrose was hydrolysed to glucose and fructose. The amount of glucose started to accumulate, and fructose was converted into FOS. The FOS content exceeded 50% (w/w) to total carbohydrates within 3 hours. The rate of FOS production decreased and gradually stopped after glucose reached about 11% (w/w) in the reaction mixture.

**Discussion**

Fructo-oligosaccharides (FOS) are indigestible, naturally occurring carbohydrates. They have drawn great attention from many laboratories because of their positive functional properties to humans and animals. Japan has the largest market; its market volume was 4000 metric tons in 1990. This market is expected to increase. Therefore, many companies are expanding their FOS market to the United States and Europe. For these reasons, Taiwan Sugar Corporation is also interested in FOS production.

In this study, we investigated the FOS-producing activity of a fungus (designated TSC-FOS1) obtained from our microorganism bank. The temperature for maximum FOS-production by the cells was determined to be 65°C; however, its residual activity was only 38% of the control after treating cells at this temperature for one hour. As shown in Figure 2A, the FOS-producing activity greatly decreased as the incubation temperature rose.

Although the activity at 55°C was only 85% of that at 65°C, the enzyme is more stable at this temperature. Its optimum reaction temperature for FOS production was determined to be 55°C. The maximum reaction pH was 6 (Figure 1B); however, the enzyme was most stable at pH 5 (Figure 2B). The enzyme was stable only within a narrow pH range. The FOS-producing activity at pH 6 was only 60% of that at pH 5. The optimum pH for producing FOS was then determined to be 5.5 after both activity and stability were included in the evaluation. The activity was inhibited by some chemicals (Table 1), including methanol, FeCl₃, and Al₂O₃. Similar observation was found in *Arthrobacter globiformis*. The fructosyltransferase activity purified from *Arthrobacter globiformis* was inhibited 82% by ferric ion at 1 mM of concentration (Seki *et al.*, 1989). This result is very different from that of *Aspergillus niger* (Masao *et al.*, 1989). The FOS-producing enzyme was not inhibited by ferric chloride at 1 mM of concentration.

It is known that fructosyltransferase has both hydrolysing and transfructosylating activities. During the reaction sucrose is quickly hydrolysed into glucose and fructose. The fructosyl residues are then transferred to sucrose to produce FOS, and glucose accumulates. Since glucose is a feedback inhibitor of fructosyltransferase (Jung *et al.*, 1989), the FOS in the syrup can only reach 55–66% of the total carbohydrates. Similar results were found in our FOS-production system (Figure 3). After three hours reaction, the rate of FOS synthesis decreased, and the glucose gradually accumulated.

The sucrose-hydrolysing activity was also inhibited. This system, therefore, is limited in its capacity to produce a product with high FOS content. Because of this, a two-enzyme system that included glucose oxidase in the reaction solution to raise the FOS content to 90–98% was developed (Jung *et al.*, 1993; Yun *et al.*, 1994). By incorporating glucose oxidase in the reaction solution, glucose was oxidized to gluconic acid, and the feedback inhibitor was eliminated.

The other means of producing high-content FOS syrup used column chromatography. We developed a system using 10 in-series columns of ion-exchange chromatography. With this system we obtained FOS syrup up to 90% from 60% sucrose.
Fig. 1—Effects of temperature and pH on the FOS-producing activity of the cultured cells. A: temperature effect; B: pH effect. The reactions were carried out in a reactor containing harvested cells and 50% sucrose with various pHs or temperatures for one hour. The amount of each carbohydrate was determined by HPLC. Activity was defined as the ratio of FOS produced to the total carbohydrates at the end of the reaction.

Fig. 2—Effects of temperature and pH on the stability of FOS-producing activity of the cultured cells. A: temperature stability; B: pH stability. The buffers used for different pH range were as following: pH 3 to 7, 0.1 M McIlvaine buffer; pH 6 to 9, Clark & Lubs buffers. After dried cells (0.05% [w/v]) were incubated at various temperatures or pH buffers for one hour, the enzyme solutions were added to 50% sucrose syrup for FOS production assay for one hour. The activities were assayed as described in Materials and Methods. Activity was defined as the ratio of FOS produced to the total carbohydrates at the end of the reaction.

Fig. 3—Reaction profile of FOS production by cultured cells. The reaction was carried out with 0.5% (w/v) of harvested wet cells at pH 5.5, 55°C, and 150 rpm agitation in a 5-L reactor containing 50% (w/v) sucrose syrup. At each time point, samples were boiled for 10 min. to stop the reaction, then subjected to HPLC analysis to quantify each individual carbohydrate.
syrup. Commercial-scale application is under development.

Conclusions

We have reported the properties of fructosyltransferase from a microorganism. The optimum condition for producing FOS by the cells was determined to be pH 5.5 and 55°C. The enzyme was sensitive to ferric chloride and aluminum oxide at 0.1 mM as well as methanol at 0.4%. We currently use it to produce FOS on a commercial scale with 10-ton reactors at Pu-Li Food Division, Product Development Department, Pu-Li, Taiwan.

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REFERENCES


LA PRODUCTION DE FRUCTOOLIGOSACCHARIDES A PARTIR DU SIROP DE CANNE PAR UN MICRO-ORGANISME TSC-FOS1

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Résumé

L'activité fructosyltransférase à partir de micro-organisme (appelé TSC-FOS1) capable de convertir le saccharose en fructooligosaccharide (FOS) fut étudiée. En utilisant la culture de cellules, il fut possible de produire du FOS à partir du sirop de canne à 50%. L'activité cellulaire dans la production de FOS était maximale à pH 6-7 et 65°C. L'activité enzymatique était stable dans une échelle de pH restreinte, pH 5 et était sensible à des températures plus élevées. Les conditions optimales de réaction pour la production de FOS avaient lieu à pH 5.5 et 55°C, après considération des facteurs de stabilité. L'activité était inhibée par FeCl₃, Al₂O₃ et le méthanol. La quantité de FOS produite par la culture de cellules se situait entre 55 et 60% des carbohydrates totaux.

Mots clés: FOS, fructosyltransférase, saccharose.
PRODUCCIÓN DE FRUCTOOLIGOSACÁRIDOS A PARTIR DE JARABE DE SACAROSA MEDIANTE EL MICROORGANISMO TSC- FOS1

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Resumen

Se investigó la actividad fructosiltransferasa de un microorganismo (designado TSC-FOS1) capaz de convertir sacarosa en fructooligosacárido (FOS).

Empleando células cultivadas fuimos capaces de producir FOS a partir de un sirope de sacarosa del 50%. La máxima actividad celular de producción de FOS se determinó estar a pH 6-7 y 65°C. La actividad enzimática fue estable dentro de un rango muy estrecho de pH, pH 5 y era sensible a mayores temperaturas. Las condiciones óptimas de reacción para la producción de FOS se determinó que estaban a pH 5.5 y 55°C después de considerar factores de estabilidad. La actividad se inhibía para FC13, Al2O3 y metanol. El contenido de FOS producido mediante el cultivo celular fue de 55-60%(FOS de los carbohidratos totales).

Palabras claves: FOS, fructosiltransferasa, sacarosa.